

EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF PLANTS FROM NORTHEAST BRAZIL AGAINST BACTERIA FROM RAW BEEF

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ABSTRACT

In Brazil, animal production constitutes a principal economic activity. To enhance productivity, antimicrobials are frequently employed, particularly as growth promoters, which contribute to bacterial resistance to these agents. Consequently, it is imperative to develop novel solutions, particularly natural ones, to mitigate bacterial resistance originating from food sources. Considering this, the present study seeks to characterize and evaluate additives with antimicrobial potential for application in the preservation of meat products. To achieve this objective, a repository of bacterial strains from packaged raw beef was established, followed by morphophysiological identification. The antimicrobial resistance profile was assessed by testing four selected bacterial strains against Imipenem, Azithromycin, Ciprofloxacin, Cefuroxime, and Nisin at varying concentrations, utilizing the Microtitre Broth Dilution Method. Concurrently, aqueous plant extracts from specimens in the Brazilian Northeast were obtained and analyzed for their phytochemical composition, antioxidant activity against DPPH and ABTS free radicals, and synergy for antimicrobial potential, conducted with the antibiotic Imipenem against four bacterial strains. The findings indicated that the packaged raw beef exhibited a microbiological profile of 60.42% Gram-negative and 31.25% Gram-positive bacteria, with increased susceptibility to ciprofloxacin. The phytochemical analysis of the extracts revealed levels ranging from 88.30 to 580.05 mg EAG/L, total flavonoids ranging from 110.04 to 387.48 mg EC/L, and condensed tannins ranging from 102.69 to 422 mg EC/L. Regarding antioxidant activity, the extract from the *Caesalpinia pulcherrima* plant demonstrated the ability to inhibit the tested free radicals. The synergism test revealed that, in combination with imipenem, the *Mimosa tenuiflora* plant extract exhibited antimicrobial efficacy against the four samples. Thus, the extracts were found to possess high levels of secondary metabolites, substantial antioxidant capacity, and the potential for use in conjunction with antimicrobials.

Keywords: Antimicrobial activity, *Mimosa tenuiflora*, Meat preservation

INTRODUCTION

Brazil has a prominent position in world agribusiness, with one of the main economic activities being the beef agro-industrial system, which in 2023 contributed 8.2% of Brazil's Gross Domestic Product (Abiec, 2024; Rodrigues; Marta-Costa, 2021). Meat is one of the foods most consumed by human beings around the world and is considered a staple in the meals of different countries. Its consumption takes place in different cultures and environments and is influenced by factors such as the economy, the environment and socio-cultural factors, in addition to bringing several health benefits, since it is an excellent protein source, as it contains 20 amino acids in its structure, among these, 9 essential amino acids for humans (Timoteo et al., 2021).

In 2021, the Brazilian Association of Meat Exporters (ABIEC) estimated in its annual report that Brazil had an animal herd of 196.47 million head. It currently has the largest commercial beef herd in the world, estimated at 197.2 million head (Abiec, 2024). However, with the search for increasing productivity and growth in agribusiness, there is an incentive for the production chain to use strategies and technologies that increase the added value of products, such as the use of antimicrobials in animal feed as growth promoters (Economou; Gousia, 2015; Repik et al., 2022).

Antimicrobials, defined as agents that inhibit microbial growth (Li et al., 2024), are used in animal production both to treat and prevent diseases and improve performance (Ribeiro; Comarella, 2015). However, their indiscriminate use as growth promoters has been associated with the selection of resistant bacterial strains (Silva; Rodrigues; Junior, 2022a).

Since the 1950s, antimicrobials have been used in animal production not only to treat infections, but also as food additives to modulate the intestinal microbiota (Gonzales; de Carvalho; Café, 2012). In Veterinary Medicine, their use encompasses: (i) prophylaxis, (ii) therapy, (iii) metaphylaxis, and (iv) growth promotion - the latter characterized by the administration of subtherapeutic doses aimed at improving zootechnical parameters (Cameron; Mcallister, 2016).

The use of antimicrobials as growth promoters in the meat production chain, despite recent attempts to ban or at least discourage its use in order to avoid antibiotic resistance, aims to improve health and provide greater resistance to

pathologies, reducing mortality, resulting in greater weight gain by the animal and, as a result, greater productivity, which has a direct impact on financial gain (Wesguerber et al., 2024).

Veterinary medicine uses 70% of the antimicrobials produced worldwide, and the concentrations used can leave residues in the meat of farm animals, a fact that can pose risks to human health, since it favors the selection of resistant bacteria, which can be transmitted to humans due to the proximity between species (Marques; Santos; Costa, 2023).

Antibiotic-resistant bacteria are a serious public health problem, as pre-existing microbial infections worsen when there is widespread antibiotic resistance. In addition to the increase in resistant strains, there is little development of new antibiotics, which is worrying given that bacterial resistance is causing a global health crisis (Almeida et al., 2023; O'rourke et al., 2020).

In this context, it is of the utmost importance to develop new antimicrobial agents, especially from natural sources, which can act against these bacteria. In this sense, a viable alternative is plant extracts, rich in secondary metabolites, which have various properties, including antimicrobial capacity. The presence of these bioactive compounds can interfere with the mechanisms of bacterial resistance, contributing to the effectiveness of antimicrobials and therefore mitigating antimicrobial resistance (Vidal; Pimentel, 2025).

