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Article

Antimicrobial Activity of Medicinal Plants Against Bacteria Causing Bovine Mastitis and Phytochemical Profiling by Paper Spray

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Abstract

Medicinal plants have become increasingly important due to the diversity and bactericidal potential of many species. They can work as an alternative to the use of antimicrobials in the treatment of bacterial infections, which may represent impairment to health. Considering the importance of alternative compounds, we aimed to evaluate the antimicrobial activity in vitro of medicinal plants *Stryphnodendron adstringens* (Mart.) Coville, known as barbatimão, *Baccharis crispa* Spreng, known as carqueja and *Azadiractha indica*, known as neem. *S. adstringens* and *B. crispa* were used as extract and obtained from plants collected in the municipality of Bambuí, state of Minas Gerais, Brazil. *A. indica* was evaluated as extract and oil, and the crushed leaves and oil were purchased from a commercial company. Antimicrobial activity was determined by the minimum bactericidal concentration (MBC) test-against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Salmonella* spp, isolated from bovine mastitis. The bacteria were submitted to the MBC test at concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0.09 mg/mL. The bacteria evaluated were sensitive to most plant extracts for at least one of the concentrations evaluated, except for Gram-negative bacteria, *Escherichia coli*, and *Salmonella* spp. There was no activity of *B. crispa* extract and *A. indica* against *E. coli* and neither of *A. indica* extract against *Salmonella* spp. even at the highest concentration evaluated. *S. adstringens* was considered the extract with the highest activity against the bacteria evaluated and *S. uberis* the most susceptible to antimicrobial action. The results indicate the antimicrobial activity of the compounds and a possible application of these for the development of biotechnological products against the main bacteria causing bovine mastitis, becoming an alternative to the use of antibiotics.

Keywords: MBC; plant extracts; antimicrobial activity; phytotherapy; spectrometry; paper spray

1. Introduction

Animal health has a major impact on the quality of the final product in the milk production chain. In this sense, bovine mastitis has great relevance since it is the main infection of dairy cattle, usually caused by bacteria, especially those of the genera *Staphylococcus* spp. and *Streptococcus* spp. [1]. Besides representing a worldwide problem, scientific reports show an increase of mastitis' cases, partly due to the selection of animals for higher milk production and use of broad-spectrum antibiotics [2–4]. Due to the high prevalence and impact of losses caused by the disease, mastitis is considered the costliest disease of dairy activity [5].

The emergence of antimicrobial resistance to commonly used antibiotics required new antimicrobial products that would ensure the efficacy of treatment [6]. Besides this necessity, a growing interest in medicinal plants as agents of new bioactive molecules against bacteria has been reported [6,7]. The bioactivity of medicinal plants is inherent to a group of compounds called secondary metabolites. These compounds are synthesized from catabolic, anabolic, and biotransformation reactions from the amino acids, carbohydrates, and lipids produced by the plant, and several crude extracts containing metabolites with antimicrobial activity that can inhibit bacterial growth are reported [6,7].

Stryphnodendron adstringens (Mart.) Coville, popularly known as *barbatimão*, stands out for being one of the most used plants in medicinal treatments, especially for the number of tannins present in the bark, which confers its main properties such as antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas* spp. and *Escherichia coli* [8–10]. *Baccharis crispa* Spreng, known by the name of *carqueja*, is popularly used as an antiseptic [11] and as an antioxidant [12]. *Azadiractha indica*, known by the name neem, has several proven benefits, such as insecticide [13], a fungicide, and controlling nematodes [14], besides proven antibacterial activity against some species [15].

Considering the potential of these medicinal plants, we aimed to determine, using the minimum bactericidal concentration (MBC), the in vitro antimicrobial effect of plant extracts of *Stryphnodendron adstringens* (Mart.) Coville, *Baccharis crispa* Spreng, and *Azadiractha indica* and of *Azadiractha indica* oil against strains of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Salmonella* spp. isolated from bovine mastitis. A complementary objective was to identify the secondary metabolites present in the evaluated extracts using the ambient ionization methodology for paper spray mass spectrometry (PS-MS). Once their antimicrobial potential is detected it is possible to investigate alternatives to the use of antimicrobials against the main bacteria that cause bovine mastitis.

