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Antibacterial activity of the released metabolites of sea anemone *Stichodactyla gigantea* (Forskal, 1775) from the coast of South Andaman, India

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Marine sessile organisms produce unique bioactive metabolites, which render a defensive barrier against microbial threats and increase survivability in the middle of predators. The earlier studies focused on isolated metabolites from marine sources, composed to exhibit antibacterial, antiviral, and cytotoxic properties. The present study aims to evaluate the antibacterial property of the anemone-released metabolites. The crude and released mucoid metabolite obtained from the sea anemone *Stichodactyla gigantea* (Forskal, 1775) assayed against five human pathogens like *Bacillus subtilis* (MTCC 121), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MTCC839), *Bacillus cereus* (MTCC 443), and *Salmonella enterica typhimurium* (MTCC 1252). The assay exhibited positive activity against two pathogens, *viz*. *B. subtilis* (MTCC 121) and *L. monocytogenes* (MTCC 839). Based on the demonstrated activity, the released metabolites were purified using Open Column chromatography. The fractions collected were subjected to an antibacterial assay, which showed a high inhibition zone of 39 mm and 23 mm in diameter against *B. subtilis* and *L. monocytogenes.* Followingly, the characterization of purified fractions through GC-MS analysis confirmed the presence of 22 compounds. This study reveals the potential power of the released mucoid metabolites against antibiotic-resistive pathogens. Further studies are essential to elucidate the role of endosymbiont's contribution to mucoid production and their responsiveness towards tackling stressed conditions.

[**Keywords**: Antibacterial activity, Released Mucoid compound, South Andaman, *Stichodactyla gigantea*]

Introduction

In the marine habitat, the sessile organisms remain endlessly exposed to environmental fluctuations and face repeated encounters with pathogenic microbes during their lifetime. Most marine organisms have evolved a critical survival mechanism to overcome this vulnerability by producing structurally unique bioactive molecules[1]. Advances in techniques led to a focused study on isolating the bioactive molecules from Cnidarians. This targeted study may help to identify pharmaceutically beneficial compounds to overcome the impact of Antimicrobial resistance[2].

The cnidarians release toxins as a mode of defense reaction while disturbed or in a stressed condition[3]. The toxins released from sea anemone have outlawed their potent capacity to block various sodium and potassium ion channels, particularly in excitable cells[4-7]. Many targeted studies focused on assessing the bioactive potential of sea anemones as in the case of *Stichodactyla helianthus*, the isolated peptide toxin ShK-186 showed a marked reaction in treating autoimmune disease[8]. Similarly, the toxins isolated from *Heteractis malu*, *Cryptodendrum adhaesivum* and *Entacmaea quadricolor* exhibited significant activity against different human cancer cell lines[9]. Likewise, the extract from *Heteractis magnifica* and *Stichodactyla mertensii* exhibited activity against human pathogens like *Staphylococcus aureus* and *Salmonella typhi*[10]. This striking power of activities comes from the stored cocktail of peptides and proteins inside the nematocyst of the sea anemone. Metabolites released from sea anemones can act as neurotoxins, cytolysins, and protease inhibitors against predators[11]. Studies encompassed assessing the bioactive potential of Cnidarians reported having an endless source of potential compounds that could become a gamechanger in biomedical studies[12].

Sea anemone harbours photosynthetic dinoflagellates and microbes that play a significant role in metabolite production[13-14]. Many studies have been undertaken on sea anemone toxicity, focusing mainly on neurotoxin, cytotoxin, sodium, and potassium channel blocking. The study on the bioactive potential of sea anemones from the Andaman Sea was lacking. The current study aims to assess the Antibacterial activity of crude extract and released metabolites from the sea anemone

Stichodactyla gigantea. Further, studies focusing on Sea anemones are necessary to understand their interaction role with endosymbionts in metabolite production and to unveil the role of symbionts in mucoid production under environmental perturbations.

Materials and Methods

Collection of Sea anemone

Sea anemone *Stichodactyla gigantea* (Forskal, 1775) (Fig. 1) was collected from the intertidal region by carefully detaching it from the substratum at Brookshabad coast, South Andaman (Lat. 11°36'59" N and Long. 92°45'10" E).

Preparation of crude extract

About 25 g of tentacles trimmed from Sea anemone (*Stichodactyla gigantea*) were dissolved in 50 ml of absolute Methanol (CH_3OH) for the crude sample preparation. The sample was further homogenized using mortar and pestle and centrifuged at 10,000 X g for 20 minutes. The supernatant collected was dried and stored at -4 °C until use.

