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Article

Antibacterial and cytotoxic potential of *Amburana cearensis* L. bark

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Abstract: *Amburana cearensis* is widely used in traditional medicine and has drawn scientific interest for its antimicrobial potential, especially amid rising bacterial resistance. Assessing the cytotoxicity of natural extracts is essential to establish safe and effective therapeutic concentrations. Thus, this study aimed to investigate the antimicrobial and cytotoxic potentials of the ethanolic extract obtained from the bark of *A. cearensis*. The extract was characterized phytochemically using qualitative reactions and UV-Vis spectroscopy. The antimicrobial activity was determined using the MIC, the well diffusion method and in silico prediction. The cytotoxic potential was evaluated in a eukaryotic cell model using *Saccharomyces cerevisiae*. The ethanolic extract of *A. cearensis* had a yield of 2.2%. Phytochemical screening revealed the presence of flavonoids, condensed tannins, coumarins and alkaloids. In the antimicrobial tests, the extract was active against *Pseudomonas aeruginosa* ATCC 27853, with a MIC of 1.875 mg/mL, while for *Staphylococcus aureus* ATCC 25923 e ATCC 6538 and *Escherichia coli* ATCC 25922 the MIC was over 15 mg/mL. In the in silico analysis, coumarin showed a binding affinity of -5.5 kcal/mol, establishing relevant interactions with residues adjacent to the catalytic site of the NDM-1 enzyme, suggesting a potential inhibitory effect. In the cytotoxicity assessment, no significant toxicity was observed up to 30 minutes, however, from 60 minutes onwards, there was an increase in cell mortality and flocculation formation, indicating time-dependent cell stress. These results reinforce the potential of *Amburana cearensis* as a source of bioactive compounds with promising therapeutic and biotechnological applications.

Keywords: Cumaru; *Pseudomonas aeruginosa*; *In silico* activity.

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1. Introduction

In the context of South America, Brazil stands out for having the greatest biodiversity in the world. It is estimated that there are more than 200,000 plant species registered in its territory, many of which have high potential for obtaining bioactive compounds capable of performing pharmacological functions. The use of plant species with therapeutic properties has been described since the emergence of the first civilizations, perpetuating empirical knowledge across generations. Over the centuries, this knowledge has been refined, integrating with scientific and technological advances, enabling the application of these resources in modern medicine, phytotherapy, and the development of new drugs [1].

Currently, it is estimated that about 80% of the Brazilian population uses plant-based products for therapeutic purposes. Similarly, approximately 85% of the population in developing countries uses medicinal plants to treat various diseases. This scenario is generally due to the difficulty of accessing conventional health services, making the use of plant species an important complementary strategy in promoting public health [2].

In this perspective, *Amburana cearensis* L., popularly known as *umburana*, *cumaru*, or *imburana-de-cheiro*, is a plant species belonging to the Fabaceae family, widely distributed in the semi-arid regions of Brazil. Traditionally, this plant is used in folk medicine, mainly in the form of teas, infusions, and decoctions, prepared from different parts of the plant, such as bark, seeds, and leaves. Its popular use is associated with the treatment of various diseases, especially respiratory dysfunction, inflammation, and gastrointestinal discomfort [3].

The presence of a chlorophyllated inner bark is responsible for this species' potential to maintain a certain level of photosynthetic activity even when it has no leaves during the dry season. Chemically, it stands out for the presence of coumarins, chalcones, flavonoids, tannins, fixed oils, phenolic glycosides, phenylpropanoids, and steroids. Pharmacological studies have proven its antimalarial, antiprotozoal, antifungal, antibacterial, analgesic, anti-inflammatory, antirheumatic, bronchodilator, and antioxidant activities [4].

Coumarins, one of the prominent metabolites of this species, are widely studied organic heterocycles known as 1,2-benzopyrones. These secondary metabolites are important compounds found in various plant species and contribute significantly to scientific development due to their ability to exert non-covalent interactions with protein structures. For this reason, they have a broad spectrum of biological activities and are relevant in the prevention and treatment of various diseases. In *Amburana cearensis*, the bark has a peculiar odor, characteristic of the presence of this metabolite [2-4].

