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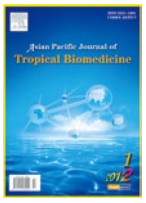

Document heading

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*

Table 1.
Antibacterial activity of crude pigment extracts of *H. magnifica* and *S. haddoni* against aquatic bacterial pathogens (mm).

Aquatic pathogens	Crude pigment extracts		Positive controls	
	H. magnifica	*S. haddoni*	Astaxanthin	Tetracycline
Aeromonas hydrophila	18.50±0.71	15.50±0.71	16.50±0.71	19.80±0.28
Enterobacter aerogens	14.50±0.71	14.00±0.00	14.15±0.21	12.70±0.42
Flavobacterium sp.	17.25±0.35	14.90± 0.14	13.30±0.42	14.50±0.71
Micrococcus sp.	13.40±0.56	16.00±0.00	14.50±0.71	14.10±0.14
Pseudomonas fluorescens	15.00±0.00	14.00±0.00	15.15±0.21	13.50±0.71
Streptococcus sp.	11.00±0.00	9.50±0.71	15.25±0.35	15.00±0.00
Vibrio parahaemolyticus	16.00±0.00	12.90±0.14	14.65±0.91	12.00±0.00
Vibrio alginolyticus	14.40±0.56	15.50±0.71	12.80±0.28	10.40±0.56
Edwardsiella tarda	16.50±0.71	9.70±0.42	10.75±1.06	16.60±0.84
Proteus sp.	15.65±0.49	13.30±0.42	8.50±0.71	14.60±0.56

Each value is the mean±SD of 3 replicates.

Table 2.
Antibacterial activity of crude pigment extracts of *H. magnifica* and *S. haddoni* against human bacterial pathogens(mm).

Human pathogens	Crude pigment extracts		Positive controls	
	H. magnifica	*S. haddoni*	Astaxanthin	Tetracycline
Staphylococcus aureus	16.00±0.00	14.90±0.14	14.25±0.35	15.50±0.71
Escherichia coli	18.30±0.42	17.00±0.00	17.70±0.42	19.75±0.35
Klebsiella pneumoniae	14.00±0.00	9.80±0.28	14.85±0.21	15.65±0.49
Vibrio cholerae	14.90±0.14	9.00±0.00	15.10±0.14	11.20±0.28
Proteus Proteus	10.00±0.00	13.90±0.14	10.65±0.49	13.85±0.21
Salmonella paratyphi	16.00±0.00	12.25±0.35	0.00±0.00	13.00±0.00
Shigella sonnie	11.40±0.56	10.00±0.00	14.50±0.71	12.10±0.14
Pseudomonas aeruginosa	17.80±0.28	15.00±0.00	13.85±0.21	15.75±0.35
Salmonella typhi	12.20±0.28	14.15±0.21	0.00±0.00	11.25±0.35
Klebsiella sp.	16.15±0.21	12.25±0.35	15.90±0.14	13.65±0.49

Each value is the mean±SD of 3 replicates.

Table 3.
Antibacterial activity of column chromatographic fractions of *H. magnifica* eluted in acetone against aquatic bacterial pathogens (mm).

Aquatic pathogens	1st	2nd	3rd	4th	5th	6th	7th
Aeromonas hydrophila	13.40±0.56	11.00±0.00	20.15±0.21	11.20±0.28	11.75±0.35	10.00±0.00	8.00±0.00
Enterobacter aerogens	14.75±0.35	7.00±0.00	18.50±0.71	12.15±0.21	14.30±0.42	9.20±0.28	10.00±0.00
Flavobacterium sp.	11.30±0.42	0.00±0.00	8.35±0.49	14.25±0.35	12.50±0.71	0.00±0.00	8.50±0.71
Micrococcus sp.	0.00±0.00	13.85±0.21	17.10±0.14	12.80±0.28	10.00±0.00	11.25±0.35	12.10±0.14
Pseudomonas fluorescens	13.50± 0.71	10.35±0.49	18.50±0.71	16.00±0.00	11.20±0.28	9.55±0.35	0.00±0.00
Streptococcus sp.	14.85±0.21	12.00±0.00	13.25±0.35	9.00±0.00	0.00±0.00	11.50±0.71	9.15±0.21
Vibrio parahaemolyticus	14.00±0.00	10.00±0.00	18.28±0.28	14.85±0.21	13.25±0.35	10.40±0.56	0.00±0.00
Vibrio alginolyticus	11.75±0.35	13.10±0.14	18.10±0.14	11.50±0.71	10.00±0.00	8.15±0.21	7.10±0.14
Edwardsiella tarda	13.00±0.00	10.50±0.71	20.20±0.28	14.85±0.21	9.30±0.42	9.30±0.42	8.20±0.28
Proteus sp.	11.50±0.71	14.15±0.21	17.30±0.42	13.00±0.00	8.50±0.71	11.20±0.28	0.00±0.00

Each value is the mean ± SD of 3 replicates.

