



RELATIONSHIP BETWEEN AGE AND CONDENSED TANNIN CONTENT IN THE BARK OF TWO FOREST SPECIES GROWN IN NORTHEAST BRAZIL

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ABSTRACT

Objective: This research aimed to evaluate the influence of tree age on the condensed tannins content in the bark of two forest species.

Theoretical Framework: Tannins are phenolic compounds present in various parts of plants and have consolidated applications in the market. This way, silvicultural studies of different species with the potential to produce are relevant.

Method: Barks of *Azadirachta indica* at 5, 6, and 7 years and *Mimosa caesalpiniaefolia* at 6, 7, and 9 years were collected from an experimental plantation located in a forest area in Rio Grande do Norte, Brazil. The barks were then air-dried and ground to obtain a material subjected to extraction. Total solids content, Stiasny index, and condensed tannin content were determined from the hot-water extracts.

Results and Discussion: The condensed tannins content in *A. indica* bark did not vary as tree age increased, while for *M. caesalpiniaefolia*, the most productive age was determined to be six years old.

Research Implications: The experimental approach showed that *A. indica* presented stable tannin content in the bark regardless of age, allowing continuous and flexible management to produce these extractives. In contrast, *M. caesalpiniaefolia* presented condensed tannins, with their reactivity decreasing with age, indicating that younger trees are more suitable for high-quality extractions and commercial production.

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Originality/Value: Tannins content in *Azadirachta indica* bark remains stable over time, facilitating continuous management and *Mimosa caesalpiniiifolia* bark, the concentration of tannins is higher in younger trees.

Keywords: Sustainable Management, Non-Wood Forest Products, Phenolic Compounds, *Azadirachta Indica*, *Mimosa Caesalpiniiifolia*.

RELAÇÃO ENTRE IDADE E TEOR DE TANINOS CONDENSADOS NA CASCA DE DUAS ESPÉCIES FLORESTAIS CULTIVADAS NO NORDESTE BRASILEIRO

RESUMO

Objetivo: Esta pesquisa teve como objetivo avaliar a influência da idade da árvore no teor de taninos condensados na casca de duas espécies florestais.

Marco Teórico: Taninos são compostos fenólicos presentes em diversas partes das plantas e têm aplicações consolidadas no mercado. Dessa forma, estudos silviculturais de diferentes espécies com potencial de produção são relevantes.

Método: Cascas de *Azadirachta indica* aos 5, 6 e 7 anos e *Mimosa caesalpiniaefolia* aos 6, 7 e 9 anos foram coletadas de uma plantação experimental localizada em uma área florestal no Rio Grande do Norte, Brasil. As cascas foram então secas ao ar e moídas para obter um material submetido à extração. O teor de sólidos totais, o índice de Stiasny e o teor de tanino condensado foram determinados a partir dos extratos de água quente.

Resultados e Discussão: El contenido de taninos condensados en la corteza de *A. indica* no varió a medida que aumentaba la edad del árbol, mientras que para *M. caesalpiniaefolia*, se determinó que la edad más productiva era de seis años.

Implicações da Pesquisa: A abordagem experimental mostrou que *A. indica* apresentou um conteúdo estável de taninos na corte independentemente da idade, o que permitiu um manejo contínuo e flexível para produzir esses extrativos. Em contraste, *M. caesalpiniiifolia* apresentou taninos condensados, cuja reatividade diminuiu com a idade, o que indica que as árvores mais jovens são mais adequadas para extrações de alta qualidade e produção comercial.

Originalidade/Valor: O teor de taninos na casca da *Azadirachta indica* permanece estável ao longo do tempo, facilitando o manejo contínuo, e na casca da *Mimosa caesalpiniiifolia*, a concentração de taninos é maior em árvores mais jovens.

Palabras clave: Gestión Sostenible, Productos Forestales no Madereros, Compuestos Fenólicos, *Azadirachta Indica*, *Mimosa Caesalpiniiifolia*.

RELACIÓN ENTRE LA EDAD Y EL CONTENIDO DE TANINO CONDENSADO EN LA CORTEZA DE DOS ESPECIES FORESTALES CULTIVADAS EN EL NORESTE DE BRASIL

RESUMEN

Objetivo: Esta investigación tuvo como objetivo evaluar la influencia de la edad del árbol en el contenido de taninos condensados en la corteza de dos especies forestales.

