

## Cytotoxic-activity of *Sapindus saponaria* L. fruits on Ehrlich Ascitic Tumor cells

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**SUMMARY.** *Sapindus saponaria* L. (Sapindaceae) is a tree of wide distribution in Brazil and its fruits are used by the population against ulcers and inflammations of the skin. The aim of this work was to evaluate the cytotoxic activity of the saponins present in the pulp of the fruits. The pulp of the dry fruits was sequentially extracted by percolation with chloroform, ethyl acetate and ethanol. The dried ethanolic extract was fractionated by column chromatography and one of the obtained fractions was hydrolysed. The obtained fractions, hydrolysed or not, were active against *in vitro* Ehrlich Ascitic Tumour (EAT). Oleanolic acid was isolated from the hydrolysed fraction.

**RESUMEN.** "Actividad citotóxica de los frutos de *Sapindus saponaria* L. sobre las células de tumor ascítico de Ehrlich". *Sapindus saponaria* L., Sapindaceae es una planta arbórea de amplia distribución en el territorio brasileño; sus frutos son usados popularmente para combatir úlceras e inflamaciones de la piel. El presente trabajo tuvo como objetivo evaluar la actividad citotóxica de las saponinas presentes en la pulpa de los frutos. La pulpa de los frutos previamente desecada y molida fue extraída secuencialmente con cloroformo, acetato de etilo y etanol, por el proceso de percolación. El extracto etanólico se evaporó a la presión reducida y el residuo llevado a sequedad fue fraccionado por cromatografía en columna y una de las fracciones obtenidas fue hidrolizada. Las fracciones íntegras y la fracción hidrolizada se probaron *in vitro* contra el Tumor Ascítico de Ehrlich, revelando actividad. Del hidrolizado se aisló el ácido oleanólico.

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### INTRODUCTION

The fruits of *S. saponaria* L., Sapindaceae, popularly known in Brazil as "sabão-de-soldado" (soldier soap) and "saboeiro" (soap-maker), are popularly used as soap, against ulcers, skin lesions and inflammation <sup>1,2</sup>.

The wide occurrence of saponins in nature has evoked considerable interest in their use and considerable data has been accumulated concerning their physiological action and other properties. In general, saponins decrease surface tension and possess emulsifying properties. They tend to alter the permeability of the cell-wall and, therefore, exert general toxicity on all organised tissues <sup>3</sup>.

A significant antimitotic activity has been observed in the glycosides and their magnesium

salts isolated from *Hedera helix*. These substances inhibit the growth of cells without destroying them and can be used for inhibiting the growth of benign or malignant tumors <sup>4</sup>.

Qin *et al.* <sup>5</sup> have studied the saponins of *Aster lingulatus* and isolated two new olean-type triterpenoid saponins, which showed inhibitory activity on DNA of human leukaemia HL-60 cells.

Some plants contain large quantities of triterpenes and the physiological function of these compounds is generally believed to be a chemical defense against pathogens and herbivores. It is expected, therefore, that triterpenes should act against certain pathogens causing human and animal diseases <sup>6</sup>.

**KEYWORDS:** Cytotoxic activity, Ehrlich Ascitic Tumor, Sapindaceae, *Sapindus saponaria* L.

**PALABRAS CLAVE:** Actividad citotóxica, Ehrlich Ascitic Tumour, Sapindaceae, *Sapindus saponaria* L.

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## MATERIALS AND METHODS

### Plant material

*S. saponaria* fruits were collected in the city of São Paulo, Brazil, and identified at the Department of Botany of the Institute of Biosciences of the University of São Paulo - São Paulo, Brazil. An exsiccate was deposited at the herbarium of the same Institute under the number SPF 77166.

### Isolation

Extracts were prepared with ground fruits previously dried. The solvents used at the percolation process were: ethanol 70% (fruit extract) and only ethanol 96 °C (ethanolic fruit extract). The ethanolic fruit extract (~20.0 g) was fractionated by column chromatography, with silica-gel 60 and eluted with chloroform (650 ml), chloroform/ethylacetate 1:1 (500 ml), ethylacetate (500 ml), ethylacetate/ethanol 1:1 (500 ml), ethanol (500ml), ethanol/water 30% (500 ml) and water (500 ml). The fraction A, obtained from the ethanolic fraction and the fractions A1, A2 and A3, obtained from fraction A by column chromatography with silica gel 60 and eluted with ethylacetate/methanol, were evaluated against Ehrlich tumor cells at the same occurrent with the fruit extract and the ethanolic fruit extract <sup>7</sup>.

### Hydrolysis

Fraction A was hydrolysed with hydrochloric acid, under reflux; the product of hydrolysis was fractionated by silica-gel 60 (70-230 mesh) column chromatography with a gradient of chloroform/acetone. A pure substance (AH36) was obtained. The structure was determined by spec-

troscopic methods <sup>8,9</sup>: IR (Jasco IR700 Spectrophotometer), <sup>1</sup>H MNR (200MHz Bruker Spectrophotometer), <sup>13</sup>C MNR (Varian FT - 80 Spectrometer), MS (Shimadzu CGMS QP 2000A) and the melting point determined by an MQAPP - 301 equipment (Microquímica Ind. Com. Ltda.).

