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Bark regeneration and tannin content in *Myracrodruon urundeuva* Allemão after simulation of extractive damages—implications to management

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Abstract Due to the current exploitation and consequent extinction of native medicinal plants around the world, new strategies have been proposed to address the sustainable use of this resource. Accordingly, the goal of this study is to assess the speed of bark regeneration of *Myracrodruon urundeuva* Allemão and to compare the tannin content before and after tissue regeneration. Twenty individuals from an area of Caatinga in Caruaru, Pernambuco State (NE Brazil), were selected. To evaluate the speed of bark regeneration, four treatments were established, according to the area of bark removed (10 × 2, 6 × 5, 8 × 5 and 10 × 5 cm), with five replicates (five individuals of *M. urundeuva* Allemão) and three treatments of tannin content (bark removed before damage, as regenerated tissue, and non-impacted bark or control). At the end of 23 months of monitoring the regeneration, we found that only seven of the 20 individuals analyzed did not fully heal. No significant correlation between the monthly percent regeneration and average monthly precipitation was found. The tannin content varied according to the quantified tissue (after damage, regenerated and control) as well as between damage classes (20 cm^2—41.64 to 63.53 mg; 30 cm^2—49.25 to 67.54 mg; 40 cm^2—31.69 to 67.44 mg; 50 cm^2—34.08 to 48.53 mg). Despite the variations found, there was no significant difference between the measurement periods ($p > 0.05$) or damage classes ($p > 0.05$). The results showed that the regeneration rate was higher in individuals belonging to the 10 × 2 and 10 × 5 cm groups and that there is no correlation between precipitation and regeneration speed. Tannin levels did not vary significantly in the tissues before damage and after regeneration.

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Introduction

The current loss of biodiversity is increasingly encouraging the conservation of natural resources. There are several issues linked to the non-sustainable use of medicinal plants, such as habitat destruction and disorganized harvesting. However, high commercial demand in recent years has had significant impact on the current rate of exploitation and has even led to the extinction of some plant species (Cunningham and Mbenkum 1993). Some authors have expressed their concerns about this extractive pressure, since plants cannot regenerate quickly enough to keep up with the high demand from local markets and phytotherapeutic industries (Williams et al. 2000; Nunes et al. 2003; Botha et al. 2004).

The *Caatinga* is one ecosystem that has been suffering from high exploitation. The rate of natural resource harvesting, especially woody plant species, is occurring at an increasing rate. The exploitation of many species is considerably affecting the stability of their plant populations. In some cases, like the *aroeira* (*Myracrodruon urundeuva* Allemão), extractive activity has caused the Brazilian Ministry of the Environment to elevate it to the vulnerable species category in the federal list of endangered species (MMA 2008). This plant is widely distributed throughout the Brazilian northeast and well studied because of the therapeutic properties (Leite 2002; Monteiro et al. 2005b). *Aroeira* is not only primarily targeted for its therapeutic proprieties but is also reputed for the high mechanical resistance of its wood, making it a preferred timber used in construction (Leite 2002; Queiroz et al. 2002).

The main medicinal proprieties of *aroeira* are generated by the considerable amount of tannins in the stem bark of its trunk (Menezes and Rao 1988; Chaves et al. 1998; Leite 2002; Viana et al. 2003).

To prevent the extinction of medicinal plants, research is required to investigate strategies for sustainable extraction. However, there are few studies on this subject throughout the world, none of which have been conducted in the Brazilian semi-arid region. Considering the high impact on species in this area, Cunningham and Mbenkum (1993) proposed several strategies for sustainable use, including the cultivation of arboreal species, the extraction of substitute species that possess similar amounts of bioactive compounds, as well as the adoption of studies on the regeneration of stem bark from the stems of medicinal species.

These suggestions prompted the central question of the present study: Do differences exist in the content of tannin compounds in the bark of *M. urundeuva* Allemão after extractive damage? This study aims to evaluate the regeneration speed of *M. urundeuva* Allemão stem barks and the level of tannins after bark extraction and in the regenerated tissue. These secondary components are present in most plants, and according to the species, vary its concentration in the plant tissue, depending on the age and size of the plant, part collected, the season, and even the site of collection (Teixeira et al. 1990; Simón et al. 1999; Larcher 2000; Monteiro et al. 2005a, b, 2006).

