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Original Article

Antimicrobial potential of extracts from leaves and culms of an Amazonian native bamboo

Potencial antimicrobiano de extratos de folhas e colmos de um bambu nativo da Amazônia

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Abstract

Antibiotics have shown less efficiency against resistance of pathogenic microorganisms. As a result, research centers have sought therapeutic alternatives against multidrug resistance of bacteria to antibiotics, one of which is using plant extracts. Bamboo extracts are used for several medicinal purposes. This study aimed to evaluate the antibacterial potential of hydroalcoholic extracts of culms and leaves of the species *Guadua* aff. *lynnclarkiae* on strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. We evaluated the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Only the leaves of *G.* aff. *lynnclarkiae* showed a bactericidal effect for all tested strains with MBC ranging from 1.55 mg ml^{-1} to 25 mg ml^{-1}. The culms had bacteriostatic action with MIC ranging from 1.55 mg ml^{-1} to 6.25 mg ml^{-1}, and bactericidal action at the concentration of 6.25 mg ml^{-1} only for *S. aureus*. This study provides bases for the use of this Amazonian native bamboo in bioprospecting.

Keywords: *Guadua* aff. *lynnclarkiae*, drugs, bactericides, bioprospection.

Resumo

Os antibióticos têm mostrado menor eficiência contra a resistência de microrganismos patogênicos. Assim sendo, centros de pesquisa têm buscado alternativas terapêuticas contra a multirresistência das bactérias aos antibióticos, sendo uma delas o uso de extratos vegetais. Extratos de bambu têm sido usados para diversos fins medicinais. Este trabalho teve como objetivo avaliar o potencial antibacteriano de extratos hidroalcoólicos de colmos e folhas da espécie *Guadua* aff. *lynnclarkiae* em cepas de *Staphylococcus aureus*, *Streptococcus pneumoniae* e *Klebsiella pneumoniae*. Avaliamos a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM). Apenas as folhas de *G.* aff. *lynnclarkiae* mostraram efeito bactericida para todas as cepas testadas com CBM variando de 1,55 mg ml^{-1} a 25 mg ml^{-1}. Os colmos apresentaram ação bacteriostática com CIM variando de 1,55 mg ml^{-1} a 6,25 mg ml^{-1}, e ação bactericida na concentração de 6,25 mg ml^{-1} apenas para *S. aureus*. Este estudo fornece bases para o uso deste bambu nativo da Amazônia na bioprospecção.

Palavras-chave: *Guadua* aff. *lynnclarkiae*, drogas, bactericidas, bioprospecção.

1. Introduction

Antibiotics have shown less efficiency against the resistance of pathogenic microorganisms (Prestinaci et al., 2015; Landecker, 2016). Although many pharmaceutical laboratories have been producing natural or synthetic antibiotics and modifying existing ones, the unrestricted consumption of antibacterials, in addition to increasing the resistance of pathogenic bacteria, has caused severe damage to human health (Yeh et al., 2022).

As a result, research centers have sought new alternatives against the multi-resistance of bacteria to antibiotics, one of which is using plant extracts (Silva et al., 2009). The bioactive constituents of plants serve as models for the synthesis of numerous drugs, while sharpening research on new molecules and substances with medicinal potential (Ledoux et al., 2018).

Natural therapeutic compounds derived from plants have antimicrobial properties commonly used to intervene in most human diseases that are susceptible to (Shinwari, 2010; Javed et al., 2023). Using drugs derived from plant extracts is effective in treating almost all types of diseases

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(Fouche et al., 2008). In addition to offering more effective molecules in combating antibiotic resistance, plant extracts also have low toxicity, which drug control agencies require globally (Lukitaningsih et al., 2020).

Bamboo species are used for this purpose among the diversity of plants used in traditional medicine to treat diseases caused by bacteria (Afrin et al., 2012; Gagliano et al., 2022). Bamboo belongs to the Poaceae family, Bambusoideae subfamily, with more than 1400 species cataloged worldwide (Clark et al., 2015). It is divided into three tribes: Arundinarieae with about 546 temperate woody bamboos, Bambuseae with 812 tropical woody bamboos, and Olyreae with 124 species of herbaceous bamboos (Clark et al., 2015). Bamboos vary in size, with small species, such as *Sasa borealis* in Japan, averaging 50 cm in height, and large species such as *Dendrocalamus giganteus* Munro in tropical Asia, reaching 40 meters or more (Benton, 2015). This plant appears more frequently in regions of hot temperatures and high rainfall such as tropical and subtropical Asia, South America, Africa, and Oceania (Benton, 2015). Brazil has the second largest diversity of bamboo species in the world, second only to China (Canavan et al., 2016), and there are 258 species of native bamboo, divided into herbaceous bamboos and woody bamboos (Filgueiras and Viana, 2017).

