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Antibacterial and hemolytic activities of *Mimosa tenuiflora* (Willd) Poir. (Mimosoidea)

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***Mimosa tenuiflora* is a shrub-sized plant native of the Northeast region of Brazil where it is popularly known as "jurema preta" and is widely used in folk medicine, especially the stem bark extract mixtures. Due to its high content of tannins and flavonoids, it is considered to have anti-inflammatory and antimicrobial activity. The antimicrobial activity of the ethanolic extracts of *M. tenuiflora* (EE*Mt*) was determined by the minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) by means of the broth microdilution technique. The MIC corresponded to the last dilution in which the presence of bacteria was not verified. In order to obtain the MBC, the spread-plating in Mueller Hinton agar (MHA) of the corresponding MIC, MIC×2 and MIC×4 dilutions was performed. The EE*Mt* presented antibacterial activity against *S. aureus* (ATCC 25.925) and *P. aeruginosa* (ATCC 25.619) where the MIC and the MBC were 128 and 256 µg/mL, respectively; concentrations inferior to the cytotoxic concentrations for human erythrocytes (A, B and O). For *S. aureus* (ATCC 25.213), the MIC and the MBC were 512 and 1024 µg/mL, respectively. As for the *E. coli* ATCC 8859 and *E. coli* ATCC 2536 they were 1024 and >1024 µg/mL, respectively. It was concluded that the EE*Mt* has a good antibacterial activity, presenting a low toxicity and a better activity against Gram positive strains. However it exhibited a good activity against the *P. aeruginosa* strain which is a Gram negative microorganism of clinical importance.**

Key words: *Mimosa tenuiflora*, ethanolic extract, antimicrobial activity, hemolytic activity.

INTRODUCTION

Currently, with the emergence of bacterial strains, resistant and multiresistant to the majority of the available antimicrobial agents, there has been a renewal of the interest in the research for new alternative antimicrobial

agents (Zhu et al., 2015; Eun-Jeong et al., 2015; Saiprasad et al., 2015). The search and use of medicinal plants with bioactive properties is an age-old practice, present in several phytotherapy treaties and pharmacopoeias of the great civilizations (Ronghui et al., 2014). The consumer has also valued the availability of more natural and healthier pharmaceutical products which may bring health benefits. These factors have contributed to increase the interest in the research of natural products which present biological activities such as the antimicrobial activity (Manuel et al., 2010; Militello et al., 2011).

M. tenuiflora (Willd.) Poir. is a species of Mimosoideae, a botanic sub-family of the Fabaceae family, and it is commonly found in the Northeast of Brazil, characteristic of the 'Caatinga' vegetation, where some of these plants are known as "jurema preta" (Diego et al., 2013; Ana et al., 2014; Silva et al., 2015). It is a shrub-sized tree disseminated in the States of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe and Bahia. The Mimosoideae has the average height of 5 to 7 m, and are composed of approximately 82 genus with 3.271 species distributed worldwide mainly in tropical, sub-tropical and temperate regions (Maria et al., 2010; Juarez et al., 2013; Cleilton et al., 2014).

The 'jurema preta' as well as other species *Mimoso* genus, has been used by indigenous tribes of the Brazilian Northeast's culture since long before the Portuguese colonization. After the colonization it was also used by Afro-Brazilians. The plant was used to make hallucinogenic drinks due to the presence of a psychoactive alkaloid called N, N- dimetiltriptamine (DMT), which is inactive when administered orally (Fernanda et al., 2010; Reinaldo et al., 2012; Alain et al., 2013; Alan and Maria, 2013).

In folk medicine, the stem barks of *M. tenuiflora* (Willd.) Poir. is used in the treatment of several diseases and pathologies, such as burns and external and internal inflammations, probably due to its elevated content of tannins and flavonoids, which is believed to have antimicrobial activity (Rafael et al., 2008; Camargo-Ricalde, 2000). Studies carried out in Mexico and in Brazil evaluated the antimicrobial properties of the stem bark of *M. tenuiflora* and demonstrated a great inhibitory action of the ethanolic and hydroalcoholic extracts, against Gram-positive, Gram-negative bacteria and dermatophyte fungi. Studies of the stem bark of the 'jurema preta' confirmed its pharmacological properties and showed an exceptional antimicrobial activity of the ethanolic extract against bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus* spp. (Itácio et al., 2010; Marcelo et al., 2012; Edilson et al., 2011; Reinaldo et al., 2014).

