

Studies on the Antifouling Properties of Some Natural Products from Goa

S.S. Sawant and A.B. Wagb

National Institute of Oceanography
Dona Paula, Goa, India

Methanol extracts of the fruits of the terrestrial plants *Randia brandisii* and *Sapindus trifoliatus* were screened for their antifouling activities on the marine fouling diatoms *Navicula subinflata* and *N. crusicula*. Both extracts inhibited the growth of these diatoms on glass surfaces. Lethal concentrations of the extracts varied with the types of plants and diatoms used. The extracts were found to act both as a stimulant and as an inhibitor depending on their concentrations in the media. Data suggest the possible potential of these extracts as antifouling agents.

INTRODUCTION

Conventional antifouling paints contain copper compounds such as cuprous oxide or copper thiocyanate as the principle biocide. The new generation of antifouling paints contain organotin compounds that are very effective in aquatic environments. However, these compounds are of environmental concern as both copper ions and organotins are very toxic to marine organisms. Thus, there is a great need to search for antifouling compounds that are not only effective in controlling fouling but are also environmentally safe. In this regard, natural products of plants, animals, and microorganisms may offer great potential as antifouling agents (Thompson et al. 1991, Maki et al. 1988).

With this in view, efforts were made in our laboratory to screen natural products from terrestrial sources for antifouling properties. The antifouling effects of methanol extracts of the fruits of two terrestrial plants, *Randia brandisii* (Gamble) and *Sapindus trifoliatus* (Vahl) were assessed on two species of marine fouling diatoms, *Navicula subinflata* and *N. crusicula*.

METHODS

Extract Preparation: Dry fruits of *R. brandisii* and *S. trifoliatum* were powdered and extracted with methanol under reflux. The crude extracts were dried by evaporating under vacuum, and a stock solution was prepared by dissolving the dried extract of known weight in sterile seawater, which was then used for preparing serial dilutions. The concentrations were expressed in terms of dry weight of extract.

Culture and Growth Conditions: The marine fouling diatoms, *N. subinflata* and *N. crucicula*, were isolated from fouled aluminum panels and maintained in a modified Guillard f/2 medium (Guillard 1975) under a 12-hour light:12-hour dark cycle at 18°C (Gotschalk and Alldredge 1989). For all experiments, mid-log phase (96-hour harvested cells) diatom cells were used.

Effects of Extracts on Growth: 1 ml of mid-log phase diatom cells (chlorophyll *a* concentration adjusted to 0.2 – 0.7 µg/ml) were aseptically transferred in a series of 50 ml flasks, each containing 14 ml of sterile f/2 medium. Increasing concentrations (10 – 200 µg/ml of medium) of crude extracts were added to three flasks, which were then incubated for 96 hours at 18°C under a 12-hour light:12-hour dark cycle. After 96 hours, the chlorophyll *a* content of the diatoms in each flask was estimated by standard procedures (Parsons et al. 1984).

Effects of Lethal Concentrations on Diatom Cells: Two set of flasks (32 flasks) each containing 14 ml sterile f/2 medium, were inoculated with 1 ml diatom cells as above. To one set (16 flasks), a lethal concentration (50 µg/ml) of *R. brandisii* extract was added and incubated for eight days under the same conditions described above. The chlorophyll *a* contents of all flasks were estimated daily. The mean of two values of chlorophyll *a* were plotted against time to obtain the growth curve.

To confirm the effectiveness of the lethal concentration, flasks with increasing cell densities (chlorophyll *a* concentration between 0.075 µg and 2.5 µg/ml) of *N. subinflata* were inoculated with a lethal concentration (50 µg/ml) of the *R. brandisii* crude extract. Chlorophyll *a* was estimated after a 96-hour incubation period.

An experiment was also conducted to assess the physical condition of the cells subjected to a lethal extract concentration. Flasks containing a known quantity of chlorophyll *a* and a lethal concentration (50 µg/ml) of the extract were incubated for 96 hours. The medium was decanted and the cells gently washed with fresh (extract free) medium. The washed cells were then incubated for 192 hours in fresh (extract free) medium.

Chlorophyll *a* was estimated after 96 hours before and after media replenishment and the cells microscopically examined.

RESULTS

The crude extract of *R. brandisii* increased the growth of *N. subinflata* and *N. crucicula* relative to the control at the initial concentrations of 30 and 75 $\mu\text{g/ml}$, respectively, after 96 hours of incubation (figures 1A, B). Further increases of concentrations, to 50 $\mu\text{g/ml}$ for *N. subinflata* and 125 $\mu\text{g/ml}$ for *N. crucicula*, resulted in sharp decreases of chlorophyll *a*, which then remained almost constant.

