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PHCOG REV.: Plant Review

Updated studies on *Ampelozizyphus amazonicus*, a medicinal plant used in the Amazonian Region

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ABSTRACT

The *Ampelozizyphus* genus (Rhamnaceae family) is endemic in the Amazonian region and in Brazil. It occurs in the states of Amazon, Pará and Roraima, and is found in undergrowth vegetation, clayey-sandy to sandy soil, and occasionally in second-growth area and in non-flooded humid forests. *Ampelozizyphus amazonicus* is a liana or shrub with large leaves and an extensive radicular system. Popularly this species is considered stimulant and energetic, increasing the organic resistance. It is known as *saracura-mirá, cervejeira* and *cerveja de índio*, among other common names. The aqueous preparations with the roots are commonly used in traditional medicine as anti-inflammatory, antidote to snake venoms and for the prevention of malaria. The phytochemical studies have reported the presence of triterpenoids, steroids and saponins. Chromatographic profiles of the beverage showed that the aqueous preparations were rich in saponins. The pharmacological studies made with this botanical species indicate activities such as: malaria prophylaxis, trypanocidal, antifungical, antiviral and antibacterial. This work presents updated data related to the botany, chemistry and pharmacology of *A. amazonicus*, which emphasize their wide use in the folk medicine in the Amazonian region.

KEY WORDS: Rhamnaceae, *Ampelozizyphus amazonicus*, malaria, chromatography, pharmacology

INTRODUCTION

Since the beginning of the human civilization, man utilizes native and exotic plants for healing purposes, whose knowledge is transmitted by oral tradition through the generations. Despite the arrival of the industry, in many countries, the majority of the population does not have access to medicines, thus making use of home preparations to cure various health problems. The considerations made on the health of all the people of the world during the Declaration of Alma-Ata in 1978 was the milestone that made the World Health Organization (WHO) stress the need to develop the medicinal plants in the world in the therapeutic scope. Presently, there are different programs supported by WHO, which integrate several preventive and therapeutic aspects of the diseases, as, for instance, the fight against malaria, a disease that can be prevented and healed, since the access to its prevention and treatment is provided (1,2). In recent years, the number of cases of malaria registered in Brazil presented a great variation, having 99% occurred in the Amazonian region (3). In this region, as a result of isolated dwelling, economic problems or even because of the healing difficulties with conventional medicines, the population appeals to nature, often through the use of folk preparations made with plants to cure or prevent malaria. In this context, various reports led to a species – *Ampelozizyphus amazonicus*, whose bitter flavor (normally associated to plants used to cure malaria) and the large amount of foam resulting from the preparation made with its roots and barks in water, is widely used in the Amazonian region mainly to prevent malaria. *Ampelozizyphus* is a genus of Rhamnaceae family with 50-60 genera and approximately 870-900 species and has a worldwide distribution. This species is a native plant from the western Amazon forest in Brazil. In the Amazonian region it is popularly known, among other common names, as "cerveja de índio" or "saracurá-mirá. In the traditional medicine, it is widely used, besides preventing and treating malaria, as an antidote to snake venoms and as anti-

inflammatory agent (4-6). Recently, a wide range of pharmacological actions have been reported, such as anti-microbial, trypanocidal, cytotoxic, antiviral, antimalarial and hepatoprotective activities (5, 7, 8). These works show the importance of other studies with *A. amazonicus* in the search of a new prophylactic agent as a therapeutic resource to fight malaria. This work is a contribution to this investigation with updated data related to the botany, chemistry and pharmacology of *A. amazonicus*.