Proteus [(10.00±0.00) mm]. *S. haddoni* showed the maximum activity against *Escherichia coli* [(17.00±0.00) mm] followed by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and the minimum activity was against *Vibrio cholerae* [(9.00±0.00) mm].

Among two positive controls, astaxanthin showed the maximum activity against *Escherichia coli* [(17.70±0.42) mm] followed by *Klebsiella* sp., *Vibrio cholerae*, *Klebsiella pneumoniae*, *Shigella sonnie*, *Staphylococcus aureus* and the minimum activity was against *Proteus proteus* [(10.65±0.49) mm]. No activity was observed against *Salmonella paratyphi* and *Salmonella tyhpi*. Tetracycline showed the maximum activity against *Escherichia coli* [(19.75±0.35) mm] followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and the minimum activity was against *Vibrio cholerae* [(11.20±0.28) mm].

The results obtained for seven fractions of purified pigments from *H. magnifica* tested against aquatic and human pathogens are shown in Tables 3 and 4 respectively. Among seven fractions, the 3rd fraction of *H. magnifica*

Table 4.
Antibacterial activity of column chromatographic fractions of *H. magnifica* eluted in acetone against human bacterial pathogens.

Human pathogens	Zone of inhibition (mm)						
	1st	2nd	3rd	4th	5th	6th	7th
Staphylococcus aureus	14.15±0.21	11.50±0.71	19.50±0.71	14.85±0.21	12.40±0.56	11.25±0.35	10.35±0.49
Escherichia coli	15.00±0.00	14.10±0.14	22.50±0.71	13.85±0.21	11.00±0.00	9.80±0.28	8.00±0.00
Klebsiella pneumoniae	12.5±0.71	10.20±0.28	15.25±0.35	11.15±0.21	8.90±0.14	0.00±0.00	7.20±0.28
Vibrio cholerae	10.00±0.00	12.20±0.28	17.90±0.14	9.00±0.00	12.50±0.71	8.25±0.35	0.00±0.00
Proteus Proteus	14.80±0.28	7.25±0.35	13.00±0.00	0.00±0.00	14.10±0.14	11.15±0.21	9.00±0.00
Salmonella paratyphi	14.00±0.00	11.75±0.35	17.50±0.71	12.00±0.00	9.30±0.42	10.30±0.42	0.00±0.00
Shigella sonnie	10.00±0.00	9.90±0.14	0.00±0.00	8.50±0.71	11.30±0.42	0.00±0.00	13.25±0.35
Pseudomonas aeruginosa	10.75±0.35	8.30±0.42	20.15±0.21	12.50±0.71	10.00±0.00	9.10±0.14	0.00±0.00
Salmonella typhi	0.00±0.00	9.30±0.42	12.15±0.21	7.90±0.14	0.00±0.00	11.20±0.28	13.30±0.42
Klebsiella sp.	10.20±0.28	0.00±0	17.20±0.28	12.00±0.00	8.25±0.35	0.00±0.00	14.15±0.21

Each value is the mean ± SD of 3 replicates.

Table 5.
Antibacterial activity of column chromatographic fractions of *S. haddoni* eluted in acetone against aquatic bacterial pathogens.

Aquatic pathogens	Zone of inhibition (mm)				
	1st	2nd	3rd	4th	5th
Aeromonas hydrophila	13.00±0.00	18.10±0.14	14.80±0.28	8.50±0.71	10.15±0.21
Enterobacter aerogens	11.20±0.28	16.85±0.21	12.00±0.00	8.20±0.28	0.00±0.00
Flavobacterium sp.	9.90±0.14	16.00±0.00	13.30±0.42	0.00±0.00	9.00±0.00
Micrococcus sp.	12.20±0.28	16.50±0.71	11.00±0.00	8.85±0.21	10.20±0.28
Pseudomonas fluorescens	13.90±0.14	15.30±0.42	0.00±0.00	7.20±0.28	11.25±0.35
Streptococcus sp.	9.20±0.28	12.25±0.35	8.50±0.71	13.20±0.28	0.00±0.00
Vibrio parahaemolyticus	14.15±0.21	15.30±0.42	11.00±0.00	9.90±0.14	7.00±0.00
Vibrio alginolyticus	9.00±0.00	14.20±0.28	12.00±0.00	0.00±0.00	8.25±0.35
Edwardsiella tarda	11.15±0.21	15.00±0.00	9.20±0.28	7.15±0.21	0.00±0.00
Proteus sp.	13.20±0.28	12.00±0.00	10.15±0.21	0.00±0.00	8.00±0.00

Each value is the mean ± SD of 3 replicates

Table 6.
Antibacterial activity of column chromatographic fractions of *S. haddoni* eluted in acetone against human bacterial pathogens.

