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Research Article

Evaluation of Antimicrobial Activity of the Crude Extract of Three Bauhinia Species from the Brazilian Amazon

Fabyanne Oliveira[1], Bruna Carvalho[1], Paula Cristina Rodrigues Frade[1], Jorddy Neves Cruz[2], Helio Longoni Plautz Junior[3], Raylson Coelho de Lima[4], Osmar Alves Lameira[5], Christian Lameira[6], Fernando Augusto Miranda da Costa[7]*

[1]Department of Biomedicine and Health Sciences, State University of Para, Para, Brazil;[2]Department of Molecular Modeling Approaches in Medicinal Chemistry, Federal University of Para, Para, Brazil;[3]Department of Microbiology and Molecular Biology, Federal University of Para, Para, Brazil;[4]Department of Computing and Information Sciences, Fiber University Center, Bethlehem, Brazil;[5]Department of Agronomy and Agricultural Research Corporation, University of Lavras, Lavras, Brazil;[6]Department of Phytocosmetics and Herbal Medicines, Fiber University Center, Bethlehem, Brazil;[7]Department of Biomedicine, Federal University of Para, Para, Brazil

ABSTRACT

The *Bauhinia ungulata*, *Bauhinia variegata*, and *Bauhinia purpurea* are commonly used in folk medicine. However, few studies have investigated its antimicrobial potential. This study evaluated the *in vitro* antibacterial effect of crude extracts from leaves of *B. ungulata*, *B. variegata* and *B. purpurea*, in addition to identifying the class of constituents. The evaluation of antimicrobial activity was determined using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods against American Type Collecti-on Culture (ATCC) strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. All crude extracts showed similar phytochemical analysis with saponins and tannins and bactericidal activity against all the bacterial strains. The extract of *B. variegata* stood out with a MIC of 3.6 µg/mL against *P. aeruginosa*. From this perspective, the extracts of *Bauhinia* spp. showed promising microbial activity and should be further investigated for the development of antibacterial agents of natural origin.

Keywords: Antibacterial activity; Microbial activity; Phytochemical analysis; Antibiotics

INTRODUCTION

Antimicrobial resistance should be discussed very seriously and carefully, as it poses a never-before-seen long-term clinical, economic, social and environmental threat, being a significant danger to the public health of countries all over the world, developed or not [1]. The economic impact will be high and recent research conducted by the world bank indicates that antimicrobial resistance would increase the poverty rate and especially affecting low-income countries [2]. Studies carried out on bacterial resistance estimate that about 10 million people will die per year in 2050 with costs about $100 trillion dollars per year. With this, the World Health Organization (WHO) and several groups around the world agree on the urgency of developing a global action plan to address the issue and especially in the development of new drugs [3,4].

The search for new antibiotics of natural origin becomes promising and an important segment of modern medicine aiming to solve the socio-economic and health impacts due to resistant bacterial infections. Several studies have demonstrated the presence of chemical groups such as coumarone, flavonoids, phenolic, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes, being used as a starting point for the development of new antibiotics for the treatment of these resistant infections [4-6].

Some plants of the Bauhinia genus are used in the treatment of diarrhea, rheumatism, pain, convulsions, delirium, septicaemia. Other studies with different species, using crude extract of Bauhinia parts, promising properties were evidenced with ample potential for antimicrobial inhibition of the plant against several bacteria of hospital origin [7]. The species, *Bauhinia ungulata L.*, *Bauhinia variegata L.* and *Bauhinia purpurea L.* are described in the literature for their use in folk medicine due to their antidiabetic, antioxidant, and anti-inflammatory effects, but little is explored on its antibacterial activity, which demonstrates the need for more detailed studies to present its actions against bacteria of clinical

Correspondence to: Fernando Augusto Miranda da Costa, Department of Biomedicine, Federal University of Para, Para, Brazil, E-mail: fernandoamc@ufpa.br

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interest [8-13].