The *S. trifoliatus* extract caused a slightly higher growth compared to the control at the initial concentrations of 10–20 $\mu\text{g/ml}$ for *N. subinflata* and 30–50 $\mu\text{g/ml}$ for *N. crucicula* (figures 2A, B). Growth of both species sharply decreased with increased concentrations, 80 and 100 $\mu\text{g/ml}$ for *N. subinflata* and *N. crucicula*, respectively. Further increase of the extract had no appreciable decreasing effect on growth.

In extract-free medium, *N. subinflata* grew slowly during the initial 48 hours. Thereafter, growth continuously increased during succeeding incubation periods, becoming stationary after eight days. On the other hand, the growth of species in media containing lethal concentrations of extract remained unaltered (figure 3).

The chlorophyll *a* concentrations of *N. subinflata* at 0 hour and after 96 hours following addition of lethal extract concentrations are shown in table 1. There was a 30 to 35% decrease in chlorophyll *a* after 96 hours.

Figure 4 shows that the growth of diatoms subjected to lethal extract concentrations remained unchanged during the initial 96 hours. However, after changing the medium, the cells were found to grow gradually during the next 96 hours. Maximum growth was attained during a second incubation period of 96 hours.

DISCUSSION

It is evident that the minimum crude extract concentration required to inhibit diatom growth varies with species. One hundred percent inhibition of the growth of *N. subinflata* was seen with a 50 $\mu\text{g/ml}$ concentration of *R. brandisii* crude extract, whereas, 80 $\mu\text{g/ml}$ of *S. trifoliatus* crude extract was necessary to inhibit 100% growth of the same species. On the other hand, 125 and 100 $\mu\text{g/ml}$ extracts of *R. brandisii* and *S. trifoliatus*, respectively, were required to inhibit the growth of *N. crucicula*. This leads to an inference that different compounds may be responsible for the inhibition of diatom species.

