



The effects of seasonal climate changes in the Caatinga on tannin levels in *Myracrodruon urundeuva* (Engl.) Fr. All. and *Anadenanthera colubrina* (Vell.) Brenan

Júlio M. Monteiro¹, Ulysses P. Albuquerque^{1*}, Ernani M.F. Lins Neto¹, Elcida L. Araújo¹,
Miracy M. Albuquerque², Elba L.C. Amorim²

¹Laboratório de Etnobotânica Aplicada, Departamento de Biologia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros s/n, Dois Irmãos, 52171-900, Recife, PE, Brazil,

²Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves s/n, Cidade Universitária, 50670-901, Recife, PE, Brazil

RESUMO: “Os efeitos da sazonalidade climática na Caatinga sobre os teores de taninos em *Myracrodruon urundeuva* (Engl.) Fr. All. e *Anadenanthera colubrina* (Vell.) Brenan”.

Examinou-se a influência da sazonalidade climática sobre as concentrações de taninos em duas espécies da Caatinga: *Myracrodruon urundeuva* (Engl.) Fr. All. e *Anadenanthera colubrina* (Vell.) Brenan. Empregou-se o método Folin-Ciocalteu para quantificar os fenóis totais e o método de precipitação da caseína para taninos. De maneira geral, existe uma forte relação entre os teores de taninos e a pluviosidade, apesar das espécies parecerem adotar diferentes estratégias de produção de compostos tânicos em resposta aos períodos de seca e chuva na caatinga.

Unitermos: Florestas secas, Folin-Ciocalteu, plantas medicinais, semi-árido.

ABSTRACT: The influence of seasonal climatic changes on tannin concentrations was examined in two *caatinga* (semi-arid) plant species (*Myracrodruon urundeuva* (Engl.) Fr. All. and *Anadenanthera colubrina* (Vell.) Brenan). The Folin-Ciocalteu method was used to quantify total phenols, and the casein precipitation method was used for tannins. In general, there is a close relationship between tannin levels and rainfall, although species seem to adopt different strategies of tannin compound production in response to periods of drought and rainfall in the *caatinga*.

Keywords: Dry forest, Folin-Ciocalteu, medicinal plant, semi-arid.

INTRODUCTION

It is well documented that some ecological variables can quantitatively and qualitatively affect tannin production in plants (Hatano et al., 1986; Teixeira et al., 1990; Furlan et al., 1999; Simon et al., 1999; Salminen et al., 2001; Paiva et al., 2002). Seasonal changes, for example, alter plant metabolism and, consequently, biosynthetic pathways (Hatano et al., 1986; Salminen et al., 2001). Periods of high temperatures are known to increase the concentration of phenolic compounds in many plants. Species of *Lotus*, for example, demonstrate seasonal variations in tannin concentration (Gebrehiwot et al., 2002), which have been shown to be significantly higher during the spring and summer. The same response was seen with *Alnus rubra* Bong (González-Hernández et al., 2000). Comparisons between plants growing in tropical sub-humid and tropical semi-arid regions of Africa have demonstrated that total phenols levels increase in regions with more severe climates and with less available humidity (Makkar; Becker, 1998).

In Brazil, only a limited number of studies have examined the influence of environmental variables

on tannin production, and most of these have focused on plant defenses in areas of *cerrado* (savannah) and Atlantic Coastal Forest vegetation (Pais; Varanda, 2003; Pansera et al., 2003; Yariwake et al., 2005). Fewer studies have been undertaken in the *caatinga* yet and it certainly remains the most incompletely studied Brazilian biome. The *caatinga* is also the least protected region in Brazil, with less than 2 % of its lands included within any type of conservation area. Research programs in this semi-arid region are therefore necessary, especially in light of the rapid alteration of this biome, and substitution of the native vegetation even before it can be effectively studied (Fernandes, 2002; Giullieti et al., 2002; Castelletti et al., 2003).

