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Botanical survey and leishmanicidal activity of grown-love

Levantamento botânico e atividade leishmanicida do amor crescido

Estudio botánico y actividad leishmanicida del amor crecido

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Abstract

Grown-love *(Portulaca pilosa* L) is used in Amazon medicine for the treatment of diseases caused by bacteria, wound care. The objective of this work was to conduct a botanical survey of *P. pilosa*, as well as to evaluate its leishmanicidal activity. For the botanical studies techniques of epidermal decoupling and histological sections were used (by hand and microtome). The ethanol extract (EE) of aerial parts of *P. pilosa* was obtained by maceration, followed by concentration in evaporator route. The EE was fractionated by chromatographic column. The EE and fractions were analyzed in thin layer chromatography (TLC) and NMR. The leishmanicidal activity was evaluated against promastigotes and amastigotes of *Leishmania amazonensis*, and promastigotes of *L. chagasi*, with prior cytotoxicity assessment in murine macrophages. In anatomical terms, *P. pilosa* presents leaf surface with adaxial epidermal cells and sinuous walls. In the phytochemical prospection of the extract, terpenes and phenols were detected, among others. The analysis of the NMR suggested the presence of terpenes. All samples were inactive against promastigotes and amastigotes of *L. (L) amazonensis*. The dichloromethane fraction was active against promastigotes of *L. (L) chagasi*. The EE, dichloromethane, ethyl acetate and methanol fractions showed no cytotoxicity to murine peritoneal macrophages (CC_{50}> 500 mg/mL). The dichloromethane fraction was promising for *Leishmania*, so futher studies may lead to the isolation of the active metabolite.

Keywords: Boctany; *Leishmania (L.) amazonensis*; Cytotoxicity; *Portulaca pilosa*.

Resumo

O amor crescido *(Portulaca pilosa* L.) é usado na medicina amazônica para o tratamento de doenças causadas por bactérias, feridas de difíceis cicatrizações. O objetivo deste trabalho foi realizar um levantamento botânico de *P.pilosa*, bem como avaliar a atividade leishmanicida.

Para os estudos botânicos foram utilizadas técnicas de dissociação epidérmica e seções histológicas (à mão e microtome). O extrato etanólico (EE) de partes aéreas de *P. pilosa* foi obtido por maceração, seguido pela concentração em rotaevaporador. O EE foi submetido a fracionamento em cromatografia de coluna, sendo este extrato e frações analisados em cromatografia de camada fina (CCD) e RMN. A atividade leishmanicida foi avaliada contra promastigotes e amastigotes de *Leishmania amazonensis*, e promastigotes de *L. chagasi*, com avaliação prévia de citotoxicidade em macrófagos. Em termos anatômicos, *P. pilosa* apresenta a superfície da folha com células epidérmicas adaxiais e paredes sinuosas. Na prospecção fitoquímica do extrato, foram detectados terpenos e fenóis, entre outros. A análise de RMN sugeriu a presença de terpenos. Todas as amostras foram inativas frente aos promastigotas e as formas amastigotas de *L. amazonensis*. A fração de diclorometano foi ativa contra promastigotas de *L. chagasi.* O extrato e frações diclorometano, acetato e metanol não apresentaram citotoxicidade aos macrófagos peritoneais murinos (CC_{50}> 500 µg/mL). Em síntese, a fração diclorometano foi promissora como leishmanicida, porém estudos adicionais podem levar ao isolamento do metabólito ativo.

