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AVALIAÇÃO DA INFLUÊNCIA DA GRANULOMETRIA NA EXTRAÇÃO DE COMPOSTOS FENÓLICOS E NA ATIVIDADE ANTIMICROBIANA EM CASCAS DE ESPÉCIES FLORESTAIS DE TRÊS BIOMAS BRASILEIROS

GRAIN SIZE INFLUENCE ON THE EXTRACTION OF PHENOLIC COMPOUNDS AND ANTIMICROBIAL ACTIVITY IN TREE BARK FROM THREE BRAZILIAN BIOMES

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Resumo

Este estudo investigou a influência da granulometria e do solvente na eficiência de extração de compostos fenólicos e na atividade antimicrobiana de extratos de cascas de *Anacardium occidentale*, *Stryphnodendron adstringens* e *Myrcia eximia*, oriundas dos biomas Caatinga, Cerrado e Amazônia, respectivamente. As extrações foram realizadas utilizando água deionizada e metanol a 70% em quatro faixas de granulometria (28, 35, 60 e 80 mesh). Foram determinados os teores de taninos condensados, fenóis totais e flavonoides, além da atividade antimicrobiana frente a cepas de bactérias Gram-positivas e Gram-negativas. Os extratos obtidos com metanol apresentaram maiores rendimentos em compostos fenólicos em comparação à água, com destaque para o Cumatê vermelho na extração de flavonoides. A atividade antimicrobiana foi mais expressiva frente às bactérias Gram-positivas (*Staphylococcus aureus* e *Listeria monocytogenes*), especialmente para os extratos de Myrcia eximia. Estes resultados reforçam o potencial de aproveitamento de resíduos florestais para a obtenção de compostos bioativos com propriedades antimicrobianas.

Palavras-chave: Compostos Fenólicos; Taninos; Biomas Brasileiros

Abstract

This study investigated the influence of particle size and solvent on the extraction efficiency of phenolic compounds and antimicrobial activity of bark extracts from Anacardium occidentale, *Stryphnodendron adstringens*, and *Myrcia eximia*, native to the Caatinga, Cerrado, and Amazon biomes, respectively. Extractions were performed using deionized water and 70% methanol in four particle size ranges (28, 35, 60, and 80 mesh). The levels of condensed tannins, total phenols, and flavonoids were measured, along with the antimicrobial activity against Gram-positive and Gram-negative bacterial strains. Extracts obtained with methanol showed higher yields in phenolic compounds compared to water, with *Myrcia eximia* showing the highest flavonoid content. Antimicrobial activity was more evident against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), especially for *Myrcia eximia* extracts. These findings highlight the potential use of forest residues to obtain bioactive compounds with antimicrobial properties.

Keywords: Phenolic Compounds; Tannins; Brazilian Biomes

1. Introduction

The use of natural products with bioactive properties has gained prominence in recent years due to their potential to replace synthetic compounds in the food, cosmetic, and pharmaceutical industries. Among these products, phenolic compounds found in tree bark stand out for their wide range of functions, including antioxidant, antimicrobial, and anti-inflammatory activities (BRUNETON, 1999; SHIRMOHAMMADLI; EFHAMISISI; PIZZI, 2018). Traditionally discarded as waste during wood processing, forest bark has been increasingly valued as a promising source of bioactive compounds, aligning with biorefinery approaches and the full utilization of biomass (MIRANDA et al., 2012).

The chemical composition of bark can vary significantly between species and biomes, being influenced by genetic, edaphoclimatic, and environmental factors (FENGEL; WEGENER, 1989). Additionally, the particle size of the biomass and the type of solvent used during the extraction process are crucial factors that directly affect the yield and quality of the obtained extracts. Previous studies suggest that finer biomass fractions tend to concentrate higher levels of extractives (MIRANDA et al., 2017), and that solvents such as methanol exhibit greater efficiency in solubilizing phenolic compounds (DO et al., 2014; DAS et al., 2020).

Despite the growing interest in bark-derived bioactive compounds, studies evaluating the combined effects of particle size and solvent polarity on phenolic extraction from native Brazilian species across distinct biomes remain limited (LORENÇO et al., 2025; ARAUJO et al., 2025). Most available studies focus on single species or isolated extraction parameters, with few addressing the interaction between granulometry and solvent type in lignocellulosic bark matrices (MIRANDA et al., 2017; DAS et al., 2020). In addition, the relationship between phenolic composition and antimicrobial activity is often discussed qualitatively, lacking robust statistical or integrative validation (HUANG et al., 2024; UCELLA-FILHO et al., 2024). It is expected that reducing particle size increases surface area and solvent accessibility, thereby enhancing phenolic extraction efficiency. Additionally, higher phenolic yields, particularly of condensed tannins, may be associated with increased antimicrobial activity against Gram-positive bacteria (LORENÇO et al., 2025; UCELLA-FILHO et al., 2024; DA SILVA ARAUJO et al., 2021).

