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Use of *Azadirachta indica* extract in the control of contaminant bacteria in fermentation processes

Uso de extrato de *Azadirachta indica* no controle de bactérias contaminantes em processos fermentativos

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ABSTRACT

Considering that gram-positive contaminant bacteria may compromise the alcoholic fermentation process for ethanol production, natural biocides are sought for use in controlling such microorganisms. In this sense, neem (*Azadirachta indica*), known for its biological properties, emerges as a solution for controlling these contaminations. This study aimed to evaluate the antimicrobial activity of neem leaf, bark, and seed extracts, correlating them to the presence of secondary metabolites, thus determining their biocide potential. The extracts were obtained by maceration in ethanol and phytochemically analyzed through thin-layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR), with the quantification of total phenolics, total flavonoids, total tannins, and antioxidant activity. The antimicrobial activity was evaluated through disk diffusion tests and the minimum inhibitory concentration (MIC) determination, using *L. fermentum* and *L. mesenteroides* as bacterial models. The tests showed that the leaf and bark extracts inhibited bacterial growth without affecting the yeast *S. cerevisiae*, with an efficacy of 50 and 30 mg/mL, respectively. A phytochemical analysis revealed the predominance of flavonoids in the leaves and a more significant concentration of tannins in the bark, both of which are recognized for their antimicrobial properties. The extracts also presented high levels of phenolic compounds, reinforcing their bacterial efficacy, while the antioxidant activity of the bark suggests a complementary role of action of the extract. The seed extract did not show antimicrobial activity. Hence, the neem leaf and bark extracts have biocide potential to be used in alcoholic fermentation.

Keywords: secondary metabolites; neem; *Leuconostoc mesenteroides*; *Lactobacillus fermentum*; alcoholic fermentation.

RESUMO

Considerando-se que o processo de fermentação alcoólica para a produção de etanol pode ser comprometido por bactérias contaminantes gram-positivas, buscam-se biocidas naturais para usar no controle desses microrganismos. Nesse sentido, o neem (*Azadirachta indica*), conhecido por suas propriedades biológicas, surge como solução para o controle dessas contaminações. Este estudo visou avaliar a atividade antimicrobiana de extratos de folhas, cascas e sementes de neem, correlacionando-a à presença de metabólitos secundários e determinando, assim, o potencial biocida. Os extratos foram obtidos por maceração em etanol, analisados fitoquimicamente por cromatografia em camada delgada (CCD) e espectroscopia na região do infravermelho (FTIR), além da quantificação de fenólicos totais, flavonoides totais, taninos totais, bem como a atividade antioxidante. A atividade antimicrobiana foi avaliada por testes de difusão em disco e determinação da concentração inibitória mínima (MIC), utilizando *L. fermentum* e *L. mesenteroides* como modelos bacterianos. Os testes mostraram que extratos de folhas e cascas inibiram o crescimento das bactérias sem afetar a levedura *S. cerevisiae*, com eficácia a 50 e 30 mg/mL, respectivamente. A análise fitoquímica revelou predominância de flavonoides nas folhas e maior concentração de taninos nas cascas, ambos reconhecidos por propriedades antimicrobianas. Os extratos também apresentaram altos teores de compostos fenólicos, reforçando sua eficácia bacteriana, enquanto a atividade antioxidante das cascas sugere um papel complementar de ação do extrato. O extrato de sementes não demonstrou atividade antimicrobiana. Assim, os extratos de folhas e cascas de neem possuem potencial biocida para serem utilizados na fermentação alcoólica.

Palavras-chave: metabólitos secundários; neem; *Leuconostoc mesenteroides*; *Lactobacillus fermentum*; fermentação alcoólica.

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Introduction

Ethanol fermentation is a biotechnological process widely used in many countries to produce high value-added bioproducts such as fuel ethanol and fermented or distilled alcoholic beverages. This process, which is based on the conversion of sugars present in the raw material into ethanol through the action of yeast *Saccharomyces cerevisiae*, is considered essential for industries seeking energy sustainability and diversification of biotechnological products (Lino et al., 2021)).

Sugarcane juice stands out among the raw materials most employed in ethanol fermentation and is widely used by biotechnological industries in Latin American, African, Asian, and Oceanic countries. However, the industries that use this raw material generally carry out fed-batch fermentation processes with yeast cell recycling, which results in high levels of contamination by bacteria such as *Lactobacillus fermentum* and *Leuconostoc mesenteroides*. These contaminants compete with yeast for the sugars and produce lactic acid and gums, which compromise yeast viability, causing yeast flocculation and reducing the fermentation rate (Brexó and Sant'Ana, 2017; Lino et al., 2024).

