Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252023v36n422rc

Histo-anatomical pattern, degradability and secondary compounds in different accessions of maniçoba (*Manihot* spp.) and different stages of maturation

Padrão histo-anatômico, degradabilidade e compostos secundários em diferentes acessoes de maniçoba (*Manihot* spp.) e diferentes estágios de maturação

Kleitiane B. da Silva[1]*, Divan S. da Silva[1], Mailson M. do Rêgo[2], Alberício P. de Andrade[3], Janieire D. C. de Bezerra[1]

[1]Department of Animal Science, Universidade Federal da Paraíba, Areia, PB, Brazil. [2]Department of Biosciences, Universidade Federal da Paraíba, Areia, PB, Brazil. [3]Department of Animal Science, Universidade Federal do Agreste de Pernambuco, Garanhuns, PE, Brazil.

ABSTRACT - The objectives of this work were to characterize the histo-anatomical pattern and degradability and to determine the secondary compounds of maniçoba in the different accessions and maturation stages (young, mature and senescent). For the research, three accessions were selected (AC002, AC004 and AC038) from the municipalities of Barra de Santa Rosa, Monteiro and Boa Vista, respectively, which showed distinct genetic characteristics, such as morphological and agronomic characteristics, chemical composition and hydrocyanic acid content. The leaf blades showed a cuticle that covers the entire epidermis, with papillose cells in the abaxial region and in a tabular shape in the adaxial region. The cells on the abaxial side are thinner than on the adaxial side. In the phytochemical screening of maniçoba leaves, the presence of steroids, tannins, flavonoids and saponins was observed. The leaves of maniçoba (Manihot ssp.) showed absence of alkaloids in all stages and accessions evaluated. All accessions evaluated showed positivity for the presence of steroids, with superiority in accession 04 in all stages, while for the other accessions it decreased with the maturation of the plant, especially in accession 02. The presence and concentration of secondary metabolites vary between accessions and maturation stages.

Keywords: Leaf anatomy. Secondary metabolites. Saponins.

RESUMO - Os objetivos com este trabalho foram caracterizar o padrão histo-anatômico e a degradabilidade e determinar os compostos secundários da maniçoba nos diferentes acessos e estádios de maturação (jovem, maduro e senescente). Para a pesquisa foram selecionados três acessos (AC002, AC004 e AC038) dos municípios de Barra de Santa Rosa, Monteiro e Boa Vista, respectivamente, que apresentaram características genéticas distintas, como características morfológicas e agronômicas, composição química e teor de ácido cianídrico. As lâminas foliares apresentaram cutícula que recobre toda a epiderme, com células papilosas na região abaxial e em formato tabular na região adaxial. As células na face abaxial são mais finas do que na face adaxial. Na triagem fitoquímica das folhas de maniçoba, observou-se a presença de esteroides, taninos, flavonoides e saponinas. As folhas de maniçoba (*Manihot* ssp.) apresentaram ausência de alcalóides em todos os estádios e acessos avaliados. Todos os acessos avaliados apresentaram positividade para presença de esteróides, com superioridade no acesso 04 em todos os estádios, enquanto para os demais acessos a presença diminuiu com a maturação da planta, principalmente no acesso 02. A presença e concentração de metabólitos secundários variam entre acessos e estádios de maturação.

Palavras-chave: Anatomia foliar. Metabólitos secundários. Saponinas.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: October 5, 2022.
Accepted in: April 25, 2023.

***Corresponding author:**
<kleitezootec@gmail.com>

INTRODUCTION

The food security of livestock is an important challenge for livestock production in arid and semi-arid regions (GODDE; LOMBARD; WOOLUMS, 2019), and with the growing world population there is a great concern about food production to meet the demands (TARRASÓN et al., 2016). In this perspective, one of the species found in the various areas that make up the semi-arid region and which stands out for being a good forage alternative for animal production is maniçoba (*Manihot* spp.) (GOMES et al., 2022), which comprises plants (wild species) of the genus *Manihot*, including *Manihot glaziovii* Müll. Arg., *Manihot catingae* Ule and *Manihot carthaginensis* (Jacq.) Müll. Arg, all native to the Brazilian semi-arid region, showing genetic characteristics and morphoagronomic diversity (CAMPOS et al., 2020; AMORIM et al., 2023).

