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Improving the extraction of *Anacardium occidentale* bark tannins by alkaline and sulphitation processes and their efficiency in coagulation: chemical characterization by HPLC

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ABSTRACT

Tannins extracted from several forest species can be successfully used as coagulants in water treatment. This study employs a high-yield tannin extract from cashew tree (*Anacardium occidentale*) bark as an eco-friendly alternative for water treatment. First, ground bark was hot-water-extracted for tannins, using two adjuvants (NaOH and Na2SO3) at three concentrations for each. The total solids content (TST), Stiasny index (I), and condensed tannins content (TTC) were determined in the tannic extracts. The proanthocyanidins extracted from the bark were separated by solid-phase extraction into oligomeric and polymeric fractions. Total phenolic compounds were measured using the Prussian blue test method. The phenolic compounds present in the original extract and in the oligomeric and polymeric procyanidin fractions were analyzed by HPLC-MS^n. The extracted tannins were cationized and applied in water treatment. The results showed that reagent-assisted extraction efficiently enhanced tannin yields and quality compared with pure hot-water extraction, with NaOH as the most effective. Total phenolic compounds and total condensed tannins in the Cashew tree bark were 154.18 mg GAE g^{-1} and 164.59 mg g^{-1}, respectively. Phenolic compounds corresponded to catechin gallate, gallic acid, catechin, epigallocatechin, epicatechin, ellagic acid glycoside, triterpenoids, and anacardic acid. Other phenolics included prodelphinidins and procyanidins, in both oligomeric and polymeric forms. Cationized tannins used as coagulants effectively decreased water turbidity. The study demonstrated the potential of Cashew tree bark tannins as a sustainable alternative for water treatment. Results showed the potential of tannins as eco-friendly coagulant bases in water treatment.

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Keywords: natural flocculant, natural products, non-wood forest products, tannins, water treatment.

Aprimoramento da extração de taninos de *Anacardium occidentale* por processo alcalino e sulfitação e sua eficiência na coagulação: caracterização química por HPLC

RESUMO

Taninos extraídos de algumas espécies florestais podem ser eficazes como coagulantes naturais, dependendo de sua composição química. Este estudo teve como objetivo aprimorar a extração de taninos da casca de Anacardium occidentale, com o emprego de solventes químicos (NaOH e Na2SO3), caracterizar quimicamente as moléculas e avaliar a eficiência desse coagulante como alternativa ambientalmente sustentável para o tratamento de água. Inicialmente, a casca moída foi submetida à extração de taninos em água quente e solventes químicos, em três concentrações diferentes (1, 3 e 5%) para cada um. Nos extratos tânicos foram determinados o teor de sólidos totais (TST), o índice de Stiasny (I) e o teor de taninos condensados (TTC). Os compostos fenólicos totais foram determinados pelo método do azul da Prússia, e as proantocianidinas extraídas da casca foram separadas por extração em fase sólida em frações oligoméricas e poliméricas, ambas analisadas por HPLC-MSn. Os taninos extraídos foram cationizados e aplicados no tratamento de água. Os resultados demonstraram que a extração assistida por solventes químicos aumentou de forma eficiente o rendimento comparado a água quente, com aumento de TTC de 3,6 e 2,4 vezes maior para NaOH e Na_2SO_3, respectivamente. Os teores totais de compostos fenólicos e de taninos condensados da casca do cajueiro foram de 154,18 mg EAG g^{-1} e 164,59 mg g^{-1}, respectivamente. Os compostos fenólicos corresponderam a galato de catequina, ácido gálico, catequina, epigalocatequina, epicatequina, glicosídeo de ácido elágico, triterpenoides e ácido anacárdico. Para as proantocianidinas, observou-se alta concentração na fração oligomérica: 47,38% (prodelphinidinas) e 79,21% (procianidinas). Esse alto PC indica eficiência no tratamento de água devido à sua correlação direta com a densidade da carga. O estudo demonstrou o potencial dos taninos da casca do cajueiro extraídos com Na2SO3 como alternativa para o tratamento de água, apresentando mais do que o dobro de taninos extraídos em água e mantendo sua eficiência, evidenciando sua viabilidade como base ambientalmente amigável para coagulantes.

Palavras-chave: floculante natural, produtos florestais não madeireiros, produtos naturais, taninos, tratamento de água.

1. INTRODUCTION

Plant tannins are polyphenols soluble in aqueous medium and characterized by high molecular mass, typically ranging from 500 to 3,000 g mol^{-1}, and have the property of precipitating proteins, gelatins, and alkaloids (Pizzi, 2019). Condensed tannins are often found mainly in trees' bark, but they can usually be obtained from the wood itself. This chemical class consists of several monomers and oligomers that contain flavan-3-ol units as components or derivatives of this structure. The formation of tannins generally involves the bond between carbon 4 of one structure and carbon 8 of the following structural unit (Chaves *et al.*, 2021).

Tannin quality may vary substantially depending on the extraction method used. Therefore, it is essential to establish technical conditions to improve extraction and standardize it, thereby lowering costs and achieving higher tannin yields. Various procedures and solvent agents are used in the tannin extraction process. This is a crucial step, as these compounds may undergo structural modifications or rearrangements of their original characteristics and composition during extraction procedures (Carneiro *et al.*, 2007). Several studies have demonstrated that

tannin extraction in hot aqueous media can be enhanced by including sodium hydroxide and some other reagents, such as sodium sulfite and sodium carbonate. By varying concentrations and operational temperatures, the extractable components from the bark of various forest species can be more readily extracted, thereby improving tannin yields. During hot-water extraction, added inorganic salts can affect the quality of tannins extracted from barks while decreasing yields of non-tannic compounds (Mori *et al.*, 2003).

Many forest species have the potential to be tannin sources. Among them is *Anacardium occidentale* Linn, popularly known as the cashew tree. It is a dicotyledonous angiosperm belonging to the Anacardiaceae family. Widely distributed in regions with tropical and subtropical climates, the cashew tree is the only species in the Anacardiaceae family to be widely cultivated and commercially cultivated worldwide (Aragão, 2015). The species in question exhibits genetic variability that can be categorized into two predominant phenotypes, distinguished by plant size: the common type and the dwarf variety (Almeida *et al.*, 2017). The specific characteristics of each plant species and the parts assessed influence the selection of the type and quantity of added chemical adjuvants, as well as the required extraction temperature. This highlights the continued importance of research in identifying the ideal extraction conditions for each plant species (Sartori, 2012).

Pollution and degradation of water resources are critical global challenges, driven by rapid urbanization and climate change, which intensify freshwater scarcity (Goonetilleke and Vithanage, 2017). In many arid and semi-arid regions, water availability is limited, leaving populations with few options for water sources, such as surface sources like ponds and reservoirs, which often have high levels of turbidity and suspended solids (Skoronski *et al.*, 2014; Vaz *et al.*, 2010). To make this resource suitable for human consumption, conventional treatment systems are necessary; however, this precarious situation makes the search for more accessible, efficient, and sustainable technologies an international priority. In Brazil, for water to be supplied for domestic use, it must meet the potability standards defined by Ordinance GM/MS No. 888, of May 4, 2021. This document establishes the procedures for controlling and monitoring the quality of water intended for human consumption, as well as the criteria that must be met to guarantee its potability (Brazil, 2021).

