

Spondias mombin L. decoction utilization as antiseptic in cats submitted to castration

Uso de decocto de Spondias mombin L. como antisséptico em felinos submetidos à castração

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ABSTRACT

This survey evaluated *mombin leaves* (*Spondias mombin* L.) decoction efficiency as an antiseptic during post-surgery period on cats submitted to orchietomy and ovariosalpingohysterectomy. For this purpose, 45 castrated mongrel cats were divided into three groups, the first group as a positive control using 0.5% chlorhexidine-alcohol solution, the second a negative control group using sterile distilled water and, finally, the test group using *mombin leaves* decocted with a concentration of 100 mg/mL. All animals, independent of age and sex, had visibly healed in most cases in a similar time. Animals treated with *mombin leaves* decoction presented a significant reduction of bacterial growth. In addition, the animals treated in the test group had better surgical wound healing. All isolated bacterial strains presented inhibition halo for chlorhexidine and for *Spondias mombin* L. Thus, the decoction of *Spondias mombin* L. leaves proved antiseptic efficacy in the surgical wounds of cats submitted to orchietomy and ovariosalpingohysterectomy.

Keywords: Medicinal plants. Orchietomy. Ovariosalpingohysterectomy. Antimicrobial.

RESUMO

Foi avaliada a eficiência do decocto das folhas de cajá (*Spondias mombin* L.) como antisséptico no pós-cirúrgico de gatos submetidos à orquiectomia e ovariosalpingohisterectomia. Para tal, foram submetidos à castração 45 gatos sem raça definida, divididos em três grupos. O primeiro grupo como controle positivo com Solução Alcoólica de Clorexidina a 0,5%; segundo grupo controle negativo com água destilada estéril; e o grupo teste com o decocto de cajá à concentração de 100 mg/mL. Todos os animais, independentemente da idade e sexo, tiveram cicatrização visível em tempo similar. Animais tratados com o decocto apresentaram uma redução significativa do crescimento bacteriano. Além disso, observou-se uma melhor cicatrização das feridas cirúrgicas dos animais tratados no grupo teste. Todas as estirpes bacterianas isoladas apresentaram halo de inibição para clorexidina e para *Spondias mombin* L. Portanto, o decocto das folhas da *Spondias mombin* L. apresentou eficácia antisséptica nas feridas cirúrgicas de gatos submetidos à orquiectomia e ovariosalpingohisterectomia.

Palavras-chave: Plantas medicinais. Orquiectomia. Ovariosalpingohisterectomia. Antimicrobiano.

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Introduction

Brazil is internationally recognized as having the largest biodiversity of plants on the planet (Soares et al., 2013; Sousa et al., 2018). The vegetation from the Caatinga biome, due to its multiplicity of use, shows economic potential related to animal feed, plant components utilization, wood production, and natural medicine (Silva et al., 2007; Sousa et al., 2018). Medicinal use of this potential has been highlighted by the scientific milieu (Soares et al., 2013).

Phytotherapeutic medicinal products are pharmaceutical preparations (syrup, elixir, dye, fluid and dry extracts, ointments, cream, gel, pills, and capsules) characterized by a wide knowledge of their effectiveness, risks of their use and constancy of their quality to treat various diseases (Dias et al., 2017).

The use of these medicinal natural origin products has emerged as an alternative and is mainly due to the great search for phytotherapeutic remedies, linked to socioeconomic factors, maintenance of cultural traditions, availability to the population and the search for drugs with fewer side effects. In addition, it is also due to the inefficiency of some synthetic products and the high cost of allopathic medicines (Magalhães et al., 2015; Matias et al., 2010).

Mombin fruit (*Spondias mombin* L.) that belongs to the Anacardiaceae family, is found in tropical regions of America, Asia, Africa, and Brazil, mainly in the northeast and north region of the country. The medicinal use of plants of the genus *Spondias* is carried out by several communities, as well several scientific works supporting its use, even for resistant bacteria. Plants belonging to this genus have a potential to synthesize molecules with bacterial activity in the infectious process (Sá et al., 2016; Silva et al., 2014; Soares et al., 2006).

In studies on healing activity and antimicrobial activity using medicinal plants, most authors suggest that tannins are responsible for the pharmacological action, because of their astringent property. Although the astringency of tannins has not been systematically studied, it is known that the antioxidant compounds positively influence wound repair (Martelli et al., 2018).

