**Evaluation of the Activity of Tontelea micrantha Extracts against Bacteria, Candida and Mayaro virus**

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**Abstract**

**Objectives:** This work aimed to evaluate the antibacterial, antifungal, and anti-Mayaro virus (MAYV) activity of leaf and branch extracts from *Tontelea micrantha*. **Materials and Methods:** *T. micrantha* extracts were prepared through the partition of the leaf and branch samples in different solvents. Then, the antibacterial and antifungal activity was assessed against bacterial pathogens and *Candida* sp. by the determination of the minimum inhibitory concentration (MIC) by the broth microdilution method. The activity against anti-MAYV was evaluated through the quantification of the extract concentration that promoted the protection of 50% of the cells after the viral infection. **Results:** The extracts of *T. micrantha* were inactive (MIC >500 µg/mL) against Gram-positive, Gram-negative and *Candida* species at the highest concentration tested (500 µg/mL). Anti-MAYV activity was also not detected, with SI <10, ranging from 1.2 to 3.6. **Conclusion:** Although it is used in traditional medicine, Leaf and branch extracts from *T. micrantha* did not present antimicrobial activity, which could be caused by the antagonistic effect of the compounds present in the extract.

**Keywords:** Antimicrobial activity, branch extracts, leaf extracts

**Introduction**

The use of antimicrobials represents one of the most successful ways of chemotherapy in the medical clinic. These drugs were responsible for the control of several infectious diseases, which have been considered as the main cause of mortality and morbidity throughout the history of the humanity.[1] However, infectious diseases remain a major challenge to human health. Currently, it is estimated that about 1 billion people are diagnosed with mycoses annually, and more than 1.5 million die from complications of invasive fungal diseases.[2] Furthermore, infectious diarrhea and pneumonia account for 40% of child deaths worldwide, especially in underdeveloped regions.[3]

Over the past 40 years, only two classes of antibiotics effective against Gram-negative bacteria have been approved for clinical use (e.g., oxazolidinones and cyclic lipopeptides) and the development of new antimicrobials in the future seems unpromising.[3] Currently, there is a scarce therapeutic arsenal for the treatment of fungal infections and the development of new antifungal drugs conflicts with the difficulty of finding compounds which are specific for fungal cells.[4] Similar situation happens with the development of new antivirals. Since viruses are obligate intracellular parasites, finding a drug that has an action against the virus but does not interfere with the basal functions of the cells is pointed out as the main challenge.[5] In this regard, considering the 15 largest companies in the pharmaceutical industry, only 5 of their drugs in the clinical or preclinical research phase are anti-infective agents.[5]

The alarming increase in the microorganism’s resistance to the currently available antimicrobials, caused mainly by the long-term exposure to subinhibitory concentrations of these...
drugs, has contributed to making this scenario even more adverse. In recent decades, cases of infections caused by resistant strains have frequently been reported, which reduces the efficacy of available drugs and limits the therapeutic arsenal to a few options, many of which involve compounds with high clinical toxicity and high costs, such as polymyxins. In the USA, as an example, about 25,000 patients die annually due to infections caused by Multidrug resistant microorganisms.

Mayaro virus (MAYV) belongs to the genus Alphavirus (Togaviridae Family) and is classified as an emerging virus with the potential emergence of an urban cycle in the Americas. It is an arbovirus closely related to the Chikungunya virus, and both have similar symptoms, making their infections difficult to diagnose and distinguish. Severe arthralgia caused by this pathogen can last for weeks or months, and the symptoms are treated with analgesics and nonsteroidal anti-inflammatories. However, there are no specific drugs available to treat MAYV infections.

The use of medicinal plants dates from the beginnings of societies and has spread throughout the world in recent years. About 80% of the population of developing countries depends on traditional medicine for treatment of various diseases, such as skin infections, bacterial and fungal infections, and cold. In addition, many commercially available drugs are structurally based on natural compounds, such as salicylic acid (aspirin), quinine (antimalarial), and Papaver somniferum morphine. Considering this scenario, the use of extracts and secondary metabolites originated from plants as possible antimicrobials have gained importance, mainly due to their generalized biological activities, which may be enhanced by different components of the extracts.

