Antibacterial, Antibiofilm, and Antischistosomal Activity of Montrichardia linifera (Arruda) Schott (Araceae) Leaf Extracts

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Abstract: With a broad ethnopharmacological tradition in Brazil, Montrichardia linifera has been reported as a potent antiretroviral, antimicrobial, and antiprotozoan agent. However, there is a lack of studies on its effect on bacterial biofilm formation and Schistosoma mansoni worms. This study reports the effects of antibacterial, antibiofilm, and antischistosomal properties of leaf extracts of M. linifera. Phytochemical screening and identification of the main compounds of the extracts were performed. All the extracts evaluated showed antibacterial activity at the concentrations tested. We checked for the presence of flavonoids and derivatives of phenolic acids by the presence of spectra with bands characteristic of these classes in the sample analyzed. The antibacterial assays showed that the best MICs corresponded to 125 µg/mL against Enterococcus faecalis ATCC 29212 in all fractions. The ethanolic and methanolic extracts showed the ability to inhibit biofilm of Staphylococcus aureus ATCC 25123. For the antischistosomal activity, only the acetone and ethyl acetate extracts had a significant effect against helminths, with potent activity at a concentration of 50 µg/mL, killing 100% of the worms after 72 h of incubation. The M. linifera leaf extracts showed antibacterial activity, biofilm inhibition capacity, and anthelmintic activity against S. mansoni.

Keywords: Montrichardia linifera; biofilm; antibacterial; Schistosoma mansoni; leaf extract

1. Introduction

Montrichardia linifera (Arruda) Schott, popularly known as “Aninga”, belongs to the family Araceae. Populations of M. linifera generally form stands of varying sizes, from a few meters to large floating islands of many hectares, which colonize open areas along the edges of rivers and lakes [1,2].

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In Brazil, *M. linifera* is traditionally used in ethnomedicine [3], wound healing, and as an antiarthritic, antidiuretic, and expectorant [4,5]. However, its overuse is considered toxic in humans, due to its ability to cause burns, skin eruptions, and spots, and, in case of eye contact, blindness [5,6]. In addition, some reports claim that the tea produced from senescent leaves attached to the plant is used to treat liver diseases [5]. Thus, due to its ethnomedicinal relevance and wide use in popular medicine, *M. linifera* has been the object of research aiming to investigate its biological activities. Several kinds of bioactivities have already been scientifically verified by standardized and validated assays, as in the case of the antiplasmodic and antibacterial activity, and low toxicity against *Artemia salina* [1,7,8].

Schistosomiasis, an illness caused by worms belonging to the genus *Schistosoma*, is common in rural low-income communities and has been relatively neglected by medical researchers. The disease, which occurs in more than 70 countries and affects more than 200 million people, is considered a severe public health problem worldwide [9]. Praziquantel (PZQ), the only drug available to treat schistosomiasis, was developed in the 1970s and has been an essential factor for the reduction in schistosomiasis mortality. However, the emergence of PZQ-tolerant or PZQ-resistant parasites remains a major concern, which points to the need to search for new drugs against schistosomiasis [10,11].

At the same time, healthcare-associated infections are a serious public health problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main human pathogens involved in community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA) infections [12]. The ability of this opportunistic pathogen to cause various infections is mainly due to the acquisition of resistance to various drugs, its diversity of virulence factors, and its ability to produce biofilm in medical devices. In the last 20 years, *S. aureus* infections have become more dangerous and expensive to treat because of the increasing prevalence of antimicrobial resistance in *S. aureus*, arising from the widespread use of antibiotics [13–16].

Given the need for and importance of seeking new drugs for the treatment of infectious diseases, coupled with the lack of research on the antimicrobial and anthelmintic activities of *M. linifera*, the aim of this study was to characterize the chemical profile and assess the antibacterial, antibiotic, and antischistosomal activity of *M. linifera* (Arruda) Schott extracts.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract

Leaves of *M. linifera* were collected from the town of Arame, Maranhão state, Brazil (latitude 04°46’08.6’’S and longitude 46°01’46.8’’W). The material was collected in the morning in March 2019 and dried in the sun. Specimens were deposited in the “Delta do Parnaíba” Herbarium, Federal University of the Parnaíba Delta (Voucher number: HDELT 1335).