In ruminant feed, maniçoba is used exclusively as hay or silage to reduce the toxicity of hydrocyanic acid, thus allowing a safe feed for animals (MOTA et al., 2011; ANDRÉ; SANTOS, 2012; AMORIM et al., 2023). It is a forage with a high degree of palatability, reasonable protein content and good digestibility, and the bromatological analyses of samples of leaves and tender branches usually show similar values (%DM), that is, crude protein (20.88), ether extract (8.30),

crude fiber (13.96), non-nitrogen extract (49.98), ash (6.88) and in vitro dry matter digestibility (62.3) (ANTÔNIO; ARAÚJO, 2018).

The plants are perennial, tolerant to cuttings and long periods of drought, with morphological adaptation related to water retention (NASSAR et al., 2010) and physiological mechanisms through the control of stomata opening to maintain a positive carbon balance even under conditions of drought (MORGANTE et al., 2020).

The evaluation of the nutritional value of both maniçoba and other forages, through anatomical studies, allows a better comparison between species and cultivars, which, through the quantification of anatomical structures, can show the arrangement and proportion of digestible and indigestible tissues, as well as monitoring the aging of these tissues with the development of the plant (BEELEN; PEREIRA FILHO; BEELEN, 2008).

On the other hand, it is known that under certain environmental conditions such as rainfall, temperature and soil fertility, some plants are capable of producing different varieties of secondary compounds, with various functions such as: defense against bacteria, fungi, viruses, environmental stress and herbivore attack, providing the plant with characteristics such as bitter taste, repulsive odor, in addition to causing intoxication or anti-nutritional effects on predators (BEELEN; PEREIRA FILHO; BEELEN, 2008; TAIZ; ZEIGER, 2013).

Like the other species of the genus *Manihot*,maniçoba (*Manihot pseudoglaziovii*) is part of the group of plants whose main characteristic is the presence of secondary compound cyanogenic glycosides, whose formation occurs through chemical hydrolysis or through the action of the main cyanogenic substances such as linamarin and lotaustralin, which, in the presence of water, come into contact with the enzyme linamarase, giving rise to hydrocyanic acid (HCN) (CASTRO et al., 2007). As secondary compounds, Silva (2016) reported the presence of steroids, saponins, tannins and flavonoids in maniçoba leaves, with differences in concentration between the evaluated accessions and leaf maturation stages. Furthermore and Beltrão et al. (2015) reported total tannins ranging from 0.2 to 5.3% DM and HCN ranging from 97.2 to 291.6 mg kg^{-1} DM in maniçoba, observed tannin levels of 1.58 and 1.87% DM, respectively, for fresh maniçoba or hay, and reported an important anti-nutritional action when tannins exceeded 5% DM in diets.

The objectives of this work were to characterize the histo-anatomical pattern and degradability and to determine the secondary compounds of maniçoba in the different accessions and maturation stages (young, mature and senescent).

MATERIAL AND METHODS

Location and Characterization of the Study Area

The study was carried out at the Federal University of Paraíba, where the collections were carried out at the Maniçoba Active Germplasm Bank (BAG), of the Animal Science Department of the Agricultural Sciences Center, Campus II – Areia, PB, located in the Brejo Paraibano micro-region, at 6° 58'12" south latitude and 35° 42' 15" west longitude, with an altitude of 684 m above sea level. The region's climate is hot and humid, with dry periods of five to six months. Rainfall in the experimental year was 1,178.3 mm, mean temperature of 24.1 °C, with mean relative humidity of approximately 81% (CCA-UFPB Meteorological Station).

For the research, three accessions were selected (AC002, AC004 and AC038) from the municipalities of Barra de Santa Rosa, Monteiro and Boa Vista, respectively, which had distinct genetic characteristics (Silva, 2016), such as morphological and agronomic characteristics, chemical composition and chemical content of HCN. Leaf collections were carried out in the morning, by means of visual observation, according to the maturation stage of the plants, collecting young, mature and senescent leaves.

Histo-anatomical assessment and tissue degradability

For the histo-anatomical evaluations, three plants were selected from each accession, from which leaves and petioles were collected at the three stages of maturation (young, mature and senescent). The young leaf was collected when it was in the beginning of its development, the mature leaf when it was completely expanded, and the senescent leaf was considered when 20% of the leaf blade area was yellow. Three leaves and petioles were removed from around the tree for histo-anatomical characterization, after preparation of histological slides. The leaves were sectioned in the middle of the blade, preserving the midrib and the petiole in order to cover the median region. Then, approximately six fragments were identified; one part was placed in a freezer (-80 °C) for evaluation of tissue digestion and the other part of the samples was stored in a polyethylene bottle containing a solution of Formaldehyde, alcohol and acetic acid 50 (90% alcohol 50º, 5% acetic acid and 5% formaldehyde). Then, each sample was removed from the solution, dehydrated in a progressive alcoholic series, embedded in paraffin, and sectioned transversally at 10 µm, using a rotary microtome (JOHANSEN, 1940).

