Original Research Article

Tannin content of the bark and branch of Caatinga species

ABSTRACT

This research aimed to determine the concentration of tannins in the bark and in the branches of ten species of Caatinga occurrence species. The Folin-Denis colorimetric method was used to determine the phenol content and the tannins are precipitated using a protein. The tannin content was obtained by the difference between the supernatant and the non-tannic phenol content. The data were subjected to the Shapiro-Wilk normality's test and after, to Analysis of Variance using a 2x10 factorial design and Turkey's test was used to detect differences. For bark sample, the species Parapiptadenia zehntneri, Parapiptadenia rigida and Libidibia ferrea presented the three highest percentages among the studied species, being 10.84%, 10.74% and 10.27%, respectively. For branch sample, Aspidosperma pyrifolium presented the highest percentage of tannins among the ten species, with 9.15% of these substances. It is possible to suggest the use of other parts of the tree to extract the tannins, such as the branches and their bark, offering an alternative for the extraction that is usually made from the main trunk and providing sustainability to the Caatinga.

Comment [AL1]: Latin names in italics.

Keywords caatinga's species, non-timber forest product, wood extractives and phenolic compounds

Comment [AL2]: You have to avoid *and* in Keywords. Correct it.

1. INTRODUCTION

The northeastern Brazilian region has a range of Non-timber forest products (NTFPs) that can be used from the diversity of species, generating employment and income. More than 70 plant species were cataloged in the Caatinga, which have potential for use as NTFPs, of which 18 are highlighted as being of intensive use. Among them, species such as *Libidibia ferrea, Myracrodruon urundeuva* and *Tabebuia aurea*, are examples of species considered to be priority for use by communities [1,2].

Among the most well-known NTFPs, the tannins are highlighted, they can be defined as substances that have the capacity to precipitate proteins and can be classified as natural or synthetic according to their origin. The plants use these substances for defense against fungi and other pathogens, due to their toxicity, which stimulates their use also as a natural preservative. The species exploited for their high percentage of tannic substances are generally used for medicinal purposes, in leather tanning process, manufacture of adhesives and paints [3,4,5].

The tannin content varies according to the species, as well as their age, part of the plant collected, season and place of collection, but in general the highest percentages are in the bark and leaves [6]. Studies show that, in addition to the stem, other parts of the trees may contain a number of tannins that can be extracted for use in, for example, the industry [7].

In general, Caatinga species are related to medicinal uses such as anti-inflammatory, healing and antioxidant, probably due to the presence of phenolic compounds and, specifically, tannins. Some studies carried out with Caatinga species that presented considerable levels of potential tannins for extraction were *M. urundeuva*, for medicinal use and leather tanning; P. pyramidalis for its anti-inflammatory, antioxidant and antimicrobial properties; *P. rigida* and *P. zehntneri*, present a high percentage of tanantes substances, which confers resistance to the attack of xylophages [8,9,10,11,12].

On the other hand, these substances can cause problems when present in species used as forage. Studies point out the relation of the tannic substances to the abortion of pregnant ruminants. This emphasizes the importance of studying the chemical composition of the species, such as Caatinga, to predict the possible effects that can cause according to the application which will be destined [13,14].

This research aimed to quantify and compare the content of tannins present on the branches and on the bark of species of occurrence in the caatinga, verifying the potential use of other parts of the tree for greater use in the logging of these species.

2. MATERIAL AND METHODS

2.1 Study Area Characterization

The material was collected in the city of Piranhas (9º 37 'S and 37º 46' W, altitude of 110 m), located in the State of Alagoas, located in the Meso-region of the Sertão and Alagoana Microregion of the São Francisco Region. The city has a Tropical semiarid climate, according to the Köppen classification, with an average rainfall of 492.2 mm / year and and average annual air temperature of 26.6 °C [15].