Among some species that occur naturally in the Acre vegetation (West Brazilian Amazonian region), there are some vegetative patches formed by populations of *Guadua* aff. *lynnclarkiae* Londoño, known as marona in Peru, and taboca, tabocão, or giant taboca in Acre (Londoño, 2013; Silva et al., 2019). It is a kind of arborescent bamboo, woody and thorny, with a pachymorphic rhizome and long reproductive cycles. Its culms are cylindrical and hollow, measuring 20-27 meters in height and 9-17 cm in diameter (Londoño, 2013). Due to the lack of knowledge about its uses, the species has been treated as an invasive and its natural populations have been reduced with the use of fire by the local population (Silva et al., 2019).

The most consolidated use of bamboo is for constructive purposes, however several studies already prospect the action of bamboo extracts for medicinal purposes, such as to produce drugs and cosmetics. It has already been proven from studies, the use of bamboo in the control of diabetes and cholesterol levels (Singhal et al., 2013), the presence of antioxidant activities and anti-inflammatory effects (Jung et al., 2009; Van Hoyweghen et al., 2014), anti-cancer effects (Lin et al., 2008) and some studies proved the bactericidal action of bamboo (Gagliano et al., 2022).

Thus, this study aimed to evaluate the *in vitro* antibacterial potential of the hydroalcoholic extract of culms and leaves of the species *G.* aff. *lynnclarkiae* on *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Klebsiella pneumoniae*.

2. Materials and Methods

2.1. Characterization of the study area

The collection of plant material from *Guadua* aff. *lynnclarkiae* was carried out in Rio Branco, in a private area adjacent to the Technology Foundation of the State of Acre (FUNTAC), in October 2021, at 9°56'46.466" S and longitude 67°52' 8.941" W, in the morning period. The area where the collected individual is located is characterized as shallow due to palm trees and lianas and because it is close to an ephemeral watercourse. The species was introduced in the area by planting seeds collected near the Purus River, on the shores of Lago do Silêncio, by FUNTAC employees in 2009. To obtain the extracts, fresh leaves and young and adult culms were collected from an individual with an estimated height of between 12 and 14 meters.

To confirm the identification of the species, specimens were made for cataloging and inclusion in the Herbarium of the Zoobotanical Park of the Federal University of Acre (UFAC), registered under the identification number 22247. It is important to emphasize that the botanical identification stage had the collaboration of the researcher Dr. Ximena Lodoño.

2.2. Obtaining plant extracts

Fragments of adult culms were cut into very fine pieces. Subsequently, the culms and leaves were placed in an air circulation oven for 5 days at 100°C. The plant materials were crushed in a knife mill, and the powder obtained (dry material) was stored in a desiccator with silica gel until the moment of extraction.

To obtain the leaf and culm extracts, the dried plant materials were immersed in 70% alcohol and submitted to the cold extraction process according to the methodology of Matos (2009) with modifications. 10 g of dry material from mature culms and leaves were weighed on a precision scale and transferred to an Erlenmeyer flask for extraction.

To produce a biologically active extract and, knowing that the more polar and hydrophilic compounds are more easily extracted by ethanol or methanol (Matos, 2009), the extraction was carried out cold using a hydroalcoholic solution (30% water, 70% ethanol). In the maceration process, a volume of 100 ml of 70% alcohol was added, and after 24 hours, filtering was performed on filter paper, identified and closed with aluminum foil. This process was performed twice on consecutive days. After the two extractions, the extract was dried in a circulation oven at 42°C for 72 h, thus obtaining the crude culm and leaf extracts of *G.* aff. *lynnclarkiae*. The dry extracts were weighed, and the yield was calculated, as shown in Supplementary Material Table S1.

