

Dataset Paper

Antitumor Activity of Leaves from *Hyptis mutabilis* (A. Rich.) Briq. (Lamiaceae) in Mice Bearing Tumor

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The *Hyptis* genus has more than 400 species, many of them being used in folk medicine to treat several conditions. Some anticancer compounds have been isolated from plants of this genus, and for that reason we decided to investigate the potential in vivo antitumor activity of extracts of leaves of *Hyptis mutabilis* with different polarities (hexane, methanol, water, and hot water) against two mice tumors: sarcoma 180 and Ehrlich solid tumor. Phytochemical analysis revealed strong presence of steroids, saponins, flavonoids (mainly dihydroflavanols), and catechins. Acute toxicity was performed according to the up-and-down method showing LD₅₀ values ranging from 100 up to 2500 mg/kg. Antitumor activity was investigated using 10% of the LD₅₀ for each extract. Methotrexate was used as positive control. Both aqueous extracts showed strong inhibition of tumor growth with values up to 70% of inhibition growth for sarcoma 180. Ehrlich solid tumor was only slight inhibited by hexane extract (38.6%). In conclusion, the aqueous extracts of *H. mutabilis* showed promising results against sarcoma 180 mice tumor.

1. Introduction

The genus *Hyptis* (Lamiaceae) has more than 400 species distributed in tropical regions of Americas and Africa [1–3]. Several species of this genus are aromatic and used in folk medicine to treat digestive disorders, pain, gastrointestinal problems, and skin infections [4]. Many pharmacological properties of the genus *Hyptis* have been reported: that is, antimicrobial [5, 6], antiulcer and gastroprotective [7], larvicidal [8], antidepressant [9], and anti-inflammatory and antinociceptive [10, 11].

From the phytochemical point of view, the genus has been extensively studied: terpenoids, including monoterpenes and sesquiterpenes present in essential oil, flavonoids, lactones, lignans, and alkaloids were isolated and identified [12]. The anticancer agents 3'-demethyldesoxypodophyllotoxin and betulinic acid were isolated from plants of this genus [13]. Based on these findings, we evaluated the antitumor activity of *Hyptis mutabilis* (A. Rich.) Briq. against murine tumors in these work.

2. Methodology

Male Swiss mice (20–25 g) were obtained from the animal facilities of the Department of Antibiotics of the Federal University of Pernambuco. All animals were kept under standard conditions, with temperature at 25 ± 2°C, 12 h light/dark cycle, and relative humidity, with food and water *ad libitum*. Experiments were carried out in accordance with the ethical principles of The Brazilian College of Animal Experiments and the National Institute of Health Guide for Care and Use of Laboratory Animals. This study was previously approved by the Committee of Experimental Animals of the Federal University of Pernambuco (004788/2005-68).

Plant material and extract were as follows. Leaves from *H. mutabilis* were collected in Aldeia (Camaragibe-PE). Botanical identification was made by Professor Dr. Alda Andrade Chiappeta of the Department of Antibiotics, Federal University of Pernambuco. Voucher specimen was deposited at the Herbarium of the Department of Antibiotics (DAUFPE 6256). Leaves were dried in open air at room temperature

($28 \pm 2^\circ\text{C}$) and crushed with an electric grinder. Powdered leaves (940 g) were subjected to serial extractions with increasing polarity solvents: hexane, methanol, water, and hot water. The sample was extracted three times with each solvent (3 hours each under stirring), filtered, and subjected to the next extraction. Hexane and methanol extracts were evaporated under low pressure at constant temperature of 45°C , yielding 17.64 g (1.88%) and 61.42 g (6.53%), respectively. Water extracts were lyophilized yielding 44.01 g (4.68%) for water and 22.00 g (2.34%) for hot water.

For the phytochemical analysis we performed phytochemical screening according to Matos [14] and Sena Filho et al. [15], based on chemical tests, to detect the presence of common secondary metabolite classes, mainly alkaloids, terpenes, and phenolic compounds, in hexane and methanolic extracts, using classical specific colour reactions.

Acute toxicity was evaluated according to the up-and-down method to determine LD_{50} values using fewer animals as possible (described in OECD 425).

Antitumor activity against Ehrlich solid tumor and sarcoma 180 was investigated. Ehrlich ascite carcinoma and sarcoma 180 were maintained in vivo in mice by transplantation of 0.2 mL of ascitic fluid (1×10^7 cells) from the infected mice to the noninfected mice [16]. The Ehrlich ascites or sarcoma 180 ascites (S180) tumor cells (1×10^7 cells/mouse) were subcutaneously implanted into the inguinal region of the mice as described by Sugiura and Stock [17]. Mice were divided into the respective groups and the treatment started after 24 hours of tumor cells' implantation.

