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Monograph of *Himatanthus sucuuba*, a plant of Amazonian folk medicine

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ABSTRACT

The importance of the genus *Himatanthus* in popular medicine is supported by reports from different local communities in the Amazonian region. *Himatanthus sucuuba* (Spruce) Woodson is a plant of the Apocynaceae family, popularly known in the northern region of Brazil as sucuuba, sucuba or janaguba, among others common names. In Brazil, latex, infusions and decoctions of the bark and poultices are used in folk medicine for the treatment of gastritis, inflammations, anaemia, arthritis, verminosis and tumor. Phytochemical studies led to the isolation of triterpene esters with anti-inflammatory activity and iridoids with significant cytotoxic activity. Currently, popular consumption of this plant has been spread out in other regions, mainly for tumor treatment purposes. This work presents not only a review of botanical, phytochemical and pharmacological publishings but also new results of monographic information about the latex, leaves and bark of *Himatanthus sucuuba*.

KEYWORDS - Apocynaceae, *Himatanthus sucuuba*, botanical aspects, pharmacology, chemical constituents, etnopharmacology.

INTRODUCTION

Apocynaceae is a tropical and subtropical family of plants with few examples in temperate regions and comprises about 200 genera and 2,000 species. Neotropic regions host about 66 genera as well as more than 700 species, being, respectively, 41 and 400 registered in the Brazilian flora (1). The importance of *Himatanthus* genus in folk medicine, in which *H. sucuuba* species stands out, is mainly reported by the communities located in different points in the Amazon region, Peru and Colombia (2, 3, 4). The repercussion of the reports on this plant consumption against ulcers, inflammatory processes and tumors, made by people from the northern and northwestern of South America, enlarged the use of these vegetable species. In Brazil, it was first restricted to people in the northern region of the country, where it can be easily found across the tropical forest that permeates the urban areas and the countryside, which has no access to the public health assistance programs. Moreover, the information on the efficacy in patients with tumors in different evolution phases or through concomitant conventional chemotherapy, and even the ones considered terminal cancer cases, lead people living miles away from the Brazilian northern region, the main center of use and distribution of the plant, to search for this plant. Although no records were found to help concluding that collecting process may prove to affect the plant supply or even to provoke its disappearance in the short-term, studies have been developed for the species conservation (5). This work is required since the scientific reports have been evidencing the pharmacological potential of *Himatanthus sucuuba* recording the presence of active constituents in the latex, bark, leaves and roots of the plant, leading people to assure the medicinal use of this vegetable species.

BOTANICAL ASPECTS

The *Himatanthus* genus, name derived from Greek, meaning "flower mantle", in a reference to the bracts that involve the flowers buds, was described by Carl Willdenow and Josef Schultes and comprises only 13 species, all of which found in South America, mainly across the Amazon basin. This genus, which was previously considered a synonym of *Plumeria*, was discussed in works which allowed a clear distinction between both (6, 7).

Geographic Distribution

Himatanthus sucuuba is a tree which colonizes the varzeas of Central Amazonia and is often found in inner wild areas where it may be under flood conditions up to 5-month long and in slopes, with no flooding incidence. In both situations, the species is associated to high light intensity areas, lasting for about 60 years (5, 8, 9).

Taxonomical Classification

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Gentianales
Family	: Apocynaceae
Subfamily	: Plumerioideae
Tribe	: Plumerieae
Subtribe	: Plumeriinae
Genus	: *Himatanthus*

Species : *Himatanthus sucuuba*

In the doctorate thesis, based on taxonomic, micro-morpholigical and philogenetic studies, Spina proposed a new circumscription and delimitation for the genus, recognizing only 9 species in the Neotropics and, among other modifications, considered *H. sucuuba* synonym of *H. articulatus* (Vahl) Woodson (10).

Morphological Characteristics

The tree of *H. sucuuba* is a medium to large-sized, 8 to 20 meters tall, latescent, with erect and round trunk, with thick, wrinkled bark. The leaves alternate-spiraled are concentrate in the branch apexes and are simple, petiolated, glabrous in both surfaces, ovate or oblong-lanceolate coriaceous, with whole and plane edges, acute apex, obtuse to oblique base. Prominent brochidodrome venation at the inferior surface, with secondary arched ribs near the edge joined by an arc of about 2-3 mm next to edge and diverging in an angle inferior to 90° as to midvein. The inflorescences are arranged in terminal cupule, with few large white flowers (Figure 1). The fruits are double follicle with several dried ellipsoid winged seeds, fully covered by a circular membrane that facilitates wind (anemochory) and water (hydrochory) dispersal (1, 9, 11, 12).