To test this hypothesis, the present study aimed to evaluate the effect of particle size (28, 35, 60, and 80 mesh) and solvent type (deionized water and 70% methanol) on the extraction yield of condensed tannins, total phenolics, and flavonoids from the bark of three native Brazilian tree species, *Anacardium occidentale*, *Stryphnodendron adstringens*, and *Myrcia eximia*, representing the Caatinga, Cerrado, and Amazon biomes, respectively; assess the antimicrobial activity of the obtained extracts against Gram-positive and Gram-negative bacterial strains; and identify qualitative patterns of association between phytochemical composition and antimicrobial response, aiming to support the valorization of forest bark residues as sources of bioactive compounds for applications in the natural products industry.

2. Theoretical Framework

2.1 Tannins

Tree bark represents a significant biomass resource, yet it is often undervalued and commonly treated as solid waste in wood processing industries. In Europe alone, approximately 12 million m³ of bark are burned annually in industrial settings. However, this byproduct is rich in valuable compounds with potential applications ranging from pharmaceutical and bioactive ingredients to green polymers and bio-based materials (MIRANDA *et al*., 2012).

Tannins, which are phenolic compounds classified as secondary metabolites, are found in both the bark and leaves of woody species. They play essential roles in plant defense mechanisms against ultraviolet radiation, herbivory, and pathogenic organisms. Their concentration is influenced by both genetic factors and environmental conditions (CARVALHO *et al*., 2014).

Biomass residues are generally heterogeneous materials that require size reduction before processing. Conventional grinding methods often fail to produce uniform fractions, and variations in particle size can affect the chemical profile of the extract. According to Miranda *et al*. (2017), studies with eucalyptus bark showed that finer fractions concentrated a greater proportion of extractives, while coarser fractions contained more cellulose and hemicellulose (BRIDGEMAN *et al*., 2010). Thus, biomass fractionation can be used as a strategy to enrich specific components by leveraging chemical and structural differences (MIRANDA *et al*., 2012).

2.2 Industrial Applications of Tannins

Tannins are widely utilized in various industries due to their complex chemical composition, which enables their application in the development of alternatives to less sustainable, non-renewable components. Their primary industrial uses include leather tanning, beverage production, pharmaceutical formulations, water treatment, adhesives, and natural wood preservatives (DA SILVA ARAUJO *et al*., 2021).

In the wood industry, tannins are particularly promising as substitutes for phenol in phenol-formaldehyde resins. Their structural similarity to synthetic phenol makes them viable for reducing formaldehyde emissions, one of the major environmental concerns associated with fossil-derived adhesives (DA SILVA ARAUJO *et al*., 2021).

2.3 Brazilian Biomes

2.3.1 Cerrado

Covering about 25% of Brazil’s territory, the Cerrado is one of the five major Brazilian biomes. It is located across the northern, northeastern, and central-western regions, with a small extension in Paraná state. As one of the most biodiverse savannas in the world, it harbors over 6,000 tree species and 800 bird species, with a high rate of endemism. More than 40% of woody plant species and 50% of bee species found there are endemic. The Cerrado is recognized as one of the world’s most important biodiversity hotspots, although it is also one of the most threatened ecosystems (BRASIL MMA, 2002).

The biome’s extent and distribution are shaped by a tropical climate with annual rainfall between 750 and 2,000 mm. It experiences two well-defined seasons: a dry season from May to October and a rainy season from October to May (BRASIL MMA, 2002).

2.3.2 Caatinga

The Caatinga is the only Brazilian biome that exists entirely within national borders, located predominantly in the northeastern region. It is home to unique biological diversity not found elsewhere in the world. Ceará holds the largest area of Caatinga, which borders the Atlantic Forest, Amazon, and Cerrado biomes. Its semi-arid climate, with annual rainfall below 750 mm and average temperatures around 26°C, makes it one of the most resilient ecosystems on the planet (BRASIL ICMBio, 2020).

Caatinga vegetation is highly adapted to drought and poor soils. During the wet season, its landscapes resemble dense forests, while the dry season reveals shrubby, twisted forms and drought-resistant plants such as cacti and bromeliads (FUNDAJ, 2019). The complex root systems and succulent stems of many species allow efficient water storage, while leaf shedding during dry periods reduces energy loss and photosynthetic activity (AC, 2021).

2.3.3 Amazon

The Amazon biome spans over 40% of Brazil’s territory and consists primarily of tropical rainforest. It encompasses the states of Acre, Amapá, Amazonas, Pará, and Roraima, with partial coverage in Maranhão, Mato Grosso, Rondônia, and Tocantins. The biome is composed of diverse ecosystems, including dense upland forests, seasonal forests, floodplain forests, wetlands, savannas, mountain refuges, and pioneer formations (IFB, 2020).

2.4 Species of Interest

2.4.1 *Stryphnodendron adstringens*

Stryphnodendron adstringens (Mart.) Coville is a native leguminous species found predominantly in the Brazilian savanna (Cerrado). The bark of this species is traditionally used in folk medicine for its astringent, anti-inflammatory, and wound-healing properties. Pharmacological studies have confirmed its anti-ulcerogenic, anti-inflammatory, and antibacterial activities (SOUZA et al., 2025). Its bark contains high levels of condensed tannins, especially flavan-3-ols such as prodelphinidins and prorobinetinidins, with concentrations exceeding 20% in dry matter (SANTOS *et al*., 2002).

