Antimicrobial activity of *Mimosa tenuiflora* (Willd.) Poir. from Northeast Brazil against clinical isolates of *Staphylococcus aureus*

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INTRODUCTION

*Staphylococcus aureus* is recognized as an important community-acquired and major nosocomial pathogen responsible for a wide range of infections. Although the introduction of antibiotics, since the early 1950s, has reduced morbidity and mortality due to *S. aureus* infections, this Gram-positive bacteria have developed/acquired resistance mechanisms to practically all antibiotics that have been introduced in clinical practice (Lowy, 2003; Hardy et al., 2004). Indeed, methicillin-resistant *S. aureus* (MRSA) are often multidrug-resistant, a serious problem worldwide. New antimicrobial agents are urgently needed and currently there has been increasing interest in evaluating the antimicrobial activity of plant natural products - extracts and phytochemicals (Gibbons, 2004, 2008; Stavri et al., 2007). *Mimosa tenuiflora* (Willd.) Poir. (Mimosaceae; Fabaceae-Mimosoideae) is a perennial evergreen tree or shrub native of Northeast Brazil where is popularly known as “jurema-preta”. It is used in folk medicine against bronchitis, coughs, fever, headache and external ulcers, and in Indigenous and Afro-Brazilian cults as entheogen (Albuquerque et al., 2007; Agra et al., 2008; Souza et al., 2008). Phytochemical investigations of *M. tenuiflora* resulted in the isolation of several classes of compounds, including indole alkaloids, tannins, chalcones, steroids, terpenoids and phenoxychromones (Rivera-Arce et al., 2007; Souza et al., 2008 and references therein).

Despite the existence of reports focusing on the antimicrobial activity of *M. tenuiflora* (Lozoya et al., 1989; Meckes-Lozoya et al., 1990; Heinrich et al., 1992), studies concerning local bacterial isolates as well as extracts from local plant specimens are warrant. In this work we evaluated the antimicrobial activity of ethanol extract of *M. tenuiflora* (EEMt) against strains of *S. aureus* isolated from a Brazilian university hospital.

MATERIAL AND METHODS

Plant material and preparation of EEMt

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Steam bark of *M. tenuiflora* was collected in a county (São José dos Espinharias) of the semi-arid region of the State of Paraíba, Brazil. The plant material was identified by Lúcio Valério Coutinho and a voucher specimen (nº HC001) is deposited at the Herbarium “Caatinga” (Universidade Federal de Campina Grande). Dried and powdered steam bark (100 g) was exhaustively extracted with ethanol using a Soxhlet apparatus. The concentrate solution (57.8 mg/mL) obtained was stored at ca. 4 °C until using.

**Bacterial strains**

Thirty isolates of *S. aureus* obtained at the Universidade Federal da Paraíba Hospital. (Freitas et al., 1999) and the reference strain ATCC 25923 were tested. All strains were maintained on blood agar base (Difco) slants and, prior to use, the cells were grown overnight at 37 °C in brain heart infusion broth (Difco).

**Susceptibility testing**

The minimum inhibitory concentrations (MIC) of the EEMt were determined by the agar dilution method using an inoculum of ca. 10⁴ cfu (colony forming unit) (Pereira & Siqueira-Junior, 1995). The extract concentration range in the Müller-Hinton (M-H agar (Difco) plates was 5.78 to 0.011 mg/mL (two-fold serial dilutions). The MIC is defined as the lowest extract concentration at which no growth is observed after 18-24 h incubation at 37 °C.

**Time kill assay**

The bacteria were grown in Müller-Hinton broth (Difco) until they reached late exponential phase (16-18 h at 37 °C) and resuspended in the same medium at a density of ca. 10⁶ cfu/mL. Extract was added to obtain final concentrations of MIC, 2x MIC, 4x MIC and 8x MIC, and the cultures incubated at 37 °C without agitation. Aliquots (100 µL) were taken at 0, 30, 60 and 120 min, serially diluted in saline and spread-plated onto Müller-Hinton agar. After 18-24 h of incubation at 37 °C, colonies were counted and the relative bacterial titer calculated (bacterial titer at time t/bacterial titer at time 0). In the control, no extract was added.

**RESULTS AND DISCUSSION**

MIC values of EEMt against the thirty clinical isolates of *S. aureus* were 0.18 mg/mL (16/30) or 0.36 mg/mL (14/30, and also the strain ATCC 25923). The steam bark ethanol extract of the same species have been mentioned in the literature for their antibacterial activity on *Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and antifungal activity on *Candida albicans* (Lozoya et al., 1989; Meckes-Lozoya et al., 1990). Studies performed by Mexican research groups support the biological activities by the existence of compounds as steroids, terpenoids, alkaloids, flavanoids, tannins, chalcones and other phenolic compounds in *Mimosae tenuiflora* (Rivera-Arce et al., 2007).

Most studies on plant extracts are based on the measurement of MIC. One limitation of this procedure is the inability of the method to determine how quickly an agent act on the organism. The present study was improved with time-kill essay.

Previous experiments demonstrated at 5.78 mg/mL a decrease in the UFC below the limit of quantification after two hours of exposure. Figure 1 shows the effect of different concentrations of EEMt on cell viability of the reference strain ATCC 25923. All time-kill assays were repeated twice, with consistent results. At concentrations up to 4x MIC, only an inhibitory (bacteriostatic) effect was observed even after two hours of exposure. At 8x MIC, a bactericidal effect [≥ 3 log₁₀ reduction in viable cells, i.e., ≥ 99.9% kill (LaPlante, 2007)] was observed already after 30 min of exposure. This concentration did not prevent a regrowth of the surviving bacteria after one hour, not exceeding, however, 1 log₁₀ increase in viable cells up to two hours.

These data are promising and could encourage further researches on phytochemical, toxicological and pharmacological aspects of *M. tenuiflora* by-products in order to support their possible rational use in the antimicrobial therapy, particularly in anti-*S. aureus* therapy.

**Figure 1.** Effect of the ethanol extract of *Mimosa tenuiflora* at different concentration on the cell viability of *Staphylococcus aureus* ATCC 25923.

**ACKNOWLEDGEMENTS**

I.Q.M.P. is very grateful to Amely Branquinho Martins for helpful discussion during the course of this work. This work was supported by the Brazilian agencies CNPq (PIBIC/UFPb) and FAPESQ-PB.
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