Figure 1. *Flowering H. sucuuba stem*

The anatomic studies on the species are restricted to the bark, leaf and stem. From a sample collected in the Reserve Ducke (Manaus, AM), the stem bark showed in a transversal section several ramified sclereids, non-articulated laticiferous channels and prismatic calcium oxalate crystals in the phloematic and cortical parenchymas (Figure 2a). The stem fragment next to the apex showed a unistratified epidermis, with stripped striate thick cuticle, which gives place to a peridermis thanks to the increase in the organ circumference. In the cortical region, there were observed isodiametric parenchymatic cells containing starch, calcium oxalate crystals in druses shape and, mainly, prisms and laticifers with lipophylic and phenolic substances. Externally to the vascular bundles there appears a discontinuous sclerenchymatic sheath of thick walls and reduced lumen. The bicollateral vascular system has phloem with sieve tube elements and laticifers immersed in parenchyma. The external phloem, with lignified fibers, is arranged in a continuous ring and presents petreous cells in lower levels of the stem, while the inner presented in the cordon shape. The xylem, totally lignified, contains tracheal radial elements separated by parenchymatic rays. The organ core is occupied by a medullar parenchyma with the same elements described in the cortical region (11). The microspcopic analysis of the leaf showed the lamina with dorsiventral mesophyll overlayed by glabrous adaxial and abaxial unistratified epidermis with striate, thick cuticle and anomocytic clustered stomata only on abaxial epidermis. The biconvex central rib presented bicollateral vascular bundles, ramified laticifiers, idioblasts with phenolic substances, calcium oxalate crystals and several cells of angular collenchyma (12). The vascular region, which is arranged in a circle, limits a medullar parenchyma that has laticifiers, starch and idioblasts containing phenolic substances. Isolated groups of phloem also may be observed in this region (Figure 2b and 2c).

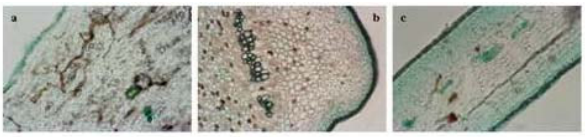

Figure 2. *(a) Barks, (b, c) Leaves*

Agronomic Study

Seeds of *H. sucuuba* were accompanied from germination until the seedling stage, in experimental conditions simulating natural field conditions (terrestrial phase and flooding period). Germination was tested in two substrates: sand and sawdust (only irrigated), and in water (submergence). The seedlings produced were then subjected, for 120 days, to three treatments: control (daily irrigation), partial submersion (root system) and total submersion. Irrespective of the flooding regime, germination rates and seedling formation were high, both above 80%. Under partial submersion, hypertrophic lenticels, adventitious roots and aerenchyma were formed in the roots while enzyme alcohol dehydrogenase activity (ADH) remained high until the 60th day of flooding, declining afterwards. Seedlings under total submersion lost all leaves, did not form adventitious roots or lenticels, but developed aerenchyma. These seedlings showed the highest values of ADH, which remained high until the end of the experiment, indicating the diversion of the aerobic metabolism to the production of ethanol as the main pathway to maintain the energetic balance. Although some totally submersed seedlings died, 70% of them survived the 120 days of flooding. These adaptive strategies of the species showed that the seedlings modulate morpho-physiologically the tolerance to flooding according to the conditions used (5).

CHEMICAL CONSTITUENTS

Terpenes

Latex: α- and β- amyrin cinnamates, lupeol, α- and β- amyrin acetates and lupeol acetate (14, 15)

Bark: α-amyrin cinnamate, lupeol cinnamate, lupeol acetate (16) and lupeol β-phenyl propionate (17)

Leaves: α- and β- amyrin cinnamates, lupeol cinnamate, α- and β- amyrin acetates and lupeol acetate (18)

Roots: lupeol, β-amyrin cinnamate (19), lupeol cinnamate, lupeol acetate (20)

Flower: β-amyrin cinnamate and germanicol (19)

Iridoids

Latex: plumericin, isoplumericin (14), plumieride, isoplumieride, 15-demethylisoplumieride and 15-demethylplumieride (14, 21)

Bark: plumericin, isoplumericin (16, 17), plumieride, 15-demethylplumieride, isoplumieride (22) and fulvoplumierin (3)

Leaves: plumericin, isoplumericin, plumieride and isoplumieride (18)

Roots: plumericin, β-dihydroplumericin, and isoplumericin (19, 20), allamandin (23)

Phenolic Compounds

Latex: catechol, gallic acid, myricetrin and quercitrin (14).