In this perspective, the phytochemical and pharmacological analysis of species of the genus Bauhinia is extremely important, with more detailed experiments, since it will allows the organization of data that could be catalogued according to the physicochemical characteristics of the plant, which may be useful for the safe commercial validation of this species herbal medicine, making it possible to minimize the serious problem currently faced with bacterial resistance, in addition to providing possible therapeutic options with less toxicity to patients [14]. In this sense, this work aims to evaluate the *in vitro* antimicrobial potential of extracts from the species *B. ungulata var.*, *B. variegata* L., and *B. purpurea* L. from the Brazilian Amazon against five bacterial strains.

MATERIALS AND METHODS

Collection of plant specimens

The leaves of *Bauhinia ungulata* (IAN 188176), *Bauhinia variegata* (IAN 184735), and *Bauhinia purpurea* (IAN 188957) were harvested at the Embrapa Amazonia Oriental, located in the municipalities of Belem and Ananindeua, Para, between coordinates 01°24'.46.14" and 01°28'4.11" south latitude and 48°20'4.60" and 48°20'31.84" west longitude of Greenwich. Approximately 50 g of leaves of species of the genus Bauhinia were collected. The project complies with national and international guidelines and legislation and is registered on the platform of the National Management and Genetic Heritage System and Associated Traditional Knowledge (SISGEN), whose provided license to collect the species under registration A2C1D3D. Moreover, according to the IUCN 2019 red list of endangered species, *Bauhinia ungulata* (no synonymy), *Bauhinia variegata* (synonymy of Bauhinia alba Wall. Bauhinia candida Roxb. Bauhinia candida aiton, *Bauhinia variegata L.* var. chinensis DC., and *Bauhinia variegata L.* var. candida (Aiton) Corner) and *Bauhinia purpurea* (synonymy of Bauhinia castrata Blanco, Bauhinia coromandeliana DC., Bauhinia platyphylla Span., *Bauhinia purpurea L.* var. violacea de Wit, *Bauhinia purpurea L.* var. corneri de Wit, Bauhinia rosea Corner, Bauhinia triandra Roxb., Bauhinia violacea Corner, Caspareopsis purpurea (L.) Pittier, and Phanera purpurea (L.) Benth) are listed as least concern.

Preparation and obtaining extracts

The extracts were obtained in the laboratory of centro universitario fibra according to the protocol used by Cruz. The leaves after being scraped were hydrated with 70% hydro alcoholic solution. Afterward, the samples were placed in a bath with agitation under low light conditions. Subsequently, the extracts were centrifuged, filtered, and stored in an amber bottle for seven days for maximum recovery of phenolic compounds.

Identification of chemical components

Qualitative analytical research techniques were used to identify the chemical components: saponins, tannins, polysaccharides, catechins, alkaloids, and flavonoids. In the identification of polysaccharides, 2 mL of the crude extract of Bauhinia species was dissolved in 5 mL of distilled water. Two drops of Lugol were added. The blue colour indicates a positive reaction; to identify tannins, 2 mL of crude extracts were dissolved in 10 mL of distilled water. A drop of 1% ferric chloride was added. Colour change indicates a positive reaction; to identify catechins, 2 mL of crude extract was dissolved in 3 mL of methanol. 1 mL of 1% vanillin solution and 1 mL of concentrated HCl was added. The formation of an intense red colour indicates a positive reaction; to identify saponins; 2 ml of the extracts were dissolved in 1 ml of 80o GL ethanol and diluted with 15 mL of distilled water. Shake vigorously in a closed test tube. If a foam layer forms and remains stable for 30 minutes, it indicates a positive reaction; to identify flavonoids, 2 mL of crude extract was dissolved in 10 mL of methanol and five drops of concentrated HCl, and 1 cm of ribbon magnesium. The pink colour formation in the solution indicates a positive reaction; to identify alkaloids, 2 mL of crude extract was dissolved in 4 mL of 5% HCl. Four 1 ml portions were separated into test tubes and the following reagents: Bouchardat (positive reaction-Orange reddish colour), Dragendorff (positive reaction-red brick colour), Bertrand (positive reaction-white), and Mayer (positive reaction–white).