Controlling bacterial contamination in fermentation reactors is a recurring challenge in the industry, with the use of antimicrobials being a necessary practice to reduce the population of such microorganisms. Traditionally, industries opt for synthetic antibiotics due to their high efficacy, with the prominence of substances such as monensin sodium, penicillin, erythromycin, chloramphenicol, and virginiamycin (Fadel et al., 2018). However, this approach has serious limitations: in addition to the high cost, the constant use of such products may entail relevant environmental impacts, such as promoting bacterial resistance and the possibility of residues in the final product. Moreover, the indiscriminate use of antibiotics may harm the performance of the yeast *Saccharomyces cerevisiae* itself, negatively interfering with fermentation efficiency (Bischoff et al., 2016).

Hence, there is growing interest in natural alternatives that can offer effective microbiological control with less significant environmental impacts and reduced toxicity. Products such as propolis (*Apis mellifera*), jambolan (*Syzygium cumini*), and hops (*Humulus lupulus*) have shown potential antimicrobial properties and are emerging as viable alternatives (Fernandes et al., 2022; Bhatti et al., 2024; Khaliullina et al., 2024).

Standing out among other promising natural alternatives is *Azadirachta indica*, known as neem, a tree native to the Indian subcontinent and widely cultivated in tropical regions of Asia, Africa, Central America, and South America (Wylie and Merrell, 2022). Studies have pointed to its traditional use in treatments for infections, inflammations, skin diseases, and parasitoses, which boosted scientific and commercial interest worldwide (Bhatti et al., 2024). More recently, it has been drawing the interest of modern medicine for its antimicrobial potential and applications in fields such as oncology, dentistry, and dermatology (Yarmohammadi et al., 2021; Baby et al., 2022; Patil et al., 2022).

Its bioactive compounds—e.g., azadirachtin, nimbin, salannin, and flavonoids—confer antipyretic, antibacterial, antiviral, antifungal, antioxidant, and antidiabetic effects (Nagano and Batalini, 2021). Studies have also shown its action against resistant microorganisms such as *Staphylococcus aureus* and *Salmonella enterica*, in addition to its safe use in pest control and food and cosmetic formulations (Wylie and Merrell, 2022; Ammara et al., 2023).

Neem extract is widely used in commercial products due to its antimicrobial, antifungal, anti-inflammatory, and insecticidal properties (Baby et al., 2022). In the personal care sector, it is employed in shampoos and facial soaps, such as the Himalaya Neem Face Wash®. In agriculture, it serves as the basis for bioinsecticides, such as NeemAzal® and Neemix®, which control pests with low environmental toxicity. In phytotherapeutic supplements, it is marketed in capsules and liquid extracts for immune system support and its hepatoprotective action. In veterinary medicine, it is used in shampoos and antiparasitic sprays for flea and tick control (Wylie and Merrell, 2022).

Despite these diversified applications, the use of neem in the context of ethanol fermentation remained unexplored, representing a significant opportunity for innovations in bacterial contamination control. Its antimicrobial properties may be effective against contaminants or even in the stabilization of the fermentation medium, reducing unwanted byproducts and optimizing industrial yield. Thus, neem has the potential to integrate sustainable solutions in the biotechnological sector, in line with the bioeconomy and environmental efficiency trends.

Hence, this study aimed to obtain and phytochemically characterize neem bark, leaf, and seed extracts, evaluating their antimicrobial potential and the minimum inhibitory concentration against bacteria *L. fermentum* and *L. mesenteroides*. Moreover, the antimicrobial activity against the yeast *S. cerevisiae* was also evaluated. Therefore, the biocide potential of this product was determined.

Methodology

Plant material collection and botanical identification

The leaves, trunk bark, and fruits were collected from a neem tree located in an urban area of the municipality of Frutal, Minas Gerais, Brazil, in 2021. The plant material was identified as *Azadirachta indica A.* Juss by comparison with voucher specimens deposited in the BHCB Herbarium of the Department of Botany from the Institute of Biological Sciences of the Federal University of Minas Gerais (UFMG).

Plant extract obtention

The leaf, trunk bark, and fruit samples were sanitized with distilled water and a 5% hypochlorite solution. The fruits were pulped, and the seeds were removed. Next, the plant materials were dried in a forced-air oven maintained at 45°C for 48 hours (leaves and bark) or 72 hours (seeds). Later, the dry leaf and bark samples were ground in a knife mill

(Willey SL-31) using a 1.0 mm mesh sieve. The seeds were processed separately in a food mixer.

For the phytochemical evaluations, ground samples of 30 g (leaves and bark) and 20 g (seeds) were subjected to the extraction process by maceration with 300 mL and 200 mL of analytical grade ethanol, respectively, for 48 hours (Corrêa et al., 2025). The ethanolic extracts of the leaves, bark, and seeds were called FEt, CEt, and SEt, respectively.

For the antimicrobial analyses and chemical characterization by Fourier transform infrared spectroscopy (FTIR), ground portions of 50 g of each plant material were used. These portions were subjected to extraction by maceration with 500 mL of analytical grade ethanol. After the maceration process, the alcoholic extracts were filtered and concentrated using a rotary evaporator until the complete elimination of the solvent (Shaheen et al., 2022). Next, the extracts were frozen at a temperature of -80°C, lyophilized, and stored in light-protected flasks under a controlled temperature of 5°C.