The *M. linifera* samples were analyzed in the Laboratory of Natural Sciences and Biotechnology at the Federal University of Maranhão/Grajaú. The samples were washed with distilled water and dried in an oven for 17 h at 40 °C with closed air circulation. Dry samples were powdered using an analytical mill and stored at −20 °C until extraction preparation. Separate extractions of 20 g of dry sample were prepared by stirring at 10 rpm in either ethanol, methanol, acetone, ethyl acetate, hexane, or chloroform (200 mL) for 72 h. After filtering using Whatman paper filters (20–25 µm particle retention) under vacuum, the extracts were stored in amber flasks. The seven solutions will be referred to as ethanol (EE), methanol (ME), acetone (AE), ethyl acetate (EAE), hexane (HE), dichloromethane (DE), and chloroform (CE) [17,18]. A phytochemical screening [19,20] was carried out before starting to perform detection using chromatography.

2.2. High-Performance Liquid Chromatography (HPLC) Analysis

In order to trace the chromatographic profile, the extract was solubilized in 2.0 mL of methanol/water (9:1) and applied to a C18 cartridge (Strata C18 –E Phenomenex, Torrance, CA, USA). The sample was filtered through membrane filters (Simplepure PTFE 0.22 µm, Allcrom, São Paulo, Brazil) and diluted in methanol/water (8:2) to obtain an approximate
solution concentration of 5.0 mg/mL. A Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan) was used, consisting of a solvent delivery module with a double-plunger reciprocating pump, Photo Diode Array (PDA) detector (SPD-20AT). The column used was a Luna 5 µm C18 100 A (250 µm × 4.6 µm). The elution solvents used were A (2% acetic acid in water) and B (2% acetic acid in methanol). The samples were eluted according to the following gradient: 95% A/5% B as initial in 50 min. The flow rate was 1 mL/min, and the run time 50 min. The column temperature was 20 °C. The sample injection volume was 10 mL. Data were collected and processed using LC Solution software (Shimadzu, Kyoto, Japan).

2.3. Antibacterial Assay

2.3.1. Bacterial Strains and Culture Conditions

The effect of *M. linifera* extracts on seven bacterial strains was determined: *Staphylococcus aureus* ATCC 25123, *Escherichia coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, and *Salmonella enterica* subsp. *enterica* ser. *Typhimurium* ATCC 14028. Bacteria were grown in Mueller–Hinton agar for 24 h and, from isolated colonies, a microbial suspension was standardized with sterile saline to a turbidity equivalent to 0.5 on the McFarland scale (approximately 1 × 10^8 CFU/mL). This solution was diluted ten times and used in the in vitro experiments described below, where each well in the 96-well plate contained the bacteria at a final concentration of 5 × 10^5 CFU/mL.

2.3.2. Determination of Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration (MIC), sterile flat-bottom 96-well plates were used to perform serial microdilution in Mueller–Hinton broth. At the end of the process, the concentrations of the seven extracts varied from 125 to 1000 µg/mL (0.01% DMSO) and the wells contained an inoculum of 5 × 10^5 CFU/mL [20]. The first column was defined as the positive control (bacterial viability—C+), the two following columns represented the negative controls (one for broth sterility and the other for the extract sterility—C−), and the remaining columns corresponded to the tested samples. All experiments were performed in triplicate. The plates were then incubated under aerobic conditions at 37 °C, for 24 h. The MIC was considered the lowest concentration able to inhibit visual bacterial growth. For a better observation of bacterial growth as well as its inhibition by the extract, 10 µL of TTC (2,3,5- tryphenil tetrazolium chloride) was added to each well. Vancomycin, Meropenem and Sulfamethoxazole + Trimethoprim were used as positive control.

2.3.3. Agar Diffusion Method (ADM)

Plates containing Mueller–Hinton agar were previously prepared. Subsequently, with the aid of a sterile swab, the bacterial inoculum, with a turbidity corresponding to 0.5 on the McFarland scale, was distributed in a uniform manner on the surface of the agar. After that, wells with a diameter of 12 mm were performed and identified. Each well received 60 µL of extract, and the plates were incubated in a hot air oven at 35 ± 1 °C for 24 h. The zone of inhibition was measured in millimeters using a metric ruler with millimetric subdivisions. For this test, the same strains as above were used [21,22].