In addition, high bacterial resistance to antimicrobials concerns professionals in many areas, as it is a problem that affects the entire population, increases treatments costs, and causes a higher number of infection mortality, which becomes a challenge for clinical handling (Magalhães et al., 2015; Matias et al., 2010).

Surgical sterilization consists of the removal of male or female gonads, called orchietomy and ovariocalpingohisterectomy, respectively. These are among the most common surgeries in small animal clinics, since they are simple surgeries, affordable and quick. Owners' main objective of this type of surgery is to avoid animals having unwanted pups, and this surgery is widely performed (Conceição et al., 2017).

This study aimed to evaluate efficiency of decoction based on mombin leaves (*Spondias mombin* L.) *in vitro* and *in vivo* as an antiseptic in the post-surgery period of cats submitted to orchietomy and ovariocalpingohisterectomy,

since it is a promising antimicrobial alternative, according to Leonez et al. (2018).

Material and Methods

The *Spondias mombin* L. decoct production and processing of collected samples were performed at the Laboratory of Veterinary Microbiology (LAMIV), while animal surgical sterilization procedures were performed at the Dix-huit Rosado Veterinary Hospital (HOVET), both located at the Federal Rural Semi-Arid University (UFERSA), in the municipality of Mossoró, Rio Grande do Norte - Brazil. This research was evaluated by the Committee on Ethics and Use of Animals (CEUA) of UFERSA, with consent n°. 20/2018.

From March to May 2018, 45 non-defined breed cats (*Felis catus*) were randomly selected, their owners received information about the experiment and signed the consent form allowing the surgery. These animals were then transferred to the HOVET, where they were subsequently anesthetized and submitted to ovariosalpingohisterectomy in females and orchietomy in males. Post-surgical follow-up occurred in sanitized cats for a period of seven days, reinforcing that the animals received daily ration and water ad libitum.

Spondias mombin L. leaves were harvested in the morning from a specimen on the university campus, then packed in bags and taken to LAMIV for processing. We noted that an exsiccate of the collection was deposited in Herbarium Dárdaro de Andrade Lima with n°. 13953.

After the collection, 100 g of leaves were weighed in analytical balance (Cauduro LTDA, Model number EK3350), crushed and placed in Erlenmeyer, adding 1000 mL of distilled water. Afterward, this solution was boiled for 10 min. Finally, the solution with a concentration of 100 mg/mL was filtered and conditioned in amber sterile glass and kept refrigerated until use.

The sample was divided into three randomized groups, comprising 15 animals each. The first group served as positive control (chlorhexidine-alcohol solution 0.5%), the second, negative control (sterile distilled water), and the third, the test group with mombin leaves decoction 100 mg/mL. The animals were treated daily for seven days, with disinfection and collection of the samples performed daily at the surgical incision spot with the aid of sterile swab after 10 min of the antiseptic action.

Swab samples were collected in a tube containing 2 mL of sterile distilled water, and subjected to the dilutions 10^1 , 10^2 and 10^3 , respectively. After the procedure, 1 mL of each dilution was seeded in plate count agar and incubated in a bacteriological oven for 24 h at a temperature range of

37 °C to 37.5 °C, the necessary time for bacterial counting by mesophilic present in each dilution (Tortora et al., 2017).

After isolating bacteria, they were cultured in BHI broth for 24 h at 37 °C to 37.5 °C until the log phase for approximately 18-24 h, adjusted by the McFarland scale. Microorganisms were identified by cytology and biochemical tests (MacFaddin, 2000).

The standard inoculum of each microorganism was cultured for diffusion testing in Mueller-Hinton agar at the concentration of 0.5 of the McFarland scale for 18-24 h. The sensitivity test to extracts by agar diffusion was performed according to antimicrobial sensitivity test for diffusion-disc (National Committee for Clinical Laboratory Standards, 2003).

Microbiological analyses results were submitted to the analysis of variance and the means were compared to each other by the Scott-Knott test, at the 5% probability level, using statistical software Sisvar[®].

Results and Discussion

Number of females was higher than number of males, in which a ratio of 1: 1.50 (male: female) was observed, adults number was higher than kittens number: 0.79 (adult: kitten). All animals, independent of age and sex, had visible healing at a similar time. Kiani et al. (2014), on the other hand, studied healing in cats submitted to ovariosalpingohysterectomy, in which they observed that adult cats during the post-surgery period formed greater granulation tissue and fibroblast maturation scores in their surgical wounds, as well as the formation of neovascularization and reepithelialization, when compared to young cats.

