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**ATIVIDADE ANTIMICROBIANA DO AÇAFRÃO (*CURCUMA LONGA*)
EM *STHAPHYLOCOCCUS AUREUS* CAUSADORES DA MASTITE
BOVINA**

COLORADO DO OESTE, RO

2024

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ATIVIDADE ANTIMICROBIANA DO AÇAFRÃO (*CURCUMA LONGA*) EM *STHAPHYLOCOCCUS AUREUS* CAUSADORES DA MASTITE BOVINA

Trabalho de conclusão de curso apresentado ao Curso Superior de Zootecnia do Instituto Federal de Educação, Ciência e Tecnologia de Rondônia, como requisito para a obtenção do título de Bacharel em Zootecnia.

Nome do Orientador: Prof. Dr. Alan Andrade Mesquita

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RESUMO

Palavras-Chave: Atividade antimicrobiana; Bactérias; Curcumina; Cúrcuma.

Extraída da *Curcuma longa*, a curcumina é descrita na literatura atual como tendo potencial para prevenir e curar diversas doenças, além de melhorar a resposta imunológica, aumentando os níveis de antioxidantes na defesa contra radicais livres e ação antibacteriana. Este trabalho teve como objetivo avaliar a atividade antimicrobiana *in vitro* da cúrcuma (*Curcuma longa* L.) em bactérias *S. aureus* originárias de mastite bovina. O extrato hidroalcoólico reconstituído de açafrão em pó, na concentração de 5%; extrato alcoólico de açafrão em pó, na concentração de 10%; extrato hidroalcoólico reconstituído de açafrão em pedaços, na concentração de 5%; extrato alcoólico de açafrão em pedaços, na concentração de 10% e tratamento controle, contendo os antimicrobianos cefoxitina, aztreonam, ceftazidima, gentamicina, cefepima, amicacina, ampicilina e tetraciclina. Os resultados mostraram que *S. aureus* foi sensível ao extrato alcoólico de açafrão em pó, apontando sua atividade antimicrobiana, bem como aos antimicrobianos comerciais cefoxitina, gentamicina, cefepima, amicacina, ampicilina e tetraciclina. Dentre todos os extratos de *Curcuma longa* testados, apenas o extrato alcoólico de cúrcuma em pó apresenta atividade antimicrobiana contra *S. aureus*, decorrente da mastite bovina, podendo, portanto, tornar-se uma alternativa à antibioticoterapia convencional. Porém, são necessários estudos que melhorem a elucidação dos mecanismos de ação da *Curcuma longa* em animais.

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Antimicrobial activity of turmeric (*Curcuma Longa*) against *Staphylococcus aureus* that causes bovine mastitis

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Abstract:

Extracted from *Curcuma longa*, curcumin is described in current literature as having the potential to prevent and cure various diseases, as well as improve the immune response, increasing antioxidant levels in defense against free radicals and antibacterial action. This work aimed to evaluate the *in vitro* antimicrobial activity of turmeric (*Curcuma longa* L.) in *S. aureus* bacteria originating from bovine mastitis. The reconstituted hydroalcoholic extract of saffron powder, at a concentration of 5%; powdered saffron alcoholic extract, at a concentration of 10%; reconstituted hydroalcoholic extract of saffron in pieces, at a concentration of 5%; saffron alcoholic extract in pieces, at a concentration of 10% and control treatment, containing the antimicrobials cefoxitin, aztreonam, ceftazidime, gentamicin, cefepime, amikacin, ampicillin, and tetracycline. The results showed that *S. aureus* was sensitive to the alcoholic extract of saffron powder, pointing out its antimicrobial activity, as well as the commercial antimicrobials cefoxitin, gentamicin, cefepime, amikacin, ampicillin, and tetracycline. Among all the *Curcuma longa* extracts tested, only the alcoholic extract of turmeric powder has antimicrobial activity against *S. aureus*, arising from bovine mastitis, and could, therefore, become an alternative to conventional antibiotic therapy. However, studies are needed to improve the elucidation of the mechanisms of action of *Curcuma longa* in animals.