To meet these regulatory requirements, the water treatment process is fundamentally based on the coagulation and flocculation stage, traditionally carried out using synthetic chemical agents. However, these generate non-biodegradable waste, representing a secondary source of pollution (Oladoja *et al.*, 2017). Natural coagulants can offer several advantages over chemical coagulants in water treatment plants. They produce less sludge, are renewable, have high raw material availability, and help reduce costs and risks in water treatment processes. Furthermore, they do not alter the pH of the treated water and do not harm human or animal health (Lima Júnior and Abreu, 2018). Recent studies have demonstrated the potential application of cashew bark tannins as a natural coagulant (Anjos *et al.*, 2022). However, few studies demonstrate the optimization of tannin extraction from this species and the long-term stability of its efficiency. Advances in research on extraction methods and their appropriate application in water treatment are therefore of great interest.

The present study aimed not only to employ cationized cashew tree tannins for water treatment but also to optimize the extraction of condensed tannins from cashew tree bark, thereby developing more efficient methods applicable to other plant species. Further, it aimed to maximize the quality and yield of the extracted tannins for use in water treatment. The research also seeks to fill existing gaps in the literature and to provide a solid scientific basis for industrial and technological applications.

2. MATERIAL AND METHODS

2.1. Tree selection and bark collection

For the development of this study, samples were collected from the base, middle, and top of the trunk of five cashew trees (*Anacardium occidentale*) grown in an orchard in the municipality of Macaíba, RN (5°51'28" S and 35°21'14" W). The trees selected for the study were chosen based on their vigor, with only those that showed no signs of pest or disease attack included. Bark collection was conducted in March, at the beginning of the rainy season. The bark was carefully and uniformly removed from the plant's main trunk using a machete, taking precautions to avoid girdling. The bark then was exposed to the air for drying. A dry bark sample of approximately 500 grams was then reduced in size using a Willey mill. The material was classified into the fraction that passed through a 40-mesh sieve (0.425 mm) and the fraction retained on a 100-mesh sieve (0.150 mm). The obtained material was homogenized, and its moisture content (dry basis) was determined to calculate tannin content for each sample.

2.2. Extraction of condensed tannins

Three 25-g samples of completely dry bark were taken for each treatment to extract tannins. The bark was extracted under reflux with water, water containing sodium hydroxide (NaOH), and water containing sodium sulfite (Na_2SO_3) at different concentrations. This resulted in seven different treatments. The samples were placed in flat-bottomed 500 mL flasks, and 250 mL of distilled water was added to each flask at a 1:10 ratio. The flasks were heated under reflux for 2 hours. Each sample was then subjected to two rounds of extraction to remove as much of the substances present as possible.

After each extraction, the material obtained was passed through a 200 mesh sieve (0.075 mm) for all treatments. Immediately after the extractions, the material was filtered through flannel fabric to retain fine particles. The resulting extract was homogenized, stored in plastic bottles, and later filtered through a sintered glass funnel with a porosity of 2. The extracts were then concentrated to 250 mL by evaporating the water using a Soxhlet-type apparatus. Subsequently, three aliquots (samples) of 50 mL were taken from each extract, two of which were used to determine the condensed tannin content (CEC), and one was evaporated in an oven at 103 ± 2ºC for 48 h to determine the percentage of total solids content (TST) (Equation 1).

$$TST(\%) = \frac{M1-M2}{M2}\ x100 \quad (1)$$

Where:

TST = total solids content (%)
M1 = initial sample weight (g)
M2 = final sample weight (g).

2.3. Determination of condensed tannin content (TTC) and Stiasny index (I)

To determine the amount of TTC in each sample, the Stiasny method (Guangcheng *et al.*, 1991) was used, with some adaptations (Anjos *et al.*, 2022). For this purpose, 4 mL of formaldehyde and 1 mL of concentrated hydrochloric acid were added to 50 mL of the crude extract. Each mixture was then boiled under reflux for 30 minutes, during which the tannins formed insoluble precipitates that could be separated by simple filtration. To perform the simple filtration process, a paper filter was placed in a Büchner funnel with a 10 cm diameter and a 4 cm depth. The material retained on the filter was dried in an oven at 103 ± 2°C for 24 hours, then weighed, and the Stiasny index was calculated. The tannins in each sample were determined by multiplying the Stiasny index by the total solids content. All analyses were performed in triplicate, following the methodologies described by Trugilho *et al.* (1997). After

obtaining the dry mass of the precipitate, the Stiasny index (I) was calculated as a percentage (Equation 2).

$$I(\%) = \left(\frac{M2}{M1}\right) x100 \quad (2)$$

Where:

I = Stiasny index (%)
M1 = solids weight in 50 mL hot-water extract
M2 = tannins precipitate weight

The amount of tannins in each sample was obtained by multiplying the Stiasny index by the total solids content (Equation 3).

$$TTC\ (\%) = \frac{TST\ x\ I}{100} \quad (3)$$

Where:

TTC = condensed tannins content (%)
TST = total solids content (from Equation 1)
I = Stiasny index (from Equation 2).

2.4. Analyses and identification of condensed tannin components

2.4.1. Extraction of methanol-soluble components

To obtain the extract enriched in phenolic compounds, including the condensed tannins, 0.5 g of *A. occidentale* bark was ground and extracted with 50 mL of methanol for 1 hour at room temperature on an orbital shaker (300 rpm). After extraction, the extracts were filtered through filter paper. Twenty milliliters of the extract were evaporated to dryness for fractionation and characterization of the condensed tannins.

2.4.2. Proanthocyanidin Fractionation

The proanthocyanidins extracted from the bark were separated by solid-phase extraction (SPE) into oligomeric and polymeric fractions, as described by Sun *et al.* (1999). Briefly, 20 mL of the methanol extract was rotary-evaporated to dryness under vacuum at 35°C. The obtained solid was dissolved in 10 mL of 0.1 M sodium phosphate buffer at pH 7. The sample was applied to a reversed-phase SPE cartridge (C-18), previously activated by passing 4 mL of methanol (2 runs) and then 4 mL of 0.1 M sodium phosphate buffer (pH 7, 2 runs). After sample elution, the column was washed with 2 x 4 mL of ultrapure water at pH 7 and dried for 2 h under vacuum. Elution was performed with 8 mL of ethyl acetate to recover flavanols and oligomeric proanthocyanidins (Fraction I), followed by 8 mL of methanol to recover polymeric proanthocyanidins (Fraction II). Finally, the eluates were evaporated by vacuum centrifugation.

