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ORIGINAL ARTICLE

Montrichardia linifera (Araceae) biological potential, phytochemical prospection and polyphenol content

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Abstract

Montrichardia linifera is commonly known in Brazil as "aninga." In November 2008 and August 2009, we collected plant samples and extracted leaves and stems using hexane or dichloromethane and methanol to evaluate its biological and chemical potential, phytochemical profile and polyphenol content. Differences in the chemical composition of the collections were evident. In the first collection, the apolar extracts were the richest in metabolite composition while the polar extracts were the richest in the second collection. In general, extracts from Aug/2009 had higher antioxidant and cytotoxic activities than extracts from Nov/2008 and the stem and leaf extracts from the Aug/2009 collection showed the highest polyphenol content. As an insecticide against *Sitophilus zeamais*, the hexane extract of the leaves (Aug/2009) presented concentration-dependent insecticidal activity. Only the methanolic extract of stems and leaves (Aug/2009) presented a small inhibition halo (9 and 7 mm, respectively) against *Aeromonas hydrophila* when evaluating its antibacterial potential. The study demonstrates that, collection period is an important factor to consider in phytochemical and biological activity studies of *M. linifera*.

Keywords: DPPH; antioxidant; *Artemia salina*; *Aeromonas hydrophila*; *Sitophilus zeamais*; polyphenols.

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Introducción

Plants metabolize substances such as proteins, carbohydrates and lipids, called primary metabolites, which are essential to their development; during their evolutive process, they produce other substances called secondary metabolites. These metabolites can act as chemical mediators, intermediating between the plant and the environment and playing a pivotal role in the protection of the plant (Taiz & Zeiger 2010). Because of this chemical potential (reaction against some organisms), these substances have been researched to find new molecules with antimalarial, insecticide, and antimicrobial, among other activities and several vegetal species have been prospected in the last years.

M. linifera is popularly known in Brazil as 'aninga,' 'aningaçu,' or 'aningaíba.' It is an herbaceous macrophyte found in river margins, mainly in the Amazonian floodplain ecosystem (Macedo et al. 2005). It is commonly used in the treatment of snake and stingray bites (Amorozo & Gély 1988), nosebleeds, abscesses and tumors (Matos 2000) and also as an expectorant (Lins 1994). Additionally, local people use specific parts of this species to treat specific diseases. The leaves are used to treat rheumatism and ulcers, and the roots are used as an antidiuretic; the sap is extracted from the trunk to treat deep cuts (Plowman 1969), however, excessive use of the sap can result in burns and even cause blindness if it comes in contact with eyes (Amarante et al. 2009). Assessments of the mineral components in the leaves and fruits have contributed to the understanding of the species chemical properties (Amarante et al. 2009, Amarante et al. 2010) as well as provided information on the nutritional value of both parts (Amarante et al. 2011d). Focused on its biological potential, Costa et al. (2009) evaluated the ethanolic extract obtained from the leaves and observed moderate activity against *Plasmodium falciparum* (clone W2) and low toxicity to *Artemia salina*. Silva et al. (2011) also observed a low biological potential against the same antiplasmodial strain for *M. linifera* hexane leaf extract. Trujillo-Trujillo et al. (2006) found alkaloids and triterpenoids and sterols, either together or individually, in ethanol extracts from leaves and stems. Costa et al. (2009) found alkaloids, flavonoids, tannins, triterpenes and steroids in ethanol extract. Amarante et al. (2011a) found alkaloids, flavonoids, tannins, triterpenes and steroids in ethanol extract. Amarante et al. (2011b) found p-hydroxybenzaldehydea, and Silva et al. (2011) found steroids in the hexane extract from leaves of *Montrichardia linifera* (**Table 1**).

Table 1. Previous chemical studies on *Montrichardia linifera*, available in literature.