Key Word: Antimicrobial activity; Bacteria; Curcumin; Turmeric.

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I. Introduction

Brazil is a significant global power in the agricultural sector, with incredible expressiveness for dairy cattle farming. It is the largest milk producer in South America, with 34.8 million tons of milk equivalent in the year 2022, behind the United States alone, which produced 102.9 million tons of milk equivalent (FAO, 2022).

However, our national production, particularly individual productivity, lags significantly behind other major milk producers. In 2020, the Brazilian average was a mere 2,192 liters/cow/year (SENAR, 2021). This alarming disparity may be attributed to bovine mastitis, a widespread issue that afflicts a significant portion of our herd. The economic losses incurred throughout the dairy chain are substantial (Duarte et al., 2020).

Bovine mastitis is an infectious process resulting from the presence of pathogenic microorganisms in the mammary gland that affect the production and composition of milk, mainly reflected in somatic cell count (SCC), total bacterial count (TBC), and casein and fat content. (Freitas et al., 2005, Santos and Fonseca 2007, Langoni et al., 2017). It can be characterized as an inflammation of the mammary gland, causing changes in several physiological functions in animals. It can cause clinical signs such as changes in the characteristics of the milk and udder, resulting in a qualitative and quantitative decrease in the milk produced (Kibebew, 2017; Braga et al., 2018).

Another negative impact of mastitis observed is the economic losses due to the costs of treatments, carried out using antibiotics and anti-inflammatories, in addition to veterinary services, and more severe cases, leading to the animal's death or early disposal due to productive losses (Oviedo-Boyso et al., 2007). Mastitis has a high prevalence in national cattle herds (Costa et al., 2012; Cunha et al., 2015), and several pathogens are involved in the cases; several studies have demonstrated multi-resistance to the antibiotics used in treatments, which may represent a risk to public health. In general (De Vlieghe et al., 2012; Forsythe, 2013; Mesquita et al., 2019).

Among the primary culprits of bovine mastitis is the bacterium *Staphylococcus aureus*, which is prevalent in both subclinical and chronic cases. Animals infected by *S. aureus* serve as crucial reservoirs for this pathogen, and transmission can occur from one cow to another during milking (Keefe, 2012; Pumipuntu et al., 2017). The primary method of treating mastitis is through antibiotic therapy, administered mainly in clinical cases and therapeutic treatments in late lactation cows (Erskine et al., 2004). However, the indiscriminate use of these drugs has led to the selection of resistant strains, posing a significant risk to public health. The failure of sanitary controls further compounds this issue, hindering the success of antibiotic therapy and potentially leading to the presence of antibiotic residues in the human food chain and the creation of multi-resistant strains of bacteria (Medeiros, 2015; Krömker and Leimbach 2017; Mohandes et al. 2021).

Some factors interfere with the effectiveness of antimicrobial treatment against *S. aureus*, such as the severity of mastitis and factors related to the cow, such as age, days of lactation, and number of infected people (Reksen et al., 2006). Furthermore, *S. aureus* has characteristics that hinder the action of antimicrobials, such as the ability to survive inside phagocytes, biofilm production, and high genetic variability between strains (Cheng and Han 2020; Ren et al., 2020).

In this sense, the development of research with natural products with antibacterial power can accelerate and make the production of new substances cheaper, generating alternatives to the conventional therapeutic control available on the market for the control of multi-resistant agents (Guimaraes et al., 2010). Considering the high cost of developing synthetic molecules, the extraction of molecules from plant species with antimicrobial activity can be a viable alternative for mastitis control (Dimasi and Grabowki, 2007).

Amidst this concern, *Curcuma longa* L., commonly known as turmeric, emerges as a beacon of hope. This member of the *Zingiberaceae* family possesses a myriad of

beneficial properties, including antibacterial, antifungal, antiviral, anti-aging, anti-malarial, anti-cancer, anti-Alzheimer's, antioxidant, and anti-inflammatory effects (Moghadamtousi et al., 2014; Boroumand et al., 2018).