Considering this, it is imperative to identify plants that possess bioactive compounds with antimicrobial properties, such as medicinal or unconventional food plants, which offer various benefits due to their secondary metabolites. The objective of this study was to evaluate phytochemical additives with antimicrobial potential for application in the preservation of meat products.

MATERIAL AND METHODS

Obtaining the raw material and extract

The plants *Talinum paniculatum* (Beldroegão), *Talinum triangulare* (Cariru), *Portulaca oleracea* (Beldroega) and *Cnidioscolus aconitifolius* (Chaya Estrela) were obtained from a family farm that grows Unconventional Food Plants for consumption and the sale of seedlings. The plants *Jatropha urens* (Cansação-de-

Leite) and *Croton heliotropiifolius* (Velame-da-Caatinga) were obtained from around the Banabuiú River, located in Morada Nova - CE. The plant *Mimosa tenuiflora* (Jurema-preta) was donated by the Zé Maria do Tomé Camp, located in Limoeiro do Norte - CE. The plant *Caesalpinia pulcherrima* (Flor-do-pavão) was collected at the Federal Institute of Education, Science and Technology of Ceará (IFCE) - Limoeiro do Norte Campus - CE. All the plants were collected in spring, in November 2024, a period of high sunshine and temperatures in Ceará.

The meat was obtained from a supermarket located in Limoeiro do Norte - CE. Raw, ground and packaged beef was selected and processed in the supermarket itself. After being obtained, the meat was transported in an ice box to the Food Microbiology Laboratory of the Federal Institute of Education, Science and Technology of Ceará (IFCE), Limoeiro do Norte campus, where the microbiological assays were assessed.

The processing of the plants began with the harvesting of the leaves, which were sanitized in a 200-ppm chlorine solution for 15 minutes and then rinsed in running water, arranged on glass plates and taken to an air circulation oven (Marconi®) for drying, which maintained the temperature at 40°C until the leaves were completely dry. After drying, the material was macerated using a pistil to obtain powder.

To obtain the extract, 0.5 g of the dried plant powder was weighed and diluted in 15 ml of distilled water. After dilution, the mixture was placed in a Dubnoff bath (Quimis®, Q226M2), stirred at speed 8 and at an average temperature of 22 °C for 24 hours. The extracts were then filtered through qualitative filter paper, freeze-dried and stored in sterile Falcon tubes.

Analysis of secondary metabolites

The plants were analyzed for the presence of total phenolic compounds, total flavonoids and condensed tannins. All the analyses used the aqueous extract, the development of which is described in section 2.1, at a concentration of 33.33 mg/ml.

The total phenolic compounds were quantified using the Folin-Ciocalteu method described by **Singleton and Rossi (1965)**, where gallic acid was used as the standard for developing the standard curve, consisting of adding 450 µl of the 33.3% Folin-Ciocalteu reagent to 450 µl of the previously diluted sample, then adding 3600 µl of distilled water, resting for 5 minutes in the dark and then adding 450 µl of 10% sodium carbonate. The absorbance was then read at 725 nm on a spectrophotometer (Biospectro®, SP-220) and the absorbance was then applied to the standard curve developed, expressing the results in milligrams of gallic acid equivalent per liter of sample (mg EAG/L).

Total flavonoids were quantified using the method described by **Zhishen et al. (1999)**, using catechin as the standard for developing the standard curve. The analysis consisted of adding 250 µl of the diluted sample, 2720 µl of 30% ethanolic solution and 120 µl of 0.5 mol/L sodium nitrite solution, with subsequent homogenization. After 5 minutes, 120 µl of 0.3 mol/L Aluminum Chloride Hexahydrate solution was added. After 5 minutes, 800 µl of 1 mol/L Sodium Hydroxide solution was added and homogenized. The absorbance was read at 510 nm using a UV-VIS spectrophotometer. The results were obtained by applying the absorbances of the samples to the standard curve developed, and these results were expressed in milligram equivalents of catechin per liter of sample (mg EC/L).

Condensed tannins were quantified using the methodology of **Hagerman (2002)**, also using catechin as a standard for developing the standard curve. Quantification was carried out by adding 400 µl of the diluted sample, 2400 µl of 4% vanillin solution and 1200 µl of 32% sulphuric acid solution. The tubes were left to stand for 15 minutes in a dark place and the absorbance was read at 500 nm on a spectrophotometer, expressing the results as milligrams of catechin equivalent per liter of sample (mg EC/L).

Antioxidant activity

Antioxidant activity was assessed by checking the ability to reduce DPPH and ABTS free radicals. The antioxidant activity against the DPPH free radical was carried out using the method proposed by **Brand-Williams et al. (1999)**, which consisted of completing the volume of a 10 ml volumetric flask after adding 1220 µl of the aqueous extract with the DPPH free radical. After resting for 30 min in a dark place, the absorbances were obtained in a spectrophotometer at 517 nm and substituted into Equation 1. The results were expressed as % antioxidant activity. The aqueous extract used was the same one used for the analysis of secondary metabolites and its development is described in section 4.2.

$$\text{Antioxidant activity (\%)} = \left[1 - \left(\frac{A_{517 \text{ sample}}}{A_{517 \text{ control}}} \right) \right] \times 100$$

The antioxidant activity against ABTS followed the methodology described by **Cai et al. (2025)**. Initially, 3 ml of the ABTS radical solution was added to 1 ml of the sample, left to stand for 6 minutes in the dark and the absorbance was read at 734 nm on the spectrophotometer. The results were obtained by calculation using Equation 2, where A0 refers to the absorbance at 734 nm of the ABTS radical solution without sample and A refers to the absorbance given with the presence of the sample. The results were expressed as % antioxidant activity.