Despite extensive research into the pharmacological properties of *A. cearensis*, few studies comprehensively integrate phytochemical characterization with functional biological data, especially with regard to antimicrobial activity correlated with cellular cytotoxicity. In addition, the use of in silico approaches to predict the interaction of its metabolites with molecular targets is still in its infancy. This gap compromises the critical evaluation of the species' therapeutic potential, since traditional use alone does not ensure efficacy or safety. Thus, studies that combine experimental and computational methods are essential to validate and guide the rational use of its bioactive constituents. With this in mind, this study aimed to investigate the antimicrobial activity, in vitro and in silico, of *A. cearensis*, in addition to characterizing its phytochemical profile and evaluating its cytotoxicity, with the aim of contributing to the identification of new therapeutic strategies.

2. Results

2.1. Phytochemical screening

The results of the preliminary phytochemical analysis of the secondary metabolite groups identified from the ethanolic extract obtained from the bark of *Amburana cearensis* (*cumaru*) are presented in Table 1. Based on the results obtained, it was possible to identify the presence of phenolic and nitrogenous compounds in the ethanolic extract obtained from the bark of *A. cearensis*. The presence of flavonoids, condensed tannins, coumarins, and alkaloids was verified.

After evaporation of the solvent, the dry extract was obtained, with a mass of 1.10 grams. Considering the mass of dried and pulverized bark (50 grams) used to obtain the extract, and applying the equation to evaluate the yield, a yield of 2.2% was identified.

Table 1. Results of phytochemical analysis of the ethanolic extract of *Amburana cearensis*.

Secondary metabolite	Reaction result	Presence/absence
Alkaloids	Orange precipitate [a]	Present
Flavonoids	Yellow coloration [b]	Present
Tannins	Precipitate formation [c]	Present
	Green coloration [d]	Present
Saponins	Foam formation [e]	Absent
Coumarins	Green fluorescence [f]	Present

[a] Dragendorff's reagent; [b] Shinoda reaction; [c] Reaction with 2% gelatin solution; [d] Reaction with ferric chloride; [e] Afrossimetric index; [f] Reaction with NaOH.

2.2. Characterization of the chemical pattern of products by UV-Vis spectrophotometry

The chemical profile obtained by spectrophotometry is shown in Figure 1, showing an intense absorbance peak in the region between 200 and 230 nm, followed by bands of lower intensity that extend to approximately 300 nm.

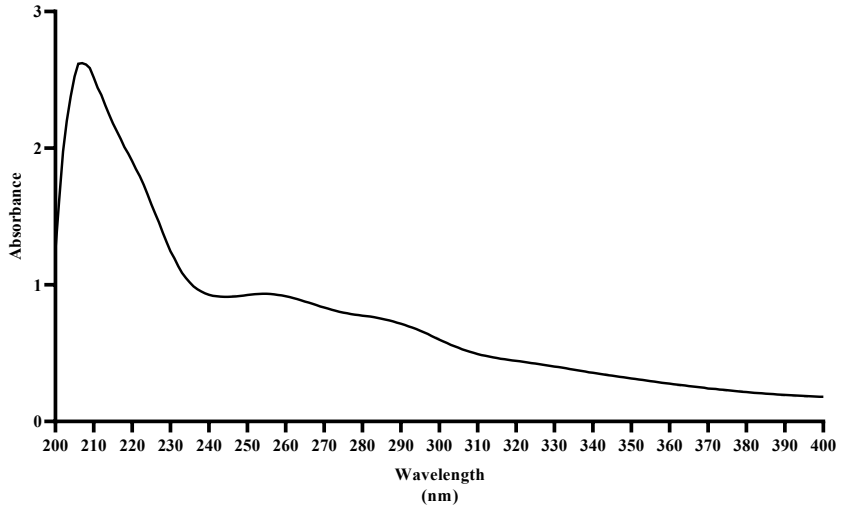


Figure 1. Characterization of the chemical pattern by UV-Vis spectrophotometry of the ethanol extract of *Amburana cearensis*.