Figure 1. Leaves and roots of *A. amazonicus*

Folk Medicine

A. amazonicus is popularly known as *saracura-mirá*, *curupira-mirá cervejeira* (brewer), *cerveja-do-mato*, *cervejinha*, (9) *cerveja-de-índio* (Indian beer) (9, 10) and *saracuracorá* (10). Widely diffuse in the popular use, this plant is utilized in the Negro River region as well as in several other Amazonian areas, including urban centers (9, 11). Le Cointe (1947) (12) described the indication of the roots saps against the malarial fever. Accounts exist on the preparation made of the roots of *cerveja de índio* by the Brazilian Army as preventive use against malaria (13). Ground roots are used to heal colds and to prevent malaria. Besides, this species is known as energetic stimulant, "which improves resistance". Reports describe a beverage prepared with the mixture of the roots phloem and the fresh stalk to recover from the effects of fatigue and famine, being also indicated before heavy tasks, long walks in the woods or just to work as stimulant. It is said that the body, being weak or tired, recovers with *saracura-mirá*. Its users in *São Gabriel da Cachoeira* (a city in the Amazonas State) mention other indications: rheumatism, liver, aphrodisiac, pneumonia, verminosis, itches, pains, diarrhea, inflammation, wounds and AIDS. In the city of Manaus (capital of Amazonas State) there were described uses against malaria, hepatic problems and sleep disorders (9). An investigation made by a resident researcher in Jaú National Park, Amazonas State, checked the therapeutic practices of local inhabitants by means of interviews from May to December 1995. 519 recorded uses were indicated for 81 therapeutics purposes, which were further grouped into 15 categories of use according to there expected effects. In this context, *A. amazonicus* was mentioned against inflammatory processes, fever, tropical diseases (roots), gastrointestinal disturbances (barks/roots) (11). In Colombia, the bark is ground, mixed with water and vigorously shaken. The resulting foamy preparation is applied as an antiseptic on wounds (7). This species is used as a febrifuge in Peru (10). There are reports stating that the plant is not hallucinogenic or toxic (9), although being narcotic when ingested in a large amount (14).

Botanical Considerations

Lianas are soil-germinating plants that are rooted in the ground for their life cycle and that require physical support so as to reach the canopy. In the tropical forests they constitute a major form of life because of their diversity and because they are part of the forest structure, besides providing food for several animals (15). In this context, in the Rhamnaceae, a cosmopolitan family of approximately 58 genera and 900 species, with around 28 genera and 170 species in the neotropical region, *Ampelozizyphus* is a taxon, which had originally been considered monospecific, but which has recently lost this condition as a new species of arboreal size was acknowledged in the Venezuelan mountain chain. In 1935, it was originally described by Ducke, who included it in the Zizypheae tribe. Later, Suessenguth, in 1953, considering the morphology of the fruit, transferred it to the Rhamneae tribe. Most recently, Richardson et al. (2000) (16), after studying the phylogenesis of the family and based on the molecular sequence, proposed the inclusion of a new tribe – Ampelozizypheae – so as to have it better positioned. In that connection, *A. amazonicus* presents the following taxonomic classification:

Kingdom: Plantae
Division: Magnoliophyta
Subdivision: Magnoliophytina
Class: Magnoliopsida
Subclass: Rosidae
Order: Rhamnales
Family: Rhamnaceae
Tribe: Ampelozizypheae
Genus: *Ampelozizyphus*
Species: *Ampelozizyphus amazonicus* Ducke (17, 18)

Habitat and External Morphology

Ampelozizyphus amazonicus is endemic in South America and found within Amazonian Brazil, Venezuela, Colombia, Peru, also reaching Ecuador. In Brazil, it is found in the states of Amazonas, Pará and Roraima, in firm land forests. It is a robust liana, unarmed, lacking tendrils, with a cylindric, striated and rust-colored stem, with brownish lenticels. The leaves are large, alternate or subopposite, petiolate, with ovate to oblong contour, and coriaceous consistency. Its blade (10-22 cm x 6.2-11 cm) presents a round or obtuse base, acuminate apex, entire revolute margin, with glabrous adaxial surface and abaxial surface from puberulus to glabrescent. Venation is clearly brochidodromous with prominent veins (3 to 5) in both surfaces. The cylindric-like petiole (1.3 – 2.5 long) is adaxially furrowed, with early deciduous lateral stipules. The thyrso-loose, rust-colored inflorescences (October to December), are axillary or terminal, with 42 – 50 cm long rachis and puberulus leafy bracts with 2.7-6.3 cm x 1.3-3.3 cm. The flowers, thick and monoclinous, with 3-4 mm long are pedicel, with sepals. Its conchiform petals, unguiculate, with long laminate claws, have stamens with thick, flattened edges; anthers about 3 mm long, thick nectariferous disc, crenate, glabrous; ovary, tricarpelar, trilocular, with three short, thick stylets, free only in the apex and obtuse stigmas. The fruits (November to

February) are capsular, obovate, angular, glabrous. Seeds present oval contour and are brilliant-brownish colored. Dispersion is made by animals and by the wind (18, 19). This species may be taken for some other lianas from the Menispermaceae family, yet the stem of the *A. amazonicus* does not present an anomalous development and its petiole does not have articulation (pulvinus), which are characteristic of the Menispermaceae (19). The new species acknowledged in Venezuela, *Ampelozizyphus guaquirensis*, was differentiated by Meier e Berry (2008) (20) because of its arborescent size, to its subsessile inflorescence, to the deciduous lobes of the calyx in the fruit and to the presence of nectarious glands at the bottom of the leaf blades.