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A}{A_0} \right) \times 100$$

Isolation and Identification of bacterial strains

Bacterial strains from packaged raw ground beef were inoculated using a swab and inoculated onto nutrient agar, followed by incubation in an incubator at 35°C for 48 hours. After bacterial growth, they were isolated onto nutrient agar and subsequently incubated in an incubator at 35°C for 24 hours.

To know the morphological aspects of all the strains isolated from beef, Gram staining was carried out to assess the cell wall, shape and arrangement (**Gephart, 1981**). Biochemical identification was also carried out, which consisted of assessing the presence of the enzyme catalase by the bacteria, according to the methodology proposed by **Goyal et al. (2012)**, with slight modifications, making it easier to identify the bacterial strains. The catalase test consisted of adding a drop of Hydrogen Peroxide (H₂O₂) to a bacterial smear isolated on a slide. The presence of the catalase enzyme in the bacteria was demonstrated by the formation of oxygen bubbles on the slide, due to the enzyme converting hydrogen peroxide into oxygen and water.

Evaluation of bacterial resistance to antimicrobials

Bacterial resistance to antimicrobials was tested using bacteria from packaged raw beef. These bacteria were obtained by inoculating them by rubbing a swab on the meat in Nutrient Agar and incubating them in a 35°C incubator for 48 hours. Four previously isolated bacterial colonies were selected based on different morphophysiological aspects and, after this selection, each bacterial strain was transferred to 5 ml of Mueller Hinton Broth and then incubated in an oven at 35 °C for 24 hours. The bacterial concentration was adjusted by inoculating 5 µl of the bacterial suspension into 5 mL of sterile Mueller Hinton Broth, obtaining a new bacterial suspension with a lower bacterial concentration.

To assess bacterial resistance, the Minimum Inhibitory Concentration (MIC) was analyzed in microplates using the least concentrated bacterial suspension against the antimicrobials Azithromycin 15 (AZI15) [from 60 µg/ml to 0.9375 µg/ml], Cefuroxime 30 (CRX30) [from 120 µg/ml to 3.75 µg/ml], Ciprofloxacin 05 (CIP05) [from 10 µg/ml to 0.3125 µg/ml], Imipenem 10 (IMP10) [from 40 µg/ml to 1.25 µg/ml] and Nisin [from 30.000 IU/ml to 0.3125 IU/ml]. The antibiotics used were eluted from antibiotic discs into Mueller-Hinton Broth following the method proposed by **Wilson et al. (1990)**, initially, the first four were diluted in Mueller-Hinton broth and only Nisin in 0.02M HCl and then filtered through a sterile 0.45 µm syringe filter.

200 µl of Mueller-Hinton broth, 10 µl of the new bacterial suspension of lower concentration and 90 µl of the antimicrobial were added to the microplate. It should be noted that positive bacterial growth controls were prepared, consisting only of Mueller-Hinton broth and bacterial suspension, and negative controls, consisting of the antimicrobial without dilution, bacterial suspension and Mueller-Hinton broth, to confirm the sterility of the material used and the environment. Finally, after incubation at 35 °C for 24 hours, it was possible to check which minimum concentration was able to inhibit the growth of the bacteria tested, by visualizing the presence or absence of turbidity in the medium present in the well, with subsequent application of 20 µl of 2% resazurin solution in the wells for colorimetric visualization of bacterial viability, according to the methodology proposed by **Sarker et al. (2007)**, with slight modifications. The bacterial resistance profile was interpreted from BrCast (2025) standards considering the highest MIC values regarding all listed genera.

Evaluation of the antimicrobial activity of the plant extracts

To find out the antimicrobial capacity of the plant extracts in isolation, a MIC test was carried out, following the same methodology used to assess bacterial resistance to antimicrobials, with the modification of using the plant extracts to replace the antimicrobials (**Sarker et al., 2007**).

Initially, the lyophilized plant extracts were diluted in Mueller Hinton Broth and then filtered through a sterile 0.22 µm syringe filter. Next, 200 µl of Mueller Hinton Broth, 90 µl of plant extract and 10 µl of less concentrated bacterial suspension, inoculated and suspended as performed in the test to evaluate bacterial resistance to antimicrobials, were added to the microplate. The microplate was incubated at 35°C for 24 hours and then 20 µl of 2% resazurin solution was added to the wells for colorimetric visualization of bacterial viability.

