

**THE MARINE PLANT *Thalassia testudinum*
POSSESSES ANTI-INFLAMMATORY AND
ANALGESIC PROPERTIES**

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Summary

The natural marine compounds represent a source of new chemical structures and of pharmacological substances with anti-inflammatory activity that will allow to deep in the knowledge of the inflammatory process and in novel mechanisms of action of therapeutic agents. In this work we carry out the study of a extract of a marine plant present in the Cuban coast, *Thalassia testudinum* (Tt) with the objective of detecting anti-inflammatory and analgesic effects by carrageenan paw oedema, croton oil mouse ear oedema, phospholipase A₂ (PLA₂) inhibition, cyclo-oxygenase (COX) inhibition and writhing tests. Three fractions, obtained from this extract, were also evaluated in the assay of croton oil-induced ear oedema, PLA₂ inhibition, COX inhibition and writhing test.

The extract obtained from the Tt plant demonstrated an inhibitory effect in the carrageenan paw oedema. Also, demonstrated inhibitory effect over PLA₂ and COX enzyme. Chloroform and butanolic fractions also inhibited mouse ear oedema assay, and COX enzyme, but only the chloroform fraction inhibited the PLA₂ enzyme. The extract, butanolic and aqueous fractions inhibited writhing test. We can conclude that the extract, chloroform and butanol fractions shows anti-inflammatory properties and the extract and two of the fractions show analgesic effect.

Key Words: anti-inflammatory, analgesic, marine plant.

MATERIALS AND METHODS

- Carrageenan paw oedema.¹
- Croton oil mouse ear oedema.²
- Phospholipase A₂ (PLA₂) inhibition.³
- Cyclo-oxygenase (COX) inhibition.⁴
- Writhing induced by acetic acid.⁵

RESULTS

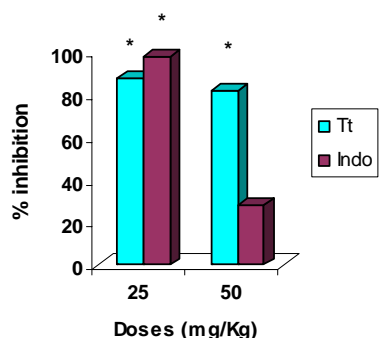


Fig. 1 Effect of *Thalassia testudinum* (Tt) extract and indomethacin (Indo) in paw oedema induced by carrageenan (* p<0.05)

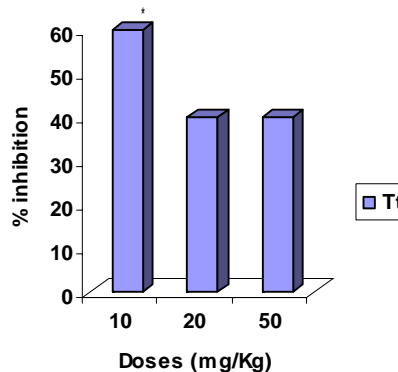


Fig. 2 Effect of *Thalassia testudinum* (Tt) extract in croton oil induced mouse ear oedema (* p<0.05)

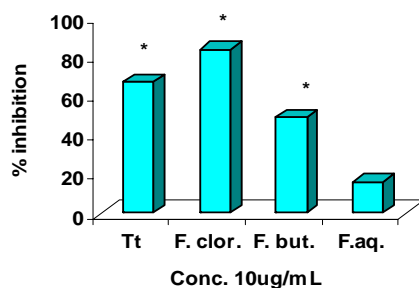


Fig. 3 Effect of *Thalassia testudinum* (Tt) extract and chloroform (F. clor), butanol (F. but) and aqueous (F.aq) fractions on the phospholipase A₂ human recombinant enzyme (*p<0.05)

The extract did not inhibit the phospholipase A₂ enzyme of bee poison, pancreatic and naja-naja sources.

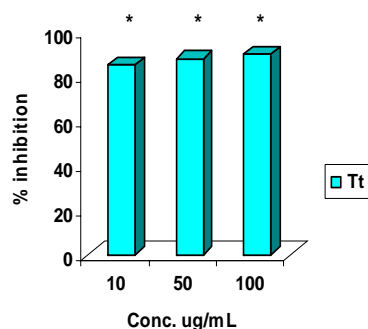


Fig. 4 Effect of *Thalassia testudinum* (Tt) extract in cyclooxygenase-1 enzyme (* p<0.05)