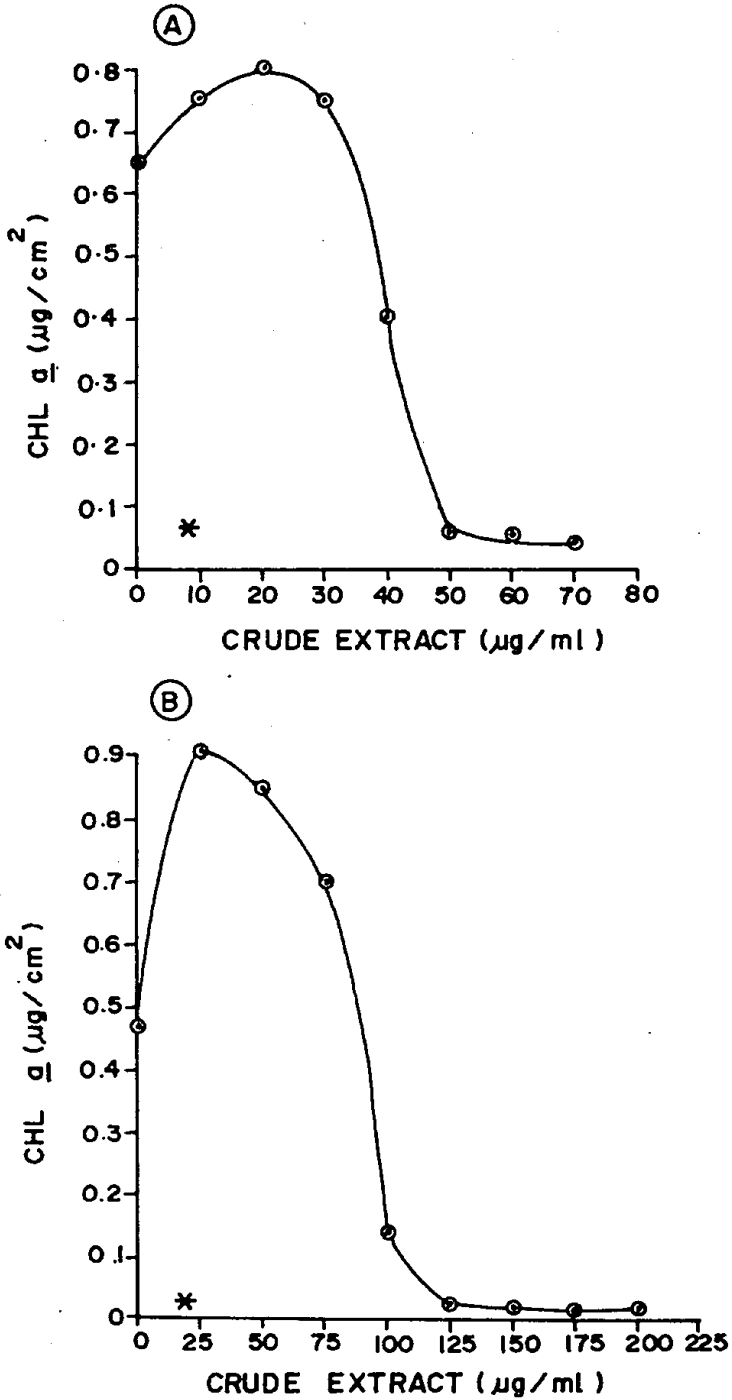


Figure 1. Effects of *Randia brandisii* crude extracts on the chlorophyll *a* contents of *Navicula subinflata* (A) and *N. crusicula* (B) following 96-hour incubation. * = chlorophyll *a* concentration with extract.

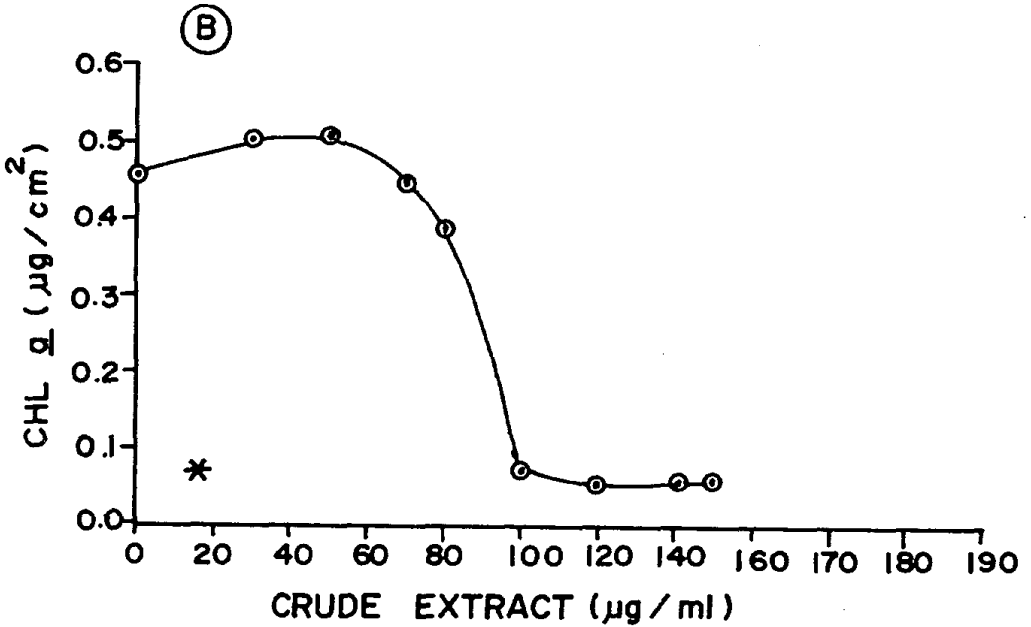
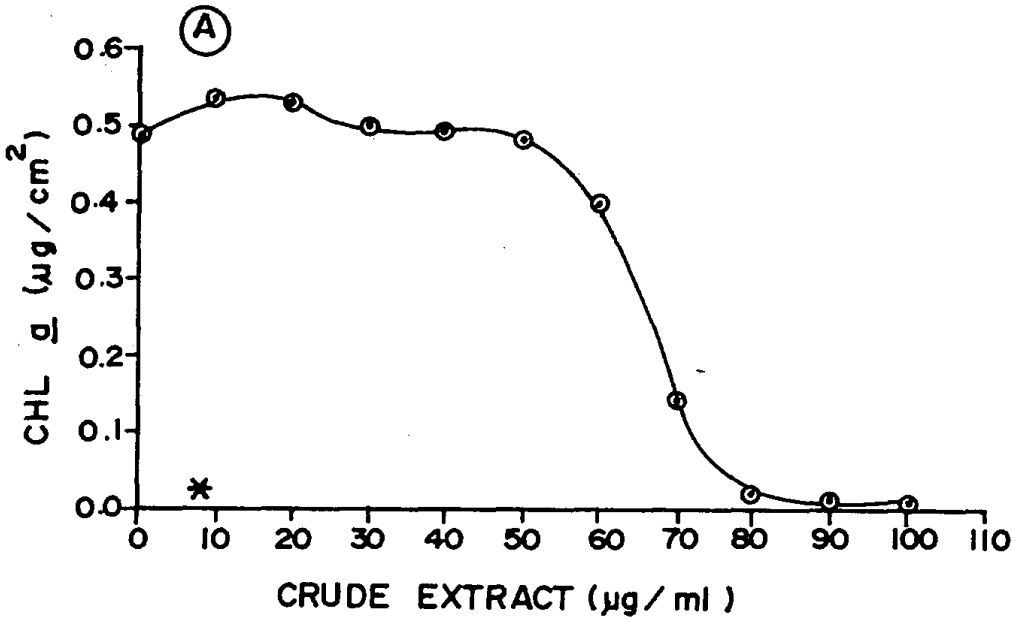