Plant Extract or Part	Period of collection	Place of collection	Chemical Composition	Biological Activity	Authors
Ethanol extract of leaves and stems and their petroleum ether and ethyl acetate phases.	July/2005	Colombia	▸ Alkaloids in ethyl acetate extract of leaves and petroleum ether phase of stems. ▸ Triterpenoids and/or sterols in ethyl acetate and petroleum ether phase of leaves and in the petroleum ether phase of stems.		Trujillo-Trujillo et al. (2006)
Leaves	Rainy (March/2008) and dry (July/2008) season	Guamá riverside, Belém, PA, Brazil	▸ Ca, Cu, Fe, Mg, Mn, Zn level. ▸ Toxic Mg level for animal food. ▸ Ca and Mg: higher level in dry season.		Amarante et al. (2009)
Ethanol extract.	Rainy season (April/2007)	Guamá riverside, Belém, PA, Brazil	Alkaloids, flavonoids, tannins, triterpenes and steroids.	Moderate antiplasmodial activity and low brine shrimp toxicity.	Costa et al. (2009)
Leaves and fruits.	Rainy season	Guamá and Maratauíra riverside, Belém, PA, Brazil	▸ Ca, Mg, Cu, Fe, Zn, Mn level. ▸ Low proteic level. ▸ Toxic Mg and Mn level for animal (turtles, cattle and buffaloes) food.		Amarante et al. (2010)
Hexane and Ethanol extracts of stems and hexane, dichloromethane and ethyl acetate fractions of ethanol extract.	Rainy season (April/2007)	Guamá riverside, Belém, PA, Brazil	Alkaloids, flavonoids, tannins, triterpenes and steroids in the ethanol extract.	Brine shrimp toxicity of the ethanol extracts and the hexane, dichloromethane and ethyl acetate fractions.	Amarante et al. (2011a)
Hexane and Ethanol extracts of stems and hexane, dichloromethane and ethyl acetate fractions of ethanol extract.	Dry season (July/2008)	Guamá riverside, Belém, PA, Brazil	*p*-hydroxybenzaldehyde	High toxicity against *A. saline* and high antiplasmodial activity	Amarante et al. (2011b)
Tea from senescent leaves.	Dry season (July/2008)	Guamá riverside, Belém, PA, Brazil	▸ Ca, Cu, Fe, Mg, Mn, Zn level. ▸ Mn toxic level to human consumption.		Amarante et al. (2011c)
Physical, chemical, and nutritional fruit characterization.			▸ Low protein value ▸ Toxic Mn level for animal (buffaloes) food		Amarante et al. (2011d)
Hexane extract from leaves.	Rainy season (April/2007)	Guamá riverside, Belém, PA, Brazil	▸ Steroids	Low antimalarial potential	Silva et al. (2011)

However, the production of secondary metabolites is not solely determined by genetic factors, its is determined mainly by environmental factors such as the presence of pathogens or herbivores, as well as plant phenological stage, locality, period of collection, water availability, among other abiotic factors (Gobbo-Neto & Lopes 2007). Considering the widespread occurrence and use of *M. linifera*, its biological potential and variance during the production of secondary metabolites, we advanced this study. The plants were collected, in the same area, at two different dates and subjected to phytochemical prospection to find any chemical variations as well as to chemical and biological assays (antioxidant, antibacterial, cytotoxic [using *Artemia salina* as model] and insecticide). Determining the phytochemical composition is fundamental when evaluating the biological potential of a species. Each biological target has a different metabolism and different molecules will interact with the active enzyme sites; chemical composition has a direct relationship with the biological effect.

Materials and methods

Apparatus and Reagents: All spectrophotometric measurements were accomplished using a spectrophotometer (Cirrus 80ST, Femto, Brazil) and using a 1.0 cm optical path length glass cell for all measurements. We used deionized water was to prepare chemical reagents of analytical grade (except when another solvent is mentioned) as well as in all dilutions. Dichloromethane (DCM), hexane, methanol (MeOH), 2, 2–diphenyl–1–picril–hydrazyl (DPPH), ascorbic acid, dimethyl sulfoxide (DMSO) and ethanol were purchased from Sigma-Aldrich, USA. A fresh 1.0×10^{-3} mol L^{-1} pyrogallic acid ($C_6H_6O_3$, Labsynth, Brazil) stock solution was prepared by dissolving 0.0126 g in 100.0 mL of water immediately before the assay. A 1.0×10^{-4} mol L^{-1} solution was obtained by appropriate dilution.

Collection of the material: *M. linifera* was collected two times, first in November 2008 (Nov/2008), during the rainy season and then in August 2009 (Aug/2009), during the dry season, in Volta Grande do Xingu, on Xingu River margins (S 03º17'945" and W052º03'487"), Altamira (State of Pará, Brazil).

Sample preparation for antioxidant, antibacterial, cytotoxic and insecticide assays: We dried the plant material (leaves and stems) from the first collection (Nov/2008) at room temperature, then pulverized and extracted it to increase solvent polarity (i.e. dichloromethane and methanol). Each extraction was performed in triplicate in an ultrasound bath (Unique Model USC–2800, Brazil, 154 Watts, 40 kHz) for 20 minutes. After filtering, both organic extracts were concentrated at 45 °C and speed of 70 rpm in a rotary evaporator (Fisatom, model 802, Brazil), then, stored in a freezer (-20 °C). We performed the same procedure with the material collected in Aug/2009 (also leaves and stems), but hexane (HEX) was used as a non polar solvent instead of DCM, because of its environmental toxicity.