Bark: *p*-coumaric acid and 4-hydroxy-3-methoxy-benzoic acid (24)

Depsides

Bark: confluentic acid and 2'-O-methylperlatolic acid (24)

Aminoacid

Latex: glutamic acid (14)

Cyclitols

Latex: *myo*-inositol (14)

Flower: *myo*-inositol (19)

Carbohydrates

Latex: arabinose, glucose, xylose, rhamnose and galactose (14, 25)

Polymer

Latex: *cis*-polyisoprene (14, 25)

Microelements

Latex: Na, Mg, Al, K, Ca, Mn, Ti, V, Cr, Co, Ni, Cu, Zn, Ba, Fe, Sr, Zr, Th, Pb (14, 25)

PHYSICAL CHEMISTRY ANALYSES

Triterpenes

The hexane fraction obtained of the latex was initially analysis by GC-MS, indicating the presence of four main substances with characteristic fragmentations of triterpenes. This mixture fractioning was performed through chromatographic process in column with silica gel impregnated with $AgNO_3$ and eluted with hexane/benzene (7:3), generating fractions that after CCD analysis were put together in groups which were characterized by spectral methods. The obtained results led to the determination of the following triterpenes esters: cinnamates of α- and β-amyrin (mixture), cinnamate of lupeol, acetates of α- and β-amyrin (mixture) and lupeol acetate (14). Other works showed that these triterpenes were also isolated and identified by GC-MS and NMR analyses of the hexane extracts of barks (16), roots (19) and leaves (18). The crude ethanol extract of the bark was subjected to column chromatography (SiO_2) and hexane/toluene (3:1) eluent system to give lupeol acetate. Other three substances were separated using preparative silica gel TLC (hexane/toluene 1:4) and gave α-amyrin cinnamate, a partially separated mixture of lupeol cinnamate and lupeol β-phenylpropionate (17).

Polymer

n-Butanol was added to the latex of *H. sucuuba* to coagulate the polyisoprene. This mixture was filtered and the filtrate was subjected to liquid-liquid extraction (n-BuOH/H_2O). The polymer obtained was submitted to Soxhlet extraction with acetone and then methanol, and then further purified by selective precipitation from hexane/isopropanol, which yielded 0.61 % of solid material. After this procedure, the polymer was re-suspended in THF and submitted to Gel Permeation Chromatography (GPC) on a styragel column, calibrated with polystyrene standards. Analysis of the polyisoprene indicates a unimodal distribution, with a median molecular weight (Mn) of 192, a weighted average (Mw) of 571 and a polydispersion index (Mw/Mn) of 2.97. The polyisoprene isolated from the latex was characterized by spectroscopic methods (^1H and ^{13}C NMR; IR) as being *cis*-polyisoprene (25).

Iridoids

The crude hexane fraction of the latex was subjected to Sephadex LH-20 column chromatography and combinations of hexane, CH_2Cl_2, acetone and methanol as eluent systems. The mixture of plumericin and isoplumericin was separated by preparative TLC (silica gel) eluted with hexane:AcOEt (6:4) of one fraction obtained of this procedure (14). The ethanol extract of the bark was partitioned between hexane and 80% aqueous MeOH and the methanol soluble fraction was further partitioned between CH_2Cl_2 and H_2O:MeOH (4:6). Purification of the CH_2Cl_2 fraction using silica gel column chromatography with 3% MeOH in CH_2Cl_2 as eluent, led to the isolation of plumericin (17). The crude aqueous latex (3L) of *H. sucuuba* was extracted with hexane and the aqueous fraction was subjected to Medium Pressure Liquid Chromatography (MPLC). This MPLC separation was performed at column packed with LiChroprep C-18 in combination with a water/methanol gradient. 26 fractions were obtained and combined after TLC analyses to 4. The preparative HPLC-UV separations were performed on a RP-18 column in combination with H_2O + 0.05% of TFA and CH_3CN gradients. Finally these procedures led to the isolation of four compounds: 15-demethylisoplumieride, 15- demethylplumieride, plumieride and isoplumieride (14, 21).

