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Evaluation of antibacterial activity of crude extracts of ascidian *Didemnum psammathodes* Sluiter, 1895 against isolated human and fish pathogens

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ABSTRACT

Objective: To evaluate the antimicrobial activities of ascidian *Didemnum psammathodes* (*D. psammathodes*) against human and fish pathogenic organisms. **Methods:** In this study antimicrobial activities were carried out by standard disc diffusion method. In this experiment 40 human, fish bacterial and fungal pathogens were isolated and assayed against 7 different solvents such as methanol, acetone, ethanol, n-butanol, chloroform, ethyl acetate and dichloromethane. Each solvent were assayed at different concentrations of 25, 50, 75, 100 mg/mL. **Results:** From this experiment solvent having higher concentrations showed high inhibition activity and the fungi are showed more resistant than the bacterial strains used. **Conclusions:** These results indicate that the ascidian *D. psammathodes* is found to have remarkable antimicrobial activities against isolated microbes. Further studies will fulfill for purification and structural elucidation of antimicrobial drugs.

1. Introduction

Natural products and their derivatives contribute more than half of all clinically administered drugs[1]. Of the natural products isolated from marine organisms, only less than 1% has been examined so far for pharmacological activities[2]. Since the early days of marine natural product discovery, a large proportion of natural compounds have been extracted from marine invertebrates, Porifera (sponges) and Chordata (including ascidians) have dominated as the major contributing phyla of novel bioactive compounds[3]. Tunicates have been reported to be rich sources of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans[4]. Although research on bioactive compounds from ascidians were recently initiated, it is significant that the first marine natural product Didemnin B is entering in to human clinical trial and it is an ascidian metabolite.

Antimicrobial peptides have recently become the focus of considerable interest as a candidate for a new type of antibiotic, due primarily to their potency against pathogenic microbes that are resistant to conventional antibiotics, as well as their broad-spectrum activity[5]. In the last two decades, the incidence of human bacterial and fungal infections has increased dramatically, in parallel with the wide spread of incurable infectious diseases associated with antibiotic- resistant bacteria. Fungal and bacterial diseases have become a growing threat, especially in immunocompromised patients, for whom few or no effective drugs are currently available[6]. Accordingly, a variety of studies have been conducted in an attempt to isolate natural anti bacterial and antifungal substances with potential pharmaceutical utility, and to develop and design new synthetic or semi-synthetic drugs[7].

Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection. The case of living marine surfaces the colonization process can additionally be affected by organic metabolites produced by the host organism. These metabolites may affect bacteria in a number of ways, ranging from the induction of chemotactic responses to the inhibition of bacterial growth or cell death. Since they accumulate chemical defenses, ascidians have been screened in a variety of pharmacological bioassays. Biological activities which have been frequently observed in ascidian crude extracts include antibiosis against both human microbial pathogens and marine microorganisms[8].

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The concentrations of the secondary metabolite plays vital role against micro organisms. Such potential ascidians need to be explored for the pharmaceutical purpose. Hence a broad based screening of ascidians for bioactive compound is necessary. This study will use to evaluate the anti microbial properties of the natural product derived from biofoulants ascidians *Didemnum psammathodes* (*D. psammathodes*) against isolated human and fish pathogenic micro organisms.

2. Material and methods

2.1. Specimen collection and identification

Ascidians were collected as common and persistent biofoulants from the rocks of Tuticorin Coast (Lattitude 8° 47′ 20″ and Longitude 78°09′ 70″), India by SCUBA diving at the depth ranging from 1 to 3 m between September, 2010. The samples were thoroughly washed with treated sea water and removed from sand, mutt and overgrowing organisms at the site collection, and then transported to laboratory. The collected specimens were identified by the standard literature and immediately shade dried.

2.2. Extraction

The extraction method was followed by Chellaram *et al*[9]. The freshly collected and dried ascidians were weighed 10 g, each 10 g of the ascidians were soaked in methanol, acetone, ethanol, n-butanol, chloroform, ethyl acetate and dichloromethane for 5 days. The extracts were filtered through Whatman® No.1 filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30 ℃) with reducing the pressure to give a dark brown gummy mass. The resultant residues were stored at 4 ℃ for further analysis.