Human pathogens	Zone of inhibition (mm)				
	1st	2nd	3rd	4th	5th
Staphylococcus aureus	11.20±0.28	17.15±0.21	10.00±0.00	8.25±0.35	12.00±0.00
Escherichia coli	9.30±0.42	19.10±0.14	8.00±0.00	0.00±0.00	13.25±0.35
Klebsiella pneumoniae	14.30±0.42	13.40±0.56	9.00±0.00	10.85±0.21	7.25±0.35
Vibrio cholerae	12.10±0.14	15.00±0.00	9.50±0.71	0.00±0.00	9.30±0.42
Proteus proteus	9.90±0.14	16.15±0.21	9.50±0.71	12.30±0.42	8.40±0.56
Salmonella paratyphi	11.25±0.35	13.20±0.28	8.00±0.00	0.00±0.00	10.00±0.00
Shigella sonnie	9.00±0.00	12.50±0.71	14.20±0.28	11.15±0.21	0.00±0.00
Pseudomonas aeruginosa	10.25±0.35	17.30±0.42	12.25±0.35	10.00±0.00	9.10±0.14
Salmonella typhi	12.85±0.21	11.00±0.00	10.15±0.21	14.25±0.35	0.00±0.00
Klebsiella sp.	12.00±0.00	17.85±0.21	9.20±0.28	0.00±0.00	11.00±0.00

Each value is the mean ± SD of 3 replicates.

exhibited highest activity against aquatic pathogens, *Edwardsiella tarda* [(20.20±0.28) mm] followed by *Aeromonas hydrophila*, *Enterobacter aerogens*, *Pseudomonas fluorescens*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Proteus* sp., *Micrococcus* sp. and the minimum activity was against *Flavobacterium* sp. [(8.35±0.49) mm]. The third fraction exhibited highest activity against human pathogens, *Escherichia coli* [(22.50±0.71) mm] followed by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella paratyphi*, *Klebsiella* sp., *Klebsiella pneumoniae* and the minimum activity was against *Salmonella tyhpi* [(12.15±0.21) mm] and no activity was observed against *Shigella sonnie*.

The results obtained for five fractions of purified pigments from *S. haddoni* tested against aquatic and human pathogens are shown in Tables 5 and 6 respectively. Among five fractions, the 2nd fraction of *S. haddoni* exhibited highest activity against aquatic pathogens, *Aeromonas hydrophila* [(18.10±0.14) mm] followed by *Enterobacter aerogens*, *Micrococcus* sp., *Flavobacterium* sp., *Pseudomonas fluorescens*, *Vibrio parahaemolyticus*, *Edwardsiella tarda*, *Vibrio alginolyticus* and the minimum activity was against *Proteus* sp. [(12.00±0.00) mm]. The 2nd fraction exhibited highest activity against human pathogens, *Escherichia coli*

[(19.10±0.14) mm] followed by *Klebsiella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus Proteus*, *Vibrio cholerae* and the minimum activity was against Salmonella tyhpi [(11.00±0.00) mm].

4. Discussion

Aquatic pathogenic bacteria are responsible for heavy mortality in wild and cultured fishes. Majority of bacterial infections are caused gram negative organisms: *Vibrio* (Vibriosis), *Aeromonas* (Motile aeromonad disease, Furunculosis), *Flavobacterium* (Columnaris disease), *Edwardsiella* (Edwardsiellosis), *Citrobacter*, *Pseudomonas* and *Mycobacterium*. Gram positive organism, *Streptococcus* (Streptococcosis) has also been shown to cause diseases in aquaculture systems[11].

Human pathogenic bacteria have potential to cause human diseases such as skin infections, pneumonia, tetanus, typhoid fever, diphtheria, syphilis, meningitis and leprosy. Most human diseases are caused pathogenic genera: *Mycobacterium*, *Streptococcus*, *Pseudomonas*, *Shigella*, *Campylobacter*, *Salmonella*, *Staphylococcus*, *Vibrio* and *Klebsiella*[12].

Over the past 20 years, various chemotherapeutics, vaccines, immunostimulants and probiotics have been used to treat bacterial infections in cultured systems and humans but the emergence of drug-resistant bacteria has become a major problem[13]. Therefore, scientists all over the world have found alternatives of natural origin to the available commercial antibiotics for controlling the human and aquatic bacterial diseases[14].

