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Antimicrobial properties of nudibranchs tissues extracts from South Andaman, India

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ABSTRACT

Objective: To evaluate the antimicrobial properties of tissues extracts of different nudibranchs such as *Phyllidia varicosa, Plakobranchus ocellatus, Phyllidiella rosans* and *Halgerda stricklandi* against bacterial and fungal pathogens.
Methods: Nudibranchs tissue samples were subjected to organic solvent extraction for antimicrobial activity by well diffusion method.
Results: The crude extract 50 µL (0.2 mg) of *Phyllidia varicosa* showed the maximum inhibitory zone (22 mm) against *Shigella flexneri*. *Plakobranchus ocellatus* extract of 50 µL (0.2 mg) showed the maximum inhibitory zone against *Shigella flexneri* (22 mm) and *Staphylococcus aureus* (19 mm) and no significant activity was found against the fungal pathogens.
Conclusions: This work reveals that nudibranch tissues contain the antimicrobial secondary metabolites, which leads the significant activity against bacterial pathogens and further emphasizes detailed study on novel drug discovery from nudibranch tissues against certain human bacterial infections.

1. Introduction

Nudibranchs are tiny organisms classified under the phylum Mollusca and are known to live in various habitats of the marine ecosystem. Bioactive properties have been reported from 21 dorid nudibranchs from Australian waters tested against six pathogens[1]. Several researchers reported the antimicrobial compounds such as terpenoids, 9-thiocyanatopupukeanane sesquiterpene, deoxymanoalide and deoxysecomanoalide phospholipids, sterols and monoalkyl-diacylglycerol from nudibranchs[2-5]. Furthermore, nudibranch tissues and its associate actinomycetes have the potential antibacterial properties against human pathogens[6]. Apart from antimicrobial compounds, dorids are known to secrete complex toxic compounds to deter potential predators, and these chemicals could have been obtained from other marine sources like sponges or ectoprocts[7]. Tetrapyrrole is a blue pigmented antimicrobial compound, derived from *Nembrotha kubaryana*, while terpenoids known as a fish antifeeding agent isolated from *Chromodoris petechialis* and *Hypselodoris infucata*[8]. A similar kind of antifeeding agent Hodsgonals produced by an Antarctic nudibranch *Bathydoris hodgsoni*[5]. Marine molluscs are known as a remarkable source for antimicrobial agent[9]. Recently polysaccharides extracted from cephalopods were found to be a good source of antimicrobial activity[10,11]. In India, marine molluscs are well known for their food importance, whereas their antimicrobial activities are not much studied. The present work was undertaken to evaluate the antimicrobial potentiality of nudibranchs collected from intertidal regions of South Andaman Islands, India.

2. Materials and methods

2.1. Sampling and identification

Nudibranchs were collected during low tide by handpicking method from the three intertidal locations including Chidiyatapu (11°29'25.91' N, 92°42'30.59' E), Burmanallah (11°33'36.24' N, 92°43'49.73' E) and Carbyn's Cove (11°37'41.39' N, 92°45'06.63' E) (Figure 1). The samples were kept in sterile seawater in containers and transported to the research laboratory. The specimens were identified based on the descriptive Guide to Opisthobranchs of Andaman and Nicobar Islands[12] and Field Guide to the Marine Life of India[13].

2.2. Preparation of methanol and hexane extracts

Phyllidia varicosa (*P. varicosa*), *Plakobranchus ocellatus* (*P. ocellatus*), *Phyllidiella rosans* (*P. rosans*) and *Halgerda stricklandi*

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(*H. stricklandi*) specimens were cut into small pieces using sterile scissors and homogenized in a mortar and pestle by following aseptic techniques. The homogenized samples were extracted with methanol and hexane at room temperature for a week. The extracts were filtered through Whatman No.1 filter paper and the filtered samples were concentrated under vacuum in a rotary evaporator (Buchi) at 30 ℃.