2.3. Minimum inhibitory concentration and minimum bactericidal concentration

The antimicrobial activity was performed by microdilution technique, using sterile 96-well microplates. *Staphylococcus aureus* (ATCC 12598), *Streptococcus pneumoniae* (ATCC 11733), gram-positive bacteria, and *Klebsiella pneumoniae* (ATCC 700603), gram-negative bacteria, were used as test microorganisms.

Three to five well-isolated bacterial colonies of the same morphological type of culture of each bacterial microorganism were selected in a Petri dish with Müller-Hinton agar (MH) medium to conduct the bioassay. Each colony was touched with a spatula and transferred to tubes

containing 5 ml of sterilized 0.9% NaCl solution to obtain an optical turbidity comparable to that of the standard solution of 0.5 McFarland scale, which corresponds to 10^8 CFU ml^{-1}.

Successive dilutions were conducted in plates containing 96 wells, starting from an initial concentration of 100 mg of extract per ml of DMSO. From the dissolved extract, a volume of 100 µL was taken and added to the first row of microwells. Next to this volume, another 100 µL of Müller-Hinton medium (MH) and 5 µL of inoculum were added, reaching a concentration of 50 mg ml^{-1} in the first row of microwells. This concentration was serially diluted to a final concentration of 1.55 mg ml^{-1}.

The control drug, Chloramphenicol 30 µg ml^{-1}, was diluted similarly to the extracts. 5 µl of the inoculum corresponding to each strain assessed were added, except for the negative control according to norm M7-A6 (CLSI, 2005). The negative control contained only 200 µl of MH medium, and the positive control 200 µl of MH medium and 5 µl of inoculum. The microplates were incubated at 37°C for 24 h, after which 50 µl of Resazurin reagent (3.0 mg ml^{-1}) was added to each well, indicating microbial growth when the color changed from blue to red (Riss et al., 2013). Assays were performed in duplicate.

To determine the minimum bactericidal concentration (MBC), to assess whether the extract had a bacteriostatic or bactericidal action, a sterile swab was used to absorb the contents of the wells that showed inhibition of microbial growth, from the lowest MIC value, a dilution above the MIC and one dilution below the MIC inoculated into Petri dishes containing MH agar medium for bacteria. The plates were incubated at 37°C for 24 h (Riss et al., 2013).

3. Results

The hydroalcoholic extracts from leaves and culms of the species *Guadua* aff. *lynnclarkiae* inhibited the growth of all analyzed bacteria, with MIC ranging from 1.55 mg ml^{-1} for *Staphylococcus aureus* to 6.25 mg ml^{-1} for *Klebsiella pneumoniae*. The lowest bactericidal concentration observed was for the leaf extract against the bacteria *S. aureus*, 1.55 mg ml^{-1}, as seen in Table 1.

The results of the microplate tests indicated that for *Klebsiella pneumoniae* the MIC was 6.25 mg ml^{-1} for leaf and culm extracts. However, it is possible to observe that for this same bacterium only the leaf extracts had a bactericidal effect at concentrations of 6.25 mg ml^{-1} (MBC) and 12.5 mg ml^{-1}. Bacterial growth was observed on the plates at a concentration of 3.1 mg ml^{-1} (concentration below the MIC). The culms had a bacteriostatic effect on *K. pneumoniae* due to small colonies that grew on the plates (Figure 1A and 1D).

Table 1. Antibacterial activity of ethanolic extracts from leaves and culms of *Guadua* aff. *lynclarkiae*. This result was obtained from the observation of two repetitions. Hyphens indicate that MBC was not detected at the concentrations studied.

Extract	Bacterium					
	Klebsiella pneumoniae		*Streptococcus pneumoniae*		*Staphylococcus aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC
Leaf (mg ml^{-1})	6.25	6.25	12.5	25	1.55	1.55
Culm (mg ml^{-1})	6.25	-	6.25	-	1.55	6.25
Chloramphenicol (30 µg ml^{-1})	1.55	1.55	1.55	1.55	1.55	1.55