Methods

Study area and evaluation of regeneration speed of stem bark from *M. urundeuva* Allemão

The study was conducted in a section of dry forest in Caruaru, a semi-arid region of Pernambuco State, Brazil. The land is protected by the State and owned by the Institute of Agricultural Research (*Instituto de Pesquisa Agropecuária*). The Caruaru municipality, the largest population center in the *agreste* region of Pernambuco State, has an economy based mainly on agriculture, handcrafts, and tourism. The population density is 229.28 inhabitants per km^2 (Prefecture of the Caruaru 2010). The climate is semi-arid, with rainfall concentrated during the months of March and April, although heavy rainfall may occur in January and February in atypical years (e.g. 2004). The local vegetation is spiny and caducifolious.

Twenty *M. urundeuva* Allemão individuals with the diameter at breast height (DBH) were selected from a total of 27 plants sampled in 1 ha that met the minimum criteria of DBH > 35 cm and height > 3.5 m. The selected specimens were monitored for 23 months, from April 2006 to February 2008, to determine the regeneration speed of stem barks after extractive damage and the content of tannins compounds before and after damage

To assess the regeneration speed of *M. urundeuva* Allemão barks, four treatments with five replicates were conducted. Bark was extracted at 1.30 m in four dimensions for each treatment: 10 × 2 cm (20 cm^2); 6 × 5 cm (30 cm^2); 8 × 5 cm (40 cm^2) and 10 × 5 cm (50 cm^2). Each treatment was replicated for five specimens of *aroeira*. The analysis for the regenerated area followed the same principles used to calculate the leaf area (Pedro-Júnior et al. 1986; Santos and Del-Claro 2001). The regenerated area in each individual was assessed once per month.

Determination of total tannin content in the bark of the stem of *M. urundeuva* Allemão

To avoid misinterpretations of the variation of tannin contents before and after damage, a control treatment used the same procedure to determine the amount of tannin compounds. Bark used for the control was removed in the smallest size class (10 × 2 cm; about 3 g) at the same height as the regenerated tissue but was extracted from the opposite side of the stem. Bark from the stem of 20 specimens was removed at the beginning of the study in April 2006. These samples, in addition to the completely regenerated tissues, were placed in paper bags and brought to the laboratory for the analysis of total tannin content.

In order to compare the tannin content after damage and in the regenerated tissue, the total phenol and tannin contents were quantified using tannic acid as a standard, according to the Folin–Ciocalteu and casein precipitation methods, respectively (Folin and Ciocalteu 1927; Hagerman and Butler 1989; Appel et al. 2001; Mueller-Harvey 2001; Schofield et al. 2001). The Folin–Ciocalteu method consists of the addition of 0.25–0.50 mL of extract to 75 mL of distilled water, 5 mL of Folin–Ciocalteu reagent (in aqueous solution at 10% *v*/*v*), 10 mL of sodium carbonate (0.75% *m*/*v*), and a remaining amount of distilled water to make a final volume of 100 mL. The collected tissue from the stem bark of the sampled specimens was placed in paper bags and taken to the Laboratory of Natural Products, Department of Pharmacy, Universidade Federal de Pernambuco. The bark samples were dried em estufa at 50°C for approximately three days. After drying, they were crushed and methanolic extracts (80% *v*/*v*) were made by combining 500 mg of the material with 50 mL of solvent. Five successive extractions were performed under heat; the material was filtered whenever the solvent started to boil. All extractions were conducted in triplicate. The solution was allowed to rest for 30 min, and the absorbance was read at a wavelength of 760 nm on a spectrophotometer, which was calibrated using control solutions of tannic acid in the following concentrations: 0.1, 0.5, 1.0, 2.5 and 3.75 μg mL^{-1}.

The total tannin concentration was determined by the casein precipitation method, which requires the addition of 1 g of casein to 6 mL of sample extract diluted in 12 mL of distilled water. The resulting solution was stirred for 3 h at room temperature (25°C), then filtered through 9-cm Whatman filter paper, and the filtrate was adjusted with distilled water for a final volume of 25 mL. Aliquots (8 to 12 mL) of this solution were tested for residual phenols using the Folin–Ciocalteu method. The amount of tannins corresponds to differences in the absorbance of the samples precipitated with casein and those obtained by the analysis of total phenols. The amounts of total phenols and tannins are expressed in mg of dry matter (Monteiro et al. 2005b, 2006).

