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Natural sanitizers pre- and post-milking in the prevention of bovine mastitis

Sanitizantes naturais pré e pós-ordenha na prevenção da mastite bovina

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ABSTRACT

Approaches focused on improving animal health are essential strategies to increase the quality and productivity of the livestock sector. Bovine mastitis is a prevalent disease in dairy farming worldwide. There are various ways to prevent infections and mammary inflammation in cows, such as using sanitizers or disinfectants before and after milking to clean the cow's udder surface and reduce contamination. Chlorine-based disinfectants are commonly used, and in some cases, antibiotics are used to prevent mastitis. This review aimed to analyze scientific articles that used plants as a sanitizer for a cow's udder in the last 5 years. There were few records of experiments with plants for disinfecting cows' udders; a total of 10 articles and 12 plant species were mentioned, including twice *Piper betle* and *Acacia nilotica*. Among the classes of metabolites indicated as active are terpenoids, flavonoids, and tannins. Studies indicate that plant extracts exhibit antimicrobial properties. Plant-based disinfectants promote sustainable and environmentally friendly sanitation in the livestock production sector, as these products are biodegradable and renewable.

Keywords: Bovine mastitis; Udder; Teat asepsis; Disinfectant resistance; Eco-friendly disinfectants

RESUMO

Abordagens que apostam na sanidade animal são estratégias para impulsionar a qualidade e produtividade do setor pecuário. A mastite bovina está entre as doenças de maior prevalência na pecuária leiteira mundial. Entre as estratégias utilizadas na prevenção da infecção e consequente inflamação mamária das vacas podemos citar os sanitizantes aplicados antes e depois da ordenha para higiene da superfície do úbere da vaca e reduzir as contaminações. Existem desinfetantes de uso longevo e comum como os a base de cloro, e até o uso de antibióticos como preventivo da mastite. O objetivo desta revisão foi fazer um levantamento de artigos científicos com plantas como saneante do úbere da vaca nos últimos 5 anos. Foram poucos os registros de experimentos com plantas para desinfecção das tetas das vacas, totalizando 10 artigos e 12 espécies vegetais citadas, e duas vezes *Piper betle* e *Acacia nilotica*. Entre as classes de metabólitos indicados como ativos os terpenoides, flavonoides e taninos. Estudos mostram que extratos vegetais apresentam propriedades antimicrobianas. Os desinfetantes à base de plantas compactuam com uma proposta sustentável e de saneamento ambiental na cadeia produtiva do setor pecuário, uma vez que estes produtos são biodegradáveis e renováveis.

Palavras-chave: Mastite bovina; Úbere; Assepsia da teta; Resistência a desinfetantes; Desinfetantes ecológicos

INTRODUCTION

Sanitizers are disinfecting agents that are used to prevent, reduce, or eliminate the microbiological load on surfaces. The use of disinfectants to clean the teats of dairy cows before and after milking is a sanitary measure. This helps reduce the local microbial load and transmission of mastitis-causing agents in the herd (CHENG; HAN, 2020). This is an important safety measure in dairy management, as it has been proven to effectively eliminate microorganisms, especially bacteria, from the surface of tissues (LOPES et al., 2013; BACH et al., 2019). According to EMBRAPA (Brazilian Agricultural Research Corporation) investing in animal health measures is strategic, especially considering that Brazil is one of the main livestock and milk-producing countries (BRITO et al., 2021).

Various parameters, including concentration or dilution, temperature, exposure duration, organic matter presence, surface type, microorganism susceptibility, and health guidelines for cow handlers, affect disinfection efficacy (MORALES-UBALDO et al., 2023). Multiple chemical disinfectants are used during sanitation procedures before and after milking. Since there is no perfect disinfectant product, several factors are considered before making a choice, such as non-toxicity for animals and humans, effective disinfectant properties, and cost-effectiveness. The most disinfectant used are chlorhexidine, iodine, sulfonic acid, chlorine, sodium hypochlorite, and chlorous acid and to avoid undesirable effects emollients such as glycerin, lanolin, propylene glycol, sorbitol, vegetable oils, minerals, and collagens are added to these substances (DOMINGUES, 2010; BACH et al., 2019). The main target microorganisms associated with bovine mastitis are *Streptococcus dysgalactiae*, *S. uberis*, *Staphylococcus* spp., and fecal coliforms such as *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli* and *Enterococcus* spp. Some of the environmental bacterial agents are opportunistic, such as *Pseudomonas aeruginosa* and *S. uberis* (PERES NETO; ZAPPA, 2011; FREITAS et al. 2020).