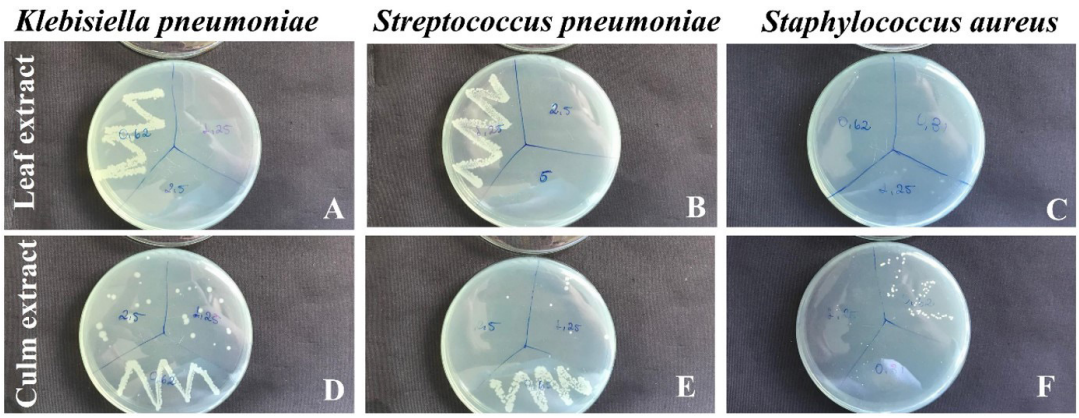

Figure 1. Result of the bactericidal/bacteriostatic test for leaf (top row) and culm (bottom row) extracts of *Guadua* aff. *lynnclarkiae*. A: *Klebisiella pneumoniae* striation on treatment with leaf extract (MBC: 6.25 mg ml^{-1} = 1.25 mg 0.2 ml^{-1}). B: *Streptococcus pneumoniae* striation on treatment with leaf extract (MBC: 25 mg ml^{-1} = 5 mg 0.2 ml^{-1}). C: *Staphylococcus aureus* striation on treatment with leaf extract (MBC: 1.55 mg ml^{-1} = 0.31 mg 0.2 ml^{-1}). D: *Klebisiella pneumoniae* striation on treatment with culm extract (MBC: not observed). E: *Streptococcus pneumoniae* striation on treatment with culm extract (MBC: not observed). F: *Staphylococcus aureus* striation on treatment with culm extract (MBC: 6.25 mg ml^{-1} = 1.25 mg 0.2 ml^{-1}).

Considering the *Streptococcus pneumoniae* species, the MIC was 12.5 mg ml^{-1} for the leaf extract and 6.25 mg ml^{-1} for the culm extract, however, only the leaves had a bactericidal effect from the concentration of 25 mg ml^{-1} (concentration above the MIC), at a concentration of 12.5 mg ml^{-1}, a bacteriostatic effect was observed due to the growth of 1 colony (Figure 1B). The bacteria grew normally at the lower concentration (in this case, 6.25 mg ml^{-1}). For this bacterium, the stem extract had a bacteriostatic effect due to the presence of some colonies, as can be seen in Figure 1E.

Regarding *Staphylococcus aureus* bacteria, leaf and culm extracts showed inhibitory action at all concentrations analyzed in the microplate test (50 to 1.55 mg ml^{-1}). Thus, for evaluating the bactericidal concentration in Petri dishes, concentrations of 1.55 mg ml^{-1}, 3.1 mg ml^{-1}, and 6.25 mg ml^{-1} were used for the two extracts. However, in the analysis of the Petri dishes, it is noted that for *S. aureus*, only the leaf extracts had a bactericidal effect at all tested concentrations of 1.55 mg ml^{-1}, 3.1 mg ml^{-1}, and 6.25 mg ml^{-1}. For the culms, a bactericidal effect was observed only at the concentration of 6.25 mg ml^{-1}, in the other concentrations, there was the development of some colonies showing a bacteriostatic effect (Figure 1C and 1F).

Thus, the results show that the leaves had the desirable bactericidal effect, making it possible to recommend their use at a concentration of 25 mg ml^{-1}, aiming at bactericidal action for the three species of bacteria studied. The most susceptible bacteria was *Staphylococcus aureus* since the leaf extracts at a concentration of 1.55 mg ml^{-1} (which was the lowest concentration used) showed bactericidal action.

4. Discussion

In the literature, several studies point to the antimicrobial capacity of bamboo leaf extract, and other plant species, against different microorganisms and fungi (Afrin et al., 2012; Silva et al., 2017; Anselmo-Moreira et al., 2021; Gagliano et al., 2022).