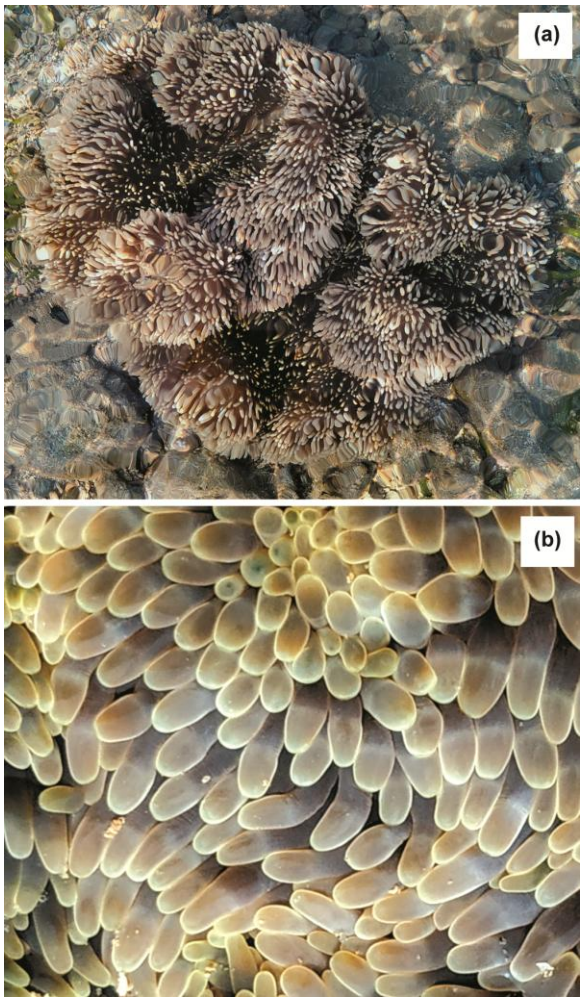

Fig. 1 — *Stichodactyla gigantea* at Brookshabad, Coast of South Andaman: a) *Stichodactyla gigantea* and b) Tentacles

Collection of released mucoid substance

The released mucoid substance from the sea anemone *Stichodactyla gigantea* was collected at the sampling site. Initially, the sea anemone was detached from the substratum and washed with seawater and subsequently with distilled water to remove epiphytes and external debris. The animal was then transferred to a five-litre glass tank with one litre of distilled water and kept for an hour to collect the released substances. After one hour, the animal was released back into its environment, and the sea anemone-conditioned water was brought to the laboratory in an icebox for further analysis. The conditioned water was filtered using Whatman (GF/C) filter paper to remove any insoluble material in the conditioned water. Concentrated filtrate was reduced to 25 ml by applying a Rotary Evaporator at 40 °C. Further, the concentrated sample was used to study its bioactive potential against human pathogens.

Bioassay for antibacterial activity

The antibacterial activity was checked for the crude extract and the released metabolite prepared using a well diffusion method against five human pathogens *viz. Bacillus subtilis* (MTCC 121), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MTCC839), *Bacillus cereus* (MTCC 443), and *Salmonella enterica typhimurium* (MTCC 1252). Muller Hinton agar (Himedia) was used for the antibacterial study. The test microorganism was spread on the plate using a sterile cotton swab and well prepared using a sterile stainless-steel borer. The well plates were inoculated with 100 µl/1 ml of crude and released cue extract. For positive control check, used a 10 µl/1 ml of antibiotic Erythromycin. In crude plates, 100 µl/1 ml of methanol for negative control to countercheck the activity did not occur due to solvent use. The inoculated plates were kept in incubation for 24 – 36 h. Antibacterial activity was confirmed based on the inhibition zone.

Purification of a released compound by open column chromatography

The released metabolite sample was purified using open column chromatography based on the bioassay activity. The purification was done through the column packed with silica gel (60 – 120 mesh;

Himedia) and mounted vertically. The sample was eluted using three gradients; 50 % methanol **(**F1), 70 % methanol (F2), and absolute methanol (F3), respectively. Eluted fractions were then concentrated using a rotary evaporator at 40 °C. The fractions were subjected to antibacterial activity against five human pathogens like *B. subtilis*, *L. monocytogenes*, *S. aureus*, *S. enterica typhimurium* and *B. cereus*.

Compound identification

The active fraction F1 was subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. General profiling was carried out, and the spectrum obtained was compared to the database of NIST08. L.