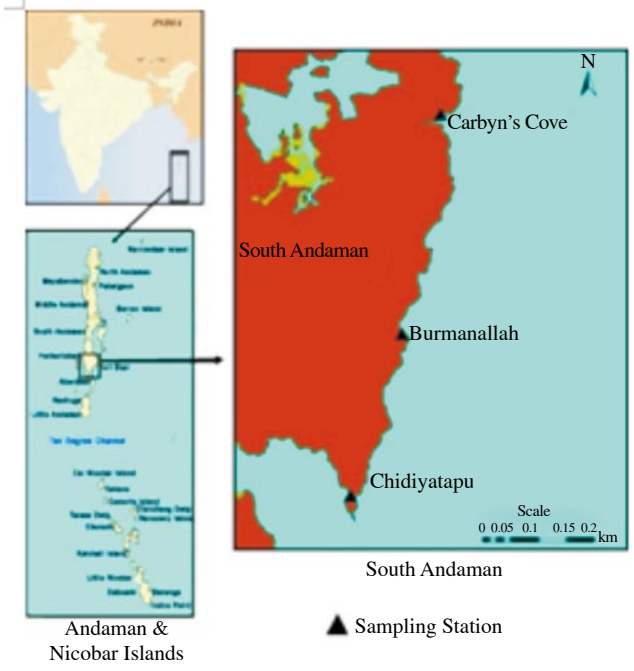

Figure 1. Map showing the sampling stations.

2.3. Preparation of crude extract for antimicrobial study

The 5 g of concentrated crude extracts were diluted with methanol and hexane and the final concentration of 5 mg/mL was then obtained. Two concentrations 25 µL (0.1 mg) and 50 µL (0.2 mg) were used to study the activity.

2.4. Microbial cultures

Five human pathogenic bacteria strains such as *Staphylococcus aureus* MTCC 96 (*S. aureus*), *Escherichia coli* MTCC 443 (*E. coli*), *Salmonella typhi* MTCC 733 (*S. typhi*), *Klebsiella pneumonia* MTCC 109 and *Shigella flexneri* MTCC 1457 (*S. flexneri*) and four fungal pathogens including *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Trichoderma* sp and *Rhizopus* sp were used for the present antimicrobial assay.

2.5. Well diffusion method

Antimicrobial activity was determined by well diffusion method described earlier[14]. Each bacterial and fungal suspension was mixed in test tube and the respective strains were cotton swabbed on Muller Hinton agar plates (Himedia, Mumbai) and potato dextrose agar plates (Himedia, Mumbai) respectively. Agar wells (8 mm) were prepared by using sterile cork-borer. The different concentrations of methanol and hexane extracts were dispensed in agar wells. The solvents of methanol and hexane were used as negative controls; the antibiotics such as gentamicin and clotrimazole were used as positive controls for bacteria and fungi respectively. To assess the inhibition over the bacterial and fungal growth, the bacterial plates were incubated at 37 ℃ for 24 h and fungal plates were incubated at room temperature (27 ℃) for 48-72 h. Growth inhibition zones were measured in diameter (mm) and all the experiments were performed in triplicates.

3. Results

Antimicrobial properties of nudibranch tissue extracts were tested against bacterial and fungal pathogens. The extracts showed significant activity against bacterial pathogens, not with fungal pathogens.

Among the two organic solvents, methanolic *P. ocellatus* crude extract (50 µL) displayed maximum inhibitory zone against *S. flexneri* (22 mm) and *S. aureus* (19 mm). A volume of 50 µL of *P. varicosa* extract showed the maximum inhibitory zone against *S. flexneri* (21 mm). All the four methanolic nudibranch tissue extracts showed the moderate inhibition zone against *S. aureus* (Table 1). About 50 µL (0.2 mg) hexane crude extract of *P. varicosa* showed maximum antimicrobial activity against *S. flexneri* (22 mm) and *S. typhi* (20 mm). *P. ocellatus* extracts showed moderate antimicrobial activity against *S. typhi* (15 mm), *Klebsiella pneumoniae* (*K. pneumoniae*) (14 mm) and *S. flexneri* (14 mm). *P. ocellatus*, *P. rosans* and *H. stricklandi* exhibited a less activity against *S. aureus* (9 mm) (Table 2).

Table 1

Antimicrobial activity of methanol tissue extracts of nudibranchs against human pathogens.

Bacterial strains	*P. varicosa*		*P. ocellatus*		*P. rosans*		*H. stricklandi*		Positive control		Negative control (methanol)
	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	1	2	
S. flexneri	14	21	16	22	16	16	-	-	25	-	-
E. coli	-	-	-	-	-	-	-	-	26	-	-
K. pneumoniae	12	14	12	12	-	-	-	-	26	-	-
S. aureus	11	13	12	19	-	12	9	14	22	-	-
S. typhi	-	-	-	-	-	-	-	-	25	-	-
A. niger	-	-	-	-	-	-	-	-	-	17	-
A. flavus	-	-	-	-	-	-	-	-	-	15	-
Trichoderma sp	-	-	-	-	-	-	-	-	-	15	-
Rhizopus sp	-	-	-	-	-	-	-	-	-	15	-

Positive control 1: Gentamicin; 2: Clotrimazole. Zones of inhibition are expressed in mm.