Comment [AL3]: Correct. 'and' is duplicated

Branches of the following species were used for the research: *Parapiptadenia zehntneri* (Harms) M.P.Lima & Lima), *Parapiptadenia rigida* (Benth.) Brenan, *Myracrodruon urundeuva* Allemão, Mimosa hexandra Micheli, Poincianella pyramidalis (Tul.) L.P. Queiroz, *Tabebuia aurea* (Silva Manso) Benth. & Hook. f ex S. Moore, *Commiphora leptophloeos* (Mart.) J.B. Gillett, *Erythrina velutina* Wild., *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz and *Aspidosperma pyrifolium* Mart. & Zucc. All the species used are of occurrence of the Caatinga biome and were chosen from the use history for de extraction of non-timber forest products. The branches were collected from adult trees, during the summer, from an area of managed native forest. No information is available regarding the age of the trees.

The branches were randomly removed from the first third of the canopy and were packed in properly sealed plastic bags. All the collected material presented perfect phytosanitary conditions

2.2 Sample Preparation

All the next steps of the methods to obtain the tannins content were made according to the methodology described by [4] under controlled laboratory conditions. The barks were removed from the branches with the help of knives and reduced to smaller pieces, then were air dried. The branches were fragmented and then milled in a knife mill (Willey) with a 2 mm particle selection screen to obtain sawdust. The barks were about 2 mm thickness and the branches were 2 cm in diameter. After processing, the materials were packaged in hermetically sealed containers and kept at room temperature.

Comment [AL4]: **Methodology** is the systematic, theoretical analysis of the methods applied to a field of study. It comprises the theoretical analysis of the body of methods and principles associated with a branch of knowledge.
Methodology is wrong.!!!!!!!!!!!!!!! Do you understend.

2.3 Obtaining the Plant Extract

For the preparation of the plant extracts, 0.2 g of sawdust or barks were used, to which 20 mL of ethanol (80%) in Erlenmeyer flask were added, remaining for a period of 30 minutes

under constant agitation. The extract obtained was filtered on filter paper into a 50 ml volumetric flask, then the residue was washed and the volume of the flask filled with distilled water. Five replicates were performed for each sample, for both bark and branches. The extract was homogenized and transferred to a bottle, identified and kept refrigerated until the following ingredients were used.

2.4 Determination of Total Phenolic Compounds

For the determination of total phenols, the following solutions were used: saturated sodium carbonate solution, standard tannic acid solution, dilute standard solutions and the Folin-Denis reagent. All of them were previously prepared.

Using a pipette, 0.2 ml of standard tannic acid solution (concentrations: 25, 50, 100 and 200 mg.L-1) was transferred to a tube, then 5 ml of distilled water, 1.0 ml of saturated sodium carbonate solution (350 g / L) and 0.5 ml of the Folin-Denis reagent were also added in the tube. The same procedure was performed for the plant extracts obtained in step “Obtaining the Plant Extract”.

The spectrophotometric reading of the vegetable extracts and standard solutions was performed in the 760 nm range, using a spectrophotometer (Spectrum brand, model SP 1105 335 at 1000 nm), to obtain the absorbance values and the standard curve preparation.

2.5 Tannin Precipitation

After the determination of the total phenols, the same samples were used to precipitate the tannins, adding in each one 50 mg of protein (bovine serum albumin), responsible for reacting with the tanic substances and performing the precipitation. The tube was sealed and shaken for one minute. The mixture was allowed to stand for a period of 24 hours in a refrigerator and then centrifugation was performed for 10 minutes at a speed of 2000 rpm. Then, the spectrophotometric reading of the plant extracts was made, also in the 760 nm range, was carried out to determine the supernatant.

Comment [AL5]: rotation speed

From the supernatant the total non-tannin phenols content was determined. The tannins present were calculated by difference between the supernatant and the precipitate.