Evaluation of synergism between plant extracts and antibiotics

After knowing the MIC of the bacteria against the antibiotics, Imipenem 10 (IMP 10) was selected to be evaluated together with the plant extracts. Initially, the lyophilized plant extract and Imipenem (sub-MIC concentration) were diluted in Mueller Hinton Broth and then filtered through a sterile 0.22 µm syringe filter. The test to assess synergism between the plant extracts and IMP 10 consisted of adding 110 µl of Mueller Hinton Broth, 90 µl of the plant extract, 90 µl of Imipenem at the subMIC concentration and 10 µl of bacterial suspension. As a way of controlling the MIC, the minimum inhibitory concentration, now known, was

repeated in the synergistic effect test microplate. In addition, this microplate also contained positive controls, consisting of 290 µl of Mueller Hinton broth and 10 µl of bacterial suspension, and a negative control, consisting of 200 µl of Mueller Hinton broth, 90 µl of undiluted Imipenem and 10 µl of bacterial suspension. Only 2 of the 4 bacterial strains were used. All the plant extracts had a concentration of 100 mg/ml (Sarker et al., 2007). The test was carried out in triplicate.

Statistical analysis

The analyses were carried out in triplicate. The results obtained were presented as means and standard deviation, evaluated using analysis of variance (ANOVA) followed by Tukey's test at 5% significance, using Statistica software version 7.0.

RESULTS AND DISCUSSION

Analysis of secondary metabolites

The total phenolic compound contents of the plant extracts ranged from 88.30 to 580.05 mg EAG/L, indicating high values of phenolic compounds using water as a solvent, even though it is known that the use of solvents such as ethanol and methanol facilitates the extraction of these compounds. Furtado et al. (2021) quantified these compounds in other plant extracts and reported a content of 14.28 mg EAG/g present in the ethanolic extract of *Mormodica charantia* leaves. Despite the use of ethanol as a solvent, the total phenolic compound contents of the plants analyzed in this study were higher compared to the study cited. According to the results shown in Table 1, it was found that Flor-do-pavão extract had a higher content of total phenolic compounds (580.05 ± 2.25 mg EAG/L) and condensed tannins (422.45 ± 5.41 mg EC/L).

Table 1 Content of secondary metabolites in the plant extracts studied.

Plant	Total phenolic compounds (mg EAG/L)	Flavonoids (mg EC/L)	Condensed Tannins (mg EC/L)
Jurema preta	171.53 ± 1.17 ^c	110.04 ± 4.49 ^c	102.69 ± 1.09 ^b
Flor-do-pavão	580.05 ± 2.25 ^a	377.11 ± 11.11 ^a	422.45 ± 5.41 ^a
Chaya Estrela	254.21 ± 0.70 ^b	387.48 ± 6.79 ^a	116.74 ± 0.41 ^g
Beldroegão	89.52 ± 0.56 ^g	378.59 ± 4.63 ^a	151.02 ± 1.09 ^c
Cariru	122.57 ± 0.58 ^e	184.48 ± 1.28 ^{bc}	166.02 ± 4.04 ^d
Cansanção	88.30 ± 0.32 ^g	156.33 ± 4.01 ^d	323.40 ± 8.28 ^b
Velame	111.86 ± 0.43 ^f	195.96 ± 1.70 ^b	132.21 ± 3.98 ^f
Beldroega	152.99 ± 0.43 ^d	171.52 ± 1.70 ^{cd}	226.74 ± 2.89 ^c

a,b,c,d,e,f,g,h Averages with different letters on the same line differ significantly (P < 0.05). Source: Own authorship (2025).

In the study conducted by Menezes Filho et al. (2019), the phenolic compounds of 17 plant ethanolic extracts were analyzed, in which the phenolic compound contents ranged from 6.40 to 23.05 mg EAG/100g, where the leaf extract of the plant *Rollinia laurifolia* presented a content of 23.05 mg EAG/100g. In both previously mentioned studies, ethanol was used as the solvent to obtain the extract, which provides better extraction of phenolic compounds. In the present study, the extract developed used distilled water as the solvent, aiming at a lower risk of toxicity, cost, and environmental impact.

As for flavonoids, the extracts of the plants Flor-do-pavão, Chaya Estrela and Beldroegão did not differ significantly, and therefore showed higher levels of total flavonoids, with levels of 377.11, 387.48 and 378.59 mg EC/L, respectively. A quantification of flavonoids in the leaves of *Mormodica charantia* revealed that they had a content of 12.16 mg QE/g, showing greater richness in flavonoids in the extracts of the plants in this study (Menezes Filho et al., 2019).

Noura et al. (2024) analyzed flavonoids from six plants using different parts of these plants and revealed that the highest flavonoid content was 456.95 µg EQ/g, present in the aqueous extract of *C. sesamoides*. However, the extract was developed using the whole plant, which explains the higher flavonoid content compared to the present work, in which flavonoid contents ranging from 110.04 to 387.48 mg EC/L were obtained, still demonstrating a high flavonoid content and, therefore, possibly, an antimicrobial potential.

Regarding condensed tannins, the highest tannin content was also found in the Flor-do-pavão extract, with a content of 422.45 ± 5.41 mg CE/L of condensed tannins. In the study by El Baakili et al. (2024), the condensed tannins of the *Retama dasycarpa* plant were quantified from its hydromethanolic extract, with a value of 12.5 ± 0.36 mg EC/g. In the current study, the condensed tannin content ranged from 102.69 to 422 mg EC/L, quantified from an aqueous extract. In the aforementioned study, the quantification of tannins was carried out using a hydroethanolic extract, so the use of methanol as one of the solvents may have favored the extraction of condensed tannins.