2.3. Antimicrobial activity

2.3.1. Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the extracts against different bacterial strains was determined using the well diffusion method, including sensitive isolates of both Gram-positive and Gram-negative bacteria. As shown in Table 2, it was observed that the extract was able to inhibit the growth of the *Pseudomonas aeruginosa* strain (ATCC 27853) at a concentration of 1875 μg/mL.

Table 2. Minimum inhibitory concentration, by diffusion in a well cavity, of the extract of *Amburana cearensis*.

Gram	Microorganisms	MIC
Positive	*Staphylococcus aureus* ATCC 25923	>15000 μg/mL
	Staphylococcus aureus ATCC 6538	>15000 μg/mL
Negative	*Escherichia coli* ATCC 25922	>15000 μg/mL
	Pseudomonas aeruginosa ATCC 27853	1875 μg/mL

2.3.2. In silico prediction

Before performing molecular docking with coumarin, the original ligand present in the active site of the NDM-1 enzyme was removed and subsequently subjected to structural optimization. Next, the optimized ligand was repositioned in the active site of the protein in order to validate the docking protocol employed. The overlap between the original and repositioned structures resulted in RMSD values lower than 2 Å (Figure 2, Table 3).

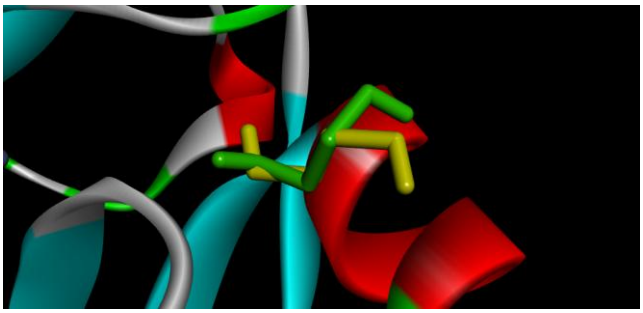


Figure 2. Validation of the fitting protocol by overlapping the connector (green) and after refitting (yellow).

Table 3. Binding affinity and RMSD of the original ligand and coumarin.

Compound	Affinity (kcal/mol)	RMSD (Å)
Original ligand	-3.3	0.7298
Coumarin	-5.5	-

Figure 3A shows the molecular interactions between coumarin and NDM-1. The binding affinity of coumarin, the major compound described for *Amburana cearensis*, was -5.5 kcal/mol. Even so, coumarin established relevant interactions with residues close to the catalytic site, suggesting inhibitory potential. Three-dimensional visualization revealed the formation of a conventional hydrogen bond with the Lys211 residue, a Pi-hydrogen donor interaction with Lys216, and Pi-Sigma and Pi-Alkyl hydrophobic interactions with Ala215. The distances between the atoms involved in the interactions ranged from 2.61 Å to 4.88 Å, indicating the stability of the complex formed.

In contrast, the co-crystallized ligand showed a lower binding affinity (-3.3 kcal/mol) but established direct interactions with residues essential for catalysis, including conventional hydrogen bonds with Glu152 and Asp192, and an unfavorable donor–donor interaction with Thr190. The short interatomic distances (2.05–2.15 Å) indicate strong anchoring within the active site, highlighting a binding mode highly favorable for inhibition despite its smaller molecular size.

Overall, these results suggest that while coumarin provides a more energetically stable interaction near the catalytic site, the co-crystallized ligand achieves direct engagement with key catalytic residues.