2.6 Experimental Design and Statistical Analysis

For the statistical analysis, the software Assistat 7.7 beta was used. The data were submitted to the Bartlett test (5%) to verify the homogeneity of the variances, and the Shapiro-Wilk test (5%) to verify its normality. The results were submitted to Variance Analysis, considering a factorial 2x10, where the sources of variation were the parts of the tree (bark of the branch and branch), the species and the interaction of the factors (parts of the tree x species). In those that presented significant difference, the Tukey’s test was used at the 95% probability level for the comparison of averages.

3. RESULTS AND DISCUSSION

By means of spectrophotometric reading, a default curve was obtained (figure 1) from different concentrations of tannic acid.

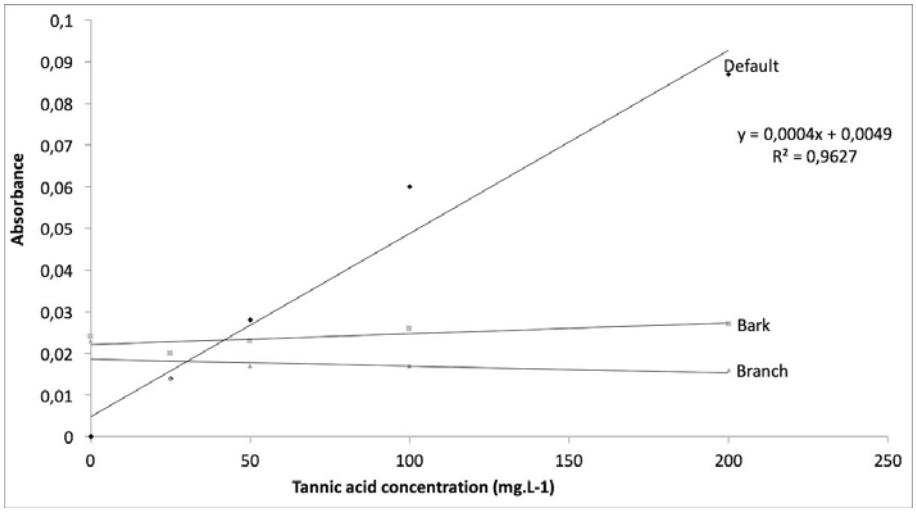

Fig. 1. Default curve of tannic acid and the resulting curves from the values obtained by the absorbances for bark and branch

Comment [AL6]: Change decimals on graphs. Instead of commas put dots. change options in the settings: region

According to the coefficient of determination (R^2), the standard curve of tannic acid fits the model. Thus, the equation: y = 0.0004 x + 0.0049, obtained from the tannic acid concentrations by the regression analysis, was used to obtain the tannin content in the extracts.

In general, the species showed higher tannin content in the bark samples, especially P. zehntneri, P. rigida and L. ferrea, as can be seen in Figure 2.

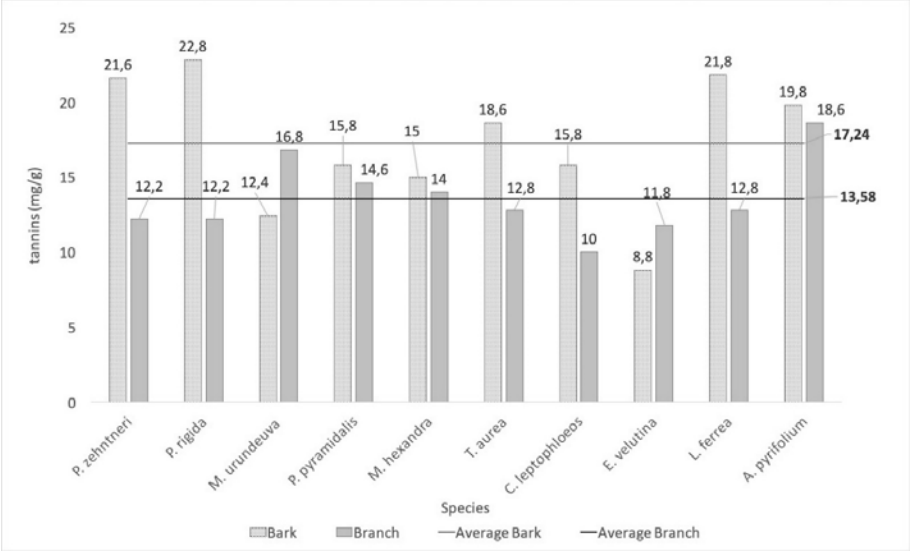

Fig. 2. Values obtained from the amounts of tannins (mg / g) with the general average of bark and branch of Caatinga's species

Comment [AL7]: Change decimals on graphs. Instead of commas put dots.