Phytochemical group determination: To determine the phytochemical groups in the extracts we used the methodology described by Matos (1997), which is briefly described below:

For all the assays, hexane and dichloromethane extracts were dissolved in chloroform and all methanol extracts were dissolved in ethanol; 2 mL of the extracts was placed in tests-tubes.

a) Steroids and triterpenes: Conducted using a Lieberman-Burchard reaction. First, 2 mL of the extract were mixed with 2 mL of chloroform. This solution was slowly filtered in a funnel with cotton with a few decigrams of Na_2SO_4 anhydrous and placed in a test tube. Then, 1 mL of acetic anhydride was added and gently agitated while three drops of concentrated H_2SO_4 carefully added and agitated; we then observed it to establish color changes. Results: Initially, an evanescent blue followed by a stable green, indicative of steroids and a brown to red color indicating triterpenes.

b) Phenols and Tannins: In a test-tube, 2 mL of the extract was mixed with three drops of an alcohol solution of $FeCl_3$, then shook vigorously and observed for any color variations or precipitate formation. Results: Color varied between blue and red indicating phenols, dark blue precipitate indicating the presence of pyrogallic tannins (hydrolysable), and green indicating the presence of flobabenic tannins (condensed).

c) Alkaloids: In a test-tube, 2 mL of the extract was alkalinized with fifteen drops of sodium hydroxide (1 %) then, we added 2 mL of water and 2 mL of chloroform. The aqueous fraction was discarded and the chloroformic fraction was mixed with fifteen drops of chloridric acid (1 %), and subsequently extracted with 2 mL of water. The water fraction was analyzed by adding three drops of Drangendorff reagent to verify the presence of alkaloids. Results: The formation of insoluble and floccule precipitates confirmed the presence of alkaloids.

d) Coumarin: Using a capilar column, we made two dark spots of 1.5 cm of diameter on a non-fluorescent filter paper and applied a drop of 1 N KOH alcoholic solution on one of the spots. The spots were partially covered with a non-fluorescent opaque card and exposed to UV light for 2-3 min. After this period, we removed the card, and still under the UV light, we checked for any fluorescence modification on the alkalinized spot. Results: Strong blue fluorescence on the alkalinized spot, which was not covered, is indicative of the presence of coumarin.

e) Anthocyanins, Anthocyanidins, Chalcones, Aurones, Flavonoids: Three test tubes, each one with 2 mL of the extract. In the first one, we added acid to pH = 3, in the second, we added an alkali to pH = 8.5 and in the third, we added an alkali to pH = 11. Results: Observed color change, and compared results with **Table 2**.

f) Flavonols, Flavanons, Flavanonols, Xanthones: Some cg of a magnesium stripe and 0.5 mL of concentrated HCl were placed in a test tube. Results: After the effervescence, the intensification of red is indicative of flavonols, flavanons, flavanonols or xanthones.

Sample preparation for total polyphenol quantification: The method described in Brazilian Pharmacopeia (1996) was employed to extract the total hydrosoluble polyphenols from plant samples. We transferred 0.75 g of dry material (leaves and stems) of *M. linifera* to a 250 mL erlenmeyer, containing 150 mL of water; this was kept in a water-bath for 30 min at 80-90 °C. After cooling, the mixture was transferred to a 250.0 mL volumetric flask and topped with water, then, the plant material was decanted. This solution was then filtered discarding the first 50 mL of the filtrate.

Quantification of total polyphenol content: For total polyphenol quantification, we used Folin Ciocalteau (FC) reagent; 5 mL of the filtrate of the aqueous extracts of the plants was transferred to a 25.0 mL volumetric flask and topped with water.

The quantification of total polyphenol content was based on the procedure recommended by Brazilian Pharmacopoeia (1996), with a 10-fold reduction of the volumes of all the reagents, described by Marino et al. 2009.

First, a calibration curve was obtained by mixing aliquots of 50 to 350 µL from a 1.0×10^{-4} mol L^{-1} pyrogallic acid standard solution, with 200 µL of FC reagent and then by completing it with a 10% sodium carbonate in 5.0 mL volumetric flasks.

Then, the multiple standard addition method (Silva et al. 2013) was used with all extract sample analysis as follows: 500 µL of the aqueous extracts sample were transferred to five volumetric flasks (5.0 mL) followed by the addition of 200 µL of FC reagent. In four out of five volumetric flasks, aliquots of 150, 200, 250 and 300 µL of a 1.0×10^{-4} mol L^{-1} of pyrogallic

Table 2. Color modification of the extract according to the medium pH, following Matos (1997).