In the present work, the tannin levels of two *caatinga* medicinal plants, *Myracrodruon urundeuva* (Engl.) Fr. All. and *Anadenanthera colubrina* (Vell.) Brenan, were quantified and compared with local seasonal rainfall data. Studies which increase our understanding of the behavior of *caatinga* plants are extremely important at this moment for conservation and sustainable. Management strategies are needed to protect and preserve many semi-arid species threatened with

* E-mail: upa@ufrpe.br; Tel. + 55-81-33021350

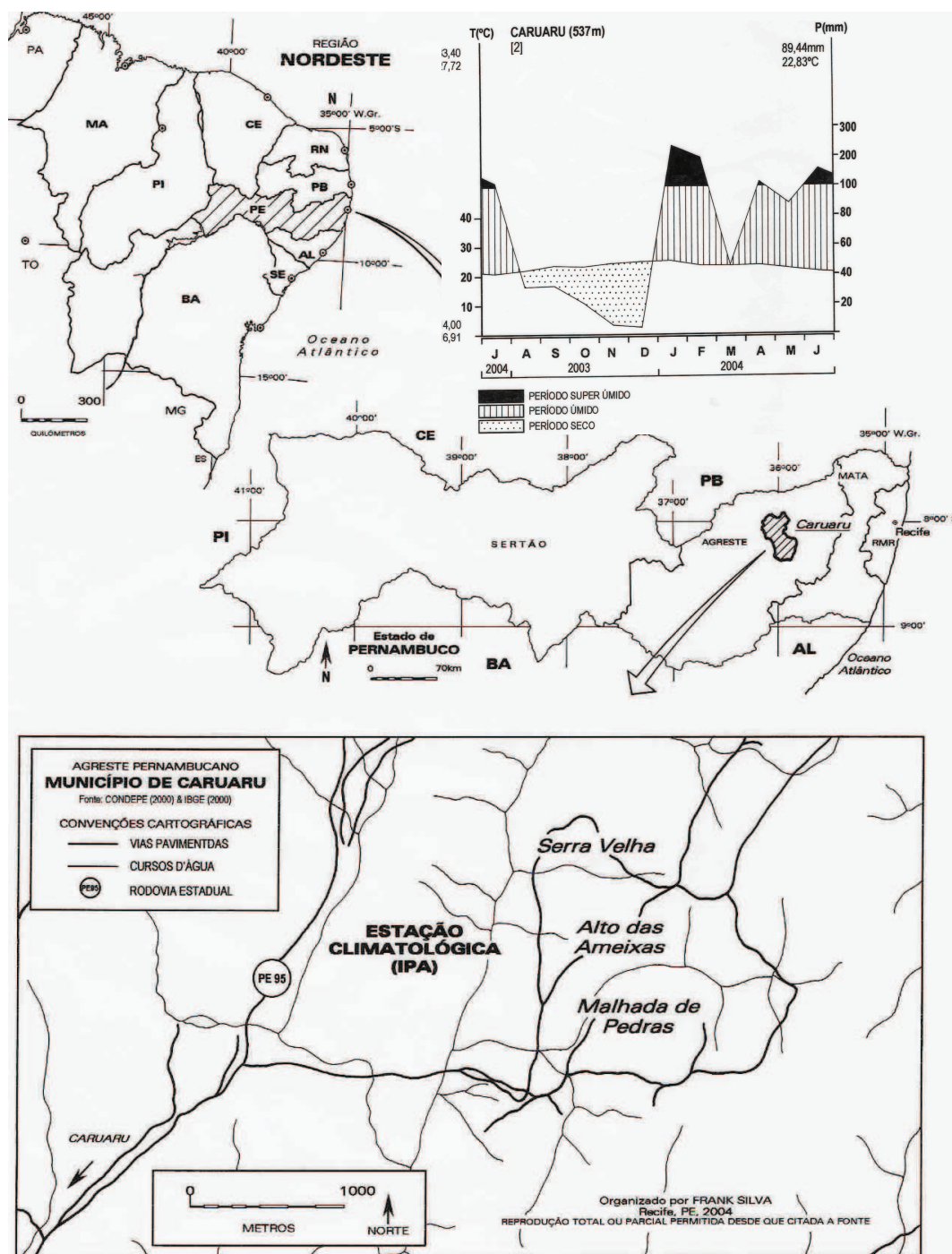


Figure 1. Localization of the communities “Riachão de Malhada de Pedra” and “Alto das Ameixas” within the municipality of Caruaru, Pernambuco State, Brazil, with climograph for the research period – August 2003 to July 2004 (data furnished by the Estação Climatológica do Instituto de Pesquisas Agropecuárias, IPA).

extinction (Monteiro et al., 2005a,b; 2006). Additionally, many secondary metabolic compounds involved in plant-herbivore interactions are active principals for industrial pharmaceuticals.

