

60 utilizando-se um gradiente de hexano-CH<sub>2</sub>Cl<sub>2</sub>. As frações 1.7 e 1.8 foram recristalizadas de hexano.

A fração 5 (4,1 g) sofreu uma extração ácido-básica. Uma alíquota de 3,5 g desta fração foi acidificada com HCl (1N), o precipitado formado foi filtrado e a fase aquosa extraída com CHCl<sub>3</sub>.

O caule subterrâneo seco e moído (18,4 g) foi submetido a 3 extrações com CO<sub>2</sub> supercrítico, em uma unidade piloto da Autoclave Engineers, extrator com capacidade de 75 ml, pressão máxima de trabalho de 10x10<sup>3</sup> psi a 238 °C nas condições de operação especificadas na tabela 2.

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## ***Physalis angulata* L. antineoplastic activity, in vitro, evaluation from its stems and fruit capsules**

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### Abstract

*Physalis angulata* L. (genus *Physalis*; family Solanaceae) is an herbaceous specimen that grows plentifully at North, Northeast and Middle East Brazilian's regions<sup>1</sup>. Its fruits are edible, roots and epigeal parts are taken as tea or infusion, all through the world as traditional medicine. Despite of this usefulness not much scientific work has been done on it. This research carried out with plant material (stems and fruit capsules) has the main aim to find out anti-neoplastic activity. The obtained results are described in Table 1. The most significant inhibition values are those for fruit capsules fractions such as 97% mouse lymphoma; 93% Erlich carcinoma strains when was assayed with MGTS-1-2ai and MGTS-1-1ai respectively. In the course on going studies on the biological response and chemical constituents of *P. angulata* some fractions were obtained from stems and fruit capsules ethanolic and methanolic extracts. The extract prepared from roots of *P. angulata* is the most clinically used by physicians for treatment of human hepatic disorders, despite the substance responsible for the efficacy still a matter of argument.

*Physalis* species have been reported as a source of steroids derivatives<sup>2</sup> such as withasteroids comprising: withanolides, ixocarpactones, withaphysalins, acnistins, perulactones, and physalins. Steroid derivatives have a broad spectrum biological activities such as anti-inflammatory, antiviral, antimicrobial, immunostimulant, trypanocidal that could be justified by the large structural diversification of this class of compounds.

Withangulatin A<sup>3,4</sup> and physalins F, D, B have been described as interfering on antitumor responses<sup>5</sup>.

Physalin F has been mentioned as an antitumor agent

showing activity against five human cancer cell lines including anti hepatoma H A 22 T as the most potent one's.

Looking for antitumor substances from plants, our group is researching for different cell lines responses working with several sections of *P. angulata*.

Fractions (IT<sub>1</sub>) and (IT<sub>2</sub>) came from modified Mabry technique<sup>6</sup> over stems extracts and contain a pool of physalins. (B, D, F, L -H and G)<sup>7-10</sup>. While MGTS -1-1ai and MGTS -1-2ai, were obtained from fruit capsules, and they are originated from the polar methanolic fractions partition of hexane and chloroform extractions.

The high percentual inhibition of proliferation cells *in vitro* experiments express the significance of those results on cancer research, deserving better studies *in vivo* laboratory assays.

**Table 1.** Percentual values of *Physalis angulata* L. fractions. ICP - Induced cell's proliferation

Fractions \ Strains	Strains						
	MK2	SP 20	Neuro 2A	P 3653	Erlich	J 774	BW
IT-1	44%	63%	5%	72%	67%	ICP*	59%
IT-2	56%	74%	79%	69%	66%	ICP*	57%
MGTS-1-ai	87%	92%	ICP*	87%	93%	61%	97%
MGTS-2-ai	53%	89%	38%	92%	63%	79%	87%

## Material and Methods

Stems and fruits of *P. angulata* L., were collected near Belém, Pará state, Brazil in 1995. The plant material was identified by Dra Lúcia de Freire de Carvalho, from Botanical Garden of Rio de Janeiro, Brazil. Voucher specimens are deposited at Herbarium of Biology Institute of Federal University at Rio de Janeiro under numbers RFA 23907 and RFA 23908.

**Chemical procedure:** The stems ethanolic extract (15 g) after been dried was dissolved with 300 ml of methanol. To this solute was added a lead acetate's solution (25 g of PbAcO<sub>2</sub> in 200 ml of hot distilled water). After two hours contact 20 g of carbon was poured into the flask under continuous stirring. The material was then filtrated and partitioned with chloroform (3x100 ml) using a separatory funnel. The chloroform phase gave (IT<sub>1</sub>) and the methanolic phase (IT<sub>2</sub>) were dried under reduce pressure giving 3,03 g of IT<sub>1</sub> and 0,470 g of IT<sub>2</sub> which have been assayed for anti tumor activity.

