

Antifouling activities of marine sedentary invertebrates on some macrofoulers

V Wilsanand, A B Wagh* & M Bapuji

Forest and Marine Products Division, Regional Research Laboratory (CSIR), Bhubaneswar, 751013, Orissa, India

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Antifouling activities of alcohol extracts from four gorgonian species (*Melitodes* sp., *Gorgonella sanguinolenta*, *Echinogorgia complexa*, *Acanthogorgia turgida*), five soft corals [*Dendronephthya* sp. 1, *Dendronephthya* sp. 2, *Dendronephthya (Roxasia)* sp., *Dendronephthya (Morchellana)* sp., *Dendronephthya (Spongodes)* sp.], three unidentified antipatharian species (coded as antipatharian sp.1, antipatharian sp.2, antipatharian sp.3) and five sponges [*Plakortis simplex*, *Axinella* sp., *Fasciospongia cavernosa*, *Azorica pfeifferae*, *Dercitopsis* sp.] against cyprids of the barnacle *Balanus amphitrite* and green mussel *Perna viridis* are reported here. All the extracts examined inhibited the settlement of cyprids of *B. amphitrite*. The EC₅₀ values of the extracts ranged from 16 ± 3 µg/ml (antipatharian sp.2) to 150 ± 8 µg/ml (antipatharian sp. 3). The LC₅₀ values of the extracts varied from 38 ± 3.8 µg/ml [*Dendronephthya (Roxasia)* sp.] to 218 ± 30.6 µg/ml (*Fasciospongia cavernosa*). A comparison of the EC₅₀ and LC₅₀ values reveal that most of the extracts are non toxic to the cyprids of *B. amphitrite*. In the case of *P. viridis* only 7 out of 17 extracts (41%) inhibited byssal production and attachment. Gorgonian coral extracts displayed highest incidence of attachment inhibitory activity (75%) followed by soft corals (40%), antipatharians (33%) and sponges (20%), when tested against *P. viridis*. The EC₅₀ values of the extracts ranged from 164 ± 12 µg/ml (*A. pfeifferae*) to 898 ± 11 µg/ml [*Dendronephthya (Morchellana)* sp.]. Toxicity revival studies indicate that the transient presence of active extracts did not cause irreversible damage to *P. viridis*. The results indicate the possible potentialities of these extracts as natural non toxic antifouling agents.

Marine fouling organisms such as barnacles and mussels cause serious problems to various industries in the marine sector particularly influencing the activities of shipping, and maintaining offshore structures, thermal power plant operation, OTEC systems, aquaculture systems and other submarine infrastructures¹. Copper and organotin compounds such as tributyltin oxide (TBTO) have been used as effective antifoulants². However, these compounds are of environmental concern. It has been observed that TBT causes sex reversal in marine gastropods³ and is also known to be harmful to a wide range of non target aquatic marine organisms including micro algae⁴, molluscs and crustaceans⁵, fish⁶ and invertebrate communities of sea grass beds⁷. The adverse impact of TBT on non-target organisms has led to the introduction of legislative control on TBT in many countries.

Various studies indicate that marine sedentary invertebrates use chemical defense against biofouling species⁸⁻¹⁰. Natural products being biodegradable show restricted toxicity, therefore, these substances are considered to have potential as antifoulants. An attempt has been made to study the antifouling

activities of some marine sedentary organisms like sponges, gorgonians, soft corals and antipatharians against the cyprids of *Balanus amphitrite* and the green mussel *Perna viridis* and the results obtained are presented here.

Materials and Methods

Samples of the sponges [*Plakortis simplex* Schulze, *Axinella* sp., *Fasciospongia cavernosa* (Schmidt), *Azorica pfeifferae* Carter, *Dercitopsis* sp.]; gorgonian corals [*Melitodes* sp., *Gorgonella sanguinolenta* Gray, *Echinogorgia complexa* Nutting, *Acanthogorgia turgida* Nutting]; soft corals [*Dendronephthya* sp. 1, *Dendronephthya* sp. 2, *Dendronephthya (Roxasia)* sp., *Dendronephthya (Morchellana)* sp., *Dendronephthya (Spongodes)* sp.] and 3 unidentified antipatharian species coded as antipatharian sp. 1, antipatharian sp. 2, antipatharian sp.3, were collected from a depth of 28-30 m off Gopalpur coast, Bay of Bengal (lat., 19°14' and 20°02' N; long., 82°02'-85°51' E) by SCUBA divers. The samples were extracted in ethanol and the vacuum dried extracts were used for the antifouling assays.