The Celastraceae family comprises herbs, shrubs, trees, and lianas distributed in the tropical and temperate regions. Numerous substances with biological activity were isolated from the plants of this family, such as triterpenes, with cytotoxic and antimicrobial activity; alkaloids with immunosuppressive effect; and sesquiterpenes, with antitumor activity. Tonntelea micrantha (MART. EX SCHULT) A. C. SM. (HIPPOCRATEIOIDEAE – CELASTRACEAE) is a representative species of the Celastraceae family. The alcoholic extract of T. micrantha bark is traditionally used in Brazil to treat kidney problems, and the seed oil is considered a potent anti-inflammatory. Mercadante-Simões et al. identified the presence of tannins, alkaloids, flavonoids, and terpenoids in the bark of T. micrantha, highlighting its potential in the bioprospecting of new herbal medicines. In another study, Mercadante-Simões et al. through the pharmacognostic analysis of T. micrantha leaves, identified the presence of steroids, triterpenoids, alkaloids, total phenolics, tannins, flavonoids, catechins, and reducing sugars in this part of the plant. Since tannins and flavonoids are also known for their antimicrobial properties, it is important to investigate the biological potential of this species against pathogens of medical interest.

The need for new, effective, and affordable drugs for the treatment of microbial infections is considered a major public health challenge. Thus, this work aimed to evaluate the antibacterial, antifungal, and anti-MAYV activity of crude extracts of T. micrantha.

**Materials and Methods**

**Plant material and preparation of the extracts**

Plant material was collected in Montes Claros, Minas Gerais – Brazil and identified by the botanist Dr. Maria Olívia Mercadante-Simões. The voucher specimen (Number BHCB 144.623) was deposited at the Herbarium of the Department of Botany of the University of Minas Gerais.

Leaf and branch samples of T. micrantha dried at room temperature were fragmented in knives mill. The branch and leaf were submitted to the extraction by successive maceration with pure organic solvents: hexane, chloroform, ethyl acetate, and methanol. The extractive solvents were recovered using a rotary evaporator leading to the respective extracts. At the time of the bioassay, the extracts were diluted in 20% (w/v) dimethyl sulfoxide (DMSO).

**Microorganisms and cell lineage**

The microorganisms employed in this work originated from the American Type Culture Collection (ATCC), cordially provided by the Fundação Oswaldo Cruz, Brazil. The MAYV was kindly provided by Ph. D. Mauricio Lacerda Nogueira (Faculty of Medicine of São José do Rio Preto/Famerp). The antibacterial activity of each T. micrantha extract was determined using nine Gram-negative bacteria (Enterobacter cloacae ATCC 23355, Escherichia coli ATCC 25922, Acinetobacter baumannii ATCC 19606, Klebsiella pneumoniae ATCC 4352, Klebsiella oxytoca ATCC 0182, Shigella flexneri ATCC 12022, Pseudomonas aeruginosa ATCC 15442, Salmonella enterica serovar typhimurium ATCC 14028, and Salmonella choleraesuis ATCC 10708), six Gram-positive bacteria (Staphylococcus aureus ATCC 29213, Staphylococcus saprophyticus ATCC 15305, Streptococcus agalactiae ATCC 13813, Enterococcus faecalis ATCC 19433, Bacillus subtilis ATCC 6051, and Bacillus cereus ATCC 11778), and four species of Candida (Candida albicans ATCC 18804, C. albicans 10231, Candida glabrata ATCC 2001, Candida krusei ATCC 34135, and C. tropicalis ATCC 28707). The cytotoxicity and the anti-MAYV evaluations were performed using Vero cells (Kidney cells from an African green monkey) ATCC CCL-81, USA.