Antioxidant activity

For the analysis of antioxidant activity, both against DPPH and ABTS (Table 2), the extracts were used at a concentration of 16.66 mg/ml, except for the Flor-do-

pavão extract, which had to be used at a concentration of 8.33 mg/ml against ABTS.

Table 2 Determination of the antioxidant activity of studied plant extracts.

Plant	Free radical inhibition DPPH (%)	Free radical inhibition ABTS (%)
Jurema preta	81.19 ± 0.36 ^b	59.27 ± 1.84 ^{bc}
Flor-do-pavão	86.87 ± 0.10 ^a	29.77 ± 0.20 ^{*a}
Chaya Estrela	74.32 ± 0.05 ^c	80.40 ± 0.27 ^a
Beldroegão	43.38 ± 1.42 ^f	38.69 ± 4.00 ^f
Cariru	34.22 ± 0.4 ^g	43.15 ± 0.66 ^{cd}
Cansanção	60.88 ± 0.15 ^d	63.64 ± 0.13 ^b
Velame	73.98 ± 0.52 ^c	47.02 ± 0.84 ^d
Beldroega	48.68 ± 0.25 ^e	58.51 ± 1.07 ^c

a,b,c,d,e,f,g,h Means with different letters on the same line differ significantly (P < 0.05). Extracts used at a concentration of 16.66 mg/mL. *: Extract used at a concentration of 8.33 mg/ml. Source: Own authorship (2025).

The results of the antioxidant activity analysis showed that the extract of the Flor-do-pavão plant had the highest antioxidant potential against both free radicals. Although it showed a lower percentage against the ABTS free radical, it should be noted that it was necessary to double the dilution to verify the antioxidant activity against the ABTS radical, thus demonstrating its high antioxidant capacity. The antioxidant capacity against the ABTS radical ranged from 38.69 to 80.40% using extracts at a concentration of 16.66 mg/mL. The Flor-do-pavão extract was used at a concentration of 8.33 mg/mL, demonstrating greater antioxidant activity.

The substantial antioxidant capacity of the Flor-do-pavão extract aligns with its elevated levels of total phenolic compounds, total tannins, and total flavonoids. It is well-established that high concentrations of total phenolic compounds are associated with significant antioxidant capacity. Moreover, the notable antioxidant activity observed in extracts with lower levels of phenolic compounds, such as the Jurema-preta extract, may be attributed to the specific flavonoid profile of the sample. Flavonoids possessing multiple hydroxyl groups exhibit enhanced antioxidant activity due to their chemical structure (Silva et al., 2020).

The study by Pessuto et al. (2009) analyzed the aqueous extracts of the leaves of *Maytenus ilicifolia* Mart. ex Reiss, known as espinheira-santa, against the free radical DPPH, where it was found that the extract had a 47.31 free radical inhibition capacity. In the present study, seven of the eight plant extracts studied had a higher antioxidant capacity against DPPH than in the aforementioned study, with radical inhibition capacity ranging from 34.22 to 86.87%, thus demonstrating the high antioxidant potential against DPPH of the extracts currently studied.

Identification of the bacterial strains

From the cultivation of bacteria from commercially obtained beef for this study, it was possible to establish 21 distinct microbiological colonies which, from a morphophysiological point of view, were mostly composed of mixed cultures - except for three bacterial strains which presented a single bacterial biotype.

Considering all analyzed strains, there was a majority of Gram-negative bacteria (65.91%), in which Bacilli without arrangement, Cocobacilli without arrangement and Streptobacilli stood out. It was also possible to observe the occurrence of Gram-positive bacteria (34.09%), especially Bacilli without arrangement; Streptobacilli; Cocci without arrangement and Staphylococci. All the bacterial strains had the enzyme catalase.

In the beef analyzed, most of the bacterial strains found were Gram-negative bacilli. The majority presence of Gram-negative bacteria in raw beef is a cause for concern, given that Foodborne Diseases are mainly caused by Gram-negative bacteria. Some of the main bacteria responsible for these diseases are *Escherichia coli* and *Salmonella* spp. both of which are Gram-negative bacilli (Soragni; Barnabe; Mello, 2019; Dias et al., 2008).

In another study carried out with ground beef, 100% of the meat analyzed in public shops showed contamination by *E. coli* and *Salmonella* spp. The meat was analyzed in natura, before grinding, meaning that it is understood that the contamination by these bacteria is not in the grinding, but probably in transportation, handling or inadequate storage (Almeida; Monteiro; Bezerra, 2015).

In addition to concerns about Foodborne illnesses, a microbiological profile composed primarily of Gram-negative bacteria may be more associated with more difficult-to-treat diseases, since Gram-negative bacteria are more likely to be resistant to antimicrobials. This is due to the outer membrane of Gram-negative bacteria, which contains lipopolysaccharide (LPS), as well as efflux pumps and other antimicrobial resistance mechanisms (Saxena et al., 2023). The meat microbiota is composed not only of foodborne pathogenic bacteria but also of spoilage microorganisms that may be responsible for the transmission of antimicrobial resistance to humans, through resistance genes (Conceição; Queiroga; Laranjo, 2023).