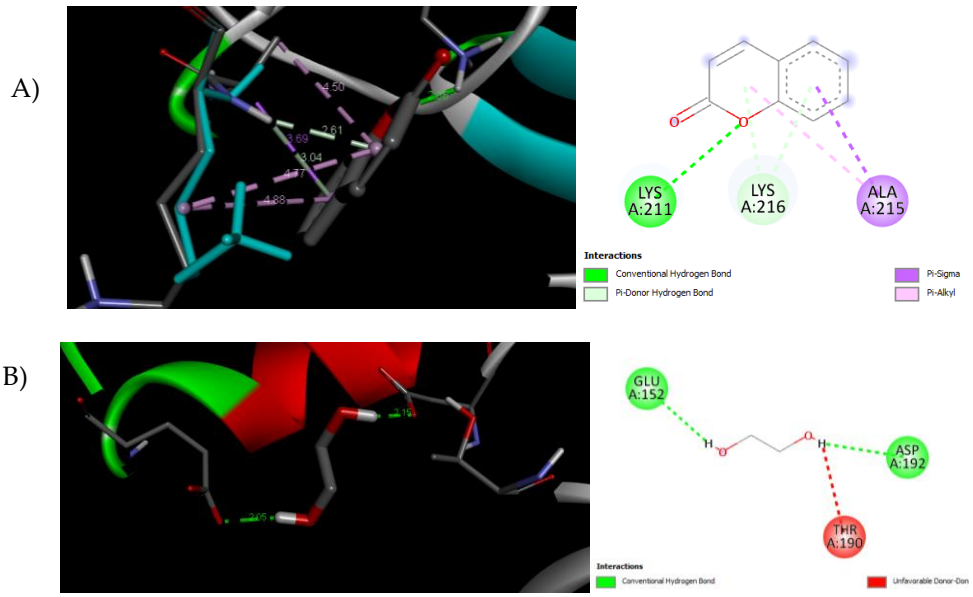


Figure 3. Ligand-protein complex between the NDM-1 enzyme and the coumarin (A) and original ligand (B) and its interaction with amino acids.

2.4. Cytotoxic activity

The yeast cell mortality data were analyzed by two-way ANOVA, revealing statistically significant effects for time, treatment, and the interaction between both ($p < 0.0001$ for all factors). At 30 minutes, it was observed that the *Amburana cearensis* extract did not show significant cytotoxic activity, since there was no statistical difference between the group treated with the extract and the growth control. However, cell mortality in the treated group was significantly lower than that of the positive control. At 60 minutes, the mortality rate in the group treated with the extract was equal to that observed in the positive control, a behavior that remained at 90 and 120 minutes. Interestingly, in the last readings, it was noted that the growth control had a mortality rate similar to that of the positive control and extract-treated groups, suggesting a possible loss of cell viability over time even in the absence of treatment (Figure 4).

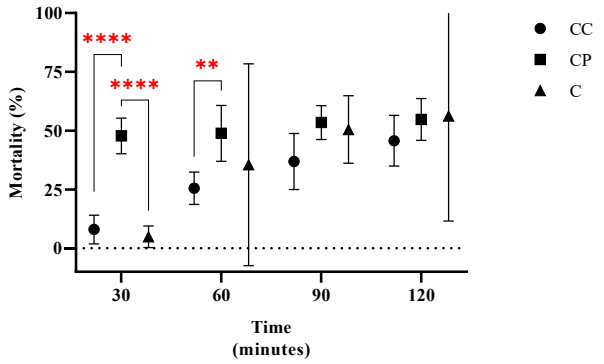


Figure 4. Temporal assessment of mortality in different treatment groups. ** = p-valor = 0,0031; **** = p-valor < 0,0001.

The progression of cell flocculation observed over time reveals a pattern consistent with stress response and possible toxicity of the extract on yeast cells. As recorded in Table 3, no flocculated cells were identified after 30 minutes of exposure. However, after 60 minutes, the formation of cell clusters began, intensifying at 90 and 120 minutes (Table 4).

Table 4. Visual records of flocculation of living and dead cells in the sample.

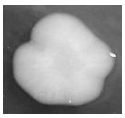
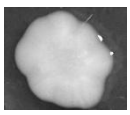
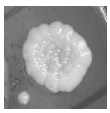
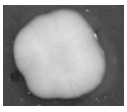
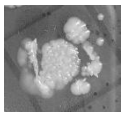
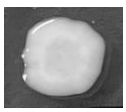
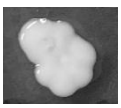
Time (minutes)			
30	60	90	120
No presence of flocculated cells			

When observing the colonies developed at different exposure times to *cumaru* extract, a progressive reduction in the number and size of colonies is noted as the contact time increases, especially in the group treated with the extract. This pattern is indicative of cumulative cytotoxic activity, in which cell viability is compromised in proportion to the exposure time (Table 5).

