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Short Communication

Antifouling activity by sea anemone (*Heteractis magnifica* and *H. aurora*) extracts against marine biofilm bacteria

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ABSTRACT. Sea anemones (Actiniaria) are solitary, ocean-dwelling members of the phylum Cnidaria and the class Anthozoa. In this study, we screened antibacterial activity of two benthic sea anemones (*Heteractis magnifica* and *H. aurora*) collected from the Mandapam coast of southeast India. Crude extracts of the sea anemone were assayed against seven bacterial biofilms isolated from three different test panels. The crude extract of *H. magnifica* showed a maximum inhibition zone of 18 mm against *Pseudomonas* sp. and *Escherichia coli* and a minimum inhibition zone of 3 mm against *Pseudomonas aeruginosa*, *Micrococcus* sp., and *Bacillus cerens* for methanol, acetone, and DCM extracts, respectively. The butanol extract of *H. aurora* showed a maximum inhibition zone of 23 mm against *Vibrio parahaemolyticus*, whereas the methanol extract revealed a minimum inhibition zone of 1 mm against *V. parahaemolyticus*. The present study revealed that the *H. aurora* extracts were more effective than those of *H. magnifica* and that the active compounds from the sea anemone can be used as antifouling compounds.

Keywords: anemones, bioactive metabolites, novel antimicrobial, biofilm, natural antifouling, India.

Actividades antiincrustantes de las extractos de las anémonas marinas *Heteractis magnifica* y *H. aurora* frente a biofilm de bacterias marinas

RESUMEN. Las anémonas de mar (Actiniaria) son solitarias, habitantes oceánicos del phylum Cnidaria y de la clase Anthozoa. En este estudio se determina la actividad antibacteriana de dos anémonas bentónicas *Heteractis magnifica* y *H. aurora* recolectadas en la costa de Mandapam, sudeste de India. Los extractos crudos de estas anémonas fueron ensayados frente a siete biofilms bacterianos aislados de tres paneles de control distintos. El extracto crudo de la anémona *H. magnifica* mostró una zona inhibición máxima de 18 mm contra *Psudomonas* sp. y *Escherichia coli* y la zona de inhibición mínima de 3 mm fue encontrada frente a *Pseudomonas aeruginosa*, *Micrococus* sp. y *Bacillus cerens* de extractos de metanol, acetona y DCM respectivamente. El extracto de butanol de la anémona *H. magnifica* mostró una zona de inhibición máxima de 23 mm frente a *Vibrio parahemolyticus*, mientras que con el estracto de metanol se observó una zona de inhibición mínima de 1 mm frente a *V. parahemolyticus*. El presente estudio mostró que los extractos *H. aurora* son más efectivos que los de *H. magnifica* y que los compuestos activos de las anémonas de mar pueden ser usados como compuestos anti-incrustantes.

Palabras clave: anémonas, metabolitos bioactivos, novela antimicrobiana, biopelícula, antiincrustantes naturales, India.

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Marine biofouling is an extensive phenomenon causing large penalties to engineered structures such as ships and offshore platforms by way of increased use of manpower, fuel, material and dry-docking time (Chambers *et al.*, 2006). Until recently, antifouling paints containing Tributyltin (TBT) and copper compounds were effectively used to combat fouling (Evans, 2001). However, these compounds, especially

TBT, were reported to be highly toxic and persistent in the marine environment, and were proved to have adverse effects on non-target marine organisms (Omae, 2003; Yebra *et al.*, 2004). Under these circumstances, search for natural antifouling compounds as ecologically compatible substitutes for chemical biocides is progressing worldwide (Sipkema *et al.*, 2005; Raveendran *et al.*, 2008).

Natural products and their synthetic analogs exhibiting anaesthetic, repellent and settlement inhibition properties, but non-toxic to the non-target organisms, are preferred as potential antifouling agents (Omae, 2003). In this regard, sessile, soft-bodied marine organisms maintaining a clean surface were identified as possible sources of natural product antifoulants (NPAs). This was attributed mostly to the production of secondary metabolites, presumably as a means of protection from predation, colonization by epizoic organisms or to reduce competition for space (Wahl *et al.*, 1994).

Sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs, many of which exhibit structural features, not found in terrestrial natural products (Ireland *et al.*, 1988). Of the natural products isolated from marine organisms, only less than 1% has been examined so far for pharmacological activity (Rao *et al.*, 1985; Fusetani, 2000). Much of the studies are warranted to find antimicrobial activity in the resistant strains of microorganisms. Sea anemones (Actiniaria) are solitary, ocean dwelling member of the phylum Cnidaria and the class Anthozoa. Among the marine organisms, Anthozoa are ecologically important animals, which need to protect themselves against the lethal or debilitating consequences of microbial or parasitic invasion (Ramalingam & Ramarani, 2006). The ability of anthozoans to display discriminatory tissue reactions to foreign grafts has been demonstrated by many workers (Jokiel & Bigger, 1994; Rinkevich *et al.*, 1994; Perma *et al.*, 2005). The present study was aimed and find out the efficacy antimicrobial activity of two benthic sea anemones against primary film forming bacteria.

Specimen collection and identification

Two species of sea anemones, *Heteractis magnifica* and *H. aurora* were collected from Mandapam (09º16'N, 72º12'E), Southeast coast of India by SCUBA diving at the depth ranging from 3 to 5 m between February and March, 2009. The samples were thoroughly washed with sea water and removed sand, mud and overgrowing organisms at the site of collection, and the sample was packaged with air in thin cover to the laboratory and maintain in culture tank. Collected specimens were identified by following the standard literature of Indo-Pacific coral reef field guide (Geraled & Steene, 1998).

Preparation of crude extract

The entire body of two different sea anemones, *Heteractis magnifica* and *H. aurora* were washed with cleaned sea water and later for extraction; 1 kg of each sea anemones were cut into small pieces and approximately 200 g of sea anemone pieces were immersed with five different solvents in separately. Methanol, Dichloromethane, Ethanol, Acetone and Butanol by using these solvents animal was homogenised, extracted with respective solvents and filtered through Whatman® Nº1. Filter paper (0.4 µm); it was then evaporated at low pressure using an R-200 Buchi Rotavapor® at 30ºC. The resultants were stored at 4ºC for further use. These crude extracts were used for antibacterial activity against biofilm bacterial strains were isolated from the different test panels.

Isolation of biofilm bacteria for antibacterial activity

The fouling bacteria used in the antibacterial assay were isolated from the biofilm formed over aluminium, fiber glass and wood panels by pour plate method (Wahl, 1995). The panels were deployed for about a month during October to November 2009 at 1m-depth in the Vellar estuary boat jetty (11º29'N, 79º46'E)**,** southeast coast of India. The panels were washed with sterile seawater before swabbing with sterile cotton swabs. The swabs were placed in a tube containing sterile seawater, serially diluted and inoculated in marine agar plates with Zobell marine agar media. The plates were incubated at room temperature for 24 h. The pure bacterial strains based on colony morphology, colour and appearance were isolated by repeated streaking and identified up to genus level (Holt *et al.*, 1994). The isolated bacterial strains were stored in slant at 4ºC for antibacterial assay.

Antibacterial activity

Antibacterial activity was carried out by using the standard disc diffusion method (Murugan & Santhana-Ramasamy, 2003). The following seven biofilm bacterial pathogens, *Pseudomonas aurogenosa*, *Micrococus* sp. *Bacillus cerens*, *Pseudomonas* sp. *Escherichia coli*, *Vibrio cholerae* and *V. parahemolyticus* were used. The extracts were applied to 6 mm sterile discs in aliquots of 30 µL of solvent allowed to dry at room temperature, and placed on agar plates seeded with microorganisms. The bacteria were maintained on nutrient agar plates and incubated

at 37ºC for 24 h. The zones of growth inhibition were measured after 24 h incubation. All extracts were tested thrice at a concentration of 30 µL disc^{-1}.