Degradability of the tissues was evaluated using the methodology described by Akin (1989) with adaptations, where three leaf and petiole fragments measuring approximately 1.0 cm^2 were placed in histological cassettes of approximately 2.0 cm long by 1.5 cm wide to include the tissues. The tissue fragments were transported in Styrofoam with ice to the incubation, placed in a nylon bag, and kept in the rumen of a fistulated cow for a period of 48 hours. After this period, the material was removed, placed in ice water to stop microbiological activity, and the residues of the fragments from each cassette were washed with distilled water and stored in identified pots containing FAA50 fixative solution, later subjected to the progressive series of alcohol,

then embedded in paraffin, sectioned approximately 10 µm in diameter in a manual microtome and deparaffinized (HAGQUIST, 1974).

Both sections were stained with safranin and toluidine blue, mounted individually between slide and coverslip with the help of a drop of Entellan™ for better adhesion of the slide to the coverslip. After preparing the slides, the quantification evaluations of each tissue were carried out with the aid of an Olympus BX-60 microscope and Zeiss AxioCam camera coupled with a Motic Image Plus 2.0 digital image capture program. To determine the total area of the section, the measurements were taken in the area between two larger vascular bundles, one of them located in the median portion between the main vein and the edge of the leaf blade. In the petiole, a region between the center and the epidermis was evaluated.

In both leaf blade and petiole, the areas (µm) were measured and the proportions (%) of the epidermis (EPI), sclerenchyma (ESC), xylem plus associated fibers (XIL), phloem (FLO) and collenchyma (COL) were calculated. The mesophyll region (MES) was calculated by the difference between the total area of the cross-section and the areas of the other tissues. For degradability, the area occupied by each tissue was measured and the disappearance of tissues by rumen degradability was analyzed. This was determined by the difference between the amount of tissues observed before and after ruminal incubation, determining the disappearance of epidermis (EPI), sclerenchyma (ESC), xylem plus associated fibers (XIL), phloem (FLO), collenchyma (COL) and parenchyma (PAR).

Tissue digestibility was considered high when the degradability was 66-95%; medium when it was 31-65%, and low when it was 1-30%.

Analysis of secondary metabolites

For the analysis of secondary metabolites through visual observation, according to the maturation stages of the plants, approximately 200 g of the leaves were collected in the morning. After collection, the samples were identified, placed to dehydrate in the sun for 2 hours, from 8:00 am to 10:00 am, and then transported to a room where they remained in the shade until drying was completed. After this period, the samples were transported to the Laboratory of Food Analysis and Animal Nutrition - LAANA, belonging to UFPB, where they were ground in a Wiley mill, using 1-mm-mesh sieves, placed in sealed plastic pots, identified and taken to the Pharmaceutical Technology Laboratory at CCS/UFPB, where the crude ethanolic extracts were prepared. Approximately 1 g of the samples was used for the phytochemical screening, where the main classes of chemical compounds were identified, with the presence (+) or absence (-) of alkaloids, steroids, saponins, flavonoids and tannins, with the abundance expressed by the number of signs, according to the methodology proposed by Matos (2009).

The following methods were used to determine each metabolic compound: alkaloids were detected by the precipitation method, using Bouchardat (A), Mayer (B), Dragendorff (C) and Bertrand reagents or silicic tungstic acid (D); steroids were detected by the Liebermann-Buchard reaction; tannins were detected by the precipitation method with iron salts and gelatin; flavonoids were detected by the Shinoda and Taubouk reactions; and saponins by stirring the aqueous extract with persistent foam formation (DESOTI et al., 2011).

Alkaloids

To detect alkaloids, 25 mL of the alcoholic extract was evaporated and basified with 0.8 ml of 1% sodium hydroxide (NaOH). 6 ml of distilled water with 6 mL of chloroform ($CHCl_3$) were filtered with cotton and placed in a separatory funnel to separate the extract from the chloroform layer. 6 mL of hydrochloric acid (1% HCl) was added to the chloroform phase, stirred and allowed to decant until clear. The supernatant hydrochloric acid phase was distributed into four test tubes, with 1 mL in each. Subsequently, the respective tests were carried out with Bouchardat (3-5 drops), Mayer (5 drops), silicic-tungstic acid (5 drops), and Dragendorff (5 drops) reagents, resulting in the formation of a precipitate in the presence of alkaloids.

