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Isolation of pigments from sea-anemones, *Heteractis magnifica* (Quoy and Gaimard, 1833) and *Stichodactyla haddoni* (Kent, 1893) and their effects against aquatic and human bacterial pathogens

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ABSTRACT

Objective: To screen inhibitory activity of pigments extracted from two sea-anemones, *Heteractis magnifica* (*H. magnifica*) and *Stichodactyla haddoni* (*S. haddoni*) against 10 aquatic and 10 human bacterial pathogens. **Methods:** Crude pigment were extracted by using acetone solvent and the pigment extracts were fractionated into 7 for *H. magnifica* and 5 for *S. haddoni* by using silica gel column chromatography and also tested for the antibacterial activity using Agar well-diffusion method. **Results:** The 3rd fraction of *H. magnifica* and 2nd fraction of *S. haddoni* displayed higher activity against eight aquatic bacterial pathogens and seven human bacterial pathogens. **Conclusions:** The 3rd fraction of *H. magnifica* showed higher antibacterial activity than the crude pigment extracts and other fractions of *H. magnifica* and *S. haddoni*. Thus the sea-anemone *Heteractis magnifica* is promising for further exploration of antibacterial drugs.

1. Introduction

Marine natural products have interesting biomedical potential, pharmaceutical relevance and diverse applications. More than 12,000 compounds have been isolated from marine sources with hundreds of new compounds still being discovered every year. Marine invertebrates that are sessile organisms like sea-anemones, sponges and soft corals provided the largest number of secondary metabolites for bio-activity including antimicrobial, anti-tumour, anti-inflammatory, antioxidant, enzyme inhibitors, cell division-inhibitors, ctotoxic or cardiovascular properties *etc*[1]. Bacterial pathogens may cause a serious snag for aquaculture and may also create health hazards to humans[2]. Great numbers of compounds with diverse structural features and strong biological activities have been found in sea-anemones, which contain a primitive immune system and produce toxic chemicals as a form of defense[3]. Sea-anemones belong to the phylum Cnidaria and class Anthozoa are generally bottom-dwelling, solitary, non-motile and attach to hard substratum using sucker-like organs. They also have a tentacle that surrounds a central mouth opening and these are used to catch and transfer food items to their mouth. The nematocysts present on the edges of the tentacles expel toxins[4]. Sea-anemones, especially those from tropical waters, are often brilliantly coloured due to photosynthetic pigments of symbiotic zooxanthellae present in different tissues of the organism[5]. Pigments are compounds that absorb particular wavelengths of light and can contribute to the colour of biological patches. Among these classes of pigments, carotenoids are most widespread. Pigments have several biological functions including antioxidants, anticancer, antidiabetic, protection against UV-light, immune response, communication, improved reproduction and disease resistance in higher animals and human[6]. In the present study, crude pigments were extracted from two sea-anemones, *Heteractis magnifica* (*H. magnifica*) and *Stichodactyla haddoni* (*S. haddoni*), purified through column chromatography and tested for antibacterial activity against aquatic and human bacterial pathogens.

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2. Materials and methods

2.1. Collection and acclimatization of sea-anemones

The two sea-anemones, *H. magnifica* and *S. haddoni* (phylum-Cnidaria & class-Anthozoa) were obtained in healthy condition from the traders at Mandapam, Tamil Nadu and packed by sterile polythene bags. The samples were transported immediately to laboratory for extraction.

2.2. Extraction of pigments

Pigments were extracted using acetone solvent by adopting the method proposed by Rodriguez[7]. These animal samples were washed in sterile phosphate buffer saline (pH 8) to remove the dust particles. The tissues of the each sea-anemone were cut into small bits and ground separately by using a mortar and pestle. The crude extracts were filtered through filter paper and two extraction processes were repeated until the samples became colourless.

2.3. Purification of crude pigment extracts by column chromatography

The two crude extracts were purified by silica gel column chromatography using acetone as solvent, as proposed by Selvameenal[8]. Silica gel G (230-400 mesh size) was from MERCK (Germany) used as a stationary phase in a glass column. The column was packed with silica gel by wet packing method wherein a padding of cotton was placed at the bottom of the column and then it was filled with eluting solvent. Silica gel was packed in to the column to form a bed of silica with a maximum height of 30 cm. The crude extracts of sea-anemones were then poured onto the bed of silica separately and eluted successively with 50 mL of acetone. Seven fractions were collected from *H. magnifica* and five were collected from *S. haddoni*. The obtained fractions were evaporated and stored at -80℃ for further use.