HPLC

The analysis by HPLC-PAD of the aqueous fraction of the latex was performed on a CarboPac PA-1 anionic exchange column and indicated the presence of the carbohydrates arabinose (37.4 %), glucose (20 %), xylose (9.2 %), rhamnose (8.4 %) and galactose (7.4 %) (25). HPLC-DAD was used for the quantitative analyses of the main iridoids plumieride (a), isoplumieride (b) and 15-demethylplumieride (c) of the bark and latex. The analyses of the extracts were performed on a Lichrospher C-18 column and the chromatograms showed a similar iridoid composition. The bark extract showed concentrations of 26.7, 4.4 and 3.8 μg/mL of the iridoids a, b and c. The concentrations of these iridoids on the latex extract were 34.7, 4.7 and 10.5 μg/mL, respectively (22).

ICP-MS

For determination of metals, an aliquot of the latex was freeze dried and submitted to acid digestion. Inductively coupled plasma mass spectrometry (ICP-MS) analysis of microelements in the aqueous phase of the latex showed the most abundant to be Ca (354 μg/g) and Mg (250 μg/g) (25).

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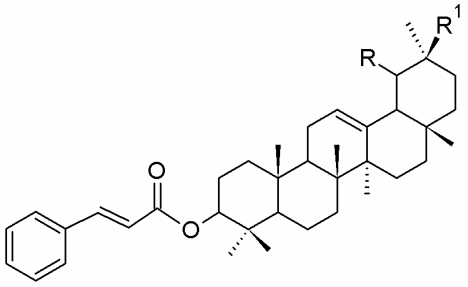
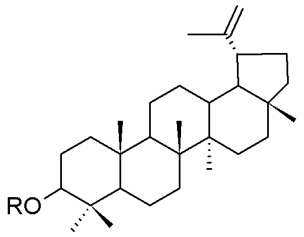
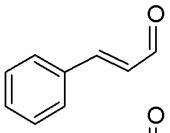


α–amyrin cinnamate, R = CH_3; R^1 = H

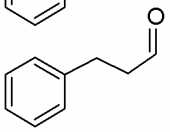
β–amyrin cinnamate, R = H; R^1 = CH_3

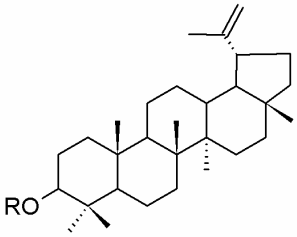


lupeol cinnamate, R =



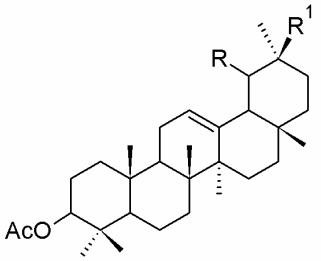
β- phenyl propionate, R =





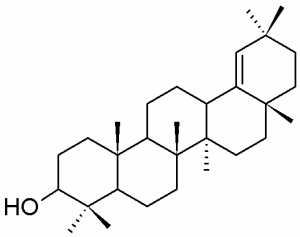
lupeol acetate, R = Acetyl

lupeol, R = H

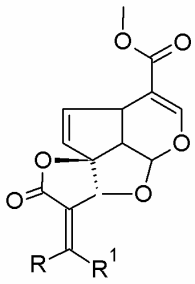


α–amyrin acetate, R = CH_3; R^1 = H

β–amyrin acetate, R = H; R^1 = CH_3

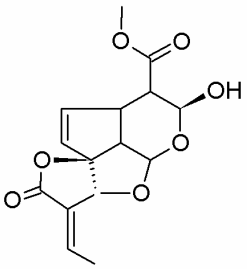


germanicol

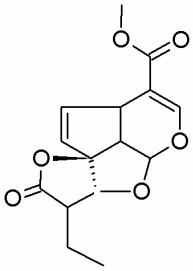


plumericin, R = H; R^1 = CH_3

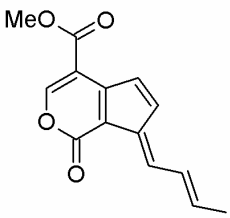
isoplumericin, R = CH_3; R^1 = H



allamandin



β-dihydroplumericin



fulvoplumierin

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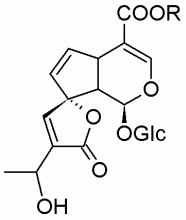
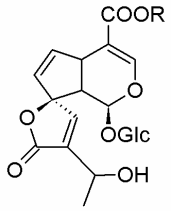