Internal Morphology – Methodology

The applied material corresponds to the leaves and secondary root of a sample collected at experimental farm of the Federal University of Amazonas in Manaus, Brazil. The voucher specimen was deposited at the Herbarium of the university under number 191532. Semi-permanent blades were made with paradermic (leaves) and transversal (leaves and roots) slices, obtained with Ranvier-type manual microtome with the help of a histological razor. All sectioned material was purified in sodium hypochlorite (10 to 50%) and submitted to a double-color stained process of blue alcian/safranin or congo red/iodine green. After rinsed in distilled water, the slices were mounted in glycerin and photographed with optical microscope coupled with microphotography system (21).

Anatomic Aspects of the Foliar Limb

The leaf in paradermic cuts is hypostomatic, with paracytic stomats, secreting glands and sinuate-contour epidermal cells (Fig. 2a). In transversal section, the limb presents unistratified epidermis, overlaying the two pages, externally covered by a thin cuticle with few unicellular covering trichomes, ramified and gland trichomes. The mesophyll maintains dorsiventral organization, with a palisade parenchyma layer and several spongy parenchyma layers where intercellular spaces are observed. The vascular bundles are covered by the sclerenchymatic sheath with thickened walls and calcium oxalate druses (Fig. 2b). The midrib, with biconvex contour, has unistratified epidermis in both surfaces. Innerly, it is observed angular collenchyma, fundamental parenchyma with round cells, calcium oxalate druses and collateral vascular bundles protected by a sclerenchymatic sheath. Medullar parenchyma appears in the middle (Fig. 2c and 2d) (22). The powdered foliar limb contain parenchymatic tissue and secretory glands (Fig. 2i).

Anatomic Aspects of the Root in Secondary Structure

Externally, the root is covered by a thick suberous tissue, innerly finds the cortical parenchyma of polygonal cells with a sclerenchymatic continuous ring and isolated sclerous cells or in groups, of thick and canaliculated walls (Fig. 2e). The phloem region is filled with parenchyma with sieve elements and companion cells. The vascular cambium limits externally the xylem made of alternate layers of fibers and uniserial parenchymatic rays where there may be found large-caliber tracheal elements isolate or gathered in small groups (Fig. 2f and 2g). The parenchyma of the axial system is vasocentric paratracheal, being proportionally smaller that the space occupied by the fibrous tissue (Fig. 2g), a detail not very common in the lianas in the tropical forests as described in Brandes and Barros (2008) (23). The powdered roots contain parenchyma, rays and sclerenchymatic tissue (Fig. 2h).

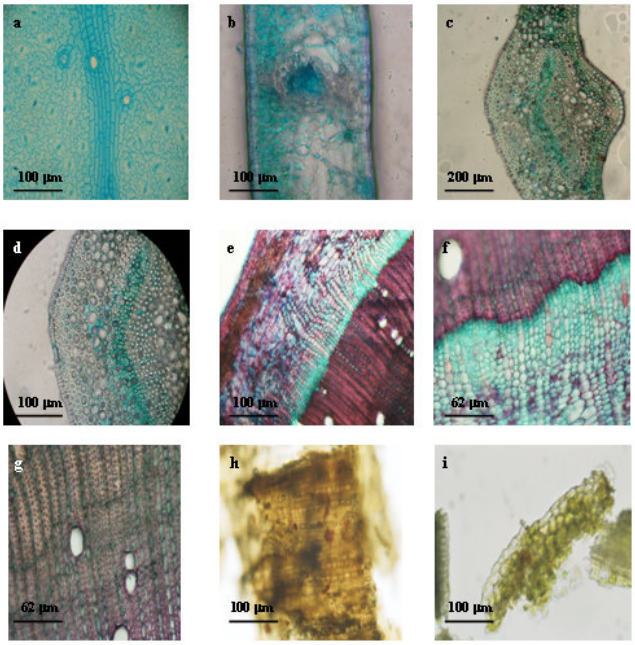

Figure 2: Leaves: a- Paradermic cut of the abaxial region of the mesophyll. b- Transversal section of the foliar limb. c- Biconvex midrib. d- Detail of the abaxial region of the midrib. Roots: e- Overview of the secondary structure. f- Detail of the vascular region. g- Detail of the xylematic region. h- Powdered roots. i- Powdered leaves.