2. Materials and Methods

2.1. Material Sampling and Extract Preparation

Plants *S. adstringens* (Mart.) Coville and *B. crispa* Spreng were collected in the municipality of Bambuí, state of Minas Gerais, and then deposited in the Herbarium of the Agricultural Research Company of Minas Gerais (EPAMIG). The crushed leaves of *A. indica* were provided by the company GoNeem, and the oil of *A. indica* (OAi) extracted from the seeds of the plant by cold maceration was acquired from the company Globo Agronegócios e Participações Ltda.

The extract of *S. adstringens* (ESa) was obtained by maceration, from 428.57 g of dry matter (DM) of the ground bark, in one liter of absolute ethyl alcohol PA for eight days, and the solvent was renewed every four days [16,17]. The extract of *B. crispa* (EBc) was prepared from the aerial part of the dry plant at room temperature and obtained by decoction for 15 minutes from 25 g of DM of the ground plant for each liter of distilled water [18,19]. The extract of *A. indica* (EAi) was obtained from the maceration of 100 g of DM of the crushed leaves in one liter of absolute ethyl alcohol PA for eight days, with the renewal of the solvent every four days [20,21]. All extracts were filtered in a paper filter with a capacity of 80 g/m² with the aid of a vacuum.

ESa and EAi were concentrated using roto-evaporator *Fisatom* 801 at 45 °C. The semi-solid residue was placed in an oven at a temperature of 45 °C for seven days for total drying. The EBc was kept in a freezer at -80 °C and then freeze-dried using the L101 Liotop®. The extracts and OAi were

used to evaluate the bactericidal activity after the sterility certification proposed by the Brazilian Pharmacopoeia (2010) [22].

2.2. Strains

Five strains isolated from bovine mastitis cases were used. They belong to the Collection of Microorganisms of Interest of Agroindustry and Livestock (CMIAP) of Embrapa Dairy Cattle. The following bacterial species were evaluated: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Salmonella* spp.

2.3. Inoculum Preparation

The bacteria were kept at -20 °C in *Skim Milk* culture medium (Difco®), plus glycerol at 10% (v/v). To reactivate them, an aliquot of frozen bacterial suspension was inoculated onto Petri dishes containing *Tryptone Soya Agar* (TSA) (Difco®), enriched with 5% (v/v) of defibrinated sheep blood. The plates were incubated at 35 °C ± 2 °C for 24 hours.

The standardization of bacterial suspensions was performed according to the method proposed by the Clinical and Laboratory Standards Institute [23]. The inoculum was prepared by suspending from four to five colonies previously grown in Brain Heart Infusion (BHI, Difco®) in a test tube containing Mueller-Hinton broth (MH). The turbidity was adjusted according to the standard of 0.5 of the McFarland scale by visual comparison, corresponding to approximately 1.5×10^8 CFU/mL. The inoculums were standardized at the concentration of 1 to 3×10^6 CFU/mL for all bacteria and then dispensed in each test tube. MH broth was used as a culture medium for MBC tests. For the bacteria *S. agalctiae* and *S. uberis*, 5% sterile defibrinated equine serum was added.

2.4. Determination of Minimum Bactericidal Concentration (MBC)

The MBCs of the extracts and the OAi were determined by the broth macro dilution method as proposed by CLSI (2008). One mL of the extracts and OAi reconstituted in aqueous solution containing DMSO and Tween 80 [24] at the concentration of 200 mg/mL was diluted twice in MH broth to obtain concentrations 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78, 0.39, 0.19 and 0.09 mg/mL for each extract and OAi.

The solution containing DMSO and TWEEN 80 was used as a negative control, and antibiotics oxacillin or neomycin were used as a positive control, the first for Gram-positive bacteria and the second for Gram-negative bacteria. One mL of the bacterial inoculum standardized in 1 to 3×10^6 CFU/mL was inoculated in each of the test tubes. Then, the test tubes were incubated at 35° C ± 2 °C for 24 hours. Subsequently, an aliquot of 10 µL of each dilution was spread out onto Petri dishes containing BHI agar and incubated at 35° C ± 2° C for 18-24h. The MBC was considered as the lowest concentration of extracts that did not present bacterial growth. The tests to evaluate the bactericidal activity of the extracts and oil were executed in triplicates.

