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ANTI-INFLAMMATORY ACTIVITY OF THE APOLAR EXTRACT FROM THE SEAWEED *Galaxaura marginata* (RHODOPHYTA, NEMALIALES)

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ABSTRACT: The red seaweed *Galaxaura marginata* (Ellis & Solander) Lamouroux, well known by the antibacterial activity of its polar extract and the cytotoxic activity of its oxygenated desmosterol, showed anti-inflammatory action in its apolar fraction. Topical anti-inflammatory activity was observed in samples collected at São Sebastião channel, northern littoral of São Paulo State, Brazil. The apolar extract and its fractions obtained through Thin-Layer Chromatography (TLC) reduced the topical inflammation produced by croton oil in mouse ear. Such data indicated that the apolar extract from the marine red alga *G. marginata* displayed anti-inflammatory activity (since 1mg/ear extract reduced 95±0.5% inflammation), which could be the result of the synergic activity of the four fractions present in the apolar extract.

KEY WORDS: seaweed, *Galaxaura marginata*, inflammation, *Croton* oil, edema, anti-inflammatory agents.

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In the last decades, seaweed metabolites presenting biological activities have been increasingly discovered. Such compounds have shown antibacterial, cytotoxic and anticoagulant activities; capability of agglutinating red blood cells and stimulating cell migration; anticancer properties; effects on the immune response; and anti-inflammatory activity (10). The latter has indicated those metabolites as a viable alternative to replace traditional drugs. Also, 6-n-tridecylsalicylic acid, isolated from *Caulocystis cephalornithos*, is an active anti-inflammatory agent in both acute and chronic animal models of inflammation and is chemically similar to the salicylic acid but less ulcerogenic – a common side effect of salicylic acid (2).

Acute and chronic inflammations are complex processes that can be induced by several means, and anti-inflammatory agents exert their effects through different modes of action (1, 4).

For the screening of new anti-inflammatory compounds, the croton oil-induced mouse ear edema test is widely used together with the *in vitro* phospholipase A_2 assay (5, 3). An acetylene-containing fatty acid derivative, isolated from the red seaweed *Liagora farinosa*, showed activity in both tests mentioned above, reducing the induced mouse ear edema and inhibiting the bee-venom phospholipase A_2 (PLA_2) activity (7, 6). Other compounds isolated from red algae *Phacelocarpus labillardieri* (Mertens) J. Agardh, *Sphaerococcus coronopifolius* Stackhouse, and *Phacelocarpus labillardieri* also showed bee-venom PLA_2 inhibitory activity (6, 11). Extracts from brown seaweed *Padina boergesenii* and *Hypnea valentia* also inhibited the action by *Naja nigricollis* venom, when inoculated into mice and when *in vitro* assayed (12).

Galaxaura marginata, a red seaweed that belongs to the same order of *L. farinosa*, presents well-known significant cytotoxic activity against carcinoma and leukemia cells, showing different desmosterol structures acting on such cells and antibacterial activity (9, 8), but its anti-inflammatory action is unknown. The present study investigated the anti-inflammatory effects of *G. marginata* apolar substances on acute inflammation using the croton oil-induced mouse ear edema test.

To obtain the extract, 1kg sample of *G. marginata* was collected by free diving in shallow waters of São Sebastião channel (45^o25'W; 26^o49'S), northern coast of São Paulo State, Brazil, during the spring of 2000. The algae were washed with fresh water to remove epiphytes and salts. Fresh plant material was exhaustively extracted

using ethanol:acetic acid (3:1 v/v) and filtered. The extract was concentrated under reduced pressure and partitioned against hexanes.

After the hexane was removed from the apolar phase, the extract was dissolved in acetone and kept at -20^{o}C until precipitate. A portion (5mg) of the precipitate was then separated through TLC (Si gel 60 F_{254}) using ethyl acetate-hexane (2:3), producing four spots. Each spot was cut, scraped off and extracted with ethyl acetate-hexane; the fraction obtained from each spot was dispensed in glass vial, dried under nitrogen and weighed.

The topical anti-inflammatory activity of the apolar extract and fractions separated through TLC was tested using the mouse ear edema assay as experimental model of topical inflammation. Croton oil (Sigma-Aldrich Co.) was dissolved in acetone (20μg/μl). The mice left ears were topically treated with 20μl croton oil solution (200μg) and apolar extract (0.25–1.0mg/ear) or 20μl of croton oil and each fraction obtained from TLC (200μg/ear). Treatments were applied in the inner surface of the left ear of mice (*Mus musculus*, n=5, 25±3g), and 20μl acetone was applied in the right ear. As control, 100% inflammatory activity was induced by applying 20μl of croton-oil solution in the left ear and 20μl of pure acetone in the right ear of mice (*Mus musculus*, n=10, 25±3g). Ear disks of 6mm diameter were taken from the animals for analysis 3h after the treatments application. The anti-inflammatory activity (AI) was measured as follows: $AI = [(W_{control}–W_{trial}) / W_{control}] \times 100\%$, where $W_{control}$ = Left ear disk weight – right ear disk weight from control animals; W_{trial} = Left ear disk weight – right ear disk weight from animals with the same concentration of the apolar extract and fractions tested.

