# CONTRIBUTION TO THE STUDY OF *Himatanthus sucuuba*: LATEX MACROMOLECULE, MICROELEMENTS AND CARBOHYDRATES

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**ABSTRACT:** The polymeric material in the latex of *Himatanthus sucuuba* (Spruce) Woodson was identified by spectroscopic methods as *cis*-polyisoprene ($M_n$ = 192; $M_W$ = 571; $M_W/M_n$ = 2.97). ICP-MS analysis of microelements in the aqueous phase showed the most abundant to be Ca (354 µg/g) and Mg (250 µg/g). Carbohydrate analysis of the aqueous phase by HPLC-PAD showed arabinose, glucose, xylose, rhamnose and galactose to be the predominant saccharides.

**KEYWORDS:** Apocynaceae, *Himatanthus*, latex, *cis*-polyisoprene, microelements, carbohydrates

# CONTRIBUIÇÃO AO ESTUDO DE *Himatanthus sucuuba*: MACROMOLÉCULA, MICROELEMENTOS E CARBOIDRATOS DO LÁTEX

**RESUMO :** O polímero do látex de *Himatanthus sucuuba* (Spruce) Woodson foi identificado por métodos espectroscópicos como o *cis*-poliisopreno ($M_n$ = 192; $M_w$ = 571; $M_w/M_n$ = 2.97). A análise de microelementos na fase aquosa por ICP-MS forneceu Ca (354 mg/g) e Mg (259 mg/g) como elementos majoritários. A detecção de carboidratos na fase aquosa por CLAE-DPA apresentou arabinose, glucose, xilose, ramnose e galactose como açúcares principais.

**PALAVRAS-CHAVE:** Apocynaceae, *Himatanthus*, látex, *cis*-poliisopreno, microelementos, carboidratos

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## INTRODUCTION

*Himatanthus sucuuba* (Spruce) Woodson (Apocynaceae) is a medium-sized tree growing on firm ground in the Amazon region, popularly known as sucuuba, sucuba or janaguba. Its wood is used for construction and other purposes. The trunk bark is popularly used for the treatment of gastritis, stomach ulcers and hemorrhoids (van den Berg, 1984), and as an analgesic (Elisabetsky *et al.*, 1990). In the Peruvian Amazon it is reportedly used for hernias, boils and tumors (Perdue *et al.*, 1978).

The latex, a bark exudate, is used *in natura* as an antihelmintic (Corrêa, 1975), antitumor, antifungal and antiphlogistic (van den Berg, 1984) agent, as well as for poultices and fractures. Chemical studies have indicated the presence of the iridoids fulvoplumierin, plumericin and isoplumericin, which possess antifungal, antibiotic (Vilegas *et al.*, 1992) and cytotoxic (Hamburger *et al.*, 1991) activities. Fractionation of the bark extract, guided by antifungal bioassay, demonstrated strong activity against *Cladosporium sphaerospermum* (Silva *et al.*, 1998). Antiphlogistic and analgesic activities were also detected in *in vivo* tests, and associated with the presence of cinnamoyl esters of hydroxy-triterpenes in the latex (Miranda *et al.*, 2000).

Several functions have been attributed to latexes and resins, including transport and storage of nutrients, regulation of water-balance, the capacity to store non-functional products of secondary metabolism and protection against pathogens. The occurrence of isoprenoid substances in latexes is already well established in the plant families Euphorbiaceae, Moraceae, Asclepiadaceae, Compositae, Guttiferae and Apocynaceae (Tanaka, 1989).

*Hevea brasiliensis* latex, for example, contains 30 to 40 % (w/v) of isoprenoid material, and about 5 % (w/v) of non-isoprenoid substances in its composition. The latter are dissolved or suspended in the aqueous medium, or are adsorbed on the surface of the rubber (Tanaka, 1989). The non-polymeric material consists mainly of minerals, lipids, terpenes, proteins, carbohydrates and amino acids (Aik-Hwee *et al.*, 1993; Moir, 1959).

The objectives of this report were to determine microelement and carbohydrate composition and characterize the polyisoprene in the latex of *Himatanthus sucuuba*.