2.3.4. Inhibition of *S. Aureus* Biofilm Adherence Assay

The *M. linifera* samples were analyzed at the Laboratory of Microbiology of Biodiversity and Biotechnology Research Center (BIOTEC) at the Federal University of the Parnaíba Delta. Previously, the minimum inhibitory concentration (MIC) of EM and EE extracts against *S. aureus* ATCC 29213 was determined, as mentioned above [20]. The biofilm assay was performed at sub-inhibitory concentrations in a 96-well plate. The *M. linifera* extracts’ effect on *S. aureus* biofilm adherence was evaluated following [23], with adaptations. The strains (5 × 10^5 CFU/mL) were grown in 96-well plates with tryptic soy broth (TSB) with
0.5% glucose and extracts at concentrations of 1/2, 1/4 MIC. After 24 h, the wells were rinsed with saline solution, and the cells that remained adhered in the wells were fixed with methanol PA for 10 min. After removal of the methanol, the wells were stained with 100 µL of 0.1% crystal violet (CV) dye for 5 min, rinsed twice with distilled water, and thoroughly air-dried under ambient conditions. For biofilm adherence quantification, 100 µL of ethanol (95%) was added to the CV-stained wells for 30 min. The resulting solutions were transferred to other 96-well plates, and their absorbance (A) was measured at 595 nm. This assay was performed in quadruplicate. The percentage of inhibition was calculated using the equation: 

\[
\text{Percentage of inhibition} = \left( 1 - \frac{A_{595 \text{ of the test}}}{A_{595 \text{ of non-treated control}}} \right) \times 100.
\]

Data were expressed as mean ± SD and evaluated using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test using GraphPad Prism 5.0. *p < 0.05 or ***p < 0.001 was considered statistically significant, compared to the control group consisting of a non-treated control biofilm (0% inhibition), according to [24].

2.3.5. AFM Biofilm Analysis

The effect of M. linifera on S. aureus ATCC 29213 planktonic aggregates was examined by atomic force microscopy (AFM). A methodology similar to that previously described for the biofilm adherence assay was performed, where the microorganism was incubated with the extract at 1/2, 1/4, and 1/8 MIC, in TSB with 0.5% glucose. The non-treated inoculated culture medium was used as a negative control. After incubation for 24 h, 15 µL of the culture media containing the treated or non-treated bacteria were deposited onto a clean glass surface followed by air-drying. The samples were then gently rinsed with 1 mL of deionized water to remove salt crystals and air-dried again under ambient conditions before analysis. All samples were prepared at the same time, accurately exposed to the same conditions, and examined within 12 h of deposition. AFM was carried out on a TT-AFM microscope from AFM Workshop (Signal Hill, CA, USA). The analysis of Neem EE effect on planktonic bacterial aggregate morphology was carried out in vibrating mode, using cantilevers (Tap300-G, Ted Pella, Redding, CA, USA) with a resonant frequency of approximately 300 kHz. Multiple areas of each sample were examined, but here we show only representative images. Images were analyzed using Gwiddion software 2.33.

2.4. In Vitro Antischistosomal Activity

2.4.1. Maintenance of the Schistosoma Mansoni Life Cycle

Female Swiss mice (3-week old, weight ca. 14 g) were purchased from Anilab (São Paulo, Brazil). All animals were maintained under controlled conditions (temperature ca. 22 °C, humidity ca. 50%, 12/12 h light/dark cycle, and free access to rodent diet and water). To maintain the parasite’s life cycle, mice were infected with S. mansoni (BH strain) by subcutaneous injection of ~120 cercariae. Cercariae were harvested from infected intermediate host snails Biomphalaria glabrata by exposure to light for 3 h, following standard procedures from our laboratory [25–27].