2.4.3. Determination of total phenolic compounds

Total phenolic compounds (TPC) were measured using the Prussian blue test method developed by Price and Butler (1977). For the analyses, 5 mL of 0.1 M iron chloride hexahydrate ($FeCl_3.6H_2O$) in 0.1 M HCl was added to 50 µL of extract, followed immediately by the addition of 5 mL of 0.008 M potassium (III) hexacyanoferrate [$K_3Fe(CN)_6$]. Absorbances of the solutions were measured after 10 min in 1 cm cells at 720 nm using a spectrophotometer (Thermo Scientific Genysis 50) zeroed with water. A calibration curve for gallic acid was prepared and analyzed under the same conditions as the extracts, and the results were expressed as gallic acid equivalents. All analyses were performed in triplicate.

2.4.4. Depolymerization with thioglycolic acid

The previously separated dry fractions (FI and FII) of each sample were dissolved in 200 µL of methanol. From Fraction I (FI), 100 µL were divided into a vial for direct analysis without depolymerization. On the other hand, the remaining 100 µL of FI was mixed with 200 µL of thioglycolic acid solution for depolymerization. Then, fraction II was further mixed with 200 µL of thioglycolic acid solution to depolymerize it. The reactions were conducted at 70°C for 6 minutes. The reaction was stopped in an ice bath, and 200 µL of 77 M sodium acetate was added. This reaction protocol was adapted with minor modifications from Mouls and Fulcrand (2012).

2.4.5. Identification of Phenolic Compounds by LC-MS^n

The phenolic compounds present in the original extract and in the oligomeric and polymeric procyanidin fractions were analyzed before (oligomeric fraction) and after thioglycolysis (oligomeric and polymeric fractions) by HPLC-MS^n using a Thermo Scientific Ultimate 3000 HPLC system coupled to a Thermo Scientific LTQ XL linear ion trap mass spectrometer, with the instruments controlled by Xcalibur software. The analysis was performed on a Macherey-Nagel C18 column with a particle size of 5 µm, a length of 250 mm, and an internal diameter of 4.6 mm, manufactured in Germany. The column temperature was maintained at 30°C throughout the analysis.

For the analysis of phenolic compounds in the extract, an HPLC C18 column was used with mobile phases A and B containing 0.1% formic acid and pure methanol, respectively. The injection volume was 10 µL. Two distinct HPLC gradient programs were used: one after the depolymerization reaction with thioglycolic acid and another for the flavanol standards in the absence of the reaction. Mobile phases A and B for both methods consisted of 0.1 M formic acid in water and a mixture of acetonitrile (80%), formic acid (0.1%), and water (19.9%), respectively. The sample injection volume was set at 5 µL.

The HPLC gradient program applied to the flavanol standards flowed at 0.5 mL min^{-1} with the following concentrations: 10% B at 5 minutes, 30% B at 20 minutes, 56% B at 35 minutes, and 90% B at 39 minutes. The cleaning step occurred at 44 minutes using 90% B. The following parameters were used to analyze samples after the thioglycolic acid reaction: the flow rate was set to 0.5 mL min-1 at 15% B from 0 to 5 min. For the 5 to 30 minute interval, the flow rate was set at 0.5 mL min^{-1} at 65% B. For the 30.1 to 35.1 minute interval, the flow rate was set at 1 mL min^{-1} at 90% B. The final interval, from 35.3 to 40.2 minutes, was characterized by a flow rate of 1 mL/min at 90% B. The flow rate was set at 1 m min^{-1} at 15% B for the 40.2 to 40.2 minute interval. The LTQ XL MS instrument was operated in positive polarity with the following parameters: source type HESI, capillary temperature 200°C, and source heater temperature 350°C. The sheath gas flow was set to 60.00, the auxiliary gas flow to 20.00, and the sweep gas flow to 0.00. The source voltage was set to 3.50 kV and the source current to 100.00 µA. The capillary voltage was set to 47 V, and the tube lens voltage to 110.00 V. The concentrations of aqueous standards for flavan-3-ols, including (-)-epicatechin, (+)-catechin, (-)-epigallocatechin, (+)-gallocatechin, (-)-epigallocatechin gallate, (+)-epigallocatechin gallate, (-)-epigallocatechin gallate, and (+)-catechin gallate, ranged from 0.025 ppm to 100 ppm and were analyzed in triplicate.

2.5. Cationization process

For tannins to be used as coagulants, they must undergo chemical modification. Thus, they were cationized based on the Mannich reaction. Initially, 5.4 g of ammonium chloride and 24.4 g of formaldehyde were placed in a volumetric flask, and the mixture was heated to 80°C for 2 hours. Then, the product obtained was mixed with 28.0 g of an aqueous tannin solution for 30 minutes at 60°C. Immediately thereafter, the post-reaction stage consisted of adding 0.2 g of

monoethanolamine and reacting for 3 hours at 50°C.

2.6. Coagulation and flocculation tests

The water used to perform the coagulation and flocculation tests was collected from a reservoir in Macaíba, Rio Grande do Norte, Brazil. The tests were performed at the Non-Timber Forest Products Laboratory. Cationized cashew tannins were evaluated as coagulant agents, and ferric chloride, a commercial coagulant commonly used in water treatment, was used for comparison. Flocculation tests were performed using 1 L of water with a turbidity of 150, and applying 150 and 200 mg L^{-1} of coagulant solution using the Jar-test apparatus. The tests were conducted in jars with two agitation periods: 135 rpm for 2 minutes and 35 rpm for 10 minutes. The turbidity and pH of the treated water were evaluated every 10 minutes for 60 minutes. The objective was to determine the sedimentation time at which the turbidity presented greater efficiency and to identify the most appropriate resolution time for the process.

2.7. Experimental results analysis

Seven experimental treatments were set: T1 = pure hot water, T2 = hot 1% sodium hydroxide (NaOH) solution, T3 = 2% NaOH solution, T4 = 5% NaOH solution, T5 = hot 1%, sodium sulfite (Na_2SO_3) solution, T6 = 3% Na_2SO_3, and T7 = 5% Na_2SO_3, composing an entirely randomized experimental design in a factorial scheme, with three replications per treatment. The factors analyzed were the extractant type (NaOH and Na_2SO_3) and the extractant concentration (0, 1, 3, and 5%). The values of all variables (TST, I, and TTC) were compared using the Tukey test at the 99% confidence level to determine whether their behavior differed. Afterward, regression models were adjusted for each salt to predict the behavior of the variables as a function of concentration (1, 3, and 5%). The best models were selected based on the following decision criteria: the correlation coefficient (r) between experimental and estimated values, the significance of the regression parameters, the biological realism of the model, and the root-mean-square error (RMSE), as recommended by Gujarati and Porter (2009). In general, a correlation coefficient closer to 1 and a lower RMSE indicate better model estimation power.

3. RESULTS AND DISCUSSION

3.1. Tannins quantification

Table 1 shows the mean values found for the total solids content (TST), Stiasny index (I), and condensed tannin content (TTC) of the tannin extract from the cashew tree bark.

Table 1. Mean values determined for total solids content (TST), Stiasny index (I), and condensed tannins content (TTC) for the tannin extract from cashew tree bark as a function of the salt concentration.

