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EVALUATION OF ANTIFOULING COMPOUNDS FROM *CHICOREUS RAMOSUS*

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ABSTRACT

Isolated fractions of the digestive gland, gonad, and hypobranchial gland were put on panels of mango timber (*Mangifera indica*) and tested against untreated controls. The ether extract of the digestive gland, and the acetone extract of the gonad possessed much antifouling substance. The ether extract of the hypobranchial gland exhibited relatively less activity. The active fractions displayed selective inhibition towards fouling barnacles, oyster spat and tubicolous polychaetes.

INTRODUCTION

Organotin compounds are efficient antifouling substances, but marine metabolites appear to be more promising antifoulants without causing any serious adverse effect on the biological systems. These active metabolites of marine origin virtually encompass all classes of chemicals (Scheuer 1978, 1979, 1981; Bakus *et al.* 1986). Furthermore, the chemical basis for settlement of fouling organisms has been of particular interest since it enables a better understanding of the processes involved (Pawlik and Faulkner 1988). However, the active chemical compounds of most marine natural antifoulants have not been scrutinized. Recent investigations showed that extracts of the gastropod *Chicoreus ramosus* exhibited promising antifouling activity. (Emerson & Ayyakkannu 1994). Therefore, efforts were made to identify the natural compound from these extracts possessing strong inhibitory properties. The present paper deals with the fractionation of these active extracts and further testing of each fraction for antifouling activity.

MATERIALS AND METHODS

The digestive gland, gonad and hypobranchial gland of *Chicoreus ramous* were extracted with 1:1 methanol : chloroform. The solvent was removed in vacuum, and the residues from each extract were partitioned between distilled water and ethyl acetate to yield the crude water soluble and the non water soluble extracts. The latter extracts were fractionated by column chromatography on silica gel. Five fractions were eluted with methanol, diethyl ether, acetone, hexane, and butanol. The fractions obtained for each extract are referred to as CRDG 1-5 (Dig.gl) CRG 1-5 (gonad) and CRHG 1-5 (Hyp.gl). The separated fractions and the crude water soluble fractions of each extract were allowed to evaporate and the concentrated fraction (5 ml) was mixed with abietic acid and dichloromethane. Abietic acid acts as a soluble matrix for slow leaching of the substances. The mixture of each fraction was poured into grooves made on plywood test panels (mango timber, *Mangifera indica*), and dried. Control panels were treated with abietic acid and dichloromethane alone (control 1). Another set was left bare (control 2). These test panels were suspended by a nylon rope and anchored in the Vellar estuary, Southeast coast of India at a depth of 1m for a period of one month (July - August 1994). The retrieved panels were analysed for the percentage coverage, biomass and extent of inhibition.

RESULTS

The crude water soluble fractions did not exhibit any significant repellent properties. Chemical fractionation of the non water soluble extracts showed that the activity is localized in both intermediate polar and non polar fractions. In the digestive gland extract (CRDG), the activity was identified in the non polar ether fractions (CRDG-2), and in a fraction of intermediate polarity (acetone) (CRDG-5). In the gonad extract (CRG), it was in the intermediate polar fraction (ACETONE) (CRG-4). In the hypobranchial gland extract (CRHG) it was confined to the non polar ether fraction (CRHG-2). However, the extent of inhibition displayed by this

fraction (8-9 mm) was not significant whereas the range of inhibition displayed by CRDG-2 (10-12 mm) and CRDG-5 (12-14 mm) and CRG-4 (11-13 mm) were significant. Other fractions did not differ from the control in terms of settlement of fouling organisms.
Fouling organisms were barnacles (dominant), oyster spat, tubicolous polychaetes, and algal species. Algal occurrence was noted on the surface of controls only. The tested fractions were most active against barnacles.
The leaching rate of the fractions was quite fast and many of the fractions were not present on the panels at the time of retrieval. Test panels suspended at the bottom were exposed to a higher rate of sedimentation, and had relatively less fouling. The percentage of recruitment of foulers and the extent of inhibition are shown in Figs. 1-4.

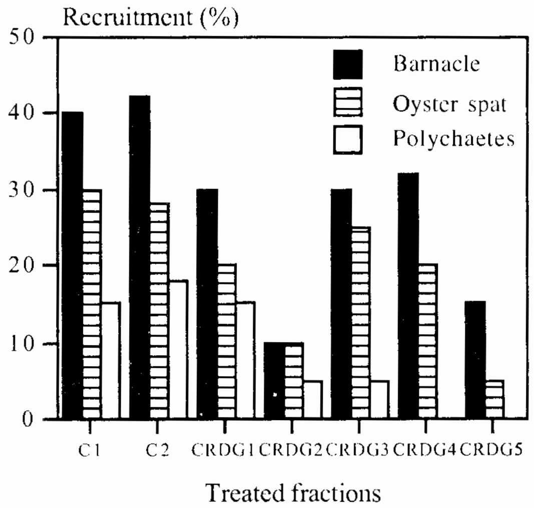

Figure 1. Pecentage of recruitment as a function of treated fractions.