Palavras-chave: Botânica; *Leishmania (L.) amazonenses*; Citotoxicidade; *Portulaca pilosa.*

Resumen

El amor crecido (*Portulaca pilosa* L) se utiliza en la medicina amazónica para el tratamiento de enfermedades causadas por bacterias, cuidado de heridas. El objetivo de este trabajo fue realizar un relevamiento botánico de P. pilosa, así como evaluar su actividad leishmanicida. Para los estudios botánicos se utilizaron técnicas de desacoplamiento epidérmico y cortes histológicos (a mano y microtomo). El extracto etanólico (EE) de partes aéreas de P. pilosa se obtuvo mediante maceración, seguida de concentración en vía evaporadora. El EE se fraccionó mediante columna cromatográfica. El EE y las fracciones se analizaron en cromatografía en capa fina (TLC) y RMN. Se evaluó la actividad leishmanicida frente a promastigotes y amastigotes de Leishmania amazonensis, y promastigotes de L. chagasi, previa valoración de citotoxicidad en macrófagos murinos. En términos anatómicos, P. pilosa presenta superficie foliar con células epidérmicas adaxiales y paredes sinuosas. En la prospección fitoquímica del extracto se detectaron terpenos y fenoles, entre otros. El análisis del espectro ultravioleta sugirió la presencia de terpenos. Todas las muestras fueron inactivas contra promastigotes y amastigotes de *L. (L) amazonensis*. La fracción de diclorometano fue activa contra promastigotes de *L. (L) chagasi*. Las fracciones de EE, diclorometano, acetato de etilo y metanol no mostraron citotoxicidad para los macrófagos peritoneales murinos

(CC_{50}> 500 mg / ml). La fracción de diclorometano fue prometedora para Leishmania, por lo que estudios posteriores pueden conducir al aislamiento del metabolito activo.

Palabras clave: Boctany; *Leishmania (L.) amazonenses*; Citotoxicidad; *Portulaca pilosa*.

1. Introduction

The *Portulaca pilosa* L. (Portulacaceae Juss.), native plant of the Amazon region, is popularly known as grown-love, Saint John's rosemary, sweet Jacobean lily, melody flower, and silk-flower. The juice obtained from the leaves is used for the treatment of erysipelas, cicatrizing, hair loss (Cauper, 2006), stomach pain, liver problems, infections in general, wounds, slackness, and fever (Barbosa, et al., 2009).

In an ethnobotanical survey carried out in the region of Igarape-Miri (Amazon Region, in the State of Para, Brazil), *P. pilosa* was found to be among the 10 most used plants in the locality (Barbosa, et al., 2009). In Brazil, *P. pilosa* is included in the National Listing of Plants of Interest to the Health System (RENISUS).

The majority of the studies were conducted with Asiatic species, *P. oleracea* L., another accepted name for *P. pilosa* (Flora of North America Editorial Committee, 2003). Phytochemical prospection performed with the methanol extract obtained from the leaves of *P. oleracea* has detected the presence of alkaloids (Xiang, et al., 2005), carbohydrates, tannins, phenols, flavonoids (Xu, et al., 2006), saponins (Garcia-Garay, 1994), steroids, proteins, and amino acids (Kumar, et al., 2008). From the ethanol extract of *P. oleracea* the alkaloids oleraceins A, B, C, D, E (Xiang, et al., 2005), and diketopiperazine (Xing, et al., 2008) were isolated (XU, et al., 2006).

Several studies have demonstrated the pharmacological potential of *P. oleracea*, highlighting the following activities: anti-inflammatory, analgesic activities (Chan, et al., 2000), muscle relaxant activity (Parry, et al., 1987, Parry, et al., 1993), with no change in the extracellular calcium (Okwuasaba, et al., 1987), or increased potassium excretion without altering diuresis (Rocha, et al., 1994), negative inotropic and chronotropic effects (Parry, et al., 1987), inhibition of diaphragm contraction (Okwuasaba, et al., 1987), gastroprotective action (Karimi, et al., 2004), memory deficit improvement (Wang & Yang, 2010), hypoglycemic activity (El- Sayed, 2011), and hepatoprotective action (Elkhayat, et al., 2008; Anusha, et al., 2011), inhibited the growth of *Aspergillus*, *Trichophyton and Candida* (Oh, et al., 2000).