	Medium color		
Constituints	**Acid[3]**	**Alkaline[8.5]**	**Alkaline[11]**
Anthocyanins, Anthocyanidins.	Red	Purple	Purpe - blue
Flavones, Flavonols, Xanthones.			Yellow
Chalcones, Aurones.	Red		Red - purple
Flavonoids.			Red - orange

Table 3. Phytochemical analysis of extracts of *Montrichardia linifera* collected in Volta Grande of the Xingu River, Altamira region, State of Pará, Brazil, following Matos methodology (1997). Legend: DCM = dichloromethane; HEX = hexane; MeOH = methanol; (+) presence; (–) absence.

Extract	Collection time (Month/Year)	Coumarins	Tannins/Phenols	Triterpenes	Steroids	Alkaloids	Anthocyanins, Anthocyanidins, Chalcones, Aurones Flavonoids	Flavonols, Flavanons, Flavanonols, Xanthones
Leaves (DCM)	Nov/2008	–	–	–	+	–	–	–
Leaves (MeOH)	Nov/2008	–	–	–	–	–	–	–
Stems (DCM)	Nov/2008	–	–	–	+	–	–	–
Stems (MeOH)	Nov/2008	–	–	–	–	–	–	–
Leaves (HEX)	Aug/2009	–	–	–	–	+	–	–
Leaves (MeOH)	Aug/2009	–	+	+	+	–	–	–
Stems (HEX)	Aug/2009	–	–	–	–	–	–	–
Stems (MeOH)	Aug/2009	+	+	+	+	–	–	–

acid standard solution were added and the 5.00 mL volumetric flasks were completed with a 10% Na_2CO_3 solution. In both curves (calibration and multiple standard additions with the samples) the absorbance was measured at 715 nm after 30 min using water as a reference solution. All results were expressed in % of pyrogallic acid.

Antioxidant assay: A qualitative extracts antioxidant assay was performed on thin layer chromatography (TLC) by using 0.2% of 2,2–diphenyl–1–picrylhydrazyl (DPPH) in methanol, which is capable of quenching free radicals. Areas where spots became whitish yellow after 30 minutes of application indicated the presence of antioxidant compounds (Cuendet et al. 1997). We selected the DPPH method because it is stable and can be used to determine the antioxidant capacity of several matrices (Molyneux 2004, Miliauskas 2004, Kadri 2013).

In the quantitative antioxidant assay, the extracts were tested to determine their ability to lighten the purple DPPH oxidant. DPPH, in the presence of a reductant, changes to pale yellow and then can be spectrophotometrically measured at 517 nm (Cirrus 80ST, Femto, Brazil). The results were compared with ascorbic acid, used as the standard. The reaction started with the addition of 990 µL of DPPH (30 µg/mL in methanol) and 10 µL of each extract sample (0.5 mg/mL), in triplicate, and readings subsequent to 30 minutes. The negative control used was a mixture of 10 µL of methanol with 990 µL of DPPH solution. The results are presented as ascorbic acid equivalents. All measurements were made in triplicate.

Antibacterial assay – *Aeromonas hydrophila*: *Aeromonas hydrophila* assay was performed, in duplicate, according to CLSI (2003, 2005). Bacteria were grown in Petri dishes containing Müeller-Hinton Agar in 6.0 mm diameter holes made in the agar. All the extracts were assayed at 3 mg/cavity. Dimethylsulphoxide (DMSO, 5%) and oxytetracycline (20 µg/cavity) were used as negative and positive controls, respectively. After, the plates were incubated at 30 °C for 18-24 hours and the inhibition halo diameter was measured with a ruler; the measurements indicated bacterial growth inhibition.

***Brine shrimp assay – Artemia salina*:** Brine shrimp assay was performed using *Artemia salina* eggs. First, artificial seawater was prepared (38 g of marine salt/L) and 10 mg of eggs was added. After 48 hours, hatching occurs by maintaining sound aeration (AP100, Dymax, 120 V, 60 Hz, 1.2 W) and under continuous light at 25 to 28 ºC. Then, 10 larvae were collected and put into a

Table 4. Biological and chemical activities of *Montrichardia linifera* extracts, collected two times (in November/2008 and August/2009) in Volta Grande do Xingu, Altamira region, State of Pará, Brazil. DCM = dichloromethane; HEX = hexane; MeOH = methanol; NT = not tested.