2.4.2. In Vitro Antischistosomal Assay

Adult S. mansoni of both sexes were collected by dissecting the intestinal veins of mice euthanized 7 weeks post-infection [11]. For the in vitro antischistosomal assay, one pair of worms was transferred to each well of a 24-well culture plate (Tissue Culture plastics, TPP, St. Louis, MO, USA) containing RPMI medium supplemented with FCS and antibiotics at 37 °C in a 5% CO₂ atmosphere, as previously described [28,29]. The S. mansoni cultures were then incubated with each drug at a final concentration of 50 µM. Parasites were then kept for 72 h, and the effects of compounds were assessed microscopically, with an emphasis on changes in worm motor activity, morphological changes, and mortality rate. Schistosomes were also incubated in the presence of the highest DMSO concentration (0.5%) as a negative control and 5 µM praziquantel as a positive control. Each concentration was tested in triplicate, and experiments were performed at least three times.
2.4.3. AFM Schistosoma Analysis

After the test to check the anti-schistosoma potential of the fractions EAE and EA, the worms were fixed with 2% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA). Worms from each sample were chosen randomly and placed on a clean glass slide. Then, the worms on the glass slide were washed with sterile distilled water (to remove residues) and were all dried at room temperature for 20 min. After this time, the center of the sample was manually positioned under the tip, to increase the probability of scanning the worm cuticle. The worms were analyzed using TT-AFM equipment (AFM, Workshop, USA) in vibrating (tapping) mode with a silicon cantilever (Tap300-G, Ted Pella, USA) with a resonance frequency of 237 kHz. Images of $25 \times 25 \mu m$ with a resolution of $512 \times 512$ pixels were obtained and processed using the Gwyddion 2.4 software. From the images, parameters that indicate the average roughness ($Ra$ and $Rms$) of the worm surface were obtained and statistically compared with GraphPad Prism 6.0 software using one-way ANOVA with Sidak post-hoc and $p < 0.05$ for significant differences.

3. Results

3.1. HPLC Analysis

With the aid of the PDA detector coupled to the HPLC system, it was detected that the extract of M. linifera presented great complexity and diversity in the matrix, as evaluated by the large number of peaks eluted along the chromatographic run. With the aid of HPLC-PDA, the chromatographic data were processed and the chromatograms obtained at selective wavelengths for a determined secondary metabolite class. We checked for the presence of flavonoids and derivatives of phenolic acids by the presence of spectra with bands characteristic of these classes in the sample analyzed, such as the chemical classes already described in the literature with reports of different biological potentials, including antimicrobial activity. Compound 1 was compared to an authentic isolated standard and was identified as p-hydroxybenzaldehyde. Compounds 2–5 were identified as derivatives of glycosylated flavonoids, and the other compounds were identified as flavonoid aglycones (Figure 1 and Table 1).

| ID | Time Retention (min) | UV max (nm) |
|----|---------------------|-------------|
| 1  | 2.1                 | 225; 280    |
| 2  | 20.1                | 299; 327    |
| 2a | 20.6                | 299; 327    |
| 2b | 21.0                | 262         |
| 2c | 22.2                | 262         |
| 3  | 28.1                | 255; 364    |
| 4  | 28.9                | 255; 364    |
| 5  | 30.0                | 256; 365    |
| 6  | 30.2                | 256; 365    |
| 7  | 35.0                | 255; 270    |
| 8  | 36.2                | 252; 265    |
| 9  | 42.0                | 253; 266    |
| 10 | 43.8                | 254; 270    |
| 11 | 44.0                | 285         |
| 12 | 45.1                | 287         |

Table 1. UV-Vis data of the chromatographic peaks of the extract and fractions obtained from M. linifera.
Figure 1. Typical chromatogram obtained by HPLC-PDA analysis (254 nm) of the EE, ME, AE and EAE of *M. linifera*.

3.2. Antibacterial Assay

All the extracts evaluated ethanol (EE), methanol (ME), acetone (AE), ethyl acetate (EAE), hexane (HE), dichloromethane (DE), and chloroform (CE), and showed antibacterial activity at the concentrations tested. The best MICs corresponded to 125 µg/mL for *E. faecalis*, 250 µg/mL for *S. aureus*, and 500 µg/mL for *S. epidermidis* (Table 2).
Table 2. Minimum inhibitory concentration (MIC) in μg/mL, obtained for the EE, EM, EA, EAE, EH, ED and EC leaf extracts from *M. linifera* in *E. coli*, *S. enterica*, *S. maltophilia*, *A. baumannii*, *S. aureus*, *E. faecalis* and *S. epidermidis* strains.