Dried and ground fruit capsules (100 g), was extracted with five liters of hexane, at room temperature, the solute was evaporated under pressure giving 3,0 g of green gummy material. The residue was extracted with chloroform (5 liters) the resultant solution, after been dried, weight 3,50 g. The hexanic and chloroformic extracts were partitioned with methanol: water (5:4:1) and the methanolic fractions were submitted to biological assays, for antineoplastic activities, after being dried yielding

0,600 g of TMGTS -1-1ai and 0,300 g of MGTS -1-2ai.

**Determination of haemolytic activity:** Sheep blood cells were washed and diluted (25%) in Hauk's balanced salt solution. The suspension (100 ml) was mixed with 100 ml of saline (negative control). A total of 100 ml of water or aqueous soap (positive control) or 250 mg/2 X10<sup>5</sup> of fractions (IT<sub>1</sub>, IT<sub>2</sub>, MGTS-1-1ai e MGTS -1-2ai) were added in to the well. Deposition of red cells after 4 and 24 h was indicative of absence of haemolytic activity.

**In vitro proliferation assay:** The cell lines used were J774 (mouse monocytic cell line), SP 2/0 (mouse myeloma), Eilich carcinoma (sarcoma induced by methyl chlolanthrene), P3653 (mouse plasmocytoma), Neuro -2 a (mouse neuroblastoma), MK<sub>2</sub> (monkey epithelial cells) and BW (mouse lymphoma). They were all cultured in complete RPMI 1640 (Sigma) medium supplemented with 10% FCS, gentamicin, pyruvate, monessential amino acids, 2 mercaptoethanol and a - glutaminic in 96 - well flat bottomed plates, at 37 °C in 5% CO<sub>2</sub>.

After two hours pre-incubation, 5X10<sup>5</sup> cells per well were treated with samples (IT<sub>1</sub>; IF<sub>2</sub>; MGTS -1 - 1- ai and MGTS -1 - 2ai) at 2,5 mg/ml in 0,2 ml - Then the cells were placed in MTT (3 - (4,5 - dimethyl thiazol - 2 yl) diphenyl tetrazolium bromide) soln. At an end concentration of 1mg/ml for 48 h and further incubated for 4 h. The supernatants were removed and 100 ml of DMSO were added to all wells and the optical density measured on a Microelisa plate reader, using a reference wavelength of 630 nm and a test wavelength of 490 mn. Data are represented as mean ± SEM of triplicate cultures.

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## Estudo botânico, fitoquímico e avaliação da atividade antimicrobiana de *Rubus rosaefolius* Sm. - Rosaceae

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### Abstract

*Rubus rosaefolius* Sm., Rosaceae, is a shrub with compound leaves, recurvate prickles, white flowers and aggregate fruit, popularly known as sylvan strawberry. The present research concerns its botany (macroscopic and microscopic studies), phytochemistry and antimicrobial properties. The presence of antraquinones, saponins, flavonoids, alkaloids and tannins on stem, root and leaves, were confirmed by specific phytochemical tests. Antimicrobial activity of aqueous and hydroalcoholic fractions were tested against *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*. The hydroalcoholic fraction revealed antimicrobial activity against all species tested and the aqueous fraction inhibited the growth of *S. aureus* and *C. albicans*.

*Rubus rosaefolius* Smith (syn. *Rubus rosifolius* Smith<sup>7</sup>) é um arbusto escandente, com poucos acúleos desenvolvidos, frutos isolados, agregados, ocos, rubros na maturidade; cada frutículo drupáceo contém uma semente. As flores axilares e terminais, isoladas, brancas, pentâmeras, com simetria actinomorfa, apresentam cálice gamossépalo e corola dialipétala, com o pedúnculo floral cônico e protuberante, contendo um gineceu apocárpico dialicarpelar. O caule é ramificado, cilíndrico, de cor verde. A raiz primária é pivotante. As folhas são compostas, alternas, imparipenadas, com 3 a 7 folíolos, e estípulas. O limbo dos folíolos é membranáceo, ovado-oblongo, ápice acuminado, base arredondada, áspera ao tato e pubescente à visão. As margens são duplamente denteadas, com nervuras pinadas<sup>8</sup>. O folíolo não apresenta regiões de transparência, à vista desarmada. Acúleos recurvados estão presentes em toda a parte aérea da planta, sendo mais freqüentes nos caules e pecíolos. A espécie floresce o ano todo.