In the treatment of leishmaniasis or wounds that are difficult to heal, the Amazon population uses plants (Veiga, et al., 2020), including *Portulaca pilosa*. American Cutaneous Leishmaniasis is an infectious non-contagious, causes ulcers on the skin and mucous membranes. The disease is caused by protozoa of the genus Leishmania and transmitted by the bite of infected female sand flies (Silva, et al., 2020).

Few studies evaluate the botanical and phytochemical aspects of *P. pilosa*. There is not of investigation against the *Leishmania*, this parasite can cause wounds. The present study describes the botanical features of samples of *P. pilosa*, as well as presents information on its chemical composition. In addition, reported the antimicrobial and leishmanicidal activities of this species for the first time.

2. Materials and Methods

2.1 Obtaining the plant material and extract and fractionation

Portulaca pilosa samples were collected in September 2012, on the Island of Combú, Belém, PA, Brazil (Latitude: 48º, 25'W Grennwich; Longitude: 1º, 25' S). The testimony sample was deposited at Herbarium John Murça Pires of the Museu Paraense "Emilio Goeldi" (Belém, PA, Brazil) under record MG. 131457.

The plants were rinsed in tap water, kept in a 40°C forced air circulation oven for one week. The dry material underwent grinding on the knives mill. The ethanol extract (EE) was obtained by maceration, followed by concentration under reduced pressure to residue.

The EE (5g) was fractionated by open column chromatography, using silica gel as stationary phase (Sigma, 35-70 mesh) and solvents of increasing polarity. Fractions were concentrated in evaporator route. These extract and fractions were further analyzed by TCL. The dichloromethane and ethyl acetate fractions were analyzed by H^1 NMR.

2.2 Botanical Studies

After rinsing the aerial parts of the plant were fixed in glutaraldehyde 2.5% in 0.1 M phosphate buffer, pH 7.2 (Gerlach, 1977), FAA 70% (Johansen, 1940), ferrous sulfate in formalin (SFF), and neutral buffered formalin (FNT). The samples were preserved in 70% alcohol. For the anatomical studies the following laboratory techniques were used: epidermal decoupling and histological sections (by hand and microtome, Gerlach, 1969)

The sections of the various parts of the medulla, obtained by the microtome, were washed in ethyl alcohol 30% and 50% and stored in ethyl alcohol 70 %. Then, the sections were dehydrated in an increasing series of butyl alcohol (75 %, 85 %, 95% and 100 %) during 24 h each, infiltrated and embedded in paraplast histological paraffin (Johansen, 1940). The sections were treated by descending series of xylene-ethanol (xylene, xylol/ethyl alcohol (1:1), ethyl alcohol 100 %, 90 %, 80 %, 70 %), stained with 1 % alcoholic solution of safranin, washed in 50% and 30% alcohol and in distilled water, and then stained with solution of 1 % Astra blue, with modifications (Braga, 1977). Then the sections underwent an increasing series of xylene-ethanol and were mounted in slides and coverslip was placed in synthetic resin. The slides were pictured by a camera coupled to the microscope (Braga, 1977).

For the achievement of histochemical tests, microtome cuts pre-fixed in FNT and SFF were used, and fresh detached epidermis, added the following dyes and reagents: Astra blue (cellulose), basic fuchsin (cutin, suberin), lugol's iodine (starch), ferric chloride, SFF (phenolic compounds), and safranin (lignin, cutin, suberin, Johansen, 1940).

2.3 Antileishmanial Activity

The present study was approved by the Ethics Committee (CEUA) of Evandro Chagas Institute (nº. 0022/2011). BALB/c males (20 ± 2 g) underwent euthanasia under a CO_2 chamber. To obtain the cells, 5 mL of culture medium RPMI 1640 was injected into the peritoneal cavity and re-aspirate and centrifugation. Cell count was performed in a Neubauer chamber with the aid of 0.4% trypan blue dye. Cell suspension was adjusted to 5 x 10^5 macrophages per well in a final volume of 50 µL of RPMI 1640 medium, enriched with 10% of SBF, 50 IU/mL penicillin and 50 g/mL of streptomycin. This suspension was in contact with coverslips for 20 minutes, in an oven at 37ºC with 5% CO_2.