Phytochemical screening

Thin-Layer Chromatography Characterization

Phytochemical prospecting was carried out using thin-layer chromatography (TLC), according to the methodology described by Wagner and Bladt (2009). Pre-coated silica gel F254 thin-layer chromatography plates on aluminum support (Merck) were used, along with a saturation chamber for ascending migration. The leaf (FEt), bark (CEt), and seed (SEt) extract samples dissolved in ethanol were applied to the 3.5 × 6 cm plates with the aid of glass capillaries. The plates were developed in a chamber equipped with a UV-A (365 nm) and UV-C (254 nm) radiation emission lamp (ALLCUV-001).

For flavonoid identification, a mixture of butanol, distilled water, and acetic acid in the ratio of 99:1:0.5 (v/v) was used as the eluent, with development carried out using aminoethyl diphenylborinate (NP) and polyethylene glycol (PEG) 4000, followed by heating.

For tannins and polyphenols, a mixture of ethyl acetate and methanol was used as the eluent in the ratio of 97:3 (v/v). The development was carried out with a mixture of the ethanolic solutions of 1% (w/v) ferric chloride and 1% (v/v) potassium ferrocyanide. Hydrolyzable and condensed tannins were analyzed using the same eluent, with 1% ferric chloride as the developer (Silva et al., 2004).

A mixture of hexane and ethyl acetate in a ratio of 80:20 (v/v) was used to analyze terpenes and steroids. The development was carried out with sulfuric anisaldehyde and with the Liebermann-Burchard reagent under UV light at 365 nm and 254 nm (Sousa, 2020).

Attenuated total reflectance/Fourier transform infrared spectroscopy characterization

The extract analysis was performed using an Agilent CARY 630 FTIR spectrometer and the attenuated total reflectance (ATR) method. The spectra were obtained in the transmission mode, in the 4000 cm^{-1} to 600 cm^{-1} range, with a 4 cm^{-1} resolution and 40 scans.

Secondary metabolite quantification

The extracts were analyzed for their contents of phenolic compounds, flavonoids, and total tannins, in addition to the antioxidant activity. The phenolics quantification followed Lazzarotto et al. (2020), with the Folin-Ciocalteu reagent and reading at 765 nm using a gallic acid standard curve (10–100 mg/L), with results in mg/g of dry extract. The total flavonoids were determined according to Araújo et al. (2020), using aluminum chloride, reading at 415 nm, and a rutin standard curve (5–30 µg/L), expressed in mg/g. The total tannins followed Mota (2023), with reading at 510 nm and an epicatechin standard curve (10–100 mg/L), expressed in mg/g. The antioxidant activity was evaluated following Silva et al. (2019), with 2,2-diphenyl-1-picrylhydrazyl (DPPH), reading at 517 nm, and a Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard curve, in the concentrations from 0.1 to 1 mM, and the results are expressed in µmol of Trolox equivalent per liter (µmolET).

Antimicrobial activity through the antibiogram test

The antimicrobial activity was evaluated using the disk diffusion test according to Balouiri et al. (2016), with pure cultures of gram-positive bacteria *L. fermentum* (CCT 0559) and *L. mesenteroides* (CCT 0605), in addition to the yeast *S. cerevisiae* (CAT-1). The lyophilized cultures were obtained from the Andre Tosello Tropical Foundation of Technological Research.

To prepare the yeast inoculum, 1 g of *S. cerevisiae* was dissolved in 10 mL of a 0.5% (w/v) glucose solution. The bacteria *L. mesenteroides* and *L. fermentum* were also cultured on Petri dishes with a Muller-Hinton medium (pH 7–7.5) using a sterile Drygalski loop.

Filter paper disks were soaked with 10 µL of each crude extract at a 100 mg/mL concentration and placed on the surface of the culture medium. Disks soaked with dimethyl sulfoxide (DMSO) were used as a negative control, while disks containing a solution of ampicillin (1 mg/mL), actidione (1 mg/mL), and commercial green propolis Propomax® (100 mg/mL) were used as positive controls.

The plates were incubated for 24 hours in biochemical oxygen demand (BOD) at 30°C for *L. mesenteroides* and *S. cerevisiae* and 35°C for *L. fermentum*. The inhibition halos were measured and the results interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2010). Microorganisms were considered resistant if the halo diameter was less than 8 mm, intermediate if between 9 and 14 mm, and sensitive if equal to or greater than 14 mm.

Minimum Inhibitory Concentration

Initially, the antimicrobials were prepared, with 500 mg of each dry extract dissolved in 1 mL of DMSO, and the concentrations were adjusted to 10, 30, 50, and 100 mg/mL.

