ANTIFOULING PROPERTIES OF THE EXTRACTS OF *CHICOREUS RAMOSUS*

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ABSTRACT

Crude extracts of the mantle, foot, digestive gland, gonad, prostrate gland, and hypobranchial gland of the gastropod *Chicoreus ramosus* were tested. Exposure trials were conducted at the Vellar estuary, on the southeastern coast of India for a period of one month with test panels of *Mangifera indica* (mango timber) treated with crude extracts mixed with abietic acid and dichloromethane. Barnacles, oyster spats and tubicolous polychaetes were common fouling organisms on the test panels. Slight inhibition was obtained with extracts of foot, mantle and prostrate gland, while extracts of hypobranchial gland, digestive gland, and gonad, exerted a potent inhibitory influence against fouling organisms.

INTRODUCTION

In the mid 1960s tributyl tin (TBT), an organotin compound, was regarded as an effective antifouling compound and was commonly used in antifouling paint. However, field studies have conclusively shown that TBT contamination has resulted in massive loss of marine life. (Reeve *et al.*, 1976; Langhlin *et al.*, 1988). Thus, it has become imperative that marine bioactive substances be used as antifouling agents since many marine antifoulants (Targett *et al.*, 1983; Rittschof *et al.*, 1984; Standing *et al.*, 1984; Gerhart *et al.*, 1988) were identified to be more promising as biological control. Employing marine bioactive metabolites as antifouling agents has the added advantage of not causing any serious adverse impact on the biological systems. Sessile marine organisms such as octocorals, sponges and ascidians are believed to possess chemical defense against epizoic recruitment. Numerous marine metabolites have been assayed in both field and laboratory tests to determine their effectiveness against amphipods, polychaetes and molluscs (Hay & Fenical, 1988). It has also been reported that marine toxins and chemical secretions of the benthic marine organisms play a significant role in antifouling (Bakus & Kawaguchi, 1984; Bakus, 1991). Significant biological activity has been revealed by the toxic extracts of the predatory marine molluscan gastropod *Chicoreus ramosus* (Emerson & Ayyakkannu, 1991). These active extracts are also presumed to possess novel antifouling compounds. Therefore, field trials were carried out at the jetty of the Vellar estuary on the south east coast of India, to examine whether the crude extracts of *Chicoreus ramosus* caused any strong inhibitory influence on the settling of fouling organisms.

MATERIALS AND METHODS

Plywood panels (17x15x1.3 cm) made of mango timber (*Mangifera indica*) were seasoned in filtered sea water for 2-3 days. Ethanol extracts of the different parts of *Chicoreus ramosus* foot, mantle, gonad, digestive gland, prostrate gland and hypobranchial gland were prepared by concentrating them at reduced pressure. The crude extracts (10 ml of each) were mixed with the antifouling agents abietic acid and dichloromethane. Abietic acid acts as a soluble matrix for slow leaching of the extract into the marine environment. Two grooves, 6 mm wide and 8 mm deep, were made on one side of the test panel. The mixture of crude extract, abietic acid and dichloromethane was poured into the grooves and allowed to dry. Control panels were made in similar manner. One set of control panels was treated with abietic acid and dichloromethane alone and the other set was left untreated. Treated and untreated panels were suspended by a nylon rope through holes made in the center of panels and anchored in the Vellar Estuary at a depth of 1 m for a period of one month (July - August 1993). The retrieved panels were preserved individually in ethanol. Fouling organisms were counted on each panel, and the percentage coverage, biomass and extent of inhibition estimated.

Clean microscopic slides were numbered and smeared with a thin layer of extracts + abietic acid and dichloromethane. A pair of control slides were treated with abietic acid and dichloromethane alone, and a pair of slides were left untreated. The slides were placed in plastic tubes 4 cm diameter, bored with holes, sealed at both sides and suspended in the estuary by a long nylon twine. The slides were retrieved after one month and analyzed to assess if the extracts inhibited growth of microbial communities.