Rearing and settlement inhibition of cyprids of *B. amphitrite* were carried out following the method of Ritschoff *et al.*¹¹⁻¹². Ten to twenty 3 day-old

*Institute of Marine Affairs, Hilltop Lane, Chaguaramas, Trinidad and Tobago, West Indies.

cyprids were pipetted into experimental beakers, in triplicate, containing 20 ml of sterilised filtered seawater with approximately 500 µg/ml of extract. Three beakers were kept as control without adding any extract. Beakers were then incubated for 24 hours at room temperature in a controlled 15:9h LD cycle. After 24 hours of incubation the beakers were carefully observed under microscope. Cyprids are scored as 1) dead—if they do not move and if they do not respond when touched gently with a needle, 2) permanently attached and 3) unattached. Extracts that completely inhibited the settlement of cyprids were subjected to more detailed assay by taking dilution series of the extracts, in triplicate, in a series of beakers with 20 ml autoclaved filtered seawater, with a control also in triplicate. Ten to 20 cyprids were added to each beaker. Incubation and scoring were done as mentioned above. Probit analysis by Reddy *et al.*¹³ was used for the estimation of EC₅₀ and LC₅₀ values.

Small green mussels of *P. viridis*, of approximately 1.5-2 cm length were collected from local environment during low tide period. The shells were cleaned for any adhered organic or inorganic deposits using seawater. The byssus threads, if any, were cut off. They were then rinsed with filtered seawater. Six mussels were then transferred to experimental beakers containing 100 ml of aged, sterilised filtered seawater containing approximately 1 mg/ml of extract concentration. Three replicates were used for each treatment. Three beakers were kept as control without adding any extract. The beakers were then incubated at room temperature with mild aeration under a 15: 9h LD cycle. The mussels were not fed during the experimental period. After 24 hours, the beakers were visually observed for attachment of byssus and were scored as 1) mussels that attached, 2) mussels that failed to attach and 3) mussels that died—if shells were open even after touching with a glass rod. Extracts which have inhibited byssus production were subjected to a detailed experiment to examine the EC₅₀ (effective concentration for 50% inhibition of attachment). Six mussels each were transferred to a series of beakers, each containing 100 ml of sterilised seawater and different concentrations of extract. Three replicates were used for each treatment. Three beakers were kept as control without adding any extract. Incubation and observation were carried out as mentioned earlier. EC₅₀ values were obtained by probit analysis¹³.

Toxicity assays were carried out following standard methods¹⁴. Mussels from the experimental beakers which completely inhibited the byssal attachment, were kept for further 72 hours in the same condition. After 72 hours, the mussels were cleaned two to three times with sterilised seawater and transferred to extract free beakers containing the same quantity of sterilised filtered seawater. They were then kept under the same conditions and were observed for byssal production and attachment after every 24 hours for a period of 4 days. Percentage revival in terms of byssal production and attachment were calculated.

Results

All the extracts under study inhibited the settlement of cyprids of *B. amphitrite*. When extracts were tested against *Perna viridis*, 3 gorgonian corals (*G. sanguinolenta*, *A. turgida*, *E. complexa*), 2 soft corals (*Dendronephthya (Roxasia)* sp., *Dendronephthya (Morchellana)* sp.), 1 antipatharian (antipatharian sp.2) and 1 sponge (*A. pfeifferae*) displayed attachment inhibitory activity (41.2%). When data are considered separately within each taxon, the gorgonians displayed maximum attachment inhibitory activity (75%); soft corals (40%), antipatharians (33.3%), and sponges (20%) ranked next to gorgonians in the order of percentage activity. Mussels displayed 100% attachment in control beakers with plain seawater. Mussels, except those in control and treatments with lower active extract concentrations remained with their shells tightly closed.