## EXPERIMENTAL

*Himatanthus sucuuba* latex was collected within the city limits of Santarém, State of Pará, by Mr. Raimundo S. Carneiro. A voucher specimen was deposited at the Herbarium of the Institute of Biological Sciences of the University of Amazonas, Manaus, Brazil, registered under the number 5436.

The pH measurement of the crude latex was made digitally (Hanna model 8417), calibrated with potassium hydrogen phthalate 0.05 M (pH 4.01) and $KH_2PO_4$ 0.025 M + $Na_2HPO_4$ 0.025 M (pH 6.86) buffer solutions.

For the determination of metals, an aliquot of the latex *in natura* was freeze dried, submitted to acid digestion as described by Pereira *et al.* (1998), and analyzed by mass spectrometry with inductively coupled Plasma mass spectrometry (ICP-MS), using a Perkin-Elmer Sciex, model Elan 5000 Å instrument, with semi-quantitative calibration and internal standard containing the elements Li, Mg, Na, P, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Al, Zr, Th, Si, Sr, Mo, Ag, Cd, Ba, Tl, and Pb. The analysis was made in duplicate.

$^{1}H$ and $^{13}C$ NMR spectra were registered on a Bruker AM-200 spectrometer (200 and 50 MHz, respectively), using $CDCl_3$ as solvent and tetramethysilane (TMS) as internal reference. Chemical shifts are expressed in ppm.

Samples were prepared as KBr pellets and their IR spectra obtained with a Nicolet spectrophotometer with Fourier transform Model Magna-IR 760. Wavelengths are expressed in reciprocal centimeters ($cm^{-1}$).

Gel permeation chromatography (GPC) was carried out with a Waters 600E apparatus using tetrahydrofuran (THF) as mobile phase; flow rate 1 ml/min, and a Waters styragel column with porosity of 500 Å. Polystyrene standards used in the calibration curve were: A-500 (500 Da); A-1000 (950 Da); A-8 (2900 Da); A-7 (3600 Da); F-1 (9700 Da); A-5 (33000 Da); A-4 (111000 Da); A-3 (200000 Da); A-2 (465660 Da). Refractive Index and ultraviolet (200-400 nm) detectors (Waters Models 410 and 991, respectively) were used.

High performance liquid chromatography, coupled to a pulsed amperometric detector (HPLC/PAD), was performed using a DIONEX Model DX-300 equipped with CarboPac PA-1 anionic exchange column.

*n*-Butanol was added to latex to coagulate the polyisoprene. This mixture was filtered and the filtrate was subjected to liquid-liquid extraction (*n*-BuOH/$H_2O$). The aqueous fraction was analyzed by the sulfuric acid - phenol method (Dubois *et al.*, 1956) and by HPLC, as described above (Corradini *et al.*, 1998), for quantification of total sugars.

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 the polymer was re-suspended in THF and submitted to GPC on a styragel column, calibrated with polystyrene standards.

## RESULTS AND DISCUSSION

Analysis of *H. sucuuba* latex by ICP-MS showed the presence of several elements. The most abundant were calcium (354 µg/g) and magnesium (250 µg/g). Polyisoprene isolated from the latex was characterized by spectroscopic methods ($^{1}H$ and $^{13}C$ NMR; IR) as being *cis*-polyisoprene. Analysis by HPLC-PAD of the aqueous fraction indicated the presence of the carbohydrates arabinose, glucose, xylose rhamnose and galactose.

### Physico-chemical aspects of *H. sucuuba* latex

The latex consists of a suspension of particles with pH 4.54, brown coloration and bitter flavor. ICP-MS analysis showed the presence of several microelements (Table 1) of which the most concentrated are related to several therapeutic properties, including the treatment of inflammatory processes and neoplasias (Larson, 1995).