Microorganisms

The inoculum preparation was carried out according to the Brazilian national health surveillance agency protocol 132. Using a sterile platinum loop, morphologically similar colonies were selected and suspended in saline solution to obtain a suspension with turbidity corresponding to McFarland's 0.5 scales. (1 × 108 CFU/mL).

Assessment of antimicrobial activity

Antimicrobial assays were performed in triplicate using the Minimum Inhibitory Concentration (MIC) method, with modifications, and the Minimum Bactericidal Concentration (MBC) method, according CLSI laboratory standard for broth micro dilution assays 33. MIC tests were performed in Mueller-Hinton broth added to 96-well micro plates. Bauhinia leaf extracts were deposited in the wells, following the serial dilution technique (range 1/2 to 1/512 dilutions). The positive and negative controls were performed as follows: 100 µL of Mueller-Hinton Broth (MHB) and 50 µL of each bacterial inoculum were added to the positive control well, and 100 µl of MHB and 50 µl of saline solution were added to the negative control. The plates were incubated at 37°C for 18 h. The reading was performed by changing the colour using 0.5% 2,3,5 Triphenyl Tetrazolium Chloride (TTC), considering the inhibition of bacterial growth the absence of reddish ping staining and bacterial growing in the presence of reddish-pink staining. The CBM was performed on the MIC cultures that did not show visible bacterial growth, as well as the negative and positive controls as confirmatory. The wells were seeded on Mueller-Hinton (MH) agar and after 18 h of incubation at 36 ± 1°C; the lowest concentration of extract capable of kill bacteria was defined.

RESULTS

Phytochemical analysis of crude extracts

Crude extracts of *B. ungulata*, *B. variegata* and *B. purpurea* showed similar phytochemical distribution (Table 1). All extracts showed the presence of saponins, tannins, and alkaloids and the absence of polysaccharides, catechins and flavonoids.

Minimum Inhibitory Concentration (MIC)

The crude extracts of the species were tested against five American Type Culture Collection (ATCC) bacterial strains, being *Staphylococcus aureus* (43300), *Enterobacter aerogenes* (13048), *Klebsiella pneumoniae* (700603), *Escherichia coli* (35218) and *Pseudomonas aeruginosa* (27853) performed in triplicate. The results are described in Table 2. *P. aeruginosa* was the most sensitive bacterium against the extracts of *B. variegata* and *B. purpurea* with MIC of 3.6 µg/mL and 4.38 µg/mL, respectively, whereas for *B. ungulata* showed less sensibility with MIC of 12, 67 ± 4,47 µg/mL. The extracts showed similar results for *E. aerogenes* and *E. coli* with MIC of 9.5 µg/mL for *B. ungulata*, 7.19 µg/mL for *B. variegata* and 11.70 ± 4, 14 µg/m for *B. purpurea* extract. *K. pneumoniae* showed MIC of 9.5 µg/mL for *B. ungulata*; 9.58 ± 3.38 µg/mL for *B. variegata* and 8.77 µg/m for *B. purpurea*. The *Staphylococcus aureus* showed less sensitivity to crude extracts of all species of Bauhinia tested, with a MIC of 19.0 µg/mL for *B. ungulata*, 14.39 µg/mL for *B.variegata* and 17.55 µg/mL for *B. purpurea*.

Minimum Bactericidal Concentration (MBC)

About the aliquots of the wells that were plated to determine the CBM of the leaf extracts of *B. ungulata*, *B. variegata* and *B. purpurea*, it was possible to detect bactericidal effects against Gram-positive and Gram-negative bacteria. The results are described in Table 3.