Environmental factors such as soil composition, moisture availability, temperature, and stress conditions significantly influence the concentration of phenolic compounds in plants. These variables can affect the development of secondary metabolites, which in turn impacts the pharmacological and industrial quality of the plant (SAMPAIO *et al*., 2011).

2.4.2 *Myrcia eximia*

Myrcia eximia DC is a species native to the Amazon biome, commonly found in secondary forests. Compared to species from the Cerrado and Caatinga, research on *M. eximia* is still limited. Nonetheless, its bark has shown promising potential as a rich source of polyphenolic compounds with antioxidant capacity. Recent studies suggest its applicability in the food and pharmaceutical industries, as well as in the production of condensed tannin-based products (ARAUJO *et al*., 2020).

2.4.3 *Anacardium occidentale*

Anacardium occidentale L., commonly known as the cashew tree, thrives in tropical climates with high temperatures and water availability. In Brazil's northeastern region, the species plays a significant economic role due to its fruit (cashew nut) and peduncle, which is rich in vitamin C and widely used in juice production (LEITE et al., 2016).

Beyond its nutritional value, *A. occidentale* possesses antioxidant, anti-inflammatory, healing, and antimicrobial properties. Traditional medicine in the region often employs various parts of the plant to treat ailments such as sore throat, bronchitis, arthritis, jaundice, and asthma (LEITE et al., 2016).

2.5 Microorganisms Tested

Among the tested microorganisms, *Listeria monocytogenes* is a pathogenic bacterium of concern in food safety. It causes listeriosis, a disease with symptoms

Received: 13/01/2026 - **Accepted:** 19/03/2026
Vol: 13.03
DOI: 10.66104/spkk9x50
Pages: 1-26

ranging from gastroenteritis to more severe conditions such as meningitis and septicemia, particularly affecting vulnerable groups (EMBRAPA, 2009).

Staphylococcus aureus is a Gram-positive bacterium commonly found in the human microbiota, responsible for various infections ranging from skin conditions to pneumonia and endocarditis. Despite being among the first bacteria targeted by antibiotics, its resistance and adaptability make it one of the leading agents in nosocomial and community infections (TOUAITIA et al., 2025).

Klebsiella oxytoca, a Gram-negative rod-shaped bacterium, has a protective polysaccharide capsule that aids in immune evasion. It is often associated with hospital-acquired infections, especially in immunocompromised patients, and presents significant resistance to antibiotic treatment (SINGH; CARIAPPA; KAUR, 2016).

Escherichia coli, although part of the normal intestinal flora, includes several diarrheagenic strains capable of causing gastrointestinal, urinary, and systemic infections. These strains are a leading cause of pediatric diarrhea worldwide, and certain pathotypes are associated with hemorrhagic colitis and hemolytic-uremic syndrome (NATARO, 1998).

Tannins exert antimicrobial effects through their ability to bind proteins via hydrogen bonding and hydrophobic interactions, promoting protein precipitation. This mechanism destabilizes bacterial membranes and inhibits growth (PIZZI, 2019; HUANG et al., 2024). Additionally, their use in medicine is linked to astringent, antioxidant, antiviral, antitumoral, antidiarrheal, and antiseptic properties (BRUNETON, 2008; FENTANES MOURA DE MELO et al., 2023).

3. Methodology

3.1 Bark Sampling and Preparation

Samples were collected from six individuals of *Anacardium occidentale* L., *Stryphnodendron adstringens* (Mart.) Coville, and *Myrcia eximia* DC, tree species commonly found in the Caatinga, Cerrado, and Amazon Forest biomes, respectively, with an average diameter at breast height (DBH) of 13.8, 10.2, and 15.9 cm,

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Vol: 13.03
DOI: 10.66104/spkk9x50
Pages: 1-26

respectively. The collected bark samples were then dried in solar ovens until no moisture was observed. Subsequently, the bark was ground using a hammer mill to obtain material with reduced particle size. After grinding, the bark was classified by sieving a portion of the material through 28, 35, 60, and 80 mesh screens (Araujo *et al.*, 2020).

3.2 Experimental Design

The experimental design was a completely randomized design (CRD) in a factorial arrangement (3 species × 2 solvents × 4 particle sizes), totaling 144 experimental units. The evaluated response variables were condensed tannins, total phenolics, and total flavonoids. Six biological replicates per species (n = 6 individuals) were included, along with analytical duplicates (n = 2) for each treatment combination. The use of six individuals per species aimed to account for intraspecific chemical variability, since secondary metabolite concentrations in bark are strongly influenced by genetic and edaphoclimatic factors (SAMPAIO et al., 2006; ARAUJO et al., 2020; MOTA et al., 2021; SOUSA et al., 2020). This sampling approach is consistent with previous studies on Brazilian forest species, which emphasize the importance of representative sampling to capture natural variation in phenolic and extractive composition (LORENÇO et al., 2025; ARAUJO et al., 2025).

3.3 Extraction of Phenolic Compounds

For each sample, 1 g of dried bark was extracted with 30 mL of solvent (deionized water or 70% methanol) in a water bath at 70 °C for 3 hours. After filtration, the extracts were stored under refrigeration (4 ºC) until analysis (Araujo *et al.*, 2019).