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Ecology and Management

The relative density of *Ampelozizyphus amazonicus* was compared to those of the main species of palms and trees found in nine properties located in the rural community of "Cristo Rei do Uatumã", a district of Presidente Figueiredo (Amazonas State), and aimed to verify the influence level among them. Additionally, some samples of *A. amazonicus* with different height cuts were harvested to provide estimates on the rates of growth, mortality and survival. The percent values of openness and coverage of the canopy were measured as well as the percentage of clay and foliar index were determined, thus having the results correlated to the density data and the rates of the growth of the species. The variation in the relative density of *A. amazonicus* was negatively influenced by the coverage percentage of the canopy, which indicates that the studied trees were at the same stage. The same patterns resulting from other studies made with lianas were verified in *A. amazonicus*, which, under increase of resources availability, should be able to grow up to reach the forest canopy. The percentage of clay (varying from 11% to 85%) has not provided relevant information to explain the density of *A. amazonicus*, but field observations have shown the tendency to find individuals with greater diameter (percentage of clay below 50%) in the sandbanks and with greater density in the interface sandbank/hillside. The rates of growth in height and diameter have not shown linear correlation with the environmental variables that have been studied, probably because of the short period of study (six months) (24).

Chemical Constituents

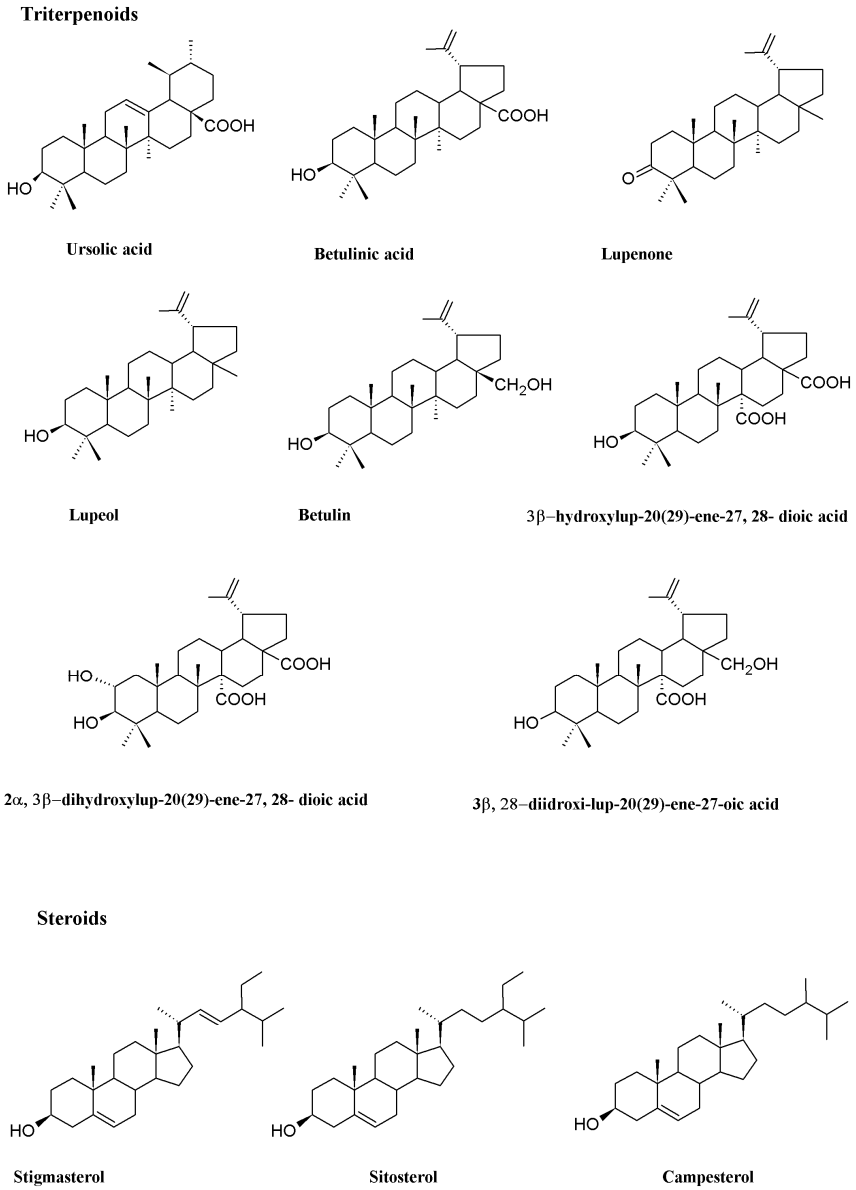

Figure 3: Triterpenoids and steroids isolated from stems and roots of *A. amazonicus* (5, 25-26).