2.5. Paper Spray Mass Spectrometry

A sample of 1 g of each extract (ESa, EBc and OAi) was weighed separately on an analytical balance and added to 8 mL of methanol (HPLC grade) in a Falcon flask. These mixtures were vortexed for 30 seconds and left at rest for one hour at room temperature.

The chemical profile of the extracts was determined according to the methodology described by Ramos et al. [18] using an LCQ Fleet mass spectrometer (ThermoScientific, USA) coupled with an ionization source for paper pulverizing. For this, chromatographic paper in the shape of an equilateral triangle (1.5 cm) was used, placed at a distance of 0.5 cm from the spectrometer entrance in a metal connector. 2 µL of the extract, and 40 µL of methanol was applied to the paper, reading in triplicate in positive and negative ionization modes.

The instrumental conditions of the analyses were: source voltage at 4.5 kV for the positive mode and 3.5 kV for the negative mode; capillary voltage of 40 V; transfer tube temperature of 275 °C; tube

lens voltage of 120 V; mass range from 100 to 1000 m/z. To identify the compounds, the charge mass ratios from literature data were compared with the instrumental signals obtained and subsequent fragmentation with collision energies of 15 to 30 eV

2.6. Statistical Analysis

The statistical analysis was performed with the help of the statistical program R [25]. For the analysis of the bactericidal action of the extracts and the OAi, the generalized linear model (GLM) of the MASS package was used, with the response modeled by Bernoulli distribution and logit link function, and ANODEV was analyzed in a completely randomized design. The value 0 was assigned when there was bactericidal action and the value 1 when there was bacterial growth. The significance level was established in $P < 0.01$. For graphical analysis, the ggplot function of the ggplot2 package [25] was used. CBM response variable is the following:

$$Y_i \sim \text{Bernoulli}(\mu_i)$$

Y_i : when there was bacterial growth (1) and when there was bactericidal action (0).

μ_i : Bernoulli distribution with logit link function.

3. Results and Discussion

3.1. Antimicrobial Activity

All evaluated extracts showed some antimicrobial activity against at least one of the tested bacteria. However, differences regarding the concentrations used and regarding the species in question were observed. The ESa showed an inhibitory effect against all bacterial species tested, but at different concentrations. The inhibitory effect against *E. coli* was observed only with the highest concentration of 100mg/mL(50 mg/mL). The same was observed with OAi since the oil proved efficient against all species under test, but at higher concentrations than those used when the test was performed with ESa. For OAi, the lowest dose considered efficient was 50 mg/mL against *S. uberis*. Against other species, OAi was only able to inhibit bacterial growth when used at maximum concentration (100 mg/mL). EBc and EAi, even when used at the maximum concentration, were not able to inhibit *E. coli* growth, and considering the *Salmonella* spp, the same was observed for the maximum concentration of EBc (Table 1). Thus, according to MBC, the pathogens most susceptible to bactericidal action were *S. aureus* and *S. uberis*, and the most effective extract was ESa; on the other hand, the least susceptible species was *E. coli*, which presented MBC only when exposed to ESa and OAi at high concentrations ($P > 0.001$).

Table 1. Minimum bactericidal concentration (MBC) of ESa, EBc, EAi, and OAi against strains of *S. aureus*, *S. agalactiae*, *S. uberis*, *E. coli*, and *Salmonella* spp. isolated from cases of bovine mastitis.

Bacterium	Concentration of extracts and oil (mg/mL)			
	ESa	EBc	EAi	OAi
<i>S. aureus</i>	1.56	100	25	100
<i>S. agalactiae</i>	3.12	100	12.5	100
<i>S. uberis</i>	1.56	50	6.25	50
<i>E. coli</i>	50	>100*	>100*	100
<i>Salmonella</i> spp.	6.25	>100*	100	100

*Showed bacterial growth in all concentrations. ESa= Extract of *Strypnodentron adstringens*; EBC=Extract of *Bacharis crispa*; EAI=Extract of *Azadrachta indica*; OAi= Oil of *Azadrachta indica*. Concentrations do not differ between columns but differ between rows (P<0.001).