At 30 minutes, the group treated with *Amburana cearensis* still has colonies with morphology and size similar to the negative control, which is consistent with the absence of statistically significant cytotoxicity at this interval, as indicated in the ANOVA analysis. After 60 minutes, however, there was a marked reduction in the density and size of the colonies in the *Amburana cearensis* group, similar to that observed in the positive control (ethanol), reinforcing the interpretation that the extract begins to exert a relevant cytotoxic effect (Table 5).

The progressive degradation of the colonies in the images (90 and 120 minutes) coincides with the findings of the mortality graph, where it is observed that the group treated with the extract has cell death rates comparable to the positive and negative (or growth) controls (Table 5).

Table 5. Development of *S. cerevisiae* colonies after 48 hours of incubation.

Time (minutes)				
	30	60	90	120
Growth control				
Positive control				
A. cearensis				

3. Discussion

The present study aimed to investigate the antimicrobial activity of *Amburana cearensis*, in addition to characterizing its phytochemical profile and evaluating its cytotoxicity, with a view to contributing to the identification of new therapeutic strategies. According to Aboud [5], plants produce secondary metabolites as a defense strategy against biotic and abiotic stresses. Among the main representatives of this class of compounds are alkaloids, flavonoids, terpenoids, phenols, and saponins. These substances have a wide range

of biological activities with pharmaceutical potential and are fundamental for the research and development of new drugs. Relevant properties such as antibacterial, anticancer, antidiabetic, anti-inflammatory, antioxidant, antiviral, cytotoxic, and hypoglycemic actions have already been identified in these metabolites [6].

The results obtained in the phytochemical screening corroborate the study by Canuto, Silveira, and Bezerra [7], who identified and isolated several secondary metabolites from the species *A. cearensis*, including: phenolic compounds such as vanillic acid; flavonoids such as quercetin, kaempferol, and afrormosine; and coumarins such as umbelliferone and apigenin. Araújo [8] evaluated the phytochemical composition of the glycolic extract from the bark of the *A. cearensis* stem and identified the presence of alkaloids and tannins. Negri et al. [9], in a study with methanolic extract from the stem bark, identified the predominant presence of coumarins, such as: simple coumarin, dihydrocoumarin, and scopoletin.

Studies indicate that hydroethanolic extracts from the stem bark have anti-inflammatory, analgesic, antispasmodic, and bronchodilator activities, with no evidence of toxicity at usual doses. Phytochemical studies show that the main chemical component found in plant derivatives obtained from the stem bark of *A. cearensis* is coumarin, which, together with other substances, is responsible for the bronchodilator activity, already validated experimentally and corroborated by popular use and home treatment, especially in children and the elderly [10].

Another coumarin found in *A. cearensis* is dicumarol, responsible for the hypoprothrombinic effect, due to its competitive action with vitamin K, which has the activity of antagonizing the hepatic enzyme that acts in the synthesis of prothrombin [11]. Thus, many studies have already attributed a considerable number of pharmacological activities to coumarins, such as hypotensive, antimicrobial, anti-inflammatory, antitumor, antimalarial, leishmanicidal, and anti-chagasic [12,2].

Regard to the chemical profile obtained by spectrophotometry, the absorbance peak observed is typical of conjugated aromatic compounds, such as coumarin, one of the main secondary metabolites present in *cumaru*. In fact, coumarin has intense absorption bands in the ultraviolet region, especially between 210 and 230 nm [13]. Thus, the signals observed in the spectrum strongly suggest that coumarin is the main compound responsible for the absorption profile detected.