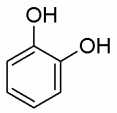
15-demethylisoplumieride, R = H

isoplumieride, R = CH_3

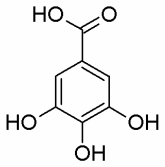


15-demethylplumieride, R = H

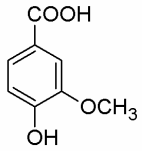
plumieride, R = CH_3



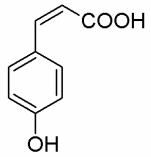
catechol



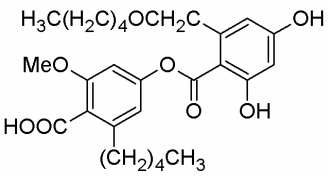
gallic acid



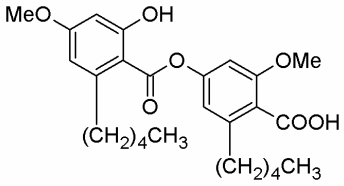
4-hydroxy-3-methoxy-benzoic acid



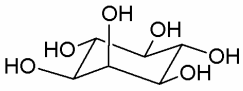
***p*-coumaric acid**



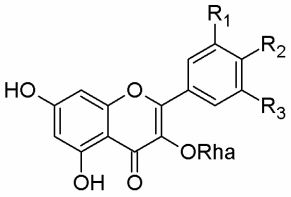
confluentic acid



2'-*O*-methylperlatolic acid



***myo*-inositol**



myricetrin, R_1=R_2=R_3=OH

quercetrin, R_1=R_2=OH, R_3=H

Rha = Rhamnose

Figure 3. *Chemical constituents isolated of H. sucuuba*

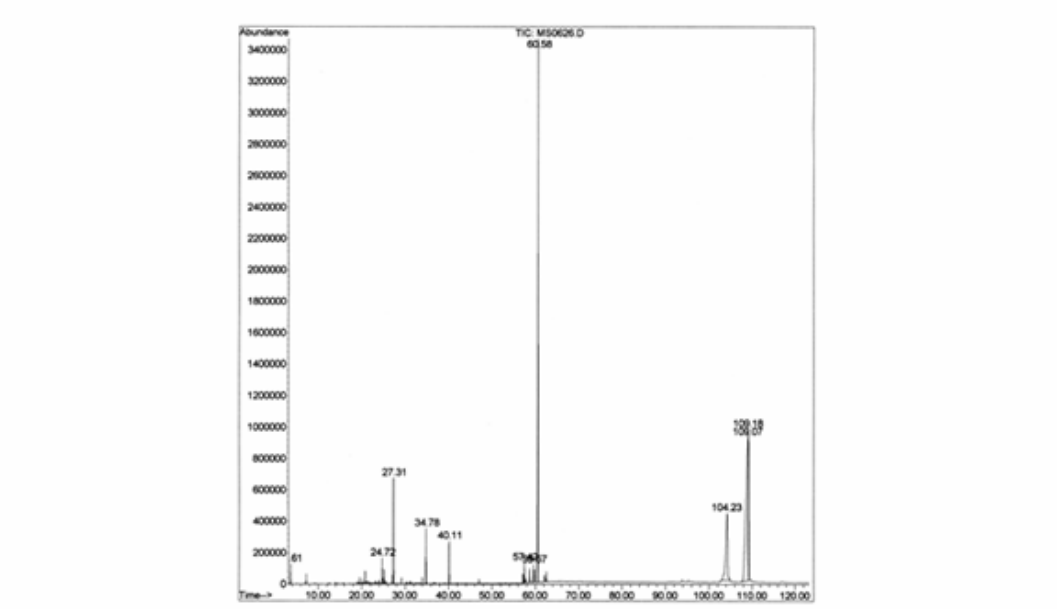


Figure 4. *GC chromatogram of hexane fraction from latex of H. sucuuba. Retention time 34.8, 60.6, 104.2, 109. 1 and 109.2 min; substances plumericin, lupeol acetate, lupeol cinnamate and α- and β- amyrin cinnamates respectively.*