3.4 Chemical Determination

3.4.1 Total phenolics

The total phenolics were determined by the Folin–Ciocalteu method, with absorbance measured at 760 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry bark (mg GAE/g) (SINGLETON; ROSSI, 1965).

3.4.2 Total flavonoids

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Vol: 13.03
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Pages: 1-26

The total flavonoids were quantified by the aluminum chloride colorimetric method, with absorbance read at 510 nm. Results were expressed as milligrams of catechin equivalent per gram of dry bark (mg CE/g) (ZHISHEN; MENGCHENG; JIANMING, 1999).

3.4.3 Condensed tannins

The total of condensed tannins were quantified by the vanillin–sulfuric acid method, with absorbance measured at 500 nm and expressed as milligrams of catechin equivalent per gram of dry bark (mg CE/g) (SUN; SILVA; SPRANGER, 1998).

3.5 Bacterial Sensitivity Test

3.5.1 Microorganisms and Inoculum Standardization

For the sensitivity test to the bark extracts, the following microorganisms were used: Gram-positive bacteria (*Staphylococcus aureus* ATCC 23925 and *Listeria monocytogenes* ATCC 19117) and Gram-negative bacteria (*Klebsiella oxytoca* and *Escherichia coli* K12). The strains were obtained from the culture collection of the Food Microbiology Laboratory at the Department of Food Science, Federal University of Lavras. Stock cultures were maintained in a cryopreservation medium (glycerol – 15 mL; bacteriological peptone – 0.5 g; yeast extract – 0.3 g; NaCl – 0.5 g; distilled water – 100 mL) at –20 °C.

The target microorganisms were reactivated in BHI (Brain Heart Infusion) broth and incubated at 37 ± 2 °C for 24 hours. After incubation, the cultures were centrifuged at 10,000 g for 5 minutes at 4 °C, resuspended in 0.85% (w/v) saline solution, and standardized to 0.5 on the McFarland scale, equivalent to 10^8 CFU/mL.

3.5.2 Disk Diffusion Method

The bacterial sensitivity test to the different bark extracts was performed using the agar disk diffusion method (NCCLS, 2003). Briefly, the standardized bacterial suspensions were inoculated onto Tryptic Soy Agar (TSA) plates by surface spreading. Sterile filter paper discs (6 mm) were aseptically placed on the agar surface after inoculation and impregnated with 10 µL of the different bark extracts.

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Pages: 1-26

The plates were incubated at 37 ± 2 °C for 24 hours. Chloramphenicol at 0.5% (w/v) was used as the positive control. The inhibition zone was measured in millimeters (mm), and all analyses were performed in triplicate. It should be noted that the disk diffusion method presents inherent limitations for complex plant extracts, particularly those rich in high-molecular-weight condensed tannins, whose limited diffusibility in agar may underestimate their true antimicrobial potential (HUANG et al., 2024; UCELLA-FILHO et al., 2024). The results reported here are therefore considered preliminary screening data (LORENÇO et al., 2025). Determination of the Minimum Inhibitory Concentration (MIC) by broth microdilution, the method recommended by Clinical & Laboratory Standards Institute (CLSI) for plant extracts, is proposed as a complementary approach in future studies (UCELLA-FILHO et al., 2024).

3.6 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), considering particle size as the main factor (four levels: 28, 35, 60, and 80 mesh), applied separately for each species–solvent combination. This approach was adopted to focus on extraction behavior within each species, given the distinct phytochemical profiles of Anacardium occidentale, Stryphnodendron adstringens, and Myrcia eximia, which may limit direct cross-species comparisons. When significant effects were detected ($p \leq 0.05$), means were compared using Tukey's test at a 5% significance level. Among the evaluated variables, a significant effect of particle size was observed only for flavonoid content in Myrcia eximia methanolic extracts ($F = 17.16$; $p = 0.0095$), while the remaining variables showed no significant differences ($p > 0.05$), and results should be interpreted with caution, representing comparative trends rather than strict inferential outcomes. All analyses were performed using SISVAR software (Version 5.8).

4. Results and Discussions

4.1 Condensed Tannins

The results indicated that methanol was more effective than water in extracting condensed tannins for all evaluated species (Figure 1). In the case of *A. occidentale*

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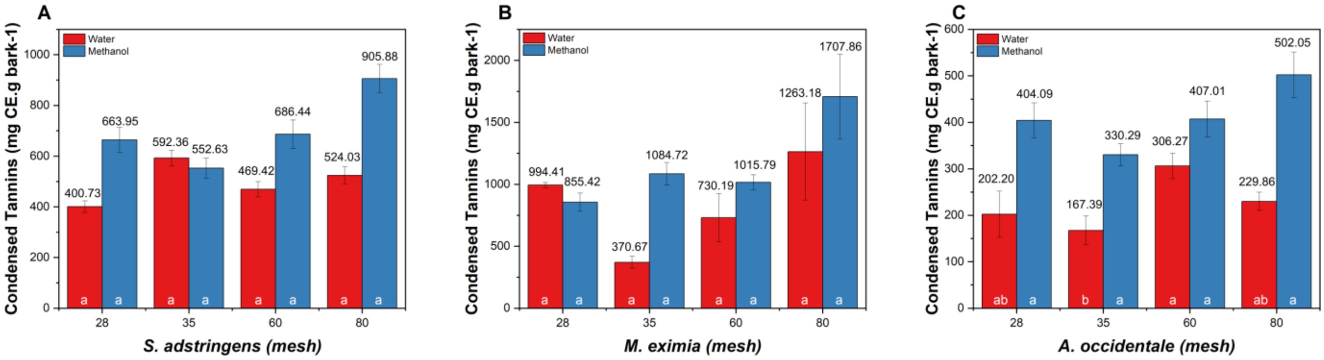
Pages: 1-26

