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CYTOTOXIC AND ANTITUMOR ACTIVITIES OF *HYPTIS PECTINATA* (SAMBACAITÁ) EXTRACT

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Summary

Plant extracts and derived active principles have served as a major source for new pharmaceuticals for treatment of malignant tumors. Sambacaitá is a plant popularly utilized in the treatment of cancer and other diseases. The chemical studies of this plant extracts showed the presence of monoterpenes (especially cymene, thymol and β-terpinene) in a greater proportion in the essential oil and of a 2(5H)-furanone and α-pyrones in a dichloromethane extract obtained from this plant. In view of the popular utilization of this plant in cancer treatment, we decided to perform an evaluation of its cytotoxic and antitumor properties. The plant was collected, identified, and the hydroethanolic extract was prepared. In the pharmacological approach, the follow assays were done: toxicity against brine shrimp *Artemia salina,* in vitro test against human tumor cell lines and in vivo evaluation using sarcoma 180 in mice. The phytochemical screening showed the presence of flavonoids, tanins, saponins and sterols. In the *Artemia salina* essay *Hyptis pectinata* was considered atoxic. In the preliminary in vitro assay *Hyptis pectinata* showed moderate cytotoxicity: 62% of inhibition against HCT-8line cells, presented no hemolytic effect and in the evaluation of the cytotoxicity activities against peripheral blood mononuclear cells, showednon-toxicity. In the *in vivo*test, *Hyptis pectinata* inhibited the sarcoma 180 (70.5%). The histopathological analysis of the liver, kidneys and spleen of animals which was treated showed absence of lesions. Taken together these results suggest that *Hyptis pectinata* used by the population showed potential anti-tumor activity, at least at the present*in vitro* and *in vivo* pharmacological models.

Keywords: *Hyptis pectinata* (L.) Poit (Lamiaceae), cancer, cytotoxicity

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Introduction

The use of medicinal plants has a long history in many countries. Medicinal plants continue to be useful tools for the treatment of diseases, especially in communities within developing countries. For instance, Brazil is one of the most promising sites for the discovery of novel, biologically active substances from its flora [1], [2].

Of the 252 drugs that are considered basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin, and a significant number of synthetic drugs are obtained from natural precursors [3].

In the treatment of cancers, the discovery of anti-infection compounds from natural products has had a major impact as templates or direct drugs [4].

Among the 92 drugs that were commercially available in the United States prior to 1983 or approved worldwide between 1983 and 1994 for the treatment of cancer, approximately 62% are related to a product of natural origin. This statistic excludes those drugs that were isolated from animal sources, such as interferon and recombinant cytokines [5].

With regards tothe recent efforts of public health-care organizations to encourage the use of the large body of popular knowledge in Brazil, this work seeks to explore the use of plants as a complementary treatment in patients undergoing chemotherapy. This work also aims to initiate the use of pharmacological models to test the anticancer activity of such plants.

Methods

Plant material

Specimen was collected in Maceió-AL, Brazil in 2006 and identified by professor Flavia B. P. Moura.

The specimen voucher was deposited at the Museu de História Natural/University Federal of Alagoas (Maceió-AL, Brazil): *Hyptis pectinata* (MUFAL 4071).

Cell lines, chemicals and biochemicals

MDA-MB435 (human breast cancer), HCT-8 (human colon cancer), SF-295 (human glioblastoma) and HL-60 (leukemia cancer) were obtained from the National Institute of Cancer, USA. Sarcoma 180 from the Departmentof Antibiotics at the Federal University of Pernambuco. Cremophor® (purity > 99%), Triton X-100 (purity > 99%) and Doxorubicin (purity > 98%) were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Thymol (purity > 99%) was purchased from Henrifarma Produtos Químicos e Farmacêuticos Ltda, São Paulo, Brazil. DMSO (purity > 99%) and Ethanol P.A. was purchased from VETEC Química Fina, Rio de Janeiro, Brazil.

Extraction

Dried powdered plant material (100.0 g) whole plant of *H. pectinata* was extracted using a percolator apparatus with ethanol 90%, for 72 h. The extract was concentrated to dryness under reduced pressure and the dried extract was re-dissolved in DMSO or water to form crude extract of known concentration. After obtaining the extract, it was sterile filtered using 0.22 µm pore size filter.

Cytotoxicity on tumoral cell lines

The cytotoxic activity of *H. pectinata* was tested against several tumor cell lines: MDA-MB435 (human breast cancer), HCT-8 (human colon cancer), SF-295 (human glioblastoma) and HL-60 (leukemia). For all experiments, the cells were plated in 96-well plates at 0.1 x 10^6 cells/well for MDA/MB-435 and SF-295 and 0.7 x 10^5 cells/well for HCT-8. After 24 h, the extract from the process described above (50 µg/mL) was dissolved in DMSO (1%), added to each well, and incubated for 3 days (72 h). Control groups received an equivalent amount of DMSO. Doxorubicin (0.3 µg/mL) was used as the positive control. Data are presented as IC_{50} values and 95% confidence intervals obtained by non-linear regression. The experiment was performed in triplicate. The general viability of the cultu-

red cells was determined according to Mosmann [6].