| Microorganisms     | EE (μg/mL) | EM (μg/mL) | EA (μg/mL) | EAE (μg/mL) | EH (μg/mL) | ED (μg/mL) | EC (μg/mL) | Control (μg/mL) |
|-------------------|------------|------------|------------|-------------|------------|------------|------------|----------------|
| *E. coli*         | 1000       | 1000       | 1000       | 1000        | 1000       | 1000       | 1000       | <0.5*          |
| *S. enterica*     | 1000       | 1000       | 1000       | 1000        | 1000       | 1000       | 1000       | <0.5*          |
| *S. maltophilia*  | 500        | 500        | 500        | 1000        | 500        | 1000       | 1000       | <2**           |
| *A. baumannii*    | 1000       | 500        | 500        | 1000        | 1000       | 1000       | 1000       | <2**           |
| *S. aureus*       | 1000       | 250        | 1000       | 2000        | 2000       | 1000       | 1000       | 1***           |
| *E. faecalis*     | 125        | 125        | 125        | 125         | 125        | 125        | 125        | 1***           |
| *S. epidermidis*  | 1000       | 1000       | 1000       | 1000        | 1000       | 1000       | 1000       | 1***           |

Antibiotics: Meropenem *; Sulfamethoxazole + Trimethoprim **; Vancomycin ***.

The solvents used in this research were the most efficient for extracting active substances from plants, according to data from the literature. First, it is important to highlight the methanolic fraction, which obtained an MIC of 250 μg/mL against *S. aureus* ATCC 25923, and 500 μg/mL, against *A. baumannii* and *S. maltophilia*. The ethanolic, acetone and hexane fractions also presented an MIC of 500 μg/mL against *S. maltophilia*.

### 3.3. Agar Diffusion Method (ADM)

The HE, DE, and CE extracts obtained from the leaves of *M. linifera* showed zones of inhibition against all the Gram-positive bacteria tested, namely *S. aureus*, *S. epidermidis*, and *E. faecalis* (Figure 2).

Figure 2. The results found by the agar diffusion method (ADM) were obtained from the leaf extracts of *M. linifera*—5: hexane (HE); 6: dichloromethane (DE); and 7: chloroform (CE). (A) *S. epidermidis*; (B) *E. faecalis*; (C) *S. aureus*.

### 3.4. Antibiofilm Assay

Atomic force microscopy was performed to observe the behavior of the aggregates after exposure to *M. linifera* extracts at sub-MIC concentrations. This test was performed with EE and ME, against *S. aureus* ATCC 29213 (strain capable of forming biofilms) and examined by AFM. Both EE and ME showed MIC at 500 μg/mL and displayed biofilm adherence inhibition, especially at the highest concentration. It is worth highlighting that the concentrations tested corresponded to 1/4 and 1/2 of the MIC found for each substance against the tested strain, and these concentrations varied from 125 μg/mL to 250 μg/mL. No substance was added to the control (0% inhibition) and, for this reason, no adherence inhibition was observed.

The adherence inhibition assay, using sub-MIC concentrations of *M. linifera* EE, showed that the extracts were able to inhibit the formation of bacterial aggregates (Figure 3). For *S. aureus* ATCC 29213, this effect was significant using only 1/2 of the MIC (Figure 4).
3.4. Antibiofilm Assay

Atomic force microscopy was performed to observe the behavior of the aggregates examined by AFM. It was possible to calculate the average biofilm inhibition by ME and EE at different concentrations (Figure 4).

With the AFM results obtained, it was possible to calculate the average biofilm inhibition by ME and EE at different concentrations (Figure 4).

It was observed that EE obtained an average inhibition of 60.67, at a concentration of 250 µg/mL, while the ME obtained an average inhibition of only 16.67. Thus, these results show a significant difference between the concentrations of EE and ME in biofilm inhibition. The control (untreated biofilm) represents 0% inhibition (not showed).

3.5. In Vitro Antischistosomal Assay

To perform the anthelmintic tests, biological screening was carried out using six different extracts: ethanol (EE), methanol (ME), acetone (AE), ethyl acetate (EAE), hexane (HE), and chloroform (EC). Initially, adult S. mansoni worms were exposed to 50 µg/mL of each extract. Praziquantel (1 µg/mL) was used as a positive control. Two out of six extracts showed activity against S. mansoni (Table 3).