The crude extracts from the two species of sea anemones, *Heteractis magnifica* and *H. aurora* was screened the antibacterial activity against seven biofilm forming bacteria. The growth inhibition zones of *H. magnifica* extracts showed (Fig. 1). The maximum inhibition zone (16.0 ± 1.2 mm) was observed against *Psudomonas* sp. and *E. coli* in the butanol extract and the minimum inhibition zone (4.0 ± 0.8 mm) was noticed against *Pseudomonas aeruginosa*, *Micrococus* sp. and *Bacillus cerens* of methanol, acetone and DCM extracts respectively. Whereas the extract of *H. aurora*, the maximum inhibition zone (22.6 ± 0.4 mm) showed against *V. parahemolyticus* of the butanol extract and the minimum inhibition zone was showed (1 ± 0.8 mm) against *Vibrio parahemolyticus* of the methanolic extract shown (Fig. 2). No inhibition zones were noticed against *B. cereus*, *Psudomonas* sp., *E. coli* and *V. cholerae* in both methanol and dichloromethane extracts.

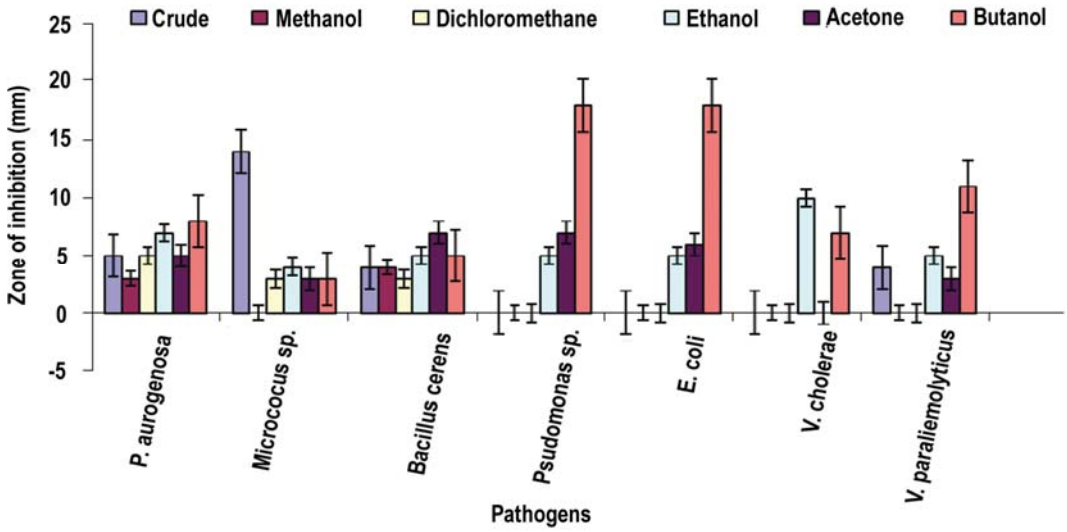

Figure 1. Antibacterial activity of *Heteractis magnifica* Quoy & Gaimard, 1833 against biofilm bacteria.

Figura 1. Actividad antibacteriana de *Heteractis magnifica* Quoy & Gaimard, 1833 frente a biofilm bacteriano.

