Original Research Article

BARK AND FRUIT EXTRACTS *Anadenanthera colubrina* (Vell.), *Mimosa tenuiflora* (Willd.) AND *Acacia mearnsii* (Wild.) SPECIES

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ABSTRACT

The aim of this study was to quantify extracts from the bark and green fruits of *A. colubrina* and *M. tenuiflora*, compared to *A. mearnsii* bark using the formaldehyde method. Ten *M. tenuiflora* and ten *A. colubrina* trees were selected to collect the studied material and five *A. mearnsii* individuals. Moisture content, total solids, Stiasny index and condensed tannin content were analyzed. The results were compared by Tukey test at 5% probability. In relation to the Stiasny index, the species *A. mearnsii* and *M. tenuiflora* did not differ statistically, with averages of 68.3 and 62.6%, respectively. The content of condensed tannins found in *A. colubrina* fruits, did not differ statistically from the content of the bark of the same species, corroborates with data referenced in the literature in research with this species that is traditionally exploited in the Northeast Region of Brazil by the tannery industry. The *M. tenuiflora* and *A. colubrina* species present potential for the production of tannic extracts, although tannin contents are inferior to those obtained for *A. mearnsii*. In addition, due to the abundance in the Brazilian semiarid region, the *M. tenuiflora* has potential for exploitation to obtain tannins.

Keywords:Vegetable components; phenolic compounds; tannins extraction; stiasny method

1. INTRODUCTION

Tannic compounds are produced by the secondary metabolism of plants, with repelling action to insect and microorganism attacks, and its oldest application is the tanning of animal skins [1], being in turn beneficial to health because they present on their antimicrobialproperties, antioxidant and anticancer actions [2].

Tannins are natural compounds framed in two distinct classes of chemical compounds and of phenolic nature, which are hydrolysable tannins and the condensates, being the first group found in the bark of species such as *Terminalia sp., Eucalyptus sp., Phyllantus sp*. and *Caesalpiniasp*., among other genders [3]. The condensates are present in the bark of all the hardwood and coniferous species are studied until today, as well as in the core of several woody species [4].

Due to the different concentrations of extracts in the distant parts of the plant, several methods have been developed to detect tannins from plant extracts, in food products and beverages [5]. The use of tannins in the manufacture of adhesives is considered recent in Brazil. However, in some countries, such as South Africa and Australia, tannins for this purpose are used on a commercial scale. Such use is related to the greater ease with

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polyphenols bind together to formaldehyde, allowing their use in the industry of plywood and particleboard, under normal bonding and pressing conditions [6].

Due to a lack of appropriate management and uncontrolled exploitation of the species *A. colubrina* and *M. tenuiflora*, for tannin production, *M. tenuiflora*, a species common in disturbed areas of Caatinga and widely used for the production of firewood, charcoal and wood for cooking, were highlighted in this research the potential of its tannin content for use in the tanning production chain using parts of the plants [7].

Therefore, the aim of this work was to quantify the condensed tannins obtained from bark and fruits of *A. colubrina* and *M. tenuiflora* as well as the *A. mearnsii* species.

2. MATERIAL AND METHODS

2.1 Collection and preparation of material

The materials (barks and fruits)used in this study were obtained in the year2016 from a StepicSavannah area in the municipality of Malta, State of Paraiba, Brazil, located at 07º 01' South latitude and 37º 17' West longitude, with average altitude of 250 m, presenting a BSh (hot and dry) climate, according to Koppen's classification, with annual average rainfall between 250 and 800 mm, mainly concentrated in the months of February to April and average temperature of 29ºC.

Ten *M. tenuiflora* and ten *A. colubrina* matrices were selected, both species being vigorous and with good phytosanitary appearance of the population, randomly selected at different points in the area and equally distributed within the study area, in order to contemplate all the variability of the local. Bark and green fruits samples of both species were taken for extraction and quantification of the tannic substances.