2.3. Test microorganisms and microbial culture

2.3.1. Human bacterial and fungal pathogens

The reference pathogens used to test antimicrobial assay were the following gram-positive and gram-negative bacteria including *Escherichia coli* (*E. coli*), *Klebsiella oxytoca* (*K. oxytoca*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* (*P. mirabilis*), *Salmonella paratyphi* (*S. paratyphi*), *Salmonella typhi* (*S. typhi*), *Staphylococcus aureus*, *Streptococcus aureus*, *Vibrio cholerae* (*V. cholerae*) and *Vibrio parahaemolyticus* (*V. parahaemolyticus*), and the fungal pathogens such as *Alternaria alternata* (*A. alternata*), *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*), *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Trichophyton mentagrophytes* (*T. mentagrophytes*) and *Trichophyton rubrum* (*T. rubrum*) were used. These microbes were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. Bacterial and fungal strains were maintained on nutrient agar and fungal agar slants at 4 ℃ respectively.

2.3.2. Fish bacterial and fungal pathogens

Fish pathogens were used for antimicrobial activity. The bacterial pathogens such as *Aeromonas hydrophila* (*A. hydrophila*), *Aeromonas* sp., *Klebsiella* sp., *Micrococcus* sp., *P. mirabilis*, *Proteus* sp.1, *Streptococcus* sp., *V. cholerae*, *V. parahaemolyticus* and *Vibrio* sp.1 and the fungal pathogens such as *Aspergillus flavus* (*A. flaves*), *Aspergillus fumigatus* (*A. fumigatus*), *A. niger*, *Aspergillus* sp. 1, *Aspergillus* sp.2, *Fusarium* sp., *Ichthyophonus* sp., *Microsporum* sp., *Rhizopus* sp. and *Rhizopus* sp.1 were used. These fish pathogens were isolated from infected fishes from Cuddalore Government fish hatchery during April and May 2010. Isolated pathogens were identified based on the morphological, cultural and biochemical characteristics following Bergey's Manual of Determinative Bacteriology and Manual of Clinical Microbiology[10,11]. These fish pathogenic bacterial strains and fungal strains were maintained on Zobell marine agar and fungal agar slants at 4 ℃.

2.4. Antibacterial activity

Antibacterial activity was carried out by using standard disc diffusion method by Dulger and Gonuz, Parekh and Chanda, and Laouer[12-14]. The test cultures (bacteria 108 CFU/mL) were swabbed on top of the solidified media and allowed to dry for 10 min. The human bacteria were maintained on nutrient agar plates. Fouling and fish pathogens were maintained on Zobell marine agar plates. Samples were tested at different concentrations of 25, 50, 75 and 100 mg/mL and applied onto the 6 mm sterile discs. The different extracts were applied onto 6 mm sterile discs in aliquots of 30 μL of solvent, allowed to dry at room temperature, and extract loaded discs were placed on agar plates seeded with isolated microorganisms and incubated at 37 ℃ for 24 h. The susceptibility of the test organisms were determined by radius of the zones inhibition around each disc. The tetracycline discs (30 mg/disc) were used as a positive control and solvents discs were used as a negative control. All the extracts were tested with triplicate at a concentration of 30 mg/disc.

2.5. Antifungal activity

Antifungal activity was carried out by using the standard disc diffusion method by National Committee for Clinical Laboratory Standards[15]. Aliquots of 30 μL of each extract samples were tested at different concentration of 25, 50, 75 and 100 mg/mL onto 6 mm sterile discs, respectively. They were allowed to dry at room temperature and placed on agar plates seeded with microorganisms. The fungus were maintained on fungal agar plates and incubated at 37 ℃ for 24 h. Zones of growth inhibition were measured in millimeters after incubation. The tetracycline discs (30 mg/disc) were used as a positive control and solvents discs were used as a negative control. All the extracts were tested triplicate at a concentration of 30 mg/disc.

2.6. Statical analysis

The results were expressed as Mean ± SD of three independent values.

3. Results

The ascidians, *D. psammathodes* (895 g in wet weight) was collected from Tuticorin fishing harbor. The specimens were identified by following the standard literature of Kott Cole and Lambert[16,17]. Solvents of *D. psammathodes* were concentrated under reduced pressure to give a dark