As observed in Figure 1, animals treated with sterile distilled water had the highest number of microorganisms found, since water has no ability to inhibit bacteria. However, animals treated with *Spondias Mombin* L. presented a significant reduction of bacterial growth.

Results with chlorhexidine at 0.5% were better than the negative control (sterile distilled water), since this antiseptic is characterized by being a cationic detergent of the biguanides class available in acetate, hydrochloride and digluconate forms. This last one is the most commonly used salt in formulas and products, which has a wide spectrum of action, acting on gram-positive, gram-negative bacteria, fungi, yeasts, and lipophilic viruses (Tortora et al., 2017). However, better healing of the surgical wounds of those treated with *Spondia mombin* L. was observed, with Castejon (2011), reporting that the healing activity can be attributed to tannins, stimulation of phagocytic cells, as well as anti-infective activities.

Regarding the bacteria found in the surgical wounds of cats treated with the negative control, *Staphylococcus aureus*, *Staphylococcus hycus*, *Staphylococcus coagulase-negative*, *Corynebacterium* sp. and Gram-positive bacilli strains, all bacterial strains isolated from the negative control were tested *in vitro* and presented inhibition halos for 0.5% chlorhexidine and for the decoction of the *mombin* leaves (Figure 2).

Similar results were described by Leonez et al. (2018), when they found that the *mombin* leaves extract was effective on inhibiting coagulase-negative *Staphylococcus* ampicillin-resistant, isolated from goat teats, demonstrating the actual antimicrobial potential of compounds in the *mombin* plant.

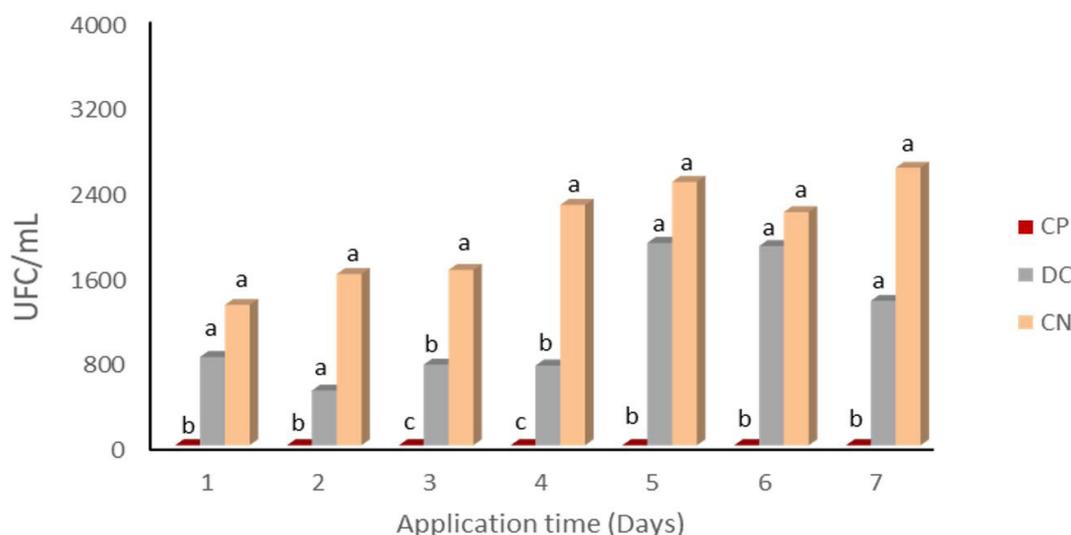


Figure 1 – Mongrel cats submitted to castration surgery. Bacterial count in the period of seven days after surgery. CP = positive control (chlorhexidine-alcohol 0.5%); DC = test group (*Spondia mombin* L decoction); CN = Negative Control (sterile distilled water). Averages followed by the same letters do not present significant difference ($p \geq 0.05$) by the Scott-Knott test. Mossoró, Brazil, 2020.