Evaluation of bacterial resistance to antimicrobials

Table 3 shows the results of the MIC tests carried out on bacteria isolated from beef against different antimicrobials at different concentrations. The results showed that all the bacterial strains did not show inhibition at any of the

concentrations tested with AZI, CRX and Nisin. There was also resistance to the antibiotic imipenem (IMP) because, according to BrCast, a dosage of less than 2 mg/L is required to be sensitive to imipenem. As a result, only Ciprofloxacin (CIP) was more effective in the bacterial strains.

Table 3 Minimum Inhibitory Concentration (mg/L) of tested antimicrobials against bacteria isolated from raw beef.

SAMPLE	AZI	CRX	CIP	IMP	NIS
Mixed Culture (G+ and G- bacilli)	-	-	0.16	≥5.00	-
Single Culture (G- bacilli)	-	-	0.16	≥5.00	-
Mixed Culture (G- Coccobacilli and G- Streptobacilli)	-	-	1.25	≥5.00	-
Single Culture (G- bacilli)	-	-	1.25	≥5.00	-

The symbol "-" means no Minimum Inhibitory Concentration even at highest tested concentration. AZI: Azithromycin; CRX: Cefuroxime; CIP: Ciprofloxacin; IMP: Imipenem; NIS: Nisin. Source: Author (2025).

Azithromycin, unlike Ciprofloxacin and Imipenem, is an antibiotic belonging to the macrolide class. It functions by binding to specific regions of RNA within the bacterial ribosome, thereby disrupting the elongation process of the peptide chain during translation and effectively inhibiting bacterial protein synthesis. Bacterial resistance to Azithromycin can be attributed to the inactivation of the molecule or the obstruction of its access to the effector site. Additionally, bacteria may develop the capability to modify the target site, potentially through the erm gene, which leads to the demethylation of the adenine nucleotide in the 23S subunit, altering its conformation and consequently preventing Azithromycin from binding to ribosomal RNA. (Freires; Junior, 2022). To be considered resistant, depending on the bacterial strain, a dose of between 0.25 and 4 mg/L of azithromycin is required, according to BrCast. In this study, the bacteria were resistant to 60 mg/L of the antibiotic.

Resistance to cefuroxime, which are β-lactam antibiotics, is explained by the production of β-lactamases, which act by hydrolyzing the β-lactam ring, thus inactivating the antibiotic. In addition, this resistance mechanism is more present in Gram-negative bacteria, which is in line with the present study, since the bacterial strains isolated from meat were mostly Gram-negative (Scherer et al., 2016). According to BrCast (2025), the dose for resistance to be considered is 8 mg/L. In this study, not even 120 mg/L was able to inactivate the bacteria.

The large presence of Gram-negative bacteria in this study may also explain the resistance to Nisin. Nisin is a peptide, used as a food additive, synthesized by bacteria of the genus Lactococcus lactis, which has high bactericidal power, however, its spectrum of action is mainly for Gram-positive bacteria, being effective for Gram-negative bacteria only when, in conjunction with complementary treatments, it is possible to break the protective membrane present in Gram-negative bacteria (María-Almudena et al., 2019).

As for imipenem (IMP 10), all four bacterial strains had MICs of 5 mg/L, which shows resistance to this antimicrobial. The high resistance to imipenem, which is a carbapenem, can be explained by the bacterial resistance mechanism of decreased permeability of the outer membrane, decreasing the functionality of porins, increased expression of efflux pumps, as well as increased production of carbapenemases, which are capable of hydrolyzing carbapenems (Coutinho et al., 2015). According to BrCast, doses of 0.03 to 4 mg/L would be necessary to configure resistance to imipenem, depending on the bacteria analyzed. In this study, only 5 mg/L was effective against the bacteria tested.

Due to the high level of bacterial resistance to antimicrobials in these bacteria, it can be said that they are multidrug-resistant, since, according to Magiorakos et al. (2012), "multidrug-resistant defined as non-susceptibility to at least one agent in three or more antimicrobial categories".

The occurrence of multidrug-resistant bacteria in meat products is a cause for significant concern. Although meat is typically subjected to cooking, there remains a risk of contamination of cutting surfaces and other utensils, thereby facilitating the transmission of these bacteria to other surfaces or food items, which may lead to human exposure. The processes involved in the slaughter and handling of these meats are associated with an elevated risk of contamination by these bacteria (Araújo et al., 2023; Costa et al., 2020; Nilo; Marin, 2022).

Consumption of raw or undercooked meat persists, resulting in the intake of meat contaminated through handling and preparation processes, thereby exacerbating contamination and microbiological risk. The consequences of infections associated with antimicrobial-resistant bacteria include an increased incidence and severity of illness, prolonged hospitalization durations, and elevated associated costs. This is correlated with heightened treatment failure, disease severity, increased hospitalization rates, and mortality (Badawy; Loffy; Shawir, 2019).

Evaluation of the antimicrobial activity of the plant extracts

Strains 13 and 3 were selected for assay development due to their elevated MIC values. Upon evaluating the antimicrobial activity of the plant extracts

individually, it was observed that the *Mimosa tenuiflora* extract (100 mg/mL) exhibited antimicrobial activity against strain 3, a mixed culture comprising coccobacilli and streptobacilli, both Gram-negative (Figure 1). However, this extract did not demonstrate activity against strain 13, an isolated Gram-negative bacillus. The antimicrobial efficacy of the *M. tenuiflora* extract can be attributed to the presence of condensed tannins, which likely possess antimicrobial specificity, targeting the bacterial cell membrane and inducing cell death. In contrast, the other plant extracts presumably lacked compounds with such antimicrobial specificity.