Extract	Collection time (Month/ Year)	Total Polyphenol content	Antioxidant assay (DPPH)		Cytotoxic assay (*Artemia salina*)		Antibacterial assay (*Aeromonas hydrophila*)		Insecticide assay (*Sitophilus zeamais*)		
										Mortality (%)	
		% Pyrogallic acid	Concentration (mg/mL)	Equiv. of Ascorbic Acid (mg extract/	Concentration (µg/mL)	Mortality (%)	mg Extract/ cavity	Inhibition halo (mm)	Concentration (mg/mL)	Contact	Ingestion
Leaves (DCM)	Nov/2008	0.23 ± 0.01	0.5	84.7 ± 3.2	250	53.3 ± 3.8	3	0	1.0	1.0 ± 0.0	0
Leaves (MeOH)	Nov/2008	0.23 ± 0.01	0.5	64.7 ± 3.3	500	16.6 ± 6.9	3	0	1.0	5.0 ± 0.7	6.0 ± 0.8
									6.0	3.0 ± 0.5	4.0 ± 0.8
Stems (DCM)	Nov/2008	0.53 ± 0.02	NT	NT	NT	NT	NT	0	1.0	0	0
Stems (MeOH)	Nov/2008	0.53 ± 0.02	0.5	59.6 ± 2.8	1000	0	3	0	1.0	3.0 ± 0.5	5.0 ± 1.7
									6.0	0	6.0 ± 1.3
Leaves (HEX)	Aug/2009	0.45 ± 0.02	0.5	15.9 ± 0.5	250	80.0 ± 0.0	3	0	0.1	0	1.0 ± 0.4
									1.0	1.0 ± 0.4	5.0 ± 0.7
									10	8.0 ± 1.1	9.0 ± 1.4
Leaves (MeOH)	Aug/2009	0.45 ± 0.02	0.5	29.0 ± 1.6	500	56.3 ± 1.9	3	7 ± 0.0	0.1	2.0 ± 0.8	2.0 ± 0.8
									1.0	2.0 ± 0.5	0
									10	1.0 ± 0.4	1.0 ± 0.4
Stems (HEX)	Aug/2009	0.76 ± 0.06	0.5	24.9 ± 1.2	250	86.0 ± 3.8	3	0	0.1	3.0 ± 0.8	0
									1.0	2.0 ± 0.5	4.0 ± 1.3
									10	2.0 ± 0.8	1.0 ± 0.4
Stems (MeOH)	Aug/2009	0.76 ± 0.06	0.5	23.8 ± 1.1	250	100.0 ± 0.0	3	9 ± 0.0	0.1	5.0 ± 1.4	1.0 ± 0.4
									1.0	2.0 ± 0.5	3.0 ± 0.8
									10	1.0 ± 0.4	4.0 ± 0.8

24 well plate. All extracts were initially tested on 1000 µg/mL concentration, following Meyer et al. (1982) methodology, and solvent used to dissolve the extract was used as a negative control. All experiments were made in triplicate. After 24 hours of incubation, the number of survivors in each well were counted and recorded. To be sure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation, we compared the dead larvae in each treatment to those used as controls. In order to determine the LC_{50} (lethal concentration to fifty percent of the individuals) the extracts that showed at least 70 % of lethality in 1000 µg/mL, were assayed in other concentrations (500, 400, 250 µg/mL).

Insecticide assays – *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae): The individuals used came from rearing stock, maintained under the conditions proposed by Tavares & Vendramim (2005). The mortality effect was evaluated two ways, by contact, per Huang et al. (1997) and ingestion of contaminated grains (Llanos et al. 2008). Each test was performed with different concentrations of extracts prepared in HEX, DCM

or MeOH (**Table 4**) we used the solvent used in the extraction as a control. Both tests were done in quintuplicate; in each one, 20 unsexed adults, 10 to 20 days old, were added. The contact test was performed by transferring 1.0 mL of each concentration of extracts and 1.0 mL of the respective solvent for its control, to a sheet of filter paper. For the ingestion test 2.0 mL of each concentration of extracts and 2.0 mL of the respective solvent control, 20 g of grains were mixed. In both trials, complete immobility was used to determine the mortality of the individuals.

Results

The differences between the chemical compositions of the extracts from the collections are shown in **Table 3**. The extracts from the second collection are richer in chemical classes than the ones from the first collection. In the extracts from the first collection (Nov/2008), steroids were only present in the apolar. In the extracts from the second collection (Aug/2009), the polar extracts, showed a greater variety of metabolites such as coumarins, tannins and triterpenes; however, none of these compounds was found in the apolar ones (Table 3), except for alkaloids in the hexane extract of the leaves.

Most of the extracts obtained from the Aug/2009 collections showed greater antioxidant and cytotoxic activities than the extracts from Nov/2008 (Table 4). All of the extracts from Aug/2009 showed an antioxidant potential of 16-29 mg extract/mg ascorbic acid, and the extracts from Nov/2008, even the methanol from stems, showed two to four times lower activity. Although the relationship between these antioxidant capacity values and the total polyphenol content cannot be firmly established, this correlation could explain the differences found between the Nov/2008 and the Aug/2009 extracts. In fact, the polyphenols content of stems (0.76 ± 0.06)% and leaves (0.45 ± 0.02)% extracts from the Aug/2009 is slightly higher than those found in the stems (0.53 ± 0.02) % and leaves (0.23 ± 0.01)% extracts from the Nov/2008 extracts.