Studies have compared disinfectants to standardize concentration and exposure time to optimize cow udder hygiene. BACH et al. (2019) reviewed the main disinfectants used in milking management in dairy herds. Other researchers conducted comparative trials applying disinfectants to bacterial cultures of strains related to bovine mastitis (Table 1) (RAMALHO et al. 2012, SILVA et al. 2015, PEIXOTO et al. 2015, and DE MENDONÇA et al. 2020). The authors carried out experimental trials with *Staphylococcus* spp. isolated from mastitis on dairy farms to different exposure times and

concentrations of disinfectant. RAMALHO et al. (2012) used chlorhexidine, iodine, chlorine, and quaternary ammonium, while SILVA et al. (2015) used the same disinfectants, replacing chlorine with sodium hypochlorite. The first study demonstrated varying susceptibility to each disinfectant at different exposure times, while the second study showed a 100% reduction in microbial load for all disinfectants tested after 30 and 60 minutes of exposure (RAMALHO et al. 2012, SILVA et al. 2015).

Ensuring proper aseptic care of cows' teats is crucial for preventing and managing mastitis among dairy cows. It is important to use disinfectants that meet quality standards and effectively reduce the quantity of microorganisms that cause bovine mastitis.

Table 1 - Sanitizers commonly used in the asepsis of dairy cows' teats and the respective concentrations for *in vitro* tests.

Sanitizers	**References**					
	1	2	3	4	5	6
Hypochlorite /Clorine (NaClO/Cl)	NaClO 2,5 %	NaClO 0,62 - 2,5 %	-	Cl 2,5 %	NaClO 2,5 %	
**** Iodine**	1 a 2 %	0,5; 1 e 2 %	0,7%	0,57 %	-	
Chlorhexidine	0,5 %	0,5; 1 e 2 %	2 %	2 %	2,5 %	
Lactic acid	-	-	-	-	3 %	
Quaternary ammonium	-	0,17; 0,34 e 0,68 %	-	4%		
Chitosan						1%

1. BACH et al. 2019, 2. DA SILVA et al. (2014), 3. PEIXOTO et al. (2015), 4. RAMALHO et al. (2012), 5. MENDONÇA et al. (2020), 6. ZHANG et al. (2021)

Among the natural sanitizers used to treat bovine mastitis are essential oils, plant extracts, bacteriocins, and phyto-derivatives (BASKARAN et al., 2009; HÖFERL et al., 2009; PIETERSE et al., 2010; MUBARACK et al., 2011). DAFERERA et al. (2003) and OLIVEIRA-TINTINO et al. (2018) in their work showed that substances of plant origin and their derivatives are a viable and efficient alternative due to the complexity of the structures of these substances that escape bacterial adaptations (CHENG; HAN, 2020).

Antimicrobial resistance is a major global health concern and poses a significant threat to health, food, and security, according to the World Health Organization (WHO, 2023). The search for plant products as antimicrobial agents has increased due to the emergence of resistance to conventional antibiotics and sanitizers, as well as the high cost of developing new synthetic molecules. Plants with demonstrated antimicrobial properties are being targeted for the development of new drugs that can overcome

microbial resistance and are economically viable, showing promising results (GUIMARÃES, 2010; NADER et al., 2018). Brazil is one of the leading countries in milk producer and exporter. Diseases like bovine mastitis result in significant economic losses and reduced production capacity (MARQUES et al. 2020, VIDAL et al. 2023). However, there are few scientific studies on natural products for veterinary use, especially concerning sanitizers for bovine udders (VIDAL et al. 2023). This review compiles researches on plant-based disinfectants for pre- and/or post-milking use, providing an overview and encouraging further research on natural sanitizers.