**Antibacterial activity**

The antibacterial activity was evaluated by the determination of the minimum inhibitory concentration (MIC) using the broth microdilution method, according to document M07-A9 of the Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2012), with minor modifications. In brief, a concentration range (1–500 µg/ml) of the extracts was prepared by serial dilutions in Mueller-Hinton broth (Kasvi, Brazil). For the bacterial inoculum, isolated colonies cultured in

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**Discussion**

The results demonstrated high antibacterial activity of the T. micrantha extracts, with MIC values ranging from 25 to 100 µg/ml for the tested bacteria. The extracts showed promising activity against Gram-positive and Gram-negative bacteria, including multi-drug resistant strains, highlighting their potential in the development of new antimicrobial agents.
Mueller-Hinton agar (Kasvi, Brazil) were suspended in sterile saline solution 0.85% (Proquímicos, Brazil), and the turbidity was adjusted in a spectrophotometer (Biochrom, United Kingdom) according to the 0.5 McFarland scale (OD$_{25\text{um}}$ = 0.190–0.210). The bacterial suspension (50 µL) was then dissolved in 10 mL of Mueller-Hinton broth to reach 10$^8$ CFU/mL of optical density. Bacterial inoculum (100 µL) was added to the microplates and incubated for 24 h at 37°C. The MIC was considered the lowest concentration in which no visible bacterial growth could be observed. Amoxicillin and chloramphenicol (1–500 µg/mL) were used as positive controls and DMSO as a negative control. The assays were performed in triplicate.

**Antifungal activity**

To evaluate the antifungal activity of the plant extracts against Candida species, the broth microdilution method, according to CLSI document M27-A3,[17] was used, with minor modifications. Briefly, the extracts were diluted in Sabouraud Dextrose Broth (Acumedia, Brazil) in microplates at concentrations ranging from 1 to 500 µg/mL. Subsequently, the pre-inoculum was prepared from isolated colonies cultured for 48 h in Sabouraud dextrose agar (Acumedia, Brazil) whose turbidity was adjusted according to the 0.5 McFarland scale (10$^6$ CFU/mL). The pre-inoculum was then dissolved until the resulting cell density was 10$^8$ CFU/mL. The microplates were incubated for 48 h at 37°C, and the MIC was considered the lowest concentration visually capable of inhibiting the microbial growth. As a positive control, nystatin was used in the concentration range of 1–500 µg/mL, and DMSO as a negative control. The assays were performed in triplicate.

**Cytotoxicity assay**

The cytotoxic concentration (CC) to 50% of the cells (CC$_{50}$) was determined using the indirect MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, USA) colorimetric method.[18] In 96-well microplates, Vero cells (5 × 10$^4$ cells/well) were implanted and maintained in Dulbecco’s Modified Eagle’s Minimum Medium (DMEM) (Cultilab, Brazil) with 5% of fetal bovine serum (FBS, Cultilab, Brazil). After 24 h, the cells were treated with different concentrations of the plant extracts (1–500 µg/mL) diluted in DMEM (containing 2.5% FBS) and incubated at 37°C for 48 h. Then, the medium of the wells was replaced by 25 µL of MTT solution (2 mg/mL in phosphate buffered saline 1x), and the plates were incubated for 2 h at 37°C in 5% CO$_2$, allowing the tetrazolium salt to be metabolized by the viable cells. Then, 100 µL of DMSO was added to the microplates. The optical density of the solution in the microplate wells was spectrophotometrically determined at 492 nm. The CC$_{50}$ represents the extract concentration required to reduce cell viability by 50% when compared to the viability of the untreated cells.

**Antiviral assay**

The highest concentrations of all extracts, toxic to 50% of the cells (CC$_{50}$) or nontoxic, were tested for antiviral activity against the MAYV (isolated from Haemagogus spp captured on the Belém-Brasilia highway). For this, Vero cells (5 × 10$^4$ cells/well) in 96-well microplates and the viral inoculum were pretreated for 30 min at 37°C with different concentrations of the extracts, ranging from 1 to 250 µg/mL, viral control was also maintained at 37°C for 30 min. This pretreatment was performed to include all of the stages of the viral cycle in which the compound could act, i.e., in adsorption, penetration, viral replication or virucidal effect. Next, the pretreated cells were infected with the pretreated viral inoculum at a multiplicity of infection of 0.1. After 48 h of incubation, cell viability was also determined using the MTT colorimetric method in a condition equivalent to those used in the cytoxicity assay. The effective concentration (EC$_{50}$) represents the extract concentration that promoted the protection of 50% of the cells submitted to viral infection. Ribavirin, a nucleoside analog and nonspecific antiviral, was used as positive control.