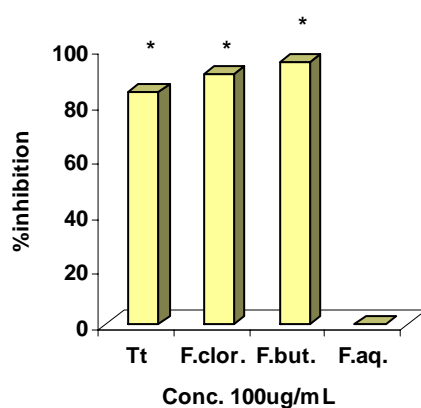


Fig. 5 Effect of *Thalassia testudinum* (Tt) and chloroform (F. clor), butanol (F. but), and aqueous (F. aq) fractions in cyclooxygenase- 2 enzyme (*p<0.05)

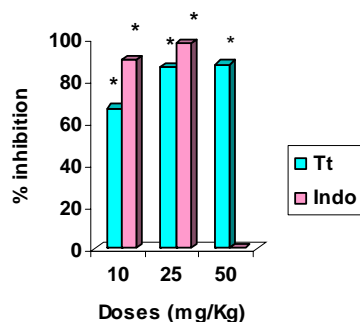


Fig.6 Effect of *Thalassia testudinum* (Tt) extract and indomethacin (Indo) in writhing test (*p<0.05)

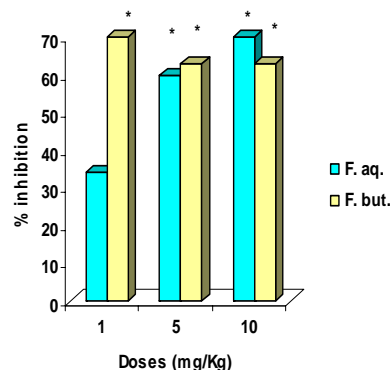


Fig.7 Effect of aqueous (F. aq) and butanol (F. but) fractions in writhing test (*p<0.05)

DISCUSSION

Marine organisms are a rich source of natural products with a high pharmacological potential. It has been demonstrated the anti-inflammatory properties of a number of marine metabolites isolated from marine algae, sponges and coelenterates, such as epitandiol, manoalide, ircinin, scalaradial, fuscoidin and others. This activity is related in some cases to the inhibition of enzymes that play an important role in the release of arachidonic acid and the formation of lipid mediators.

In this study we demonstrated that *Tt* extract show antioedema activity in paw and ear oedema models, which could be expressed as an inhibition of prostaglandin E_2 (PGE₂) formation or release. In studies with several seaweed and sponge extracts have been proved this effect⁶. It is interesting to observe that *Tt* extract in carrageenan paw oedema shows similar inhibition percent, at 25 mg.kg⁻¹, as the positive control indomethacin (88 and 98 % respectively), and in 50 mg.kg⁻¹ it is not toxic and indomethacin was.

When we assayed the effect of the *Tt* extract and its fractions over PLA₂ human recombinant enzyme, is observed a clear inhibition of this enzyme in the lower concentration (10µL) produced by the extract and by the chloroform fraction. The inhibition of PLA₂ enzyme is characteristic of anti-inflammatory marine substances, as has been demonstrated by several authors^{7, 8, 9} remarking the difference with many of the anti-inflammatory compounds present in the market.

The *Tt* extract inhibited COX-1 enzyme in a concentration-dependent manner with high inhibition percents. However, the COX-2 enzyme only is inhibited at 100 µL by the *Tt* extract and by the chloroform and butanol fractions. COX enzyme is part of the route of prostaglandins formation. COX-1 is constitutive and forms prostanoids, participating, fundamentally, in homeostatic functions, whereas COX-2 is induced rapidly upon stimulation of cells and synthesizes high levels of prostaglandins¹⁰. We can conclude that the inhibition of COX enzyme by *Tt* extract and two of the derived isolated fractions contribute to the anti-inflammatory activity.

Pain is closely associated with inflammation. That is why most of the anti-inflammatory compounds are analgesic too. For this reason we evaluated *Tt* extract in the writhing test. Pretreatment of mice with the extract reduced writhing induced by acetic acid in a dose-related manner, similarly to indomethacin. This result suggests that the extract might induce analgesic effects through peripheral action as reported for the non-steroidal anti-inflammatory agents such as aspirin and indomethacin. These drugs are known to relieve pain by inhibition of the synthesis of prostaglandins¹¹ and decrease of the sensitivity of peripheral nociceptive receptors¹². COX-1 is the enzyme involved in the analgesic effect, because in 30 min this enzyme forms the prostaglandins whereas COX-2 requires more time to form them¹³.

We can conclude that the extract, the chloroform and the butanol fractions shows anti-inflammatory properties by inhibiting PLA₂ and prostanoids production, and the extract and two of the fractions shows analgesic effect in the model assayed.

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