MATERIAL AND METHODS

Study area

Research was carried out in a 20 ha. fragment of arboreal *caatinga* within the “Empresa Pernambucana de Pesquisa Agropecuária” (IPA) Experimental Station, Pernambuco State, Brazil (Figure 1) (8°14’18” S, 35°55’20” W) at 537 m a.s.l. The area is located ca. 9 km

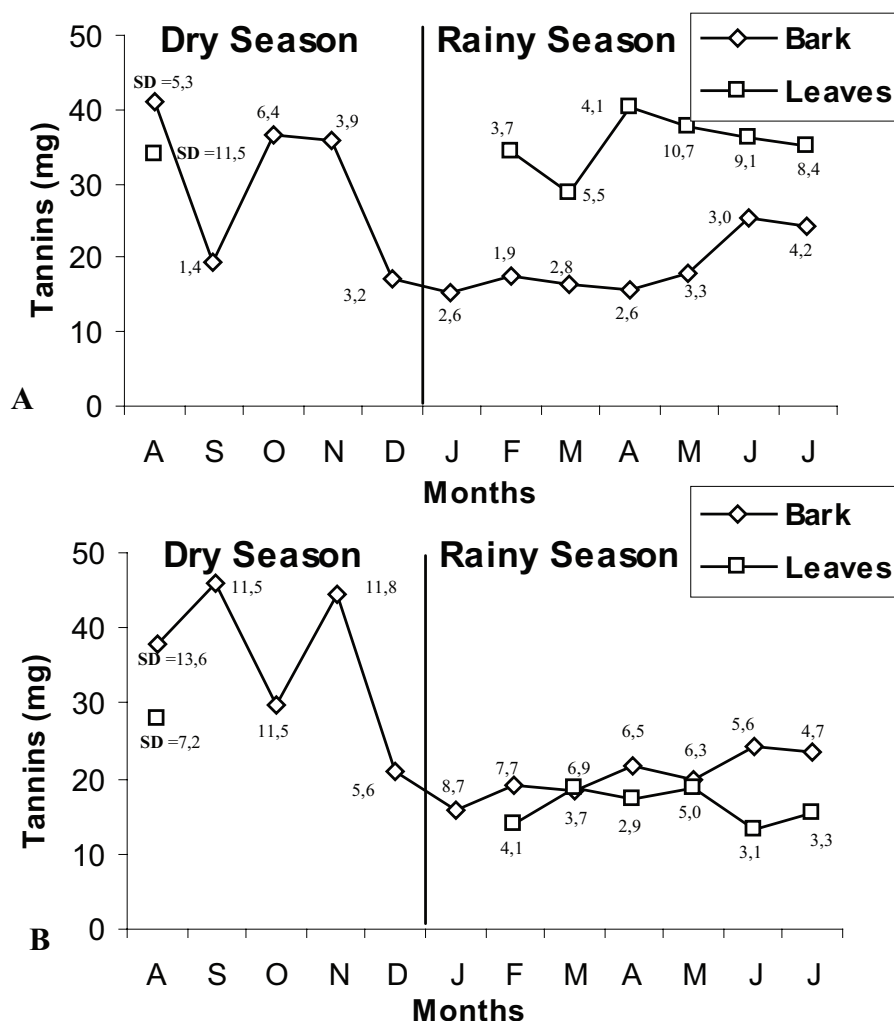


Figure 2. Variation in tannin levels in 500 mg samples of bark and leaves of *Myracrodruon urundeuva* (Engl.) Fr. All. (A) and *Anadenanthera colubrina* (Vell.) Brenan (B) in response to seasonal changes during 2003/2004 in a caatinga fragment in Pernambuco State, Brazil. **SD** = Standard deviation.

northeast of the city of Caruaru, and approximately 150 km from the state capital of Recife. The soils there are predominantly eutrophic, yellow Podzols tb, abrupt, A moderate (Alcoforado-Filho et al., 2003). The dry season was considered as falling between August and December 2003, as in January of 2004 there was a total rainfall of 240 mm, with only slightly less rainfall occurring in the following months (Figure 1) of the rainy season. It is important to note that the uncharacteristically heavy rainfall during this wet season was quite atypical for the region. The vegetation at the research site is deciduous and spiny, and comprises ca. 105 species, distributed among 41 families, including herbaceous plants, vines, subshrubs, shrubs, and trees. Some of the most conspicuous arboreal elements belong to the families Anacardiaceae and Caesalpiniaceae (Alcoforado-Filho et al., 2003). Vegetative material was collected at monthly intervals between August 2003 and July 2004, with the exception of mature leaves, which were collected in August 2003

and from January to July of 2004.