When comparing the minimum inhibitory concentration result with the study by Silveira et al. e Lima et al. [3,14], whose objective was to systematically review the phytochemical composition, uses in traditional medicine, and bioactivities associated with *Amburana cearensis*, as well as some of its isolated compounds, it was found that *cumaru* also exhibited antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853, although at a higher concentration (6900 μg/mL), using bark extract obtained with chloroform. However, the study also showed that extracts obtained from other parts of the plant exhibited antimicrobial activity against all strains tested at a concentration lower than 1024 μg/mL.

The difference in MICs observed can be attributed to seasonality, which directly influences the biosynthesis of secondary metabolites. Depending on the collection period, plant material can produce different amounts of these metabolites, which are responsible for the plant's pharmacological activities. In addition, the extraction method can also impact the results, and it is possible that the ethanol extraction used in this study was more efficient than chloroform extraction [15].

Considering the worrying advance of bacterial resistance, this study evaluated the interaction of coumarin, described in the literature as one of the major compounds of *cumaru*, with key enzymes involved in the antimicrobial resistance of *Pseudomonas aeruginosa*, with emphasis on NDM-1. The analysis was based on the use of bioinformatics as a strategic initial screening tool for identifying new compounds with potential antibacterial activity. The NDM-1 enzyme has been widely studied due to its epidemiological relevance and the urgent need for new therapeutic approaches capable of neutralizing it.

P. aeruginosa is currently one of the leading causes of Gram-negative bacterial infections worldwide, especially in immunocompromised patients. The global spread of carbapenem-resistant strains, often associated with high-risk epidemic clones widely disseminated in hospital settings, poses a serious threat to public health. Carbapenem resistance in this species results from a combination of factors, including the production of β-lactamases, mutations in porins, overexpression of the MexA-MexB-OprM efflux pump, and alterations in penicillin-binding proteins [16, 17].

Among these β-lactamases, NDM-1 (New Delhi metallo-β-lactamase-1) stands out as one of the main carbapenemases responsible for conferring resistance to *P. aeruginosa* on a global scale, with reports of its occurrence in several countries, such as Spain, Ukraine, Greece, Iran, South Korea, Morocco, the United States, and the Netherlands [16,17,18,19,20,21,22]

Regard to the validation of molecular docking, the overlap between the original and repositioned structures resulted in RMSD values lower than 2 Å, which indicates high accuracy and reliability of the method adopted, as recommended by Castro-Alvarez, Costa, and Vilarrasa [23], who consider RMSD ≤ 2 Å as an adequate criterion for validating molecular docking studies.

Although coumarin interacted with residues from the active site, no direct interactions were observed with the amino acids that coordinate the Zn^{2+} ions essential for the enzyme's catalytic activity, specifically His120, His122, Asp124, His189, Cys208, and His250. Furthermore, according to the literature, other compounds anchored to NDM-1 have predominantly shown hydrogen bonding interactions with the Lys211 residue, a pattern also observed in the present study [24].

Few studies have focused on performing molecular docking simulations to identify potential inhibitors of metallo-β-lactamases. However, with the aim of developing new therapeutic candidates, Barón [25] designed 140 benzimidazolyl acetamide derivatives using in silico approaches. Among these molecules, 3-(5-(3-hydroxybenzyl)-1H-pyrrol-2-yl)-N-(5-nitro-1H-benzo[d]imidazol-2-yl)propanamide stood out as the most potent inhibitor identified against the NDM-1 enzyme.

Regarding the toxicity of medicinal plants, Pereira et al. [2] emphasize the importance of subjecting herbal preparations and herbal medicines to rigorous testing to ensure their safety and prevent potential health risks to users. In this context, the hydroalcoholic extract of the stem powder of A. cearensis has demonstrated cytotoxicity against different cell lines, confirming the findings of the present study [3]. Notably, a time-dependent increase in cell mortality was observed, with values exceeding 70% at 90 and 120 minutes, and statistically significant effects of time, treatment, and their interaction ($p < 0.0001$). These results reinforce the previously reported cytotoxic effects of A. cearensis, including promising activity against several cancer cell lines. Furthermore, flocculation may be related to a cellular defense mechanism against the toxic action of the extract, as flocculation is recognized as an adaptive response that aims to isolate damaged cells and protect the remaining cell population. Therefore, the presence and gradual increase in flocculation suggest that Amburana cearensis extract induces time-dependent cellular stress, corroborating the cell mortality data. It should be noted that the presence of flocculation suggests that the cells that did not die activated defense mechanisms, which may also impact the ability to replicate and form viable colonies.