In the test conducted by Crepaldi et al. (2022), the antimicrobial activity of the methanolic extract of the *Mimosa tenuiflora* plant (40 µg/mL) was verified against *Aeromonas* strains, which can also be found in animal products. Silva et al. (2021). Conversely, the study demonstrated the antimicrobial efficacy of the ethanolic extract derived from the bark of the *M. tenuiflora* plant against *Staphylococcus aureus* strains, utilizing the disk diffusion method, which resulted in a halo measuring 9.85 mm in radius. It is noteworthy that the employment of ethanol or methanol in the extraction process may enhance the extraction of compounds with antimicrobial properties, such as phenolic compounds. Moreover, inadequate or incomplete evaporation of these solvents may leave residues in the extract, potentially leading to antimicrobial effects with a reduced concentration of the plant material utilized.

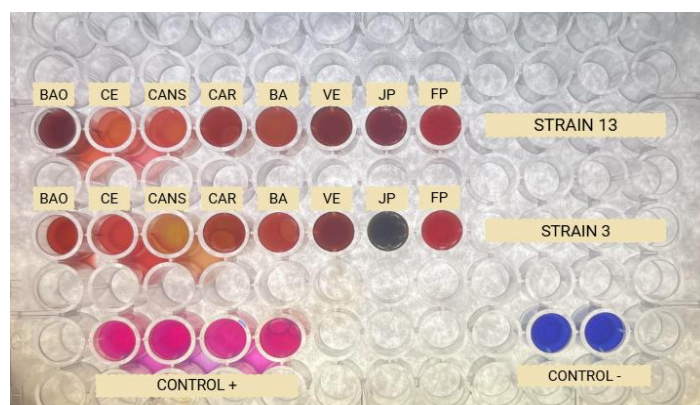


Figure 1 Evaluation of antimicrobial activity of plant extracts against strain 13 (Gram-negative bacillus) and strain 3 (Gram-negative Coccobacilli and Streptobacilli).

BAO: Beldroegão, CE: Chaya Estrela, CANS: Cansanção, CAR: Cariru, BA: Beldroega, VE: Velame-da-Caatinga, JP: Jurema-preta, FP: Flor-do-pavão, Control + : Positive control, Control - : Negative control, Strain 13: Bacterial colony 13, Strain 3: Bacterial strain 3. Source: Author (2025).

Evaluation of synergism between extracts and antibiotics

The increasing resistance of bacteria, particularly those associated with foodborne illnesses, to antimicrobial agents necessitates the exploration of alternative solutions, ideally of natural origin, that can effectively counteract this resistance. Such solutions have potential applications in various food technologies, including antimicrobial materials like films, coatings, or active antimicrobial packaging for meat products. Notably, plants, which are widely utilized globally for the treatment of bacterial and fungal infections, exemplify these natural solutions.

Because they have a high and diverse content of secondary metabolites, such as alkaloids, terpenes and phenolic compounds, plant extracts can have antimicrobial capacity (Singh et al., 2023). As a result, it was possible to observe that the extract of the plant *M. tenuiflora* (Jurema-preta) showed synergism with Imipenem (Figure 2 and 3). *M. tenuiflora*, native to the north-east of Brazil and popularly known as Jurema-preta, is resistant to periods of drought. In popular medicine, it is used to treat infectious diseases, as it has anti-inflammatory, healing and antioxidant activity (Santos et al., 2022).

Some studies report the antibacterial capacity of *M. tenuiflora*, however, this capacity is mainly present in the bark of the plant (Bezerra et al., 2009). Padilha et al. (2010) reported that the bark extract of Jurema-preta presented a Minimum Inhibitory Concentration of 0.18 mg/mL against 16 bacterial strains. However, ethanol was used as a solvent, which can significantly improve the extraction of the compounds. Studies using the leaf extract of *M. tenuiflora* are scarce, with greater studies on other parts of the plant.

By helping to stabilize and enhance the antimicrobial effect, in the study by Brito et al. (2022), the bark extract of Jurema-preta, combined with synthesized silver nanoparticles, contributed to improved action against strains of *Staphylococcus aureus* (ATCC 25923), thus demonstrating the possibility of using the synergistic effect of the extract with other antimicrobial molecules to collaborate in antibacterial activities.

In studies with other plants, it was observed that the use of essential oils obtained from *Ammodaucus leucotrichus* Cosson, *Thymus vulgaris* L., and *Lavandula maroccana* revealed synergistic interactions with the antibiotics gentamicin and

amoxicillin, reducing the Minimum Inhibitory Concentration by 2- to 64-fold (Soulaïmani et al., 2022).

In synergism with Imipenem, the Jurema-preta leaf extract, at a concentration of 100 mg/L, was able to potentiate the antibiotic's action. Knowing that imipenem is a carbapenem and therefore acts by inhibiting the synthesis of the bacterial cell wall by inhibiting penicillin-binding proteins, which are essential for the development of the cell wall structure, the adjuvant effect of the plant's leaf extract with imipenem suggests that the plant may have acted by potentiating the antibiotic's mechanism of action, acting on the cell wall, contributing to bacterial cell death (Aurilio et al., 2022).