The species *Myracrodruon urundeuva* (Engl.) Fr. All. (common name: Aroeira) and *Anadenanthera colubrina* (Vell.) Brenan (common name, Angico), were selected for analysis because of the large number of individuals present in the area, and their importance in local traditional medicine (Albuquerque and Andrade, 2002a,b; Macedo; Ferreira, 2004). Ten individuals of each species within previously established permanent plots (covering a total of one hectare) at the experimental station (Araújo, 1998) were designated for monitoring. Individuals were selected according to the following criteria: DBH > 35 cm, height > 3.5 m. Monthly collections of trunk bark and mature leaves (ca. 5 grams each) were made to analyze tannin levels. Leaves were preferentially collected from the same position on all trees, and care was taken in selecting material that was intact and without signs of predation, and in the same development stage. Bark was harvested from between 50 and 120 cm above

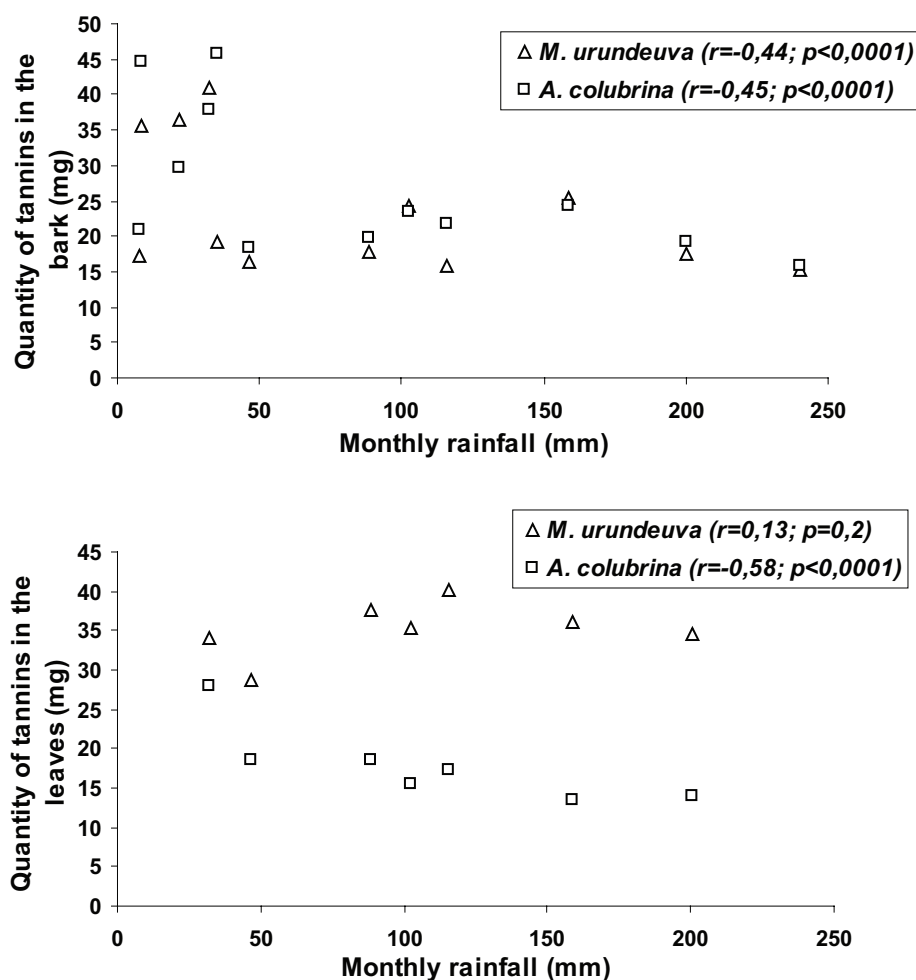


Figure 3. Correlation between the quantities of tannins in 500 mg samples of the bark and leaves of *Myracrodruon urundeuva* (Engl.) Fr. All. and *Anadenanthera colubrina* (Vell.) Brenan in relation to the rainfall during 12 months (2003/2004) in a *caatinga* fragment in Pernambuco State, Brazil.

ground level.

