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MICRO-ALGAL LETHALITY POTENTIALS OF MARINE ORGANISMS COLLECTED FROM THE INDIAN LITTORAL

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ABSTRACT

Microalgal lethality bioassay was developed to detect the toxic profile of organic extract of marine organisms and their possible significance in the context of antifouling activities. Organic extracts of seaweeds, Ulva fasciata and *Hypnea musciformis*, sponges, *Dendrilla nigra*, *Axinella donnai* and *Clathria gorgonoides* and a holothurian *Holothuria scabra* were used for the detection of microalgal lethality potential. The microalgae such as *Isochrysis galbana*, *Chlorella salina* and *Nanochloropsis* sp. were used for the assay. The findings revealed that *H. scabra* contained toxic secondary metabolites, which might have the reason for its potent antifouling activity. Invariably all extracts inhibited the growth of microalgae at various concentrations except *H. musciformis* and *A. donnani*, which induce the growth of microalgae to certain extent. Based on the present findings, it could be inferred that the 'microalgal lethality bioassay' could be used as a primary screening assay system for the detection of biotoxic and antifouling agents from marine organisms.

Key words: Sponge, Seaweeds, Holothuria, organic extract, *Isochrysis galbana*, *Nanochloropsis sp*. *Chlorella salina*, Microalgal-lethality.

RESUMEN

Se desarrolló un bioensayo de letalidad en microalgas para detector los perfiles de toxicidad de organismos marinos y su posible significado en el contexto de las actividades antiincrustantes. Se utilizaron extractos orgánicos de algas marinas, *Ulva fasciata* y *Hypnea musciformis*, esponjas, *Dendrilla nigra, Axinella donnai* y *Clathria gorgonoides* y una holoturia, *Holothuria scabra* para detector posibles efectos letales sobre microalgas. En el ensayo se utilizaron las microalgas *Isochrysis galbana*, *Chlorella salina* y *Nanochloropsis sp*. Los experimentos probaron que *H. scabra* contiene metabolitos tóxicos, lo que podría ser la razón para su potente actividad antiincrustante. Invariablemente todos los extractos inhibieron el crecimiento de las microalgas a distintas concentraciones excepto *H. musciformis* y *A. donnani*, que indujeron el crecimiento hasta cierto punto. Basándose en los resultados obtenidos, se propone el bioensayo de letalidad de microalgas como un sistema de ensayo para cribado primario en la detección de agentes biotóxicos y antiincrustantes obtenidos a partir de organismos marinos.

Palabras clave: Esponja, Algas marinas, Holothuria, Extractos orgánicos, *Isochrysis galbana, Nanochloropsis sp. Chlorella salina,* Mortalidad microalgas.

INTRODUCTION

The search for novel marine bioactive secondary metabolites has taken new dimension particularly on the bio-screening models. Recent reports are focusing on micro-bioassays particularly more relevant to explore ecological phenomena of marine natural products (Hellio *et al.,* 2002; Nagle *et al.,* 2004). Micro-algal toxicity is an established index for the testing of xenobiotics and toxicants (Radix *et al.,* 2000; Theegala *et al.,* 2001; Imail *et al.,* 2002). Algal toxicity based testing systems were also used for the evaluation of biotoxicity of antibacterial agents (Halling-Sørensen, 2000). In addition, xenobiotics and secondary metabolites were screened for the development of potent anti-cyanobacterial agents, which is helpful to prevent algal blooming and eutrophication in aquatic ecosystems (Schrader *et al.,* 2000; Nagle *et al.,* 2003). It is well known that microalgae are a part of biofilm, which pave the way for the colonization of macrofouling communities (Thiyagarajan *et al.,* 1999). It was presumed that the metabolite, which showed selective toxicity against microalgae, might have potent antifouling and antipredatory properties. Keeping this hypothesis in mind, the present work was initiated to explore biocidal properties of organic extract of marine organisms against micro-algae. The chosen sponges, seaweeds and holothuria showed one or more bioactivity such as antibacterial, antifungal, brineshrimp cytotoxicity, insecticidal, anticoagulant, anti-fouling and anti-predation (ichthyotoxic) activities (Selvin, 2002). In this background, the 'microalgal lethality bioassay' was developed to detect the toxic profile of these bioactive secondary metabolites and their possible significance in the context of ecological and bioprospecting approaches.

