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Preliminary phytochemical studies of the latex of *Sapium haematospermum* Müll. Arg. used in popular medicine

Erica Mandón[1], Adriana Cortadi[1], Ma. Laura Martinez[1], Nicolás Blanco[2] and Martha Gattuso[1]

[1]Cátedra de Botánica, Área de Biología Vegetal, Departamento de Ciencias Biológicas; [2]Instituto de Biología Molecular y Celular de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas. U.N.R., Suipacha 531 (S 2002 LRK) Rosario, Argentina. e-mail: emandon@fbioyf.unr.edu.ar

INTRODUCTION

Sapium haematospermum (Euphorbiaceae) is popularly known as 'curupí' or 'lecherón' and the latex is commonly used like antiodontalgic, and to cure grains and hurts owing to antimicrobial and anti-inflammatory properties.
Although latex function is ignored, laticifer localization either in the most external stem bark layers or in the external phloem phase suggests a defensive function. On the other hand, there are reports that show latex as a source of enzymes (proteases and glycosidases).
The aims of the present work were to determine localization and type of laticifers, and to analyse the latex of *Sapium haematospermum* in order to validate its vernacular use.

METHODOLOGY

Material fixed in FAA (formaldehyde, alcohol, acetic acid) and included in paraffin was used for anatomical studies. Transverse and longitudinal sections of the shoot organs and flowers were carried out with a type Minot microtome. Stains were safranine-fast green, hematoxylin Harried's eosin, and cresyl violet.
Macerates were performed according to Boodle and Hellram test in order to recognize laticifers (Monacelli, 2005).
Starch was identified by conventional histochemical reactions.
Observations were made under OM (optical microscope) and PL (polarized light). The dimensions of starch grains were estimated with the drawing tube, considering 20 grains in each of the 5 fields selected at random.
To detect proteolytic *in situ* activity native tissue sections were incubated in contact with a coloured film (Denker, 1974).
The latex obtained by stem incisions was collected on 0.1M citric-phosphate buffer pH = 6.9 with EDTA and 5 mM cysteine, a 10% latex dispersion being obtained. It was centrifuged at 10,000 *g* for 30 minutes at 4°C to eliminate cell remains and other insoluble materials, thus obtaining the *raw extract* (RE), in which glycosidase activity was tested using the corresponding *p*-nitrophenyl-sugars. After incubating at 30°C for 30 minutes, the reaction was stopped, and the released *p*-nitrophenol was spectrophotometrically measured.
Electrophoreses in polyacrylamide/gelatin (PAGE/gelatin) were carried out. In this technique proteins were first solved by SDS-PAGE and then electrotransferred to a polyacrylamide gel containing 0.1% w/v gelatin. Proteolytic activity was visualized as clear bands against a blue background after staining with Coomasie Brillant Blue the gel that contained gelatin (Visal-Shah, 2001).
The method of Bradford was used to quantify the content of total proteins.

RESULTS AND DISCUSSION

Histological analysis revealed that non articulated laticifers in Y-form ramified were located in the leaves in subepidermic position, and in the parenchyme surrounding the vascular sheaves.
In stems the laticifers of the primary plant body were distributed in the pith and in the cortical parenchyme, and in the secondary structuration were limited to the internal cortical area.
In latex rod-shaped starch grains were identified in aggreement with those reported for laticifers of some genera of Euphorbiaceae. The dimensions of these grains were similar to those observed in the latex of some species of the genus *Euphorbia* (Rudall, 1987).
In latex manosidase and NacGlucosaminidase activity was observed. Two enzymes that could be involved in the degradation of fungal cell walls

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(Giordani, 2002).

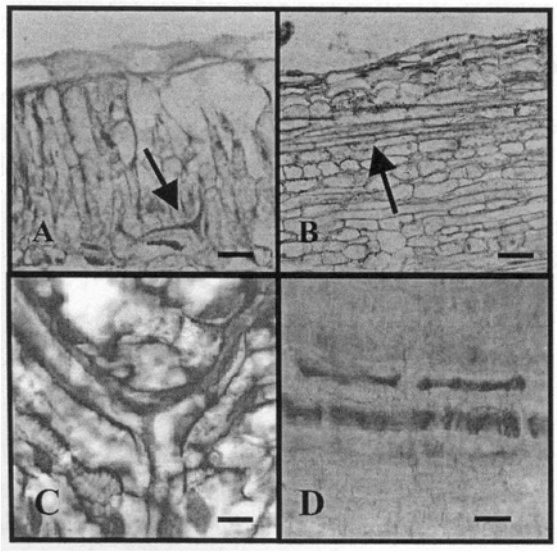

Figure 1. A-D Photomicrographies with OM. **A**: Transverse section of leaf with laticifer Y configuration (arrow). **B**: Longitudinal section of stem with laticifer (arrow). **C**: Transverse section of flower with laticifer Y configuration. **D**: Longitudinal section of stem with Hellram test. Scale = 25 µm: A; 50 µm: B and D; 10 µm: C.