After this time, coverslips were washed with supplemented RPMI 1640 medium (37ºC), being kept on a 24 wells cell culture plate. Adherence was confirmed through inverted microscopy. The plates were kept on an oven at 37°C with 5% CO_2 for 24 hours. The promastigota of *Leishmania (L.) amazonensis* (record MHOM/BR/2009/M26361) have been added. After 4 hours, the test sample was added. After 72 hours of incubation 37°C with 5% CO_2, coverslips were removed from the wells, fixed with methanol and stained with Giemsa and observed under an optical microscope (100x), determining the number of amastigotes per

100 macrophages. Thus, the index of infected macrophages and antiamastigote activity were determined.

The antipromastigote activity was determined through the parasitic viability assay (MTT). The pomastigotes of *L. (L.) amazonensis* and *L.* (*L.*) *chagasi* (record MCAN/BR/2011/M27289) were cultivated in RPMI 1640 medium supplemented with fetal bovine serum. The pellet containing promastigote forms in logarithmic phase were resuspended in complete RPMI medium, adjusted to $5x10^7$ parasites/ 100 µL. This suspension was distributed in 96 wells plates containing tests samples at different concentrations and controls. After 24 hours of exposure, 10 µL of MTT (bromide (3-(4,5-dimethylthiazol-2-yl)-2,5-difeniltetrazólio); 5mg/mL) was added, plates were incubated for 4 hours in an oven at 26°C, and the optical density was read at 490nm. The viability of the promastigotes was evaluated based on MTT metabolism and the percentage of viable cells was calculated by the formula adapted from (Ngure, et al., 2009).

The 50% inhibitory concentration (IC_{50}) was determined by the program GraphPad Prism version 5.04. In the interpretation of the results the following criteria was adopted: $IC_{50} \leq$ 100 mg/mL- active, IC_{50} between 101 to 200 mg/mL- moderately active; IC_{50} > 200 mg/mL- inactive (Mota, et al., 2015).

2.4 Citotoxicity assay

The suspension of peritoneal macrophages from BALB/c mice was adjusted to 4 x 10^5 macrophages per well in a final volume of 100 µL of RPMI 1640 complete medium and distributed in 96-well flat-bottomed plates. The plates were incubated at 37ºC n 5% CO_2 for two hours. After the incubation period, the cultures were visualized in inverted microscope and wells washed 3 times with RPMI 1640 medium previously heated to 37ºC (Tempone, et al., 2004).

After rinsing, plates were incubated in the presence of the tested samples and incubated at 37 ºC with 5% CO_2 for 24 h. After the incubation period, 10 µL of MTT (5mg/mL) was added per well, the plate was protected from light, and further incubated (4 h x 37 ºC). At the end of this incubation, 10 µL of dimethyl sulfoxide (DMSO) was added, stirring for 5 minutes. The optical density (O. D.) of the samples was read in an ELISA plaque reader at 490 nm. The viability of the cells was evaluated based on the metabolism of MTT and percentage of viable cells (macrophages), calculated by the formula adapted from (Ngure, et al., 2009).

3. Results

3.1 Botanical Studies

The leaf surface of *P. pilosa*, in front view, shows slightly thickened epidermal cells of sinuous anticlinal walls. The sinuosity is more pronounced in the adaxial than the abaxial epidermis (Figure1A). The stomata are located above the level of other epidermal cells, surrounded by two subsidiaries cells positioned so that its longitudinal axis is parallel to the stomata slot, characterizing the paracytic type (Figure 1B). Both adaxial and abaxial leaf surface present randomly distributed clusters of glandular trichomes, which are present from the apex to the base of the leaf, giving the leaf a velvety aspect (Figure 1C).