In order to compare, five individuals of *A. mearnsii*, barks were already stored in the Technology of Forest Products Laboratory, Health and Rural Technology Center, in form of large fragments (splinters). These barks were derived of five tree individuals from a forest stand located in the municipality of Pelotas-RS, Brazil.

In the *M. tenuiflora* and *A. colubrina* species, the bark and fruit were removed with aid of hand tools (machetes, hammers and knives). The material was collected and kept in a ventilated environment for natural drying; later was stored in dark plastic bags. For *A. mearnsii*, the barks were already in this condition.

After the drying stage, the materials (barks and/or fruits) from trees of each species were homogenized, fragmented in a hammermill and milled in a Willey type mill, with constant stops to avoid heating. Subsequently, the particles were subjected to the vibrating sieve, selecting the portion that got through the 35 mesh (0.42 mm) sieve and was retained in the 60 mesh (0.25 mm) sieve.

Finally, the classified particles were stored in identified hermetically sealed bottles, protected from the light and humidity.

2.2 Generation of analytical solution

The extraction was carried outunder boiling in a volumetric flask with capacity of 500 ml, in which 300 ml of distilled water and 12.5 g of air-dried sample were added, following a similar methodology used by [9] and [11]. Posteriorly, the mixture was boiled under reflux for 2

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hours. After the boiling period, the mixture was submitted to a 150 mesh (0.105 mm) sieve, being the liquid part (liquid extract) stored in a plastic bottle, while the solid part (particles) was again subjected to three more boils, 2 hours each, in order to remove the most of the extractives. The filtrates from the sample (900 ml total) were packed in the same bottle. After this procedure, they were strained in a flannel and filtered through a sintered glass crucible of porosity 2, having then the volume completed to 1000 ml by the addition of distilled water.

2.3 Determination of particle humidity

Simultaneously to the removal of the sample for the generation of the analytical solution (primary sample), a secondary sample (air-dried) of 3 g was obtained, which was placed in an oven (100℃) until its anhydrous mass was obtained, all in order to calculate its humidity content (Equation 1).

$$TU\% = ((Mus - Mas) / Mus) \cdot 100 \qquad \text{(Equation 1)}$$

In which:

TU% = Humidity content of the secondary sample, in %;
Mus = Air dry mass of the secondary sample (3 g), in grams;
Mas = Anhydrous mass of the secondary sample, in grams.

2.4 Determination of anhydrous mass of the particles subjected to extraction

Knowing the humidity content (secondary sample) and the air-dry mass of the portion transferred to the volumetric flask (primary sample), the anhydrous mass of the sample undergone to the extraction and was calculated by the equation 2:

$$Mae = Mue \cdot [1- (TU\%/100)] \qquad \text{(Equation 2)}$$

In which:

Mae = Anhydrous mass of the sample used in the extraction, in grams;
Mue = Air dry mass of the sample used in the extraction, in grams;
TU% = Humidity content of the secondary sample, in %.

2.5 Determination of Total Solids

For the determination of the total solids content, a 50 ml aliquot of the analytical solution was evaporated in an oven (103 ± 2℃) until its anhydrous mass was obtained and the total solids content (TST) was calculated, according to equation 3, being the initial anhydrous mass corresponding to an anhydrous mass of 12.5 g of the air dried sample and the final anhydrous mass obtained from 50 ml (residue after evaporation in the oven) and extrapolated to 1000 ml.

$$TST = (Mf / Mi) \cdot 100 \qquad \text{(Equation 3)}$$

In which:

TST = Total solids content of the solution, in %;
Mf = Final anhydrous mass of the sample, in grams;
Mi = Initial anhydrous mass of the sample, in grams.

2.6 Determination of the Stiasny Index

The method of Stiasny 2016 was employed, with modifications suggested by [8]. In a 100 ml sample of the analytical solution were added 4 ml of formaldehyde (37%) and 1 ml of concentrated HCl. The material was heated under reflux for 30 minutes. In this condition, the tannins formed insoluble complexes, which were separated by filtration.

