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Short Communication

Antibacterial activity of fractions and isolates of *Maytenus guianensis* Klotzsch ex Reissek (Celastraceae) Chichuá Amazon

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Abstract

Introduction: This aim of this study was to evaluate the antibacterial activity of fractions and isolates of *Maytenus guianensis*, a plant species used in Amazonian folk medicine. **Methods**: A disk diffusion technique was used to investigate the antibacterial potential. **Results**: The hexanic fractions and tingenone B isolate showed inhibitory effects against *Staphylococcus aureus* and *Streptococcus pneumoniae*. **Conclusions**: These results indicate the antibacterial potential of this species and will enable future studies to identify novel therapeutic alternatives from this species.

Keywords: Ethnopharmacology. *Staphylococcus aureus*. *Streptococcus pneumoniae.*

The discovery of antimicrobials has been beneficial to mankind and has allowed the treatment of several diseases that were previously considered incurable. However, the indiscriminate use of antimicrobials has led to an increase in the number of resistant bacteria[1], necessitating the search for new, more potent, and more effective antibiotics.

The field of ethnopharmacology has emerged as one of the most promising sources for the discovery and production of new drugs. The species *Maytenus guianensis*, belonging to the family Celastraceae, is an excellent natural candidate, as it is used in traditional medicine, predominantly as an antiparasitic and antibacterial[2], to treat various diseases. Therefore, the present study aimed to evaluate the antibacterial activity of the fractions and isolates of *M. guianensis*.

The samples of *M. guianensis* were collected in February 2008 at the Adolpho Ducke Forest Reserve, located at kilometer 26 of the Manaus Road - Itacoatiara (AM-010) (Latitude 02º53'S, Longitude 59º58'W). The species was identified at the Herbarium of the National Research Institute of the Amazon [*Instituto Nacional de Pesquisas da Amazônia* (INPA)], and exsiccate no. 188,485 was sent to the laboratory of Natural Products Chemistry at the Federal University of Rondônia [*Universidade Federal de Rondônia* (UNIR)].

The fractions were prepared from 50g of crude acetone extract from the binder and foil and fractionated on silica gel column chromatography by elution with hexane, ethyl acetate, ethanol, and chloroform until exhaustion. The obtained fractions were as follows: the hexanic fractions of bast (HFB) and hexanic fractions of leaf (HFL); the ethyl acetate fractions of bast (ECFB) and ethyl acetate fractions of leaf (ECFL); the ethanolic fractions of bast (EFB) and ethanolic fractions of leaf (EFL); and the chloroform fractions of bast (CFB) and chloroform fractions of leaf (CFL).

Secondary metabolites were obtained only from the HFB to achieve clearer results from the fractions, according to the data shown in the **Table 1**. The HFB was separated by using silica gel column chromatography, eluted with n-hexane, and then with a mixture of n-hexane: $CHCl_3$, which had greater polarity. The structures of all the isolated compounds were elucidated by the analysis of their spectral data (IR, MS, ^1H and ^{13}C, including COSY, HMQC, HMBC, and NOESY spectra) and through comparison with the literature data[3].

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TABLE 1: Biological activity of the hexane fraction and tingenone B of *Maytenus guianensis.*

Samples	*Staphylococcus aureus*	*Streptococcus pneumoniae*
Clorofenicol control 30mg	18.6mm	15mm
HFB 20µg/mL	12mm	20.4mm
Tingenone B 20µg/mL	15.6mm	14.4mm

mg: microgram; **µg/mL**: microgram per milliliter; **mm:** millimeter; **HFB**: hexanic fractions of bast.

Four secondary metabolites were isolated: friedelin, friedelinol, tingenone B (22β-hydroxytingenone), and 29-hydroxyfriedelin. These compounds have all previously been reported from *M. guianensis*[3].