Marine invertebrates especially sedentary sea-anemones are rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs[3]. Sea-anemones have brilliant colours such as green, brown, pink and yellow and it is based on the presence of algal pigments. The presence of the pigments is linked to the chemical defences of marine invertebrates and to the biosynthetic activity of the symbiotic bacteria[4]. Most researches focus on symbiotic algae of sea-anemones, rather than bacteria associated with them[15].

Many cnidarians exist in an obligatory mutualism with dinoflagellates commonly called zooxanthellae. When these symbioses are stressed, zooxanthella densities often decrease (*i.e.*, bleaching), resulting in reduced host fitness or mortality. Because zooxanthellae play a prominent role in the colouration of hosts[16]. Johnson[16] discussed that the comparison of photographic analyses used to quantify zooxanthella density and pigment concentrations in cnidarians. The photosynthetic and respiratory physiology of the sea-anemone, *Anthopleura elegantissima* (Brandt, 1835) living in association with the endosymbiotic dinoflagellate, *Symbiodinium* was investigated[17]. Alan verde[18] have analysed the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperature clonal anemone, *Anthopleura elegantissima*.

Carotenoids pigments are responsible for color polymorphism of the sea anemone, *Bunodosoma granulifera* has also been reported[19]. Several colour-variants of the pacific coast anemone, *Metridium senile fimbriatum* and the red form of the British species, *Metridium senile senile* yielded astaxanthin esters as the preponderant carotenoid fraction stored in somatic or in gonadal tissues were reported. These pigments are responsible for colour and protect the sea anemones against UV-radiation and also provide a filter for the protection of the symbiotic algal cells within the tissues of the animal[20]. Stoletzki[21] have tested the genetic and colour morph differentiation in the Caribbean anemone, *Condylactis gigantea*. Needham[22] reported that the properties of the connective tissue pigment of sea-anemone, *Lithobius forficatus*. The pigment shows the properties of a biuret compound and similarities to the copper proteins. It may function in respiration under hypoxia, or in the oxidative darkening of the exoskeleton. A C37 carotenoid pigment, peridininol, isolated from a marine *Zoanthus* sp. exhibits promising anti-spasmodic activity against nicotine and serotonin *in vitro* studies using guinea pig ileum[23].

Bacteria associated with anemones possess the antibacterial activity. Antimicrobial, antiprotozoal and toxic activities of crude extracts obtained from six cnidarian species from the mexican caribbean sea were studied[24]. Lee seong wei[25] have found the antimicrobial property of 12 species and methanol extract of ornamental sea-anemone, *Radianthus ritteri* against *Edwardsiellosis* agent and other bacteria. This is perhaps the first report on antimicrobial property of *Radianthus rittei* against pathogenic bacteria isolated from aquaculture. Jia yi har[26] have tested the anemone, *Nematostella vectensis* as a model for investigating microbial mediation of health and disease in hexacorals.

Prakash Williams[11] have tested the antimicrobial activity of tissue and associated bacteria from benthic-anemone, *S. haddoni* against microbial pathogens. Ghosh[4] has been described that the toxic proteins obtained from the sea anemones, *H. magnifica* and *Stichodactyla mertensii* and also sea anemones extracts are tested for the antibacterial activity against human pathogens, *Staphylococcus aureus* and *Salmonella typhi*. Bragadeeswaran[3] studied that the antifouling activity against marine biofilm bacteria using crude extracts of sea-anemones, *H. magnifica* and *Heteractis aurora*.

Thangaraj[27] has been investigated that the antimicrobial activities of the methanol and aqueous extracts of sea-anemones, *Stichodactyla mertensii* and *Stichodactyla gigantea* against bacterial and fungal pathogens. The results are revealed that the antibacterial activity of *Stichodactyla gigantea* exhibited significantly inhibitory activity against *Pseudomonas aeruginosa* than the *Stichodactyla mertensii*. In antifungal activity, *Stichodactyla mertensii* showed good activity against *Aspergillus niger* compared with other strains. This study results are supported that the sea-anemones, *Stichodactyla mertensii* and *Stichodactyla gigantea* extracts for treatment of some bacterial and fungal diseases.

However, detailed analyses of the sea-anemone pigments have not been undertaken and also antibacterial activity remains largely unknown. Compared with previous studies, the present study revealed that the purified pigments extract of *H. magnifica* showed potential activity against both aquatic and human bacterial pathogens. To the best of our knowledge, this is the first report demonstrating the

antibacterial activity of sea-anemone pigments against aquatic and human bacterial pathogens. The sea-anemone pigments must play a role in colour of the sea anemones, host defence and also a good source of antibacterial compounds. Further studies are also need to encouraging the isolating biologically more potential pigment from the mixture of purified pigments of sea-anemone for drug discovery. The present study will support the antibacterial compounds are present in pigments of other marine resources also.

Conflict of interest statement

We declare that we have no conflict of interest.

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