For the test, 1 mL of the liquid MRS culture medium (yeast extract—5 g/L; peptone—10 g/L; meat extract—5 g/L; glucose—20 g/L; dipotassium hydrogen phosphate—2 g/L; tween 80 – 1 g/L; sodium acetate—5 g/L; magnesium sulphate—0.05 g/L) was added to test tubes, which were later sterilized in an autoclave at 121°C for 15 min. Next, 50 µL of each microorganism culture (10^8 Colony-Forming Units [CFU]/mL) was added. The samples were placed in a shaker at 140 rpm at 30°C (*L. mesenteroides*) or 35°C (*L. fermentum*) for 2 hours. Next, the extracts were incorporated into the test concentrations, and the tubes were returned to the shaker.

To evaluate the bactericidal effect of the antimicrobials, we quantified the microorganism population soon after the extracts were added and after 2 and 8 hours of agitation. For this, aliquots were removed from the tubes and added to Petri dishes containing the solid MRS medium (liquid MRS plus the addition of agar—15 g/L). The dishes were incubated in BOD at 30°C (*L. mesenteroides*) or 35°C (*L. fermentum*) for 24 hours.

Statistical analysis

The quantifications and antimicrobial evaluations were performed in triplicate. The collected data were submitted to an analysis of variance (ANOVA) using the F test, and the means were compared through the Tukey test (5%). The statistical analyses were processed using AgroEstat and Minitab software — version 17.1.0 (2024).

Results and Discussion

Phytochemical characterization

The phytochemical characterization of the ethanolic extracts of leaves (FEt), bark (CEt), and seeds (SEt) of *A. indica* indicated the presence of the different classes of phytochemicals, such as phenolics, flavonoids, tannins, and terpenes in all obtained products. The phytochemical screening is illustrated in Figures 1 and 2 for TLC and Figure 3 for ATR-FTIR, and the data are synthesized and presented in Table 1.

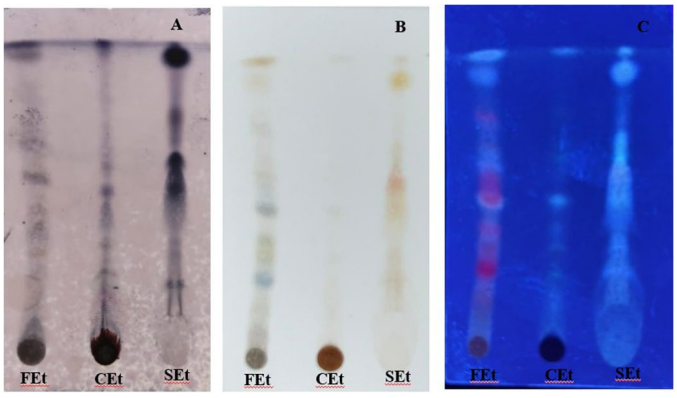

Figure 1 – Chromatographic profile of the leaf, bark, and seed extracts. Mobile phase: hexane/ethyl acetate (80:20). Developers: (A) sulfuric anisaldehyde and heating, (B) Liebermann-Burchard, (C) Liebermann-Burchard under 365 nm UV light.
FEt: leaf; Cet: bark; Set: seed.

The phytochemical analysis of the FEt, CEt, and SEt extracts of *A. indica* revealed the presence of various classes of bioactive compounds, including phenolics, flavonoids, tannins, and terpenes in all analyzed extracts. The screening results are represented in Figures 1 and 2 (TLC) and Figure 3 (ATR-FTIR), with the data synthesized in Table 1.

The TLC analysis of the neem FEt, CEt, and SEt extracts revealed the presence of different classes of secondary metabolites. Purple coloration signals observed on the chromatographic plates indicated the presence of terpenes in the three extracts, with this identification being reinforced by bluish fluorescence under UV light (Figures 1A and 1C), as described by Alexandre and Rocha (2017). The presence of steroids was confirmed by blue-greenish colorations and fluorescence varying from orange to red (Sousa, 2020), in addition to specific reddish spots on the leaves detected by the Liebermann-Burchard test (Figure 1B), suggesting a more significant concentration of this group in this matrix.

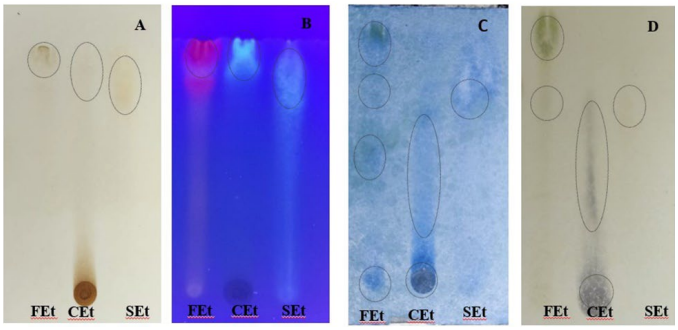

Figure 2 – Chromatographic profile of the leaf, bark, and seed extracts. Mobile phase for A and B: butanol/water/acetic acid (99:1:0.5, v/v). Developers: A. NP+PEG with heating—visible; B. NP+PEG—365 nm UV; Mobile phase for C and D: ethyl acetate/methanol (97:3). Developers: C. 1% (w/v) ferric chloride and 1% (w/v) potassium ferrocyanide, D. 1% (w/v) ferric chloride.
FEt: leaf; Cet: bark; Set: seed.