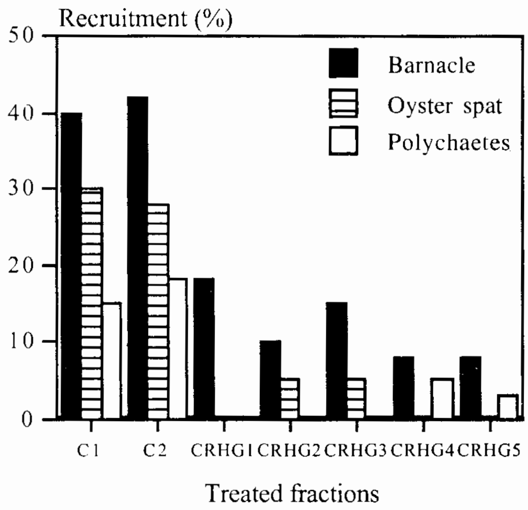

Figure 3. Pecentage of recruitment as a function of treated fractions.

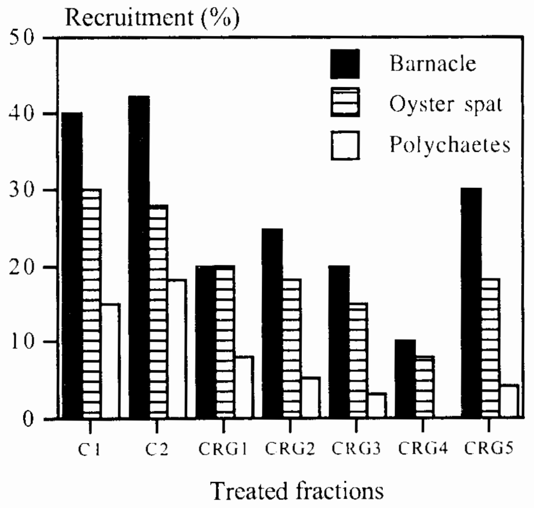

Figure 2. Pecentage of recruitment as a function of treated fractions.

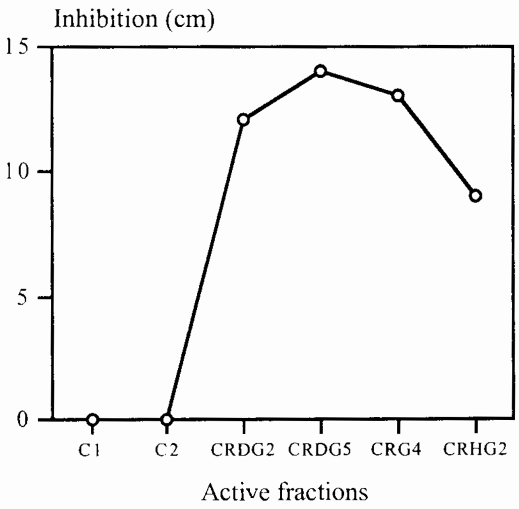

Figure 4. Extent of inhibition, expressed as the distance of inhibition relative to the active fractions.

DISCUSSION

Chemical fractionation of the water non-soluble extracts revealed that antifouling substances are localized only in specific fractions. The effect of these fractions decreased with time, suggesting that the active constituents undergo degradation (Targett 1988). The assumed degradation paves way for the settlement of foulers.

The number of barnacles settling on all panels (controls and treated) were considerably smaller compared to panels tested for antifouling activity during July-August 1993 (Emerson and Ayyakkannu, 1994). The low number of settling foulers may be due to pronounced fluctuations in salinity, temperature, rainfall, sedimentation, and turbulent water during the exposure in the Vellar estuary. Environmental conditions play a decisive role in settling success (Bakus 1968; Crisp 1974). Therefore, it becomes apparent that the growth of barnacles depends on local hydrographical conditions (Meenakumari & Nair 1988; Newell 1979).

REFERENCES

Bakus, G. J. 1968. Defensive mechanism and ecology of some tropical holothurians. - Marine Biology **2**(1): 23-32.

Bakus, G. J., N. M. Targett & G. Schulte. 1986. Marine Chemical Ecology: an overview. - Journal of Chemical Ecology **12**: 951-987.

Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. - *In* P. T. Grant & A. M. Mackie (eds.). Chemoreception in marine organisms. Academic press, New York.

Emerson Kagoo, I & K. Ayyakkannu. 1994. Antifouling properties of the extracts of *Chicoreus ramosus*. - Phuket marine biological Center Special Publication **13**: 195-198.

Meenakumari, B. & N. Balakrishnan Nair. 1988. Growth of the barnacle *Balanus amphitrite communis* (Darwin) in Cochin Backwaters. - *In* Mary Frances Thompson *et al.* (eds.). Marine Biodeterioration. Oxford & IBH Publishing Company, New Delhi.

Newell, R. C. 1979. Biology of intertidal animals. - Marine ecological surveys Ltd., Faversham, U.K.

Pawlik, J. R. & J. Faulkner. 1988. The gregarious settlement of sabellarid polychaetes: New Perspectives on chemical cues. - *In* Mary Frances Thompson *et al.* (eds.). Marine Biodeterioration. Oxford & IBH Publishing Company, New Delhi.

Scheuer, P. J. 1978. Marine natural products: Chemical and biological perspectives. Vol. I. - Academic Press, New York.

Scheuer, P. J. 1979. Marine natural products: Chemical and Biological perspectives, Vol. III. - Academic press, New York.

Scheuer, P. J. 1981. Marine Natural products: Chemical and Biological perspectives, Vol. IV. - Academic press, New York.

Targett, N. M. 1988. Allelochemistry in marine organisms: Chemical fouling and antifouling strategies. - *In* Mary Frances Thompson *et al.* (eds.). Marine Biodeterioration. Oxford & IBH Publishing Company, New Delhi.