MATERIAL AND METHODS

This article presents the findings of research on sanitizers that are used to prevent bovine mastitis. Our research followed a well-defined protocol. Firstly, we selected relevant databases and conducted a thorough search for scientific articles published in the last 5 years (2019-2024). Secondly, we carefully selected articles that met our specific criteria. Finally, we extracted and organized the data from those articles.

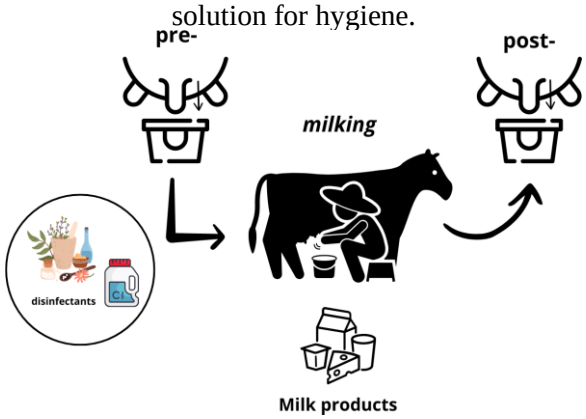
The searches were conducted in the following databases: SciELO (Scientific Electronic Library Online), Lilacs (Latin American and Caribbean Literature in Health Sciences), PubMed (National Library of Medicine), ScienceDirect and Scopus. Scientific article surveys were performed using a combination of keywords - “teat dipping” AND “plant extract”, “teat dipping” AND “natural product”, teat AND milking AND plant extract, teat AND dipping AND plant, milking AND “plant extract” AND sanitizer, milking AND “plant extract” AND disinfectant. We did not consider review articles. After excluding articles that did not involve cow, teat, plant, bovine mastitis, disinfectants, and/or sanitizers, a data table containing plant species, plant organs, extract types, biological assays, descriptions of tests, and bioactives was done.

RESULTS AND DISCUSSION

Cow teat disinfection was first documented in 1916, when natural mixture such as pineapple (*Ananas comosus* L. Merril) essential oils were used to reduce microbial proliferation (RASOOL et al., 2021). Disinfection has been a routine procedure applied to dairy cow since the 1960s when the National Institute for Research in Dairying (NIRD, England) improved methods in the dairy industry, made the first scientific evaluations showing the efficiency of teat disinfection to minimize bovine mastitis (VALA et al., 2013; RASOOL et al., 2021). The decontamination process involves more than simply

washing with water and drying the teats. It includes immersing the teats in a disinfectant solution for a few seconds pre- and post-milking (Figure 1). This practice reduces the risk of intramammary infections, maintains the health of the teat tissue, and prevents the entry of microorganisms through skin lesions (VALA et al. 2013; SILVA et al. 2015). Asepsis products may include natural disinfectants like extracts or essential oils with antimicrobial properties, peptides, as well as chemical solutions such as potassium permanganate, zinc, copper, iodine, and others (KOVACEVIC et al., 2023).

Figure 1 – Pre- and post-milking stages where the cow's teats are immersed in the sanitizing solution for hygiene.



Source: Own Authorship (2024)

Conventional chemical methods have been effective, but there are some important factors to consider. Chlorine-based detergents are commonly used for daily disinfection of surfaces, from household cleaning to washing fruits and vegetables. However, there have been reports of public health issues linked to chlorine, including a potential association with an increased risk of certain cancers (MEYER, 1994, SHI et al. 2024). Recent studies have shown that microbial communities forming biofilms can develop resistance to chlorine-based substances (MEHDIPOUR et al. 2023, SHAN et al. 2024). The addition of sodium hypochlorite increases the risk of antibiotic resistance genes (ARGs) to human health even after dosing was discontinued, indicating a persistent response (ZHANG et al., 2023). Research indicates that there are issues with iodine-based antiseptics, including poor tolerability, inactivation of organic matter, and the development of cross- and antimicrobial resistance (BARRETO et al., 2020). Chlorhexidine and iodine-based antiseptics are used to clean skin, wounds, and mucous membranes prior to surgery or other medical procedures, according to internationally recognized standards (KOBURGER et al., 2010). In addition to microbial resistance problems, there are cases of adverse effects such as allergies, irritations, and toxicity.