Notably, curcumin and the oil fraction have demonstrated the ability to inhibit the growth of various bacteria, such as *E. coli*, *Streptococcus*, *Salmonella*, *Typhimurium*, *Staphylococcus*, *Yersinia enterocolitica*, *Bacillus subtilis*, and *B. cereus* (KaiKai et al., 2020). The objective of this study was to evaluate the in vitro antimicrobial activity of reconstituted or unreconstituted extracts obtained from turmeric (*Curcuma longa* L.) against bacterial inoculums of *Staphylococcus aureus* from bovine mastitis.

II. Material And Methods

The experiment was conducted at the Bromatology Laboratory of the Federal Institute of Science and Technology - IFRO, Campus Colorado do Oeste, which is located in the municipality of Colorado do Oeste/RO, at coordinates 13° 07'39" S; 60° 29'68" W, 410 m high, climate according to Koopen's classification is Awa type, hot and humid tropical, characterized by two well-defined seasons: dry and hot between April to September and rainy and hot between October to March.

To manufacture the extracts, fresh rhizomes of *Curcuma longa* L. were used, cleaned, and cut into small pieces and subsequently dried and subjected to alcoholic and hydroalcoholic extraction processes (Avancini, 2002; Souza and Wiest, 2007; Paim et al., 2010). The treatments were constituted as follows:

Treatment 1 - Reconstituted hydroalcoholic extract of saffron powder, consisting of 100 grams of saffron powder and another 1000 mL of ethyl alcohol 96° GL, discarding 50% of the volume and adding 50% distilled water to reconstitute the extract, being considered a concentration of 5% saffron in its constitution.

Treatment 2 - Alcoholic extract of saffron, consisting of 100 grams of saffron powder and 1000 mL of 96° GL ethyl alcohol, considering the concentration of 10% of saffron in its composition.

Treatment 3 - Reconstituted hydroalcoholic extract of saffron in pieces, consisting of 100 grams of saffron in pieces and 1000 ml of ethyl alcohol 96° GL, discarding 50% of the volume and adding 50% of distilled water to reconstitute the extract, which is considered a concentration of 5% saffron in its composition.

Treatment 4 - Alcoholic extract of raw saffron, consisting of 100 grams of saffron pieces and 1000 ml of 96° GL ethyl alcohol, considering the concentration of 10% of saffron in its composition.

Treatment 5 - Control treatment containing the antimicrobials cefoxitin, aztreonam, ceftazidime, gentamicin, cefepime, amikacin, ampicillin, and tetracycline.

The extracts were stored and kept closed for 30 days. To permanently control the asepsis of these extraction and reconstitution procedures, the sterility of all extracts was determined by removing a 5 mL aliquot, seeding it in tubes of BHI Broth, and incubating it at 37 °C for up to 48 hours.

To isolate *Staphylococcus aureus*, five individual milk samples were collected from cows diagnosed with mastitis using the California Mastitis Test (CMT). Subsequently, a 50µL aliquot of each sample was sown in a Petri dish containing the Baird-Parker agar selective medium supplemented with egg yolk solution with potassium tellurite, incubated at 37 °C for 24-48h. Samples that showed suggestive colony growth were subjected to phenotypic differentiation.

Colonies were considered positive for *S. aureus* if, after incubation, they presented a dark gray to black color, a glossy appearance, and a convex shape, measuring 1-5 mm in diameter, with a clear halo measuring 2-5 mm wide on their periphery.

To confirm the pathogen, three isolated colonies were used, placed in tubes containing slanted BHI Agar, incubated for 24 hours at 35 °C, and, subsequently, catalase production and staining tests were carried out using the Gram method. After these initial tests, the tube coagulase test was performed (Lancette and Bennett, 2001).