**Table 1:** Microelements from the latex of *H. sucuuba*

| Elements | Na | Al | Ca | Fe | Ti | Cr | Ni | Zn | Zr | Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/g) | 70.5 | 3.88 | 354 | 4.45 | 1.03 | 0.04 | 0.04 | <0.01 | 0.35 | 0.08 |
| Elements | Mg | K | Mn | Sr | V | Co | Cu | Ba | Th | |
| Conc. (mg/g) | 250 | 123 | 8.88 | 3.90 | <0.001 | <0.01 | 0.08 | 0.26 | 0.03 | |

Calcium and magnesium are very important minerals to life in general. In laboratory animals, increased calcium intake suppresses hyperproliferation of the cells of the colon and, in humans, the ingestion of calcium supplements reduces hyperplasia of colon cells (Lipkin *et al.*, 1995). In humans magnesium participates in important biological functions, promoting good immune defense and taking part in enzymatic reactions. It is indicated for the treatment of stress and for nervous diseases in general, including all well-known forms of epilepsy, besides exhibiting anti-allergic, antiphlogistic and analgesic properties. The magnesium ion is indispensable in RNA and DNA synthesis, is important in the prevention of disturbances of the digestive system and also in general of malignant diseases (Wood *et al.*, 1995).

On the other hand, the trace elements, especially manganese, iron, copper and zinc, which are also important to the pharmacological properties of this plant (Silva, 1997), were present in much lower quantity in the latex than in the bark (Silva, 1997). They were also lower in *H. sucuuba* than in other medicinal plants with healing properties (Pereira *et al.*, 1998).

The analysis of sugars revealed a significant amount (1.9 mg/ml) of total sugars in the crude latex, consisting of arabinose (37.4 %), glucose (20 %), xylose (9.2 %), rhamnose (8.4 %) and galactose (7.4 %). The first two are involved in a series of life maintenance functions, serving, for example, as a plant nutrient reserve during growth and development (Agrawal, 1992).

Analysis of the polyisoprene indicates a unimodal distribution, with a median molecular weight ($M_n$) of 192 and a weighted average ($M_w$) of 571. The polydispersion index ($M_w/M_n$) was 2.97, a value comparable to that of natural rubber from *Hevea brasiliensis* (Aik-Hwee *et al.*, 1993).

The infrared spectrum showed bands between 3036 and 2726 $cm^{-1}$, corresponding to axial deformations of the methyl, methylene and methine C-H groups. The absorption at 1664 $cm^{-1}$ was attributed to the axial deformation of the double bond of the *cis*-polyisoprene. The absorptions at 1376 $cm^{-1}$ and 1216 $cm^{-1}$ corresponded to the symmetrical angular deformations of the methyl group and the asymmetric out-of-plane deformation of the methylene group, in accordance with other data for *cis*-polyisoprene (Aik-Hwee *et al.*, 1993; Richardson *et al.*, 1953). The $^1H$ NMR spectrum showed signals at 1.68 ppm, attributed to methyl group hydrogens, 2.05 ppm, corresponding to the methylene hydrogens, and 5.13 ppm, for the vinylic hydrogens. The $^{13}C$ NMR spectrum showed signals at 23.3 ppm (methyl carbon), 26.3 ppm (methylene group adjacent to an unsubstituted olefinic carbon); 32.2 ppm (methylene group adjacent to a substituted olefinic carbon), 125.0 ppm (olefinic carbon linked to a methyl group) and 135.1 ppm (olefinic carbon). Comparison of the IR spectra, $^1H$ and $^{13}C$ NMR of the polymer, with spectra described in the literature, shows that this *H. sucuuba* latex polymer is *cis*-polyisoprene (Aik-Hwee *et al.*, 1993; Richardson *et al.*, 1953; Tanaka *et al.*, 1995).

## CONCLUSION

The polymer in the latex was identified as *cis*-polyisoprene, whose occurrence in many plants accompanies that of other isoprene based secondary products, such as triterpenes and their esters. The most abundant elements,

calcium and magnesium, can be considered as contributors to the antiphlogistic and antitumoral medicinal properties attributed by popular use to the latex.

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## LITERATURE CITED

Agrawal, P.K. 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry*, 31:3307-3330.

Aik-Hwee, E.; Tanaka, Y. 1993. Structure of natural rubber. *Trends in Polymer Science*, 493-513.