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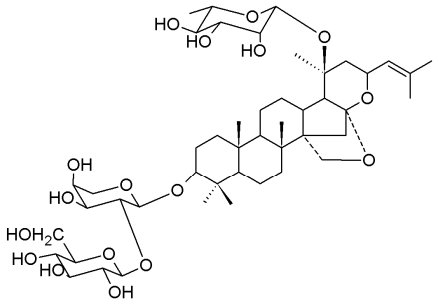
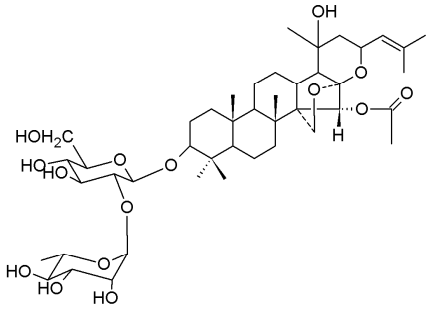
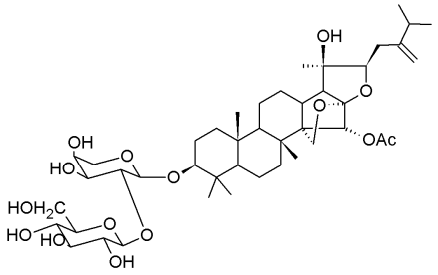


3-*O*-[β-D-glucopyranosil-1(1➔ 2)-α-L-arabinopyranosil]-20-*O*-α–L-rhamnopyranosil-jujubogenin



3-*O*-β-D-glucopyranosil-1 (1 2)-α-L-rhamnopyranosil-15-α-acetil-ampelozigenin



Ampelozigenin-15-α-*O*-acetil-3-*O*-α-L-rhamnopyranosil-(1➔2)- β–D-glucopyranoside

Figure 4: Saponins isolated from roots of *A. amazonicus* (25-26).

CHROMATOGRAPHY

Gas Chromatography Mass Spectrometry (Gc-Ms)

Method

The analyses of the samples were performed in EI mode on a Agilent 5973 gas chromatograph coupled to a mass spectrometer (GC-MS), with a DB-5 MS capillary column (crossbond 5% phenyl-95% dimethylpolysiloxane, 30 m). The conditions were: injector split at 280°C (split ratio 1/10), helium as carrier gas at 0.5 mL/min, ion source at 280 °C and electron impact ionization at 70 eV; oven temperature programmed from 70 °C to 300 °C at 5 °C/min. Each constituent was characterized by comparison of mass spectra with the Wiley Library on the instrument.

Sample Preparation

2.0 g of ground dried vegetal material (root barks and stem barks) was submitted to extraction under maceration with hexane (10 mL). After 24 hour, an aliquot of each hexane extract was treated with diazomethane (ether solution) and analyzed.

Results

The analyses of the chromatograms and mass spectra indicated as major substances triterpenoids, steroids and methyl esters. Table 1 showed the constituents of each hexane extract analyzed.

High Performance Liquid Chromatography (HPLC)

Method

Analytical HPLC analysis was performed on a Shimadzu LC-8Avp gradient chromatograph equipped with two LC-8Avp

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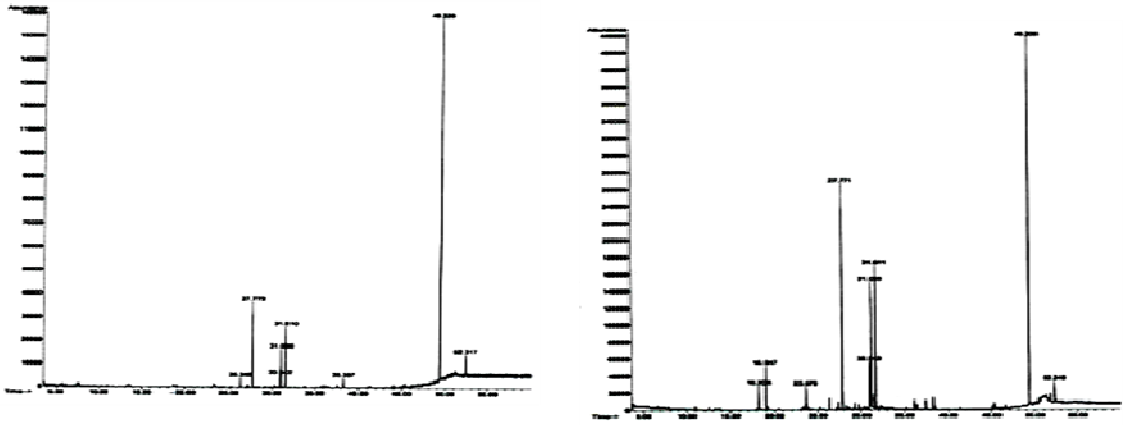