A significant statistical difference was observed between position and species, as well as the respective interaction of mean tannin contents for the ten species.

It was verified that the effect of the interaction of the factors part of the plant x species was significant for the factors individually and in the interaction, which shows that they are not independent. In this way, the test of comparison of means of interaction was verified, verifying tannin contents within the species in the bark and branch samples, according to Table 1.

Table 1. Mean values of total tannin content (%) present in the bark and branch, respectively, of ten tree species of the Caatinga

Comment [AL8]: What is respectively for bark and branch. Nothing. Correct sentence.

Species	Parts of the plant					
	Bark			Branch		
	Mean	Standard Deviation	Coefficient of variation (%)	Mean	Standard Deviation	Coefficient of variation (%)
P. zehntneri	10,84 aA	2,91	26,89	5,93 aB	2,05	34,63
P. rigida	10,74 aA	1,46	13,57	5,69 aB	1,76	31,00
L. ferrea	10,27 aA	1,89	18,40	6,20 aB	1,99	32,14
A.pyrifolium	9,59 aA	2,67	27,85	9,15 aA	2,65	28,97
T.aurea	9,00 abA	2,75	30,60	6,04 aA	2,60	43,01

Comment [AL9]: Instead of commas put dots. Is it so difficult?

P. pyramidalis	7,74 abA	1,57	20,32	7,13 aA	2,91	40,84
M. hexandra	7,23 abA	1,25	17,36	6,31 aA	2,43	38,53
C. leptophloeos	6,75 abA	4,30	63,63	4,95 aA	0,92	18,52
M. urundeuva	6,07 abA	4,12	67,86	7,85 aA	3,05	38,90
E..velutina	4,02 bA	1,49	37,05	5,80 aA	2,35	40,54
Average overall	8,23	3,23	49,60	6,50	2,42	37,27

Means followed by the same letter do not differ statistically from each other, according to the Tukey test at the 5% level of significance. Upper case letters in horizontal correspond to the factor part of the tree (bark of the branch or branch) and lowercase letters in vertical corresponds to the factor species.

**Values in parentheses correspond to the standard deviation and coefficient of variation (%), respectively.*

Considering the part of the tree used to determine the tannin content (bark and branch), a statistical difference was observed between these factors. This behavior is within the expected range since many studies confirm the presence of tannins in higher percentages in tree barks [6] which may occur due to its use as a mechanism of defense against pathogens and its cicatrizing effect.

The tannin content obtained for the bark varied between 4.02% and 10.84%, presenting the general average value of 8.23%, being the species *A. pyrifolium, P. zehntneri, P. rigida* and *L. ferrea*, similar among them and differed only from the *E. velutina* species. The other species did not present statistical differences among themselves. It was observed for the branch factor, mean values from 4.95% to 9.15%, with an overall mean of 6.50%.

Comment [AL10]: But why?

The *Acacia mearnsii* De Wild. is a species used for the commercial extraction of tannins in Rio Grande do Sul (Brazil) and can produce on average 27% of tannin only in the bark. When comparing the values obtained with the tannin content of the black acacia species, it is perceived that they are inferior. However, it is worth mentioning that the concentration of tannins decreases in the base-top direction, in addition to being present in a larger quantity in the older structures of the trees [16]. This distribution justifies the lower percentages found in the present study, since the material used came from branches, which are structures younger and closer to the tree canopy. Although producing a smaller percentage, the extraction of tannins from tree branches could promote greater sustainability to commercial plantations.