Concentrations (%)		TST (%)	I (%)	TTC (%)
How water (T1) – C	0	16.97 ± 0.95	64.04 ± 1.85	10.88 ± 1.97
Sodium hydroxide (NaOH) –A	1	28.34 ± 0.58	90.44 ± 5.67	24.80 ± 1.10
	3	42.24 ± 1.51	95.41 ± 0.52	37.60 ± 1.39
	5	52.51 ± 2.30	75.10 ± 5.15	39.54 ± 1.36
Sodium sulfite (Na_2SO_3) – B	1	26.35 ± 1.55	76.24 ± 2.27	20.07 ± 0.58
	3	31.25 ± 0.71	74,74 ± 1.10	23.35 ± 0.19
	5	37.34 ± 4.93	70.62 ± 4.64	26.52 ± 5.21

*Letters A, B, and C after the salts' names in the first column indicate that they are statistically different by Test F at a 99% probability.

The experimental results for extraction with chemical solvents were significantly better than those for extraction with hot water alone. As indicated in Table 1, the values for all dependent variables (TST, I, and TTC) increased in the order of pure hot water, Na_2SO_3, and NaOH. This means that the pure water extraction presented the lowest values for all parameters analyzed. Thus, the descending hierarchical order for the extraction type is A > B > C. For example, total solids content (TST) refers to the total amount of extracted substances found in the samples analyzed in solid form (Medeiros *et al.*, 2019). The data collected in this research show that as salt concentration increases, total solids content also increases. These values are similar to those reported by Carneiro *et al.* (2007), who analyzed the effect of sodium sulfite on the bark of *Anadenanthera peregrina* and observed a statistically significant difference in tannin extraction at other concentrations. This phenomenon may be associated with increased levels of sugars and amino acids and increased pectin solubility, as reported by Gonçalves and Lelis (2001).

Figure 1 displays the quadratic regression models used to explain and predict the behavior of total solids content (TST), Stiasny index (I), and condensed tannin content (TTC) as a function of the extractor salt concentration. For TST, both curves exhibited a similar shape (half-parabolic), with close values at 1% and more distinct values at 3% and 5%. Regarding I, the curves showed a similar parabolic shape for both extractor salts, with a maximum at 3% concentration and a significant decrease thereafter for Na_2SO_3. The NaOH showed the same parabolic pattern, but with a slightly different trend. For both extractor salts, a similar behavior was observed for TTC, with a maximum at 4% and then decreasing. Those results suggest that concentrations of 3 or 4% are sufficient for both salts to achieve acceptable extraction of condensed tannins from cashew tree bark.

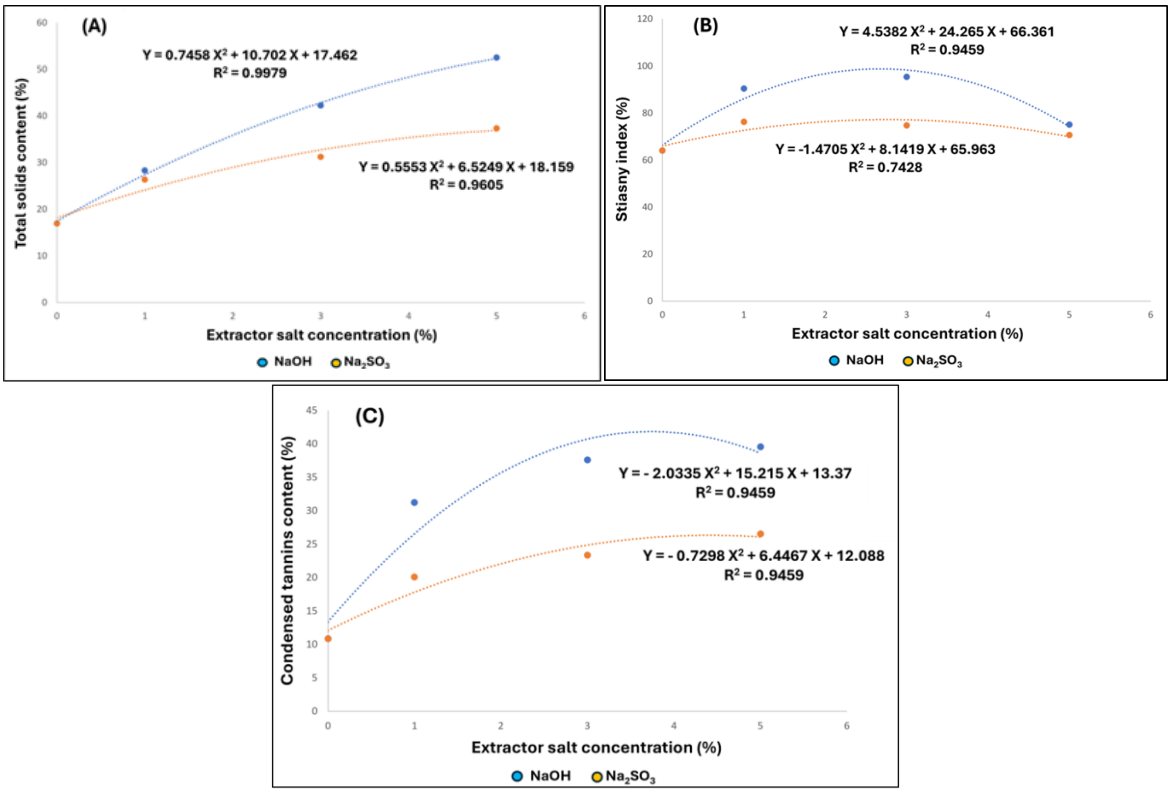


Figure 1. Regression models adjusted to predict the behavior of the dependent variables determined for the hot-water extracts from the Cashew tree bark – total solids content – TST (A), I – Stiasny index – (B), and total condensed tannins content – TTC (C), as a function of the salt concentration.

For all variables, the values from pure hot-water extractions are lower than those from the

other treatments. This confirms that hot water has a limited capacity to extract tannins compared to solutions with added salts. (Almeida, 2010), who evaluated the addition of different salts to *Pinus oocarpa*, also obtained a similar result when comparing the TST of the water extraction with that of the other solvents. This comparison revealed statistically significant differences in the evaluated parameter, consistent with the findings of this study. The Stiasny index (I) represents the concentration of polyphenols in a sample; higher values indicate greater purity of condensed tannins. Thus, it directly provides the proportion of other extractive components, such as gums, sugars, and hemicelluloses, which are not reactive to formaldehyde (Anjos, 2021; Souza *et al.*, 2021). In this experiment, the addition of salt lowered the extracts' non-tannin content. Then, pure hot water had the lowest I and was statistically different from saline solutions. Sodium hydroxide at 3% has the highest Stiasny index (95.41%), indicating a high efficiency in extracting mostly condensed tannins rather than other extractives, as previously reported (Ferreira, 2004). The efficiency differences between hydroxide and sulfite are evident, as hydroxide-extracted values are statistically higher than sulfite-extracted values at all concentrations tested. However, the decrease in I and TTC reduction at the highest sodium hydroxide concentration suggests that tannins may be degraded or interact in an undesirable manner.