Only the polar extracts from Aug/2009 showed inhibition halos against the bacteria *A. hydrophila*. Similarly, the extracts from Aug/2009 showed more insecticidal effect against *S. zeamais* than the ones from Nov/2008. The apolar extracts from Aug/2009 were the most active while the polar extracts from Nov/2008 were most active and no activity was found in the apolar extracts from this date. The most active extract was the hexane from the leaves (Aug/2009), which was active both by contact and ingestion.

Discussion

In this work, we established a clear difference between the chemical compositions of the extracts of the two collections; the extracts from the second collection were richer in chemical classes and had more biological activities than the extracts from the first one. In order to understand why the plant produced such a variety of compounds, we analyzed the temperature and the pluviosity (**Figure 1-2**). When comparing the temperature during the two periods, Nov/2008 was slightly warmer the minimum temperature only reached 24-26 °C, and the maximum was 34-36 °C while Aug/2009 had a minimum temperature of 22-24 °C, and the same maximum temperature. As for pluviosity, there is a clear difference; in Nov/2008, it rained between 50-100 mm while in Aug/2009 it rained between 1-25 mm.

According to Macedo et al. (2005), *M. linifera* has primitive anatomical features that protect it against high light radiation increasing its photosynthetic efficiency and reducing polyphenolic compounds. We could expect that if this is the only protection mechanism, there should be no difference between the two periods of collection; however, this did not occur. The second collection was richer in phenolic compounds. Because these compounds are related to the antioxidant activity of plants (Lima et al. 2004), the results yielded by the material collected in Nov/2008 may have been influenced by the time of collection. Some studies have attempted to correlate the presence of phenolic compounds (mainly flavonoids) with the period of highest light intensity (Tattini et al. 2004, Gobbo-Neto & Lopes 2007) or with water deficit (Van Staden & Jäger 1998, Abreu & Mazzafera 2005). Comparing the results of the antioxidant assays (Table 4) with Figure 1 (a,b) and Figure 2 (a,b) we can infer that pluviosity was the determining factor

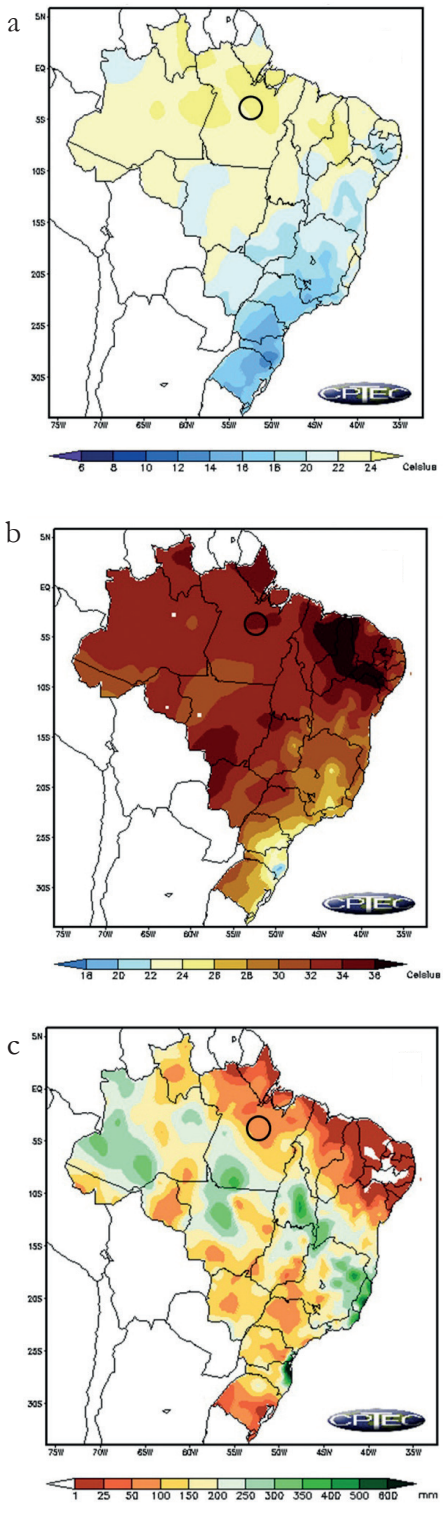

Fig. 1. Minimum (**a**) and maximum (**b**) temperatures and rainfall (**c**) for the first collection (November 2008). The red circle indicates the location of collection (Volta Grande do Xingu, in Altamira, State of Pará, Brazil). Source: Centro de Pesquisas Tecnológicas - Instituto Nacional de Pesquisas Espaciais/CPTEC-INPE (last atualization: 09/Jan/2009).

in the synthesis of photo-protective compounds, as there was a significant dissimilarity between the two collections periods. In the Amazon, less rain means no clouds and more light; this could stimulate the production of photo-protective compounds.