Corradini, C.; Canali, G.; Cavazza, A.; Delfino, D.; Teti, G. 1998. Compositional analysis of the major capsular polysaccharides of *Cryptococcus neoformans* by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). *J. Liq. Chromatogr. R.T.*, 21:941-951.

Corrêa, M.P. 1975. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Imprensa Nacional, vol VI, Ministério da Agricultura, Rio de Janeiro, Brasil, pp. 154-155.

Dubois, M.; Gilles, A.; Hamilton, J.K.; Rebers, P.A.; Smith, S. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28:350-355.

Elisabetsky, E.; Castilhos, C. 1990. Plants used as analgesics by Amazonian caboclos as basis for selecting plants for investigation. *Int. J. Crude Drug Res*, 28:309-320.

Hamburger, M.O.; Cordell, G.A.; Ruangrungsi, N. 1991. Tradicional medicinal plants of Thailand. XVII. Biologically active constituents of *Plumeria rubra*. *J. Ethnopharmacol.*, 33:289-292.

Larson, R.A. 1995. The antioxidants of higher plants. *Phytochemistry*, 27:969-978.

Lipkin, M.; Newmark, H. 1995. Calcium and the prevention of colon cancer. *J. Cell. Biochem.*, 22:65-73.

Miranda, A.L.P.; Silva, J.R.A.; Rezende, C.M.; Neves, J.S.; Parrini, S.C.; Pinheiro, M.L.B.; Cordeiro, M.C.; Tamborim, E.; Pinto, A.C. 2000. Anti-inflammatory and analgesic activities of extracts from *Himatanthus sucuuba* (Spruce) Woodson. *Planta Med.*, 66:284-286.

Moir, G.F.J. 1959. Ultracentrifugation and staining of *Hevea* latex. *Nature*, 184:1626-1628.

Perdue, G.P.; Blomster, R.N. 1978. South American plants. III: isolation of fulvoplumerin from *Himatanthus* sucuuba (M. Arg.) Woodson (Apocynaceae). *J. Pharm. Sci.*, 67:1322-1323.

Pereira, C.E.B.; Felcman, J. 1998. Correlation between five minerals and healing effect of Brazilian medicinal plants. *Biol. Trace Elem. Res.*, 65:251-259.

Richardson, W.A.; Sacher, A. 1953. Infrared examination of various polyisoprenes. *J. Polym. Sci. Part A: Polym. Chem.*, 10:353-370.

Silva, M.S. 1997. Relação entre os teores de elementos traço e as propriedades terapêuticas de algumas plantas da região amazônica. *Dissertação de mestrado,* – Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, 73 p.

Silva, J.R.A.; Rezende, C.M.; Pinto, A.C.; Pinheiro, M.L.B.; Cordeiro, M.C.; Tamborim, E.; Young, C.M.; Bolzani, V.S. 1998. Ésteres triterpênicos de *Himatanthus sucuuba* (Spruce) Woodson. *Quím. Nova*, 21:702-704.

Tanaka, Y. 1989. Structure and biosynthesis mechanism of natural polyisoprene. *Prog. Polym. Sci.*, 14:339-371.

Tanaka, Y.; Kawahara, S.; Aik-Hwee, E.; Shiba, K.; Ohya, N. 1995. Initiation of biosyntheses in *cis* polyisoprenes. *Phytochemistry*, 39:779-784.

van den Berg, M.A. 1984. Ver-O-Peso: The ethnobotany of an Amazonian market. *In*: Prance, G.T.; Kallunki, J.A. (Eds). Advances in Economic Botany 1, *Ethnobotany in the Neotropics.* New York Botanical Garden, Bronx, New York. pp.140-149.

Vilegas, J.H.Y.; Hachich, E.M.; Garcia, M.; Brasileiro, A.; Carneiro, M.A.G.; Campos, V.L.B. 1992. Antifungal compounds from Apocynaceae species. *Rev. Latinoamer. Quím.,* 23:44-45.

Wood, R.J.; Suter, P.M.; Russel, R.M. 1995. Mineral requirements of elderly people. *Am. J. Clin. Nutr.,* 62:493-505.

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