Chitosan is a non-toxic and biodegradable biopolymer alternative to disinfectant teat cows with sustainable characteristics (ZHANG et al., 2021). Biopolymers inhibit biofilm formation in mammary epithelial tissue due to toxicity to the strains or by providing a physical barrier that prevents infection (MORALES-UBALDO et al., 2023).

Various types of disinfectant formulations and non-antibiotic strategies have been employed over the years to prevent bovine mastitis. These include aerosol sprays, ointments, nanocomposites, nutraceuticals, probiotics, bacteriophages, bacteriocins, peptides, and phytochemicals whose aim to reduce infectious processes in the teats of dairy cows (LOPES, 2022; TOUZA-OTERO et al., 2024). In addition to disinfectant products, many antibiotics are used in dry-cow therapy. They are placed in the cow's udder to prevent or treat initial infections, yielding satisfactory results for the animal's health. However, this practice increases the risk of developing microorganism resistance (GARCIA et al., 2019; LOPES, 2022). It is estimated that 700,000 people die annually due to antimicrobial resistance. In 2050, 10 million fatalities are predicted if there are no scientific alternatives (Neculai-Valeanu et al., 2021).

The use of plants and their bioactive compounds in preventing bovine mastitis offers alternative treatments when faced with microbial resistance or other limitations. Additionally, there is a growing interest in developing environmentally friendly disinfectant products. VIDAL et al. (2023) cited a phytomedicine derived from *Phytolacca decandra* (*Phytolaccaceae*) and *Pulsatilla nigricans* (*Ranunculaceae*) for veterinary use against bovine mastitis, particularly after inflammation has occurred. Table 2 lists the natural herbal products used for cleaning cows' teats. Some studies are fundamental and involve plate diffusion tests in the laboratory to assess their effectiveness against the causative agents of bovine mastitis. Researchers from Asian countries and Brazil conducted the most recent studies. The publications covered *ex vivo*, *in vivo* and *in vitro* researches. One of the plants used as a disinfectant is an aquatic macrophyte from the fern group (Pteridophyte) - *Salvinia auriculata* (*Salviniaceae*), with a broad phytoremediation profile for aquatic ecosystems (LUBIS et al., 2022). Another plant, *Acacia nilotica* (*Fabaceae*), mentioned in two articles, has been used for a long time in various medicinal systems, such as Ayurvedic, Unani and Chinese. It has multiple biological effects, including anti-inflammatory and antibacterial. The main secondary metabolites identified in its extracts are tannins (RATHER et al. 2015). Another plant cited in two research studies, *Peper betle*, has monoterpenes among its active ingredients in the leaves that were tested *in vitro* and *in vivo* directly on a cow's teat

(SUNGKATAVAT et al. 2023). Root extracts from *Salvinia auriculata* improved aseptic conditions in *ex vivo* test models of teats excised from killed cows (PURGATO et al. 2023). Some commonly used plants for the sanitation of cow teats include lavender, rosemary, oregano, and cinnamon (Table 2).

The bioactives of plants used to sanitize cows' udders include tannins, terpenoids such as cineole, eugenol, carvacrol, and thymol, as well as flavonoids (Table 2). The main types of plant extracts used as sanitizers are polar or essential oils. Plant extracts are complex mixtures containing different substances related to classes of secondary metabolites such as terpenoids, flavonoids, alkaloids, etc. (KHARE et al., 2020; VICTÓRIO et al., 2022). Variations in metabolites among species were observed in quality and concentration, often depending on the plant organ used. Different organs involved with the extracts obtained for the tests include leaves, flowers, fruits, rhizomes, stems, roots, and bark (Table 2).

The evolutionary origin of plant secondary metabolism pathways involves species' defense responses or the promotion of benefits that culminate in reproductive and population survival processes to deal with the nuances of ecological conditions (KHARE et al., 2020; MAYNARD et al., 2020; VICTÓRIO et al., 2022). The major metabolites in a plant may not always correspond to the bioactive components for a particular usage, especially if the action is linked to the complexity of the extract and the metabolites' synergy or, conversely, to an isolated substance (VICTÓRIO et al., 2022). Nevertheless, studies using medicinal plants are guided by knowledge of secondary metabolites and their biological activity.