RESULTS

Barnacles, oyster spats and tubiculous polychaetes settled on the test panels. The test panels treated with the extracts of the mantle (CRM) exhibited no significant inhibitory influence. However, a slight inhibitory influence was observed near the periphery of the grooves with an inhibition zone of 5-6 mm. Apparently CRM enhanced the settling of barnacles, oyster spats and tubiculous polychaetes. Considerable inhibition was observed on the panels treated with the extracts of foot (CRF) and the prostrate gland (CRPG) with an inhibitory range of 9 mm and 7 mm respectively. The prostrate gland extract inhibited the growth of barnacles and polychaetes but not that of the oyster spats. A very high range of inhibition of foulers was observed on panels treated with extracts of the gonad (CRG - 16 mm), the digestive gland (CRDG - 14 to 15 mm) and the hypobranchial gland (CRHG - 14 mm). Less and scattered settlement of oyster spats and barnacles was found on the panels treated with extracts of the digestive gland and the hypobranchial gland. Inhibition by extracts of the gonad was considerable since there was no settlement of barnacles and polychaetes and only one or two oyster spats were found. Both the controls (bare and resin treated) were heavily fouled by barnacles, oyster spats and polychaetes. The leaching rate of extracts was slow. Some of the treated extracts still remained on the panel after one month. The results of microbial fouling were interesting since the slides treated with extracts of the digestive gland, gonad and the hypobranchial gland also showed considerable inhibition of microbial mats whereas the other extracts did not exhibit any pronounced effects. The percentage of recruitment of foulers and the extent of inhibition are shown in Figs. 1 & 2.

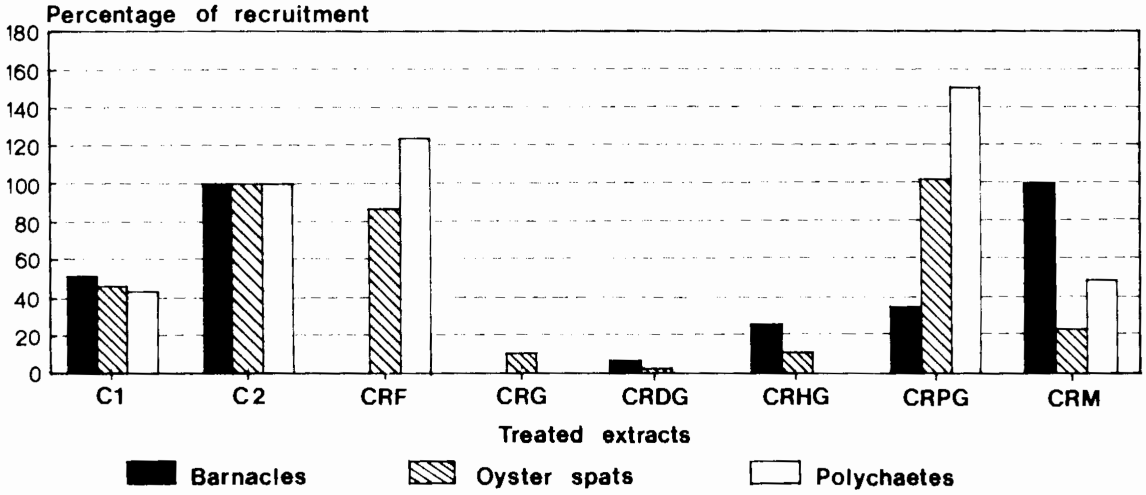


Figure 1. Percentage of recruitment of fouling organisms on treated panels. (Legend: c_1, c_2 = control; extraction of *C. ramosus*, CRF = foot, CRG = gonad, CRDG = digestive gland, CRHG = hypobranchial gland, CRPG = prostate gland, CRM = mantle.

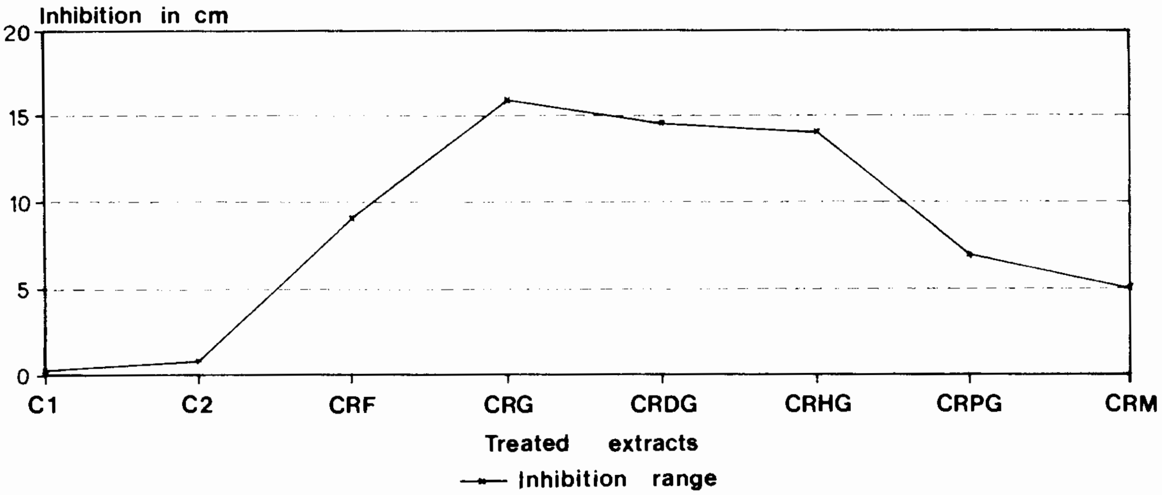


Figure 2. Extent of inhibition on treated panels. Legends are the same as Fig. 1.