Considering the broad potential of application of the unrefined extracts of the stem barks of the mentioned plant species, as well as the fact that a plant species may present a variable chemical composition, and, therefore, also a variable biological activity according to the geographical localization, the aim of the present work was to evaluate the "*in vitro"* antibacterial activity of *Mimosa tenuiflora* (Willd) Poir. by means of the microdilution in plates technique and therefore the determination of the MIC and MBC as well as to determine the hemolytic activity.

MATERIALS END METHODS

Samples of plants and substances

The collection of the 'jurema-preta' was carried out in the rural area of the municipality of Santa Terezinha, interior of the 'sertão' region of the State of Pernambuco, located in an area denominated 'Alto Pajeú'. The plants were identified and stored in the Microbiology Research Laboratory (MRL) Of the Integral Faculties of Patos-PB in exsiccate codified as: *M. tenuiflora*-2735-LPM. The parts of the plants used in the studies were the stem barks. The following substances used in this work were commercially obtained: dimethylsulfoxide (DMSO) and Tween-80 were, respectively, bought from Labsynth Products for Laboratories Ltd. (Diadema, SP, Brazil) and Vetec Fine Chemicals Ltd. (Duque de Caxias, RJ, Brazil), respectively.

Ethanolic extract of *Mimosa tenuiflora* (EEMt)

A quantity of 250 g of the stem bark of *M. tenuiflora* was dried at room temperature and kept away from light; at night it was submitted to artificial drying in a kiln with temperature not superior to 35ºC and posteriorly pulverized (Correa Junior et al., 1994; Furlan, 1998). The powdered material was extracted by maceration using 1 L of ethanol (EtOH) at 95% as a solvent, at room temperature, and homogenized, and then it was left to rest for 72 h at room temperature. After that, the extracts were filtered and concentrated in vacuum in a rotary evaporator at an average temperature of 35°C (Beatriz et al., 2006). For the tests, the material of the dry extract was dissolved in DMSO at 0.5%. The DMSO was chosen due to its lower toxicity when compared to the ethanol.

Culture medium

To test the biological activity of the ethanolic extracts, Brain Heart Infusion broth (BHI-Difico) and Müller-Hinton agar (MHA-Difico) were acquired from Difco Laboratories (Detroit, MI, USA). They were prepared and used according to the manufacturer's instructions.

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Bacterial strains

The tests were performed with 5 standard bacterial strains: *S. aureus* 25,213 and 25,925, *E. coli* 2536 and 8859 and *P. aeruginosa* 25,619 obtained from the American Type Culture Collection (ATCC) that originated from the Department of Molecular Biology of the Federal University of Paraiba (DMB, UFPB). The strains were maintained in MHA at 37 and 4°C until they were used.

Preparation of inoculum

The suspensions were prepared of recent bacterial cultures, plated on MHA, and incubated at 37°C for 24 h in a microbiological incubator. After the incubation, about 4-5 bacterial colonies were transferred with a sterilized microbiological strap to test tubes containing 5 mL 0.9% saline solution (Farmax-Distributor Ltd., Amaral, Divinópolis, MG, Brazil). The resulting suspensions were stirred for 15 s with the aid of a vortex (Fanem Ltd., Guarulhos, SP, Brazil).

The turbidity of the final inoculum was standardized using a suspension of barium sulphate and sulfuric acid at 1% (tube 0.5 in the McFarland standard). The final concentration obtained was of around 1-5 × 10^6 Colony Forming Units per Milliliters (CFU/mL). The confirmation of the final concentration was carried out by microorganism counting in a Neubauer chamber (Cleeland and Squires, 1991; NCCLS, 2008).

Determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The determination of the MIC of the ethanolic extracts against the five strains used in the biological tests was done by the broth microdilution method (Cleeland and Squires, 1991; NCCLS, 2008). One hundred microliters (100 µL) of BHI broth were transferred to the wells of a U-shaped bottom 96-well microdilution plate (Alamar, Diadema, SP, Brazil). After that, 100 µL of the EE*Mt* was inoculated in the wells of the first horizontal line of the plate. Dilutions were duplicated in series, where an aliquot of 100 µL was removed from the most concentrated well into the following well, and originated concentrations of 1024-1 µg/mL. Finally, 10 µL of the inoculum of the bacterial suspensions was added to each well of the plate, in which each column represents a bacterial strand. At the same time, positive and negative controls were made for the bacterial viability. The plate was incubated at 37°C during 24 h in a microbiological incubator. After the adequate incubation time, the presence (or absence) of growth was visually observed. The formation of cellular aggregates or "buttons", as well as the turbidity of the means in the plate wells was considered. The MIC was defined as the lowest concentration which produced a visible inhibition of bacterial growth faced with the unrefined ethanolic extract of *M. tenuiflora* (Willd.) Poir.

To determine the MBC, we subcultivated aliquots of 1 µL of the MIC and two immediately anterior (MIC × 2 and MIC × 4) of the contents of the wells of microdilution plates in Petri dishes containing MHA. After 24 h of incubation at 37°C, a reading was performed to evaluate the MBC, which was considered the lowest concentration which impeded the formation of up to three CFU. The concentrations immediately superior to MIC were sufficient to demonstrate the bactericidal effect of the natural products, seen as the bacteriostatic effect that was determined by the absence of growth in the wells of the microdilution plates (Glauco et al., 2008; Ernst et al., 1996; Espinel-Ingroff et al., 2002; Patrícia et al., 2010).

The biological activity tests were carried out in duplicate, and the results were expressed as the arithmetical average of MIC and the MBC.

Determination of the hemolytic activity

The human erythrocytes were obtained from samples to be disposed from the Clinical Hematology Unit of the Clinical Analysis Teaching Laboratory (BIOLAB) of the Integral Faculty of Patos / FIP.

Aliquots of human blood (type A, B and O) were mixed with NaCl at 0.9% at a ratio of 01:30, under slow and constant stirring. After that, the samples were centrifuged (FANEM) at 3000 rpm for 5 min in order to obtain the erythrocytes. This procedure was repeated twice and the sediment of the last centrifugation was resuspended in 0.9% of NaCl 0.5% up to a final concentration of 0.5%. The faction of the EE*Mt* was added to a 2 ml of erythrocyte suspension at various concentrations (1, 10, 100, 1000 and 2000 µg) in different preparations for a final volume of 2.5 mL. The erythrocyte suspension was the negative control (0% of hemolysis) and the erythrocyte suspension plus 50 mL of Triton X-100 (SIGMA) at 1% was the positive control (100% of hemolysis). The samples were incubated for 1 h at room temperature under slow and constant stirring (100 rpm). After this time, they were centrifuged at 3000 rpm during 5 min and the hemolysis was quantified by spectrophotometry at 540 nm (Beckman DU model – 640, USA) (Mebs et al., 1985; Dresch et al., 2005). The tests were carried out in triplicate. The results were expressed as a percentage which represents the arithmetical average of three measurements.

RESULTS AND DISCUSSION

Antibacterial activity

The results of the antibacterial activity of the EE*Mt* fractions were determined using the MIC and the MBC of the broth microdilution. The EE*Mt* was capable of inhibiting 99.9% of the growth of the used strains, showing specific MIC and MBC for each of the tested strains (Table 1). The ethanolic extracts of some *Mimosa* species have been mentioned in literature because of their antibacterial activity against *S. epidermidis*, *E. coli*, *P. aeruginosa* and activity against *Candida albicans*. The tannins probably are the majority components with antimicrobial activity (Lozoya et al., 1989; Meckes-Lozoya et al., 1990). Studies carried out by research groups in Mexico report the biological activity of the EE*Mt* and the existence of components such as steroids, terpenoids, alkaloids, flavonoids, tannins and others phenolic components (Rivera-Arce et al., 2007).