4. Materials and Methods

4.1. Collection and preparation of botanical material

The bark of *Amburana cearensis* was purchased from a herbal store located in the municipality of Governador Dix-Sept Rosado, in the state of Rio Grande do Norte, Brazil. The plant material was subjected to hygienization and artificially dried in an oven at 35 °C for seven days, as described by Melo, Radünz, and Melo [26]. After drying, the bark was ground using a knife mill to obtain the powdered plant material (vegetable drug).

4.2. Preparation of ethanolic extract

To obtain the extract, 50 g of the bark powder were macerated in 250 mL of 99.5% ethanol (v/v) in a closed system and kept at room temperature for 72 hours. The resulting solution was then filtered. The ethanolic extract was placed in an oven at a controlled temperature of 35 °C until complete evaporation of the solvent. The extract yield was calculated using the following formula:

$$Re = \left(\frac{Pext}{Pcascas}\right) x\ 100$$

Where: Re = Total extract yield (%); Pext = Weight of dry extract (g); Pcascas = Weight of fresh or dried bark (g).

4.3. Phytochemical screening

The phytochemical screening was carried out through qualitative tests based on colorimetric and precipitation reactions in order to characterize the phytochemical profile of the ethanolic extract of *Amburana cearensis* L. (Table 5).

Table 5. Phytochemical screening reactions of the ethanolic derivative of *Amburana cearensis* L. Source: Matos et al., 2014. [27]

Metabolite Class	Identification Method	Expected Result
Alkaloids	Dragendorff's Reagent	Formation of orange precipitate
Flavonoids	Shinoda Reaction	Appearance of red, pink, or yellow coloration
Tannins	2% Gelatin Solution Reaction	Formation of a precipitate
	Ferric Chloride Reaction	Appearance of blue or green coloration
Saponins	Froth Index	Formation of persistent foam
Coumarins	Sodium Hydroxide Reaction	Fluorescence under UV light

4.4. Characterization of the chemical profile by UV-Vis spectrophotometry

In this assay, the dried extract of *Amburana cearensis* was directly diluted in test tubes to obtain solutions at a concentration of 234.25 μg/mL. UV-Vis spectrophotometric analyses were performed using a UV-Vis spectrophotometer (Bel Photonics UV M-51), scanning from 200 to 400 nm with a 1 nm interval, using the diluent solution as the blank. The absorbance values obtained were processed and graphically represented using GraphPad Prism software, version 9.5 [28].

4.5. Antimicrobial activity

4.5.1. Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of the ethanolic extract of *Amburana cearensis* was evaluated using the agar diffusion method with the well-diffusion technique, as described by Helmy et al. [29]. The assay was performed against the bacterial strains *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 27922, and *Pseudomonas aeruginosa* ATCC 27853.

Petri dishes (90 × 15 mm) containing sterile Mueller-Hinton agar were used, and the medium surface was inoculated with a sterile swab soaked in a bacterial suspension adjusted to 1.5×10^6 CFU/mL. Six wells of 10 mm diameter were made in each plate. Each well received 100 μL of the extract at concentrations of 15, 7.5, 3.75, 1.875, and 0.9375 mg/mL, along with a vehicle control, namely: dimethyl sulfoxide, tween, and distilled water. Plates were incubated at 37 °C for 24 hours. After incubation, inhibition zones were measured in millimeters. All tests were performed in triplicate.

4.5.2. In silico prediction

The in silico antimicrobial activity was evaluated using coumarin, one of the major secondary metabolites identified by Canuto, Silveira, and Bezerra [29] in the bark of A. cearensis, targeting the NDM-1 protein (PDB ID: 5ZGZ), whose 3D structure was retrieved from the Protein Data Bank [31].