DISCUSSION

The crude extracts of *Chicoreus ramosus* displayed promising biological potency in inhibiting the settlement of the marine foulers. The results indicate that tropical marine organisms are active in producing antifouling compounds. In comparison, Bakus & Kawaguchi (1984) and Berguist & Bedford (1978), found evidence of more prevalent antibacterial activity in temperate than in the tropical latitudes. However, the development of a fouling community is a complex process influenced by temporary variability in growth, physical disturbances, competitive interaction of different species, grazing and predation (Sarma *et al.*, 1991). The random growth of the primary film in most of the slides may be due to the fact that smooth surfaces could not retain the test compound which got leached away soon. But, as mentioned above, the study of fouling on surfaces is a complex process, and interpretation of the results is always a difficult matter.

REFERENCES

Bakus, G.J. 1991. Studies on Bioactive substances. **In**: Mary Frances Thompson *et al.*(*eds.*) *Bio-active compounds from marine organisms*. p.387-391. Oxford Publishing Co. Pvt. Ltd., New Delhi.

Bakus, G.J. and Kawaguchi. 1984. Toxins from marine organisms: Studies on antifouling. **In**: Bolis *et al.*(*eds.*): *Toxins, drugs and pollutants in marine animals*. p. 43-46.Springer-Verlag, Berlin.

Berguist, P.R. and J.J. Bedford. 1978. The incidence of antibacterial activity in marine Desmospongiae: systematic and geographic considerations. *Mar. Biol.* **46**: 215-221

Emerson Kagoo, I. and K. Ayyakkannu. 1991. Bio-active compounds from *Chicoreus ramosus*: a status report. *Phuket mar. biol. Spec. Publ. no.***10**: 225.

Gerhart, D.J., D. Rittschof and S.W. Mayo. 1988. Chemical ecology and the search for marine antifoulants: studies of a predator - prey symbiosis. *J. Chem. Ecol.* **14**: 1905-1917.

Hay, M.E. and W. Fenical. 1988. Marine plant - herbivore interactions: the ecology of chemical defense. *Ann. Rev. Ecol. Syst.* **19**: 111-145.

Langhlin Jr., R.B., A.B. Cobet and H.E. Guard. 1988. Mechanisms of triorganotin toxicity to macro-invertebrates and the role of the biofilm as a controlled release medium. **In**: Mary Frances - Thompson *et al.(eds.)*. *Marine Biodeterioration*. Oxford publishing Company, New Delhi.

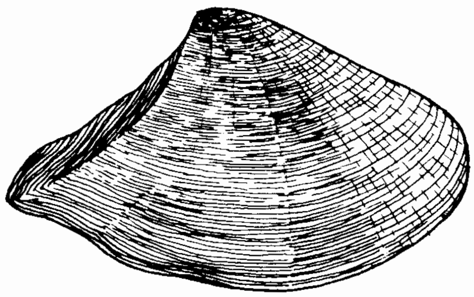
Reeve, M.R., G. D. Grice, N.R. Gibson, M.A. Walter, K. Darcy and T. Ikeda. 1976. A controlled environmental pollution experiment (CEPEX) and its usefulness in the study of larger marine zooplankton and toxic stress. *Soc. Exp. Biol. Semin. Ser.* **2**: 145-162.

Rittschof, D., E.S. Branscomb and J.D. Costlow. 1984. Settlement and behaviour in relation to flow and surface on larval barnacles, *Balanus amphitrite,* Darwin. *J. Exp. Mar. Biol.* **82**: 131-146.

Sarma, N.S., K. Satyanarayana Rao and B. Viswanadhan. 1991. Settling responses and progression in community development of selected macrofouling organisms to a recently isolated sponge metabolize, herbacin, at Visakhapatnam harbor, Bay of Bengal. **In**: Mary Frances Thompson *et al. (eds.)*.*Bioactive compounds from marine organisms*. Oxford Publishing Company, New Delhi.

Standing, J., I.R. Hooper and J.D. Costlow. 1984. Inhibition and induction of barnacle settlement by natural products present in octocorals. *J. Chem. Ecol.* **10**: 823-834.

Targett, N.M., S.S. Bishop, D.J. McConnell and J.A. Yoder. 1983. Antifouling agents against the benthic marine diatom, *Navicula virgulata*, homarine from the gorgonians, *Leptogorgia virgulata* and *L. setacea* and analogs. *J. Chem. Ecol.* **9**: 817-829.



Donax (*Hecuba*) *scortum* L., 1758. PMBC 4321.
Drawing by Patairat Singdam.