The EC₅₀ values of the extracts against cyprids (Fig. 1) ranged from 16.25 ± 3µg/ml (antipatharian sp.2) to 150 ± 7.9µg/ml (antipatharian sp. 3). The LC₅₀ values of the extracts varied from 38 ± 3.8µg/ml [*Dendronephthya (Roxasia)*] sp. to 218 ± 30.6µg/ml (*F. cavernosa*). Excepting *P. simplex*, *A. pfeifferae* and *Dercitopsis* sp., the EC₅₀ values were much less than the LC₅₀ values. The EC₅₀ values of the active extracts against *P. viridis* (Fig. 2) ranged from 164 ± 12µg/ml (*A. pfeifferae*) to 898 ± 11 µg/ml (*Dendronephthya (Morchellana)* sp.). The EC₅₀ values for *G. sanguinolenta*, *A. turgida*, *E. complexa*, *Dendronephthya (Roxasia)* sp., and antipatharian sp.2 being 794 ± 18, 198 ± 21.4, 787 ± 27.67, 812 ± 18.7 and 722 ± 18.43 µg/ml respectively. The EC₅₀ values of *A. pfeifferae* and *A. turgida* were about 4 to 5 times less than that of other active extracts. EC₅₀ values for

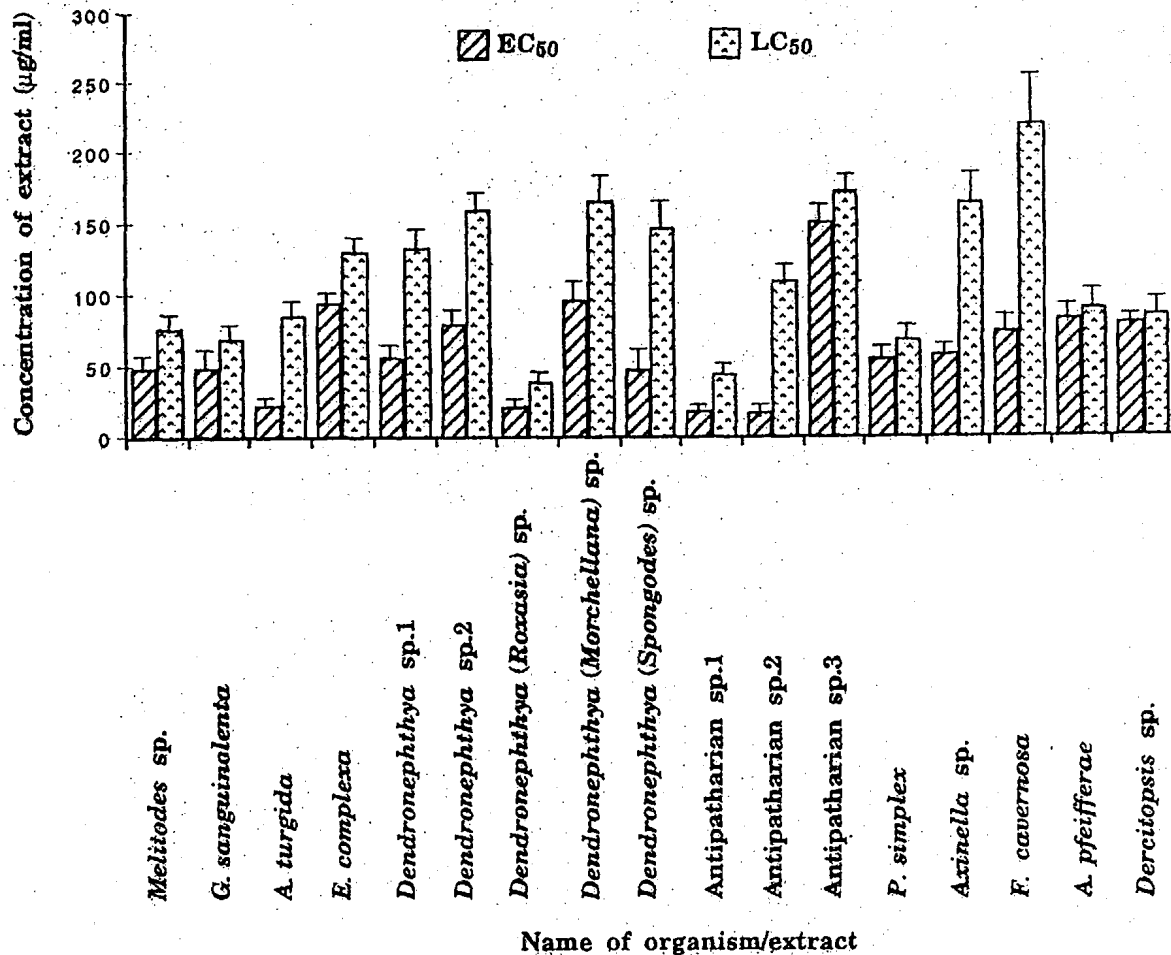


Fig. 1—Comparison of EC₅₀ and LC₅₀ values (\pm SE) of various extracts against cyprids of *B. amphitrite*.

the mussels were significantly higher than those recorded for cyprids of *B. amphitrite*. This shows that mussels are highly resistant to the extracts under study.