Data analysis

Differences in tannin production in the bark samples removed before and after bark regeneration, and during the monitoring of tissue regeneration, were verified through variance tests. First, data were submitted to the Shapiro–Wilk test for normality. In the cases where the data were distributed normally, a parametric one-way ANOVA was applied; otherwise, the equivalent non-parametric Kruskal–Wallis test and Wilcoxon test (dependent samples; for 5% probability) were used. To assess significant differences between monthly rates of regeneration for the different classes of damage over two years, variance tests were done on the percentage data transformed into angular values (arcsin $\sqrt{x/100}$). The Spearman coefficient was used to evaluate the correlation between the monthly tissue regeneration and average monthly precipitation, in the 23-month period (April 2006 through to February 2008). Bioestat 5.0 software (Ayres et al. 2007) was used for all statistical analyses.

Results and discussion

Regeneration in the bark of *M. urundeuva* Allemão stems

The regeneration speed was different for each damage class analyzed. The specimens in the 20, 30 and 40 cm^2 damage classes three months after damage (June 2006) showed more than 50% of regenerated area (Table 1); notably, two specimens from the 20 cm^2 damage class had 89.17% tissue regeneration (Fig. 1). Five months after the beginning of the experiments (August 2006), the majority of the specimens in all of the damage classes showed more than 70% regenerated area, with three of five individuals from the 20 cm^2 class showing the highest rates, 100% of regenerated area (the remaining two showed 94.50% and 93.83%) and one individual from each of the 30 and 50 cm^2 classes showing 55.28 and 43.67 cm^2, respectively (Fig. 1). At the end of a year of monitoring, all specimens belonging to the 20 cm^2 damage class showed full bark regeneration, whereas the remaining classes still had specimens in the regeneration phase (Table 1). At the end of the 23 months of monitoring tissue regeneration, we found that only seven of the 20 specimens analyzed from all of the classes had not healed fully. These were one from the 30-cm^2 damage class, with 88.89% regenerated area; three from the 40 cm^2 damage class, one with 97.50% and two with 99.17% regenerated area and three from the 50 cm^2 damage class, with 96.97%, 97.67% and 98.07% regenerated area.

Table 1 Regeneration means of *Myracrodruon urundeuva* Allemão bark in cm^2 per month among the four damage classes (20 to 50 cm^2) over 23 months of monitoring

Months	20 cm^2	30 cm^2	40 cm^2	50 cm^2
1	6.87 (34.33)	4.47 (14.89)	11.6 (29.00)	6.00 (12.00)
2	8.10 (74.83)	9.8 (97.56)	9.63 (53.08)	16.70 (45.40)
3	2.54 (87.52)	2.30 (55.20)	4.79 (65.00)	2.20 (49.80)
4	1.10 (93.00)	3.24 (66.02)	2.60 (71.50)	6.77 (63.34)
5	0.93 (97.67)	2.09 (72.98)	2.79 (78.53)	2.11 (67.56)
6	0.05 (97.90)	1.36 (77.51)	1.13 (81.36)	1.99 (71.55)
7	0.03 (98.03)	0.03 (77.61)	0.00 (81.36)	0.33 (72.21)
8	0.01 (98.08)	0.03 (81.03)	0.42 (82.30)	0.21 (72.63)
9	0.17 (98.92)	0.30 (82.03)	0.15 (82.68)	0.48 (73.58)
10	0.14 (99.60)	0.07 (82.26)	0.12 (82.98)	0.28 (74.13)
11	0.02 (99.67)	0.01 (82.29)	0.00 (82.98)	0.06 (74.26)
12	0.00 (99.67)	1.85 (88.46)	2.44 (89.09)	3.46 (81.17)
13	0.06 (100)	1.13 (92.21)	1.06 (91.73)	1.19 (83.55)
14	0.00 (100)	0.45 (93.70)	1.39 (95.22)	1.87 (87.29)
15	0.00 (100)	0.19 (94.33)	0.35 (96.09)	0.85 (88.99)
16	0.00 (100)	0.35 (95.51)	0.21 (96.61)	0.47 (89.93)
17	0.00 (100)	0.19 (96.13)	0.26 (97.27)	0.17 (90.27)
18	0.00 (100)	0.49 (97.78)	0.21 (97.80)	1.39 (93.05)
19	0.00 (100)	0.00 (97.78)	0.19 (98.28)	0.16 (93.37)
20	0.00 (100)	0.00 (97.78)	0.08 (98.49)	2.24 (97.85)
21	0.00 (100)	0.00 (97.78)	0.06 (98.65)	0.29 (98.43)
22	0.00 (100)	0.00 (97.78)	0.21 (99.17)	0.00 (98.43)
23	0.00 (100)	0.00 (97.78)	0.00 (99.17)	0.05 (98.54)