All fractions and isolates were tested for antibacterial activity against the bacteria *Staphylococcus aureus* ATCC 12598, *Streptococcus pneumoniae* ATCC 11733*, Escherichia coli* ATCC 10536, and *Klebsiella pneumoniae* ATCC 700603 by using the disk diffusion technique[4]. The samples that prevented bacterial growth around the disk were considered to have antibacterial activity; chloramphenicol (30mg) was used as the positive control. The inhibition halos produced were measured by using a digital caliper.

The plant extracts with antibacterial activity were subjected to new tests to determine the minimum inhibitory concentration (MIC) for each of the bacterial species that showed inhibitory activity over the concentration range 0,18mg/mL[5] by using the disk diffusion test.

The data represent the mean of the inhibition halos of triplicate experiments and were compared with the chloramphenicol data.

Among these fractions, only the HFB fraction presented antibacterial activity, with activity demonstrated against *S. aureus* and *S. pneumoniae* (**Table 1**).

The antibacterial tests showed that HFB exerted higher activity against *S. aureus* and *S. pneumoniae*, which resulted in inhibition halos of 12.0mm and 20.4mm, respectively, than the control drug, which resulted in 18.6mm and 15mm inhibition halos, respectively, despite being used at a higher concentration (30mg). These results contrasted with those of other studies performed using the hexanic extract of *Maytenus rigida* bast, which indicated no antibacterial activity against Gram-positive *S. aureus* or Gram-negative *E. coli*[6]. These variations in antibacterial activity may not be related only to the characteristics of the plant, as several species contain different active components, but also related to the characteristics of the test strains[7].

HFB did not present satisfactory results against *K. pneumoniae* and *E. coli*, the Gram-negative bacteria tested. This was thought to be attributable to the double membrane of the cells; although all bacteria have an inner membrane, Gram-negative bacteria have a single external membrane that prevents the penetration of certain drugs and antibiotics into the cell[8].

Among the isolated secondary metabolites, tingenone B (**Figure 1**) exhibited antibacterial activity, as shown in **Table 1**.

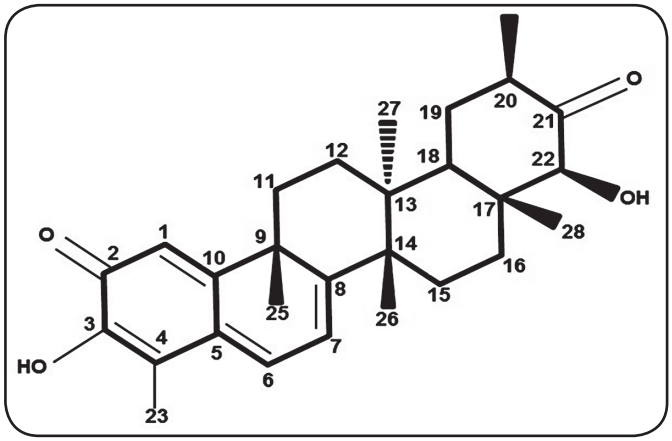

FIGURE 1: Chemical characteristics of tingenone B isolated from *Maytenus guianensis.* **O:** oxygen; **HO:** hydroxyl; **OH:** hydroxyl.

Tingenone B isolate showed better antibacterial activity against the *S. aureus* and *S. pneumoniae* strains than the positive control. In tests performed with 20µg/mL tingenone, satisfactory results against *S. aureus* strains were observed, with an 18mm inhibition halo[9]. Another study performed on tingenone isolate demonstrated better antibacterial potential than azithromycin, an antibiotic that suppresses protein biosynthesis and retards bacterial growth, with an MIC of 0.12µg/mL and 4.0µg/mL[10].