Table 1 indicates that 50 - 100% of the mussels treated with EC₁₀₀ values of the active extracts revived byssal production and attachment after media replenishment. Within 24 hours of media replenishment, 100% recovery was observed in *G. sanguinolenta* and antipatharian sp.2 treatments. However, in the case of *Dendronephthya (Roxasia)* sp., *Dendronephthya (Morchellana)* sp., and *E. complexa* only 83% of the mussels were recovered and the rest died. In the case of *A. turgida* only 67% recovered, the rest were alive but did not revive even after 96 hours of media replenishment.

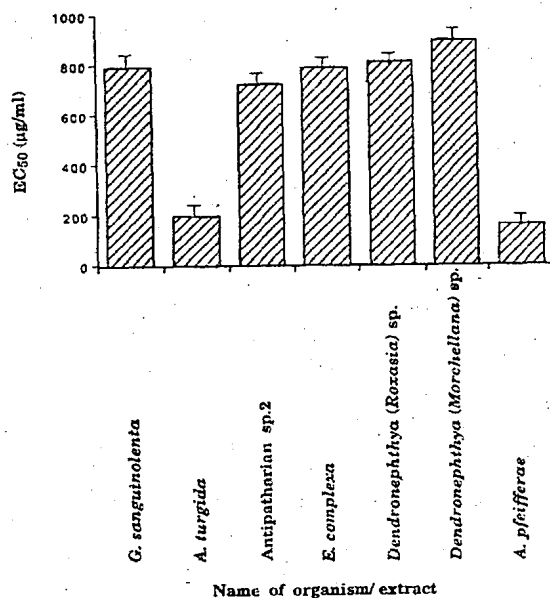
Discussion

All the 17 extracts under study inhibited the settlement of cyprids of *B. amphitrite*. This is the first report of antissettlement activities by antipatharian colonies against cyprids of *B. amphitrite* and the green mussel *P. viridis*. Thus, hypotheses concerning the use of chemical defense mechanisms against biofouling organisms could be extended to hexacorals. Furthermore we propose that these previously neglected taxa demonstrate potential source of natural antifouling compounds. The EC₅₀ values exhibited by crude extracts under study are comparable to extracts from marine sponges reported by Willemsen¹⁵.