Although a clear relation between the presences of phenolic compounds and water stress cannot be established (Tattini et al. 2004, Huber & Rodriguez-Amaya 2008), in this study, we observed a close relationship between the results of antioxidant assay (Table 4) and rainfall at the time of collection (Figure 1c and 2c). There is also an inverse relationship between precipitation and production of antioxidant compounds. Thus, less rain (more bright days) may have been the agent that influenced the plant to produce a higher amount of phenolic compounds, and caused the increased of the values obtained in the antioxidant assay.

The higher cytotoxic activity results for the Aug/2009 extracts (Table 4) and the higher number of metabolites (Table 3) indicates that this collection has a higher biological potential. In fact, the more toxicity to *A. salina*, the more likely they are to have antitumor, antibacterial, antifungal and trypanocidal activities (Costa et al. 2009). This study confirms these statements; the most active extracts against *A. salina* were the same with high antibacterial and insecticide activity (Table 1 and 2). Amarante et al. (2011a) tested the hexane, dichloromethane, methanol and ethyl acetate fractions of the leaf extract of *M. linifera* to verify which one was most toxic to *A. salina* and found that all of the fractions were active. On the other hand, Costa et al. (2009) observed low toxic effect on *A. salina* of the *M. linifera* ethanolic leaf extract.

The activity against the bacteria, *A. hydrophila*, may be due to the presence of tannins, triterpenes and steroids, because only the methanolic extracts of the second collection presented these phytochemical classes and showed some activity, even low activity. Besides the antioxidant and cytotoxic effects (Table 4), the insecticidal activity may also be related of the presence of alkaloid classes (Riedell et al. 1991, Trindade et al. 2008, Terezan et al. 2010) although several studies cite terpenes as toxic to insects (Siddiqui et al. 2003, Pungitore et al. 2005, Nathan et al. 2008,

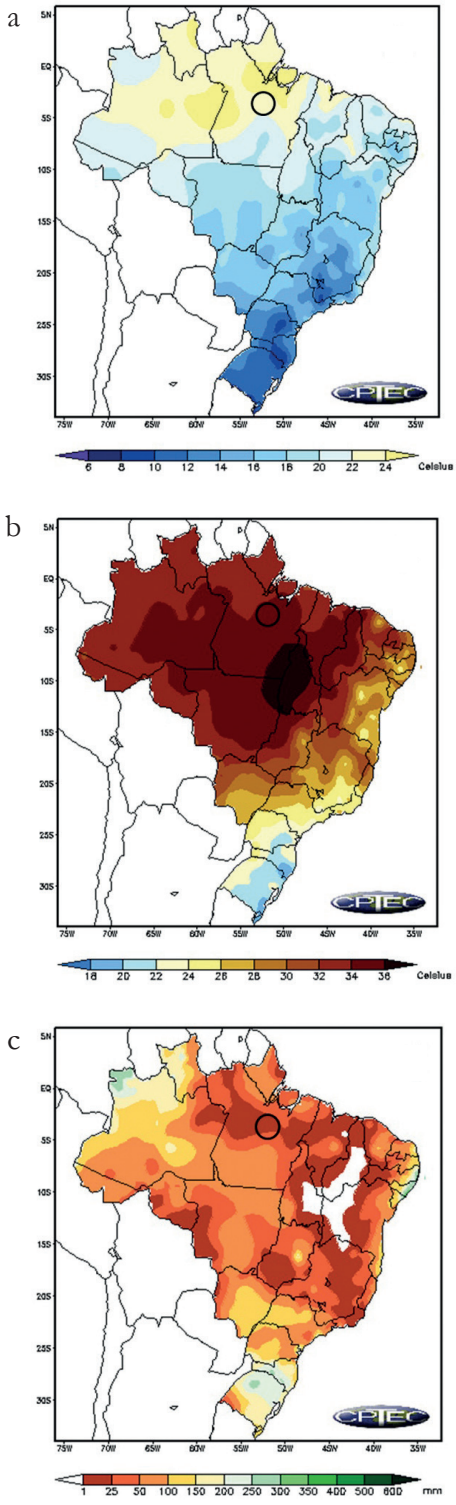

Fig. 2. Minimum (**a**) and maximum (**b**) temperatures and rainfall (**c**) for the second collection (August 2009). The red circle indicates the location of collection (Volta Grande do Xingu, in Altamira, State of Pará, Brazil). Source: Centro de Pesquisas Tecnológicas - Instituto Nacional de Pesquisas Espaciais/CPTEC-INPE (last atualization: 09/Jan/2009).