Marco Teórico: Los taninos son compuestos fenólicos presentes en diversas partes de las plantas y tienen aplicaciones consolidadas en el mercado, por lo que los estudios silviculturales de diferentes especies con potencial para producirlos son relevantes.

Método: Se recolectaron cortezas de *Azadirachta indica* de 5, 6 y 7 años y de *Mimosa caesalpiniaefolia* de 6, 7 y 9 años de una plantación experimental ubicada en una zona forestal de Rio Grande do Norte, Brasil. Luego, las cortezas se secaron al aire y se molieron para obtener un material sujeto a extracción. El contenido de sólidos totales, el índice de Stiasny y el contenido de taninos condensados se determinaron a partir de los extractos de agua caliente.



Resultados y Discusión: El contenido de taninos condensados en la corteza de *A. indica* no varió a medida que aumentaba la edad del árbol, mientras que para *M. caesalpiniaefolia*, se determinó que la edad más productiva era de seis años.

Implicaciones de la investigación: El enfoque experimental mostró que *A. indica* presentó un contenido estable de taninos en la corteza independientemente de la edad, lo que permitió un manejo continuo y flexible para producir estos extractivos. En contraste, *M. caesalpiniaefolia* presentó taninos condensados, cuya reactividad disminuyó con la edad, lo que indica que los árboles más jóvenes son más adecuados para extracciones de alta calidad y producción comercial.

Originalidad/Valor: El contenido de taninos en la corteza de *Azadirachta indica* se mantiene estable en el tiempo, facilitando un manejo continuo y en la corteza de *Mimosa caesalpiniaefolia* la concentración de taninos es mayor en los árboles más jóvenes.

Palabras clave: Gestión Sostenible, Productos Forestales no Madereros, Compuestos Fenólicos, *Azadirachta Indica*, *Mimosa Caesalpiniaefolia*.

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1 INTRODUCTION

Plant tannins are phenolic compounds widely distributed in the plant kingdom and found in various parts of plants, such as roots, bark, leaves, and fruits (Sharma, 2019). Tannins have an astringent flavor, which helps defend the plant against attacks by fungi, bacteria, and viruses and protects against herbivores due to their harmful effects (Silva *et al.*, 2022). They have versatile industrial applications, from producing natural adhesives for wood panel bonding to generating products for water treatment (Szcurek, 2021, Iqbal & Poór, 2024, Medeiros *et al.*, 2024) and leather tanning, a purpose widely practiced in Northeast Brazil.

Anadenanthera colubrina var. *cebil.* is a native forest species in Northeast Brazil. Its bark is the primary source of tannins for the local industry, especially for leather tanning. These compounds are essential in this process since they interact with and stabilize proteins, transforming animal skins into resistant and durable leather (Divekar *et al.*, 2020). However, the decline in *A. colubrina*'s natural populations has resulted in growing concern about the environmental sustainability of tannins' production. Because of that, there is an urgent need to seek alternative sources for this native species. Nevertheless, sustainable production of tannins requires the identification of new forest species that can supply industrial demand without compromising ecological equilibrium. Therefore, it is essential to determine the ideal extraction age for potential new forest species for tannins production. This knowledge is crucial to



managing likely forest species, contributing to efficiency, and mitigating environmental impacts due to the overexploitation of native species (Malok *et al.*, 2020; Adel *et al.*, 2024).

Several factors may influence the concentration of condensed tannins in trees' bark, including climatic conditions, silvicultural practices, and harvest time. Souza *et al.* (2021) when analyzing the seasonality of tannins content in the bark of *Acacia mangium* grown in Northeast Brazil, observed that the tannin content was significantly higher at the end of the rainy season, regardless of the soil preparation method. Paiva *et al.* (2023) when studying the effect of thinning in *Mimosa caesalpiniaefolia* trees, concluded that this practice decreases the bark tannin contents and the Stiasny index of the hot-water extracts. However, thinning can help increase the production of trunks for specific uses, such as posts and firewood. Notwithstanding, there is still a lack of studies evaluating the influence of age on the production of condensed tannins in the bark of tree species.