Against *S. aureus* and *E. aerogenes*, the crude extract of *B. ungulata*, *B. variegata* and *B. purpurea* demonstrated bactericidal activity at concentrations of 19.0 µg/mL, 14.37 µg/mL and 17, 55 µg/mL, respectively. Bacteriostatic activity occurred at concentrations of 9.5 µg/mL, 7.19 µg/mL and 8.77 µg/mL, respectively.

Only against Gram-negative strains did the crude extract show bactericidal activity at higher concentrations. The extract of *B. ungulata* obtained only bacteriostatic action at a concentration of 38 µg/mL against *E. coli* and *P. aeruginosa*, while against *K. pneumoniae* it obtained bactericidal action at a concentration of 19.0 µg/mL and bacteriostatic action at a concentration of 9.5 µg/mL.

For *E. coli*, the extract of *B. variegata* showed only bacteriostatic action at a concentration of 28.75 µg/mL, whereas its action against *P. aeruginosa* was bactericidal with CBM of 7.19 µg/mL and bacteriostatic at a concentration of 3.60 µg/mL, and as for *K. pneumoniae*, its action was given as bactericidal at a concentration of 28.75 µg/mL and bacteriostatic at a concentration of 14.37 µg/mL.

Regarding the extract of *B. purpurea*, its action against *E. coli* and *P. aeruginosa* was bactericidal at a concentration of 8.77 µg/mL and bacteriostatic at a concentration of 4.38 µg/mL, while against K pneumoniae, its action was only bacteriostatic at a concentration of 35.1 µg/mL.

Table 1: Phytochemical profile of extracts of *B. ungulata var.*, *B. variegata* L., and *B. purpurea* L.

Crude extract	**Chemistry groups**					
	Saponins	**Tannins**	**Polysaccharides**	**Catechins**	**Flavonoids**	**Alkaloids**
B. ungulata var.	+	+	-	-	-	+
B. variegata L.	+	+	-	-	-	+
B. purpurea L.	+	+	-	-	-	+

Table 2: MIC of extracts of *Bauhinia ungulata* var., *Bauhinia variegata L.*, and *Bauhinia purpurea L.* against ATCC strains of *S. aureus* (43300), *E. aerogenes* (13048), *K. pneumoniae* (700603), *E. coli* (35218) and *P. aeruginosa* (27853).

Crude extract	**Minimum inhibitory concentration (µg/mL)**				
	-	-	-	-	-
B. ungulata var.	19.0 ± 0,0	9.5 ± 0,0	9.5 ± 0,0	9.5 ± 0,0	12.67 ± 4,47
B. variegata L.	14.39 ± 0,0	7.19 ± 0,0	9.58 ± 3,38	7.19 ± 0,0	3.6 ± 0,0
B. purpurea L.	17.55 ± 0,0	11.70 ± 4,14	8.77 ± 0,0	11.70 ± 4,14	4.38 ± 0,0

Data are Mean ± SD (Standard Deviation).

Table 3: CBM of extracts of *Bauhinia ungulata* var., *Bauhinia variegata L.* and *Bauhinia purpurea L.* against ATCC strains of *S. aureus* (43300), *E. aerogenes* (13048), *K. pneumoniae* (700603), *E. coli* (35218) and *P. aeruginosa* (27853).

Crude	**Minimum Bactericidal Concentration (MBC)**				
Extract	***S. aureus***	***E. aerogenes***	**K.pneumoniae**	***E. coli***	***P. aeruginosa***
B. ungulata var.	19.0*	19.0*	19.0*	38.0**	38.0**
	9.5**	9.5**	9.5**	38.0**	38.0**
B. variegata L.	14.37*	14.37*	28.75*	28.75**	7.19*
	7.19**	7.19**	14.37**	28.75**	3.60**
B. purpurea L.	17.55*	17.55*	35.1**	8.77*	8.77*
	8.77**	8.77**	35.1**	4.38**	4.38**

* Bactericidal Activity.