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The filter paper containing the material was transferred to a 250 ml Becker beaker and dried at 103 ± 2℃ for 24 hours. Knowing the mass of the filter paper, Stiasny Index was calculated by the following equation 4):

$IS\ (\%) = (M2 / M1) \cdot 100$ (Equation 4)

In which:

IS = Stiasny Index, in %;

M1 = Mass of solids in 100 ml of extract, in grams;

M2 = Mass of tannin-formaldehyde precipitate, in grams.

2.7 Determination of the condensed tannins content

After obtaining the TST and IS, the condensed tannin content of the material (TTC) was calculated, according to equation 5:

$TTC\ (\%) = (TST \cdot IS) \cdot 100$ (Equation 5)

In which:

TTC = Condensed tannins content, in %;

TST = Total solids content (Equation 3);

IS = Stiasny Index (Equation 4).

2.8 Experimental design

Ten matrices of*M. tenuiflora* and *A. colubrina* were evaluated, combined in their different parts (bark and fruits), in addition to the *A. mearnsii* bark, totalizing five treatments.Mixtures of the materials related to the treatments after collection of the different trees and materials (bark and/or fruits) were carried out, and afterwards, a completely randomized design was employed.

2.9. Data analysis

Three replicates (extractions) per treatment were evaluated and all sub-replicates (humidity content, total solids, Stiasny index, condensed tannin content) were analyzed in duplicate. The results were interpreted through comparison of means by the Tukey test, considering a 5% probability of error.

3. RESULTS AND DISCUSSION

The variance analysis of the humidity content of the particles did not indicate a significant statistical difference between the treatments ($P < .2371$), which varied from 10.1% (*M. tenuiflora* fruit) to 12.5% (*M. tenuiflora* bark), which is probably associated with the collection season, since it was carried out in the same period.

The total solids content (TST) of the analytical solution can be understood as the gross yield of the material in powdered extract. It was observed that there was no significant statistical difference in relation to the TST of the bark of studied species, being the highest value obtained for the bark of *A. mearnsii* (47.9%), followed by the *A. colubrina* (44.3%). The lowest mean (27.7%) was obtained by the *M. tenuiflora* fruit, presenting a significant difference in relation to the others (Table 1).

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Table 1. Comparisons of averages of the total solids content obtained from different forest species and parts of the plant.

Treatment**	TST %*	IS %*	TTC %*
ANb	47.9a	68.3a	32.5a
JPb	42.0a	62.6a	26.1a
AVb	44.4a	47.1b	20.2c
JPf	27.7b	9.9c	2.7d
AVf	40.3a	50.2b	20.2c

*Means followed by the same letter in the column do not differ statistically by the Tukey test (P < .05).

**ANb = *A. mersiani* bark; JPb= *M. tenuiflora* bark; AVb = *A. colubrina* bark; JPf = *M. tenuiflora*fruit; AVf = *A. colubrina* fruit.

For condensed tannins, the highest mean was obtained by *A. mersiani* bark with 32.5%, followed by *M. tenuiflora* and *A. colubrina* barks, with means of 26.2 and 20.3%, respectively. The *M.tenuiflora* fruits showed, on the other hand, mean of 2.7%, statistically differing from its bark, emphasizing their tannic potential (Table 1).

For the Stiasny index, the extract of the *A. mearnsii* bark obtained a superior mean in relation to the others, with 68.3%, however, it did not differ statistically from the *M. tenuiflora*, which presented 62.6%, indicating the latter's potential for the production of tannin-formaldehyde-type adhesives (Table 1), in the other hand, the lowest mean was obtained by *M. tenuiflora* fruit (9.9%).

4. DISCUSSION

It was observed a tendency of the *M. tenuiflora* bark humidy content to be higher. This is mainly due to the higher humidity of the air during the period in which these barks were put to dry (rainy period), being that for the other cases, a more homogeneous humidity was obtained. Nevertheless, the existing humidity facilitated the grinding of the material, with little loss in the form of fines and, consequently, few incrustations in the mill knives.