Preparation of extracts and quantification of phenol and tannin levels

All plant material collected in the field was placed in paper envelopes for transport to the laboratory. Bark was dried for three days at 50 °C in a drying oven, while leaves were dried at room temperature (except on rainy days, when these also were treated in a drying oven at 50 °C). Methanol extracts (80% v/v) were made of the bark and leaf samples, at the proportion of 500 mg of plant material to 5 mL of solvent. Five successive extractions were performed, heating the solvent each time to the initiation of boiling. All extractions were performed in triplicate. Total phenolic compounds present in the extracts were analyzed using the Folin-Ciocalteu method, and total tannins assayed by the casein precipitation technique (Folin; Ciocalteu, 1927;

Seigler et al., 1986; Mueller-Harvey, 2001; Readell et al., 2001; Queiroz et al., 2002). The Folin-Ciocalteu method employed in this study to measure total phenolic compounds consisted of adding 0.25-0.5 mL of the extract to 75 mL of distilled water, 5 mL of the Folin-Ciocalteu reagent (in aqueous solution at 10% - v/v), 10 mL of aqueous sodium carbonate (0.75% - m/v), and then completing the final volume to 100 mL with distilled water. The resulting solution was stirred mechanically for three hours at room temperature, and then left to settle for 30 minutes. Absorbance of the supernatant was measured at 760 nm in a spectrophotometer. Absorption measurements were calibrated against control solutions of tannic acid at concentrations of 0.1; 0.5; 1.0; 2.5 and 3.75 µg.mL⁻¹.

Total tannin concentrations were determined by the casein precipitation method, which consists of adding 1 g of powdered casein to 6 mL samples of extracts diluted with 12 mL of distilled water. The resulting solution was

agitated for three hours at room temperature (25 °C), after which it was filtered through 9 cm Whatman filter paper and the filtrate adjusted to 25 mL final volume. Aliquots (8 mL – 12 mL) of this solution were then tested for residual phenolic compounds using the Folin-Ciocalteu method. The quantity of tannins corresponds to the difference in the absorption of these casein precipitated samples and those obtained in the total phenol analysis. The quantity of total phenols and tannins are expressed per mg of dry material.

Data analysis

The Kolmogorov-Smirnov test was used to examine the normality of the data (Zar, 1996). The Kruskal-Wallis test was employed to reveal differences in tannin concentrations of the two species during different seasons. In order to evaluate the correlation between tannin concentrations in the two species and rainfall averages, the Spearman correlation coefficient was used. All statistical analyses were performed using BioEstat 2.0 software (Ayres et al., 2000).

RESULTS

Tannin x season

The bark and leaves of *A. colubrina* concentrate more tannins during the dry season than during the rainy season ($H = 33.51$; $P < 0.0001$ and $H = 18.89$; $P < 0.0001$, respectively). The bark shows higher tannin concentrations than do the leaves during the rainy season ($H = 14.43$; $P = 0.0001$) (Figure 2). Likewise, trunk bark of *M. urundeuva* concentrated more tannins during the dry season than during the rainy season ($H = 29.97$; $P < 0.0001$). There was no observed difference in tannin concentrations in the leaves of this species during the different seasons ($H = 0.11$; $P = 0.73$). The leaves of *M. urundeuva* demonstrated higher tannin concentrations than the bark during the rainy season ($H = 79.64$; $P < 0.0001$) (Figure 2).

The bark of *A. colubrina* had greater tannin concentrations than the bark of *M. urundeuva* during the dry season ($H = 3.98$; $P = 0.04$), however, the leaves of *M. urundeuva* had greater tannin concentrations than *A. colubrina* leaves during the rainy season ($H = 82.54$; $P < 0.0001$) (Figure 2). The tannin concentrations in the bark of the two species were similar during the rainy season.