Generally the Gram positive bacteria are more sensitive to the antibiotics than Gram negative ones. This is expected, as the Gram negative bacteria have an already known external structural membrane which provides a type of barrier to the penetration of numerous molecules which could cause cellular damage, and the periplasmic space contains enzymes capable of hydrolyzing strange substances introduced from the exterior (Madigan and Martinko, 2004; Fabíola et al., 2002).

Evaluation of the hemolytic activity on human erythrocytes

The EE*Mt* fractions did not present any hemolytic activity

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the EE*Mt* Fraction against Gram positive and Gram negative bacterial strains.

Bacterial strains	Fractions EE*Mt*	
	MIC(µg/mL)	MBC (µg/mL)
S. aureus ATCC 25.925	128	256
S. aureus ATCC 25.213	512	1024
E. coli ATCC 8859	1024	>1024
E. coli ATCC 2536	1024	>1024
P. aeruginosa ATCC 25.619	128	256

Table 2. Hemolytic effect of the ee*mt* on human erythrocytes.

Erythrocytes	Hemolytic activity (%)	
Type	EE*Mt* fraction	
	1000 µg	2000 µg
A	3.0	23.1
B	0.0	5.17
O	0.0	1.08

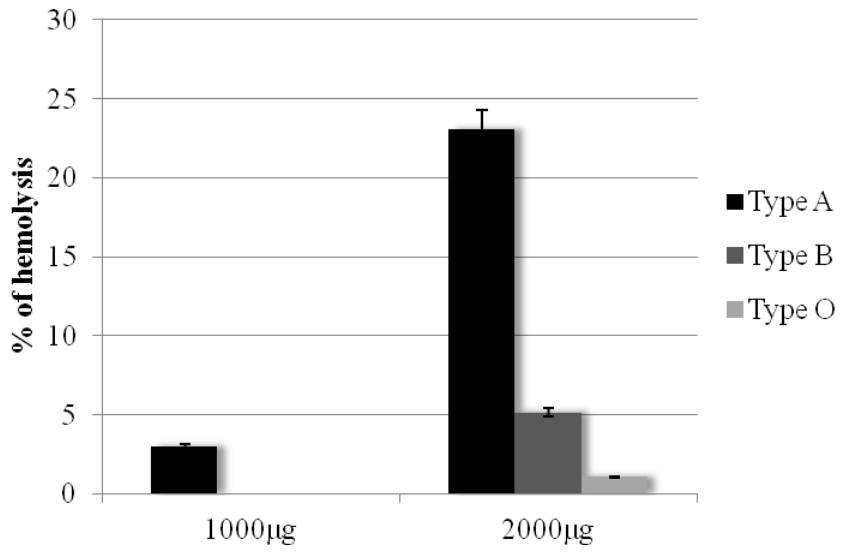

Figure 1. Hemolytic effect of the EE*Mt* on human erythrocytes. At the concentration of 1000 µg, EE*Mt* presented respectively 3.0, 0.0 and 0.0% haemolysis and at the concentration of 2000 µg, EE*Mt* presented 23.1, 5.17 and 1.08% hemolysis on the human erythrocytes (A, B and O), respectively.

up to the concentration of 1000 µg and in 2000 µg presented low toxicity as reported by Mekces-Lozoya et al. (1990) (Table 2 and Figure 1). The tritepenic saponins are considered to be substances probably responsible for this activity, causing the rupture of the erythrocytes membranes (Banerji et al., 1981). Thereby, the extract fractions showed to have a good antibacterial potential against Gram positive and Gram negative bacteria and low toxicity for the human erythrocytes cells.

Conclusions

Based on these results, the present study demonstrated that the EE*Mt* has a good antibacterial activity, with better activity against Gram positive strains; but, however, it

had a good activity against the *P. aeruginosa* strain which is a Gram negative microorganism and which may be isolated in several infections, above all in systemic cases and which commonly presents an elevated profile of resistance to many antibiotics of long-standing use. The low toxicity to the human host of the tested product may be promising and could encourage new research about the phytochemical, toxicological and pharmacological aspects, in order to support its possible rational use in the antimicrobial therapy, especially as anti *S. aureus* and anti *P. aeruginosa.*

Conflict of interests

The authors did not declare any conflict of interest.

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