Figure 2. Effects of *Sapindus trifoliatus* crude extracts on the chlorophyll *a* contents of *Navicula subinflata* (A) and *N. crusicula* (B) following 96-hour incubation. * = chlorophyll *a* concentration with extract.

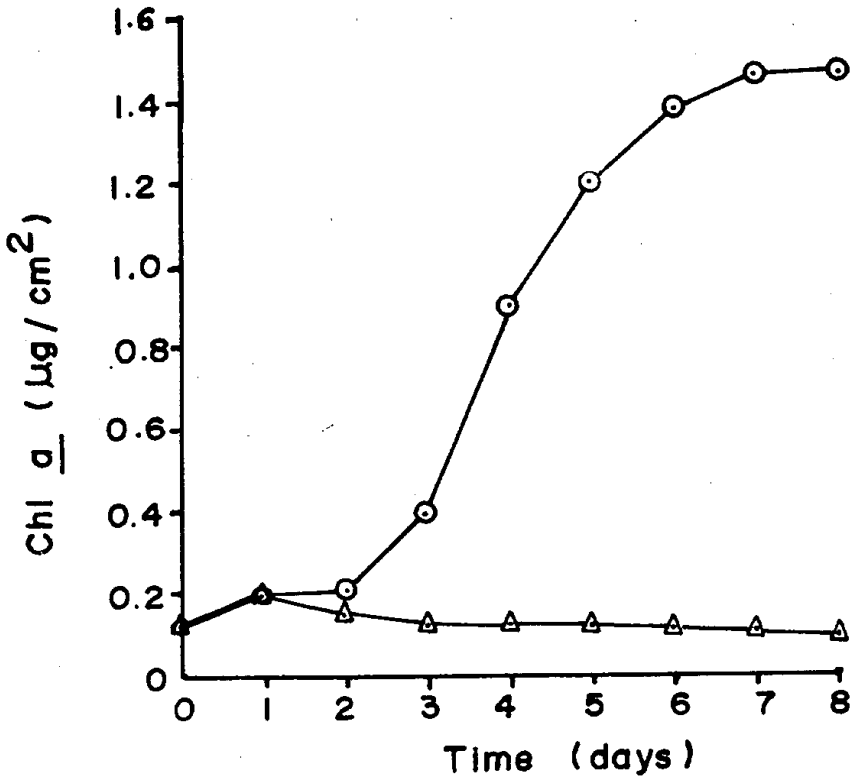


Figure 3. Daily growth (chlorophyll *a*) of *Navicula subinflata* in f/2 medium with lethal concentration of extract (△—△) and without extract (○—○).

Table 1. Different cell densities (chlorophyll *a* concentration µg/cm²) of *Navicula subinflata* before and after exposure to lethal concentrations of *R. brandisii* crude extract.

Hours	Cell Density								
0	0.087	0.17	0.25	0.32	0.40	0.82	1.23	2.00	2.45
96	0.075	0.17	0.20	0.22	0.28	0.53	0.95	1.36	1.76

The enhanced growth of both diatom species at lower extract concentrations suggests that the compound may be acting as a stimulant at those concentrations. However, higher extract concentrations caused inhibition of growth. Thus, it is evident that the extracts can act both as stimulants and as inhibitors, depending on their concentrations. Similar results were reported by Standing et al. (1984) and Vitelina et al. (1991) regarding the effects of extracts of marine organisms on barnacle larvae and microalgae. The actual mechanisms involved are not known, but are probably due to multiple activities in the medium.