Understanding how tree age influences bark tannins content is essential to optimize production and ensure sustainable exploitation (Skowrya *et al.*, 2015). Furthermore, the lack of detailed knowledge about the best ages for tannin harvesting and collection prevents the adoption of strategies and rational use of forest resources. Identifying the ideal age for tannin extraction can significantly improve the sustainability of forestry operations and contribute to preserving biodiversity since using alternative forest species saves the conventional ones from the native biodiversity (Desalegne, Ayele, Wasse, 2019; Ye *et al.*, 2012; Fraga-Corral *et al.*, 2020).

Within this context, this work aims to evaluate the influence of age on the concentration of condensed tannins in the barks of *Azadirachta indica* and *Mimosa caesalpiniaefolia* for the extraction of tannins, contributing to the diversification of sources of this substance in the market and promoting sustainable management practices.

2 MATERIAL AND METHODS

2.1 STUDY AREA AND MATERIAL COLLECTION

The study was conducted in an experimental plantation (5°51' 36" S and 35°20' 59" W) in Macaíba, RN, Brazil. The region has a rainy tropical climate, transitioning between the As and BSw types, according to the Köppen classification, with an average temperature of 27°C, a maximum of 32°C and a minimum of 21°C, an average annual relative humidity of 76% and rainfall ranging from 863.7 to 1,070.7 mm (Alvares *et al.*, 2014).



For each year of experimental assessment, bark samples were collected from five trees for each species, randomly along each individual's trunk. The collection was carried out when *A. indica* was five, six, and seven years old and *M. caesalpiniiifolia* was six, seven, and nine years old. All collections were carried out in the same period of each year. Using machetes, bark samples were taken homogeneously from different parts of the plant, avoiding girdling and the consequent death of the tree. The trees studied were selected based on their vigor and freedom from pest and disease attacks.

The barks were then air-dried and ground in a Willey-type mill to obtain a material with a low particle size. The powder was sieved, and the portion that passed through the 16-mesh (1.00 mm) and was retained in the 60-mesh (0.25 mm) sieve was used for further experimental procedures. The power obtained was homogenized, and its moisture content was determined (% based on the dry weight of the samples) to calculate the condensed tannins content from each sample.

2.2 EXTRACTION AND QUANTIFICATION OF TANNINS

The bark samples were subjected to hot-water extraction. For the extractions, three samples of 25 g of dry bark powder were taken from each forest species and transferred to flat-bottomed flasks with a capacity of 500 mL. In each flask, 250 mL of deionized water (ratio 1:10—bark weight to water volume) was added and heated until boiling. The system was kept under reflux for two hours. Each powder sample was subjected to two sequential extractions to yield the maximum amount of extractives possible. After each extraction, the aqueous extract was passed through a 150 mesh (0.105 mm) sieve and a flannel cloth to remove powder particles. The extract was homogenized and filtered through a sintered glass funnel with porosity 2. Each sample was then made up to 500 mL with distilled water, and three 50 mL aliquots were separated from each extract. Two aliquots were used to determine the condensed tannins content (TTC), and the other was evaporated in a laboratory oven at 103 ± 2 °C for 48 hours until absolute dryness to determine the total solids content (TST) based on equation 1.

$$\text{TST}(\%) = (\text{Mi} - \text{Mf}) \times 100 \quad (1)$$

where:

TST(%) = total solids content (%);

Mi = initial weight of extract (g);

Mf = dried material after water evaporation (%).



To determine the condensed tannin content (TTC) present in each sample, the Stiasny method, was used. For this purpose, 4 mL of formaldehyde (37% weight/weight) and 1 mL of concentrated hydrochloric acid were added to 50 mL of the raw extract. Each mixture was heated until boiling and kept under reflux for 30 minutes. Under these conditions, tannins formed insoluble complexes and were separated by simple filtration. In this case, a paper filter placed in a 10-cm diameter Büchner funnel was used. The precipitate retained on the filter was oven-dried at 103 ± 2 °C for 24 h, weighed, and the Stiasny index was calculated based on Equation 2.

$$I (\%) = (M2 / M1) * 100 \quad (2)$$

where:

I(%) = Stiasny index (%);

M1 = solids weight of 50 mL of raw extract (g);

M2 = precipitate weight (g).