### Animals

Male Swiss Albino mice, weighing from 25 to 35 g, were used in the experiments.

### Maintenance and preparation of the tumor cells for assay

Mice were inoculated with Ehrlich Tumor cells. 10 days after the inoculation, about 3.0 ml of ascitic fluid were intraperitoneally collected from mice, and diluted in HBSS (Hank's balanced salt solution); the viability was verified by exclusion test with 0.1% Tripan Blue solution <sup>10</sup>.

Cells were counted in a Neubauer hemocytometer. The cells that acquired a blue colour with Tripan Blue were counted as not viable <sup>11</sup>.

Washed cells were obtained by washing and centrifuging the ascitic fluid at 1000 rpm during 10 min. Cytotoxicity of washed and unwashed Ehrlich Ascitic Tumor cells was evaluated by incubation with samples (fruit extract; ethanolic fruit extract; fraction A; fraction A1, fraction A2 and fraction A3) at 37 °C for 1 h and compared with a 25% DMSO control solution <sup>11</sup>.

## RESULTS

The extracts and fractions that demonstrated significant activity against washed and unwashed EAT cells are shown in Table 1.

The spectral data of the substance AH36 are identical to that of the oleanolic acid.

Samples	concentration (mg/mL)	% viability	
		Washed cells	Unwashed cells
Fruit extract	0.3	0	0
Ethanolic fruit extract	3.0	0	0
Fraction A	0.5	5.0	5.0
	1.0	0	0
Fraction A1	0.5	0	11.0
	1.0	0	3.7
Fraction A2	0.5	0	91.0
	1.0	0	0
Fraction A3	0.5	0	95.0
	1.0	0	95.0
Control	DMSO 25%	96,7	97,0

**Table 1.** Viability of washed and unwashed cells of EAT, submitted to *S. saponaria* L. fruit extracts and fractions.

Oleanolic acid: **3-Hydroxyolean-12-en-28-oic acid**; mp.: 246-248 °C; **I<sub>max</sub> (KBr) cm<sup>-1</sup>**: 3400, 1680; **MS m/z(%)**: 456 (m+;1.4), 438 (2.9), 410 (1.6), 300 (2.0), 255 (2.0), 248 (100.0), 203 (87.2), 133 (20.8), 69 (30.2), 55 (32.6), 43 (47.4); **<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 200MHz)**: 0.76 (s,Me); 0.77(s,Me); 0.90(s,Me); 0.91(s,Me); 0.93(s,Me); 0.99(s,Me); 1.14(s,Me); 5.26(m;H-12); **<sup>13</sup>C-NMR (CDCl<sub>3</sub>; 200MHz)**: 15.3(C-25), 15.6(C-24), 17.1(C-26), 18.3(C-6), 23.4(C-16), 23.6(C-11), 27.2(C-27), 27.7(C-2), 28.1(C-15), 29.7(C-23), 30.7(C-20), 32.5(C-7), 32.7(C-22), 33.1(C-29), 33.8(C-21), 37.1(C-10), 38.5(C-1), 38.8(C-4), 39.7(C-8), 41.1(C-18), 41.7(C-14), 45.9(C-19), 46.5(C-17), 47.7(C-9), 55.3(C-5), 79.1(C-3), 122.8(C-12), 143.8(C-13), 181.3(C-28).

## DISCUSSION AND CONCLUSIONS

The cytotoxic activity of *Sapindus saponaria* was evaluated using the Ehrlich Ascitic Tumor cells, a neoplasm derived from mammary adenocarcinoma of mice and transformed into the ascitic form.

In the present assay, cytotoxic activity showed significant difference between washed and unwashed cells, where ascitic fluid was removed. During the incubation period cells degenerated, acquiring an aspect of cellular disintegration very similar to that observed in hae-

molysis, a characteristic attributed of saponins.

This fact suggests that ascitic fluid contains substances that modify the action of the studied fractions. This may occur by physical-chemical mechanisms, such as the difficulty of active substances contacting the cell, or as a result of chemical reactions between substances of the ascitic fluid and the samples.

Tokuda *et al.*<sup>12</sup> demonstrated the cytotoxic activity of the oleanolic acid in the inhibition of the growth of skin tumors of rats.

The mechanism of action of the saponins of *S. mukorossi* L. fruits was proposed by Quetin-Leclerq<sup>13</sup>, as related to the activity directly to the ribosomic one and to the inhibition of elongated factors EF1 and EF2 in the protein synthesis.

These results open new perspectives for the use of *S. saponaria* L. saponins as antineoplastic agents, however for the employment of such substances more studies in experimental models *in vivo* are necessary. Absorption, distribution, metabolism, excretion and toxicology of this saponins have to be studied.

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