The condensed tannin content (TTC) represents the concentration of tannins in the bark and serves as the primary indicator for characterizing tannic substances in the analyzed sample (Paiva *et al.*, 2024). The results for TTC confirm the trend observed in the other parameters. Distilled water presents the lowest content, with an average content significantly lower than that of saline solutions. Sodium hydroxide again stands out with the highest contents, especially at the maximum concentration tested (39.54%), which is statistically higher than the other treatments. The increase in TTC with increasing concentration is evident and significant, as indicated by the statistical test performed in the present research. (Mori *et al.*, 2003), studying the influence of the addition of sodium hydroxide and sulfite on the extraction of tannins from the bark of Stryphnodendron adstringens, obtained at a temperature of 70°C, found that the content of condensed tannins was 30.10% for extraction by adding 3 g of sodium sulfite and 34.03% by adding 3 g of sodium hydroxide. (Paes *et al.*, 2013) analyzed the tannin content of Anadenanthera colubrina extracted with 3% sodium hydroxide and sulfite solutions, obtaining 21.49% and 19.18%, respectively. In the present study, at a sodium hydroxide and sulfite concentration of 3%, the values were 37.60% and 23.35%, respectively. Such variations can be attributed to tannin characteristics, which can differ between species and even within the same species (Chaves *et al.*, 2021).

3.2. Characterization of the condensed tannin components

Total phenols obtained from the bark and extract of *A. occidentale* and total condensed tannins after isolation and thioglycolysis depolymerization are presented in Table 2. The values found for total condensed tannins were higher than those for phenolic compounds due to the method employed for each determination, in which total condensed tannins are the result of the sum of oligomeric and polymeric values related to the proanthocyanidins presented later.

Table 2. Determination of the contents of total phenolic compounds and total condensed tannins from the Cashew tree bark.

Material	Total phenolic compounds (mg GAE g^{-1})	Total condensed tannins (mg g^{-1})
Bark	154.18 ± 6.12	164.13
Extract	103.21 ± 3.07	109.87

Literature reports similar studies using the Folin-Ciocalteu method for identifying total phenols in the bark of A. occidentale. Encarnação *et al.* (2016) performed water extraction and found a total phenolic content of 58 ± 0.4 mg GAE g-1 in cashew stem bark. In the research by Chaves *et al.* (2010), which extracted ethanol three times and concentrated, they found 345.16 ± 16.24 mg GAE g-1, a value higher than that found in this study. Another study is that of Santos *et al.* (2018), where, using the Folin-Ciocalteu method, they evaluated total phenols in the bark of *A. occidentale*, using maceration in ethyl alcohol, solvent removal by rotary evaporation, changing the solvent every 48 h for 1 week, and using 0.0025 g of the dry extract, and found 0.0664 mg GAE g^{-1}, a value considerably lower than that found in this research. The contradictions in the values found in the literature may be directly related to the different methods applied to obtain the extract (different solvents, exposure time of the material to the solvent, presence or absence of agitation, number of extractions, and use of temperature), which makes fair comparisons between the results difficult. In addition to the methodology employed, factors that can vary the total phenols in samples with similar methodologies include plant age, site, climatic conditions (Souza *et al.*, 2021), and even the time of bark collection (Azevêdo *et al.*, 2017).

For the methodology employed in this research, no quantitative data on total condensed tannins were found; only qualitative data were found, which record their presence in the bark and stem of *A. occidentale*. However, the literature reports data on the skin and fruits of the cashew tree, with values ranging from 149 to 802 mg 100 g^{-1}, depending on the clone analyzed. Therefore, the value found in this research is comparable to that found in the fruit of the same species analyzed (MICHODJEHOUN-MESTRES, 2009). It is suggested that the presence of phenolic compounds, including condensed tannins, in plant extracts is an indicator of coagulant potential, since phenolic groups are directly associated with the adsorption, complexation, and bridging mechanisms responsible for coagulation-flocculation (IBRAHIM *et al.*, 2021).

3.2.1. Identification of the phenolic compounds

Figure 2 shows the total ion chromatogram of the methanol extract from *A. occidentale* bark obtained by HPLC-MS/MS, with the retention times and corresponding identifications of the phenolic compounds.

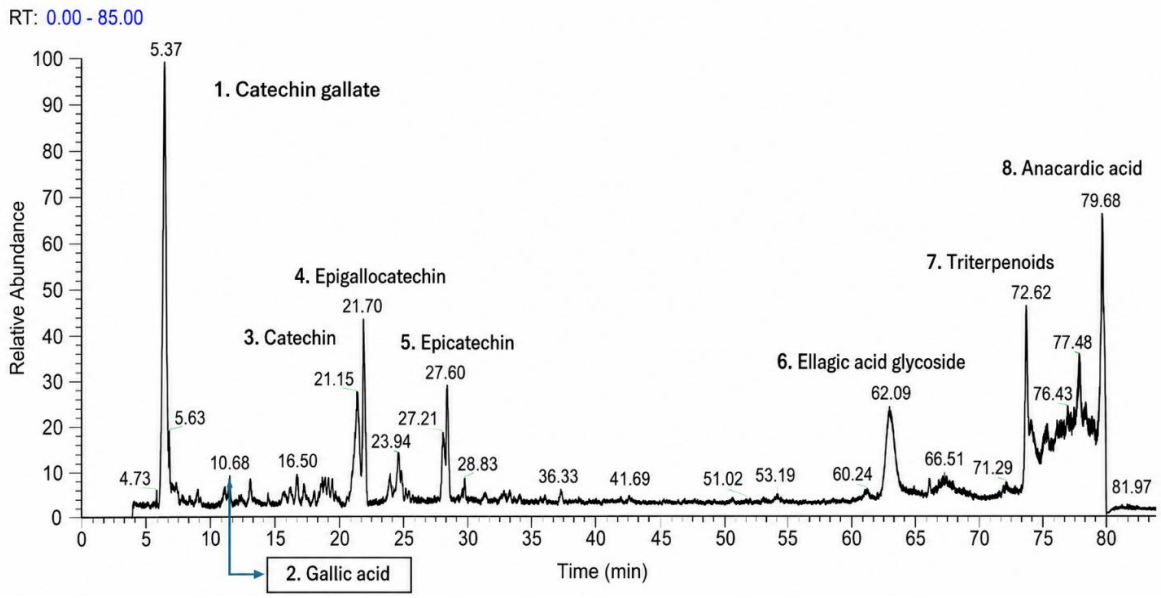


Figure 2. Chromatogram of the methanol extract from the Cashew tree bark obtained by HPLC-MS/MS.

Comparison of the retention times (RT) and UV absorption spectra of the phenolic compound standards, and the MS^2 spectrum, shows that the peaks marked as 1, 2, 3, 4, 5, 6, 7, and 8 in Figure 2 correspond to catechin gallate, gallic acid, catechin, epigallocatechin, epicatechin, ellagic acid glycoside, triterpenoids, and anacardic acid, respectively. The RT and peak areas of interest resulting from the analysis are presented in Table 3. Still, references that corroborate the identification of the phenolic compounds were included in Table 3.