When used at concentrations 0.097, 0.195, 0.39, and 0.78 mg/mL, ESa proved inefficient in inhibiting all bacteria studied. As the concentration was increased, the bactericidal effect of ESa increased, showing different susceptibility of the bacteria studied to this extract. When used at a concentration of 50 mg/mL, ESa was effective against all species studied (Figure 1). Among the bacteria studied, *E. coli* was the least susceptible (growth inhibited at a concentration of 50 mg/mL), while *S. aureus* and *S. uberis* were the most susceptible to this extract (growth inhibited at concentrations of 1.56 mg/mL (Table 1).

Costa et al. [26] evaluated higher concentrations of ESa against bacteria isolated from milk samples, reaching 400 mg/mL, and classified *E. coli* as not susceptible to the extract since no bactericidal action was observed even at the highest concentration evaluated. The result obtained by these researchers differs from that observed in our study and showed the difference in susceptibility of microorganisms isolated from different sources, especially the environmental pathogen, which may originate from a variety of sources, including cattle bedding, manure, pastures and water [27].

Despite limiting the growth of both Gram-positive and Gram-negative species, lower ESa concentrations have more inhibitory activity in the growth of Gram-positive species, indicating the greater susceptibility of this group to the extract. The difference in sensitivity between Gram-positive and Gram-negative has been discussed previously, and may be related to structural differences between these microorganisms. Gram-negative bacteria have an external phospholipid membrane with lipopolysaccharides, which makes the cell wall more complex and resistant to certain antimicrobial components, different from Gram-positive bacteria, which have only one outer layer of peptidoglycan and may be are, more permeable [26,28,29].

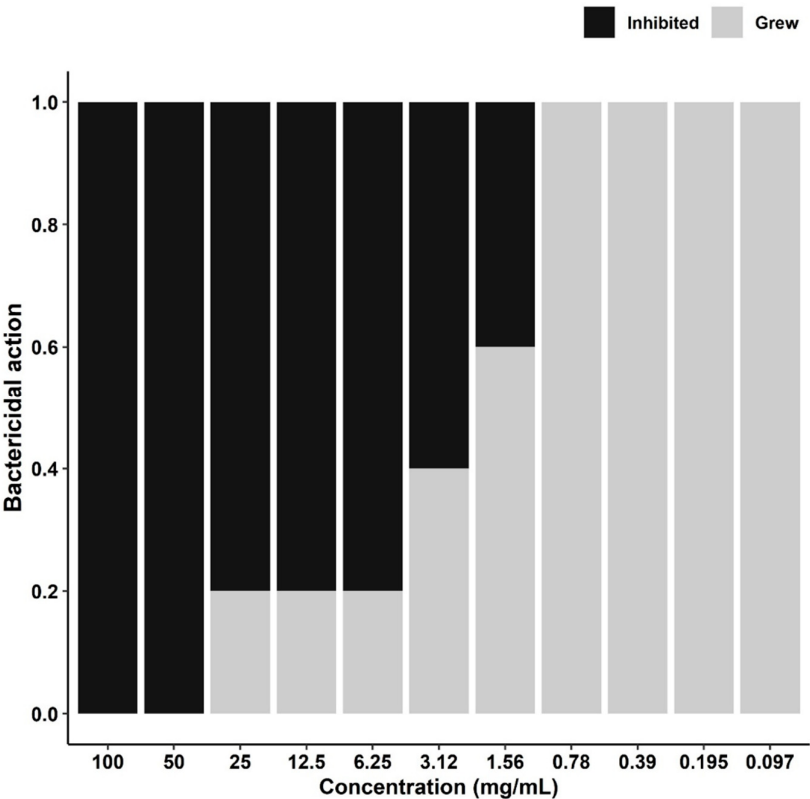


Figure 1. Bactericidal action of the different concentrations of *Strypnodentron adstringens* against *S. aureus*, *S. agalactiae*, *S. uberis*, *E. coli*, and *Salmonella* spp.

Unlike ESa, Ebc did not exhibit significant antibacterial activity against the microorganisms tested. It was not possible to find the MBC for *E. coli* and *Salmonella* spp. (Table 1). For these two species, MBC was considered to be higher than 100 mg/mL and was considered not susceptible to the extract. Since Ebc did not present bactericidal action against Gram-negative bacteria due to the lower susceptibility of these bacteria, the difference ($P<0.001$) was observed in the concentrations evaluated against *S. aureus*, *S. agalactiae*, and *S. uberis*. Ebc at the concentration of 100 mg/mL presented higher bactericidal potential when compared with the use at the concentration of 50 mg/mL, which in turn was effective only against *S. uberis* (Figure 2).