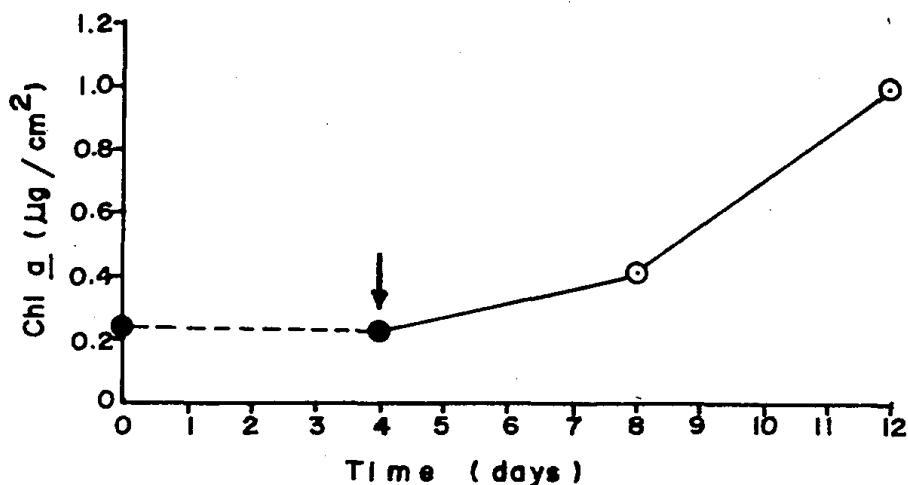


Figure 4. Growth (chlorophyll *a*) of *Navicula subinflata* subjected to lethal concentration of extract (●-----●) and growth of same cells following washing in extract-free medium (○-----○).

The experiment to examine the effectiveness of lethal extract concentrations on varying diatom cell densities indicated that the lethal concentration was independent of diatom cell densities, and that its concentration was dependent upon the volume of medium. A 30 to 35% reduction in the initial concentration of chlorophyll *a* was observed after 96 hours. This was attributed to detachment of cells from the base of the flask due to the effect of the extract.

The experiment conducted to determine the effects of lethal extract concentrations on the physical condition of the diatom cells indicated that there was no physical damage. Diatom growth was inhibited in media containing lethal concentrations; however, after media replenishment, the cells exhibited gradual increases in growth during the first four days, followed by sharp increases in growth. This indicates that the lag phase of diatom growth was prolonged due to the lethal concentration of extract. These observations suggest that there is also a nonlethal concentration effect of extract on the diatom cells. This leads to an inference that the compound present in the extract may be temporarily suppressing growth by acting as a narcotizing agent.

These preliminary laboratory results indicate that the extracts contain some compound that can act both as a stimulant and as an inhibitor depending on its concentration in the environment. Because the compound is not lethal, these extracts have a high potential as an antifouling agent.

ACKNOWLEDGEMENTS

We thank A. Garg for help in maintenance of the cultures and analyses of samples, and Dr. Bhosle for his valuable suggestions.

LITERATURE CITED

- Gotschalk, C.C., and A.L. Alldredge. 1989. Enhanced primary production and nutrient regeneration within aggregated marine diatoms. *Mar. Biol.* 103: 119-129.
- Guillard R.R.L. 1975. Culture of phytoplanktons for feeding marine invertebrates. Pages 29-60 in W.L. Smith and M.H. Chanter, eds. *Culture of Marine Invertebrate Animals*. Plenum Press, New York.
- Maki, J.S., D. Rittschof, J.D. Costlow, and R. Mitchell. 1988. Inhibition of attachment of larval barnacles *B. amphitrite* by bacterial surface film. *Mar. Biol.* 97: 199-206.
- Vitellina, M., M. Evelin, D. Rittschof, R. Sarojini, and R. Nagabhushanam. 1991. Compounds from octocorals that inhibit barnacle settlement: isolation and biological potency. Pages 331-339 in M.F. Thompson, R. Sarojini, and R. Nagabhushanam, eds. *Bioactive Compounds from Marine Organisms*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.
- Parsons T.R., Y. Maita, and C.L. Lalli. 1984. Pages 104-109 in *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York.
- 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.
- Thompson M.F., R. Sarojini, and R. Nagabhushanam. eds. 1991. *Bioactive Compounds from Marine Organisms*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.