Figure 5: GC profile of hexane extracts from stem barks (A) and root barks (B) of *A. amazonicus*.

Table 1. Percentage composition from the hexane extract of stem barks and root barks of the *A. amazonicus*

Compounds	Stem barks – A (%)	Root barks - B (%)
Lauric acid methyl ester	-	3.36
Methylmiristate	-	1.97
Palmitic acid methyl ester	9.86	19.44
Linoleic acid methyl ester	-	3.73
Oleic acid methyl ester	5.35	12.10
Octadecanoic acid methyl ester	7.09	13.60
Lupenone	52.19	39.23
Betulin	3.39	3.49

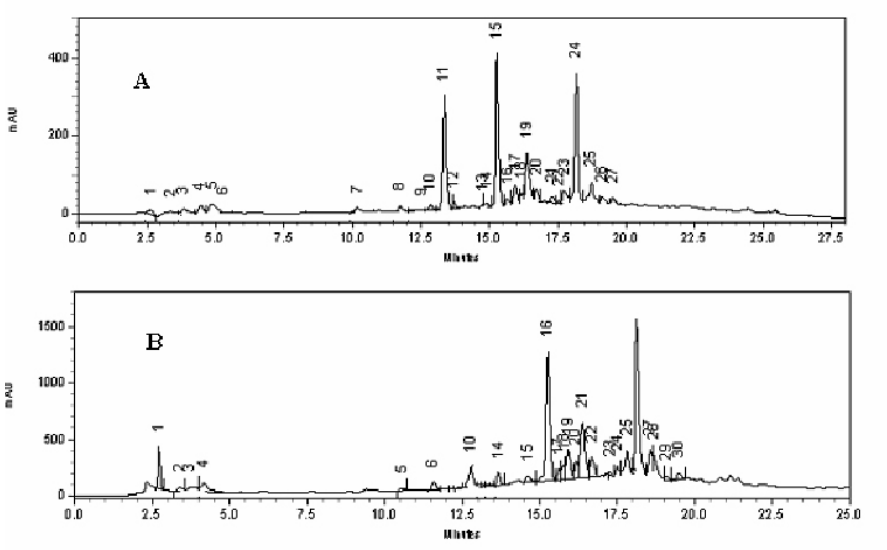

Figure 6: HPLC profile of aqueous extracts from stem barks (A) and root barks (B) of A. amazonicus.

pumps, controlled by an interface module, an automatic injector and an SPD-M10Avp photodiode array detector (PAD). The chromatographic separation was carried out on a RP18 column (250 mm x 4.6 mm, 5μm; Lichrospher, Merck). The mobile phase consisted of 0.05% aqueous trifluoracetic acid (A) and acetonitrile (B) and the composition gradient was: (0-2 min) 1% (B), (2-16 min) 1%-25% (B), (16-26 min) 25-45% (B), (26-30 min) 45-75% (B). 40 μL of the sample were injected and monitored at 222 nm. A constant flow-rate of 1.0 mL. min^{-1} was used during analysis and a PAD range 200 – 600 nm. HPLC grade solvents and Milli-q water were used in this study. All chromatographic experiment was performed at room temperature.

Sample Preparation

1.0 g of the dried vegetal material (root barks and stem barks) was added to 100 ml of deionized water, the mixture was agitated (5 to 10 min) and the formed foam was removed. Each result extract was immediately analyzed after the preparation. This procedure was based on the popular use of *A. amazonicus*.

Results - In the chromatogram obtained by HPLC/DAD at 222 nm were identified the peaks with similar profile based on the characteristic UV spectrum of saponins, previous isolated compounds from *A. amazonicus* (25, 26). The analyses of the chromatograms (Fig. 6) related to the UV spectra showed a higher percentage of saponins in the sample of the stem barks (A) [50.7%] when compared to the root barks (B) [37.3%].