** Bacteriostatic Activity.

DISCUSSION

Currently, the number of therapeutic failures growing at alarming rates due to the rapid expansion of antibiotic-resistant pathogens [15]. For this reason, the use of natural products such as medicinal plants such as those of the genus Bauhinia has been widely publicized as a raw material for the synthesis of bioactive substances [16]. The present study evidenced the phytochemical composition of the plants *B. ungulata*, *B. variegata* and *B. purpurea*. And the potential antibacterial effect of their extracts.

The crude extracts of *B. ungulata*, *B. variegata* and *B. purpurea* presented saponins, tannins, and alkaloids as the main classes of chemical constituents. Oliveira and Lima [17] carried out a phytochemical study of the ethanolic extract of the stems of Bauhinia forficate and showed positive results for tannins, when evaluating the leaves of B. forficate the authors also observed the presence of flavonoids [18]. Neto et al., showed the presence of flavonoids and alkaloids from leaves of *B. ungulata* [18]. Silva who evaluated the aqueous extract obtained from the leaves of *B. ungulata* also obtained a positive reaction for the presence of tannins and alkaloids, however when evaluating the presence of saponins both studies showed negative results [19]. Another research that aimed to evaluate the presence of phenolic compounds by comparing the phytochemical analyses of three species of B. forficata with a species of *B. variegata* also obtained similar results, confirming the presence of tannins in other species of this genu [20].

The scientific literature reports that the classes of secondary metabolites saponins and tannins are responsible for several actions of pharmacological importance, being saponins, emulsifiers, leading to intestinal relaxation, in addition to having expectorant, diuretic, and anti-inflammatory action. While tannins are classified as phenolic and water-soluble complexes acting pharmacologically as antiseptics, antidiarrheal, and antioxidants [17].

The MIC assay showed a strong antimicrobial activity of the three Bauhinia species studied since all assays showed growth inhibition of the tested strains. This fact is evidenced in the literature, due to the broad antibacterial potential of the Bauhinia genus [19-27]. This study showed promising results in the inhibition of *P. aeruginosa*, *E. aerogenes*, *K. pneumoniae*, *E. coli*, and *S. aureus*. The crude extract of *B. variegata* and *B. purpurea*, achieved better results in inhibiting the growth of *P. aeruginosa*, requiring the lowest concentration (3.6 μg/mL) to inhibit it. The MIC presented by plant extracts against strains of *E. aerogenes*, *K. pneumoniae*, and *E. coli* showed similar growing inhibition results, except for *S. aureus* which needed the highest concentration to be able to inhibit bacterial growth. The crude extract of *B. ungulata* showed a MIC of 19.0 μg/mL (*S. aureus*) and 9.5 μg/mL (*E. aerogenes*, *K. pneumoniae*, and *E. coli* and *P. aeruginosa*).

A study with *B. ungulata*, the extract inhibited *S. aureus* at all concentrations tested (250-1.95 μg/mL)28. In another study, the crude extract of *B. purpurea* had a MIC of 0.22 μg/mL and 1.31 μg/mL, for *S. aureus* and *E. coli*, respectively. The presence of alkaloids could explain the antimicrobial activity of the extract, since this compound is known to have an antimicrobial effect against various microorganisms [28,29], confirming the inhibitory potentials as expressed in our research, however, and the MIC values differ.

Regarding the extract of *B. variegata*, the MIC was 14.39 μg/mL (*S. aureus*), 7.19 μg/mL (*E. aerogenes*, *K. pneumoniae* and *E. coli*), and 3, 6 μg/mL (*P. aeruginosa*). In a study of the antimicrobial activity through the MIC technique, the authors reported that extracts of Petroleum Ether (PE), Benzene (BZ), Chloroform, and Ethyl Alcohol (ET) from the leaf of *B. variegata* obtained a MIC of 6.72 μg/mL (EP) for *K. pneumoniae*, 3.5 μg/mL (ET) for *Pseudomonas aeruginosa* and 28.40 μg/mL (ET) for *E. coli* (30). Results that are similar to our tests performed with *K. pneumoniae* and *P. aeruginosa*, however, differ from the results presented for *E. coli*.