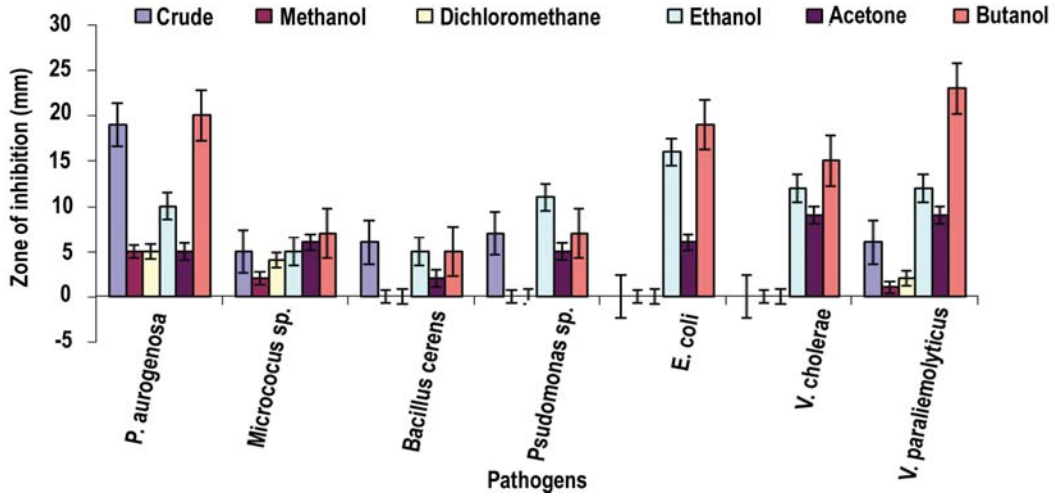

Figure 2. Antibacterial activity of *Heteractis aurora* Quoy & Gaimard, 1833 against biofilm bacteria.

Figura 2. Actividad antibacteriana de *Heteractis aurora* Quoy & Gaimard, 1833 frente a biofilm bacteriano.

The present investigation, the *H. magnifica* was showed the maximum inhibition zone (16.0 ± 1.2 mm) against *Psudomonas* sp. and *E. coli* in the butanol extract and minimum inhibition zone (4.0 ± 0.8 mm) was noticed against *P. aeruginosa, Micrococus* sp. and *B. cerens* of methanol, acetone and DCM extracts. Whereas the *Heteractis aurora*, the maximum inhibition zone (22.6 ± 0.4 mm) showed against *V. parahemolyticus* of the butanol extract and the minimum inhibition zone was showed (1 ± 0.8 mm) against *V. parahemolyticus* of the methanolic extract. A similar result was considered with previous authors work.

The crude extract of diethyl ether showed good activity against Gram positive and Gram negative bacteria (Ravikumar *et al.*, 2002). Of the human pathogens tested, *Klebsiella pneumonia* is highly inhibited (20 mm) by the tissue extract of sea anemone by using chloroform and methanol extract and 24 mm with the hexane tissue extract against fish pathogen reported by Prakash-Williams *et al.* (2007). Bhosale *et al.* (2002) have reported antimicrobial property of marine organisms against biofilm bacteria isolated from test panels. As an earlier report has been made, the crude extract of *Stichodactyla haddoni* showed good activity against Gram-negative bacteria (Sureshkumar *et al.*, 2002). Hutton & Smith, (1996) has reported the amoebocytes from the sea anemone *Actinia equine* showed considerable inhibitory activity against Gram-negative bacteria within 3 h.

Patterson-Edward & Murugan (2000) have reported broad-spectrum antibacterial activity of aqueous ink extract of the cephalopods *Loligo duvauceli* and *Sepia pharaonis* against nine human pathogens. Murugan *et al.* (1991) have reported the antibacterial activity of the hypobranchial gland of *Rapana rapiformis* eight human against pathogens. Such like my result considered with previous authors results. In the present study have shown moderate antibacterial activity against seven marine biofilm bacterial strains. In future the active compounds of sea anemones can be used as a natural antifouling compounds.

Sea anemones collected along the southeast coast of India. This preliminary assay indicated that sea anemones are interesting source for antibacterial metabolites and also that *H. magnifica* and *H. aurora* are shows the strong antibacterial activity against marine biofouling bacteria. In order to minimize the economic loss, fuel consumption and metal corrosion due to the formation of biofilms, there is an urgent need for identifying suitable natural antifouling agents to control the biofouling bacteria which is abundant in the marine environments. The sea anemone extract is a good source of antifouling compounds so further studies are being continued to purify and identify the active fraction.

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