Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity

Xavier Devanya Rosaline[1], Shanmugavel Sakthivelkumar[2], Kuppu Rajendran[3], Sundaram Janarthanan[2*]

[1] *Department of Zoology, Thiagarajar College (Autonomous), Madurai-625009, India*
[2] *Department of Zoology, University of Madras, Guindy Campus, Chennai-600025, India*
[3] *Department of Botany, Thiagarajar College (Autonomous), Madurai-625009, India*

ARTICLE INFO

Article history:
Received 22 February 2012
Received in revised form 4 March 2012
Accepted 2 April 2012
Available online 28 April 2012

Keywords:
Antibacterial activity
Brown and green algae
Human bacterial pathogens
Marine algae
Seaweeds

ABSTRACT

Objective: To screen the antibacterial efficacy of various solvent extracts of marine algae such as *Sargassum wightii (S. wightii)*, *Chaetomorpha linum (C. linum)* and *Padina gymnospora (P. gymnospora)* against some selected gram-positive and gram-negative human pathogenic bacteria. **Methods:** Crude extracts were prepared from the selected marine algae using different solvents namely, hexane, ethyl acetate, acetone and methanol and were tested for their antibacterial activity against human pathogenic bacteria using disc diffusion method. Minimum inhibitory concentration (MIC) was also performed for selected solvent extracts for all the bacterial species. A suitable positive control was also maintained. **Results:** Among the three marine algae screened *P. gymnospora* and *S. wightii* were found to be more active than *C. linum*. It was observed that the acetone extracts of all the three marine algae showed higher inhibitory activity for the selected bacterial species than other solvent extracts. The results revealed that the crude acetone extracts seem to be a good source material in identifying the effective pure antibacterial compound(s) in all the three marine algae and particularly, *S. wightii*. **Conclusions:** The present study showed that the acetone extracts of marine algae such as *S. wightii*, *C. linum* and *P. gymnospora* exhibited good antimicrobial activity. But the acetone extracts of *S. wightii* possessed highest antibacterial activity than others and so it could be useful in seeking active principles against human pathogenic bacteria.

1. Introduction

Despite the remarkable progress in the field of human medicine, the infectious diseases caused by bacteria, virus, fungi and parasites are still a major threat to public health and universal economies. They are caused by different types of infections such as drug-resistant infections, mostly involving bacteria, and many emerging pathogens are increasing significantly over time. These diseases are the most important cause of early death and killing of about 50 000 people worldwide every day[1,2]. The bacterial pathogens mainly cause severe problems to human beings by spreading various diseases, as they are found in multiple environmental habitats[3,4]. Bacterial pathogens like *Bacillus subtilis (B. subtilis)* are accountable for causing food borne gastroenteritis. *Escherichia coli (E. coli)*, *Staphylococcus aureus (S.aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)* are responsible for diseases like mastitis, endocarditis, meningitis and upper respiratory complications. Various species of *Salmonella* cause diarrhea, typhoid and enteric fever[5,6]. Enteric infections are major public health problems in developing countries and contribute to the death of 3.3-6.0 million children annually[7]. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* are the major etiological agents of sporadic and epidemic diarrhea both in children and adults[8]. People mostly use synthetic drugs to prevent or control the infectious diseases caused by microbes. Regular use of these drugs leads to development of resistance by the microbes against the drugs[9-11]. It is not only the resistance but also the cost of synthetic chemicals lead to search for alternate medicine such as antimicrobial compounds from natural sources. Plant derived natural products and antibiotics are found to be the effective alternative recognized from natural environmental resources. At this

*Corresponding author: Sundaram Janarthanan Associate Professor, Department of Zoology, University of Madras, Guindy Campus, Chennai-600 025, India.
Tel. +91-44-22202842; Fax. +91-44-22352494;
E-mail. janarthanans@rediffmail.com

time, essential pharmacological and therapeutic products are being obtained and actively sought from the ocean[12-18]. One of the potential groups of natural resource is algae which are known to possess promising novel bioactive substances[19-21].