The condensed tannins content from each sample was obtained by multiplying the Stiasny index by the total solids content through Equation 3.

$$TTC (\%) = TST * I / 100 \quad (3)$$

where:

TTC = condensed tannins content (%);

TST = total solids content (%).

The experiment followed an entirely randomized design with two forest species and three ages. To verify whether the differences among treatments observed values were statistically significant, an analysis of variance (ANOVA) was performed, comparing the means of condensed tannin contents between the different ages of the species. When the ANOVA indicated significant differences, the Tukey test compared the means, at a 95% probability. So, multiple comparison tests were performed to identify which ages and species presented significantly different concentrations.

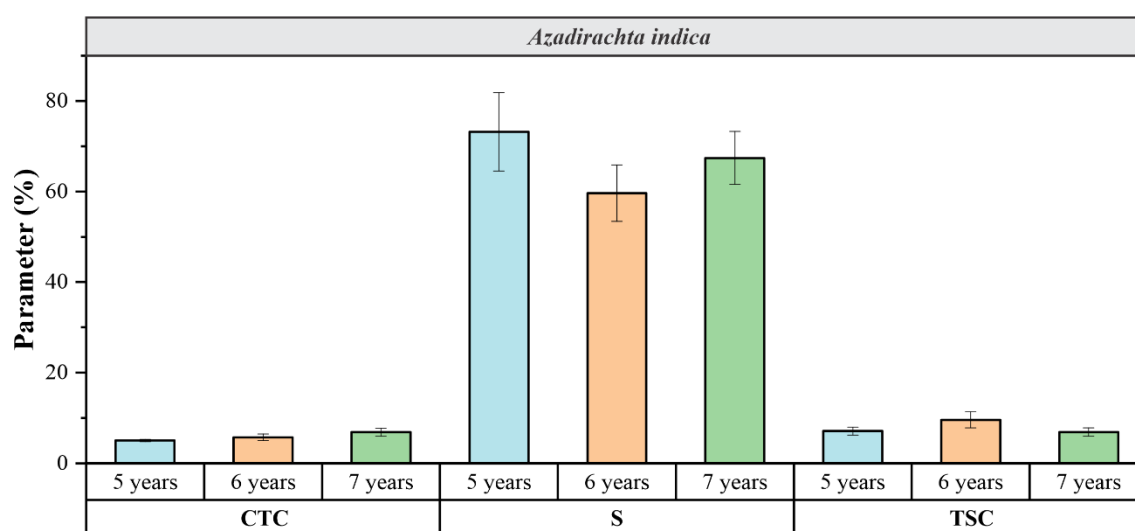


3 RESULTS AND DISCUSSION

As presented in Table 1, the values observed for total solids content (TST), condensed tannins content (TTC), and Stiasny Index (I) in *Azadirachta indica* bark at different ages (5, 6, and 7 years) did not show statistically significant differences. In terms of forest management to collect bark for tannins production, no matter the age of trees age, the collection can be done without modification in the aftermath (Figure 1).

Figure 1

Total solids content (TST) and Stiasny index (S) found for hot-water extracts and total condensed tannins determined for *A. indica* bark at five, six and seven years.



*Means followed for the same letters in the same column are statistically equal at a 95% probability

Conclusively, the research on *A. indica* bark reveals that the age of the trees, whether 5, 6, or 7 years, does not significantly affect the quantity and quality of tannins extracted. This implies that trees of any of these ages can be utilized for bark collection and subsequent tannins extraction without compromising the efficiency or quality of the product. The potential of tannins from *A. indica* bark in developing new therapeutic agents is substantial and promising. They have significant potential as natural antimicrobial agents with antibacterial, antifungal, antiviral, and antiparasitic activities. They are effective against antibiotic-resistant pathogens and biofilms, have antioxidant properties, and have low toxicity compared to synthetic drugs. Therefore, their use is promising in developing new therapeutic agents in medicine, dentistry, food safety, and agricultural pest control. These practical implications should reassure and inspire confidence in the potential of this research.