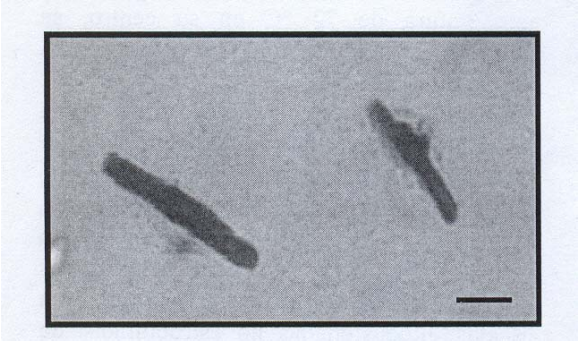

Figure 2. Photomicrography with PL. Starch grains in latex. Scale = 10 µm.

Table 1. Size of starch grains.

	Length (µm)	Width (µm)
Mean	30.7 ± 0.8	7.2 ± 0.2
Standard Deviation	7.7	1.7

Table 2. Glycosidase activity:

Tested enzyme	Specific Activity U/mg
alpha-Manosidase	4.716
NAcGlucosaminidase	2.829

In situ protease detection allowed to observe clear areas in the coloured film. In the electrophoretic RE analysis by SDS-PAGE four major bands of apparent molecular weight of 97, 66, 45 and 40 KDa were detected. Proteolytic activity was evidenced in PAGE/gelatin as clear bands so much under reductive as non-reductive conditions. Bands were also noticed, which demonstrated activity in the presence of PMSF, thus indicating that these enzymes didn't suffer inhibition.

CONCLUSIONS

The parenchyma of all organs showed ramified non articulated laticifers.

Latex showed: rod-shaped starch grains, proteic content, glycosidase and proteolytic activity.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

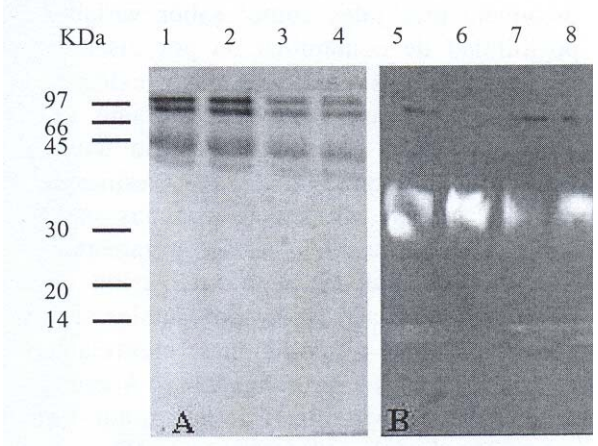

Figure 3. A: SDS-PAGE, **B**: PAGE/Gelatin. Lanes 1 and 5: RE (20 µg total proteins). Lanes 2 and 6: RE + *beta*-mercaptoethanol (20 µg). Lanes 3 and 7: RE + PMSF (15 µg). Lanes 4 and 8: RE + PMSF + *beta*-mercaptoethanol (15 µg). At the left the molecular mass of markers is indicated.

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REFERENCES

Denker H.-W. (1974) Protease substrate film test. *Histochemistry* **38**: 331-338.

Giordani R., Regli P. and Buc J. (2002) Antifungal effect of *Hevea brasiliensis* latex with various fungi. Its synergistic action with amphotericin B against *Candida albicans.* [Antimyzetische Wirkung von *Hevea brasiliensis*-Latex auf verschiedene Pilze und seine synergistische Wirkung mit Amphotericin B auf *Candida albicans*]. *Mycoses* **45**: 476-481.

Monacelli B., Valletta A., Rascio N., Moro I. and Pasqua G. (2005) Laticifers in *Camptotheca acuminata* Decne: distribution and structure. *Protoplasma* **226**: 155-161.

Rudall P. J. (1987) Laticifers in Euphorbiaceae - a conspectus. *Botanical Journal of the Linnean Society* **94**: 143-163.

Visal-Shah S., Vrain T. C., Yelle S., Nguyen-Quoc B. and Michaud D. (2001) An electroblotting, two-step procedure for the detection of proteinases and the study of proteinase/inhibitor complexes in gelatin-containing polyacrylamide gels. *Electrophoresis* **22**: 2646-2652.