bark, the 60-mesh particle size achieved the best performance with water, yielding 80% more than the 35-mesh size (Table 1). However, with methanol, no statistically significant differences were observed between particle sizes, and the extraction yields were consistently higher, on average, 87% greater than those obtained with water.

Figure 1 - Condensed tannin content for the barks of the species *S. adstringens*, *M. eximia* and *A. occidentale* extracted in two different solvents (water and 70% methanol).



(n = 6 biological samples, analytical duplicate). Identical letters in the same solvent and the same species do not differ statistically according to Tukey's test at 5% significance.

Table 1 - Percentage variation in the amount of Condensed Tannins extracted in water to those extracted in 70% methanol.

Granulometry	***S. adstringens* (%)**	***M. eximia* (%)**	***A. occidentale* (%)**
28	99.8	-14.0	99.8
35	97.3	192.6	97.3
60	32.9	39.1	32.9
80	118.4	35.2	118.4
Average Gain %	87	63	87

For *Myrcia eximia* bark, yields were high for both solvents, but methanol still outperformed water by an average of 63%. *S. adstringens* followed the same pattern, with methanol showing an average increase of 87% over water, although no

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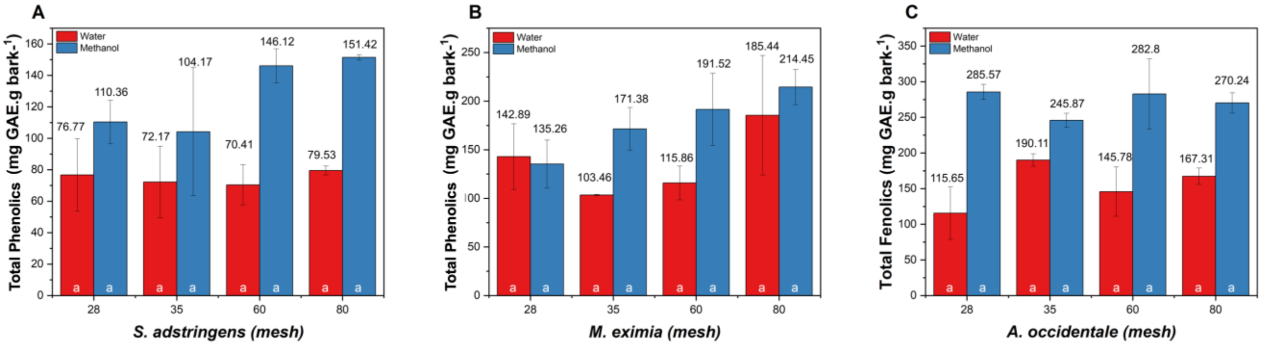
Pages: 1-26

significant differences were observed among the particle sizes.

4.2 Total Phenolics

Although no statistically significant differences were observed among particle sizes, methanol extraction resulted in higher yields for all species (Figure 2). *A. occidentale* bark showed an average increase of 83%, *M. eximia* 35%, and *S. adstringens* 72% in total phenolic content when extracted with methanol, again confirming the superior efficiency of this solvent (Table 2).

Figure 2 – Total phenolic content for the barks of the species *S. adstringens*, *M. eximia* and *A. occidentale* extracted in two different solvents (water and 70% methanol).



(n = 6 biological samples, analytical duplicate). Identical letters in the same solvent and the same species do not differ statistically according to Tukey's test at 5% significance.

Table 2 - Percentage variation in the amount of total phenolic extracted in water to those extracted in 70% methanol.

Granulometry	***S. adstringens* (%)**	***M. eximia* (%)**	***A. occidentale* (%)**
28	43.8	-5.3	146.9
35	44.3	65.7	29.3
60	107.5	65.3	94.0
80	90.4	15.6	61.5
Average Gain %	72	35	83