This antimicrobial capacity of the Jurema-preta leaf extract, together with imipenem, can possibly be explained by the presence of a high content of secondary metabolites. Phenolic compounds, especially flavonoids and tannins, are strongly linked to antimicrobial potential. However, the content of total phenolic compounds varies according to the place of collection and time of year (Oliveira et al., 2022).

Flavonoids are biologically active compounds that exhibit both antibacterial and antioxidant properties. Condensed tannins exert their antimicrobial effects by acting directly on the organelles and cell membranes of microorganisms (Hernandez et al., 2021; Santos et al., 2022; Silva et al., 2020; Zhou et al., 2015). While the Jurema-preta extract did not exhibit a higher concentration of total flavonoids and condensed tannins compared to the other plant extracts in this study, its synergistic antimicrobial efficacy may be attributed to the specific nature of the flavonoids and condensed tannins present in this sample, which demonstrated enhanced antimicrobial potential when combined with the antibiotic imipenem.

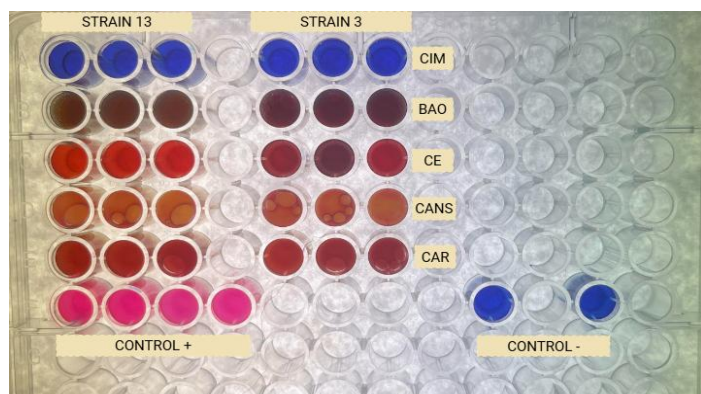


Figure 2 Synergism test between plant extracts (BAO, CE, CANS and CAR) and antibiotic Imipenem.

BAO: Beldroegão, CE: Chaya Estrela, CANS: Cansanção, CAR: Cariru, Control + : Positive control, Control - : Negative control, CIM: Minimum Inhibitory Concentration (MIC), Strain 13: Bacterial colony 13, Strain 3: Bacterial colony 3. Source: Author (2025).

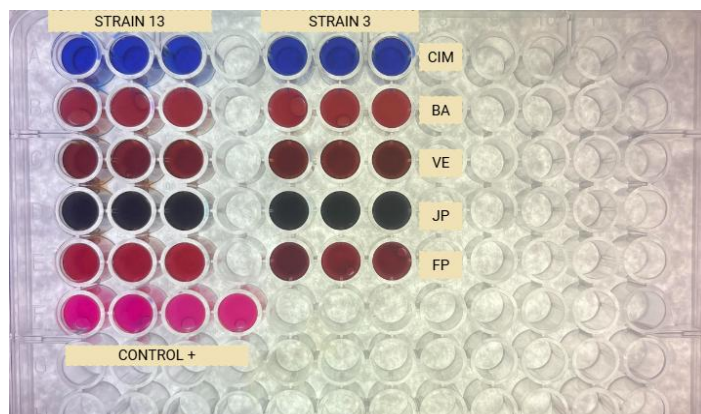


Figure 3 Synergism test between plant extracts (BA, VE, JP and FP) and antibiotic Imipenem.

BA: Beldroega, VE: Velame-da-Caatinga, JP: Jurema-preta, FP: Flor-do-pavão, Control + : Positive control, Control - : Negative control, CIM: Minimum Inhibitory Concentration (MIC), Strain 13: Bacterial colony 13, Strain 3: Bacterial colony 3. Source: Author (2025).

CONCLUSION

From this screening, it was observed that the plant extract with the highest content of secondary metabolites was the extract from the *C. pulcherrima* plant, with the highest levels of total phenolic compounds and condensed tannins. In terms of flavonoids, the extracts from the plants *C. s aconitifolius*, *C. pulcherrima* and *T. paniculatum* had the highest flavonoid levels. In terms of antioxidant activity, the

extract from the *C. pulcherrima* plant also showed a higher rate of inhibition against the DPPH radical and the ABTS radical.

As for bacterial isolation, 65.91% of the bacteria were Gram-negative and 34.09% Gram-positive. About the bacterial resistance of the 4 bacterial strains isolated from the meat, a high level of resistance to the antimicrobials tested was observed, with susceptibility only to Ciprofloxacin 05 by two bacterial strains. As for the antimicrobial effect of plant extracts and synergism with the antibiotic Imipenem 10, the extract of the plant *M. tenuiflora* (Jurema-preta) showed an isolated antimicrobial effect against one of the two bacterial strains, and a synergistic effect with the antibiotic against the two strains tested.

Thus, the results indicate the potential use of the *M. tenuiflora* plant extract as a potentiator of the effect of Imipenem, a broad-spectrum carbapenem. Furthermore, more studies are needed to verify the use of the *M. tenuiflora* plant extract in the preservation of meat products, either alone or together with other antimicrobial molecules, as well as studying its toxicity against eukaryotic cells.

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