Analyzing the humidity of the airdried bark of the *A. colubrine* (10.4%) and *M. tenuiflora*(12.5%) species, [8] obtained lower results, with mean values of 7.93% and 9.30%, respectively. These differences in humidity may be reflect the time when such authors carried out the study (drier), of the method or storage site.

Lower results were obtained by [9] when analyzing the TST of barks from *A. colubrina* (22.48%) and *M. tenuiflora* (26.32%). [11], in an analysis of the *A. colubrina* barks, obtained a mean of 23.30% in the total solids content, a result much lower than the one found in this study for the same species. This difference can be due to the method used to separate the solid fraction (material under extraction) from the liquid extract.

In a similar way [12], when analyzing the quality of *A. mearnsii* and *M. tenuiflora* peels from the same sources of the present study, observed total solids contents between 56.8 and 39.9%, values close to the one observed in this study, probably because of the similarity in the methodology used. The author points out that this difference between the results found in the literature may arise from the period of collection of the bark, plant phenophases, site characteristics or age of the trees.

The *A. mearnsii*, a species known worldwide for its high tannin yield and use, mainly for skin tanning, presented a higher mean than the others studied, with a value of 32.6%. The

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Brazilian company [13] described that it presents approximately 28% of tannins in its bark, however, without describing the methodology used.Lower yield of condensed tannins was also obtained by [14] for the barks of *A. colubrina*and *M. tenuiflora*, with 11.89 and 17.74% respectively.

When comparing the TTC of forest species occurring in the semiarid region, [8] observed 17.7% for the *M. tenuiflora* bark, indicating its potential for leather and tanner industries. Already [15] found that the tannins obtained from this species present good characteristics for the production of tannin formaldehyde adhesive.

Research conducted by [11] obtained 13.95% of tannin in the *A. colubrina* bark, from composite samples obtained in three positions equidistant in the trunk, in three positions in the main branches and three in twigs.

For the *M. tenuiflora* species, in comparison to the *A. colubrina*, which is constituted in a species commercially used by tanneries in the Brazilian northeast, it reveals its potential as a tannin producer, indicating that it must be tested for use in adhesives for wood, due to considerable content of condensed tannin present in its barks.It was observed a tendency of the *M. tenuiflora* bark humidy content to be higher. This is mainly due to the higher humidity of the air during the period in which these barks were put to dry (rainy period), being that for the other cases, a more homogeneous humidity was obtained. Nevertheless, the existing humidity facilitated the grinding of the material, with little loss in the form of fines and, consequently, few incrustations in the mill knives.

In relation to the Stiasny index, in this reaction only tannins of the flavanol type are precipitated by condensation with formaldehyde in an acid medium, these products being of high molecular weight and of difficult dissolution, where the greater the number of Stiasny, higher the quantity of polyphenols (tannins) present in the extracts [16-17].Tannins are quite chemically reactive because they are phenolic substances. The Stiasny method is characterized by the determination of the content of reactive polyphenolic components (condensable tannins). Condensed tannins or proanthocyanidins are composed of flavonoid units, especially flavone-3-ols (catechin) and flavan 3,4-diols (leucoanthocyanins), which are precipitated by condensation with formaldehyde in acid medium [18].

The Stiasny index value obtained by [8] of 52.88%, in the bark of the *A. colubrina* species was superior to the one found in this study, a fact that may be associated with the time the barks were collected, since [9] found that the same index varied with the plant phenotypes and trunk positions, in which it obtained values that presented a total variation of 32.2 to 68.3%.

With the obtained data it can be observed the significant difference in the content of condensed tannins between the barks of *M. tenuiflora* and *A. colubrina* and the fruits of these same species. It can also be observed the low index of the *M. tenuiflora* fruits and the considerable index obtained by the *A. colubrina* fruits, a behavior also observed by [9] when studying *A. colubrina* fruits. Regarding the amount of fruits produced by *A. colubrina* trees, in relation to the proportion of tannins found in them, taking into account the demand of traditional tanneries, studies to test the viability of the tannins present in the fruits for skin tanning and other purposes become indispensable, to the example of researches aimed at their use in the manufacture of adhesives for wood.