MATERIALS AND METHODS

Microalgal lethality bioassay

The organic extract of seaweeds (Selvin & Lipton, 2004a), *Ulva fasciata* and *Hypnea musciformis*, sponges (Selvin & Lipton, 2004b), *Dendrilla nigra, Axinella zonnai* and *Clathria gorgonoides* and a holothurian (Selvin & Lipton, 2004c) *Holothuria scabra* were used for the detection of microalgal lethality potential. In brief, Seaweeds were collected from the inter-tidal rocky areas along southeast and southwest coast of India (Selvin, 2002; Manilal *et al*., 2011). During collection, most abundant green alga *Ulva fasciata* and red alga *Hypnea musciformis* were collected in bulk (100 kg wet weight of each) and washed in fresh seawater to remove the epiphytes, sand and other extraneous matter. After draining off the water, the algae were wiped with a blotting sheet and air-dried under shade. Completely dried material was ground finely in a mechanical grinder and extracted in a bulk extraction process as described by Manilal *et al*. (2010).

The microalgae such as *Isochrysis galbana, Chlorella salina* and *Nanochloropsis* sp. obtained from CMFRI, Kochi (India) were used for the assay. The filter (0.2µ) sterilized seawater was autoclaved at 121ºC-15 Ibs for 15 min. The stock culture was maintained in seawater enriched with modified Walne's medium (Laing, 1990). *The media composition includes trace metal solution (TMS)*, vitamin solution and nutrient solution which were prepared separately. The TMS was prepared with $ZnCl_2$ - 2.1 g, $CoCl_2.6H_2O$ - 2.0 g, $(NH_4)_6Mo_7O_{24}.4H_2O$ - 0.9 g, and $CuSO_4.5H_2O$ - 2.0 g in 100 mL distilled water. The laresultant cloudy solution was acidified with a few drops of concentrated HCl to give a clear solution. The *vitamin solution* was prepared with cyanocobalamin - 10.0 mg, thiamine - 10.0 mg, and biotin - 200.0 µg 100 mL distilled water. The *nutrient solution* was prepared with $FeCl_3.6H_2O$ - 1.3 g, $MnCl_2.4H_2O$ - 0.36 g, and H_3BO_3 - 33.6 g, EDTA (disodium salt) - 45.0 g, $NaH_2PO_4.2H_2O$ - 20.0 g, $NaNO_3$ - 100.0g, and TMS stock solution - 1.0 ml in one litre distilled water. Finally the algal culture medium was prepared with vitamin solution - 0.1 ml, and nutrient solution - 1.0 ml in 1 L sterilised seawater. The experimental and stock cultures were maintained in optimum light (1100 ,lux), 12 h L/D and 25ºC. Growth was monitored using a Petroff-Hausser counting chamber. A stock culture of the algal culture was inoculated at a density of 5×10^4 cells mL^{-1} into flasks containing 10 mL enriched seawater with modified Walne's medium to which appropriate extract was added. Both positive (vehicle control) and negative control was maintained along with the experimental cultures. Deviations noticed in the negative control were considered in the calculation of net multiplication rate of experimental cultures. Algal growth/decline was measured at every 24 h for a period of 7 days. The experiments were repeated 7 times to achieve statistically validated data. The algal growth in the control was considered as 100% and growth/decline trend over the control flasks were plotted in the graph to extrapolate the efficacy of various concentrations of extractives on the algal growth.

