

*MIMOSA TENUIFLORA* EXTRACT REDUCES THE NUMBER OF *STAPHYLOCOCCUS AUREUS* WITH LOW TOXICITY IN *BUBALUS BUBALIS* WITH MASTITIS

**Andréia Vieira Pereira<sup>1,\*</sup>, Marcelo Biondaro Gois<sup>2</sup>, Fabiana Nabarro Ferraz<sup>3</sup>,  
Jozinete Vieira Pereira<sup>4</sup>, Sérgio Santos Azevedo<sup>5</sup>, Ednaldo Queiroga de Lima<sup>6</sup>,  
Onaldo Guedes Rodrigues<sup>6</sup>, Elizabeth Sampaio de Medeiros<sup>7</sup>, Rinaldo Aparecido Mota<sup>7</sup>  
and Maria do Socorro Vieira Pereira<sup>8</sup>**

**ABSTRACT**

The aim of this study was evaluate the treatment of *Bubalus bubalis* (dairy buffaloes) affected with clinical mastitis, through the use of *Mimosa tenuiflora* botanical extract, using as parameters for this study: the semiotic examination and strip cup test, number of colonies count by using Petrifilm plates; isolation and identification of microorganisms found in the animal's milk before and after treatment, toxicological testing and bactericidal kinetics with strains of *Staphylococcus aureus* collected from buffalo milk. We observed that the extract of *M. tenuiflora* exerts bactericidal effect on *S. aureus*, at the concentration of 107.5 mg/mL as low acute toxicity. In this context, it can be concluded that *M. tenuiflora* extract presents an antimicrobial activity about *S. aureus*, with relatively low toxicity. Thus, these outcomes suggested further studies about alternative economically viable for control and prevention of infections in veterinary medicine.

**Keywords:** phytotherapy, *Mimosa tenuiflora*, clinical mastitis, dairy buffaloes

**INTRODUCTION**

Buffalo (*Bubalus bubalis*) is a rustic animal, resistant to diseases and parasites, however, it presents similar to bovines sanitary problems, such as: mastitis (Carvalho *et al.*, 2007). In mastitis process an intense inflammatory response occurs, causing clinical manifestation, alterations in udder and milk secretion and changes in chemical composition of milk (Deb *et al.*, 2013). Among the microorganisms involved in the mastitis process, *Staphylococcus aureus*, is the most common, being an inexhaustible source of studies in various countries of the world. *S. aureus* is a natural inhabitant of human and animal skin and mucosal epithelia (De Los *et al.*, 2014). This microorganism has specific dispersion characteristics among herds, besides the high rate of resistance to drugs used in disease treatment (Troncarelli *et al.*,

---

<sup>1</sup>Department of Experimental Pathology, State University of Londrina, Londrina, Brazil,

\*E-mail: andreiavet@hotmail.com

<sup>2</sup>Department of Morphological Sciences,

<sup>3</sup>Department of Basic Health Sciences, State University of Maringá, Maringá, Brazil

<sup>4</sup>Department of Dentistry, State University of Paraiba, João Pessoa, Brazil

<sup>5</sup>Department of Veterinary Medicine,

<sup>6</sup>Department of Biological Sciences, Federal University of Campina Grande, Patos, Brazil

<sup>7</sup>Department of Veterinary Medicine, Federal Rural University of Pernambuco, Recife, Brazil

<sup>8</sup>Department of Biological Sciences, Federal University of Paraiba, João Pessoa, Brazil

2013). Moreover, indiscriminate use of antibiotics promotes the passage of antibiotic residues to milk, in the treatment of lactating females (Freitas *et al.*, 2005). These facts support the search for therapeutic alternatives, i.e., development of new products based on active ingredients from natural origin, in which safety and efficacy are scientifically proven (Troncarelli *et al.*, 2013).

*Mimosa tenuiflora* is a *Mimosoidae* subfamily legume, typical of semiarid region of Brazil, and it is widely used by the population because it presents anti-inflammatory and antimicrobial, analgesic, cell regenerating, antipyretic and pectoral astringent activity (Maia 2004). Moreover, *M. tenuiflora* is rich in tannins and flavonoids, substances responsible for the antimicrobial activity (Meckes- Lozoya *et al.*, 1996). In this context, it is aimed to evaluate the use of *M. tenuiflora* extract in animals affected with clinical mastitis, aiming to control main microorganisms responsible for bubaline clinical mastitis.