Haroun (2022) evaluated the use of tannins extracted from *Acacia nilotica* pods



(hydrolyzable tannins) and *A. indica* bark (condensed tannins) as alternatives to chrome tanning in leather processing. The results showed that tanning with tannins from *Acacia nilotica* and leather retanning with *A. indica* tannins produced wet-white leather with better physical and mechanical properties. Konai *et al.* (2020) extracted tannins from *A. indica* bark using an aqueous solution with sodium bisulfite and sodium bicarbonate, obtaining a yield of 35%. However, these tannins presented poor-quality results in the development of resins and biocomposites when compared to tannins from other plant species used in this same research, such as *Daniellia oliveri*, *Ficus sycomorus*, and *Butyrospermum parkii*.

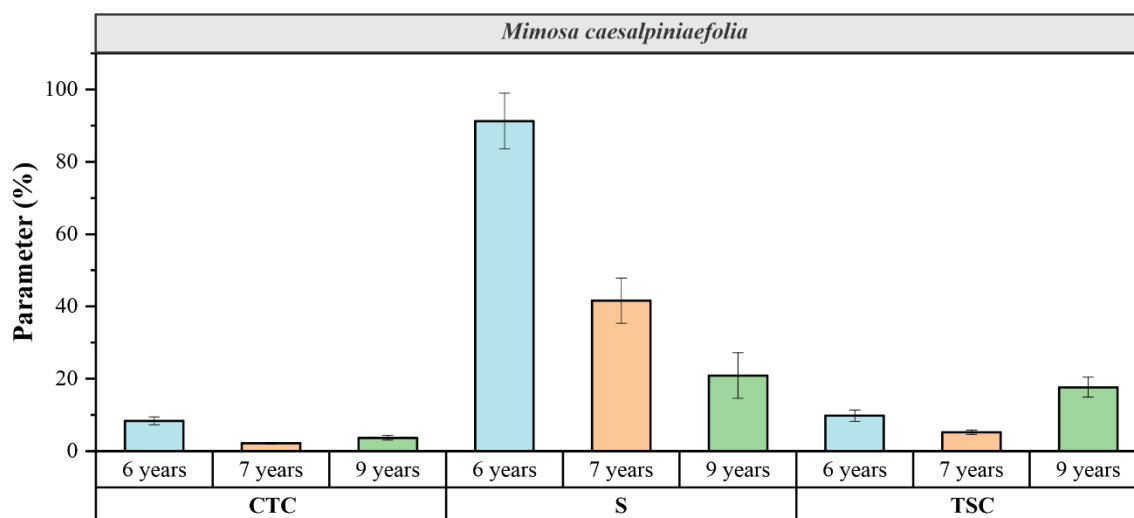
Tannins have several applications that depend on their characteristics and properties. In the case of *Azadirachta indica*, it is crucial to evaluate the best way to use these compounds. Studies indicate that tannins from this species are particularly effective for medicinal applications, highlighting their potential as a therapeutic agent for health treatments due to their antimicrobial and antioxidant properties (Mahmoud *et al.*, 2011; Patil *et al.*, 2013; Patel *et al.*, 2013). According to the results found in this research, the fact that tannins concentrations are not affected by the age of the plant allows for adequate management of *A. indica*. The constancy in tannin production provides a continuous and predictable harvest, benefiting the industry by ensuring a stable supply of high-quality raw materials. This contributes to the sustainability of forestry operations, reducing the need for intensive monitoring and promoting the environmental conservation of native forest resources.

For *M. caesalpiniifolia*, the average percentages of total solids content, the Stiasny index of the extract, and the total condensed tannin content of the bark of the species at six, seven, and nine years of age are presented in Figure 2.



Figure 2

Total solids content (TST) and Stiasny index (S) found for hot water extracts, and total condensed tannins determined for bark of *M. caesalpiniiifolia* at six, seven and nine years.