RESULTS AND DISCUSSION

Results of the microalgae lethality bioassay indicated that the organic of sponges and holothuria were relatively more toxic than the seaweeds. The organic extract of *H. musciformis* inhibited the growth of microalgae *I. galbana* at 5, 2 and 1 mg/ml (Fig. 1). The growth of *Nanochloropsis sp.* was influenced at lower concentration (1 mg/ml) (Fig. 2). On the 5th day of post-exposure, the growth was increased considerably to 35.9% over the

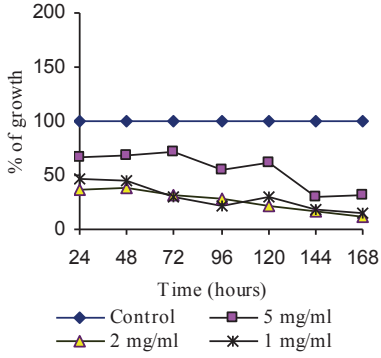

Figure 1:
Effect of Hypnea musciformis on the growth of microalgae Isogrysis galbana.

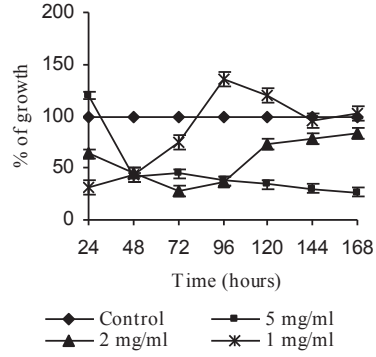

Figure 2:
Effect of Hypnea musciformis on the growth of Nanochloropsis sp.

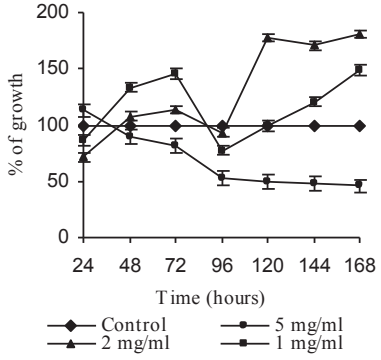

Figure 3:
Effect of Hypnea musciformis on the growth of Chlorella salina.

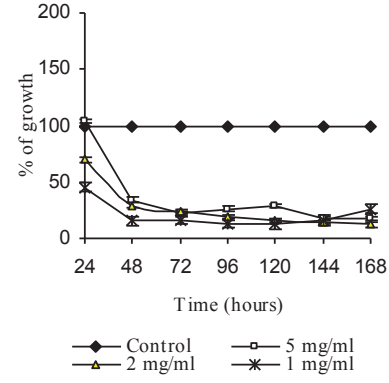

Figure 4:
Effect of Ulva fasciata on the growth of Isogrysis galbana.

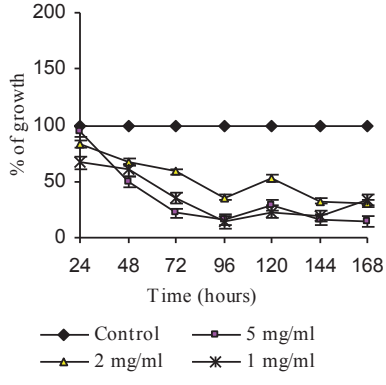

Figure 5:
Effect of Ulva fasciata on the growth of Nanochloropsis sp.

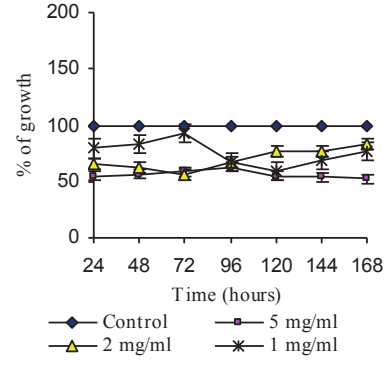

Figure 6:
Effect of Ulva fasciata on the growth of Chlorella salina.

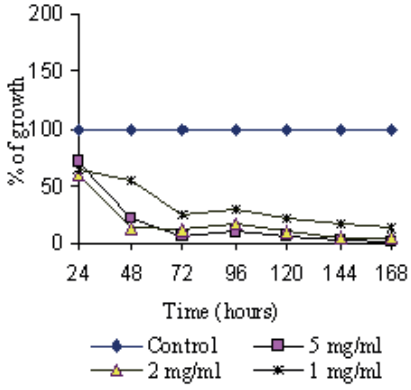

Figure 7:
Effect of D. nigra on the growth of Isogrysis galbana.