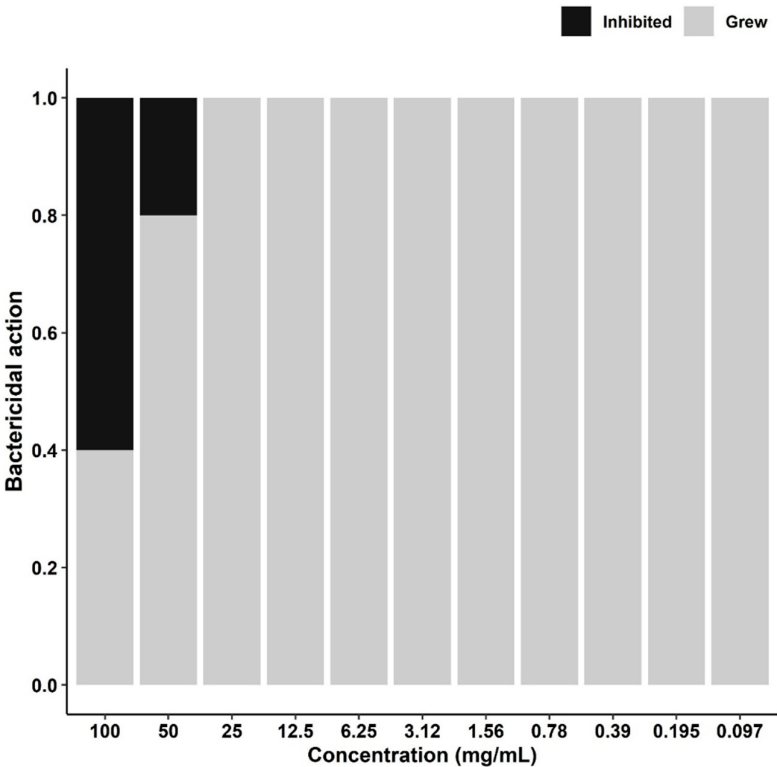


Figure 2. Bactericidal action among the different concentrations of *Bacharis crispa* against *S. aureus*, *S. agalactiae*, *S. uberis*, *E. coli*, and *Salmonella* spp.

Palacios et al. [30] evaluated the antimicrobial power of *B. crispa* oil by the presence of a disk diffusion test and also found the antimicrobial potential against *S. aureus*. They also did not find bactericidal action against *E. coli*. These authors were the first to describe the antimicrobial potential of *B. crispa* and concluded that this potential is partial and variable by the action of the compounds present according to the species.

The growth inhibition of *S. uberis*, *S. aureus*, and *S. agalactiae*, even at maximum concentration, promoted by Ebc is considered important since the extract inhibited the growth of three pathogens commonly associated with bovine mastitis. The species *S. aureus*, *S. uberis*, *S. agalactiae*, and coliforms can represent up to 80% of isolated pathogens [31]. Considering the importance of inhibiting these pathogens, Avancini et al. [19] evaluated the bactericidal power of *Baccharis trimera* and suggested the possibility of using plant extracts as a natural disinfectant and biological antiseptic in certain problem situations in animal production, specifically related to agents *S. aureus* and *S. uberis*.

The medicinal efficacy of the Indian plant *Azadiracantha indica*, popularly known as neem has been the target of several studies, and the biologically active extracts obtained from its leaves, fruits, seeds, and trunk are recognized by their multiple therapeutic properties, insecticides, nematocides, and fungicides [32,33]. In this study, the bactericidal power of the plant was evaluated in oil (OAi) and extract (EAi).

The bactericidal action of the EAi was detected for most of the species studied, except for *E.coli*, for which MBC was not determined (Table 1). The concentrations of EAi used promoted different bactericidal efficacy according to the bacteria ($P<0.01$). At a concentration of 100 mg/mL, the bactericidal action was effective against *S. ureus*, *S. agalactiae*, *S. uberis*, and *Salmonella* spp. As observed when in contact with EBC, the most susceptible pathogen was *S. uberis*, being inhibited with 50 mg/mL. When used at a concentration of 3.12 mg/mL or lower, EAi was not efficient against any of the species evaluated (Figure 3).