The cross-sections of the leaf of *P. pilosa* display mesophyll with unistratified epidermis on both sides (Figure 1D). The adaxial epidermal cells are heterodimensional, of many formats, being some spherical and other elliptical or elongated, covered by a thin cuticle. The walls are anticlinal and periclinal with thickening (Figure 1 E). The mesophyll is dorsiventral with stockade cells parenchyma (Figure 1 F) extending up to the leaf margins. Collateral vascular bundles encompass the entire leaf, and are surrounded by a sheath of parenchymatous cells (Figure 1G). Continuously to the bundles it is possible to observe a filling parenchyma containing crystal idioblasts in the format of druses. The central vein presents concave-convex section and main vascular bundle with phloem towards the adaxial side and xylem to the adaxial side, characterized as of collateral open kind.

The cells of the parenchymatous sheath display the following classes of chemicals: starch evidenced by lugol solution, pectic substances with pink color evidenced by ruthenium red.

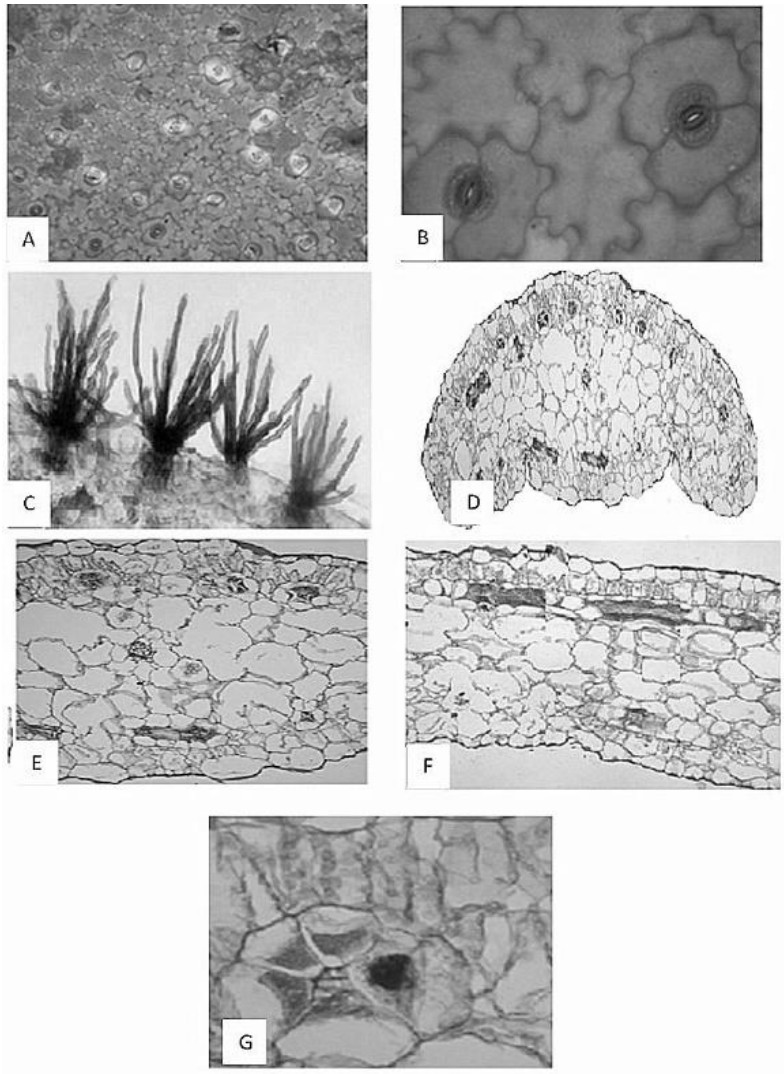
The stem of *P. pilosa* presents beginning of secondary growth characterized by a pluristratified epidermis, ranging from 5 to 7 layers of cells, with straight anticlinal and periclinal walls covered by thin cuticle. Adjacent to the periderm, 1 or 2 layers of colenchymatic cells can be seen, with thickening of lamellar type.