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5. CONCLUSION

The *M. tenuiflora* and *A. colubrina* species present potential for the production of tannic extracts.

Although it was possible to compare the contents of condensed tannins present in the different parts of the *M. tenuiflora* and *A. colubrina* species, it is suggested to carry out new studies in order to improve the extraction and quantification parameters.

COMPETING INTERESTS

Authors have declared that no competing interests exist

REFERENCES

1. Covington AD. Modern tanning chemistry. Chemical Society Reviews, Cambridge. 1997;26(1):111-126.

2. De Bruyne T, Pieters L, Deelstra H, Vlietinck A. Condensed vegetable tannins: biodiversity in structure and biological activities. Biochemical Systematics and Ecology. 1999;27(4):445-459.

3. Pizzi A. Advanced wood adhesives technology. M. Dekker: New York. 1994.

4. Wollenweber E, Dietz VH. Occurrence and distribution of free flavonoid aglycones in plants. Phytochemistry. 1981;20(1):869-932.

5. Hagermann AE, Zhao Y, Johnson S. Methods for Determination of Condensed and Hydrolyzable Tannins. Antinutrients and Phytomedicine in Food.1997;662(1):209-222.

6. Pizzi A. Wood adhesives: chemistry and technology. New York: Marcell Dekker; 1983.

7. Medeiros JX,Calegari L, Silva GH, Oliveira E,Pimenta A. Measurement of Tannic Substances in Forest Species. Floresta e Ambiente. 2018;25(3):1-8.

8. Paes JB, Diniz FCE, Marinho IV, Lima CR. Evaluation of the taniferous potential of six forest species occurring in the Brazilian semi-arid region. Cerne. 2006a; 12 (3): 232-238. Portuguese.

9. Paes JB, Marinho IV, Lima RA, Lima CR, Azevedo TKB. Technical viability of the tannins of four forest species occurring in the Brazilian semi-arid region in tanning. Forest Science. 2006b; 16 (4): 453-462. English.

10. Paes JB, Santana GM, Azevedo TD, Morais RDM, Calixto Júnior JT. Tannic substances present in several parts of the tree *A. colubrina*-red (*Anadenanthera colubrina* (Vell.) Brenan. 2010; 38 (87): 441-447. Portuguese.

11. Guangcheng Z,Yunlu L, Yazaki Y. Extractives yields, Stiasny values and polyflavanoid contents in barks from six Acacia species in Australian Forestry, Queen Victoria. 1991;3(54):154-156.

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12. Calegari L, Lopes PJG, Oliveira E, Gatto DA, Stangerlin DM. Quantification of tannins in the bark of *M. tenuiflora* and black acacia. Brazilian Forest Research. 2016; 36 (1): 61-69..

13. Tanac S.A. Taninos da Acácia Ltda. Accessed 9 January 2015. Available: <www.tanac.com.br>.

14. Lima CR, Paes JB, Lima VL, Delgado MF, Lima RA. Potentiality of the tannic extracts of three forest species in the tanning of caprine skins. Brazilian Journal of Environmental Engineering. 2014; 18 (11): 1192-1197. English.

15. Azevedo TKB. Relationship between wood, bark and tannin content and tannin quality of (*Mimosa tenuiflora* (Willd.) Poir.) For the production of tannin formaldehyde adhesives [dissertation]. Patos: Federal University of Campina Grande; 2010. English.

16. Mori FA. Use of tannins from Eucalyptus grandis bark for the production of adhesives. 1997. Viçosa. Dissertation (Master in Forest Science) - Federal University of Viçosa, Viçosa, 1997. English.

17. Gonçalves CA, Lelis RCC, Brito EO, Nascimento AM. Production of agglomerated wood sheets with adhesive Urea modified formaldehyde with Mimosa caesalpiniaefolia Bentham (sabiá). Forest and Environment. 2003; 10 (1): 1-9. English.

18. Gonçalves CA, Lelis RCC. Tannin content of bark and wood of five tree legumes. Forest andEnvironment 2001; 8 (1): 167-173. Portuguese.

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