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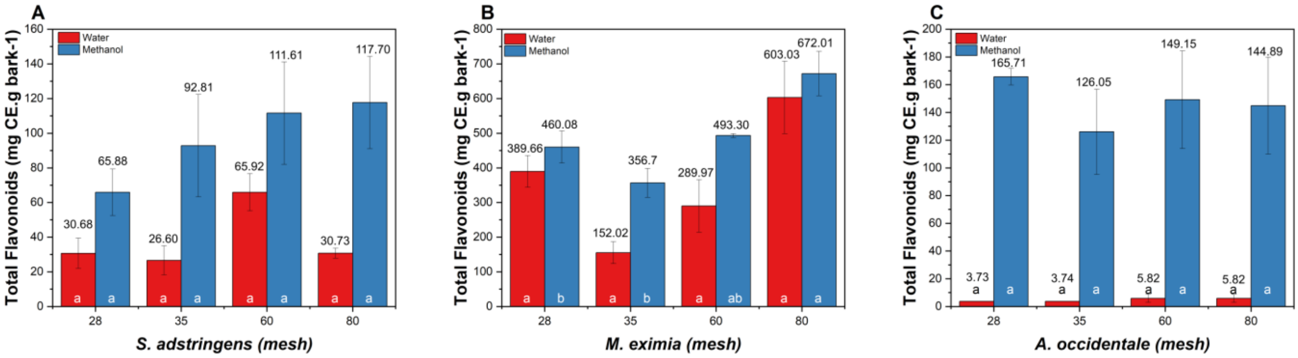
4.3 Total Flavonoids

Regarding flavonoid content, the extracts obtained with methanol also demonstrated better performance (Figure 3). For *M. eximia* bark, statistically significant differences were observed among particle sizes only in the methanol extraction, with the 80-mesh fraction standing out, showing up to a 79% increase compared to the other sizes (Table 3). *S. adstringens* bark showed the highest relative gain when methanol was used (179% compared to water), while *A. occidentale* bark exhibited a more modest average increase of 31%.

Figure 3 – Total flavonoids content for the barks of the species *S. adstringens*, *M. eximia* and *A. occidentale* extracted in two different solvents (water and 70% methanol).



(n = 6 biological samples, analytical duplicate). Identical letters in the same solvent and the same species do not differ statistically according to Tukey's test at 5% significance.

Table 3 - Percentage variation in the amount of total flavonoids extracted in water compared to those extracted in 70% methanol.

Granulometry	***S. adstringens* (%)**	***M. eximia* (%)**	***A. occidentale* (%)**
28	114.7	18.1	43.41
35	248.9	130.1	32.69
60	69.3	70.1	24.64
80	283.0	11.4	23.91
Average Gain %	179	57	31

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The observed patterns in phenolic compound extraction across particle sizes and solvents can be interpreted through the physicochemical principles governing solid-liquid mass transfer. Particle size reduction increases the specific surface area of the bark matrix, thereby expanding the contact interface between the solid material and the extracting solvent and facilitating the solubilization of phenolic compounds. Additionally, finer particles present reduced internal diffusion path lengths, meaning that solvent molecules must travel shorter distances to reach phenolic compounds complexed within the lignified cell wall structures of the bark, and that extracted molecules diffuse more readily into the bulk solvent (LORENÇO et al., 2025; ARAUJO et al., 2020). This is particularly relevant for condensed tannins and flavonoids, which are often physically entrapped within or covalently associated with lignocellulosic components, requiring sufficient solvent penetration to be released (XU et al., 2025; DA SILVA ARAUJO et al., 2021). The superior performance of 70% methanol over water in all three species is consistent with these principles, as its intermediate polarity provides a favorable balance between surface tension and affinity for the hydroxyl-rich aromatic structures of phenolic compounds, reducing the resistance to mass transfer at the solid-liquid interface (LORENÇO et al., 2025; CHEN et al., 2024). The absence of a consistent significant effect of particle size on extraction yield across most variables, despite the expected surface area gains, suggests that in these bark matrices the limiting factor for extraction may be the chemical affinity between solvent and analyte rather than diffusional resistance alone, which would explain why solvent type exerted a more decisive influence on yield than particle size in the present study (LORENÇO et al., 2025; ARAUJO et al., 2025).

4.4 Antimicrobial Activity

When comparing the extracts used in this study, it was evident that those obtained using methanol showed superior results compared to the aqueous extracts. *S. aureus* was the most sensitive bacterium to tannins extracted with methanol, with all three studied species exhibiting antimicrobial activity (Table 4). Among them, the *M. eximia* extract produced the largest inhibition zones, with an average diameter of 8.71 mm, followed by *S. adstringens* and *A. occidentale* extracts, with average

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Pages: 1-26

inhibition zones of 8.56 mm and 7.83 mm, respectively (Figure 4). In contrast, for the aqueous extracts, *S. aureus* was only sensitive to the *M. eximia* extract, which exhibited an average inhibition zone of 8.09 mm in diameter. This difference among species may be attributed to the higher concentration of phenolic compounds found in *M. eximia* bark, as the levels of condensed tannins in this species were up to twice as high as those observed in the bark of the other two species.

These differences in phenolic compound concentration may also help explain the results observed for *L. monocytogenes*, which was sensitive only to the phenolic compounds extracted from *M. eximia*. The inhibition zones were 8.09 mm and 7.95 mm for the aqueous and methanolic extracts, respectively. Tannins from cashew and barbatimão bark were not effective in inhibiting *L. monocytogenes* growth under either extraction condition.