Stevenson et al. 2009, Terezan et al. 2010, Conti et al. 2011, Huang et al. 2011). The insecticidal assay demonstrated small differences between the extracts, regardless of the time of collection (Nov/2008 or Aug/2009), the material collected (leaves or stems) or the solvent used (DCM, MeOH or HEX). However, for the hexane leaf extract (Aug/2009) a concentration dependent increase was observed in the insecticidal activity (Table 4); this was the most active extract.

There are few studies reporting phytochemical studies of *M. linifera* (Table 1). Silva et al. (2011) observed the presence of steroids in hexane extracts from leaves. Costa et al. (2009) found alkaloids, flavonoids, tannins, steroids and triterpenoids in ethanolic in leaf extracts. This study is concurs with Costa et al. (2009) in demonstrating that the chemical composition of extracts prepared from the same species but collected in different locations may vary.

Conclusion

The extracts obtained from *M. linifera* collected in the dry season showed higher activities (antioxidant potential on DPPH assay, insecticidal activity against *Sitophilus zeamais*, cytotoxic potential against *Artemia salina* and antibacterial activity against *Aeromonas hydrophila*) than the extracts obtained from the species collected in the rainy season. Moreover, the higher the amount of secondary metabolites present in the extracts obtained from the species, the higher the toxicity and their chemical and biological potential as well as their polyphenol content. Consequently, the collection period (season of the year, temperature and pluviosity) is an important factor to be considered when conducting phytochemical studies and studies on the biological activity of *M. linifera*. This study shows that, for this species, water stress is a more important factor than temperature.

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Conflict of Interest

The authors declare no conflicts of interest.

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Potencial biológico, prospección fitoquímica y contenido de polifenoles de *Montrichardia linifera* (Araceae)

Resumen. *Montrichardia linifera* es comúnmente conocida en Brasil como "aninga". Fue colectada dos veces (Nov/2008 y Ago/2009), y sus hojas y tallos fueron extraídos con hexano o diclorometano y metanol. El potencial biológico y químico, perfil fitoquímico y contenido de polifenoles de sus extractos fueron evaluados. Fueron encontradas diferencias en la composición química: de la primera colecta, los extractos apolares fueron más ricos, mientras que de la segunda colecta, los polares fueron los más ricos. Extractos de Ago/2009 mostraron, en general, actividades antioxidante y citotóxica mayores que los de Nov/2008. Los extractos de los tallos y hojas de Ago/2009 mostraron un mayor contenido de polifenoles. Cuanto al ensayo insecticida sobre *Sitophilus zeamais*, el extracto hexánico de las hojas (Ago/2009) presentó actividad insecticida concentración-dependiente. Cuando evaluado el potencial antibacteriano, apenas los extractos metanólicos de los tallos y hojas (Ago/2009) presentaron un halo de inhibición pequeño (9 y 7 mm, respectivamente) contra *Aeromonas hydrophila*. Este estudio muestra que el período de la colecta es un factor importante a ser considerado cuando los objetivos son estudios fitoquímicos y de actividades biológicas de *M. linifera*.

Palabras clave: DPPH; antioxidante; *Artemia salina*; *Aeromonas hydrophila*; *Sitophilus zeamais*; polifenoles.

Potencial biológico, prospecção fitoquímica e teor de polifenóis de *Montrichardia linifera* (Araceae)

Resumo. *Montrichardia linifera* é comumente conhecida no Brasil como "aninga". Foi coletada duas vezes (Nov/2008 e Ago/2009), e suas folhas e caules foram extraídos com hexano ou diclorometano e metanol. O potencial biológico e químico, perfil fitoquímico e teor de polifenóis de seus extratos foram avaliados. Foram encontradas diferenças na composição química: da primeira coleta, os extratos apolares forem mais ricos, enquanto que da segunda coleta, os polares foram os mais ricos. Extratos de Ago/2009 mostraram, em geral, atividades antioxidante e citotóxica maiores que os de Nov/2008. Os extratos dos caules e das folhas de Ago/2009 mostraram um maior teor de polifenois. Quanto ao ensaio inseticida sobre *Sitophilus zeamais*, o extrato hexânico das folhas (Ago/2009) apresentou atividade inseticida dependente da concentração. Quando avaliado o potencial antibacteriano, apenas os extratos metanólicos dos caules e folhas (Ago/2009) apresentaram um halo de inhibição pequeno (9 e 7 mm, respectivamente) contra *Aeromonas hydrophila*. Este estudo mostra que o período da coleta é um fator importante a ser considerado quando os objetivos são estudos fitoquímicos e de atividades biológicas de *M. linifera*.

Palavras-chave: DPPH; antioxidante; *Artemia salina*; *Aeromonas hydrophila*; *Sitophilus zeamais*; polifenóis.