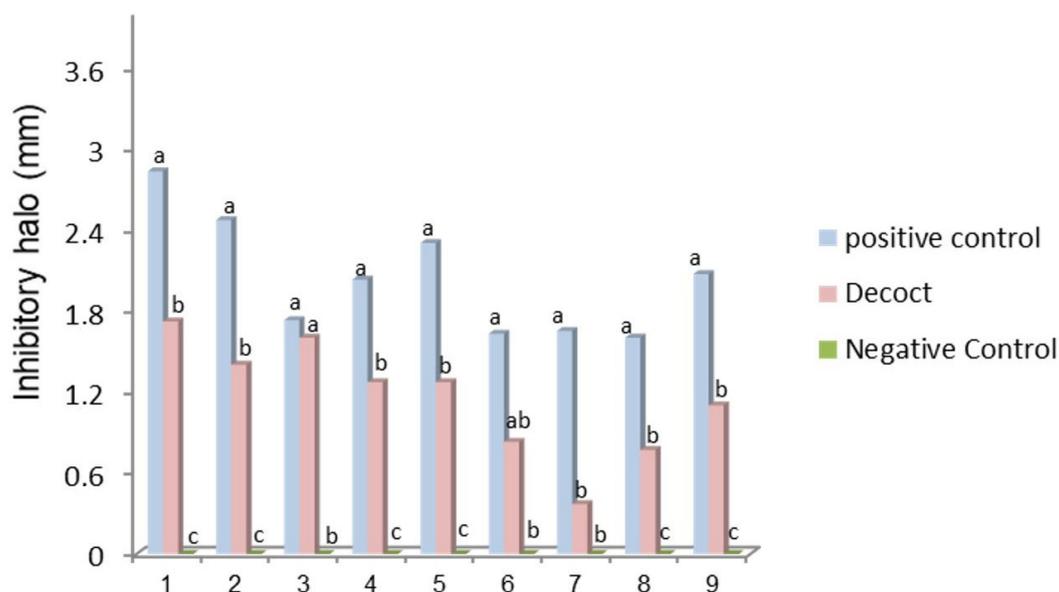


Figure 2 – Mongrel cats submitted to castration surgery. Inhibitory halo diameters formed after diffusion in well test for bacteria found in the negative control animals. The numbers in sequence represent: (1) *Staphylococcus hyicus*; (2) *Staphylococcus* -coagulase-negative strain 1; (3) *Staphylococcus* coagulase-negative strain 2; (4) Gram positive bacillus strain 1; (5) *Corynebacterium* sp. strain 1; (6) *Corynebacterium* sp. Strain 2; (7) Gram positive bacillus strain 2; (8) *Staphylococcus*-coagulase-negative strain 3; (9) *Staphylococcus* - coagulase-negative strain 4; *Staphylococcus aureus*. Positive Control (chlorhexidine-alcohol, 0.5%); Negative Control (sterile distilled water); Test Group (*Spondia monbin* L decoct). Mossoró, Brazil, 2020.

Table 1 – Mongrel cats submitted to castration surgery according to sex, experimental group, and evaluation of healing evolution after seven days of surgery. Mossoró, Brazil, 2020

Variable	Positive Control*		Negative Control**		<i>Spondias mombin</i> L.	
	Male	Female	Male	Female	Male	Female
Hyperemia	-	-	-	-	-	-
Edema	-	-	-	-	-	-
Secretion	-	-	-	3	-	-
Crusts	-	-	-	-	-	-
Dehiscence	-	-	-	-	-	-
Without changes	7	8	6	6	5	10

*chlorhexidine-alcohol, 0.5%. **sterile distilled water.

Regarding the clinical feature of the animals under study, we observed that the animals submitted to care with the decoction of *Spondia mombin* L., distilled water and chlorhexidine did not present significant differences regarding hyperemia, edema, secretion, crusting, and dehiscence, with the exception of the presence of secretion in 20% (3 females) of the negative control (Table 1).

These data were similar to the results found by França et al. (2008), when using ointment based on *Symphitum officinalis* (*confrei*) on surgical wounds of cats. This plant is popularly used for its healing and tissue regenerating properties, which confer healing and anti-inflammatory effects similar to the results found in the present study.

França et al. (2008), reported satisfactory results in almost 80% of the animals, presenting normal healing, but some with crust formation, due to the astringent action of the tannin. However, in the present study, none of the animals

treated with *Spondias mombin* L. presented differences in the normal cicatricial process.

Conclusion

It can be concluded that *Spondias mombin* L. leaves decoction presents antiseptic efficacy in surgical wounds of cats submitted to orchietomy and ovariosalpingohysterectomy, proving its use as an alternative technology to industrialized antimicrobials.

Conflict of Interest

We declare that we do not have conflicts of interest.

Ethics Statement

The study was approved by the Committee on Ethics and Use of Animals (CEUA - UFERSA) n°. 20/2018.

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