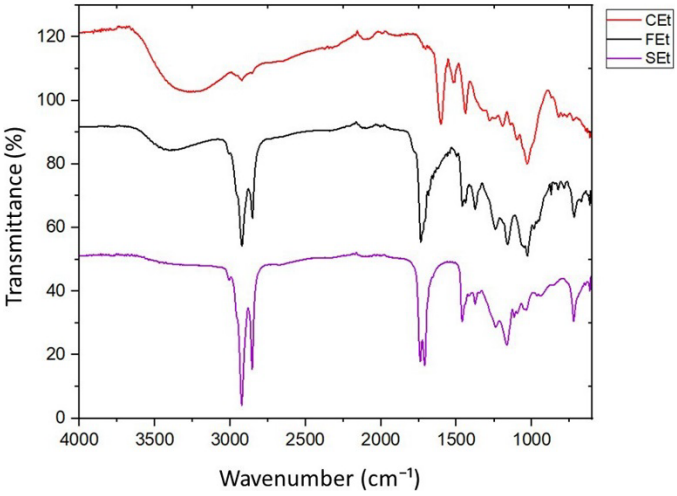

Figure 3 – Attenuated total reflectance/ Fourier transform infrared spectra of the leaf, bark, and seed extracts.
FEt: leaf; Cet: bark; Set: seed.

Flavonoids and phenolic compounds were identified through yellowish bands and orange and yellow-greenish fluorescence under UV light at 365 nm, as well as bluish fluorescence, respectively (Von Zuben and De Souza, 2022). These observations were obtained after development with NP+PEG reagent under heating (Figure 2A) and UV light exposure (Figure 2B). The presence of tannins was evidenced by bluish spots and the formation of black-bluish and green colorations after a reaction with 1% ferric chloride, indicating hydrolyzable tannins in the bark and condensed tannins in the leaf extract (Figure 2D) (Silva et al., 2004).

The spectroscopic analyses by ATR-FTIR of the FEt and CEt extracts (Figure 3) revealed bands at 3,395 cm^{-1} and 3,283 cm^{-1}, attributed to hydroxyls (-OH) associated through hydrogen bonds, with additional bands at 1,233 cm^{-1} and 1,033 cm^{-1} (FEt) and 1,234 cm^{-1} and 1,033 cm^{-1} (CEt), indicating the presence of polyhydroxylated structures. In the spectra, a band was observed at 1,455 cm^{-1}, characteristic of the C=C stretching in aromatic rings. In the SEt extract (Figure 3), a weak band at 3,383 cm^{-1} indicated a less significant concentration of hydroxylated compounds, corroborating the total phenolics, flavonoids, and tannins contents previously identified.

Additionally, the CET spectrum presented a strong band at 1,580 cm^{-1}, typical of C=C bond vibrations in aromatic rings or conjugated carbonyl (C=O) groups, associated with flavonoids and tannins (Silverstein et al., 2019). These findings corroborate the TLC results and the quantification of tannins and flavonoids, confirming the presence of such classes in the extract.

The spectra for the FEt and SEt extracts also showed weak signals at 3,030 cm^{-1}, attributed to the =C-H stretching of aromatic compounds, confirmed by the bands at 1,455 and 1,464 cm^{-1}, related to the C=C stretching of the aromatic ring. Signals at 2,933, 2,915, 2,853, 2,843, 1,455, and 1,454 indicated functional C-H groups of the hydrocarbon skeleton, suggesting the presence of fatty acids (Pavia et al., 2015). In the CEt extract, weak signals at 2,924 and 2,842 cm^{-1} pointed to a low concentration of aliphatic compounds. The absorption around 1,740 cm^{-1}, associated with the carbonyl (C=O), and the bands at 1,375 cm^{-1}, attributed to C-O bonds, confirmed the presence of esters in the FEt and CEt extracts, possibly derived from saturated and unsaturated fatty acids, as well as triterpenic structures.

The presence of phenolic compounds, flavonoids, hydrolyzable tannins, and terpenes was found for all studied neem matrices. This result is interesting because not all studies in the literature achieved this identification. For example, Galeane et al. (2017) did not find terpenes or tannins in hydroethanolic extracts of leaves of the plant. Bappah et al. (2022) determined flavonoids and alkaloids in leaf and bark extracts. In turn, studies by Fernando and Dissanayake (2020), Nagano and Batalini (2021), and Adaramola et al. (2023), using phytochemical tests by wet method, confirmed the presence of tannins, flavonoids, and terpenes in all matrices of the plant.