## MATERIALS AND METHODS

The study was conducted in department of Chemical and Biological Sciences, Universidade Federal de Campina Grande-UFCG. Parts of the plant used in this study were deposited in the form of voucher specimen in the Herbarium Caririensis Dárdano de Andrade Lima, Universidade Regional Cariri-URCA, Crato-EC, under registration #3275. This research was submitted and approved by CSTR ethics committee (protocol 36-2010).

### Collection and preparation of material

To obtain the *M. tenuiflora* extract were used 500 g of stem bark eluted in 1.000 mL ethanol

P.A. for 72 h. The material was concentrated on rota-evaporator and the *M. tenuiflora* extract concentration was carried out according to Matos methodology (Matos 1997). The concentration of the extract was determined considering their masses, extract concentration and yields, resulted in the following data: 860 mg, 75.43 mg / mL and 1.72%. From which dilutions were made: 430 mg / mL; 215 mg / mL; 107.5 mg / mL; 53.75 mg / mL; 26.87 mg / mL; 13.43 mg / mL; 6.71 mg / mL; 3.35 mg / mL; 1.67 mg / mL.

### Microbiological evaluation of milk

Acute toxicological assay was made using forty Swiss mice, males, 6 to 8 weeks old obtained from Universidade Federal de Campina Grande (UFCG). The animals were divided into 4 groups and treated with 0.25 mL of the plant extract, intraperitoneally in a single dose. Each group was treated with the *M. tenuiflora* extract diluted in proportions 430 mg / mL; 215 mg / mL; 107.5 mg / mL and 53.75 mg / mL. The groups were observed for 24 h, in order to determine the LD<sub>50</sub>. Moreover, the skin irritation test also was evaluated. Twenty-four hours prior to the test, animals were shaved, in a field of 1.5 and 1.5 cm in mid-dorsal region for application of *M. tenuiflora* extract. Three mice for each dilution: 107.5 mg / mL and 53.75 mg / mL were used (the choice of these dilutions was determined from the results obtained by MIC and acute toxicological assay) and 0.5 mL of the dilutions was applied. Records were obtained after observation at 3 minutes, 1, 4, 24, 48 and 72 h, registering the skin reactions, according to the index in OECD Guide No. 404 (2002) for sensitivity and irritation test.

Posteriorly, the treatments were performed in 24 mammary quarters of buffalos at different lactation stages, with clinical mastitis detected

by strip cup test. For treatment with *M. tenuiflora* extract (107.5 mg / mL and 53.75 mg / mL concentrations associated with 5% glycerin as fixer (v/v) was utilized 10 mammary quarters and 10 mammary quarters for treatment with antibiotic Cefquinome (Intervet®). For the control group it was stipulated four mammary quarters as non-inoculated controls. In both treatments, it was used an intramammary injection for 5 days (15 mL of the *M. tenuiflora* extract or Cefquinome 8g) (Costa *et al.*, 1999). Before each application, two daily milkings for total mammary glands depletion and collection of milk samples for further microbiological evaluation, pre and post-dipping were carried out, and the experimental animals were maintained in semi-extensive regime during the whole treatment. Milk samples were collected and analyzed 5 days before treatments, 24 h and 5 days consecutive after administration of treatments.

The physical evaluation of mammary glands and milk udders after the treatments were daily performed through palpation and visual observation, observing color alterations, temperature, edema presence and mammary parenchyma consistency. Black bottom strip cup test for observing milk secretion color, secretion consistency and presence of masses or lumps was conducted in order to determine the occurrence of clinical mastitis (Almeida *et al.*, 2005).