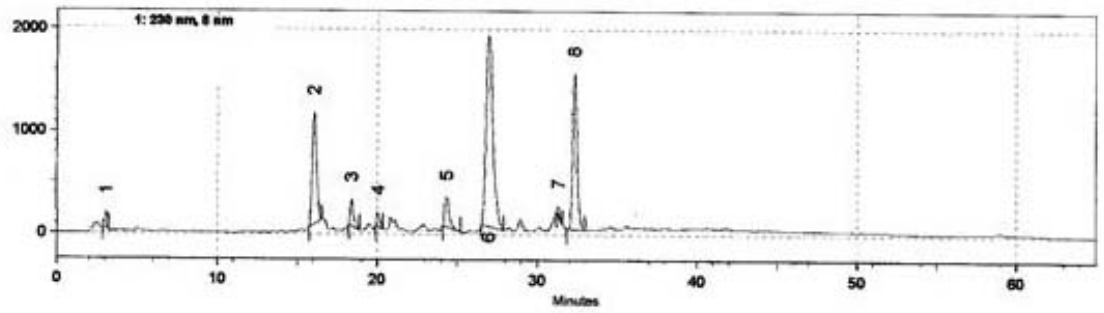


Figure 5. *HPLC chromatogram from the latex of H. sucuuba. Peaks 3, 6 and 8 iridoids 15- demethylplumieride, plumieride and isoplumieride, respectively.*

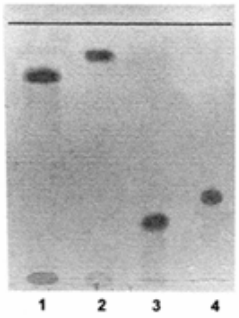


Figure 6. *TLC - C-18 analysis (H_2O with 0.05% of trifluoracetic acid : MeOH, 7:3) of the iridoids 15-demethylplumieride (1), 15-demethylisoplumieride (2), plumieride (3) and isoplumieride (4).*

CHROMATOGRAPHIC PROFILES

Examples of the general chromatographic profiles of the latex and iridoids glycosides of *H. sucuuba* collected in 2004, in campus of the Federal University of Amazonas, Manaus (Amazonas state) were showed below. The chromatographic conditions were the same of other works with this plant (14, 18, 22).

The substances above were also found in the bark (16, 17, 21, 22), leaves (18) and roots (19, 20) of the plant and vary according to place and period of the vegetal material collection.

MEDICINAL USES

Ethnopharmacology

Himatanthus sucuuba shows a wide range of use in folk medicine, mainly the latex or bark in the oral treatments or by local compression bandages. This species is popularly known as sucuúba, sucúba, sucuba, leite branco, leite de janaguba, leite de sucuuba, bellaco-caspi among others common names (2, 3, 26, 27). The table 1 shows the principal ethnopharmacological data of this plant. In spite of the long cancer-healer history of *Himatanthus* genus in Brazil, unfortunately literature lacks its register. According to collected declarations, this plant use started by the 70's with the disclosure of healing registries made by doctors, which referred to inoperable cases of lung and lymphatic cancers. As a consequence, there began the search for the miraculous "milk" to answer to the needs of the afflicted people all over the country (27).

General aspects of the latex

The latex consists of a suspension of particles with bitter flavor (pH 4.54) and brown coloration (25).

Withdrawal of the "Sucuuba Milk" (Leite de Sucuuba) of the Tree

The bark is partially stripped in a 10 x 30 cm area, taking off latex with the help of some water and a table spoon. Milking operation is concluded when the mixture "milk + water" preserved in a one-liter bottle results in a white deposit corresponding to 1/4 to 1/3 of full bottle and presents a pinky-floating coating. According to the users of this recipe, the mixture must be preserved at low temperature (preferably under refrigeration), with intakes recommended three times a day (27).

Folk Recipe for the Administration of the "Sucuuba Milk" (latex)

Mode of Administration: 3 (three) table spoon-dosing straight by mouth, followed by a glass of water/milk, three times a day, immediately before meals (breakfast, lunch, dinner).

Note: Sucuuba milk bottle must be stored under refrigeration and shake before administration.