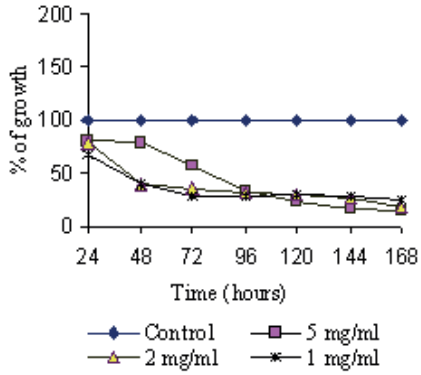

Figure 8:
Effect of D. nigra on the growth of Nanochloropsis sp.

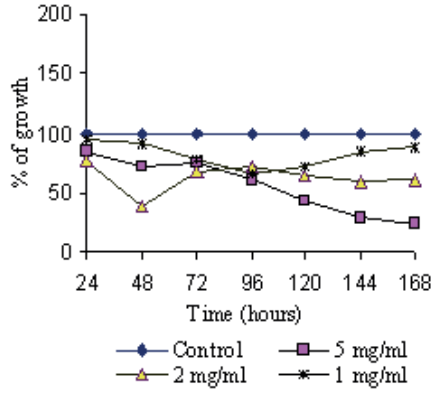

Figure 9:
Effect of D. nigra on the growth of Chlorella salina.

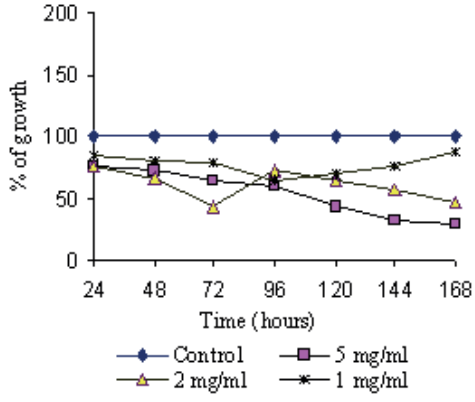

Figure 10:
Effect of Ulva fasciata on the growth of Isogrysis galbana.

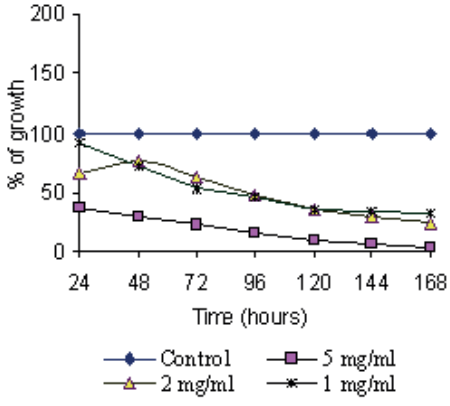

Figure 11:
Effect of C. gorgonoides on the growth of Nanochloropis sp.

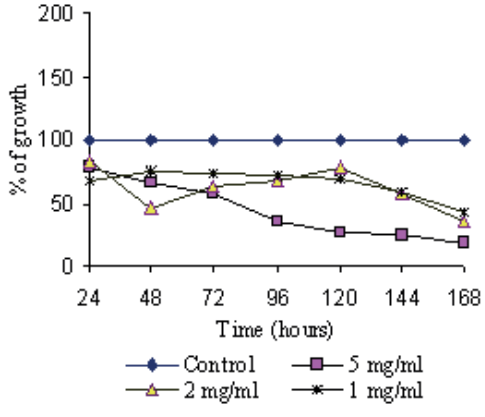

Figure 12:
Effect of C. gorgonoides on the growth of Chlorella salina.

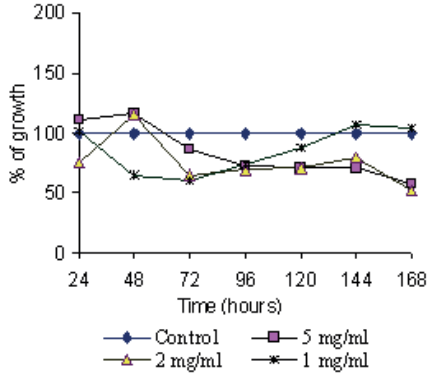

Figure 13:
Effect of A. donnani on the growth of Isogrysis galbana.