Pharmacological Considerations

Antiviral activity:

The methanol extract from dried leaves (1% p/v) of *A. amazonicus* was tested against HSV1 (Herpes Simplex Virus type 1) and Poliovirus (8). The antiviral activity of the extract was evaluated by periodical inspection of virus – induced cytopathic effects on the culture. In the case of HSV, complete cell destruction required 4 days, while for poliovirus only 2 days were required. The minimum inhibitory concentration (MIC) of the extract corresponded to the complete inactivation of the virus infectivity and partial inhibition corresponded to a non-active extract. A MIC of 22 μg/mL of the studied extract of *A. amazonicus* was active against HSV1 but showed no activity against the Poliovirus (8).

Antibacterial and antifungal activities

The antibacterial and antifungal actions of the methanol extract from dried leaves (1% p/v) of *A. amazonicus* was determined, using the method of disk diffusion assay, and using gentamicin and nystatin as controls for growth inhibition. The antifungal and antibacterial activities were studied against *Candida albicans*, and *Bacillus subtilis*, *Streptococcus faecalis*, *Staphylococcus aureus* K147, *Escherichia coli* DC10, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* 187, *Salmonella typhimurium* and *Mycobacterium phlei*, respectively. Results were reported based on the evaluation of growth inhibition. These studies were also carried out in duplicate, one in the dark and another under UV exposure to test light-activation of the extract activity. The methanol extract in the concentration of 100 mg/mL has no antibacterial or antifungal activity against the studied microorganisms species, either in the dark or under UV activation (7).

The methanol extract from leaves of *A. amazonicus,* using the paper disc agar diffusion method under anaerobic conditions, was tested *in vitro* for growth inhibiting activity against the human intestinal bacteria *Bifidobacterium longum*. A sample of this extract was applied using a glass microcapillary to a paper disc (20 mg/disc). After evaporation of the solvent, the disc was placed on the agar surface inoculated with the test bacteria. All tests were performed in triplicate. All plates were incubated anaerobically at 37°C for 2 days. This extract concentration strongly inhibited the growth of the bacteria under test (+++, zone diameter 21–30 mm) (27).

Antiprotozoal activity

Trypanocidal

Crude extracts (hexane, EtOAc, EtOH, MeOH and H_2O) of *A. amazonicus* stems and their fractions were tested *in vitro* against the trypomastigote blood forms of *T. cruzi*. The bioassays were carried out on infected blood of mice in the peak of parasitaemia (7 days of infection with *T. cruzi*) diluted to a concentration of ca. 2 x 10^6 trypomastigotes forms/mL. The extracts and fractions were tested in final concentrations of 100, 250 and 500 μg/mL. Results demonstrated that all samples (extracts and fractions) have trypanocidal activity against the trypomastigote blood forms in the tested concentrations. They presented, however, different levels of activity, with several extracts and fractions, exhibiting more than 50% of parasite lysis at the highest concentration (500 μg/mL):

a) stem bark aqueous extract with froth; b) three fractions (designated by the authors by: E_2P, E_2P_3 and E_2P_9) obtained from extract produced by ethyl acetate extraction; c) fraction resultant from the partition of ethanol extract of stem with *n*-buthanol and d) fraction resultant from *n*-buthanol partition of a extract obtained by primary partition of methanol extract.

The last two fractions (c and d) induced higher percent parasite lysis (59.53% and 61.3%, respectively) with a lower concentration studied (100 μg/mL). In some samples, although less effective, they presented active compounds, namely one of the fractions obtained from ethyl acetate and MeOH extracts, whose trypanocidal activity were mainly due to betulinic acid, 3ß-hydroxylup-20(29)-ene-27,28-dioic acid and 2α, 3ß-dihydroxylup-20(29)-ene-27,28-dioic acid (5).

Antimalarial

There were carried out assays using rodent and avian blood stages of *P. berghei* and *P gallinaceum*, respectively, with aqueous and ethanol extracts from freshly ground roots, and purified fractions with or without saponins. The results of these studies showed no activity, also against blood stages of *Plasmodium falciparum*, in *in vitro* cultures. These extracts and fractions were tested against sporozoite (invasion or development) in chickens infected with *P. gallinaceum* previously treated orally during 5 to 8 days with 200 mL/Kg of the samples. This work showed that freshly prepared ethanol extract reduced the tissue parasitemia about 67%, while sulfadiazine (used as reference drug) reduced 97% (4).