Data analysis was carried out using the Graphpad Prism 4 software. The activity was quantitatively evaluated by determining the 50% Effective Concentration (EC_{50}), using nonlinear regression with sigmoidal dose-response curve fit. Statistical analysis consisted of analysis of variance, and differences were evaluated using the Student's *t*-test; $p<0.05$ indicated statistical significance.

The apolar extract and the four fractions obtained from TLC separation showed inhibition of the edema induced by croton oil in mouse ear. The extract reduced 95±0.5% inflammation when administered at the highest dose (1mg/ear). The EC_{50} was reached when mice ears were treated with 0.31mg/ear (95% confidence intervals: 0.24–0.41mg/ear; Fig. 1). The four TLC substances inhibited inflammation

by 55%, 75%, 100%, and 100%, respectively, considering the chromatography speed order.

Extracts as well as structurally diverse compounds obtained from marine red algae have been shown to inhibit inflammation (6, 7). In the present work, such findings were extended by reporting that marine red alga *G. marginata* displayed anti-inflammatory activity in its apolar extract, which was ten-fold more potent than the apolar substance obtained from *L. farinosa* (7). Thus, as phospholipase A_2 controls inflammatory responses, the edema reduction in the mouse ear indicated that the apolar extract from *G. marginata* influences PLA_2 activity.

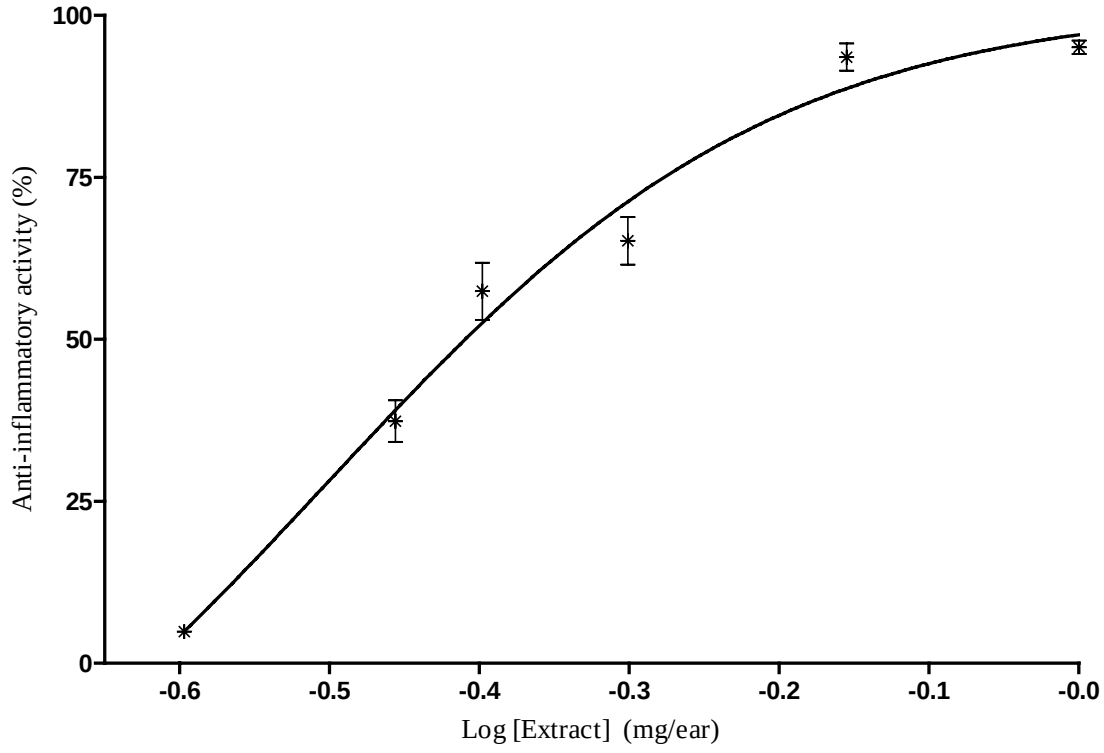

Figure 1. Anti-inflammatory activity of the apolar extract from *Galaxaura marginata*. Mice left ears were topically treated with croton oil and apolar extract. After 3h incubation, edema inhibition was determined. The EC_{50} value of 0.31mg/ear was calculated from quintuplicate measurements.

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