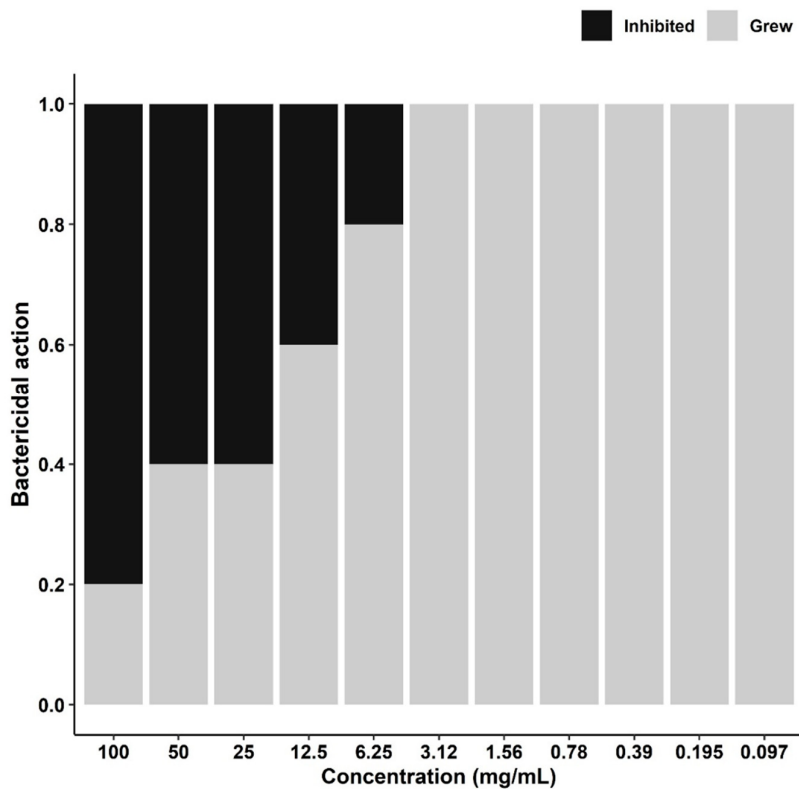


Figure 3. Bactericidal action between the different concentrations of *Azadiracantha indica* in front of *S. aureus*, *S. agalactiae*, *S. uberis*, *E. coli*, and *Salmonella* spp.

The *A. indica* oil was more efficient than the extract since lower concentrations were used to inhibit bacterial growth (Table 1). OAi showed satisfactory antibacterial activity only if used at the maximum concentration (100 mg/mL) for most bacteria, except for *S. uberis*, in which MBC was 50mg/mL ($P<0.001$, Figure 4).

Although EAi is generally considered more efficient, we found that OAi was more efficient against *E. coli* than EAi since *E. coli* was considered not susceptible to the extract. This indicates that oil compounds can be more efficient in certain situations. Arroiteia et al. [33] evaluated the effect of *A. indica* on the inhibition of a mycotoxin production found in apples and reported a higher potential of *A. indica* in oil, indicating that liposolubility would be an activating potential of its activity. As reported here, the action of oil is not always more efficient, and besides the scope of action against microorganisms, production costs should be considered. The greatest use of the extract may be associated with lower costs since obtaining the oil requires the use of greater resources [34].