In vitro antibiotic resistance and *in vitro* susceptibility tests for *Curcuma longa* were carried out using the disc diffusion method in accordance with the specifications of the Clinical and Laboratory Standards Institute (CLSI 2020). With sterilized swabs, twenty-five plates were sown, representing five replications of each treatment of the five isolated colonies of each animal infected with *S. aureus*. For this, the samples kept in each BHI were transferred to test tubes with 2 mL of Muller Hinton broth incubated at 37°C for 18-24 hours and subsequently diluted in sterilized saline solution until a turbidity equivalent to 0 was obtained 5 on the McFarland standard scale. Next, the samples were seeded on the surface of plates with a culture medium containing Mueller-Hinton Agar. Samples of *S. aureus* (ATCC-25923) and *Escherichia coli* (ATCC-25992) were used as controls for antibiogram tests.

The results of susceptibility tests for *Curcuma longa* were evaluated by reading a minimum inhibition halo of four centimeters. If this occurs, it is considered sensitive; otherwise, it is considered resistant (Paludo et al., 2019; Sales et al., 2020).

III. Results

The different extracts of *Curcuma longa* differed in their results, with only treatment 2 having an antibacterial action identified. In contrast, in the other treatments, bacteria of mastitic origin grew without any inhibition being seen in their growth, assuming no antibacterial action had occurred (Table 1).

Table 1: In vitro antimicrobial activity of turmeric on *S. aureus* from bovine mastitis.

Treatments	Assessment result according to the diameter of the inhibition halo				
	I1	I2	I3	I4	I5
Treat 1	Resistant	Resistant	Resistant	Resistant	Resistant
Treat 2	Sensitive	Sensitive	Sensitive	Sensitive	Intermediary
Treat 3	Resistant	Resistant	Resistant	Resistant	Resistant
Trat 4	Resistant	Resistant	Resistant	Resistant	Resistant

Treat 1 - Reconstituted hydroalcoholic extract of saffron powder; Treat 2 - Alcoholic extract of saffron powder; Treat 3 - Reconstituted hydroalcoholic extract of saffron pieces; Treat 4 - Alcoholic extract of saffron pieces.

Source: Prepared by the authors.

The antibiotics cefoxitin, gentamicin, amikacin, ampicillin, and tetracycline showed a positive effect on antimicrobial activity, differing from the antibiotics aztreonam, ceftazidime, and cefepime, which showed little or no antimicrobial effect (Table 2).

Table 2: In vitro antimicrobial evaluation of control treatment in bovine mastitis *S. aureus*.

Control/Antibiotics Treatment	Assessment result according to the diameter of the inhibition halo				
	I1	I2	I3	I4	I5
Cefoxitin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Aztreonam	Resistant	Resistant	Resistant	Resistant	Resistant
Ceftazidime	Resistant	Resistant	Resistant	Resistant	Resistant
Gentamicin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Cefepime	Intermediary	Intermediary	Intermediary	Sensitive	Sensitive
Amikacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Ampicillin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Tetracycline	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

Source: Prepared by the authors.

IV. Discussion

The results observed in treatments 1, 3, and 4 (Table 1) may have occurred due to a low concentration of *Curcuma longa's* active ingredient, as its antibacterial activity has already been proven (Goel et al., 2008). However, due to the way the extract was prepared, only Treatment 2 achieved this concentration.

In treatment 2 (Table 1), the non-reconstituted extracts (raw) can be seen when compared to the reconstituted ones; they showed better results, and this antibacterial action was also observed by Paim et al. (2010), in which he researched 50% ethanolic extracts, from different sources, such as fresh rhizomes, and different commercial samples of Turmeric (*Curcuma longa* L.) rhizome powder, pointing out that hydroalcoholic extracts have low inhibition activity, while the alcoholic extraction form without rehydration (crude extract) showed selective and significantly more intense antibacterial activity.