Steroids

Steroids were detected by evaporating 10 mL of the alcoholic extract until dryness, and 2.5 ml of $CHCl_3$ (chloroform) was added and dissolved. This solution was distributed in three test tubes, with 0.12, 0.25 and 0.5 mL, respectively, in each. 2.0 ml of $CHCl_3$ and 1.0 ml of acetic anhydride were added to each tube, stirring slightly, and then 2 mL of H_2SO_4 (sulfuric acid) was added in each tube, shaking slowly and observing the reaction.

Saponins

To detect saponins, 2 mL of the alcoholic extract was dissolved in 10 mL of water in a 20 mL test tube, shaken for one minute, then placed at rest for 10 minutes and monitored to check whether foam was still present (positive test). The presence of saponins indicates that the substance is highly soluble in water.

Flavonoids

The detection of flavonoids was carried out by placing 15 mL of the alcoholic extract in a separatory funnel and adding 15 mL of distilled water while stirring. After allowing the mixture to stand, 15 mL of $CHCl_3$ (chloroform) was added. It was again left to rest until the layers were separated, where the chloroform layer. The remaining extract was concentrated and 3 mL of methanol was added, 2 ml of this solution was dissolved and distributed in 2 test tubes. In the first tube, 2.0 mL of 10% HCl (hydrochloric acid) and 1 cm of magnesium tape were added, allowing it to react until the tape

disappears, observing the color that appeared (pink color = positive). In the second tube, the solution was dried in a water bath with 1 mL of acetone, 0.05 mg of oxalic acid and the same of boric acid, and placed again in a water bath for 5 minutes. After this time, 10 mL of ether ethyl alcohol was added, observing in ultraviolet light the existence of fluorescence or not.

Tannins

Tannins were detected by evaporating 50 mL of the alcoholic extract until dryness. Then, 10 mL of distilled water with 6 mL of $CHCl_3$ (chloroform) were added, dissolved well and filtered through a separatory funnel with cotton wool. The filtrate was distributed in six test tubes, where the 0.5% gelatin was tested in the first 3 tubes and the other 3 were tested with 2% FeCl, placing 0.5, 1.0 and 2.0 mL of the latter, respectively.

Statistical analysis

The design used was completely randomized in a factorial scheme (3x3), with 3 accessions (AC002, AC004 and AC038), 3 stages of maturation (young, mature and senescent), and the evaluations were carried out in two structures (leaf and petiole), with 3 replications, totaling 54 experimental units. The data obtained were subjected to analysis of variance (ANOVA) and, when significant ($p \leq 0.05$), the means were compared by the Tukey test at 5% probability level by the PRO GLM of SAS (2001).

RESULTS AND DISCUSSION

In cross-sections of the leaf blades, it was possible to observe their anatomical structures at different stages of maturation (young, mature and senescent) (Figure 1).