Values in parentheses represent the percentage of regeneration per month

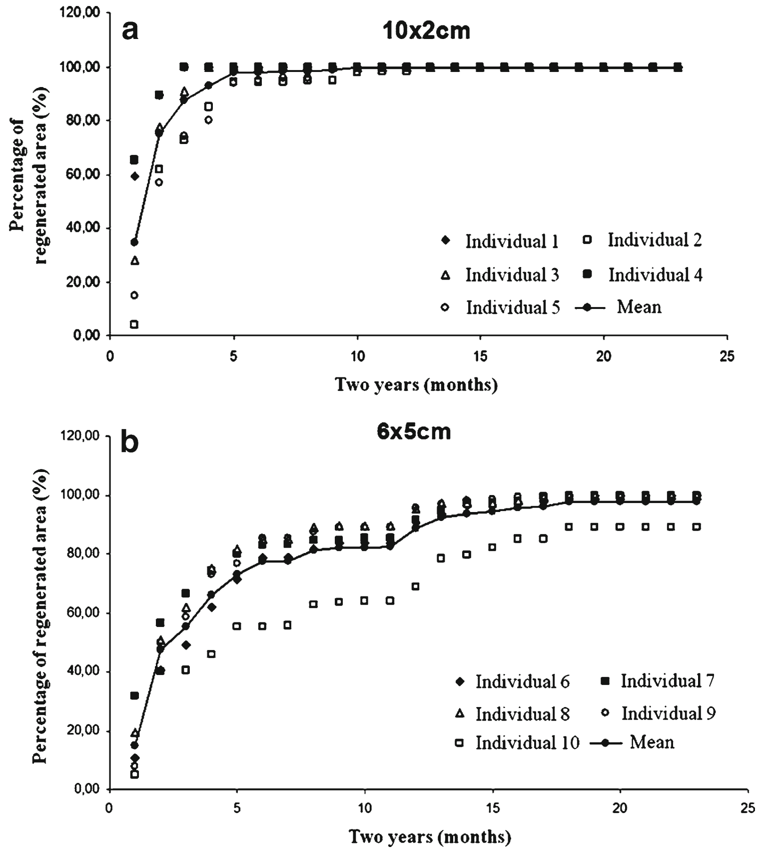
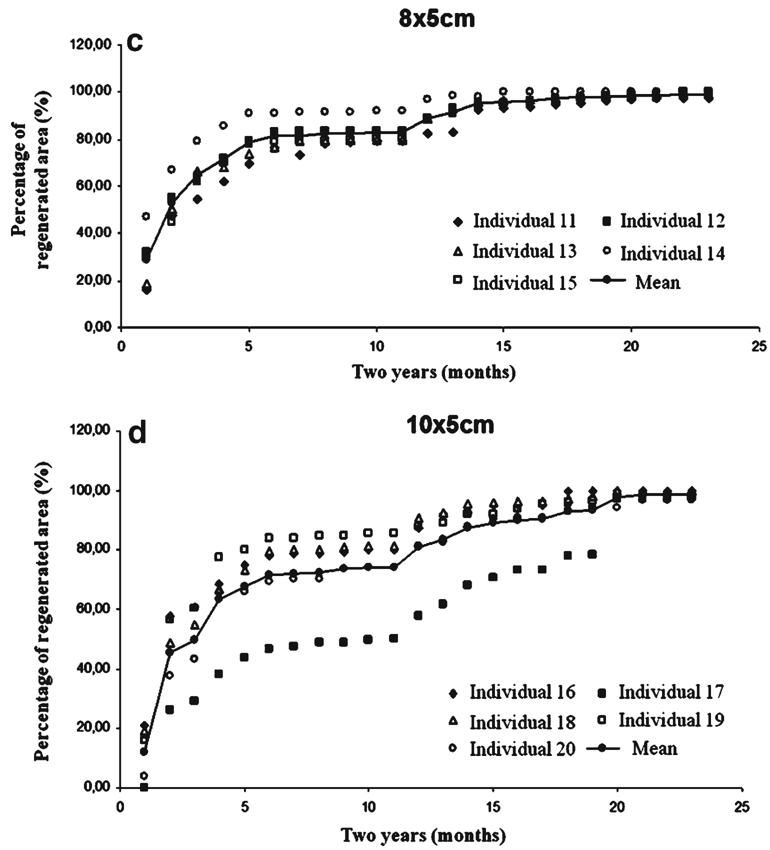