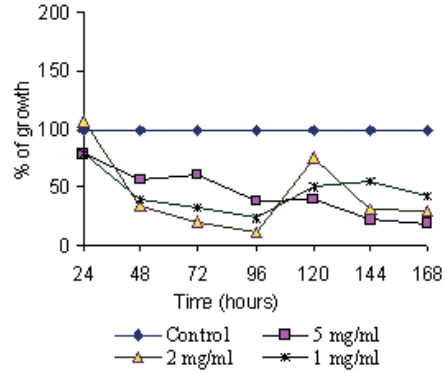

Figure 14:
Effect of A. donnani on the growth of Nanochloropsis sp.

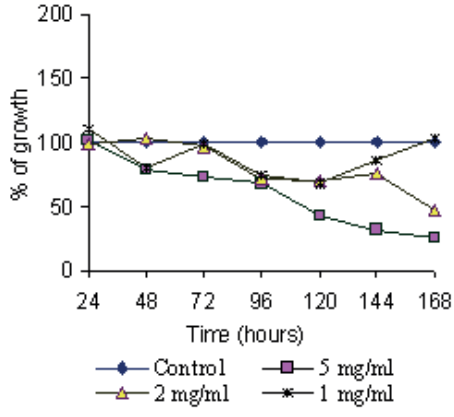

Figure 15:
Effect of A. donnani on the growth of Chlorella salina.

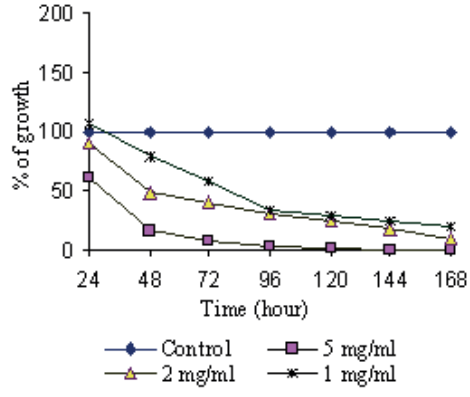

Figure 16:
Effect of Holothuria scabra on the growth of Isogrysis galbana.

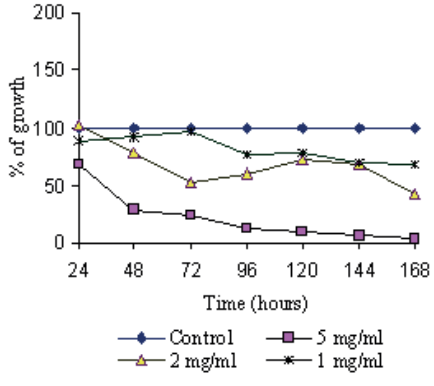

Figure 17:
Effect of Holothuria scabra on the growth of Nanochloropsis sp.

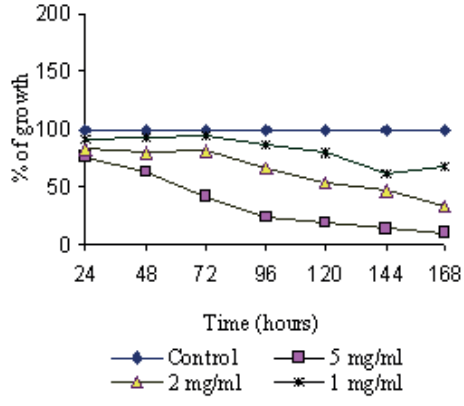

Figure 18:
Effect of Holothuria scabra on the growth of Chlorella salina.

control group. However, the following days, growth was reduced but not below the control level. It was noteworthy that the higher concentration (5 mg/ml) inhibited the growth whereas at 2 mg/ml, the growth was slightly increased. *H. musciformis* induced the growth of *C. salina* to the extent of 80.69% over the control group on the 7th day of post-exposure at 2 mg/ml (Fig. 3). Albeit, the growth of *C. salina* was increased at the lower dose (1 mg/ml), the higher dose (5 mg/ml) was found to be inhibitory to the extent of 45.36%. The organic extract of *U. fasciata* inhibited the growth of microalgae such as *I. galbana*, *Nanochloropsis* sp. and *C. salina*. The inhibitory potential was greater against *I. galbana* followd by *Nanochloropsis* sp. and *C. salina* (Fig. 4-6). A lower growth inhibition was observed against *Nanochloropsis* sp. at 2 mg/ml compared to 1 and 5 mg/ml. The inhibition potential of *U. fasciata* was reduced against *C. salina*. At 1 mg/ml, initially the growth was restored near the control group and then declined.