2.4. Bacterial cultures

Aquatic bacterial pathogens such as *Aeromonas hydrophila*, *Enterobacter aerogens*, *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas fluorescens*, *Streptococcus* sp., *Vibrio parahaemolyticus*, *Vibro alginolyticus*, *Edwardsiella tarda*, *Proteus* sp., were obtained from the Microbiology laboratory of the Marine ornamental fish hatchery of this Centre[9].

Clinical pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Proteus Proteus*, *Salmonella paratyphi*, *Shigella sonnie*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella* sp., were obtained from the Department of Microbiology, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu.

2.5. Agar well-diffusion method

In vitro antibacterial activity was determined by agar well-diffusion method[10]. Muller Hinton Agar (HIMEDIA, MUMBAI) medium was prepared and poured in to sterile petridishes. After solidification, 24 hours old bacterial broth cultures were inoculated by using a sterile cotton swab and then created wells in the media. About 75 μL containing 150 μL of the crude pigment extracts and purified pigments of two sea-anemones were placed in different wells and allowed to diffuse for 2 hours. Tetracycline and commercial Astaxanthin used as positive controls. Plates were incubated at 37℃ for 24 hours and activity was determined by measuring the diameter of the inhibition zones. Triplicate samples were maintained for each bacterial strain.

3. Results

The extraction of pigments from *H. magnifica* was 12%: total of 75 g of crude extract was obtained from 600 g; and that from *S. haddoni* was 11%: total of 55 g of crude extract was obtained from 500 g. The percentage of extraction was calculated using the following formula:

Extraction (%) = Weight of the extract (g)/ Weight of the total material (g)×100.

The crude pigment extracts were further subjected for purification by column chromatography. Totally seven fractions were collected from *H. magnifica* and five fractions from *S. haddoni*. Antibacterial activity of crude and purified pigments of two sea-anemones were tested against aquatic and human bacterial pathogens and is shown in Tables 1- 6.

In the present study, the results of two crude pigment extracts and positive controls tested against aquatic bacterial pathogens are mentioned in Table 1. *H. magnifica* showed the maximum activity against *Aeromonas hydrophila* [(18.50 ±0.71) mm] followed by *Flavobacterium* sp., *Edwardsiella tarda*, *Vibrio parahaemolyticus*, *Proteus* sp., *Pseudomonas fluorescens*, *Enterobacter aerogens*, *Vibrio alginolyticus* and the minimum activity was against *Streptococcus* sp. [(11.00± 0.00) mm]. *S. haddoni* showed the maximum activity against *Micrococcus* sp. [(16.00±0.00) mm] followed by *Aeromonas hydrophila*, *Vibrio alginolyticus*, *Flavobacterium* sp., *Enterobacter aerogens*, *Pseudomonas fluorescens* and the minimum activity was against *Streptococcus* sp. [(9.50±0.71) mm].

Among two positive controls, commercial astaxanthin showed the maximum activity against *Aeromonas hydrophila* [(16.50±0.71) mm] followed by *Streptococcus* sp., *Pseudomonas fluorescens*, *Vibrio parahaemolyticus*, *Enterobacter aerogens*, *Micrococcus* sp. and the minimum activity was against *Proteus* sp. [(8.50±0.71) mm]. Tetracycline showed the maximum activity against *Aeromonas hydrophila* [(19.80± 0.28) mm] followed by *Edwardsiella tarda*, *Streptococcus* sp., *Proteus* sp., *Flavobacterium* sp., *Micrococcus* sp. and the minimum activity was against *Vibrio alginolyticus* [(10.40± 0.56) mm].

The results of two crude pigment extracts and positive controls tested against human bacterial pathogens are shown in Table 2. *H. magnifica* showed the maximum activity against *Escherichia coli* [(18.30±0.42) mm] followed by *Pseudomonas aeruginosa*, *Klebsiella* sp., *Staphylococcus aureus*, *Salmonella paratyphi*, *Vibrio cholerae*, *Klebsiella* pneumonia and the minimum activity was against *Proteus*