These results point to the vast diversity of bioactive compounds in *A. indica*, with variations depending on environmental factors inherent to the raw material, as well as the physicochemical techniques employed for extract preparation.

Table 2 presents the quantification data for total phenolics, total tannins, flavonoids, and antioxidant activity in the FEt, CEt, and SEt ethanolic extracts. Significant variations were observed in the concentrations of such metabolites and in the antioxidant capacity between the different matrices analyzed. Among the extracts, the CEt presented the most significant phenolic compound contents, followed by the FEt, while the SEt showed the lowest values. These results align with the idea that the differentiated distribution of phenolic compounds in the analyzed plant matrices directly influences their properties and antioxidant activities (Ortolan et al., 2019).

Table 1 – Phytochemical screening of the ethanolic neem extracts by thin-layer chromatography in silica gel.

Extracts	Secondary metabolites					
	Total	Flavonoids	Hydrolyzable tannins	Condensed tannins	Terpenes	Steroids
FEt	+	+	+	+	+	+
CEt	+	+	+	-	+	-
SEt	+	+	+	-	+	-

FEt: leaf; Cet: bark; Set: seed; + presence; - absence.

Table 2 – Results of the quantification of total phenolic compounds, total tannins, flavonoids, and antioxidant activity of the neem leaf, bark, and seed extracts.

Samples	Total Phenolics (mg/g)	Total Tannins (mg/g)	Total Flavonoids (mg/g)	Antioxidant (μMET)
FEt	0.88 ± 0.10^{B}	0.19 ± 0.02^{B}	0.16 ± 0.00^{A}	0.81 ± 0.00^{B}
CEt	4.10 ± 0.18^{A}	1.59 ± 0.30^{A}	0.01 ± 0.00^{C}	5.28 ± 0.17^{A}
SEt	0.31 ± 0.02^{C}	0.06 ± 0.00^{B}	0.03 ± 0.00^{B}	0.88 ± 0.00^{B}

FEt: leaf; Cet: bark; Set: seed; Different letters in the same column indicate significantly different means according to the application of the Tukey multiple comparison test at $p < 0.05$.

In the total tannins analysis, the CEt extract showed a higher concentration than the other ones. On the other hand, in the flavonoid evaluation, the FEt extract registered the highest concentration, followed by the SEt and, finally, the CEt. According to the literature, tannins identified in bark include types such as gallocatechol and tannic acid (Haroun and Ahmed, 2022). In turn, leaves present a diversity of flavonoids, including catechins, flavones, flavonoids, and xanthones, as well as tannins such as leucoanthocyanidin. The seeds contain compounds such as quercetin, rutin, and tannic acid (Nagano and Batalini, 2021; Baby et al., 2022).

Regarding the antioxidant activity, there was no significant difference between the FEt and SEt extracts, which significantly differed from the CEt. The antioxidant activity of the CEt extract may be related to the superior content of phenolic compounds compared to the other matrices. However, the FEt and SEt extracts presented significant differences in the contents of total phenolics, yet did not show significant differences in antioxidant activity. Hence, the presence of other compounds, such as triterpenes and fatty acids in the seeds, may have contributed to their antioxidant activity (Atta et al., 2015).

The results for phenolic compounds align with the data reported by Abdulkadir et al. (2017), who found higher concentrations in the bark than in other parts of the plant. For flavonoids, the leaves presented the highest concentration, surpassing seeds and bark, as also found by Fernando and Dissanayake (2020).

Airaodion et al. (2019) observed higher concentrations of tannins in the leaves of *A. indica* relative to flavonoids, whereas Adamarola et al. (2023) reported the opposite for bark. These variations suggest that environmental factors, e.g., temperature, water availability, UV radiation, and nutrients, influence the concentration of secondary metabolites (Chen et al., 2023). Moreover, solvents and extraction methods distinctly impact plant organs (Lezoul et al., 2020).

Antimicrobial evaluation

The results of the preliminary antibiogram test for evaluating the antimicrobial potential of the extracts are presented in Figure 4, while the data regarding the determination of the MIC are described in Figures 5 and 6. The inhibition halos (Figure 4) evidenced that the FEt and CEt extracts presented antimicrobial activity superior to propolis against *L. mesenteroides*, yet inferior to ampicillin. In the case of *L. fermentum*, the FEt extract presented activity comparable to propolis, while the CEt extract did not exhibit significant activity against this bacterium. Based on the CLSI scale (CLSI, 2010), *L. mesenteroides* was classified as sensitive to the FEt and CEt extracts, while the FEt extract showed intermediate antimicrobial activity against *L. fermentum*. It is important to highlight that the SEt extract did not present an inhibition halo for the studied bacteria. Additionally, inhibition halos were not observed for the FEt, CEt, and SEt compounds for *S. cerevisiae*, demonstrating that the extracts do not affect yeast. Hence, only the FEt and CEt extracts were used for the MIC evaluation.

The MIC tests allowed validating the antibiogram results, indicating significant interactions between the extract dose and the exposure time.

