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Growth inhibition of fouling bacteria and diatoms by extract of terrestrial plant, *Derris scandens* (Dicotyledonae:Leguminocae)

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Methanol extract of terrestrial plant, *Derris scandens* Benth. was found to inhibit growth of four diatoms and 7 bacterial species of fouling community. The concentrations required to bring about 100% inhibition of growth of the diatoms ranged between 200 to 300 μg.ml^{-1}, whereas, the bacterial inhibition zone around the disc containing about 600-800 μg of extract ranged between 1-5 mm. The results suggest that the crude extract of the plant is nontoxic and possesses both antibacterial and antialgal properties. Therefore, the extract promises to be a potential source of natural biocide for fouling organisms.

In the wake of restrictions to use toxic chemicals in antifouling formulations, efforts are on to search alternate compounds which would be environment friendly. It has been observed that natural compounds from marine organisms, algae and terrestrial plants could be the most promising antifouling agents[1-8]. In view of this, the present work deals with the screening of a terrestrial plant species *Derris scandens* Benth. (Dicotyledonae; Leguminocae) for probable antibacterial and antialgal properties.

Methanolic extract of bark of *Derris scandens* was obtained following standard procedure[7,8] and dried under vacuum. A known weight of the dried extract was dissolved in sterile natural seawater (salinity 34×10^{-3}; *p*H 8.2), which was then used for preparing serial dilutions. The concentration was expressed in terms of dry weight of the extract.

Bacteria and diatoms belonging to fouling community were used as test organisms for bioassays. The isolates of bacteria were *Chromobacterium* sp., *Alkaligenes* sp., *Moraxella* sp., *Arthrobacter* sp., *Vibrio* sp., *Pseudomonas* sp. and *Flavobacterium* sp. The diatom species used were *Navicula subinflata, N. crucicula, Amphora* sp. and *Nitzschia* sp. Disc method[9] was adopted for assessing antibacterial property of the extract. Whatman paper discs (7 mm diam.), impregnated with approximately 600-800 μg per disc extract, were placed in triplicate on Zobell marine agar containing thick film of individual species of bacteria. After 24 h the plates were observed for the degree of inhibition of bacterial growth around the disc. The area around the disc covering inhibition zone was measured in millimeter.

As regards to the bioassay experiment with diatoms, a known number of the diatoms was added to a series of conical flasks (50 ml capacity) containing 15 ml Guillard F/2 medium with increasing concentration of extract (0-400 μg.ml^{-1}) of *D. scandens*. The flasks were then incubated at 18°C with 12 h light and 12 h dark cycle. The growth of cells in each flask was assessed after 72 h by estimating[8,10] the chlorophyll-*a*. The amount of diatom cells (chl-*a*) at zero hour is considered as zero growth. The experiment was conducted in triplicate. The concentration of the extract required to bring about 100% inhibition in the growth of diatom has been expressed as effective concentration[8,11] (Ec).

In addition to the bioassay experiments, toxicity of the effective concentration of the extract on the diatom *Navicula subinflata* was studied. For this purpose, a set of flasks containing equal amount of diatoms were incubated in medium with effective concentration (225 μg.ml^{-1}) of the extract. Two flasks were removed daily over a period of 4 days for assessing diatom cell growth. Thereafter, the algal cells in the remaining flasks were rinsed and maintained in plain medium for further 8 days. The algal cell growth in these flasks was also assessed daily.

Bacterial growth inhibition around the disc impregnated with extract was observed in all the seven species under study (Table 1). The degree of inhibition of growth varied with the species and ranged between 1-5 mm. Maximum inhibition was observed in case of *Arthrobacter* sp. (4-5 mm) whereas, minimum was observed in *Chromobacterium* sp. (1-2 mm). These results suggest that the extract contains antibacterial compound.

As regards to the results on the bioassays with dia-

Table 1—Degree of inhibition of fouling bacterial species around the discs impregnated with crude extract

Bacterial isolates	Inhibition (mm) around the disc
Chromobacterium sp.	1-2
Alkaligenes sp.	2-3
Moraxella sp.	2-3
Arthrobacter sp.	4-5
Vibrio sp.	2-3
Pseudomonas sp.	2-3
Flavobacterium sp.	2-3

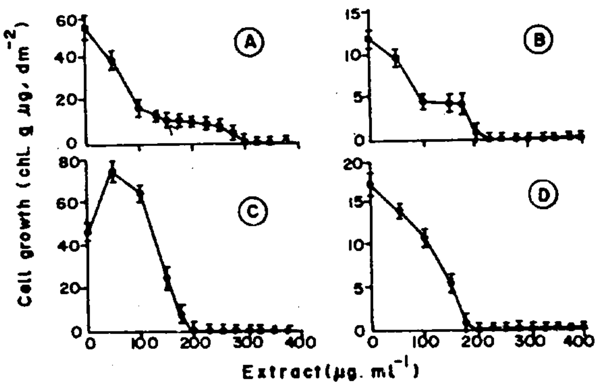

Fig. 1—Cell growth in terms of chlorophyll-*a* of diatoms: A) *Navicula crucicula*, B) *N. subinflata*, C) *Nitzschia* sp. and D) *Amphora* sp. against varying concentrations of extract.

toms, the growth of all the species under study decreased gradually with increasing concentration of the extract. The concentration required to bring about 100% inhibition (Ec) in the cell growth varied with the diatom species under study and ranged between 200-300 $\mu g.ml^{-1}$ (Fig. 1). The effective concentration for diatom *Navicula crucicula* was found to be higher than the rest of diatom species under study. These bioassay studies suggest that the crude extract of the plant probably contains compounds having antialgal property. However, the degree of activity vary with diatom species.

The results of the toxicity assay showed that the diatom cells failed to grow in the medium containing Ec of the extract, however, the same cells exhibited normal growth when transferred in extract-free medium (Fig. 2). This suggests that the cells treated with Ec of the extract were alive, but were unable to grow. From this, it could be assumed that the extract probably contains non-toxic compounds. It is suspected

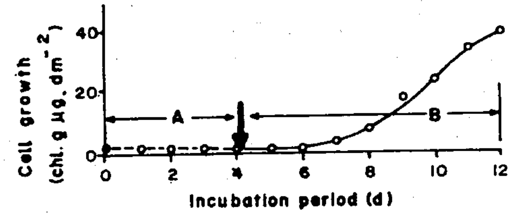

Fig. 2—Growth (Chl-*a*) of *N. subinflata* cells subjected to effective concentration of the extract A) before and B) after transferring them in extract-free medium.

that the extract might have interfered with any of the enzyme activities of the diatom, which might have resulted in the inhibition of growth[12].

From the present investigation it could be concluded that the methanolic extract of the terrestrial plant *D. scandens* possesses both antibacterial and antialgal properties. Both these properties of the extract could be used to achieve antifouling action on microfoulers especially bacteria and diatoms. Therefore, the plant *D. scandens* serves as a potential source of non-toxic antifouling agent.

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