Subsequent to colenchyma a cortical parenchyma occurs composed of heterodimensional spherical cells filled with starch grains evidenced by lugol iodine. The cortical layer next to the vascular bundles presents cells filled with starch grains. The vascular system consists of concentric collateral vascular bundles with already installed exchange and beginning of radiation from elements of xylem and phloem. Both cortical and medullar parenchyma display several crystal idioblasts in the format of druses. The medulla is

composed of parenchyma cells similar to those of cortical parenchyma, but larger and more spherical.

Stem shows onset of secondary growth by multi-stratified periderm, ranging from five to seven cell layers, with anticycline walls and straight pericyclins covered by thin cuticle. Also, there are two collenchymal layers with laminar-type thickening full of starch grains, evidenced by lugol. Starch grains were also evidenced in parenchymal sheath cells.

Figure 1. Some morphological characteristics of *P. pilosa.*



A: General View of the adaxial surface without tricmas; B: Paracytic Stomata of the adaxial surfaces; C: General View of the abaxial surfaces with Tector tricmas; D: General View of the Mesophyll; E: Detail of the cross section of the Mesophyll; F: Detail of the longitudinal section of the Mesophyll; G: Cross-sectional Section of the vascular bundle wrapped by sheath of parenchymatic cells.
Source: Authors.

3.2 Phytochemical Characterization

The ethanol extract underwent phytochemical prospecting, being detected the following metabolites: cardiotonic heterosides, flavonic heterosides, polyphenols, saponins, triterpenes, and tannins.

H^1 NMR analysis of dichloromethane and ethyl acetate fractions revealed bands characteristics of terpenes. For the dichloromethane fraction, H^1 NMR spectrum allowed the visualization of absorption signs characteristic of terpenes in δ 0.6 - δ 1.61 ppm. The signal in δ 1.617 ppm corresponds to methyl groups hydrogens bound to carbon Sp2, probably due to the isoprophenyl group. The absorption signs between δ 1.53 - 2.366 ppm correspond to methylene and methynic hydrogens. At δ 2.84 ppm there are signs of hydrogens bound to methylene groups neighboring double bonds. Another important relationship refers to absorption signs in the region of 4 - 6 ppm, which correspond to oleaphynic hydrogens, and include the signs at δ 5.39 ppm and δ 5.34 ppm.

The analysis of H^1 NMR spectra suggested the presence of terpenes in the ethyl acetate fraction, which showed signs in the region between δ 0.75ppm and δ 0.12 ppm, corresponding to methyl groups. The absorption signs at δ 1.5 ppm - δ 2.8 ppm refers to methylene and methynic hydrogens. The signal in δ 2.45 ppm might correspond to hydrogens in position alpha of carbonyl groups. The signs at δ 3.86 ppm and δ 3.89 ppm represent hydrogens in carbinolic carbons.

3.3 Leishmanicidal Activity

The ethanol extract *P. pilosa* and its methanol fraction were inactive against *L. (L) chagasi* and *L. (L) amazonensis* (IC_{50} > 200 µg/mL). On the other hand, despite inactive for *L. (L) amazonensis* (IC_{50} > 200 µg/mL), the dichloromethane and ethyl acetate fractions displayed activity against *L. (L) chagasi* (Table 1).