The results obtained for the anthelmintic activity of the extracts against S. mansoni showed that AE and EAE had positive and potent activity at a concentration of 50 µg/mL, killing 100% of the worms after 72 h of incubation. As shown in Figures 5 and 6, compared with control worms, incubated only in culture medium, EA and EAE extracts caused morphological changes in the worms’ tegument. Swelling was visible on the helminth tegument, and this parameter is considered an indicator of stress. The average amount of
swelling was visible on the AE extract at approximately 600 nm and on the EAE extract at approximately 1000 nm.

Table 3. Effect of the extracts obtained from the *M. linifera* against adult worms of *Schistosoma mansoni*.

| Group  | Period of Incubation (h) | Dead Worms (%) | Motor Activity Reduction (%) | Slight | Significant |
|--------|--------------------------|----------------|-------------------------------|--------|--------------|
|        |                          | M | F | M | F | M | F | M | F |
| Control| Immediate                | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 24                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 48                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 72                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5% DMSO | Immediate              | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 24                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 48                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 72                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Praziquantel (1 µg/mL) | Immediate          | 100 | 100 | 0 | 0 | 100 | 100 |
|        | 24                       | 100 | 100 | 0 | 0 | 100 | 100 |
|        | 48                       | 100 | 100 | 0 | 0 | 100 | 100 |
|        | 72                       | 100 | 100 | 0 | 0 | 100 | 100 |
| EAE (50 µg/mL) | Immediate        | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 24                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 48                       | 60 | 60 | 0 | 0 | 60 | 60 |
|        | 72                       | 100 | 100 | 0 | 0 | 100 | 100 |
| AE (50 µg/mL) | Immediate       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 24                       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|        | 48                       | 60 | 60 | 0 | 0 | 60 | 60 |
|        | 72                       | 100 | 100 | 0 | 0 | 100 | 100 |

*a* Percentage compared to the 20 worms.

Figure 5. Atomic force microscopy (AFM) images of control (A) and concentrations of EAE (B) and AE (C) *M. linifera* extracts in worm teguments of *S. mansoni*. 
Figure 5. Atomic force microscopy (AFM) images of control (A) and concentrations of EAE (B) and AE (C) M. linifera extracts in worm teguments of S. mansoni.

Figure 6. Median roughness tegument of S. mansoni treated with EAE and AE by AFM technique. **p < 0.05 in relation to control group (untreated).

4. Discussion

Montrichardia linifera presented different metabolic pathways originating from several types of compounds. However, studies in the literature are found reporting the presence of alkaloids and triterpenoids in M. linifera extracts [7].

According to [30], plant secondary metabolites may show considerable variation according to different factors, such as seasonality, circadian rhythm and development, temperature, hydric availability, nutrients, altitude, atmospheric pollution, and induction by mechanical stimuli or pathogens. These factors may explain the differences found in the tests performed in the present study and the results reported in the literature.

Among the compounds studied, flavonoids have stood out due to their wide range of biological and therapeutic activities, demonstrated in vitro and even in humans [31]. These include their antioxidant, anti-cancer, anti-ulcer, antithrombotic, anti-inflammatory, antiallergenic, immunologic system modulator, antimicrobial, vasodilator, and analgesic activities [32,33]. Another use attributed to this group of phenolic compounds is the taxonomic approach as markers, among which flavonoids, such as flavones, 6- and 8-hydroxylavonoids, and flavonoid sulfates, stand out.

Saponins, in aqueous solution, form a persistent and abundant foam. This behavior happens because these substances present a lipophilic part in their structure, called aglycone or sapogenins, and a hydrophilic part, constituted by one or more sugars [34]. This foam is stable under the action of diluted mineral acids, which makes it different from common soaps.

The methanolic extract showed the best results but similar results were also found, where the same type of extract stands out in M. linifera [1]. Besides, other studies have been performed with methanolic extracts of plants from the Araceae family, which also presented promising results in antibacterial assays: the authors of [35], for example, used extracts from Colocasia esculenta (Araceae) and obtained an MIC of 125 µg/mL against E. faecalis and 250 µg/mL against S. aureus.