Fig. 1 Percentage of regeneration of the bark *Myracrodruon urundeuva* Allemão over 23 months. Damage 10×2 (**a**), 6×5 (**b**), 8×5 (**c**) and 10×5 (**d**)

Individuals with a damage level of 20 cm^2 had an average monthly regeneration of 3.22 cm^2/month, followed by individuals of the 50 cm^2 class (2.13 cm^2/month) and those of the 30 cm^2 and 40 cm^2 classes, both which had rates of 1.40 cm^2/month. The first class, 20 cm^2, differed significantly from the subsequent classes, 30 cm^2 ($p < 0.05$; $F = 9.13$) and 40 cm^2 ($p < 0.05$; $F = 9.30$). Similarly, the 50-cm^2 class differed significantly from the 30-cm^2 ($p < 0.05$; $F = 34.45$) and 40-cm^2 ($p < 0.05$; $F = 59.09$) classes. The highest and lowest damage classes had the highest monthly regeneration rates and did not differ significantly. This is probably due to the variations of due to individuals whose high regeneration rates increased the treatment means, since the individuals were located close to each other and were subjected to similar environmental variables. Moreover, factors such as plant physiology, seasonality, microclimate, presence of exudates, intensity and frequency of harvesting may influence bark regeneration (Cunningham 2001). For example, in the families Moraceae and Euphorbiaceae, the cambial cells are protected by exudates after bark removal, which affects the regeneration of tissues (Cunningham 2001).

No other studies have used the same approach as this study to investigate the period required for the complete regeneration of stem bark after damage. A similar technique was developed by Cui and Li (2000), who evaluated the regeneration time of the bark of *Eucommia ulmoides* Oliv. stems after the extraction of 1 to 2 m of bark. Based on their results, the authors recommended a period of 4 to 5 years for the total regeneration of stem bark of this species, depending on the extent of the damage and the specimen. They also verified that preserving the cambial cells could protect the species from extinction (Li et al. 1983; Cui and Li 2000). Studies conducted with *Rhizophora mangle* L., in mangroves in Espírito Santo State, Southeastern Brazil, showed that the

Fig. 1 (continued)



removal of bark from the stem in longitudinal strips on up to 50% of the trunk perimeter did not cause irreversible damage to the trees (Carmo et al. 1997).

The percent monthly regeneration was correlated to the average monthly precipitation over 23 months; however, a significant correlation between these variables was not found for individual damage classes: 20 cm^2 (rs = −0.050; $p = 0.87$), 30 cm^2 (rs = −0.045; $p = 0.84$), 40 cm^2 (rs = −0.019; $p = 0.93$) and 50 cm^2 (rs = −0.048; $p = 0.83$; Fig. 2). Conversely, when studying the lignification and suberisation processes of renewed bark of *Prunus persica* (L.) Bastch., Biggs (1986) argued that the availability of water, in addition to temperature and nutritional status of the plant, might influence the regeneration of bark after damage (Biggs 1986). In the present study, we verified that the regeneration speed varied between individuals in the studied population. A hypothesis that could be easily tested in future studies is that plant gender could be an important variable. *M. urundeuva* Allemão is a dioecious species and the female individuals probably invest more energy in reproduction than the male individuals do, possibly by diverting resources that would be used in regeneration.

Tannin content in bark of *M. urundeuva* Allemão stems

The amount of tannin varied as a function of the tissue measured: after damage (31.69 to 67.54 mg), regenerated (36.19 to 65.10 mg) and control (31.58 to 77.70 mg). Tannin also varied between damage classes (20 cm^2—41.64 to 63.53 mg; 30 cm^2—49.25 to 67.54 mg; 40 cm^2—31.69 to 67.44 mg and 50 cm^2—34.08 to 48.53 mg; Table 2). Despite the variations, there were no significant

