

TITLE: SYNERGISTIC EFFECT OF ETHANOL EXTRACT OF *Mimosa tenuiflora* AGAINST *Staphylococcus aureus* OF BOVINE MILK

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ABSTRACT:

Staphylococcus spp. cause persistent intramammary infections due to the production of resistance mechanisms, such as biofilm and efflux pump, that protect them from the action of antibiotics. The development of efficient and cost-effective therapies is extremely necessary, especially when it is proposed to associate an antimicrobial, commonly used in the treatment of bovine mastitis, with a plant that has medicinal properties, aiming to obtain synergistic effect and, consequently, cost reduction. The aim of this study was to analyze the synergistic effect of crude ethanolic extract (CEE) of *Mimosa tenuiflora* against *Staphylococcus aureus* of bovine milk, besides evaluating the capacity of the microorganism to produce efflux pump. Ten isolates of *S. aureus* that presented the highest Minimum Bactericidal Concentration (MBC) for oxacillin (312.5µg/mL) were used. The CBM for the CEE of the isolates was 0.976µg/mL. The presence of efflux pump was evaluated by culturing in petri dishes containing Mueller Hinton agar (MH) plus ethidium bromide (0.5 µg/mL) at 37°C for 24h. For the synergism test, a bacterial suspension of 1x10⁶ CFU/mL in MH broth was prepared. 100µL of oxacillin (4xMBC) was added in column 6 of a microplate containing 100µL of sterile MH broth and a 1:2 serial dilution was performed. In the "A" line, 100µL of CEE (2xMBC) was added and dilution was performed. Then, 10µL of the bacterial suspension was added. The microplates were incubated at 37°C for 24h and the contents of each well were inoculated onto a plate containing MH agar and incubated at 37°C for 24h. The reading was performed by observing the bacterial growth. The 10 isolates were negative for the presence of efflux pump. In combination with oxacillin, there was a reduction of 96.87% of the CBM value of CEE for all isolates. Regarding oxacillin, its association with CEE resulted in a reduction of 93.75% for three isolates, 87.5% for four isolates and 75% for three isolates. Although the isolates are negative for efflux pump in the presence of ethidium bromide, the presence of other proteins should be evaluated. The association of CEE with oxacillin showed a synergistic effect at different concentrations, giving a significant reduction in the MBC values of both. Thus, these results support the use of antimicrobial combinations with plant extracts, establishing the capacity of this extract as a probable base of alternative antibiotic resistance compounds.

Keywords: antimicrobial activity, synergism, oxacillin, crude ethanolic extract

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