When the antibacterial activity of the methanolic extract obtained from the dry leaves of M. linifera, collected in Parnaíba, Piauí, Brazil, was evaluated, growth inhibition of S. aureus ATCC 25123 was seen at a concentration of 500 µg/mL. Thus, it is possible to note that the present study showed promising results, principally concerning the methanolic extract, which can be a strong candidate for natural antimicrobial agents against gram-negative bacterial strains.

According to [1,36], MICs with results from 100 to 500 µg/mL are moderately active, while results from 500 to 1000 µg/mL are considered less active. The results obtained in
the present study, using the ME, demonstrated that this was moderately active against *S. aureus* ATCC 25123, *A. baumannii*, and *S. maltophilia*.

Biofilm formation is an important bacterial virulence factor that causes serious problems in medical practice, affecting prostheses, tubes, and principally, heart valves, requiring high doses of antibiotics to prevent recurrence [37,38]. This aspect is even graver in the case of infections with resistant bacteria, such as MRSA, pointing to the necessity for new agents able to act against this microorganism or its virulence mechanisms [39]. *S. aureus* is known to grow in small groups of 5 to 20 cells, but in some circumstances, it may form large aggregates by the formation of polysaccharide intracellular adhesin (PIA) [40,41].

This test was performed in Parnaíba, Piauí, with EE and ME, against *S. aureus* ATCC 29213 and examined by AFM. *S. aureus* ATCC 29213 is a strain capable of forming biofilms. Thus, the antibiofilm potential of the extracts and their fractions was evaluated. To form a biofilm, it is necessary for the bacteria to adhere to surfaces. Therefore, the nonstick potential of the tested substances was first investigated with the aid of crystal violet, a basic stain that binds to polysaccharides and negatively charged surface molecules in the extracellular matrix of living or dead cells [42]. The observed effect may be due to the presence of phenolic compounds and flavonoids in the extracts used. It is proposed that these molecules have the ability to inhibit biofilm formation, by inhibiting matrix formation [24].

To perform the anthelmintic tests, the concentration of DMSO applied in most in vitro studies varies from 0.2% to 2% [43–47]. However, some in vitro studies have also used higher concentrations, such as 10% DMSO, for example [48–50]. In the present study, all the experiments were performed with two simultaneous controls, one of the controls in the absence of solvent and the other with 0.5% DMSO, showing that the solvent concentration used did not affect the viability of the worms.

Few studies have dealt with differences in the susceptibility of male and female *S. mansoni* worms. The research performed by [51] reported that, in some cases, the difference in the mortality rates between male and female *S. mansoni* is evident. Female worms are more susceptible to *Piper crassinervium*, *Piper diospyrifolium*, and *Piper fulineum* extracts, while the male helminths presented more susceptibility to the *Piper gaudichaudianum* and *Piper umbellatum* extracts.

However, the results found in the present study indicate that there was no difference between the male and female groups in terms of motor activity reduction after exposure to the tested extracts. In fact, the extracts caused alterations in the adult worms’ viability, reduced their motility, and caused the parasites’ death in a concentration-dependent manner.

Studies performed by [52–54], where anti-Schistosoma assays were also carried out to investigate the potential of other substances, reported results at values similar to those found in the present study, even though these authors used isolated substances. Thus, it is necessary to investigate which chemical compounds present in the leaves of *M. linifera* are responsible for this biological activity.

The development of new drugs, derived from natural products, to treat neglected diseases, including schistosomiasis, has been highly encouraged [53]. Besides, these results show a significant reduction in the motor activity of 100% of the worms (Table 3). These are promising results, since the tested substances were in the condition of crude extracts. It is believed that isolating and testing the pure substance responsible for parasite death would be reflected in even better results.

5. Conclusions

This work presents three important properties of *M. linifera* leaf extracts. Its extracts showed antibacterial activity against gram-positive and gram-negative bacteria as well as the ability to inhibit biofilm formation, an important bacterial virulence factor, at least in ethanolic and methanolic extracts. The action against *S. mansoni* worms, the helminth that causes the important and neglected schistosomiasis, was evidenced by acetone and ethyl acetate extracts. Finally, the phytochemical screening of the extracts evidenced the presence of condensed tannins, flavonoids, steroids, saponins, and coumarins. The results
obtained in the present work justify future studies on the biological effects of the isolated components of these extracts in the face of diseases that are neglected and of medical importance, such as schistosomiasis and infections by bacteria resistant to antibiotics.

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