To microbiological evaluation of buffalo milk, approximately 5 mL, were aseptically collected (Bouchot *et al.*, 1985). *S. aureus* identification was performed by macromorphological characteristics of the colonies (Gram stain) and biochemical tests (Quinn *et al.*, 1994; Santana & Azeredo, 2005; MacFaddin, 1980). Bacterial curve in face of *M. tenuiflora* extract was evaluated by Peyret *et al.* method (Peyret *et al.*, 1990). Three representative *S. aureus* strains were incubated in nutrient broth

at 37°C / 20 h and subcultured in Muller Hinton-DIFCO/1 h (inoculum of 10<sup>6</sup> CFU / mL). In 9 mL of bacterial culture, it was added 1 mL of extract and 1 mL of sterile distilled water was added to control tube. The tubes were maintained at 37°C / 24 h, and aliquots were taken after 2, 4, 6, 8, 10 and 24 h and plated on Mueller Hinton / 48 h. Bactericidal effect was defined as the decrease in 3log of CFU / mL or 99.9% cell death over the specified time (May *et al.*, 2000). Moreover, the rapid count of *S. aureus* was performed by petrifilm® method. The plates were inoculated in duplicate with 1.0 mL of milk samples by the traditional dilutions method 10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup> (35 to 37°C / 24 h and further transferred to an incubator at 62±2°C / 1 to 4 h).

For statistical analysis of the data, Student's t test was used for data with normal distribution, and Mann-Whitney U test was used for data with non-normal distribution. Differences were considered statistically significant at P<0.05.

## RESULTS AND DISCUSSION

The results of acute toxicological assay conducted with extract of *M. tenuiflora* presented LD<sub>50</sub> of 215 mg / mL. Animals exposed to this dose showed “pre-death” symptoms, as constant urination (urinary incontinence), piloerection, defecation, cyanosis, salivation, corneal opacity, tail relaxation, tachypnea, among others. These symptoms were observed within 24 h after application of the extract. Moreover, no significant presence of edema or erythema was observed in animals in the skin irritation test, obtaining primary irritation index of 0.7 and 0 for dilutions 107.5 mg / mL and 53.75 mg / mL respectively, corresponding to nonirritating classification according OECD guide.

Cup test was daily performed in all treated mammary quarters, being possible to observe the disappearance of lumps on the third day after inoculation in both 107.5 mg/mL and 53.75 mg/mL concentrations of the *M. tenuiflora* extract tested, the same was observed in evaluated mammary quarters treated with antibiotic (Cefquinome). However, in the first inoculation with 107.5 mg

/ mL the mammary quarters showed sensitivity to touch, increased volume and swelling. On the fourth day no sensitivity process was observed.

The bactericidal effect of the hydroalcoholic extract of *M. tenuiflora* in concentration of 107.5 mg/mL was observed within 4 h after contact for all *S. aureus* strains isolated from bovine (Table 1). From this result, we selected the concentration of

Table 1. Number of *S. aureus* colonies in buffalo milk treated with *M. tenuiflora* extract in 107.5 mg / mL concentration.

Sample	Extract Action Time (hours)						
	Bacterial			UFC / mL			
Time/hours	0	2	4	6	8	10	24
Sample 1 (C)	2.2 x 10 <sup>6</sup>	6.9 x 10 <sup>6</sup>	2.2 x 10 <sup>7</sup>	8.5 x 10 <sup>7</sup>	1.1 x 10 <sup>8</sup>	1.4 x 10 <sup>8</sup>	1.9 x 10 <sup>8</sup>
Sample 1 (T)	2.2 x 10 <sup>6</sup>	1.1 x 10 <sup>5</sup>	0	0	0	0	0
Sample 2 (C)	3.9 x 10 <sup>6</sup>	5.2 x 10 <sup>6</sup>	4.5 x 10 <sup>7</sup>	6.1 x 10 <sup>7</sup>	8.7 x 10 <sup>7</sup>	1.2 x 10 <sup>8</sup>	1.4 x 10 <sup>8</sup>
Sample 2 (T)	3.9 x 10 <sup>6</sup>	1.4 x 10 <sup>5</sup>	0	0	0	0	0
Sample 3 (C)	2.1 x 10 <sup>6</sup>	7.1 x 10 <sup>6</sup>	3.1 x 10 <sup>7</sup>	4.3 x 10 <sup>7</sup>	1.4 x 10 <sup>8</sup>	1.6 x 10 <sup>8</sup>	2.3 x 10 <sup>8</sup>
Sample 3 (T)	2.1 x 10 <sup>6</sup>	1.1 x 10 <sup>6</sup>	0	0	0	0	0