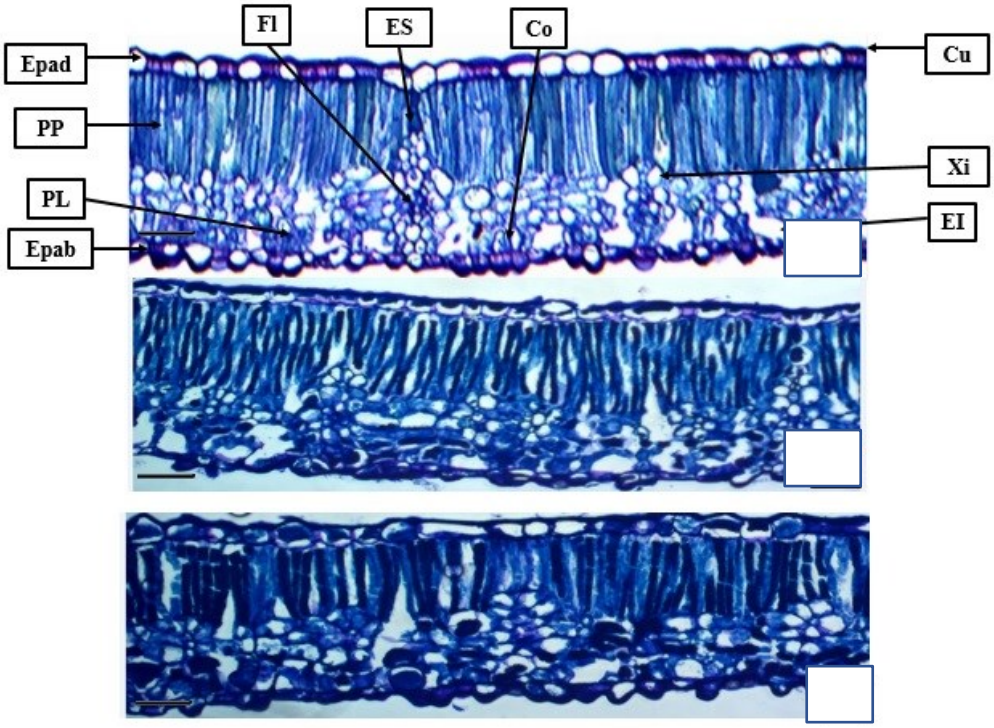

Figure 1. Cross-sections of the leaf blades of maniçoba, Young (A), Mature (B), Senescent (C). Detail of adaxial epidermis (Epad), abaxial epidermis (Epab), palisade parenchyma (PP), spongy parenchyma (PL), xylem (Xi), phloem (Fl), sclerenchyma (Es), collenchyma (Co), intercellular spaces (EI) and Cuticle (Cu). Stained with toluidine blue. Bars: A-C= 100 µm.

The leaf blades showed a cuticle that covers the entire epidermis, with papillose cells in the abaxial region and in a tabular shape in the adaxial region. The cells on the abaxial side are thinner than on the adaxial side. Regarding the mesophyll, it consists of a dorsiventral structure, where the spongy parenchyma, composed approximately of four layers of isodiametric cells, had abundant intercellular spaces, which are loosely organized and the palisade parenchyma, composed of a layer of juxtaposed cells, occupying more than half of the mesophyll with few intercellular spaces. Amid the epidermis and the larger vascular bundles, there are usually some sclerenchyma cells arranged, forming a supporting structure called the girder structure.

The greater proportion of the adaxial epidermis

observed in the senescent leaf is a negative factor, since the greater its thickness, the lower the degradability. One of the reasons for the increase in the thickness of the epidermis, especially in senescent leaves, may be related to the intensity of radiation (CASTRO et al., 2007; SOUZA et al., 2007). The lower percentage of adaxial epidermis of the leaf blade observed in the young stage can be explained by its structural development, which implies that this structure has a lower proportion of this tissue, which favors greater degradability when compared to the senescent leaf.

In cross-section of the petiole, it was observed that its epidermis is uniseriate, covered by a thin cuticle (Figure 2).

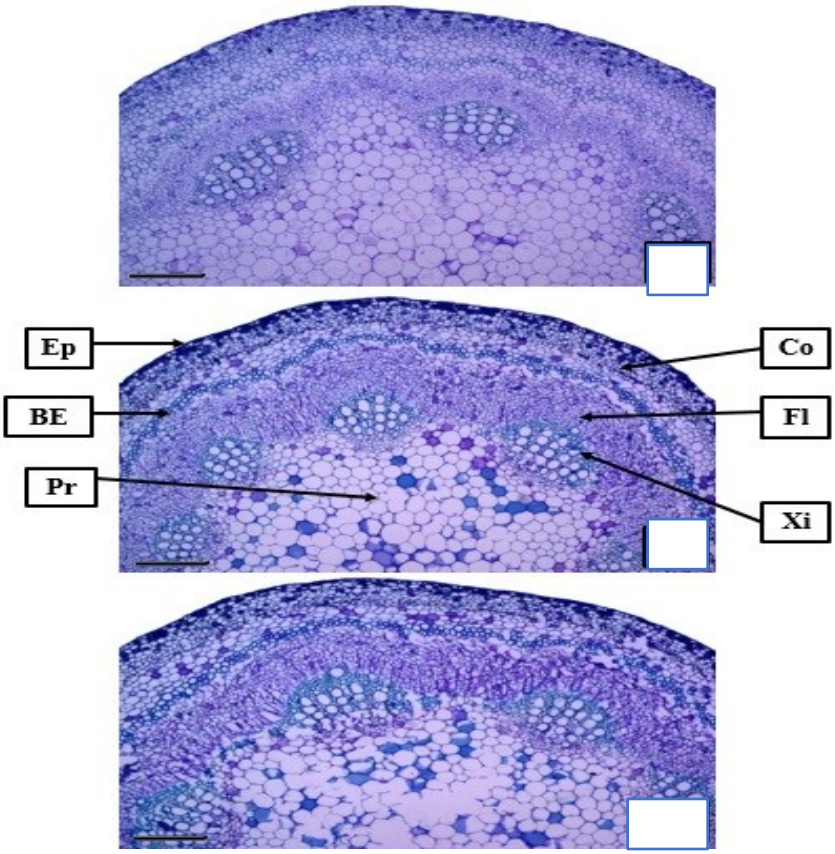

Figure 2. Cross-sections of maniçoba petioles, Young (A), Mature (B) and Senescent (C). Detail of the epidermis (Ep), Collenchyma (Co), sclerenchyma sheath (BE), phloem (Fl), xylem (Xi) and parenchyma (Pr). Stained with toluidine blue. Bars: A-C=100 µm.