According to the results found in the present study, it was verified that the bamboo leaf and culm extracts provided antimicrobial action for gram-positive bacteria (*Streptococcus pneumonie* and *Staphylococus aureus*) and gram-negative bacteria (*Klebsiella pneumoniae*).

Among the microorganisms under analysis, *S. aureus* was the most susceptible to all leaf extract concentrations evaluated in the experiment. This pattern, however, was not observed for culm extracts, where MBC was only observed at 6.25 mg ml^{-1}.

Similar results were obtained in a study by Austria et al. (2017), who verified the eradicating action and protective activities of the ethanolic extract of *Bambusa blumeana* Schult.f. and *B. vulgaris* against *S. aureus*. Singh et al. (2012) analyzed the effect of the methanolic extract of the fermented buds of *Bambusa balcooa* Roxb. Observed antimicrobial potential, not only for gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) as well as antifungal action against *Fusarium oxysporum*.

Fujimura et al. (2005) indicated the antimicrobial action of proteins isolated from *Phyllostachys pubescens* shoots against phytopathogens. Protein extract concentrations ranged from 2 to 25 µg ml^{-1}. In the study by Tanaka et al. (2011) MIC values of 200 µg ml^{-1} were found when assessing the antimicrobial potential of the skin of bamboo shoots *P. pubescens* in dichloromethane extracts applied against *S. aureus*.

Mori et al. (2019) used extracts from superheated culms of "Moso" bamboo of the species *Phyllostachys heterocycla* f. *pubescens* (Houz.) D.C. McClint to assess as a natural pesticide against plant bacteria and fungi, showing significant inhibitory effects against the tested phytopathogens.

Anselmo-Moreira et al. (2021) evaluating the antibacterial capacity of the leaf extract of seven bamboo species, native to Brazil, demonstrated its antibacterial potential. However, none of the extracts showed bactericidal activity, it only inhibited the growth of microorganisms analyzed in the study. The MIC of extracts from bamboo leaves of *Olyra glaberrima*, *Parodiolyra micranta*, *Aulonemia aristulata*, *Filgueirasia arenicola*, *Filgueirasia canavieira*, *Merostachys neesii,* and *M. pluriflora* ranged from 0.39 mg ml^{-1} to 1.87 mg ml^{-1}. The authors observed that hexane was more efficient than hydroalcoholic extracts.

Several aspects can interfere with the antimicrobial capacity of plant species extracts. The extraction method, solvents, the base of the plant material (fresh or dry), the part of the plant used (leaf or culm) and the chemical compounds present in these organs (Tanaka et al., 2011).

Research carried out by Mulyono et al. (2012), with extracts from leaves of *Dendrocalamus* asper (Schult.) *Backer* (Bambuseae) against *E. coli*, reported that the presence of fatty acids plays a key role in the antimicrobial activity of the extracts.

Among the classes of compounds with antibacterial action, linoleic acid has been described as the main responsible for giving bamboo extracts antimicrobial potential, along with benzoquinone, chitin-binding peptides, other fatty acids and phytosterols (Fujimura et al., 2005; Tanaka et al., 2013).

Soumya et al. (2014) analyzed the chemical composition and antimicrobial action of *Bambusa bambos* seed oil and found that the inhibitory effect against gram-positive and gram-negative bacteria was due to the presence of high linoleic acid content in the seeds.

The hydroalcoholic extracts of leaves and culms of *G.* aff. *lynnclarkiae*, showed antibacterial activity against the tested bacteria. The leaf extract was bactericidal for the three tested bacteria. However, the culms showed a bacteriostatic effect against *Klebsiella pneumoniae* and *Streptococcus pneumoniae* at the concentrations studied, except for *Staphylococcus aureus*, which was more susceptible at the concentration of 6.25 mg ml^{-1}, demonstrating to be bactericidal.

The hydroalcoholic extracts of *G.* aff. *lynnclarkiae* have potential for future uses as phytopharmaceuticals, but more studies are needed regarding determining their chemical constituents, to guide the development of new antibacterial products. The results are promising, especially for gram-negative bacteria, responsible for nosocomial infection problems, as they are susceptible to the studied extracts, causing inhibition in the growth and death of the strains.

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Supplementary Material

Supplementary material accompanies this paper.
Table S1. The yield of biologically active extracts from leaves and culms of Guadua aff. lynnclarkiae.
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