CONCLUSION

Some microorganisms pose a threat to the stability of livestock systems. Efforts are needed to control diseases in dairy cows and ensure high-quality production to meet national and international demand. Sanitary measures such as cleaning the cow's teats before and after milking, and guidance to cattle keepers and milkers are critical steps.

Investing in bovine health and environmental research has contributed to increasing productivity and improving the country’s economy. The diverse flora provides a rich source of secondary metabolites with potential eco-friendly antimicrobial properties, and there are no reports of microbial resistance. This article presents an overview of plant-based sanitizers used to prevent cow mastitis and suggests further study in this field.

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Table 2 - Studies on the use of natural plant-based disinfectants to control bovine mastitis from 2018 to 2024.

Plant species	Extract	Part	Biological assay	Results	Bioactives	Ref
Salvinia auriculata *(Salviniaceae)*	Hexane (spring)	Root powder	Determination of the Minimum Inhibitory Concentration (MIC) to evaluate the effectiveness of the antiseptic formulation using an excised teat model (*ex vivo*) and comparing it with commercial formulations.	The formulation disrupted biofilms of all S. aureus isolates, with reduction ranging from 90 to 100% at the concentrations MIC, 2 × MIC, and 4 × MIC.	Stigmast-22-ene-3,6-dione, β-sitosterol and octadecanoic acid 6	1
Eucalyptus globulus *(Myrtaceae)*	Essential oil	**ND**	An *in vivo* model was used, in which twenty-four dairy cows were found positive for subclinical mastitis, were selected for chemotherapeutical trials after treatment with essential oils of *Eucalyptus* and Lavender at 2 and 4% applied twice a day for 28 days.	Plant essential oils used as teat dips have better results for the reduction in somatic cell count, pH, milk yield, and colony forming units and increased milk yield in Friesian dairy cattle.	**ND**	2
Lavandula hybrida *(Lamiaceae)*		**ND**			**ND**	
Acacia nilotica *(Fabaceae)*	Methanol, chloroform, distill water and petroleum ether extracts	Fruits	The Minimum Inhibitory Concentration was determined using the plate diffusion method. .	The results demonstrated that the aqueous and methanolic extracts inhibited the growth of all microorganisms.	Glycosides, flavonoids and terpenoids. The tannins were present in methanol and aqueous extracts. Saponin petroleum ether and methanol.	3
Acacia nilotica *(Fabaceae)*	Aqueous	**ND**	Minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) tests were applied to evaluate the sensitivity for each bacterial isolate to tested commercial disinfectants and *A. nilotica* plant extract by using the broth dilution method	The bactericidal activity of *A. nilotica* plant extract was compared with four tested disinfectants (Tek-Trol, TH4+, Virkon S and peracetic acid) against *E. coli*, *S. aureus and S. agalactiae*. The plant extract has good antibacterial activity against isolated pathogens.	Saponins, anthraquinones, tannins, flavonoids, terpenoids, alkaloids and glycosides	4
Curcuma longa *(Zingiberaceae)*	Methanolic	Rhizome	The antibacterial effects of turmeric extract in combination with various antiseptics (5% povidone iodine, 0.5% v/v hydrogen peroxide (H_2O_2), 0.5% v/v chlorine (Cl_2), and 0.5% v/v chlorhexidine) were determined using the agar well diffusion method	After combination with turmeric extracts with Cl_2 and Chlorhexidine, a significant reduction in antibacterial activity was observed against almost all strains tested. Combination with H_2O_2 did not reduce its antimicrobial effect.	Curcumin	5
Piper betle *(Piperaceae)*	Ethanolic	Mature leaves	The MIC and MBC values of the extract and BSP (bovine teat dipping solution containing *P. betle* extract) against the clinical isolates of staphylococci were investigated using a broth microdilution assay	Bovine teat dipping solution containing *P. betle* extract showed strong anti-staphylococcal activity against *S. aureus*, *S. chromogenes*, and *S. haemolyticus*. The extract's MIC and MBC values ranged from 0.03 to 2 mg/mL	Hydroxychavicol and Eugenol	6