Table 3. Characterization of compounds identified in peak areas of interest by HPLC-MS/MS in the methanol extract of the Cashew tree bark.

Id	Rt min	UV (max)	Compound	MS/MS2	Reference
1	5.35	224	Catechin gallate (flavan-3-ol)	455 (409)	(Chaves *et al.*, 2010)
2	9,82	222	Gallic acid	169 (125)	(Chaves *et al.*, 2010; Abreu *et al.*, 2019; Ferreira *et al.*, 2020; Aquino, 2017; Silva *et al.*, 2012; Vilar *et al.*, 2016)
3	18,90	239	Catechin	289 (245, 137)	(Vilar *et al.*, 2016; Chaves *et al.*, 2010)
4	20,82	275	Epigallocatechins (ent-gallocatechin 3-gallate)	457 (331, 305, 287, 169)	(Chaves *et al.*, 2010)
5	27,06	277	Epicatechin 3-gallate	441 (169, 303, 289, 137)	(Chaves *et al.*, 2010; Aquino, 2017; Silva *et al.*, 2012; Kajdžanoska *et al.*, 2010; Abreu *et al.*, 2019)
6	57,84	267	Ellagic acid glycoside	463	-
7	78,50	409	Triterpenoids (β-amirina and lupeol)	426 (407)	(Chaves *et al.*, 2010)
8	79,83	302	Anacardic acid (2-hydroxy-6-pentadecylbenzoic acid)	347 (303)	(Amorim *et al.*, 2022)

*Rt = retention time.

The identified phenolic components in this experiment are common to the barks of most other forest species. Indeed, they are also consistent with those reported in the literature for phenolic compounds in the stem bark of *A. occidentale*, as in the works of Chaves *et al.* (2010) and Vilar *et al.* (2016). (Fujita, 2008) also reported the presence of catechins and tannins, among other compounds, in the same material. Anacardic acids were reported by Chaves *et al.* (2010) and Itokawa *et al.* (1987), who confirmed the presence of monoene and diene anacardic acids. (Vilar *et al.*, 2016) also found them in the stem bark of *A. occidentale*. Triterpenoids were reported by Chaves *et al.* (2010), who determined the presence of lupeol and β-amyrin. They also identified flavan-3-ol, in addition to the previously known compounds, in this study. Anacardic acid has been reported in the liquid of the cashew nut shell, presenting high quantities, and is also present in the nut and fruit juice (Toyomizu *et al.*, 2000). In the work of Amorim *et al.* (2022), the chemical data suggest the presence of anacardic acid as the main compound of the bark extract, where they were analyzed by proton nuclear magnetic resonance (1H-NMR), which corroborates what was found in this research, with anacardic acid being the second most abundant compound.

3.2.2. Proanthocyanidins

Table 4 displays the percentage composition of the monomer, oligomer, and polymer fractions, and the results obtained for the content of proanthocyanidins, which are composed of

prodelphinidins and procyanidins, in mg g^{-1} for the monomer, oligomeric proanthocyanidin, and polymeric proanthocyanidin fractions.

Table 4. Absolute values in mg g^{-1} and percentage (%) for the compounds found in the bark of *A. occidentale* – prodelphinidin and procyanidin, and the monomeric, oligomeric, and polymeric fractions.

Chemical composition	F1 – Monomers (mg g^{-1} and %)	FII – Oligomers (mg g^{-1} and %)	FIII - Polymers (mg g^{-1} and %)
GC	1.99 (7.49)	1.20 (0.95)	2.18 (5.74)
EGC	1.28 (4.79)	1.14 (0.90)	1.97 (5.19)
C	1.95 (7.34)	2.50 (1.97)	3.04 (7.99)
EC	2.27 (8.54)	9.00 (7.11)	5.05 (1329)
EC3G	5.77 (21.67)	7,94 (6.27)	0.37 (0.98)
GC-3-G	13.36 (50.17)	7.80 (6.16)	0.45 (1.19)
EGC-Der	-	9.95 (7.86)	1.68 (4.42)
C-Der	-	1.26 (0.99)	0.84 (2.22)
EGC-3G-Der	-	27.30 (21.56)	9.99 (26.30)
EC-Der	-	44.21(34.93)	11.85 (31.20)
EC3G-Der	-	14,30 (11.30)	0.56 (1.47)
Total	26.61	126.60	43.53
Prodelphinidins (PD)	16.62 (62)	47.39 (37)	15.82 (42)
Procyanidins (PC)	9.99 (38)	79.21 (63)	21.71 (57)

*GC = gallocatechin, EGC = epigallocatechin, C = catechin, EC = egalocatechin, EC3G = egalocatechin-3-gallate, GC = 3-G- gallocatechin-3-gallate, EGC-Der = derivatized epigallocatechin, C = derivatized catechin, EGC-3G-Der = derivatized epigallocatechin-3-gallate, ECDer = derivatized egalocatechin, EC3GDer-derivatized egalocatechin-3-gallate.

Ferreira (2020), evaluating the bark of A. occidentale, observed a UV profile with absorption bands characteristic of proanthocyanidins; however, there is no report in the literature on the detailed characterization of prodelphinidin and procyanidin for the stem bark of this species. PC and PD units were identified in the crude extracts of this research. In the study by Zhang and Lin *et al.* (2008), evaluating the bark of *Canario album*, MALDI-TOF mass spectra showed the presence of epi-catechin and epi-gallocatechin. (Michodjehoun-Mestres *et al.*, 2009) extracted tannins from the skin and pulp of cashew apples from Brazil and Benin (West Africa) and separated them from the monomeric phenols. The tannins were subjected to acid-catalyzed degradation in the presence of phloroglucinol, and the products were analyzed through HPLC-DAD/ESI-MS. The tannins in the skin and pulp contained high percentages of (-)-epigallocatechin (57%) and (-)-epigallocatechin-O-gallate (35%). The latter, a high value also found in this study (47.86%) for the stem bark of *A. occidentale*, contained 18.37% epigallocatechin. Followed by small amounts of (-)-epicatechin (4%) and (-)-epicatechin-3-O-

gallate (4%). In the current study, higher values were found for the same compounds, namely epicatechin-3-O-gallate (20.02%) and epicatechin (86.53%).

(Trox *et al.*, 2011) evaluated the skin covering the cashew nut, known as 'testa', which contained catechin and epicatechin at concentrations of 5.70 and 4.46 g kg^{-1} of DM, respectively. This study found 7.6 and 70.11 mg g^{-1}, or 0.007 and 0.07 g kg^{-1}, for catechin and epicatechin, respectively. (Vilar *et al.*, 2016) evaluated the activity of the ethyl acetate (EtOAc) phase of *A. occidentale* bark, catechin, epicatechin, epigallocatechin, and gallic acid. They found 6.89 µg g^{-1} (0.00689 mg g^{-1}) for catechin and 11.62 µg g^{-1} (0.01162 mg g^{-1}) for epicatechin. These values are significantly lower than those found in this study, which may be related to the method used, plant age, species phenology, and other factors.