*Means followed for the same letters in the same column are statistically equal at a 95% probability

The total solids content (TST) in *M. caesalpiniiifolia* extracts varied significantly with tree age. At six years, the TST was 9.18%, decreasing to 5.2% at seven years and increasing to 17.66% at nine years, representing an increase of 92% compared to the initial age of evaluation (Table 1). The condensed tannins content (TTC) also varied with age. The TTC was 8.38% at six years, dropping drastically to 2.16% at seven years and increasing to 3.69% at nine years (Table 1). This parameter directly quantifies the amount of tannins in the assessed material. This variation between ages for this species is most likely due to changes in the metabolism of trees as the aging process occurs, which affects the production and accumulation of secondary metabolites, such as tannins.

Also, in Figure 2, the Stiasny index observed for *M. caesalpiniiifolia* at six was 91.27%, followed by 41.60% at seven and 20.90% at nine years. The high S value at six years indicates the bark is free of other impurities. This parameter reflects the proportion of condensed tannins that can be precipitated with formaldehyde, indicating the quantity and quality of tannins extracted. The significant reduction in the Stiasny index with increasing age may be related to the change in tannins composition or the presence of other phenolic compounds that interfere with precipitation. At six years of age, the high concentration of tannins and the high S value for *M. caesalpiniiifolia* indicate a phase of high tannins production, possibly as a defense against herbivores and pathogens. The TST at this age suggests a robust output of solid compounds, with the tree prioritizing the synthesis of defensive secondary metabolites.

At seven years of age, the sharp decline in both S and TTC and a reduction in TST may



indicate a metabolic transition phase. In this phase, the plant appears to be allocating resources to vegetative growth and other essential physiological functions to the detriment of tannins production. This redirection of resources is standard during critical periods of development when the plant needs to focus its efforts on structural growth. At nine years of age, there is a significant increase in TST and a partial recovery of TTC, although IS decreases. This increase in TST and the recovery of TTC suggest that, after stabilizing its vegetative growth, the plant redirects its resources to producing defensive compounds such as tannins. The lower percentage of IS may indicate a change in the composition of tannins or the presence of other phenolic compounds that interfere with tannin precipitation, reflecting a metabolic adaptation of the plant.

The levels of condensable tannins in the bark of *M. caesalpiniiifolia* were evaluated and showed significant values. The bark of this species proved to be a potential source of tannins for industrial applications, such as the production of adhesives and leather tanning, due to the high levels of reactive polyphenols obtained. Tannins in the bark of *Mimosa caesalpiniiifolia* were analyzed in a study that evaluated the effect of thinning on biomass volumes and tannin content. The study revealed that thinning significantly affected the S, reducing tannin reactivity, but did not change the total concentration of condensed tannins (TTC). Dry wood productivity was higher in plots without thinning. These results highlight the importance of adequate management to maximize the quantity and quality of tannins extracted from the bark of *M. caesalpiniiifolia* (Paiva *et al.*, 2023).

The study of leaf chemistry in *Populus tremuloides* revealed significant age-related changes in several chemical constituents. Condensed tannins content doubled in the first five years and then stabilized, while concentrations of phenolic glycosides were high in younger branches and decreased with age. Nitrogen and carbohydrate concentrations also varied among age classes, with little change in more mature branches, and mean sugar concentrations decreased with tree age. The authors attribute these changes in the development of chemical defenses to evolutionary factors related to herbivory and ecosystem function. The findings highlight the complex interplay between plant development, chemical defenses, and ecological interactions, contributing to a better understanding of plant-herbivore dynamics and ecosystem function.

In the present research, the relationship between tree age and tannin extraction was crucial for *M. caesalpiniiifolia* and *A. indica*. Optimizing tannin production and quality is critical to contributing to sustainable management practices and diversifying tannin sources in the market.



4 CONCLUSION

A. indica maintains stable tannin concentrations throughout the ages analyzed (five, six, and seven years), allowing flexible and continuous species management. In contrast, *M. caesalpiniifolia* decreases condensed tannins' concentration and reactivity with increasing age, indicating that younger trees (six years) are more suitable for high-quality extractions.

These findings reinforce the importance of sustainable management practices that consider tree age to optimize tannin production and quality. Thus, identifying ideal ages for condensed tannin extraction maximizes production efficiency and simultaneously promotes the preservation of biodiversity, the health of forest ecosystems, and the development of new products.

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