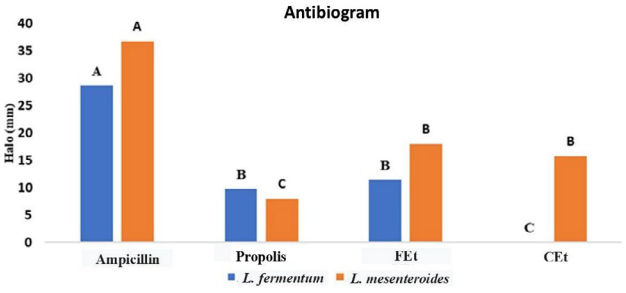

Figure 4 – Antibiogram test performed with ampicillin, propolis, leaves, and bark. Letters in the columns differ according to the Tukey test (5%).
FEt: leaf; CEt: bark.

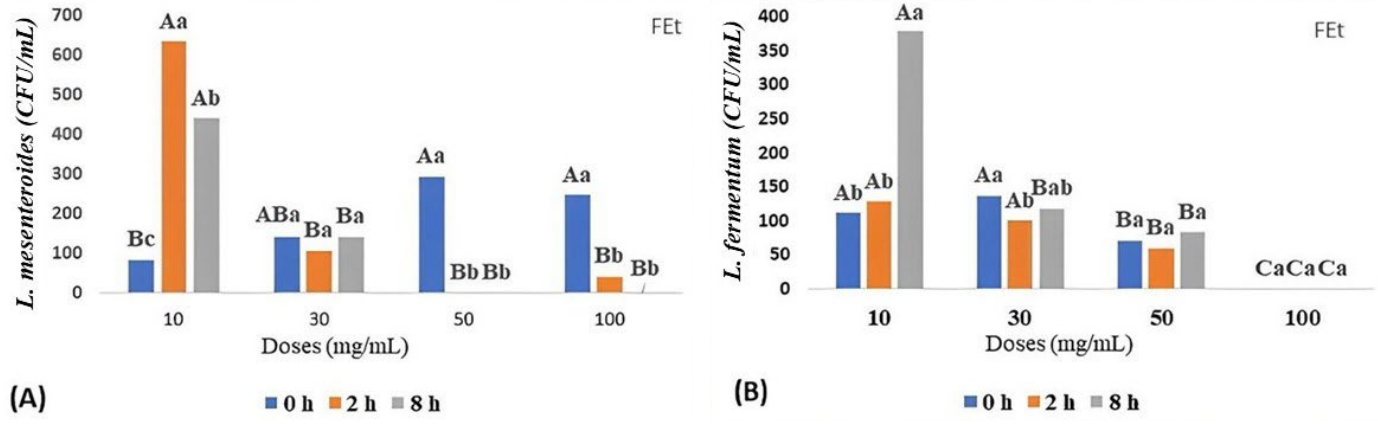

Figure 5 – Interaction between doses and exposure times for the minimal inhibitory control using the leaf extract and microorganisms *L. mesenteroides* (A) and *L. fermentum* (B). Uppercase letters compare doses. Lowercase letters compare times.
FEt: leaf.

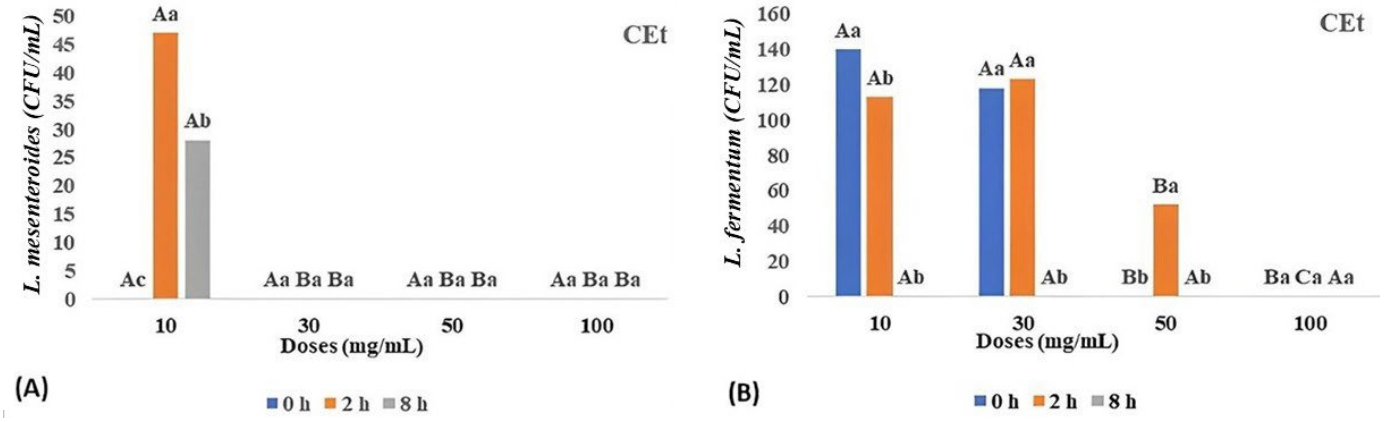

Figure 6 – Interaction between doses and exposure times for the minimal inhibitory control using the bark extract and microorganisms *L. mesenteroides* (A) and *L. fermentum* (B). Uppercase letters compare doses. Lowercase letters compare times.
CEt: bark.