Results and Discussion

Crude extract and released compound obtained from *Stichodactyla gigantea* were assayed for antibacterial activity against five human pathogens like *Bacillus subtilis* (MTCC 121), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MTCC 839), *Bacillus cereus* (MTCC 443), and *Salmonella enterica typhimurium* (MTCC 1252). Crude extract showed activity against *Bacillus subtilis* (MTCC 121) and *Listeria monocytogenes* (MTCC 839), with less zone of inhibition of 12 and 10 mm, respectively. Further, it did not show any effect on other pathogens, *i.e*., *S. aureus*, *S. enterica typhimurium* and *B. cereus*. However, the released compound showed a higher zone of inhibition in comparison to the crude extract (Table 1). The zone of inhibition in the case of *Bacillus subtilis* and *Listeria monocytogenes* was 28 and 16 mm, respectively. Moreover, there was no effect on *S. aureus*, *S. enterica typhimurium* and *B. cereus* pathogens.

Based on the activity, the released compound sample was purified via open column chromatography. The three fractions eluted were subjected to antibacterial assay. The assay of purified fraction showed a comparatively better inhibition zone than the crude extract and released compounds. As shown in Table 2, the first fraction (F1), eluted with 50 % methanol, showed higher inhibition zones with 39 mm against *B. subtilis*, and 23 mm against *L. monocytogenes*. In contrast, the second fraction (F2) eluted with 70 % methanol, which showed activity against *B. subtilis* with a 28 mm zone of inhibition and had no activity against *L. monocytogenes* (Fig. 2). The final fraction (F3) eluted with absolute methanol showed no activity against any of the human pathogens.

Further, GC-MS analysis of the F1 fraction confirmed presence of 22 compounds (Table 3) with ten major peak area comprises of Dimethylamine Rt (3.181), of 5.85 %, Acetaldehyde Rt (3.229), of 19.25 %, Bicyclo [3.1.0]hexane, 6-Methylene Rt (3.585), of 22.65 %, 3-Phenyl-2H-Chromene Rt (7.222) of 8.35 %, Hexahydropyridine, 1-Methyl-4-

Table 1 — Antibacterial activity of *Stichodactyla gigantea* extract against human pathogens

Sample	Pathogens	Zone of inhibition (mm)	Positive control (mm)	Negative control
Crude extract	*Bacillus subtilis*	12	40	-
	Listeria monocytogenes	10	22	-
Released compound	*Bacillus subtilis*	28	36	-
	Listeria monocytogenes	16	20	-

Table 2 — Antibacterial activity of purified fractions the against human pathogens

Fraction	Zone of Inhibition (mm)	
	Bacillus subtilis	*Listeria monocytogenes*
F1	39	23
F2	28	-
F3	-	-

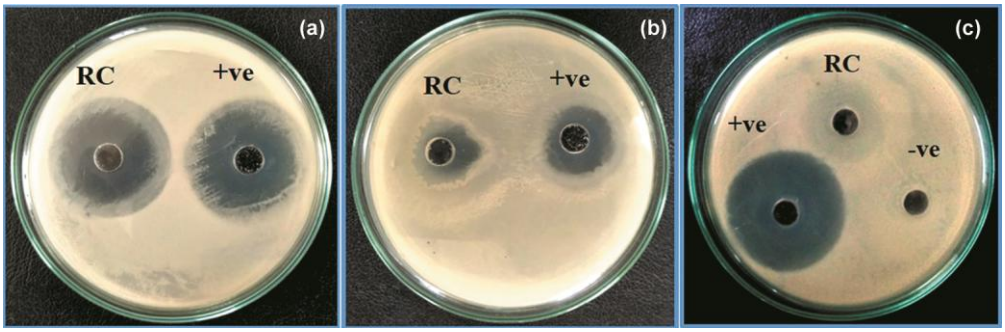

Fig. 2 — Antibacterial activity of the column purified fraction against the human pathogens: F1 [a) *B. subtilis* and b) *L. monocytogenes*] and F2 [c) *B. subtilis*]. RC - Released compound, +ve - Erythromycin, -ve – Methanol