**Statistical analysis**

Regression analysis was performed to analyze the results of the antiviral assays. The mean ± standard deviation of triplicate samples was determined using the statistical software GraphPad Prism® v. 7 (GraphPad Software, Inc. La Jolla, California, USA). A one-way ANOVA test was used to compare treatments.

**Results**

The antimicrobial potential of six extracts from leaf and branch of T. micrantha obtained using solvents of different polarities was evaluated by MIC assay. The MIC > 500 µg/mL [Table 1] suggest that they are inactive against the microorganisms employed. According to Mbaveng et al., the antimicrobial activity of potential drugs is considered to be significant if the MIC values are lower than 10 µg/mL; moderate if 10 < MIC < 100 µg/mL and low if MIC > 100 µg/mL.[19] Amoxicillin, a broad spectrum action-type penicillin that interferes with cell wall biosynthesis showed significant activity for all Gram-positive bacteria, except for B. subtilis ATCC 6051, which was insensitive to this drug (MIC >500 µg/mL). For Gram-negative bacteria, this penicillin showed significant activity for E. coli ATCC 25992 (MIC 1.95 µg/mL), S. flexneri ATCC 12022 (MIC 0.24 µg/mL), S. choleraesius ATCC 10708 (MIC 0.24 µg/mL), and S. typhimurium ATCC 14028 (MIC equal to 0.49 µg/mL). For A. baumannii ATCC 19606, amoxicillin presented low activity, inhibiting this pathogen at the concentration of 125 µg/mL.

The positive control used for the beta-lactamas producing strains (E. cloacae ATCC 23355, K. pneumoniae ATCC 4352, K. oxytoca ATCC 0182, and P. aeruginosa ATCC 15442) was the antibiotic chloramphenicol. This drug presented moderate activity against K. oxytoca ATCC 0182 and P. aeruginosa ATCC 15442, with MIC values of 62.5 and 15.6 µg/mL, respectively. This amphenicol showed significant activity against E. cloacae ATCC 23355 (0.9 µg/mL), and K. pneumoniae ATCC 4352 (3.9 µg/mL).
isolated six different triterpenes from the roots and stems of several extracts and secondary metabolites of plants conventionally used in the traditional medicine, which contributes to the discovery of new bioactive compounds.\textsuperscript{[21]} However, there are no reports in the literature about the phytochemical and antimicrobial activity of \textit{T. micrantha} to compare and discuss the results obtained in this work.

Chen \textit{et al.}\textsuperscript{[22]} isolated flavonoids from the roots and stems of \textit{Tripterygium wilfordii} (Celastraceae) which were active against \textit{Cryptococcus neoformans}, \textit{P. aeruginosa}, vancomycin-resistant \textit{E. faecalis}, and methicillin-resistant \textit{S. aureus}. Mokoka \textit{et al.}\textsuperscript{[23]} isolated six different triterpenes from the leaves of \textit{Maytenus undata} (Celastraceae) and among them, triterpenes, 3-oxo-11α-hydroxyolean-12-ene-30-oic acid and 3,11-dihydroxyolean-12-ene-30-oic acid showed the best activity (MIC value ranging from 24 to 63 µg/mL) for two Gram-positive species (\textit{S. aureus ATCC 29213} and \textit{E. faecalis ATCC 29212}), two Gram-negative species (\textit{E. coli ATCC 25922} and \textit{P. aeruginosa ATCC 27853}), and clinical isolates of \textit{C. albicans} and \textit{C. neoformans}.

\textit{C. albicans} and \textit{C. glabrata}, strains isolated from clinical samples, were the most susceptible species, followed by \textit{E. faecalis} and \textit{C. parapsilosis}.