The two "angico" species showed the highest percentage of tannins determined from the bark, with 10.84% in *P. zehntneri* and 10.74% in *P. rigida*. In general, the species of "angico" are of great importance in the exploitation of the bark that is rich in tannins [17] point out that *P. rigida* species presents between 15% and 25% of tannins in their bark. The authors pointed out that "angico" species present greater potential to be exploited in productive models with this objective, due to their great potential for timber and multiple use, constituting a better use of the species. However, there is still insufficient information to confirm the effective recovery of the species after bark extraction. Therefore, it is suggested the extraction at the time of cutting of the adult trees associated with the use of the individual for other purposes, besides stimulating the use of the barks coming from the management of the branches instead of the bark of the shaft.

The species *A. pyrifolium* presented the highest percentage of tannins in the branches (9.15%) and the fourth species with the highest percentage of tannins in the bark (9.59%). This species is on the list of the most toxic plants in the Caatinga, with reports of abortion cases in goats, sheep and cattle due to their chemical composition [13]. However, substances found in this species can also have benefits, as [18] states, in which he showed in his study the anti-inflammatory action of the plant extract related to the presence of tannins and flavonoids. The author also states that in Juazeiro do Norte, a city located in northeastern Brazil, it is common practice to extract these components that are present in the bark of the trees of this species to be used in the treatment of urinary inflammations and dermatitis.

The family Anacardiaceae, which *M. urundeuva* belongs to, is well known for the presence of phenolic compounds. In studies with species of the Caatinga to evaluate the content of tannic substances, the authors [19] found higher percentages for species M. urundeuva and *P. pyramidalis*. Superior results were found for *M. urundeuva* (67.89 mg), L. ferrea (53.95 mg), and *C. leptophloeos* (31.63 mg) [20]. However, the results for the species *E. velutina* (8.80 mg) and *T. aurea* (18.60 mg) were higher than those obtained by the same author, being 0.24 mg for *E. velutina* and 5.91 mg for a species of the genus Tabebuia.

The species *C. leptophloeos* presented one of the lowest percentage of tannins among the ten species of this study, both in the bark (6.75%) and in the branch (4.95%). However, this species belongs to the genus Mimosa, which usually presents species with potential use in folk medicine for the treatment of bronchitis, cough, ulcers, among others, due to the antibiotic properties of the tannins [21]. The same occurred with *M. urundeuva*, which presented one of the lowest percentages (6.07%).

In general, the values obtained were lower than those generally found in the literature [16,18,21], especially for species that are economically exploited for the extraction of tannins, such as those of the genus Parapiptadenia. However, the use of younger tree structures (branches) may justify the results obtained, since higher tannin contents are found in older structures of the tree, such as the main trunk, which accumulate larger amounts of tannins in their husks and heartwood [6].

4. CONCLUSION

The species *P. zehntneri* (10,84%), *P. rigida* (10,74%) and *L. ferrea* (10,27%) presented the highest potential for extraction of bark tannins, being the most suitable for this purpose among the others. Considering the branches, *A. pyrifolium* (9,15%) was the most suitable species for tannin extraction, since it presented the highest percentage of this substance.

The use of parts of the tree for the extraction of tannins instead of using the trunk, such as the branches and the bark, can be suggested. This can provide greater sustainability to the planting, as an alternative for the extraction, which is usually carried out from the main trunk. It is suggested that other researchers that work with NTFPs may develop further research to study the potential use of other parts of the tree to extract tannins and thus provide more information that may facilitate the sustainable management of Caatinga species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors TRSL, TCS and ECGA conceived and designed the research. Authors TRSL, TCS, ECGA and CAR performed the experiments. Authors TRSL and TCS analyzed the data. Authors TRSL, TCS, ECGA, RLB and JXM wrote and edited the manuscript. All authors read and approved the final manuscript.

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