Procyanidins and prodelphinidins were found in all fractions, with procyanidin predominating in FII and FIII, unlike FI, which had a predominance of prodelphinidin. In the study (Ferreira *et al.*, 2020), evaluating the bark of *A. occidentale*, they observed a UV profile with absorption bands characteristic of proanthocyanidins. However, there is no report in the literature on the characterization of prodelphinidin and procyanidin in the stem bark of this species. (Michodjehoun-Mestres *et al.*, 2009) Reinforce that cashew tannins (nut and fruit) are of high molecular weight, mostly prodelphinidins (>70%) (Macheix *et al.*, 1990); They have been reported qualitatively, but not quantitatively, as such (Satyanarayana *et al.*, 1978). (Wei *et al.*, 2010) evaluated an extract from *Acacia confusa* bark; although they did not quantify, they identified the compounds and reported a predominance of procyanidins. In a study by Zhang and Lin *et al.* (2008) evaluating the bark of the species *Canarium album*, they found a predominance of signals corresponding to procyanidins and prodelphinidins.

It is believed that in the water treatment process, monomeric coagulants initiate interaction, oligomeric coagulants stabilize aggregates, and polymeric coagulants provide the final bridging, resulting in faster coagulation, larger flocs, and better impurity removal. The studied extract is rich in proanthocyanidins with a higher quantity of PC units. Previous studies by Bello *et al.* (2022) and Bello *et al.* (2020) established a positive correlation between higher proanthocyanidin content and charge density. Dou *et al.* (2024) report that coagulants with higher charge density (PC) are generally preferred for modifying tannin-based coagulants because charge neutralization is required in most water treatments, corroborating the results of this research.

3.3. Coagulation assays

As shown in Table 5, the tannins extracted with sodium hydroxide did not improve the satisfactory efficiency in water purification. However, the tannins extracted with sodium sulfite presented results comparable to those obtained using ferric chloride. In both experiments, the initial turbidity of the water was standardized at 150 nephelometric turbidity units (NTU).

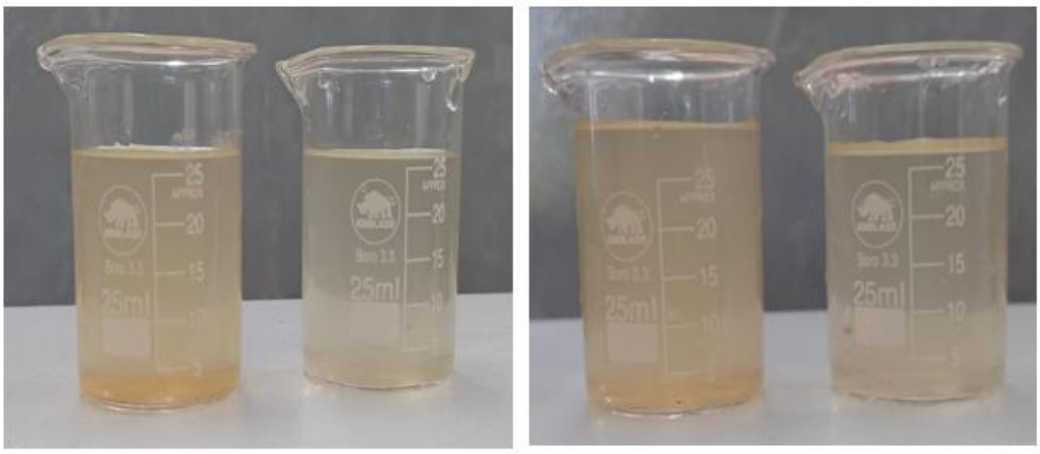
The results obtained with the cashew coagulant supplemented with 3% sodium hydroxide show a decrease in turbidity over time. Specifically, the final turbidity at the 150 mg L^{-1} concentration decreased from 101.59 NTU to 81.96 NTU over a 60-minute interval. Similarly, for the 200 mg L^{-1} concentration, the turbidity decreased from 106.37 NTU to 86.63 NTU. Analysis of the final turbidity data for both concentrations indicates that the results meet the criteria established by Resolution 357/05 of the National Environmental Council (CONAMA). This resolution determines turbidity standards for different classes of water bodies. In particular, for Class II freshwaters: those intended for supply for human consumption after conventional treatment, for the protection of aquatic communities, for primary contact recreation, for the irrigation of vegetables and fruit plants, as well as for use in parks, gardens and sports and leisure fields, in addition, the maximum turbidity limit in this resolution is set at 100 NTU (CONAMA, 2011).

Table 5. Mean values found for turbidity after water treatment with different concentrations (150 and 200 g mL^{-1}) of cashew tree tannins, having ferric chloride as a comparison.

Cationized tannins from material extracted with 3% sodium hydroxide		
	150 g mL^{-1}	**200 g mL^{-1}**
Sedimentation time (minutes)	**Final turbidity (NTU)**	**Final turbidity (NTU)**
10	101.59 ± 1,83	106.37 ± 3,06
20	98.54 ± 1,92	100.18 ± 2,37
30	90.52 ± 2,06	97.78 ± 3,30
40	85.94 ± 2,77	92.68 ± 2,27
50	83.95 ± 1,84	90.09 ± 0,79
60	81.96 ± 1,80	86.63 ± 0,45
Cationized tannins from material extracted with 3% sodium sulfite		
10	8.04 ± 1,28	8.27 ± 0,56
20	5.75 ± 0,96	7.14 ± 1,10
30	4.50 ± 0,41	4.35 ± 0,72
40	3.86 ± 0,47	3.43 ± 0,20
50	3.17 ± 0,16	3.25 ± 0,36
60	2.57 ± 0,32	2.66 ± 0,13
Ferric chloride (commercial product)		
10	6.12 ± 2,99	48.37 ± 2,51
20	5.28 ± 2,51	44.57 ± 3,03
30	4.99 ± 2,50	42.27 ± 2,75
40	5.60 ± 1,91	39.77 ± 1,99
50	4.95 ± 2,44	39.00 ± 2,17
60	4.93 ± 1,81	38.23 ± 2,14

*NTU = nephelometric turbidity unit.

Figure 3 shows the initial and final turbidities for the coagulation test using cationized tannins extracted with sodium hydroxide from cashew tree bark.