(C) Control; (T) Treated with *M. tenuiflora* extract

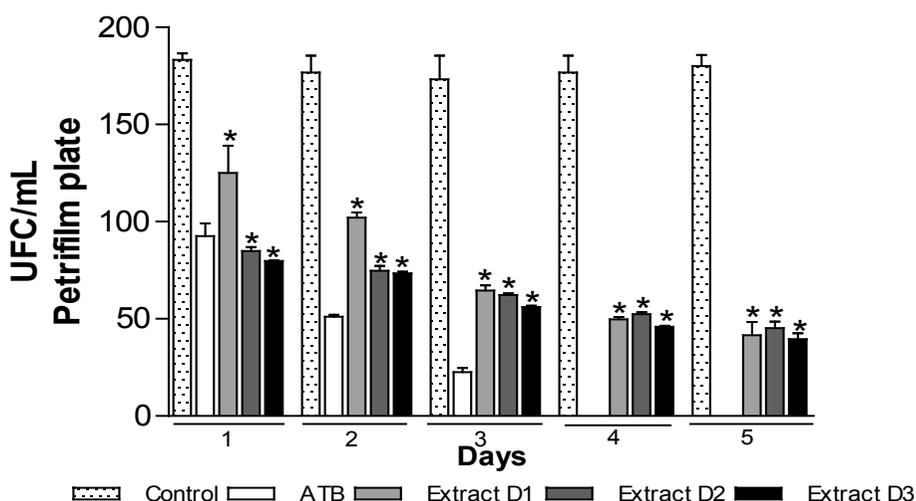


Figure 1. Count of number of *S. aureus* colonies (Mean±SD) in buffalo milk. Microbiological evaluation was made by Petrifilm method.\*Significant difference (P<0.05) of *M. tenuiflora* extract 107.5 mg / mL when was compared with antibiotics (ATB) and control.

107.5 mg / mL to evaluate the kinetics and bacterial count of the number of microorganisms in petrifilm RSA® system.

In the microbiological evaluations before and after to treatments (extract and antibiotic), the isolated microorganisms were classified as *S. aureus*. However, a significant decrease in the number of *S. aureus* colonies was observed for *M. tenuiflora* extract 107.5 mg / mL when compared with the control in all the dilutions, mainly, from the second day of treatment (Figure 1).

Antimicrobial activity of *M. tenuiflora* hydroalcoholic extract according to Meckes-Lozoya *et al.* (1996) can be connected to the presence of tannins and flavonoids. *M. tenuiflora* extract was the subject of several studies which aimed to verify its in vitro effectiveness, testing it on microorganisms such as: *Staphylococcus epidermidis*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Acinetobacter calcoaceticus*, as well as fungi such as: *Microsporum gypseum*, *M. canis*, *Trichophyton mentagrophytes*, *T. rubus* and *Chaetomium indicum* (Gonçalves *et al.*, 2005; Lozoya *et al.*, 1989 and Bezerra *et al.*, 2009).

This study contributed to clinical practice innovation, since it suggests an alternative for the treatment of mastitis through clinical use of phytoterapeutic medicines. In summary, the results suggest the clinical importance of evaluating alternative and economically viable means for infections control in veterinary medicine, showing that *M. tenuiflora* extract presents an antimicrobial activity with low toxicity in *Bubalus bubalis* with mastitis.

## REFERENCES

- Almeida, L.A. do B., M.A.V.P. Brito, J.R.F. Brito, M. de F.A. Pires and N.R. Benites. Almeida LAB, Brito MAVP, Brito J RF, Pires MFA, Benites NR. 2005. Tratamento de mastite clínica experimental por meio de ordenhas múltiplas em vacas leiteiras inoculadas com *Staphylococcus aureus*. *Arq. Inst. Biol.*, **72**(1): 1-6.
- Bezerra, D.A.C., Pereira A.V. Pereira, Lôbo K.M.S. Lôbo, Rodrigues O.G. Rodrigues, Athayde A.C.R. Athayde, Mota R.A. Mota, Medeiros E.S. Medeiros and, Rodrigues S.C. Rodrigues. 2009. Atividade biológica da jurema-preta (*Mimosa tenuiflora* Wild. Poir.) sobre *Staphylococcus aureus* isolado de casos de mastite bovina. *Rev. Bras. Farmacog.*, **19**: 814-817.
- Bouchot, M.C., C.J. Catel, C.C. Chirol, G.J.P. Ganiere and M.M.L. Menec. 1985. Diagnostic bactériologique des infections mammaires des bovins. *Rec. Med. Vet.*, **72**: 567-577.
- Carvalho, L.B., F.R. Amaral, M.A.V.P. Brito, C.C. Lange, J.R.F. Brito and R.C. Leite. 2007. Contagem de células somáticas e isolamento de agentes causadores de mastite em búfalas (*Bubalus bubalis*). *Arq. Bras. Med. Vet. Zootec.*, **59**: 1.
- Costa, E.O., R. Sá, H. Ponce, E.T. Watanabe and C.R. Valle. 1999. Avaliação da terapia de mastite clínica: eficácia terapêutica em número de dias em tratamento. *Napgama.*, **2**: 10-14.
- Deb, R., A. Kumar, S. Chakraborty, A.K. Verma, R. Tiwari, K. Dhama, U. Singh and S.A.K. Kumar. 2013. Trends in diagnosis and control of bovine mastitis: a review. *Pak. J.*