Except for extracts from *P. simplex*, *A. pfeifferae* and *Dercitopsis* sp., EC₅₀ values were much less than respective LC₅₀ values against cyprids of

Table 1—Percentage revival of *P. viridis*, previously treated with active extracts, after media replenishment

Name of organisms	24 hours			48 hours			72 hours			96 hours		
	Revival (%)	Alive but not revived (%)	Dead (%)	Revival (%)	Alive but not revived (%)	Dead (%)	Revival (%)	Alive but not revived (%)	Dead (%)	Revival (%)	Alive but not revived (%)	Dead (%)
<i>G. sanguinolenta</i>	100	0	0	—	—	—	—	—	—	—	—	—
<i>A. turgida</i>	33	67	0	33	67	0	67	33	0	67	33	0
Antipatharian sp. 2	100	0	0	—	—	—	—	—	—	—	—	—
<i>E. complexa</i>	67	33	0	83	17	0	83	17	0	83	0	17
<i>Dendronephthya</i> (<i>Roxasia</i>) sp.	83	0	17	—	—	—	—	—	—	—	—	—
<i>Dendronephthya</i> (<i>Morchellana</i>) sp.	83	17	0	83	17	0	83	0	17	—	—	—
<i>A. pfeifferae</i>	50	—	50	—	—	—	—	—	—	—	—	—

Fig. 2—Comparison of EC_{50} values (\pm SE) of various active extracts against *P. viridis*.

B. amphitrite. This suggests that most extracts tested are not toxic. A comparison of the LC_{50} and EC_{50} data provides the relationship between toxicity and settlement inhibition¹¹. Compounds that are known to act by toxic mechanisms (copper and tributyltin) have similar LC_{50} and EC_{50} values. In contrast, compounds such as renillafoulins (natural product from the octocoral *R. reniformis*) have low EC_{50} and high LC_{50} values. These assays therefore provide some insight into the mechanism of action of a compound(s) yet to be elucidated¹¹. Compounds exhibiting lower or

negligible toxicity, but which continue to inhibit settlement are potentially more environmentally acceptable solution to fouling.

Mussels are the most dominant macrofouling organisms, yet few marine natural product investigators concerned with antifouling studies have examined them¹⁰. A review of the published literature¹⁶ revealed no report of antifouling activities of marine sedentary organisms against *P. viridis*. However, antifouling activities of sponges against the blue mussel *M. edulis*¹⁰ demonstrated that the fatty acid found in the sponge *Phyllospongia papyracea* inhibited the byssal production of *M. edulis*.

Of the 17 extracts assayed, only 7 extracts inhibited byssal production completely. Gorgonians showed appreciable activity against mussels. However, apart from the gorgonian coral *A. turgida* and the sponge *A. Pfeifferae*, the EC_{50} values were high. The results imply that the mussels are relatively insensitive to extract at lower concentrations. It is evident that 50 to 100% of the mussels (Table 1) recovered immediately after media replenishment implying that the transient presence of extracts had not caused any irreversible damage to the test organisms. It is obvious that mussels closed their shells with increasing concentration of active extracts. Several workers have also reported that larvae and adult *Mytilus* close their shell valves to isolate their tissues from the environment when exposed to high copper concentrations¹⁷. Davenport¹⁷ showed that this response could allow mussels to survive intermittent exposure to lethal copper concentrations, provided that animals were able to gain regular access to clean seawater. Davenport¹⁷

and Martin *et al.*¹⁸ have indicated that mussels exposed to higher concentrations secrete fewer byssal threads. Similar observations are also noticed in the present studies. When an average of 30 byssal threads were produced by control mussels, mussels kept in active extracts (lower concentrations) produced only an average of 10 byssus, the number of byssus decreasing with increasing concentrations. However, in the present study, the number of byssus produced was not considered as a criteria for assessing activity, rather, total inhibition of byssal production was taken into consideration.

Sedentary organisms like gorgonians, soft corals and sponges are known to contain variety of novel and highly bioactive compounds which have been hypothesized to function in chemical defense¹⁹. Chemical defence may be species specific i.e bacterial²⁰, microalgal⁸, macroalgal²¹ and larval⁹ or may exhibit broad spectrum activity²². Our earlier studies²³ indicate that all the five sponge extracts used in the present studies have shown moderate antibacterial activity against four dominant marine fouling bacterial strains pointing towards broad spectrum activity. Further studies on identification of the active compounds and field trials, which are in progress would probably give a clear picture on the possible potentialities of these extracts as natural antifouling compounds.

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