Amongst approximately 50 000 known plant species[22] and 30 000 species of algae only a small percentage is known to possess potential bioactive compounds[23]. The chemical forms of these compounds include haloforms, halogenated alkenes, alkenes, alcohols, aldehydes, hydroquinones and ketones that are used in the treatment of most of the diseases as antibiotic materials[24]. According to a survey by National Cancer Institute, USA, about 64% of the 974 small-molecule new chemical entities identified from natural resources in the past 25 years were introduced as drugs in the market worldwide during 1981 and 2006[25]. Especially, marine algal species serve as a rich source of several novel biologically active compounds but a very few species have been investigated for their medicinal properties. Likewise, certain marine algae like *Ulva lactuca (U. lactuca)*, *Sargassum wightii (S. wightii)* and *Gracillaria edulis (G. edulis)* are known to be active against certain pathogenic and non-pathogenic bacterial strains[26]. Thus, there is an interest in phytomedicine from marine algae and therefore many marine algal species are now examined for their pharmacological properties. Marine algae or seaweeds are potential renewable source of marine environment and also known to produce a variety of secondary metabolites with broad spectrum biological activities. There are numerous reports with reference to several pathogen inhibitory compounds from marine macroalgae against human viral, microbial, fungi and yeast pathogens. The secondary metabolites with cytostatic[27,28], antiviral[28,29], HIV antiviral agents[30-32], antihelminthic[33-36], antiproliferative[34], antimycobacterial[37,38], antifungal[39,40] and antibacterial[41-45], antimicrobial[46-51], antileishmanial and anti-trichomonal[52,53], anticoagulant[54], antitumor[55], antiprotozoal[56], nematicidal and fungicidal activities[57] have been detected in marine algae. In the present study, antibacterial efficacy of various organic solvent extracts of the seaweeds *S. wightii*, *Chaetomorpha linum (C. linum)* and *Padina gymnospora (P. gymnospora)* against some clinically important gram-positive and gram-negative human pathogenic bacteria species is reported.

2. Materials and methods

2.1. Plant material

Three species of marine algae [*S. wightii* Greville brown algae (Phaeophyceae), *C. linum* green algae (Cladophoraceae) and *P. gymnospora* - light brown algae (Dictyotaceae)] were collected during low tide by hand picking from the coast of Tuticorin, Tamil Nadu, India. The collected marine algae were identified and used for antibacterial studies.

2.2. Preparation of solvent extracts

The collected marine algae or seaweed samples were cleaned to remove the epiphytes and extraneous matter. The necrotic parts of the plants were also removed. The samples were washed carefully for about 3 to 4 times with sea water and then in fresh water. The algal samples were then transported to the laboratory in sterile plastic bags under ice. Voucher specimens of the collected samples were deposited in the department herbarium and some of them were also frozen at -20 ℃ for future reference. The samples were once again rinsed with sterile distilled water and shade dried. The dried samples were cut into small pieces and ground into fine powder in a clean mixer grinder. The powdered samples were soaked with hexane (100g/300mL) for 48 hours at room temperature. The extract was then filtered through a Buchner funnel with Whatmann No. 1 filter paper. The filtrate was evaporated to dryness under pressure using rotary vacuum evaporator at 50 ℃. The remains of the plant material were extracted using ethyl acetate, acetone and methanol sequentially in a similar manner using cold percolation method. These crude extracts were then tested for their antibacterial activity against selected human pathogens.

2.3. Test microorganisms and media

The gram-negative bacterial strains used for this experiment were *P. aeruginosa* (ATCC27853), *S. typhi*-B, *Erwinia amylovora* (MTCC2760) (*E. amylovora*), *Enterobacter aerogenes* (MTCC111) (*E. aerogenes*), *Proteus vulgaris* (MTCC1771) (*P. vulgaris*), *Klebsiella pneumonia* (ATCC15380) (*K. pneumonia*) and *E. coli* (ATCC25922). The gram-positive bacterial strains were Methicillin resistant *S. aureus*, *B. subtilis* (MTCC441) and *Enterococcus faecalis* (ATCC29212) (*E. faecalis*). These human pathogenic microorganisms were obtained from the Laboratory of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Mueller-Hinton Broth (MHB) was obtained from Hi-Media while solvents used were of HPLC grade.

2.4. Preparation of inoculums

Bacterial inoculums were prepared by transferring a huge number of bacterial strains from fresh culture plates to tubes containing 10 mL of Mueller Hinton Broth (Hi-media) and incubated for 24 hours at 37 ℃. The tubes were shaken occasionally to aerate and promote growth. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 10^8 CFU/mL.

2.5. Antibacterial activity

Antibacterial activity was carried out using the disc-diffusion method[58]. The petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations (5 mg/disc, 2.5 mg/disc and 1.25 mg/disc) of the crude extracts were prepared and loaded on the sterile discs (Hi-media) which were placed on the surface of the