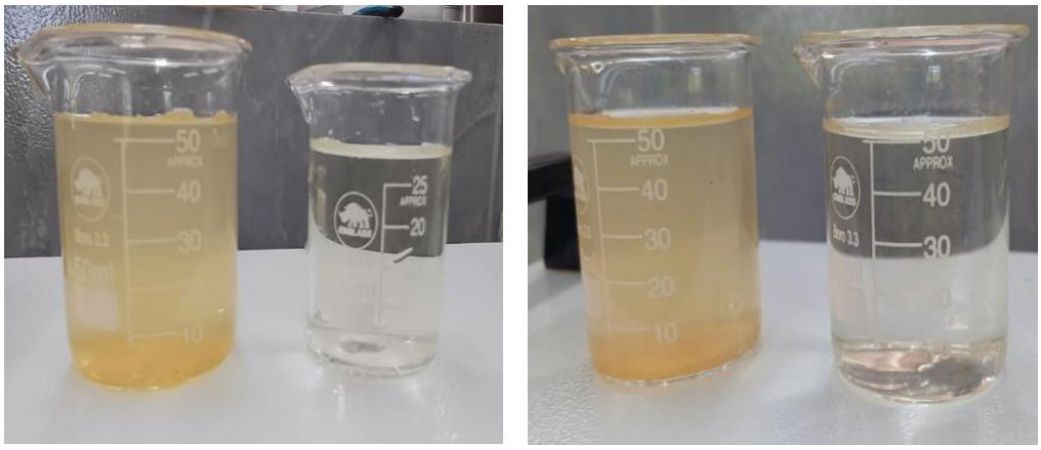


(a) (b)

Figure 3. Effect of the concentration of cationized tannins extracted with 3% sodium sulfite on decreasing water turbidity (a. 150 to 81.96 NTU at 150 g L^{-1}, and b. 150 to 86.63 NTU at 200 mg L^{-1}).

The cashew coagulant extracted with 3% sodium sulfite demonstrated higher efficiency in reducing turbidity at both concentrations (150 and 200 mg L^{-1}). After 10 minutes, turbidity was significantly lower at 8.04 and 8.27 NTU and continued to decrease until reaching minimum values of 2.57 and 2.66 NTU at 60 minutes. It is essential to mention that the initial turbidity of the water, before the addition of any coagulant, was 150 NTU, evidencing the excellent performance of this method in reducing water turbidity. (Anjos *et al.*, 2022), analyzing the treatment of water with cationized coagulants from Anacardium occidentale, obtained a final turbidity of 2.23 NTU at a concentration of 100 mg L^{-1}, which was close to the data analyzed in the present study.

The results obtained in this experiment, using the coagulant extracted from the cashew tree with 3% sodium sulfite added, meet the criteria established by Resolution No. 357/2005 of the National Environmental Council (CONAMA, 2011). This resolution, in Section I on freshwater, defines in Article 4 the classification of waters for various purposes, including human consumption after simplified treatment, the protection of aquatic communities, the irrigation of vegetables that are consumed raw, and fruits that grow close to the ground and are eaten with the peel, in addition to ensuring the preservation of aquatic communities in Indigenous Lands. According to the standards and conditions established by the resolution, the maximum permitted turbidity limit is 40 UNT (CONAMA, 2011). The initial and final turbidity of the coagulation test using sodium sulfite are shown in Figure 4.



(**a**) (**b**)

Figure 4. Effect of the concentration of cationized tannins extracted with 3% sodium sulfite on decreasing water turbidity (a. 150 to 2.57 NTU at 150 g L^{-1}, and b. 150 to 2.56 NTU at 200 mg L^{-1}).

The addition of ferric chloride, used as a chemical coagulant in the experiment, yields different data depending on its concentration, unlike the extracted tannins. At a concentration of 150 mg L^{-1}, the reduction in turbidity is similar to that observed with tannins extracted with sodium sulfite. However, at a 200 mg L^{-1} concentration, the final turbidity values remain very high, reaching 48.37 NTU at 10 minutes and slowly decreasing to 38.23 NTU at 60 minutes. However, this coagulant, in addition to posing environmental and health risks, is highly corrosive, significantly reducing the durability of equipment and pipes used in water treatment plants (Anjos, 2021). It was observed that using cationized tannins resulted in minimal changes in water pH (Table 6), providing a significant advantage by reducing the costs associated with correcting this parameter.

After the treatment tests, it was found that cationized coagulants (sodium hydroxide and sulfite) caused a slight change in water pH, which remained within the values established by Ordinance GM/MS No. 888. This ordinance specifies that the pH value of water in distribution systems must be maintained between 6.0 and 9.0 (Brazil, 2021). The results obtained in this

research corroborate those of Anjos *et al.* (2022). The authors reported a mean pH of 7.01 in their work using cationized cashew coagulants. However, in that work, the tannins were extracted without the addition of salts. This comparison is relevant since both studies used tannins from the same forest species, allowing the evaluation of the additional effects of salts on pH stability. Tannins do not alter the pH of treated water because they do not consume alkalinity, and they are effective over a pH range of 4.5 to 8.0 (Silva, 1999). According to Skoronski *et al.* (2014), this wide range of action eliminates the need for alkalizing agents, thereby reducing sludge production. Sludge treatment and disposal are high-cost activities, and growing concerns and regulations aimed at preserving environmental quality have limited, and in some cases even prohibited, sludge disposal in nearby watercourses (Richter and Netto, 2021).

Table 6. Initial and final pH of water subjected to treatment with cationized tannins of coagulants, with the addition of sodium hydroxide and sodium sulfite, as well as ferric chloride, in the water treatment test.

Type of tannins	Concentrations (mg L^{-1})	Initial pH	Final pH
Cationized cashew tannins extracted with 3% of sodium hydroxide	150	8.20	8.17
	200	7.88	8.17
Cationized cashew tannins extracted with 3% of sodium sulfite	150	8.48	7.77
	200	8.98	8.11
Ferric chloride (commercial product)	150	7.00	2.41
	200	7.00	4.42

When analyzing the water pH at both ferric chloride concentrations, a significant change was observed compared to the initial pH. This coagulant causes a sharp reduction in the final pH, especially at 150 mg L^{-1}, where the pH drops from 7.00 to 2.41. At 200 mg L^{-1}, the final pH remains very acidic at 4.42. (Silva, 2024), analyzing natural and chemical agents, also observed this same variation in pH values. These values do not comply with Ordinance GM/MS No. 888. Therefore, higher investment is required to correct these parameters when using ferric chloride. Using chemical coagulants requires adjusting the water's pH to keep it within the regulated range. In water distribution systems, this correction requires time and financial resources. On the other hand, using a natural coagulant that does not significantly alter pH allows for system optimization and reduces operating costs associated with water distribution (Silva, 2024).

4. CONCLUSIONS

This study demonstrated that chemically assisted extraction efficiently improved yield, but only 3% Na2SO3 maintained quality. The simple phenolic compounds in the peel were catechin gallate, gallic acid, catechin, epigallocatechin, epicatechin, ellagic acid glycoside, triterpenoids, and anacardic acid. Other complex phenolic compounds identified were the condensed tannins prodelphinidin and procyanidin, present in oligomeric and polymeric structures. The cationized tannins used as coagulants effectively reduced water turbidity while maintaining minimal pH variation, unlike the chemical coagulant ferric chloride, which made the water acidic. The use of tannins extracted from cashew peel with 3% Na2SO3 is a viable and environmentally friendly alternative for water treatment, with the extract's efficiency being chemically proven, especially when compared to traditional chemical products.

5. DATA AVAILABILITY STATEMENT

Data availability not informed.

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