Another study with sesquiterpenes, laurinterol, isolaurinterol, allolaurinterol, and cupalaurenol presented a broad spectrum of activities against gram-positive bacteria, including methicillin-resistant *S. aureus* and penicillin-resistant *S. pneumoniae*[11]. Similar results were found in studies in which a mixture of tingenone B and tingenone isolates (20µg/mL) showed satisfactory results against methicillin-resistant *S. aureus* with a 12mm inhibition halo[9]. These results were similar to those obtained in this study, in which the tingenone B isolate showed activity against the bacterium *S. pneumoniae* and *S. aureus*, which confirmed the potential of the triterpene group.

Tingenone B showed no biological activity against the *E. coli* and *K. pneumoniae* strains. These results were similar to those of tingenone B isolates and tingenona isolates of *M. guianensis*, which did not show satisfactory effects against *E. coli* strains[9]. Another study performed using triterpenes isolates, ursolic acid, betulinic acid, and carnosol, a phenolic diterpene, against β-lactam-producing bacteria, *E. coli* and *K. pneumoniae*, the compounds presented moderate antibacterial

TABLE 2: Minimal inhibitory concentration.

Sample/MIC		Tested microorganisms/inhibition halo in millimeter.	
		Staphylococcus aureus	*Streptococcus pneumoniae*
HFB	10µg/mL	12.2mm	8.3mm
	5µg/mL	12.7mm	9.2mm
	2.5µg/mL	12.8mm	-
	1.75µg/mL	12.1mm	-
	0.37µg/mL	10.3mm	-
	0.18µg/mL	-	-
Tingenone B	10µg/mL	12.6mm	9.4mm
	5µg/mL	19.9mm	9.5mm
	2.5µg/mL	12.7mm	8.5mm
	1.75µg/mL	12.2mm	-
	0.37µg/mL	11.5mm	-
	0.18µg/mL	-	-

MIC: minimum inhibitory concentration; **HFB**: hexanic fractions of bast; **µg:** microgram; **mL**: milliliter; **mm:** millimeter.

action; these results were considered relevant as these bacteria are often resistant to various antibiotics[12]. Even though the tingenone B isolate did not present satisfactory results against *E. coli* and *K. pneumoniae*, other results showed the promising potential of the triterpene group for antibacterial activities.

The determination of the antibacterial activity enabled the evaluation and determination of the MIC based on the measurement of the diameter of the inhibition halos (**Table 2**).

The MIC of HFB against *S. aureus* and *S. pneumoniae* was 0.37µg/mL and 5µg/mL, respectively. This can be explained by the fact that the triterpenes present in the plants are generally concentrated in the most nonpolar fraction; in this case, in the hexane fraction, these substances are known to have various biological activities, particularly antibacterial activity[13].

However, when HFB was compared with the tigenin B isolate, the latter showed better activity against both *S. aureus* and *S. pneumoniae*, with MIC values of 0.37µg/mL and 2.5µg/mL, respectively, and inhibition halos of 11.5mm and 8.5mm, respectively. These results are in agreement with a study conducted on the roots of *Maytenus blephorodes*, which showed that isolated compounds exhibited better activity than the extracts against *S. aureus*[14].

It was considered that the effect of tigenin B may be related to the inhibition of tubulin-protein polymerization that is associated with the cellular support (cytoskeleton), performing antimitotic activity, this was observed in studies with *Maytenus chuchuhuasca*, where the possible components responsible for this inhibition were the quinolamine triterpenes of tingenone, 22β-hydroxytingenone, pristimerine, and celastrol[15], which showed the potential of this isolated compound for both antibacterial activities and other antimicrobial and parasitic activities.

It was reported that HFB and the tingenone B isolate showed antibacterial potential against the *S. aureus* and *S. pneumoniae* strains. The results of this study may assist future studies aiming to deeply explore the antibacterial potential and analyze the bacteriostatic effects to develop a new prototype drug or phytotherapy that may offer better therapeutic alternatives. Future research into other plant species, predominantly plants of the family Celastraceae, which are used as ethnopharmacological remedies in the Amazon region, is suggested.

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Conflict of interest

The authors declare that there is no conflict of interest.

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