- Biol. Sci.*, **23**: 1653-1661.
- De Freitas, M.F.L., J.W.P. Júnior, T.L.M. Stamford, S.S. de A. Rabelo, D.R. Da Silva, V.M. da S. Filho, F.G.B. Santos, M.J. Sena and R.A. Mota. 2005. Perfil da Sensibilidade *in vitro* de *Staphylococcus* coagulase positivos isolados de leite de vacas com mastite no Agreste do Estado de Pernambuco. *Arq. Inst. Biol.*, **72**: 171-177.
- De Los Santos, R., M. Fernández, S. Carro and P. Zunino. 2014. Characterization of *Staphylococcus aureus* isolated from cases of bovine subclinical mastitis in two Uruguayan dairy farms. *Arch. Med. Vet.*, **46**: 315-320.
- Gonçalves, A.L., A.A. Filho and H. Menezes. 2005. Estudo comparativo da atividade antimicrobiana de extratos de algumas árvores nativas. *Arq. Inst. Biol.*, **72**: 353-358.
- Lozoya, X., V. Navarro, J.T. Arnason and E. Kourany. 1989. Experimental evaluation of *Mimosa tenuiflora* (Willd) Poir. (tepescohuite) I - Screening of the antimicrobial properties of bark extracts. *Arch. Invest. Med.*, **20**: 87-93.
- Maia, G.N. 2004. *Caatinga: Árvores e Arbustos e Suas Utilidades*, 1<sup>st</sup> ed. D and Z. São Paulo, Brasil. 101p.
- MacFaddin, J.F. 1980. *Biochemical Test for Identification of Medical Bacteria*, 2<sup>nd</sup> ed. Baltimore: Lippincott Williams and Wilkins. 56p.
- May, J., C.H. Chan, A. King, L. Willians and G.L. French. 2000. Time -kill studies of tea tree oils on clinical isolates. *J. Antim. Chemother.*, **45**: 639-643.
- Matos, F.J.A. 1997. *Introdução à Fitoquímica Experimental*, 1<sup>st</sup> ed. Fortaleza, CE, Brasil: edições UFC., 74p.
- Meckes-Lozoya, M., X. Lozoya, R. Marles, C. Soucy-Breau and A.J. Avalokitesvarasen. 1990. N, N-Dimethyltryptamine alkaloid in *Mimosa tenuiflora* bark (Tepescohuite). *Arch. Inv. Med.*, **21**: 175-177.
- Peyret, M., G. Carret, C. Carre, G. Fardel and J.P. Flandrois. 1990. Etude mathématique de la curve de mortalité d' *Echerichia coli* exposés aux polymyxines. *Path. Biol.* , **38**: 441-445.
- Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter. 1994. *Clinical Veterinary Microbiology*, 1<sup>st</sup> ed. London, England: Mosby Wolfe. England. 256p.
- Santana, A.S. and D.R.P. Azeredo. 2005. Comparação entre o sistema petrifilm RSA® e a metodologia convencional para enumeração de estafilococos coagulase positiva em alimentos. *Ciê. Tecnol. Alimen.*, **25**: 531-535.
- Troncarelli, M.Z., H.M. Brandão, J.C. Gern, A.S. Guimarães and H. Langoni. 2013. Mastite bovina sob nanocontrole: a própolis nanoestruturada como nova perspectiva de tratamento para rebanhos leiteiros orgânicos. *Vet. e Zootec.*, **20**: 124-136.