Table 2

Antimicrobial activity of hexane tissue extracts of nudibranchs against human pathogens.

Bacterial strains	*P. varicosa*		*P. ocellatus*		*P. rosans*		*H. stricklandi*		Positive control		Negative control (hexane)
	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	1	2	
S. flexineri	-	22	10	14	-	-	-	-	25	-	-
E. coli	-	-	-	-	-	-	-	-	26	-	-
K. pneumoniae	14	-	12	14	-	-	-	-	26	-	-
S. aureus	-	-	-	9	9	9	9	9	22	-	-
S. typhi	15	20	12	15	-	-	-	-	25	-	-
A. niger	-	-	-	-	-	-	-	-	-	17	-
A. flavus	-	-	-	-	-	-	-	-	-	15	-
Trichoderma sp	-	-	-	-	-	-	-	-	-	15	-
Rhizopus sp	-	-	-	-	-	-	-	-	-	15	-

Positive control 1: Gentamicin; 2: Clotrimazole. Zones of inhibition are expressed in mm.

About 25 µL (0.1 mg/mL) methanolic extract of *P. ocellatus* and *P. rosans* exhibited moderate antimicrobial activity against *S. flexneri* (16 mm). *P. varicosa* extracts showed a less activity against *S. flexineri* (14 mm), *K. pneumoniae* (12 mm) and *S. aureus* (11 mm). *H. stricklandi* tissue extracts exhibited a least activity against *S. aureus* (9 mm). All the four methanolic nudibranch tissue extracts did not show antimicrobial activity against the *E. coli* and *S. typhi*

(Table 1). Hexane extract of *P. varicosa* tissue showed moderate activity against *S. typhi* (15 mm) and *K. pneumoniae* (14 mm). *P. ocellatus* tissue extracts showed antimicrobial activity against *K. pneumoniae* (12 mm), *S. typhi* (12 mm) and *S. flexneri* (10 mm). *P. rosans* and *H. stricklandi* tissue extracts showed least activity (9 mm) against *S. aureus* (Table 2).

4. Discussion

Marine resources are known for the potential novel bioactive compounds. Since past decades, antimicrobial properties have been reported from different marine resources such as marine plants, animals and microbes. The marine environment is a remarkable source for discovery of potential antibiotics and bioactive compounds to cure several human diseases. Especially, marine invertebrates are considered as major drug source in the marine environment, molluscs being reported a good source of antimicrobial peptides. Blue pigmented tetrapyrrole isolated from *Nembrotha kubaryana* is known as potent antimicrobial agent[8]. This present study focused on dorid nudibranchs for the extraction of antibacterial compounds using two different solvents and tested against certain pathogens. Crude extracts showed antibacterial properties against bacterial pathogens, but did not show any activity against fungal pathogens. The present results reveal that nudibranch is a potential organism with antimicrobial compounds. Similar kind of studies were studied earlier from various marine molluscs sources, particularly bactericidal activity was found in the tissue extracts obtained from *Cerithidea cingulata*[15] and oyster *Pteria penguin*[16]. Aqueous molluscan ink extracts of *Dolabella auricularia* inhibit growth of Gram-positive and Gram-negative bacteria[17]. A broad spectrum of antimicrobial agents have been obtained from marine invertebrates[18] particularly tissue extract of *Siphonaria* was found to exhibit potent antibacterial activity[19]. And tissue extracts of *Sepioteuthis lessoniana*, *Sepia brevimana* and *Octopus cyaneus* had displayed broad spectrum of antibacterial activity against human pathogens[14] and the haemocyte extracts obtained from oysters exhibited bactericidal activities[20]. Earlier findings from different molluscan extracts were supportive with our present findings from the tissue extracts of nudibranchs.

The present result was accordant with earlier studies about antibacterial properties of methanol and hexane crude extracts of nudibranch tissues against both Gram negative and Gram positive bacterial pathogens[1]. Present findings revealed that nudibranch tissue extracts were potential source of antimicrobial properties, and it could also be a source of novel compounds. Further research is needed to explore the secondary metabolites responsible for the activity against the human pathogens.

Conflict of interest statement

We declare that we have no conflict of interest

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