For *L. mesenteroides* (Figure 5A), the FEt extract showed a bacteriostatic effect at 30 mg/mL and a bactericidal effect from 50 mg/mL, with a significant population reduction from 2 hours. For *L. fermentum* (Figure 5B), doses of 50 and 100 mg/mL resulted in faster bactericidal effects, while smaller doses (30 mg/mL) had bacteriostatic action.

The CEt extract showed high efficacy against *L. mesenteroides*, with doses of 30 mg/mL or higher resulting in a bactericidal effect for all evaluated exposure times (Figure 6A). For *L. fermentum*, only doses of 50 mg/mL or higher showed consistent bactericidal effects (Figure 6B). It is important to note that the MIC results differed from the antibiogram in the case of *L. fermentum*, evidencing the importance of time and concentration in antimicrobial performance.

The results highlight the relationship between the chemical composition of the *A. indica* extracts and their antimicrobial efficacy, with evidence that the ethanolic extracts of FEt and CEt presented significant activity against *L. mesenteroides*, surpassing the efficacy of propolis. This activity may be attributed to the concentration of phenolic compounds and tannins present in the extracts, which play a fundamental role in altering the functionality of the bacterial cell wall (Prasad et al., 2023). The phenolic compounds interact with the cell wall, altering its permeability and causing failures in the chemiosmotic control and DNA synthesis, while the tannins form complexes with proteins, inhibit essential enzymes, and deprive the microorganisms of nutrients indispensable for their growth (Prasad et al., 2023).

The absence of an outer membrane in *L. mesenteroides*, characteristic of gram-positive bacteria, facilitates the interaction of the bioactive compounds with the cell wall, increasing its sensitivity. Flavonoids, present in the FEt, potentiate this action through the inhibition of the synthesis of nucleic acids and the induction of structural alterations in the cell membrane (Francolini and Piozzi, 2020). In contrast, *L. fermentum* presented greater resistance, with only the FEt extract demonstrating antimicrobial activity comparable to propolis, while the CEt and SEt extracts did not show significant results. This sensibility may be explained by structural differences in the cell wall of *L. fermentum* that modulate its resistance to phenolic compounds and antioxidants (Tasanarong et al., 2021).

The chemical composition of the SEt extract, which is predominantly composed of terpenes and fatty acids, helps explain its low antimicrobial efficacy against the evaluated bacteria. While these compounds show greater affinity for gram-negative bacteria due to the interaction with the outer membrane, they have reduced efficacy against gram-positive bacteria (Mahizan et al., 2019). The selectivity of the extracts also proved evident in their inability to inhibit *S. cerevisiae*, suggesting a potential use in fermentation processes in which yeast preservation is essential. This specificity is attributed to the selective interactions of the bioactive compounds with bacterial structures, without affecting the integrity of yeast cells.

The neem extracts demonstrated performance superior to propolis, reinforcing their potential as a source of natural antimicrobial compounds, especially in controlling contamination in fermentation processes. Studies such as those by Alqahtani (2020) and Adaramola et al. (2023) confirm this efficacy against *Lactobacillus spp.* and *Leuconostoc spp.*, which is attributed to tannins, flavonoids, and phenolic compounds, as also reported by Abdulkadir et al. (2017) and Prasad et al. (2023).

Conclusion

This study evidenced the diversity of bioactive compounds in the ethanolic extracts of *A. indica* and their influence on the antioxidant and antimicrobial activities. The CEt extract presents a higher content of phenolic compounds and tannins, with superior antioxidant activity and antimicrobial efficacy against *L. mesenteroides* at the 30 mg/mL dose. The FEt extract is rich in flavonoids and phenolic

compounds, with antimicrobial action against *L. fermentum* and *L. mesenteroides* from 50 mg/mL. The SEt extract is rich in fatty acids and triterpenoids but did not show antimicrobial activity against these bacteria. Moreover, none of the extracts interfered with the growth of yeast *S. cerevisiae*, demonstrating selectivity in the bioactive action. *A. indica* presents potential as a natural biocide, with extracts effective against gram-positive bacteria, indicating promising applications in biotechnology and industry.

Authors' Contributions

Rocha, L.P.: investigation, data curation; formal analysis, writing – original draft. **Oliveira,** M.M.: investigation, formal analysis. **Silva**, E.A.: investigation, formal analysis. **Costa**, G.G.C.: project administration, resources, validation, methodology, writing – review. **Correa**, T.A.: methodology, formal analysis; supervision, validation; writing – original draft, writing – review & editing.

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