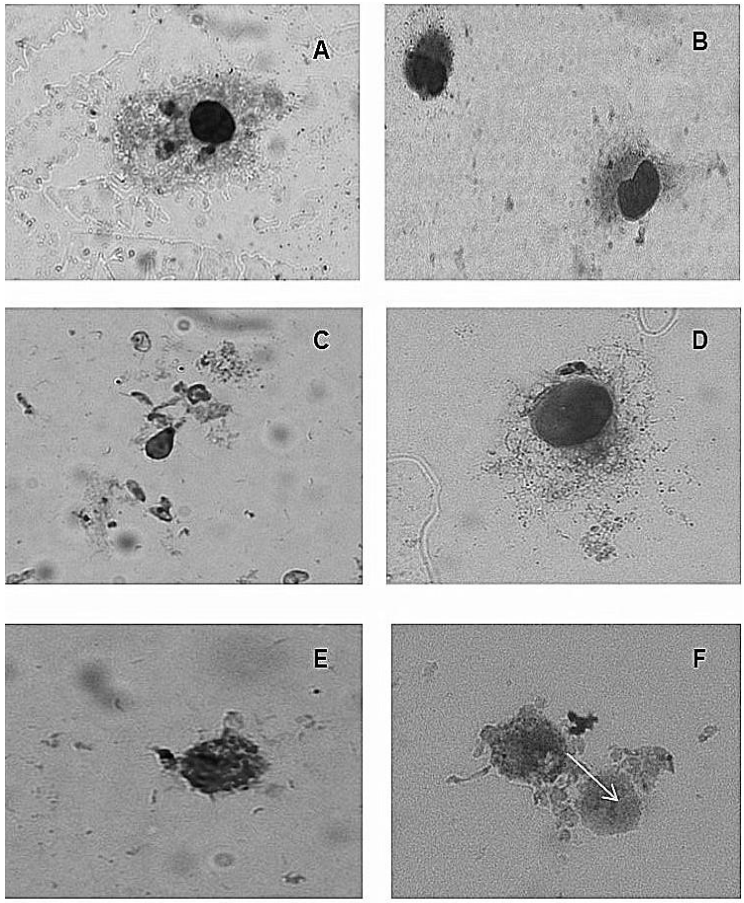
Table 1. Evaluation of the cytotoxicity of *P. pilosa* extract, its fractions in macrophages and anti-antileishmania activity.

Sample	Macrophages	*L. chagasi*	*L. amazonensis*
	CC50 (µg/mL)	IC_{50} (µg/mL) (1)	CI_{50} (µg/mL) ±DP (1)
Ext.EtOH	>500	>200	>200
Fr. Diclo	>500	65,60	>200
Fr. Acet	>500	103,6	>200
Fr. Met	>500	>200	>200
ANF.B	>100	0,062	0,1699 ±0,0004

Legend: CC_{50}=Cytotoxic concentration 50%; CI_{50} = Inhibitory concentration 50%; Ext EtOH= Ethanol extract; Fr. Diclo= Dichloromethane fraction; Fr. Acet. = Ethyl Acetate Fraction; Fr. Met = Metanolic fraction; ANF B= Anfotericicin B; (1) 95% confidence interval. Source: Authors.

In relation to toxicity to non-Leishmania-infected peritoneal macrophages, no sample showed *in vitro* cytotoxicity (CC_{50} > 500 µg/mL; Table 1). Macrophages infected with *L. (L) amazonensis* were treated with ethanolic extract and its fractions. None of the samples tested reduced the number of amastigotes present in infected cells when compared to the negative control group (Figure 2A, 2B). Nevertheless, an intense destruction of infected macrophages treated with *P. pilosa* occurred (Figure 2C, 2D, 2E and 2F), therefore, it was not possible to determine the infection rate.

Figure 2. Antiamastigote Activity in *L. Amazonensis* of *P. pilosa*.



A: Control of non-infected macrophages (increase of 100X); B: amphotericin B in the concentration of 100 M g/mL (positive); Control; increase of 100X); C: Ext. EtOH of P. Pilosa at the concentration of 250 µ g/mL; D: Fr. Dichloromethane; E: Fr. Met.; F: Fr. Acet. Source: Authors.