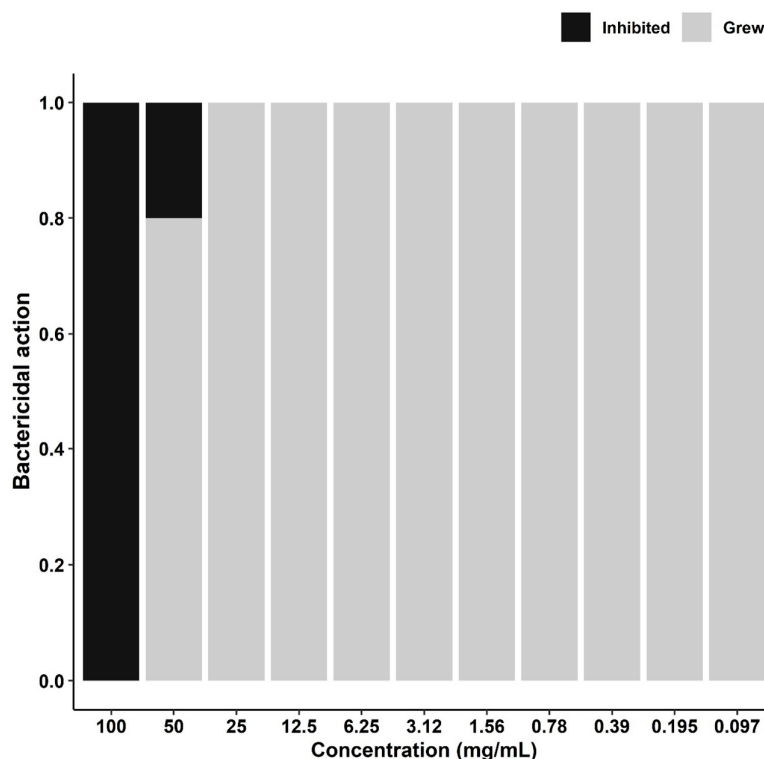


Figure 4. Bactericidal action of different concentrations of *Azadirachta indica* oil against *S. aureus*, *S. agalactiae*, *S. uberis*, *E. coli*, and *Salmonella* spp.

3.2. Mass Spectrometry with Paper Spray Ionization

3.2.1. *Stryphnodendron adstringens* (Mart.) Coville

Fourteen compounds in negative ionization mode and 8 in positive ionization mode were found for *Stryphnodendron adstringens* (Mart.) Coville detected compounds suggest a phytochemical profile particularly composed of polyphenolic and flavonoid derivatives, which are commonly associated with antioxidant and anti-inflammatory activities. These findings align with previous studies indicating the ethnopharmacological relevance of *Stryphnodendron adstringens* (Mart.) Coville, traditionally used in antimicrobial and wound-healing applications [35,36]. Identification of multiple hydrolyzable tannins, flavonoid glycosides, and phenolic acids further supports its potential use in functional foods and medicinal applications [37,38].

Related to paper spray analysis, in the positive ionization mode, ellagic acid (m/z 303) was identified, a known polyphenol with strong antioxidant and anticancer properties, commonly found in medicinal plants. Its presence in *Stryphnodendron adstringens* (Mart.) Coville may contribute to oxidative stress mitigation [39]. HHDP-digalloylglucoside, also known as tellimagrandin I (m/z 787), was detected and tentatively identified based on its fragmentation pattern $[M+H]^+$. This compound has been studied for its antiviral and antimicrobial activities, which could contribute to explaining the traditional use of this plant in wound healing [40]. Riparin III (m/z 288), an alkaloid, has been previously reported for its anxiolytic and neuroprotective properties [41]. Casuariin (m/z 785), a hydrolyzable tannin, further supports the plant's astringent properties, which are linked to its medicinal uses in gastrointestinal disorders [42].

The negative ionization mode identified several flavonoid and phenolic glycosides, substantiating the bioactive potential of *Stryphnodendron adstringens* (Mart.) Coville. Caffeic acid-hexoside (m/z 341) was detected, a derivative of caffeic acid known for its anti-inflammatory and

neuroprotective properties [43]. Rutin (m/z 609), a well-characterized flavonoid glycoside, has been widely studied for its vascular protective effects [44]. Punicalin (m/z 781), a hydrolyzable tannin, was also identified, contributing to the presence of antioxidant constituents. Quercetin-3,4'-O-diglucoside (m/z 625) and cyanidin-O-glucosyl-O-acetylpentoside (m/z 623) were also tentatively identified and can be considered to build on the plant's polyphenolic complexity, with quercetin derivatives playing a key role in cardiovascular health and cyanidin derivatives being linked to anti-inflammatory and neuroprotective effects [45,46].

3.2.2. *Baccharis crispa* Spreng

For *Baccharis crispa* Spreng, 8 compounds were identified in negative ionization mode and 5 in positive ionization mode, majorly composed of hydrolyzable tannins, flavonoids, and phenolic acids. This phytochemical composition is within the expected profile makeup of species used in traditional hepatoprotective and anti-inflammatory treatments, such as the case for *Baccharis crispa* Spreng [47,48]. The presence of bioactive polyphenols suggests potential applications in functional foods and nutraceuticals [49,50].