Recently, ethanol extracts were assayed *in vitro* for their exoerythrocytic schizontocidal activity using concentrations between 5 – 100 μg/mL and the results measured on the effect on the growth of liver cell schizonts. The results showed that in hepatoma HepG2 cell cultures infected with *P. berghei* sporozoites and incubated with *A. amazonicus* extracts, there were fewer exoerythrocytic parasites. These were significantly smaller than the parasites present in drug free culture used as controls. In the same work, mice were previously treated with 100, 200 and 400 mg/Kg of body weight, for 6 days, then infected with sporozoites by natural exposure and feeding, in an laboratory insectary. The results were evaluated on a daily basis by microscopy in search of red blood cell invasion and parasite development, and showed an extension of the prepatent period now extended from 6 days in untreated control groups to 10-16 days in the higher doses; there was also reduced parasitemia and mortality. In the second week of infection, when all the non-treated controls had died, the treated mice presented low parasitemia; some pretreated animals with higher doses never became infected, indicating that *A. amazonicus* extracts had a prophylactic activity (6).

Diuretic and Antidiuretic Activities

The ethanol extract from the dried roots of *A. amazonicus* and the saponins fraction obtained after partition and chromatography of the EtOH extract were evaluated on renal functions in rats. The extract increased diuresis and the saponins fraction decreased in a dose-dependent manner. In this study, rats previously kept under two hydration conditions (12-hour water restriction or free access to water) were analyzed as to different concentrations of the ethanol extract and the saponins fraction. A 150-mg/Kg dose of the extract provoked a discreet increase in the volume of urine, when the group of rats was compared to the control (without expansion) and with expansion just with NaCl 0.9%. The saponins fraction, even under water restriction, presented the inhibition of the increase, even discreet, of the urinary volume induced by the volume expansion (4%) with NaCl 0.9%. This inhibition was dose-dependent and reached the maximum value with the 200-mg/kg dose of saponins. In the second experiment (rats with free access to water), the extract increased the diuresis produced by the expansion with NaCl 0.9%. Similarly to what was observed in the first experiment, the saponins fraction inhibited the diuresis produced by the expansion with NaCl 0.9% in the rats with free access to water. The maximum antidiuretic effect of the saponins fraction was observed at the 90 min of expansion in the 50 and 1000-mg/Kg concentrations (28).

Larvicidal activity - The methanol extract from the leaves (2.5%p/v) of *A. amazonicus* was dried and a stock solution was prepared in ethanol (10mg/mL). Concentrations from 400 to 25 ppm of this solution suspended in distilled water with Triton X-100 (10%) were put in contact with groups of 25 early 4th-instar larvae of *Aedes aegypti* and *Culex pipiens pallens*. The larvicidal activity was evaluated 24 h after treatment. Among these two larvae species, only a moderate activity was observed with the *C. pipiens pallens*, which achieved a 53.4%-mortality with 400 ppm of the stock solution of the extract (29).

Cytotoxic activity

The cytotoxicity of the extract from the leaves (10g/100mL) of *A. amazonicus* was evaluated by Vero cell monolayers of African green monkey kidney, which were exposed to serial dilutions of the sample, starting at 2.0 mg/mL of the crude extract. These treated cells, after a 24-hour incubation, were examined microscopically and showed no toxic effects, such as cellular damage or lysis (7).

Crude extracts (hexane, EtOAc, EtOH, MeOH and H_2O) from the stems of *A. amazonicus* and 3ß-hydroxylup-20(29)-ene-27,28-dioic acid [1] and 2α,3ß-dihydroxylup-20(29)-ene-27,28-dioic acid [2] isolated of the EtOAc extract (concentrations 10, 1 and 0.1 mg/mL) were tested *in vitro* against SKBR-3 human adenocarcinoma and C-8161 melanoma tumor cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The compound 1 showed a selective cytotoxicity against SKBR-3, while the EtOAc extract and compound 2 demonstrated this activity against both tumor cell lines. At 10mg/mL, the extract and compounds 1 and 2 displayed cytotoxic activity of 53.3%, 45.6% and 42.5% against SKBR-3 and against C-8161, the extract and compound 2 showed 76.3% and 68.1%. The reference drug used was Methotrexate, which at 10 mg/mL, exhibited 36.8 % (SKBR-3) and 63.7% (C-8161) of cytotoxic activity (5).

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