4. Discussion

The leaf surface of *P. pilosa* presents randomly distributed tector trichomes clusters (Figure 1), can create an environment favorable to gas exchange, reducing water loss through perspiration (Uphof, 1962). Trichomes are a feature of the genus Portulaca (Prabhakar, 1975; Coelho & Giulietti, 2010).

Histochemicaly, the presence of starch in leaf and stem cells was observed. In many young stalks of eudicotiledons, the parenchyma of stem cortex may have photosynthetic function, through the temporary storage of starch grains and other metabolites, but in older regions of the stem starch disappears (Fahn, 1974).

In study addressed aspects of morphological characterization of tracheary elements of vegetative organs of four species of Portulaca (Hernandes-Lopes & Melo-de-Pinna, 2008),

vessel elements observed are all small (< 25 µm) and with simple perforated plaque. These results characterize the phenomenon of paedomorphosis in Portulacaceae, demonstrating that characteristics of tracheary elements in Portulacaceae represent part of adaptive strategy to juiciness (Carlquist, 1962; Gibson, 1978).

Portulacaceae species as well as other families of succulent plants have a high degree of xylem specialization, such as paedomorphosis (Carlquist, 1962), which is characterized by the occurrence of secondary vessel elements with characters typical of primary xylem (such as the default of secondary wall thickening, size of the elements, tilt of terminals walls, and type of perforated plaque).

Phytochemical prospection of *P. pilosa* performed in this study showed similar results to other studies (Garcia-Garay, 1994) and the presence of terpenes was confirmed by NMR. From P. *pilosa* have been isolated terpenes pilosanone A, pilosanone B and pilosanone C (Ohsaki, 1987; Ortega, et al., 2001) and believe that biological activities may be related to this metabolite.

The fractionation of the ethanol extract of P. *pilosa* has contributed positively to the antipromastigote activity. The dichloromethane and ethyl acetate fractions were active against promastigotes of *L.chagasi* (Table 1). Phytochemical studies indicate that these fractions contain terpenes and some studies describe the leishmanicidal activity of terpenes. The 3-O-acetyl oleanolic acid displayed activity against promastigotes of *L. donovani* (Fokialakis, et al., 2006). Lupane, showed concentration-dependent inhibitory activity against *L. amazonensis* (Lima, et al., 2011). These results reinforce the hypothesis that the antipromastigote activity against *L. (L) chagasi* is probably related to terpenes.

In the present study, we observed a selective activity for *L. (L) chagasi* (Table. 1). Other studies have demonstrated test sample selective responses (Tempore, et al., 2008; Moreira, et al., 2007), which can be explained by differences between species.

The fact that the sample is active against promastigotes does not assure activity against amastigotes, fact observed in the present study. The dichloromethane and ethyl acetate fractions were active against promastigotes and inactive against amastigotes. Amastigotes are located inside the cells, within the phagolysosome, making difficult the access of molecules due to the existence of various barriers (Veiga, 2013).

The cytotoxicity assessment of the ethanol extract of *P. pilosa* and its dichloromethane and ethyl acetate fractions showed no cytotoxic activity (CC_{50} > 500 ug/mL; Table. 1). Similar situations have been identified in other studies of *Portulaca werdermannii* and *Portulaca hirsutissima* (Costa, et al., 2007).

5. Conclusions

In anatomical terms, *P. pilosa* presents leaf surface with adaxial epidermal cells and sinuous walls. On the adaxial surface there are stomata complex scattered in leaf, the epidermal cells are covered by a thin cuticle with parallel striae.

The main chemical constituent of this species should be terpenes. The dichloromethane and ethyl acetate fractions were active against promastigotes of *L. chagasi*, but inactive against *L. amazonensis*. Moreover, both displayed low cytotoxicity. In summary, the dichloromethane fraction proved to be promising as a basis for the isolation of more effective and low cytotoxicity antileishmanial drugs

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