Table 3 — Compounds detected GC-MS

S. No.	Isolated compound	Molecular formula	MW (g/mol)	Retention time	Peak area
1	Dimethyl amine	$(CH_3)_2NH$	45.08	3.181	5.85
2	Acetaldehyde	C_2H_4O	44.05	3.229	19.25
3	Oxirane methanol,(S)	$C_3H_6O_2$	74.0792	3.229	19.25
4	Bicyclo[3.1.0]hexane, 6-Methylene	C_7H_{10}	95.15	3.585	22.65
5	CycloPropene, 3- Methyl-3-vinyl-	C_6H_8	80.1277	3.585	22.65
6	2-Cyclopentene-1-propanol	$C_8H_{12}O$	124.18	3.585	22.65
7	3-Phenyl-2H-Chromene	$C_{15}H_{12}O$	208.25	7.222	8.35
8	Pyrazolo [1,5-a]pyridine 3-Methyl-2-phenyl-	$C_{14}H_{12}N_2$	208.263	7.222	8.35
9	1-Methyl-2-Phenylbenzimidazole	$C_{14}H_{12}N_2$	208.26	7.222	8.35
10	Hexahydropyridine, 1-Methyl-4-[4,5-di hydroxyphenyl]-	$C_{12}H_{17}NO_2$	207.12	7.347	8.40
11	9-Borabicyclo[3.3.1] nonane, 9-[3-(dimethylamino)propyl]-	$C_8H_{14}B$	121.01	7.347	8.40
12	3, 5-Ethanoquinolin-10-one, decahydro-1, 7 dimethyl-, [3R-(3. alpha., 4a.beta., 5.alpha., 7.beta, 8a.beta.)]	$C_{13}H_{21}NO$	207.31	7.347	8.40
13	2, 4-Diamino-N, N, 5-trimethyl-6-quinoline sulfonamide	$C_{15}H_{24}N_6O_2S$	352.5	12.548	7.23
14	6, 7- Benzo-phenothiazine-5, 5-Dioxide	$C_{16}H_{11}NO_2S$	281.329	12.548	7.23
15	Morphinan, 7, 8-didehydro-3-methoxy-17-methyl-6-methylene-,(-)	$C_{19}H_{23}NO$	281.4	12.548	7.23
16	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	296.61	12.710	13.91
17	2H-1,3,4-Benzotriazepine-2- thione, 5-Benzyl-1,3-dihydro-3-methyl	$C_{16}H_{15}N_3S$	281.4	12.710	13.91
18	Phenol, 4- [2- (5-nitro-2-benzoxazolyl) ethenyl]-	$C_{14}H_{13}NO$	211.26	12.710	13.91
19	Formamide, N, N-dimethyl	C_3H_7NO	73.094	17.509	3.37
20	N- Ethylforamide	C_3H_7NO	73.094	17.542	6.64
21	Guanidine, methyl	$C_2H_7N_3$	73.1	17.542	6.64
22	Formamide, N, N-dimethyl	C_3H_7NO	73.094	34.126	4.36