Important:

1. Sucuuba intake must be accompanied by a fiber-based meal.
2. Increase the intake of soluble fibers: fruits (papaya), dried beans, oats, barley, legumes and vegetables, to develop good bowel habits.
3. Avoid alcohol concomitant consumption (14).

PHARMACOLOGY

Antifungal Activity

Bioactivity guided fractionation from hexane extract of *H. sucuuba* barks using direct bioautography on thin layer chromatoplates led to the isolation of iridoids mixture, plumericin and isoplumericin, which showed higher inhibition (bellow 1μg) against *Cladosporium sphaerospermum* than the reference drug nystatin (5μg) (16).

Table 1. Ethnopharmacological data of Himatanthus sucuuba

Part Used	Medicinal Indication	Mode of Use	Local	Reference
Bark	external wounds	Aqueous preparation	Peru	26
Stem bark	tumors, boils, swellings, arthritis, worms	Aqueous preparation	Peru	3
Latex	tumor, ulcers	Fresh and dried latex	Brazil	2
Stem bark	pains, coughs	Various	Amazon	28
Stem bark	tumors	Various	Peru	29
Bark and latex	herpes, wounds, leishmaniasis ulcers	Fresh bark and latex direct on the affected area	Peru	30
Latex	boils, wounds, splinter	Direct on the affected area	Bolivia	31
Latex	coughs	Direct oral ingestion	Brazil	32
Latex	body pains	Direct on the affected area	Brazil	32
Bark	stomach ulcers, inflammation in the uterus	Aqueous preparation	Pará, Brazil	32
Bark	tumors, diarrhea, worms	Aqueous preparation	Pará, Brazil	30

The crude methanol extract of the roots and hexane, $CHCl_3$, ethyl acetate and n-butanol fractions obtained from the partition of this extract were tested against various microorganisms. The $CHCl_3$ fraction showed a good broad spectrum of antimicrobial activity. A bioassay guided of this fraction using the bioauthography method led to the isolation of two iridoids, allamandin and plumericin. The latter substance showed a pronounced activity against the three tested fungi [*Saccharomyces cerevisiae* ATCC 2601 (2.5 μg), *Candida albicans* (1.25 μg), *C. dubliniensis* (2.5 μg)] which was comparable or higher than the control drug nystatin (2.5, 2.5 and 3.0 μg, respectively) (23).

Antibacterial Activity

Plant material (wood) was extracted at room temperature with ethanol and the crude extract was prepared for assay in DMSO/water (1:1). The antibacterial activity of the extract was determined using an agar-diffusion method and the inhibition was reported based on diameter of growth inhibition ring (d). This ethanol extract showed activity against bacterial strains *Clostridium histolyticum* ATCC c6282

(d > 0.7cm) and *Bacteroides fragilis* ATCC c23745 (d > 0.7cm) comparable to the positive control vancomycin (33).

Antiprotozoal Activity

The ethanol extract obtained from the barks was active on the amastigote model (IC_{50} =5μg/mL) and showed a moderate activity on the promastigote stage (IC_{50} =20μg/mL). A bioassay guided isolation of this extract evaluated *in vitro* against promastigote (p) and axenic amastigote (aa) forms of *Leishmania amazonensis* afforded the iridoids isoplumericin [0.28 μM (aa)] and plumericin [0.21 μM (aa)]. The latter showed a reduction of macrophage infection similar to that of the reference drug Amphotericin B (IC_{50} = 0.9 and 1 μM, respectively). Isoplumericin showed toxicity against infected macrophages which did not allow an evaluation of its activity against intracellular amastigotes (34). The ethanol bark extract was reported as inactive against another protozoan parasite, *Plasmodium falciparum* (35).

Anti-Inflammatory and Analgesic Activities

Fractions of the latex were pharmacologically evaluated in the carrageenan-induced rat paw edema and in the acetic acid-induced mouse constriction tests. The hexane fraction inhibited the edema formation by 35.9% at an oral dose of 200 mg/kg (*p.o.*). The fraction containing only cinnamates inhibited the edema and the abdominal constrictions by 54.5% and 57.9%, respectively, at 100 mg/kg (*p.o.*), which led the authors suggest that these compounds were responsible for the activity of the crude extract (15).