The parenchyma was arranged in 5-6 layers of parenchyma cells. In sequence, there is a thin layer of angular-type collenchyma, followed again by parenchymal tissue. In sequence, there is a thick-walled sclerenchymatous sheath surrounding the phloem and forming a ring externally to the cambium layer. The xylem is organized, and the parenchyma is located internally to the vascular system.

The greater proportion of petiole xylem tissues in the senescence stage of accession 04 becomes indigestible for rumen microorganisms due to the increase in lignin content (AKIN, 1989). The difference in the variation of this tissue occurs with the maturation of the plant, which implies that the stage that had the greatest amount of this tissue may have its digestibility affected. The presence of collenchyma does not interfere with digestion as intensely as sclerenchyma, as its cells are not lignified, thus their digestion can be facilitated.

Changes in tissue proportions were more pronounced when *Manihot pseudoglaziovii* leaves were analyzed in relation to the petiole. There was interaction between the genotypes and development stage in six of the eleven variables analyzed in the leaves, while in the petiole there was no variation. In the comparison between the genotypes, AC004 stood out in general for showing higher FL, FLR and ESC, and this last character did not differ in relation to AC038 (Table 1).

In the comparison between the stages of development of the leaves, the senescent ones present greater upper epidermis, lower epidermis, FL, FLR, XI and COLR, whereas ESC and MESO were lower in the senescent leaves, regardless of the evaluated genotype (Table 1).

Table 1. Histo-anatomical evaluation and tissue degradability of maniçoba petiole and leaf.

Petiole											
Source of variation	ES	ESR	EI	EIR	FL	FLR	XI	ESC	COL	MESO	COLR
Genotype (G)	0.335	0.490	0.420	0.720	0.0129	0.210	0.163	0.105	0.581	0.660	0.379
Stage (S)	0.000	0.140	0.0008	0.630	0.097	0.006	0.002	0.081	0.007	0.001	0.0005
G x S	0.980	0.610	0.260	0.190	0.195	0.720	0.219	0.527	0.890	0.623	0.534
C.V. (%)	14.09	11.35	52.59	34.7	29.84	55.03	15.27	21.17	28.03	8.67	25.93
Genotype											
AC002	2.92	8.61	2.43	7.12	0.38 b	0.11	9.36	2.55	0.50	51.44	1.59
AC004	2.67	8.11	3.14	8.03	0.39 b	0.17	8.1	3.01	0.56	51.44	1.82
AC038	2.91	8.54	2.33	7.25	0.57 a	0.14	8.86	3.19	0.57	53.20	1.88
Stage											
Young	4.20 a	7.92	4.28 a	8.15	0.52	0.10 b	8.29 b	2.68	0.65 a	55.98 a	1.22 b
Mature	2.77 b	8.51	2.36 b	7.07	0.38	0.10 b	7.76 b	2.76	0.57 ab	53.58 a	1.79 a
Senescent	1.54 c	8.85	1.26 b	7.17	0.44	0.22 a	10.26 a	3.32	0.40 b	46.71 b	2.28 a
Leaf											
Source of variation	MER	EPP	EPPR	FLP	FLPR	XIP	ESCP	COP	COPR	MEP	MEPR
Genotype (G)	0.121	0.297	0.984	0.81	0.001	0.000	0.47	0.007	0.35	0.324	0.211
Stage (S)	0.000	0.000	0.0009	0.102	0.000	0.022	0.028	0.000	0.795	0.000	0.000
G x S	0.537	0.037	0.031	0.003	0.22	0.000	0.268	0.031	0.622	0.0002	0.786
C.V. (%)	8.73	25.39	19.35	13.63	18.29	7.74	25.59	22.15	35.35	4.54	14.66
Genotype											
AC002	11.64	0.21	0.89	9.89	2.83 a	8.91 b	3.89	0.83 b	3.08	57.68	11.75
AC004	11.91	0.24	0.89	10.12	2.99 a	10.35 a	4.18	1.13 a	2.52	56.39	10.46
AC038	10.91	0.21	0.88	10.31	2.06 b	8.53 b	3.6	1.20 a	3.22	58.25	11.67
Stage											
Young	4.93 c	0.32 a	0.75 b	10.04	1.27 c	8.80 b	3.20 b	1.66 a	2.88	66.10 a	4.53 c
Mature	11.59 b	0.21 b	0.82 b	9.40	2.31 b	9.16 ab	3.88 ab	0.98 b	2.81	58.37 b	11.76 b
Senescent	17.94 a	0.12 c	1.10 a	10.88	4.31 a	9.830 a	4.59 a	0.52 c	3.13	47.85 c	17.59 a