These findings are consistent with the literature, as Huang et al., (2024) reported that tannins tend to be more effective antimicrobial agents against Gram-positive bacteria. Accordingly, positive results in this study were only observed for the Gram-positive strains (*S. aureus* and *L. monocytogenes*). None of the extracts tested were able to inhibit the growth of the Gram-negative bacteria *K. oxytoca* and *E. coli*. Bacterial resistance remains one of the greatest concerns in various health-related fields and motivates the search for new compounds capable of inhibiting bacterial growth and preventing associated infections.

Phenolic compounds extracted from tree bark are capable of covalently binding to proteins and precipitating them due to their chelating ability (Lorenço et al., 2021). Through this mechanism, tannins bind to proteins in bacterial membranes, causing structural destabilization, which in turn prevents cell division and bacterial growth. Additionally, tannins possess high antioxidant capacity (SHIRMOHAMMADLI; EFHAMISISI; PIZZI, 2018).

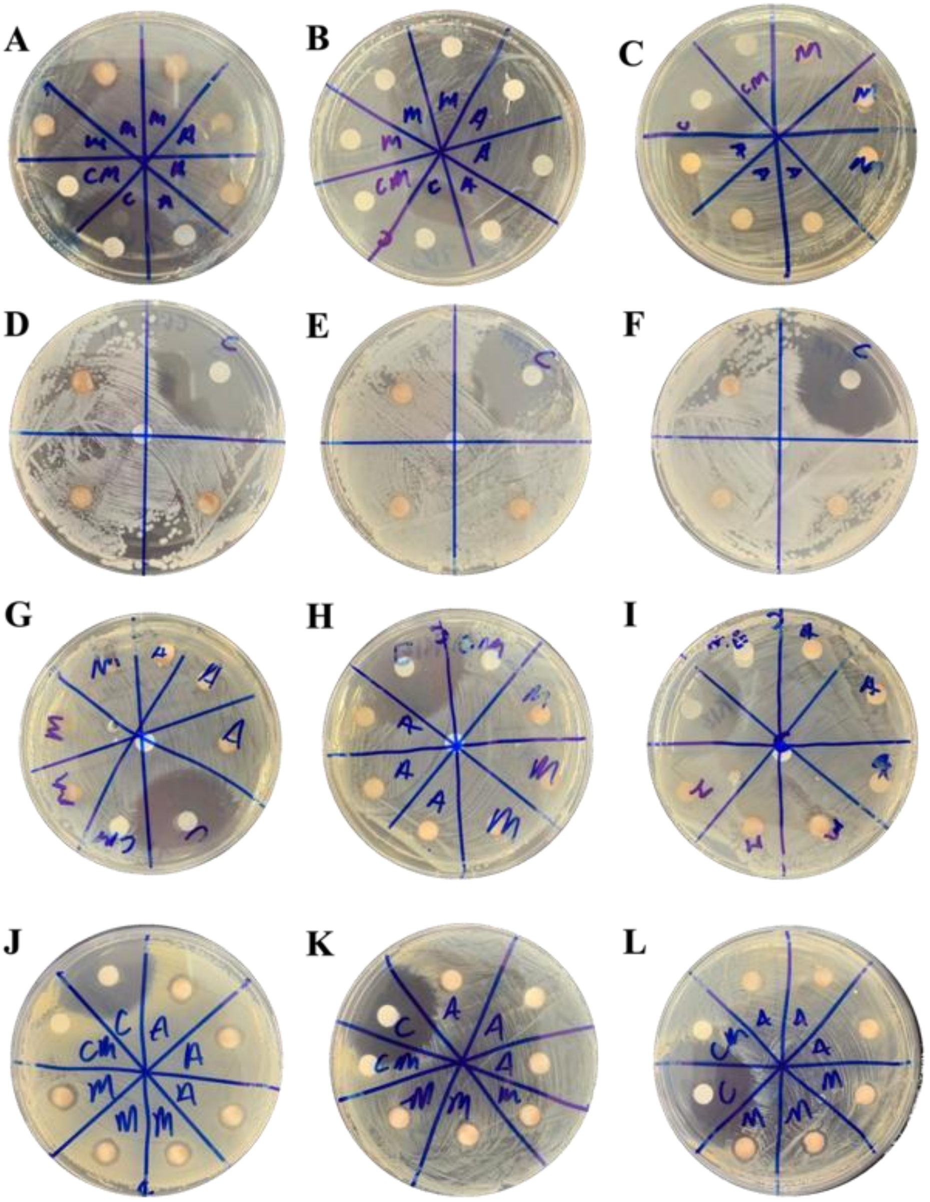
Received: 13/01/2026 - **Accepted:** 19/03/2026
Vol: 13.03
DOI: 10.66104/spkk9x50
Pages: 1-26

Table 4 - Determination of bacterial sensitivity (Disk diffusion) of microbial strains to tannin extracts from different tree bark species.

Water				
Microorganisms	***A. occidentale*** **(mm)**	***M. eximia*** **(mm)**	***S. adstringens*** **(mm)**	**Control**
S. aureus ATCC 23925	-	8,59±0,13	-	31,41±2,23
L. monocytogenes ATCC 19117	-	8,09±0,26	-	34,90±2,31
K. oxytoca	-	-	-	32,85±2,45
E. coli K12	-	-	-	31,86±2,63
Methanol				
Microorganisms	***A. occidentale*** **(mm)**	***M. eximia*** **(mm)**	***S. adstringens*** **(mm)**	**Control**
S. aureus ATCC 23925	7,83±0,84	8,71±0,38	8,56±0,49	31,41±2,23
L. monocytogenes ATCC 19117	-	7,95±0,38	-	34,90±2,31
K. oxytoca	-	-	-	32,85±2,45
E. coli K12	-	-	-	31,86±2,63

Values are expressed as mean ± standard deviation (SD). – = no inhibition zone observed.