For the treatment of each tumor group, animals ($n = 6$) were divided in six groups: negative control (received 1% DMSO dissolved in saline solution 0.9%, i.p.), positive control (received 10 mg/kg of methotrexate, i.p.), and four groups treated with *H. mutabilis* extracts (10% of LD_{50} found for each extract). Hexane extract group were treated with 250 mg/kg (i.p.), methanol extract group received 230 mg/kg (i.p.), and water and hot water extracts received 150 mg/kg and 10 mg/kg, respectively, both intraperitoneally to avoid food-extract interactions. Animals were treated once a day during seven days. On the eighth day, mice were weighed and killed and the tumors were removed. The inhibition of tumor growth was calculated by percentage of reduction of the tumors when compared with the negative control group.

3. Dataset Description

The dataset associated with this Dataset Paper consists of 3 items which are described as follows.

Dataset Item 1 (Table). Phytochemical screening of methanolic and hexanic extracts of *H. mutabilis* leaves revealed the presence mainly of steroids, saponins, flavonoids (mainly dihydroflavonols), and catechins. In the table, “+” means weak; “++”, medium; “+++”, strong; and “0”, absent. These results are similar to those found for other *Hyptis* species [12].

Column 1: Secondary Metabolites Classes

Column 2: Methanolic Extracts

Column 3: Hexanic Extracts

Dataset Item 2 (Table). LD_{50} values obtained for *H. mutabilis* extracts according to OECD 425 protocol. Single-dose acute toxicity tested showed values of LD_{50} ranging from 100 mg/kg to 2500 mg/kg. The dose used to test the antitumor activity of the extracts was 10% of the LD_{50} ; at these doses, no histological changes were observed in mice treated for seven days. So, these doses were considered safe for use in the following in vivo experiments [18].

Column 1: Extract

Column 2: LD_{50} (mg kg^{-1})

Dataset Item 3 (Table). In vivo growth inhibition percentual of sarcoma 180 (S180) and Ehrlich solid tumor (EST) of *H. mutabilis* extracts. Significant reduction of sarcoma 180 tumor mass was obtained with water extracts (72.4 and 74.3% of inhibition of tumor growth for water and hot water extracts, resp.) after 7 days of treatment. Methotrexate was used as standard drug, used as positive control, and completely inhibited sarcoma 180 tumor growth. Hexane and methanol extracts just slightly inhibit sarcoma 180 growth. Ehrlich solid tumor was only inhibited by hexane extract (38.6% of inhibition), while methotrexate inhibited 98.2% of tumor growth. Data were expressed as mean \pm S.E.M. No difference in the weight of the animals was observed among the tested groups. These results were obtained after 7 days of treatment, during which the animals were monitored for the appearance of adverse reactions, common to major commercial anticancer drugs. In the mice treated with *H. mutabilis* extracts, no strong adverse effects were observed, while in methotrexate groups, animals presented behavioral changes and enterocolitis. In the table, “-” indicates no inhibition.

Column 1: Group

Column 2: Dose, i.p. (mg kg^{-1})

Column 3: S180 Inhibition (%)

Column 4: EST Inhibition (%)

4. Concluding Remarks

Plants are rich source of antitumor agents and many of the antitumor drugs in the market are derived from them [19–21]. Both water and hot water extracts of *H. mutabilis* showed promising antitumor activity against sarcoma 180 murine tumor. These data point to the presence of polar compounds present in these extracts that are probably responsible for this activity. As no adverse effect or any histopathological changes were observed during the 7 days of treatment, these data will serve to put forward other research groups to make a phytochemistry approach to isolate polar compounds in others to characterize the polar active compounds present

in this plant. Hexane extract showed a slight inhibition of Ehrlich solid tumor probably due to nonpolar compounds that were not tested in phytochemical screening, such as carotenoids and fatty acids.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at <http://dx.doi.org/10.7167/2013/169357/dataset>.

Conflict of Interests

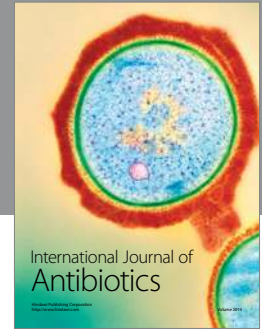
The authors have no conflict of interests to disclose.

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