Lytic activity on mouse erythrocytes

The hemolytic activity in the erythrocytes of mice (*Mus musculus* Swiss) was analyzed in a 96-well plate. The blood from the mice was collected by cardiac puncture of anesthetized, healthy mice. The erythrocytes were washed with a saline solution (NaCl 0.85% $CaCl_2^+$10 mM) by centrifugation (3000 rpm/5min) and the supernatant was discarded and resuspended in saline to obtain a 2% suspension of erythrocytes. Each well received 100 µL of 0.85% NaCl solution containing 10 mmol of $CaCl_2$. The first well was the negative control that contained only the vehicle (distilled water or DMSO 10%). The compound was tested at concentrations ranging from 3.9 to 200 µg/mL. Triton X-100 (0.1%) was used to obtain 100% hemolysis (positive control). Each well received 100 µL of the 2% suspension of mouse erythrocytes. After incubation at room temperature (26 ± 2°C) for 60 min and centrifugation, the supernatant was removed and the liberated hemoglobin was measured spectroscopically at 540 nm.The 50% effective concentration (EC_{50}) and its 95% confidence interval were determined by non-linear regression using the program SigmaPlot version 11.0. Extract with EC_{50} value < 200 µg/mL was considered active.

Assays of antitumor activities *in vivo*

Experimental protocols and procedures were performed in accordance with the principles of laboratory animal care and were approved by the ethical committee at the Federal University of Alagoas (CEP/UFAL), process number 014868/2006-31. The antitumor effects on sarcoma 180 were observed in young Swiss albino mice that weighed 25 g each, were housed under controlled temperature conditions (21 ± 1°C) and exposed to a daily 12-h light-dark cycle. The test was performed by observing the effect on the growth of the tumor in the ascite form at a dose of 0.20 mL (about $3x10^6$ cells) that were implanted subcutaneously in the right groin [7]. The tumor ascite was always 7–10 days old. At 24 h after the tumor implantation, chemotherapy was initiated with the extract that was dissolved in sterile distilled water and was injected intraperitoneally in daily doses of 100 mg/kg for 7 days. The selected dose was based on a previous acute toxicity test [8]. The experiments were performed with groups of six animals. The mice were fed a standard vivarium diet and water *ad libitum*. After 7 days of chemotherapy administration, the animals were sacrificed with a fatal dose of Xylazine, and the livers, spleens and kidneys were dissected and fixed for histopathological analyses. The tumor was removed and weighed to evaluate the tumor inhibition. The same procedure was adopted for the control group of animals. The tumor inhibition was calculated according to [9]. Data are given as means ± S.E.M. The IC_{50} or EC_{50} values and their 95% confidence intervals (95% CI) were obtained by non-linear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA). The differences between the experimental groups were compared by Student's t test. The significance level was $p < 0.05$.

Results and Discussion

The extract was classified as possessing no activity (blank space), low activity (up to 50% growth inhibition), moderate activity (between 51% and 75% growth inhibition) and high activity (over 75% growth inhibition) for each cell strain tested [10]. Although the 61% inhibition obtained using *H. pectinata* was not an acceptable value, this extract demonstrated specificity to the colon (HCT-8) tumor cell line (**Table 1**). Pereda-Miranda [11] reported nonspecific cytotoxicity using methanol extracts of several species of *Hyptis* against a panel of cell lines consisting of various types of human cancer cells (breast, colon, fibrosarcoma, lung, melanoma, KB and KB-VI, and P-388).

see Table 1.

The data presented in **Table 2** demonstrate that the SF-295 and MDA-MB-435 cell lines were the most sensitive to inhibition for the tested extract. *H. pectinata* showed toxicity to PBMCs and no selectivity to any tumor cell line.

see Table 2.

Pereda-Miranda [11] reported significant cytotoxic activity (ED_{50} <4 µg/mL) from the pectinolides of *H. pectinata* against several cancer cell lines.

Lytic activity on mouse erythrocytes

None of the extracts examined in this study was hemolytic, even at the highest tested concentration (1000 µg/mL). This result suggests that the mechanism of cytotoxicity is not a result of membrane damage.

Assay of antitumor activityin vivo

The *H. Pectinata* extracts showed inhibition of sarcoma 180 (70.5%) at dose of 100 mg/kg. The activity of *H. pectinata* toward sarcoma 180 appears to be a novel finding because in this genus, as only *H. fruticosa* and not *H. pectinata* has reported antitumor activity [12, 13]. The histopathological analysis of the liver, kidneys and spleen of the animals treated in this study showed an absence of lesions.

Conclusions

We conclude that *Hyptis pectinata* extract showed anticancer activity in the in vitro and in vivo-pharmacological models used. This work provides preliminary data for investigations into the popular use of medicinal plants, which are cited by the population as having anticancer properties. Further studies are required to confirm or refute their effectiveness and to provide support for the rational use of medicinal plants.

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Plant	SF295	HCT-8	MDA-MB435
Hyptis pectinata	49.56 ± 8.15	61.57 ± 1.19	49.59 ± 1.56
Doxorubicin	87.38 ± 5.50	97.33 ± 3.78	99.88 ±1.34

Table 1. Percent inhibition of cells grown (IC%) on three tumor cell lines at a concentration of 50 µg/mL.

Plant	**HL-60**	**HCT-8**	**SF-295**	**MDA-MB-435**	**PBMC**
Hyptis	41.75	26.92	25.56	> 50	>50
pectinata	(38.04 – 45.82)	(18.83 – 38.49)	(17.55 – 37.23)		
Doxorubicin	0.04	0.02	0.48	0.96	0.96
	(0.03-0.05)	(0.02-0.03)	(0.34-0.72)	(0.68-1.32)	(0.51-1.71)

Table 2. Cytotoxic activity of *Hyptis pectinata* extracton tumoral cell lines and PBMC.