In a study of the antibacterial activity of the extract of *B. purpurea*, the authors described a MIC of ≤1,500 μg/mL for *S. aureus* and no inhibition for *E. coli* and *P. aeruginosa*, contradicting the results presented in this study, in which the crude extract of *B. purpurea* showed a lower MIC of 17.55 μg/mL (*S. aureus*), 8.77 μg/mL (*E. aerogenes*, *K. pneumoniae*, and *E. coli*) and 4.38 μg/mL (*P. aeruginosa*). No studies were founded in literature about the MIC of *B. ungulata* against *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa*; *B. variegata* against *S. aureus* and *E. aerogenes*, as well as *B. purpurea* against *E. aerogenes* and *K. pneumoniae*, which demonstrates the scarcity of studies related to the Gram-negative species studied.

Regarding the CBM assay, the test with Gram-positive bacterium showed lower sensibility to crude extracts of Bauhinia, showing inhibition at concentrations of 19.0 μg/mL, 14.37 μg/mL and 17.55 μg/mL of extracts of *B. ungulata*, *B. variegata* and *B. purpurea* respectively. On the other hand, against Gram-negative bacteria, some crude extracts showed only bacteriostatic action: the crude extract of *B. ungulata var*. (28.75 μg/mL) for *E. coli* and *P. aeruginosa*; the crude extract of *B. variegata* L. (38 μg/mL) for *E. coli* and the crude extract of *B. purpurea* L. (35, 1 μg/mL) against *K. pneumoniae*.

Analogous to this, the study by Mishra et al., who evaluated extracts of leaves of *B. variegata* extracted with chloroform also showed resistance when tested against *E. coli* and *P. aeruginosa*. However, against *K. pneumoniae* its action was bactericidal with CBM of 20.20 mg/mL [30]. This sensitivity variation between Gram-positive and Gram-negative specimens can be attributed to the different chemical-physical composition of the cell wall of these two groups of bacteria [31]. Another study that analysed benzene extracts of *B. variegata* found a CBM of 60 mg/mL30. These CBM disparities may be associated with the chemical composition and concentration of active principles that differ among tree species and their extracts. In addition, characteristics resulting from soil, climate, and seasonality can also corroborate these differences [31].

Therefore, the evaluation of the antimicrobial activity of the crude extracts of *Bauhinia ungulata*, *Bauhinia variegata* and *Bauhinia purpurea* proved to be potent inhibitors of bacterial growth, with the extract of *B. variegata* standing out with MIC of 3.6 μg/ml. against *P. aeruginosa* and the crude extract of *B. purpurea* which was effective in inhibiting the *P. aeruginosa* strain with a Cubic Meter (CBM) of 4.38 μg/mL [32,33]. In this perspective, the investigation of new extracts obtained from plants is suggested, as they are potential bases for the development of new antibacterial agents.

CONCLUSION

Taken together, this study clearly introduces a rapid method and simple way to reduce costs in production of DNA ladder which is applicable for laboratory experiences. Moreover, most of the essential components for synthesizing DNA ladder were made in our own lab. Therefore, produced Deoxyribonucleic Acid (DNA) ladder in this study is entitled as “Home-made DNA ladder”.

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AUTHOR CONTRIBUTIONS

Conceptualization, F.A.M.C., F.O., B.C; investigation, F.O., B.C., P.C.R.F., H.L.P.J., R.C.L., O.A.L., C.L., F.A.M.C; writing-original draft preparation, F.O., B.C., J.N.C., F.A.M.C.; writing-review and editing, P.C.R.F, O.A.L, C.L., H.L.P.J., J.N.C., F.A.M.C. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY

Readers can access our data at Laboratorio de Microbiologia, Instituto de Ciencias Biologicas, Federal University of Para (Belem/Para/Brazil). The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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