Among the positively ionized compounds tentatively identified, casuariin (m/z 785) was found, a tannin with antimicrobial and antioxidant activities. Octadecanoic acid (m/z 285), a saturated fatty acid, was also detected, known for its emollient properties and potential cardiovascular benefits [42,51]. Tellimagrandin I (m/z 787), a hydrolyzable tannin, was also tentatively identified, indicating a potential yield of a significant result of *Baccharis crispa* Spreng employment in traditional medicinal use [40,52].

The negative ionization mode tentatively identified ferulic acid (m/z 193), a phenolic compound known for its antioxidant and anti-inflammatory properties. HHDP-digalloylglucose (m/z 785) and procyanidin trimer A (m/z 863) were also identified, both of which have been implicated in cardiovascular protection and metabolic health [53–55]. Galloyl-bis-HHDP-glucose (m/z 935), also known as casuarinin, tentatively indicates the presence of potent antioxidant tannins in *Baccharis crispa* Spreng, presenting hepatoprotective potential [56,57].

3.2.3. *Azadirachta indica*

The analysis of *Azadirachta indica* was able to identify 6 compounds in negative ionization mode and 3 in positive ionization mode. Identified compounds suggest antimicrobial, anti-inflammatory, and neuroprotective properties, consistent with the traditional medicinal uses of neem [58–60].

In the positive ionization mode, octadecanoic acid (m/z 285) was tentatively identified, a compound known for its dermatological applications and potential role in lipid metabolism [51,61]. Tellimagrandin I (m/z 787) was also identified, with literature citing this compound as possessing antimicrobial properties [40,52].

The negative ionization mode tentatively identified dihydroquercetin-3,5-rhamnoside (m/z 449), a flavonoid with strong antioxidant properties, frequently studied for its cardioprotective effects [62]. Delphinidin-3-O-glucoside (m/z 465), an anthocyanin, has been associated with anti-inflammatory and neuroprotective benefits [63]. Procyanidin trimer A (m/z 863) was also detected, a compound widely studied for its cardiovascular and metabolic health effects [64]. Galloyl-bis-HHDP-glucose (m/z 935), also known as casuarinin, indicates the presence of bioactive tannins in *Azadirachta indica*, and may contribute to understanding its traditional use and role in antimicrobial treatments [65].

Several compounds were identified across multiple plant extracts, including tellimagrandin I (m/z 787), casuariin (m/z 785), procyanidin trimer A (m/z 863), and galloyl-bis-HHDP-glucose (m/z 935). These shared constituents suggest a common phytochemical basis for their reported biological activities, particularly in antioxidant, antimicrobial, and anti-inflammatory effects. The identification of these recurring bioactive compounds underscores their pharmacological relevance and potential therapeutic applications. These results provide a valuable basis for further pharmacological and clinical investigations [40,42,52,55–57].

4. Conclusions

The extracts showed bactericidal activity against bacteria isolated from bovine mastitis. However, the extract of *S. adstringens* presented a lower MBC for all bacteria. Thus, we can consider it the most efficient among the extracts evaluated. *S. uberis* strain was shown to be the least resistant, regardless of the extract or oil evaluated. Despite the higher efficiency of *S. adstringens*, both the extracts and the oil evaluated demonstrated their potential as antimicrobials. Paper Spray Mass Spectrometry (PS-MS) was fundamental for identifying the secondary metabolites responsible for the bactericidal action. Therefore, studies that promote the use of these compounds, preventively or in a combination of pharmaceutical and antibiotic therapy, against the main pathogenic bacteria isolated in cases of bovine mastitis should be disclosed.

5. Patents

In the context of intellectual property protection, the invention patent “Herbal Solution for Pre- and Post-Dipping” also known as “Natural Dipping” has been filed under the Brazilian National Application for invention, utility model, certificate of addition of invention, and entry into the national phase of the Patent Cooperation Treaty (PCT). The patent application, registered under Process BR 10 2021 009874 0, was officially submitted on May 21, 2021.

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