Bhatt et al., (2013) observed the antimicrobial effect of *Curcuma longa* using crude powder extract at a concentration of 100mg/ml in a hydroalcoholic solution, mainly on bacteria of the genus *Streptococcus* and *Staphylococcus*.

However, several studies (Péret-Almeida et al., 2008; Moghaddam et al., 2009) corroborate the lack of inhibition of extracts from treatments 1, 3, and 4 of *Curcuma longa* and observe the ineffectiveness of extracts from *Curcuma longa*. Such results were indicated due to the concentrations researched, and the antibacterial activity can also be altered if the concentrations are modified (Tønnesen and Karlsen, 1987).

Sedky et al., (2022) reported a significant antimicrobial effect for the alcoholic extract of Turmeric at concentrations of 20 and 50 mg/ml, corroborating the present study, in which the alcoholic solution was able to better extract the antimicrobial compounds from *Curcuma longa*.

Nander et al., (2018) observed the antibiofilm activity of the crude extract and fractions of *Croton urucurana* on *Staphylococcus aureus* bacteria isolated from bovine mastitis. The *C. urucurana* extract at a concentration of 5 mg/ml was able to eradicate 50.03% of the *S. aureus* matrix, while the antibiotics gentamicin (30 mg/ml) and vancomycin (4µg/ml) eradicated 13.94% and 100%, respectively. Among the substances extracted from crude extracts of *C. urucurana*, the α -Costol isolate was able to reduce the amount of colony-forming units of *S. aureus* in 6 logarithmic cycles at a concentration of 125 µg/ml.

Santos et al., (2019) demonstrated the efficiency of propolis extract in antimicrobial activity against *Staphylococcus* spp. In this study, the minimum concentrations for antimicrobial effect for propolis extracts in ethanol, ethyl acetate, and hexane were 6,250 µg/mL, 3,125 µg/mL, and 1,562 µg/mL, respectively. The ethanolic propolis extract showed better efficacy (72.67%) than the ethyl acetate extract (56.49%) and the hexane extract (10.29%) against *Staphylococcus* spp. bacteria.

The susceptibility results (Table 2) of the commercial antibiotics tested in the control treatment corroborate several studies already carried out, and cefoxitin, commonly used in hospital cases of *S. aureus* infection, was effective against bacteria originating from bovine mastitis. It can be observed that, although it has been shown to be effective, tetracycline, ampicillin, and amikacin have several studies that place important considerations on their use, as results have been demonstrated with differences in the characteristics of resistance to these antibiotics. These differences have also been associated with methods of use, dosage, and treatment control, as the resistance of bacteria has been related to careless use, being an essential factor in the emergence of resistant bacteria (Zafalon et al., 2008; Zanette et al., 2010; Noel et al., 2016).

Gentamicin showed sensitivity and efficacy in inhibiting bacterial growth (Table 2). The antibiotics amikacin, ampicillin, and cefepime show a similar sensitivity rate in several antibiotic therapy studies, with the first two being variants in matters of care in management to avoid the emergence of resistant bacteria (Nader Filho et al., 2007; Mesquita et al., 2020).

Araújo (1998) and Fontana et al., (2010) found results of resistance of the *S. aureus* strain to ampicillin. In contrast, the antibiotics Aztreonam and Ceftazidime, which are commonly associated with the control of Gram-negative bacteria, are not effective against Gram-positive bacteria, as observed in this job.

The possibility of treatments with most of the antibiotics tested was highlighted (Table 2), mainly gentamicin, which showed greater efficacy. Thus, this demonstrated the need for regular analyses of antimicrobial sensitivity for a more effective treatment, reducing costs and the possibility of bacteria resistant to antibiotic therapy.

V. Conclusion

Among all the *Curcuma longa* extracts tested, only the alcoholic extract of turmeric powder has antimicrobial activity against *S. aureus* arising from bovine mastitis. Therefore, it could become an alternative to conventional antibiotic therapy. However, studies are needed to improve the elucidation of the mechanisms of action of *Curcuma longa* in animals.

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