The ease of digestion of a forage plant can be related both to the chemical composition and to the histological composition. In terms of histological composition, the digestibility potential is related to the different tissues that compose them, which have specific digestion. Thus, the nutritional value of these plants depends, in part, on the proportion of digestible and indigestible tissues (BRITO et al., 1999; VALENTE et al., 2011), cell wall thickness and density with which the cells are arranged, which also influence the digestibility of these forages (AKIN, 1989; PACIULLO et al., 2002). Lower fiber digestibility results in rumen filling, which limits feed intake, while high fiber contents reduce ruminal degradation and decrease the passage rate of the fiber fraction. According to Allen (2000), the forage NDF content and the digestibility of forage NDF are the main factors affecting the rumen filling effect (AMORIM et al., 2023).

The stage of development and the level of leaf insertion in the tiller significantly influence the anatomical and nutritional characteristics (WILSON, 1976; QUEIROZ; GOMIDE; MARIA, 2000). With the physiological maturation of plants, structural and chemical changes are observed in the tissues, which are mainly reflected in the reduction of digestibility.

The first cells to be digested by rumen bacteria are the phloem and mesophyll cells, which is attributed to the presence of a thin non-lignified primary wall (AKIN; AMOS; BARTON, 1973). This in turn is rapidly broken down into smaller particles and readily digested (CHESSON et al., 1986). As the parenchyma is one of the tissues that have a smaller proportion of lignin in their composition, it was observed that this tissue was present in a large proportion, contributing for the digestion to occur first in this tissue. It was also verified that in the different stages of plant development, with the advancement of maturation, the other tissues were more developed; consequently, in the senescent phase it was compromised both in the tissues of the leaf blades and in the petioles.

Regarding the xylem and sclerenchyma, the resistance to degradation is related to the constitution of the cell wall that forms these tissues, since both have a secondary cell wall

with a thickness ranging from 1 to 3 µm, whose function is to provide resistance to plant cells (WILSON; BROWN; WINDHAM, 1983; GLÓRIA; GUERREIRO, 2006). According to Akin (1989), cell wall thickness also interferes with the digestibility of these tissues, contributing to indigestibility.

Ruminal microorganisms can digest cellulose, but they do not digest lignin. Thus, tissues with high proportions of lignin influence the quality of forages. It is noteworthy that the xylem and sclerenchyma tissues did not undergo degradation, regardless of their stage of maturation. As evidenced in this work and described by Akin (1989), the cells that constitute the epidermis of the leaf blade, in general, are of slow to partial digestion, and the cells of the petiole behaved in the same way, the young stage was the one with the highest degradation for all tissues.

Sterols are derived from triterpenes, essential components of cell membranes, which are stabilized by the interaction of these components with phospholipids. In addition, these compounds stand out for promoting the growth and development of plants, so they can be considered primary rather than secondary metabolites (TAIZ; ZEIGER, 2013).

In the phytochemical screening of maniçoba leaves, the presence of steroids, tannins, flavonoids and saponins was observed (Table 2). The leaves of maniçoba (*Manihot ssp.)* showed absence of alkaloids in all stages and accessions evaluated. The absence of this metabolite in a forage is very important for animal nutrition, since, when present, it can cause toxicity to the animal, in addition to impairing forage intake and digestibility.

Table 2. Phytochemical profile of crude ethanolic extract of leaves of maniçoba accessions at different stages of maturation.

Maturation Stages	Alkaloids				Steroids (*Liebermann-Burchard*)	Tannin		Flavonoids		Saponin
	A	B	C	D		Gelatin 0.5%	$FeCl_3$ 2%	phytamagnesium (*Shinoda*)	Fluorescence (*Taubouk*)	Foam
Accession 02										
Young	-	-	-	-	+++	++	+++	++	+++	+
Mature	-	-	-	-	++	++	+++	++	+++	+
Senescent	-	-	-	-	++	++	+++	++	++	++
Accession 04										
Young	-	-	-	-	+++	++	+++	++	+++	+++
Mature	-	-	-	-	+++	+	++	+++	+++	++
Senescent	-	-	-	-	+++	+	++	++	++	-
Accession 38										
Young	-	-	-	-	+++	++	++	++	+++	++
Mature	-	-	-	-	+++	+	++	+++	+++	++
Senescent	-	-	-	-	++	+	++	++	++	+

Legend: (A) Bouchardat, (B) Maye, (C) Dragendorf, (D) Bertrand or silico-tungstic acid; "+++" (high intensity), "++" (medium intensity), "+" (low intensity), "-" (negative reaction).