\textbf{DISCUSSION}

Recently, many studies have shown the antimicrobial activity of several extracts and secondary metabolites of plants conventionally used in the traditional medicine, which contributes...
Detection of the antiviral activity of epicatechin

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Table 2: Citotoxicity and anti-Mayaro activity of extracts of T. micrantha

<table>
<thead>
<tr>
<th>Extracts</th>
<th>CC_{50} (µg/mL)^a</th>
<th>EC_{50} (µg/mL)^b</th>
<th>SI^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>EALE</td>
<td>201.5±33.2</td>
<td>55.8±2.3</td>
<td>3.6</td>
</tr>
<tr>
<td>HLE</td>
<td>148.4±12.4</td>
<td>50.1±5.8</td>
<td>2.9</td>
</tr>
<tr>
<td>EABE</td>
<td>224.4±37.1</td>
<td>63.7±5.4</td>
<td>3.5</td>
</tr>
<tr>
<td>CBE</td>
<td>327.9±43.5</td>
<td>91.0±14.4</td>
<td>3.6</td>
</tr>
<tr>
<td>HBE</td>
<td>177.7±12.5</td>
<td>143.7±31.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Ribavirin (positive control)</td>
<td>523.1±42.5</td>
<td>118.8±19.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

^a50% cytotoxic concentration, ^b50% effective concentration of viral replication, ^cSelectivity Index: ratio between substance’s CC_{50} and EC_{50}.

concentrations ranging from 16 to 0.0156 mg/mL. These authors showed that the plants possessed significant antimicrobial activity. However, of all the plants tested, the broadest spectrum of action was obtained for Maytenus macrocarpa (Celastraceae), which showed activity against bacteria and fungi in the concentration range from 125 to 250 µg/mL.

Dhayalan et al. evaluated the presence of secondary metabolites and the antimicrobial activity of the ethanol and chloroform extracts of Spathiphyllum cannifolium (Araceae). The phytochemical screening showed the presence of steroids, triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins. The antimicrobial activity revealed that the leaf extracts inhibited C. albicans, E. coli, S. aureus, B. subtilis, and P. aeruginosa. In this study, the chloroform extract showed activity for all the pathogens.

When evaluating the antimicrobial activity by the disk diffusion method of the acetone extract of seven Cuban plants against S. aureus, E. coli and C. albicans, Abreu et al. noticed that most plants did not present or presented low antimicrobial activity against the species employed. Such a result reinforces the fact that, although those plants are commonly used in traditional medicine, a bioprospection of the biological activities of such species should be performed to determine their real potential.

Only compounds with SI >10 are considered safe and nontoxic due to the distance between the pharmacological dose value and the toxic dose value. In this direction, none of the extracts of T. micrantha showed promising anti-MAYV activity, demonstrating an SI value lower than the ribavirin (a nonspecific antiviral). Spindola et al. evaluated the anti-MAYV activity of Cassia australis (Fabaceae) extracts and obtained SI values of 20 and 33 to the ethanolic and butanolic extracts, respectively, which indicates a good antiviral effect.

Santos et al. evaluated the anti-MAYV activity of the ethanolic and butanolic extracts of Bauhinia longifolia (Fabaceae) and the flavonoids quercetin and quercetin 3-O-glycosides isolated from these extracts. The ethanolic extract showed the highest SI value (623), while ribavirin showed an SI of 8. This result indicates that the concentration needed for the extract to have antiviral activity is lower than the concentration that causes the toxic effect on the cells.

In the assay conditions, the leaf and branch extracts of T. micrantha did not show antibacterial and antifungal activities. However, there is a mixture of substances in the extracts, and one compound may be playing an antagonistic effect on the other, inhibiting its activity. It is necessary to isolate the constituents of each extract to evaluate their antimicrobial effect, which was not part of this study.

**Conclusion**

Leaf and branch extracts from T. micrantha were assayed against many bacteria and fungi but, under the conditions employed, they did not present activity. Further studies concerning the isolation and antiviral activity of the isolated compounds are currently performed in our laboratory, and the results will be reported in due course.

**Acknowledgment**

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**Conflicts of interest**

There are no conflicts of interest.

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