Antitumor Activity

The ethanol extract of the bark [IC_{12}= 4000 μg/mL (RS321)] were screened in a mechanism based yeast bioassay using DNA repair- or recombination-deficient mutants of the yeast *Saccharomyces cerevisiae*. This extract was partitioned between hexane and 80% aqueous methanol, resulting in enhanced activity in the latter fraction. Further partitioning (60% aqueous methanol/CH_2Cl_2) concentrated the activity in the dichloromethane fraction (IC_{12}=380 μg/mL). Chromatographic purification of this fraction led to the isolation of plumericin, responsible for the DNA damaging activity of the extract. The activity observed was relatively weak, with an IC_{12} value of 70 μg/mL for the RS321 yeast strain (reference drug streptonigrin IC_{12} value of 0.65 μg/mL against RS321). The hexane fraction was evaluated to the *in vitro* differential cytotoxic activity using various cancer cell lines (H460, ME180, DU145, MCF-7, HT29). The resulting 50% growth inhibition (GI_{50}) values were greater than the control (BALB/c 3T3, GI_{50} 0.125 mg/mL) for all cell lines except for minor activity against human non-small lung cell carcinoma (H-460, GI_{50} 0.080 mg/mL). Lupeol acetate, one of the triterpenes isolated, was tested against the same tumor cell lines above and showed no cytotoxic activity (GI_{50} > 1 mg/mL) for all cell lines (17).

Monoamine oxidase B (MAO-B) Inhibitor

Two known lichen depsides found in the bark of *Himatanthus sucuuba,* confluentic acid and 2'-O-methylperlatolic acid, have shown inhibition against monoamine oxidase-B. Confluentic acid showed selective inhibition of MAO-B with IC_{50} value of 0.2 μM (24).

Immunoregulatory Activity

The hexane, methanol and acetone extracts of the root as well as the iridoid plumericin inhibited *in vitro* nitric oxide (NO) production by more than 80%. Hexane and methanol extracts alone were not able to inhibit interferon-γ (IFN-γ) production. The activity shown by these extracts is probably related to the presence of plumericin and its geometric isomer, ß-dihydroplumericin, which was cytotoxic at 100 μg/mL and inhibited NO and IFN-γ at 10 μg/mL or higher (20).

Capillary Permeability Activity

The decoction of the bark was injected intradermally onto marked sections on the animal 90 min prior to intravenous infusion of 2/mL of 1% Evans Blue dye solution. The extract dosage range of 2.5 to 5.0 mg increased capillary permeability in rats (26).

TOXICOLOGY

In order to evaluate the toxic potential of the stern bark decoction *H. sucuuba*, pregnant rats were treated from day 6 to day 15 of pregnancy with this extract (40 mg per rat, twice a day) or distilled water (1.0/mL, twice a day) by gastric intubation. Maternal and fetal data suggest low reproductive toxicity and teratogenic potentiality (36). An acute toxicity screening of the decoction of the bark after intraperitoneal administration of 0.1 to 1 mg of extract per gram of mouse weight determined that *Himatanthus sucuuba* was non-toxic in this range (26). To determine the acute toxicity of the crude aqueous latex and of the aqueous extract prepared from the dried barks of the *H. sucuuba*, there were used 140 adult male and female mice, with 24 g as average weight, divided in groups of ten animals, with same number of males and females. At first, preliminary tests were made with oral administration of the latex and the aqueous extract in 1 mL/animal dose, corresponding to 1100 mg e 1280 mg, respectively, having no death been attested in a 72-hour period. Intraperitoneal (i.p.) administration of the latex was made applying doses: 55, 110, 165, 220 and 275 mg/animal and the aqueous extract in doses: 57, 114, 171, 228 and 285 mg/animal. During the first observation hours, after the i.p. administration, animals presented a high level of depression, lethargy, followed by tachycardia and dyspnea. Medium letal dose (LD_{50}) of the i.p. via was 131.52 mg as to the latex and 153.48 mg as to the aqueous extract (37). Cytotoxicity of the plumericin and isoplumericin were determined using BALB/3T3 (non-tumorogenic, BALB/c mouse embryo cells) and Vero (African green monkey kidney cells) cells. In these tumoral assays isoplumericin (1.10 μM and 1.03 μM) was more toxic than plumericin (2.0 μM and 1.72 μM). These two compounds were evaluated for their toxicity on mice peritoneal macrophages and showed IC_{50} of 1.86 μM for both which was higher than the reference drug Amphotericin B (>10 μM) (34).

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