Figure 4 - Disk diffusion test.



Where: A= *M. eximia* in *L. monocytogenes;* B= *A. occidentale* in *L. monocytogenes*; C= *S. adstringens* in *L. monocytogenes;* D= *M. eximia* in *K. oxytoca;* E= *A. occidentale* in *K. oxytoca*; F= *S. adstringens* in *K. oxytoca;* G= *M. eximia* in *E. Coli;* H= *A. occidentale* in *E. Coli*; I= *S. adstringens* in *E. Coli;* J= *M. eximia* in *S. aureus;* K= *A. occidentale* in *S. aureus*; L= *S. adstringens* in *S. aureus.*

Received: 13/01/2026 - **Accepted:** 19/03/2026
Vol: 13.03
DOI: 10.66104/spkk9x50
Pages: 1-26

Antimicrobial activity was observed only against Gram-positive bacteria, particularly *Staphylococcus aureus* and *Listeria monocytogenes*. The extracts obtained with methanol were the most effective. All extracts from the three species demonstrated activity against *S. aureus*, with *Myrcia eximia* bark standing out, showing inhibition zones up to 8.71 mm. Only *Myrcia eximia* bark exhibited activity against *L. monocytogenes* with both solvents, reinforcing its superior bioactive potential.

None of the samples showed activity against Gram-negative bacteria (*E. coli* and *K. oxytoca*), which aligns with the literature reporting greater resistance of these microorganisms to the effects of tannins (Huang et al., 2024).

Beyond the mechanistic aspects described above, a comparative analysis of the phytochemical data across Tables 1, 2, and 3 with the antimicrobial results presented in Table 4 supports these findings and reveals consistent qualitative patterns between chemical composition and biological activity. The integrative analysis of phytochemical composition data (Tables 1, 2, and 3) alongside antimicrobial activity results (Table 4) indicates that methanolic extracts, which showed higher yields of total phenolics, condensed tannins, and flavonoids, were also associated with greater antimicrobial activity, particularly against S. aureus. These results should be interpreted as comparative trends rather than quantitative correlations.

In particular, the methanolic extract of *Myrcia eximia*, which produced the largest inhibition halos for both *S. aureus* (8.71 ± 0.38 mm) and *L. monocytogenes* (7.95 ± 0.38 mm), was also the species that exhibited the highest relative increments of total flavonoids in methanol compared to water, reaching 130.1% at the 35-mesh particle size (Table 3), alongside increases in total phenolics (65.3–65.7%) and condensed tannins (39.1–192.6%) at intermediate granulometries (Tables 1 and 2). This pattern suggests that the methanolic solvent not only increases the overall yield of phenolic compounds, but also favors the extraction of flavonoid fractions and condensed tannins of higher polarity, which are associated with protein precipitation capacity and bacterial membrane destabilization (XU et al., 2025; CHEN et al., 2024). In contrast, aqueous extracts, which showed lower or even negative percentage variations, such as *M. eximia* in total phenolics at -5.3% for the 28-mesh fraction, resulted in limited or absent antimicrobial activity, with inhibition of S. aureus and L. monocytogenes restricted to *M. eximia*, and no activity observed for *A. occidentale* and *S. adstringens*. The inability of extracts from any of the three species to inhibit Gram-negative bacteria (*K. oxytoca* and *E. coli*), regardless of solvent or particle size, is consistent with the observed phytochemical profile (TAKÓ et al., 2020), as condensed tannins act preferentially on bacterial membranes lacking the outer lipopolysaccharide layer characteristic of Gram-negative bacteria (XU et al., 2025). Taken together, these results indicate that the relationship between phytochemical profile and antimicrobial activity is influenced by the structural class of the extracted compounds, and not solely by total phenolic content (DEMBIŃSKA et al., 2025), with solvent selection playing a key role in determining this selectivity (LORENÇO et al., 2025).

5. Conclusion

The results of this study demonstrate that both particle size and solvent type have a significant influence on the extraction of phenolic compounds from tree bark originating from different Brazilian biomes. Seventy percent methanol proved to be more effective than water in extracting condensed tannins, total phenolics, and flavonoids, regardless of species or particle size used.

Among the species analyzed, *Myrcia eximia* stood out for its higher flavonoid content and significant antimicrobial activity against Gram-positive bacteria. The extracts obtained with methanol demonstrated greater efficacy in inhibiting *Staphylococcus aureus* and *Listeria monocytogenes*, while no effect was observed against Gram-negative bacteria.

These findings reinforce the potential of utilizing forest residues as sources of bioactive compounds for applications in the pharmaceutical, cosmetic, and food industries, and highlight the importance of standardizing extraction processes to maximize both yield and functional activity.

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