All accessions evaluated showed positivity for the presence of steroids, with superiority in accession 04 in all stages, while for the other accessions the presence decreased with the maturation of the plant, especially in accession 02. The presence of tannins was observed in the three accessions studied, but accession 02 showed a greater intensity both in the gelatin precipitation method and in the iron salts method, with no difference in the presence of this compound in the three stages of maturation of the plant, thus showing that this accession has a higher concentration of this phenolic compound. For the other accessions evaluated, there was a greater presence of this compound in the $FeCl_3$ precipitation method, with no difference in the different stages, except for accession 04, for which the intensity was higher in the initial stage (+++).

In the tests performed for the identification of flavonoids, medium to high intensity was observed in all accessions. It should also be noted that for the Shinoda method, accession 02 had medium intensity in all stages, whereas in the other accessions there was a greater intensity in the mature stage, and medium concentration in the other stages. In the Taubouk method, all accessions showed the same behavior, high intensity in young and mature stages and medium when senescent.

It is noteworthy that tannins play an important role in animal nutrition, and may exert beneficial and/or adverse effects when at high concentrations, behaving as an anti-nutritional factor, in relation to the digestion and absorption of nutrients by the gastrointestinal tract, as well as health and consequently animal production. In monogastric animals, the effects are more pronounced, especially when the contents of condensed tannins are above 1% in the diet, causing losses in production, due to the impairment of consumption and digestibility of protein and essential amino acids (MCDONALD et al., 1995), in addition to toxicity. On the other hand, ruminants are more tolerant to tannins due to the action of rumen microorganisms, which decrease the negative effects, as they are able to degrade anti-nutritional compounds

into simpler and non-toxic substances.

According to Slimestad (1998), younger tissues generally have higher biosynthetic rates of metabolites, such as flavonoids. The age and development of the plant, as well as the different plant organs, can also influence not only the amount of metabolites produced, but also the proportions of each component (BOWERS; STAMP, 1993; EVANS, 1997; HENDRIKS et. al., 1997). In studies with leaf samples of *Tournefortia paniculata Cham*, where the harvest time was taken into account, Moraes, Sousa and Yamamoto (2007) found variations in the average levels of flavonoids, demonstrating that the production of these constituents may be restricted to a specific stage of plant development or to certain ecological or environmental conditions.

The accumulation of flavonoids occurs in the surface tissues (epidermis, subepidermis, cuticle hairs and epicuticular material) and these substances are used by the plant as UV filters, since they absorb UV-B radiation without altering the photosynthetically active radiation, protecting leaf cells, like also chloroplasts, from UV radiation (TAIZ; ZEIGER, 2013). In this perspective, the presence of flavonoids in the species of the Caatinga was already expected, since the light intensity is high in the semi-arid region. In addition to UV radiation, temperature may have possibly contributed to the concentration of this compound. Studies report the intensity of this metabolite in Catingueira (BAHIA; DAVID; DAVID, 2010).

As for the presence of saponin, all accessions evaluated were positive, except accession 04 in the senescent stage. Accession 02 at the same stage of maturation showed medium intensity (++). According to Garcia and Carril (2009) and Lima Júnior et al. (2010), this compound has the ability to form complexes with steroids, proteins and phospholipids from the membranes of rumen fungi and protozoa, acting by disrupting the action of these membranes, in addition to preventing the growth of gram-positive bacteria of the rumen flora. Kamra (2005) points out that saponins also have a deleterious effect on rumen fermentation, causing a reduction in total fatty acids and the acetate: propionate ratio from 1.93 to 1.37 in the presence of 1% saponin in the diet.

Possibly, the production of secondary metabolites may be restricted to a specific stage of plant development or to certain environmental conditions. In addition, there may be differences in the metabolic profile between species and even within the same species. The factors that contribute to this difference are seasonality, temperature, plant age, water availability, as well as plant development, isolated or associated with each other (GOBBO-NETO; LOPES, 2007). Probably, some of these factors have affected the content of chemical compounds in the accessions used in this study.

CONCLUSIONS

With the advancement of physiological maturation of tissues in leaves and petioles of maniçoba accessions, there is a reduction in degradability.

Accession 38 has higher digestibility as it has a higher proportion of mesophyll.

The presence and concentration of secondary metabolites vary between accessions and maturation stages.

ACKNOWLEDGMENTS

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq).

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