The molecular structures of the compounds were obtained in 2D from the ChemSpider database [32], converted into 3D, protonated, adjusted to pH 7.4, energy-minimized, and saved in PDB format. In AutoDockTools, Gasteiger charges were assigned, rotatable bonds were defined, and files were converted to PDBQT format. The same protocol was applied to the reference inhibitor of the target protein.

Protein preparation included removal of water molecules and cofactors, addition of polar hydrogens, and exclusion of co-crystallized ligands. The docking region was defined based on the protein's active site with the following coordinates: X = –2.022; Y = –7.864; Z = –22.915.

Protocol validation was performed through redocking of the co-crystallized ligand using AutoDock Vina, and accuracy was evaluated based on RMSD values using Discovery Studio software. After methodological validation, molecular docking simulations were carried out, binding energies (kcal/mol) were recorded, and the most favorable conformations were selected for detailed analysis of interactions within the protein's active site.

4.6. Cytotoxicity activity

4.6.1. Culture conditions

The cytotoxicity of the ethanolic extract of *A. cearensis* was assessed using *Saccharomyces cerevisiae*, a eukaryotic cell model. Yeast cells were cultured in Erlenmeyer flasks containing 0.1 g of lyophilized yeast, 2 g of glucose, and 18 mL of sterile distilled water. The mixture was homogenized using a Vortex mixer. Then, 2.0 mL of the *A. cearensis* extract at a final concentration of 3 mg/mL was added. For the positive control flask, 2 mL of ethanol (96° GL) was added, and for the growth control, 2 mL of sterile saline solution (0.85%) was used. The flasks were incubated at 30 °C with shaking at 250 rpm for 120 minutes. The effects of cell exposure were assessed every 30 minutes for a total of 120 minutes, as described by Sarabia et al. [33].

4.6.2. Mortality assessment

To analyze the mortality rate, 100 μL aliquots were collected every 30 minutes and transferred to Eppendorf tubes containing 900 μL of methylene blue. After homogenization, the mixture was applied to a Neubauer chamber and cells were observed under an optical microscope (10x and 40x objectives) for the count and identification of viable, non-viable, and flocculating cells, following the methodology adapted from Lee et al. [34].

4.6.3. Colony growth

To assess the colony growth of *Saccharomyces cerevisiae* after exposure to the *A. cearensis* extract, Petri dishes containing Sabouraud agar were inoculated with 10 μL from each cultured flask. Aliquots were collected every 30 minutes and immediately plated on the culture medium. Plates were incubated at 30 °C for 48 hours. Colony growth was assessed visually and qualitatively, based on the methodology adapted from Mueller et al. [35].

4.7. Statistical analysis

Statistical analysis was performed using two-way ANOVA to evaluate the main effects of time, treatment, and their interaction on yeast cell mortality. Data were expressed as mean ± standard deviation, and a significance level of $p \leq 0.05$ was adopted. Tukey's

post-hoc test was used for multiple comparisons between groups. All analyses were performed using GraphPad Prism software, version 9.5.

5. Conclusions

In summary, *Amburana cearensis* L. is a promising species for the development of new products containing bioactive compounds with therapeutic potential. Phytochemical screening confirmed the presence of relevant secondary metabolites, particularly coumarins, which are recognized for their wide range of biological activities. The ethanolic bark extract demonstrated effective antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853, an opportunistic pathogenic microorganism of great clinical relevance. Molecular docking analysis suggests a potential inhibitory effect of coumarin, evidenced by its interactions with residues adjacent to the catalytic site of the NDM-1 enzyme, which is directly associated with bacterial resistance mechanisms.

Furthermore, cytotoxicity assays indicated that although no significant toxicity was observed within the first 30 minutes of exposure, from 60 minutes onwards there was a progressive increase in cell mortality and flocculation formation, characterizing a scenario of time-dependent cellular stress. These findings reinforce the biotechnological and pharmacological potential of *A. cearensis*, highlighting its relevance as a source of bioactive compounds and justifying further research, including the isolation of active principles, in vivo assays, and the development of new therapeutic approaches.

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