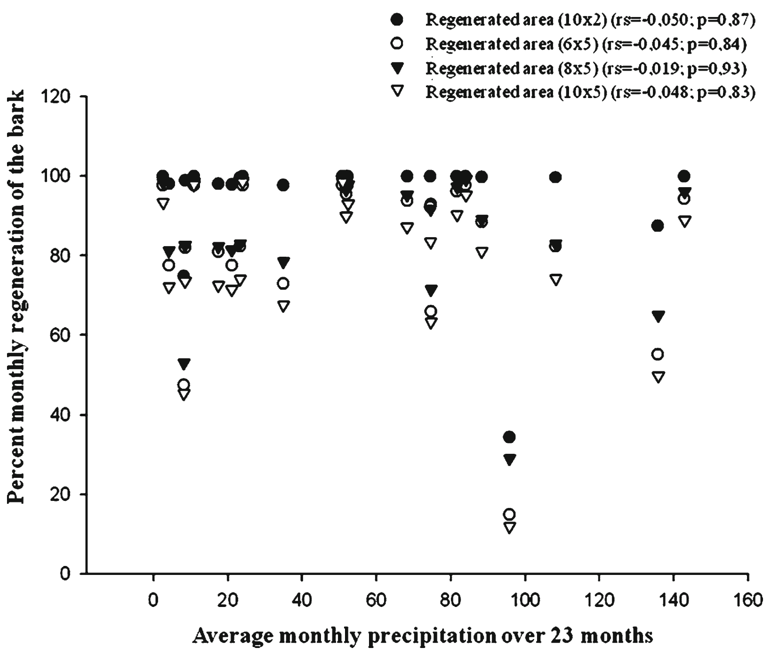


Fig. 2 Relationship between the percent *Myracrodruon urundeuva* Allemão bark regeneration and the monthly precipitation (mm) over 23 months

differences between the measurement periods (Kruskal–Wallis test, $H = 0.10$; $p > 0.05$) or damage classes (Wilcoxon test, $Z = 0.48$; $T = 92$; $p > 0.05$).

Few studies have evaluated the strategies of plants in the production and synthesis of bioactive compounds, such as tannins, in response to a physical injury, especially in the semi-arid region of Brazil. In India, researching anatomical and histochemical factors in the regeneration of barks of *Hevea brasiliensis* (Willd. ex Adr. Juss) Muell. Arg. (Euphorbiaceae) stems, Thomas et al. (1995) found that there were higher amounts of tannins and crystals in renewed bark, particularly in the outer cells of the renewed bark. However, the present study did not find significant differences in the tannin content in the renewed tissue. This fact, in addition to the information obtained by Monteiro et al. (2005b), whose biometric factors such as diameter and height did not influence tannin deposition in the stem barks of three medicinal plants, among them *M. urundeuva* Allemão, led to the conclusion that these variables were not related to the concentration of tannin compounds. However, in the Monteiro study, the tannin content in *M. urundeuva* Allemão varied because of the strong climate seasonality of the Caatinga (Monteiro et al. 2006).

Table 2 Average amount of tannin followed by standard deviation before and after regeneration of stem bark tissues of *Myracrodruon urundeuva* Allemão in a *Caatinga* area, Caruaru municipality, PE, Brazil

Damage area (cm^2)	Individuals	Tannins content after damage (mg)	Tannins content in regenerated tissues (mg)	Tannins content control (mg)
20	1–5	52.63 ± 9.30Aa	51.97 ± 7.76 Aa	51.45 ± 9.00 Aa
30	6–10	55.21 ± 7.51 Aa	55.55 ± 5.84 Aa	63.67 ± 11.68 Aa
40	11–15	46.62 ± 14.47 Aa	47.18 ± 7.13 Aa	45.31 ± 5.42 Aa
50	16–20	45.09 ± 6.20 Aa	41.44 ± 14.41 Aa	44.10 ± 9.98 Aa

Means followed by the same capital letter (column) in the vertical and lower case letter (line) in the horizontal did not significantly differ by the Kruskal–Wallis (control) and Wilcoxon (damages) test at 5% of probability

Conclusions

According to the results, individual variations seem to explain the differences found between the damage classes. There was no relationship between precipitation and regeneration processes for the monitored individuals. There was also no significant difference in tannin content between the damage classes, showing that the healed tissue could have a similar biological effect as that of the original bark.

Considering that this is the first study looking to elucidate the relationship between the regeneration process of the stem bark and the production of bioactive compounds for a species from the semi-arid region of Brazil, this information could help shape future strategies for sustainable use and management of medicinal plants. Research with a similar focus may lead to the sustainable collection of stem barks for addressing: (a) an appropriate harvesting season in terms of the production of bioactive compounds and climate seasonality; (b) areas from which to collect stem bark without harming the plant; (c) the relationship between the extent of injury to the stem bark and the healing speed, where the information on the latter is provided by this study, indicating that distinct damage classes regenerate at different speeds.

New studies using the approach applied in this study could consider the effects of climate seasonality of the *Caatinga*, sex and phenology on the regeneration speed of the studied species, and the extent of extractive damage caused by the harvesters.

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