Tannins x rainfall

The concentrations of tannic compounds in the bark of *M. urundeuva* and *A. colubrina* were negatively correlated with rainfall ($r = -0.44$; $P < 0.0001$ and $r = -0.45$; $P < 0.0001$, respectively) (Figure 3). The same negative correlation was observed with the leaves of *A. colubrina* ($r = -0.58$; $P < 0.0001$) during the rainy season.

There was no observed relationship between tannin levels and rainfall in leaves of *M. urundeuva* (Figure 3).

DISCUSSION

Tannins and seasonality

The resource availability hypothesis (Coley et al., 1985) appears to explain the fact that the greatest concentrations of tannins were found in the trunk bark of the two species examined, as well as in the leaves of *A. colubrina*, during the dry season. It is not able to explain, however, the lack of seasonal differences in leaf tannin concentrations in *M. urundeuva*. Although this hypothesis attempts to explain this phenomenon in a very general manner (Horner, 1990; Kouki; Manetas, 2002; Barone; Coley, 2002), its predictions have generally been borne out. It has been observed that plants growing on fertile soils produce higher levels of phenolic compounds than plants growing on poor soils, although no variations have been reported in levels of condensed tannins under these conditions (Barone; Coley, 2002). Makkar and Becker (1998), for example, encountered high tannin levels in species belonging to the group Leguminosae *strictu sensu* in areas where the climate is severe and water resources are limited. In *Ceratonia siliqua* L. Kouki and Manetas (2002) found that leaves of water stressed plants had higher levels of total phenolics and tannins, especially in young leaves. However, only condensed tannins were decreased by nutrient addition while both condensed and gallotannins were increased by water stressed (Kouki; Manetas, 2002). Periods of high temperature may stimulate the synthesis of tannins by favoring the shikimate pathway, a precursor of these compounds (Makkar; Becker, 1998; González-Hernández et al., 2000; Gebrehiwot et al., 2002; Riipi et al., 2002).

During the rainy season, *M. urundeuva* exhibited elevated tannin concentrations in its leaves, as compared to its bark. This strategy seems similar to that encountered in *Didymopanax vinosum* (Cham. & Scltd.) Seem. (a Brazilian *cerrado* plant that demonstrates its highest levels of tannin concentrations when growing in forest formations under more humid conditions) (Pais; Varanda, 2003). However, the behavior of *A. colubrina* was exactly the opposite of that observed for *M. urundeuva*, as greater amounts of tannins would have been expected to be found in the leaves during the rainy season due, to both a greater photosynthetic capacity and greater herbivore pressure. Perhaps the results observed for *A. colubrina* were due to investments in non-tannin plant defenses during this period, as toxicity in this plant is more related to the presence of nitrogen-based cyanogenic glycosides (Tokarnia et al., 1999). In the case of *A. colubrina*, however, the observed synthesis of cyanogenic glycosides may well be related to the fact that this group of plants harbors bacterial and fungal symbionts. These organisms fix atmospheric nitrogen that can subsequently be used

for defensive purposes (Mendonça; Schiavinato, 1996; Gross et al., 2002; Patreze; Cordeiro, 2004).

Tannins may respond quantitatively or qualitatively to season patterns, and it would be interesting to investigate how condensed and hydrolyzed tannin compounds vary in each of the species examined. Hatano et al. (1986) observed that telimagrandin II (a hydrolysable tannin) is abundant in leaves during the spring in *Liquidambar formosana* Hance, but decreases rapidly to minimal amounts in the summer. On the other hand, casuarinin and pedunculagin (also hydrolysable tannins) are abundant during the summer and fall. In species of *Quercus*, an increase in the composition of low molecular weight compounds and a decrease in elagitanin monomers and dimmers occurs before and after one year (Simon et al., 1999).

ACKNOWLEDGEMENTS

The authors would like to thank the Empresa Pernambucana de Pesquisa Agropecuária (IPA) experimental station in Caruaru, Pernambuco State, and especially its director, Mr. Jair Pereira for logistical support; Ruth R. Strattmann, technician of the “Núcleo de Controle de Qualidade e Medicamentos Correlatos”, Depto. de Ciências Farmacêuticas da Universidade Federal de Pernambuco, for her valuable contributions in the techniques of tannin analyses; to the CNPq/FACEPE for financial support for U.P. Albuquerque as part of the Primeiros Projetos project; and to CAPES for a research grant to the first author.

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