Lippia origanoides (Verbenaceae)	Essential oils	Leaves	*In vivo* test in which the performance of conventional disinfectants (chlorhexidine 1% and Iodine 2500 ppm) compared with the application of essential oil in applications before and after milking, and the milk samples were also subjected to microbiological analysis, as well as the surface of the teats and udder.	The results revealed that the essential oil (120 µl/mL) was efficient against microorganisms compared to disinfection by conventional product demonstrating the effectiveness of the alternative product in preventing new intramammary infections in dairy cows	Carvacrol, Cymene and Methyl thymol ether	7
Piper betle (Piperaceae)	Ethanolic extract and bacteriocins isolated from lactic acid bacteria *Lactobacillus plantarum* IIA-1A5	Leaves	The study was carried out *in vivo* with 12 cows selected during their normal lactation period, specifically from the 3rd to the 5th month. Teat immersion was performed in a liquid of iodine, betel leaf extract, or bacteriocin for 5 sec per nipple. Teat dip treatment was applied during the milking process over 21 days on a dairy farm in Cijeruk village, BogorWest Java-Indonesia	Treatment improved the microbiological quality of fresh cow's milk and reduced the incidence of subclinical mastitis in cow udders. Betel leaf extract and bacteriocin can replace the chemical iodine.	Phenolic components (chavicol, hydroxyl chavicol), hydroxyl fatty acids (stearate, palmitic, myristic), and fatty acids (stearic and palmitic)	8
Eugenia caryophyllata (Myrtaceae)	Essencial oil purchased from FERQUIMA, Brazil.	**ND**	Xanthan gum nanoemulsion (FSN) with 20% of the essential oils of the tested plants were tested in bacterial suspension of standard strains by diffusion in agar wells and quantitative assays on the agar surface. Three microbial species associated with bovine mastitis were tested using the following standard strains: *S. aureus*, *E. coli* and *Candida albicans*.	The quantitative tests revealed that both FSN and filmogenic suspension, through simple dispersion, were highly effective at inhibiting the growth of all microorganisms at oil concentrations above 1.0 wt%.	Eugenol, Caryophyllene. Humulene, Isocaryophyllene	9
Origanum vulgare (*Lamiaceae*)		**ND**			Caryophyllene, p-cymene, Terpinene, Carvacrol	
Cinamomum cassia (*Lauraceae*)		**ND**			Cinnamaldehyde, Benzaldehyde, Cinnamyl Acetate	
Eucalyptus urograndis (Myrtaceae)	Pyroligneous extract of *Eucalyptus urograndis* clone I144 (EU)	Firewood logs (wood vinegar)	The in vivo assay involved fifteen cows; specifically lactating females aged 3 to 6 years old. Wood vinegar was applied to the cows' teats for 28 days, and cellular debris was collected every 7 days. In vitro assays were conducted to determine the minimum inhibitory concentration using the broth microdilution method. Cytotoxicity was assessed by culturing bovine teat skin fragments and using the MTT reduction assay.	The cells incubated with 1% pyroligneous extract did not show any morphological changes. In vitro data showed that the extract has antimicrobial activity against S. aureus, *S. agalactiae*, *Salmonella*, *E. coli*, and *P. aeruginosa*. When used in vivo for post-dipping of dairy cows, the extract reduced the microbiological load in the mammary glands from 4.74 to 2.54 CFU, indicating its potential use as an antiseptic. Furthermore, the extract did not exhibit toxicity in mammary gland cells derived from lactating cows, as evaluated by MTT colorimetric assay.	Phenolic compounds	10

MIC - *Minimum Inhibitory Concentration* — MBC - *Minimum Bacterial Concentration* — CRD - *Completely Randomized Design.* ND – not detected. References: 1. PURGATO et al. (2023), 2. RASOOL et al. (2021), 3. ABDALLAH et al. (2020), 4. OBID ALLAH et al. (2020), 5. CHAISRI et al. (2019), 6. SUNGKATAVAT et al. (2023), 7. MARCELO et al. (2020), 8. KOMALA et al. (2023), 9. BARREIROS et al. (2022). 10. DA SILVA et al. (2023).

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