In the case of sponges, *D. nigra* and *C. gorgonoides* were extremely toxic whereas *A. donnani* was less toxic. *D. nigra* effectively prevented the growth of *I. galbana* and *Nanochloropsis* sp. at all concentrations (Fig. 7 and 8). However, it exhibited lower level of toxicity against *C. salina* and the growth was restored about near to the control group at 1 mg/ml (Fig. 9). In the case *C. gorgonoides,* extreme toxicity was observed against *Nanochloropsis sp.* (Fig. 11). The toxicity was extended against the growth of *I. galbana* and *C. salina* at all concentrations (Fig 10 and 12). Notably, the organic extract of *A. donnani* induced the growth of *I. galbana* and *C. salina* at 1 mg/ml (Fig. 13 and 15). On the 7th day of post-exposure, the growth of *I. galbana* was influenced to the extent of 4.25% over the control value. However at 5 and 2 mg/ml, the growth was declined after 48 h of exposure. A drastic decline of growth was noticed at all concentrations against *Nanochloropsis* sp. (Fig. 14).

In the case of *H. scabra*, the growth of *I. galbana* declined drastically at all concentrations (Fig. 16). Moreover, the algal growth was depleted after 96 h of post exposure at 5.0 mg/ml. A dose-dependent chronological decline of growth was noticed against *C. salina* (Fig. 18). Notably, the growth of *Nanochloropsis* sp. was restored up to 72 h at 1 mg/ml (Fig. 17). The culture was completely depleted at 5 mg/ml on the 7th day. The findings revealed that *H. scabra* contained toxic secondary metabolites, which might have responsible for the potent antifouling activity (Selvin & Lipton, 2004c). Other researchers also reported the presence of potent antifouling secondary metabolites in various holothurian species. According to Mokashe *et al.,* (1994), the methanol extract of a *H. leucospilota* effectively prevented the growth of biofilm forming marine diatoms, *Navicula subinflata* and *N. crucicula*. When we consider overall results for discussion, it could be concluded that the tested extracts were invariably biotoxic, though *H. musciformis* and *A. donnani* induced the growth of microalgae to certain extent. Mary *et al.*, (1991) reported the methanol and methylenechloride extract of mollusc *Onchidium verruculatum* induced the growth of *Dunaliella tertiolecta* at a concentration of 10 mg/ml. However the same concentration inhibited the growth of *I. galbana*. The exact mechanism of growth inhibition was not known. However, the organic compounds present in the extract may act as chelators. Naturally occurring organic chelators are involved in growth regulation of microorganisms (Neilands, 1967). It was reported that the tissues of some red, brown and green seaweeds do contain recognized plant hormones (Chapman, 1987). It may be the reason for growth enhancing activity noticed in the case of *H. musciformis*. Moreover, reports available on seaweed-based cytokinins have functional roles of promoting terrestrial plant growth (Stirk *et al.,* 2002). Considering the impacts of TBT and heavy metal based antifouling paints, efforts are being undertaken by several researchers to develop novel antifouling compounds from marine organisms. The compounds based on this assay may demonstrate the principle that chemical defense of marine organisms against the colonisation of epibionts on their own surfaces. Microalgal based anti-settlement assay was found effective to detect antifouling agents from algal extracts (Hellio *et al.,* 2002). Iken *et al.,* (2003) developed a novel bioassay, based on the swimming pattern of algal spores. This assay was used to evaluate antifouling properties of echinoderm extracts. Based on the present findings, it could be inferred that the 'microalgal lethality bioassay' could be used as a primary screening assay system for the detection of biotoxic and antifouling agents from marine organisms.

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