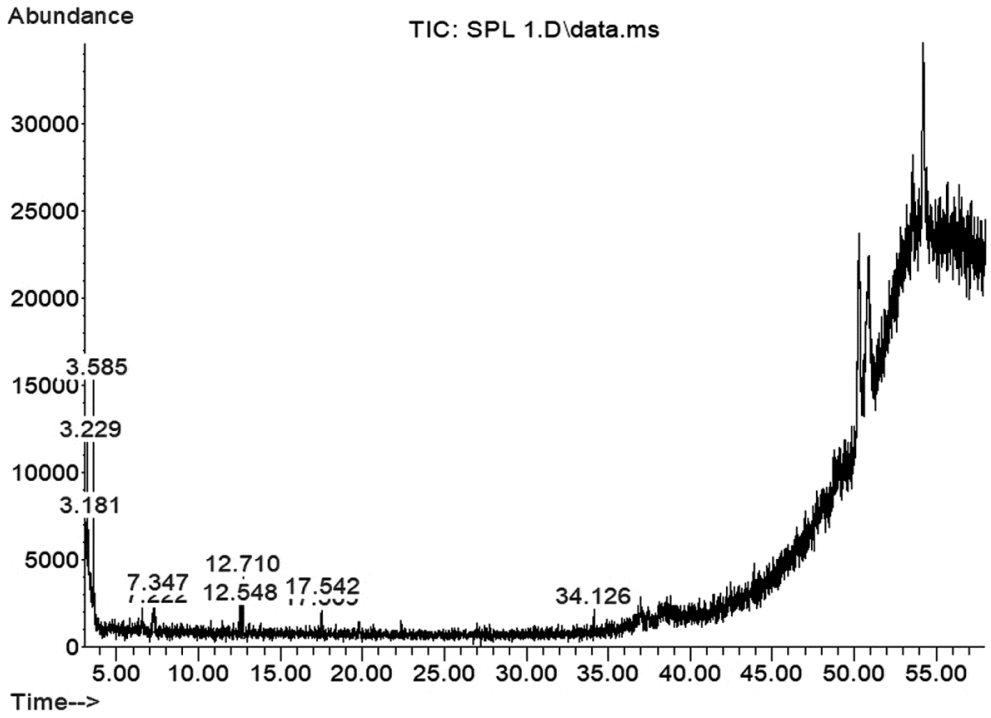

Fig. 3 — GC chart showing major peak area from F1 column purified fraction of released substance from Sea anemone *Stichodactyla gigantea*

[4,5-di hydroxyphenyl]- Rt (7.347) of 8.40 %, 2, 4-Diamino-N, N, 5-trimethyl-6-quinoline sulfonamide Rt (12.548) of 7.23 %, Cyclotetrasiloxane, octamethyl Rt (12.710) of 13.91 %, Formamide, N, N-dimethyl Rt (17.509) of 3.37 %, N- Ethylforamide Rt (17.542) of 6.64 %, Formamide, N, N-dimethyl Rt (34.126) of 4.36 % are the major peak area depicting compounds as shown in (Fig. 3).

Discussion

In marine habitat, the cnidarian secretes mucus as a part of their defence mechanism to protect themselves in a complex ecosystem[15]. In the present study, the methanolic extract of tentacle and the released mucoid metabolites of *Stichodactyla gigantea* were tested for antibacterial activity against five human pathogens. The result exhibited more prominent activity towards released metabolite than the crude extract, indicating the high action potential of mucoid against microbial attack. This congruence character has also been on the mucus of other Sea anemone *Actina equina*, which exhibited antibacterial lysozyme and cytotoxic activity against human erythromyeloblastoid leukaemia[16]. Besides this, in a broad study, the mucus-associated bacteria isolated from the coral *Acropora palmata* were shown to possess antibacterial activity and could inhibit white pox disease[17]. In contradiction with earlier findings, it was evident that the metabolites produced during stress significantly promote immunity against invaders.

Moreover, the study on *Heteractis aurora* metabolites reported antimicrobial, antioxidant, and hemolytic activity. One of the active compounds in the metabolites was reported to block enoyl-acyl carrier protein reductase[18]. Another study employed in assessing the antimicrobial activity of mottled anemone water extracts *Urticina crassicornis* showed antimicrobial characteristics and notable activity against fish pathogens like *Aeromonas hydrophila*, validating the presence of a proteinaceous compound in the mottled water extract[19]. Similarly, the methanol extract of sea anemone *Stichodactyla gigantea* and *Stichodactyla mertensii* assessed for hemolytic, neurotoxic, and analgesic activity showed better results[20]. Besides, another study reported that released venom from sea anemone *Heteractis magnifica* exhibited apoptosis in non-small cell lung cancer A549 cells[21]. In this study, the GC-MS analysis on the active fraction was carried out to validate the result further, resulting in 22 compounds. Few of the compounds recorded in this study have biological activity; CycloPropene, 3- Methyl-3-vinyl- can block ethylene-mediated response and act as a growth regulator[22]. Aside from that, Phenylbenzimidazole-related compounds play a significant role in therapeutic agents against antiulcer and anthelmintic drugs. Apart from this, it also exhibited various pharmacological activities such as antimicrobial, antihypertensive, analgesic, anticancer properties, etc[23]. The present study on sea anemone *Stichodactyla gigantea* confirms that the released metabolite has antibacterial properties. Further, studies are required to draw a deep understanding of the source of production and how endosymbionts and microbe's interaction influence metabolite production.

Conclusion

The present study investigated on antibacterial activity of sea anemone *Stichodactyla gigantea* from the coast of South Andaman. The released metabolites produced prominent activity against *Bacillus subtilis* (MTCC 121) *and Listeria monocytogenes* (MTCC 839). Subsequently, the active fractions elucidated for the GC-MS analysis have shown the presence of 22 compounds indicating the bioactive potential of released metabolites. The findings suggest that further investigation is required to uncover the role of symbionts in Sea anemone towards metabolite production. The application of omics technology will provide a deeper understanding of the metabolite role within the organism.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

The authors declare that no live organism harmed during the study.

Authors Contributions

NS designed the study and prepared the manuscript; MC and SS helped with sampling and laboratory work. JKM supervised the study and helped in manuscript editing.

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