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Evaluation of antibacterial activity of some selected green seaweed extracts from Muttam coastal areas, Kanyakumari, Tamil Nadu, India

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PEER REVIEW

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Comments

The background information and methodology of the present paper was very clear. The selected seaweed extracts were most effective against Gram-negative strains (*P. aeruginosa* and *E. coli*) compared to Gram-positiveve strains (*B. subtilis* and *S. aureus*).
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ABSTRACT

Objective: To investigate the antimicrobial activity of the selected marine green algae *Ulva lactuca* (*U. lactuca*), *Cheatomorpha linoides* and *Helimeda macroloba* against six strains of Gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* and *Lactobacillus acidophilus* (*L. acidophilus*)] and Gram-negative bacteria [*Escherichia coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus mirabilis*].

Methods: The selected green seaweed extracts were experimented with four different solvents (acetone, ethanol, methanol and chloroform) against the selected pathogens by using agar disc diffusion method.

Results: The maximum activity (7 mm) was observed by the extract of *U. lactuca* against *Proteus mirabilis* by using methanol as a solvent and the lowest activity (2 mm) was recorded by the extract of *U. lactuca* against *L. acidophilus* by using chloroform as a solvent and ethanol extract against *P. aeruginosa*. The lowest activity (2 mm) was seen in the extract of *Cheatomorpha linoides* by using ethanol and methanol as a solvent against *S. aureus*. In *Helimeda macroloba* extract, the lowest activity was recorded against *Escherichia coli* by using chloroform as a solvent. The microbial strains *S. aureus*, *P. aeruginosa*, *Bacillus subtilis* and *L. acidophilus* were resistant to the chloroform and methanol of all selected seaweeds.

Conclusions: Further study should be needed to identify the prime compound which is responsible for the activity against the selected pathogens especially those causing the human diseases.

KEYWORDS
Seaweeds, *Ulva*, *Cheatomorpha*, *Helimeda*, Antimicrobial activity

1. Introduction

Seaweeds are multicellular macroalgae used as potential renewable resource in the field of medical and commercial environment. The seaweed contains numerous pharmacologically important bioactive constituents such as flavanoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals and they used to found attached to the bottom in relatively shallow coastal waters. Nowadays seaweeds are used as dietary food supplements in daily life and it regulates the human health. As well it enhances human health and controls the various pathogenic conditions. The seaweeds are grouped under three divisions, namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae) depending on their nutrient and chemical composition[1]. They are abundant on hard substrates and commonly extending to depths of 30-40 m. About 624 species have been reported in India with a potential of 77000 tons (wet weight) per annum. The red seaweeds contribute 27.0%, brown 0.2% and others 72.8%. About 206 species of algae have been reported from the mangrove environment. Seaweeds are the only source of phytochemicals, namely, agaragar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents. They are also used for human consumption, animal feed and as manure in several countries. The greatest use of agar is in association with food preparation and in the pharmaceutical industry as

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a laxative or as an outer cover of capsules. Being rich in minerals, vitamins, trace elements and bioactive substances, seaweeds are called medical food of the 21st century. In recent years, there are numerous reports of macro algae derived compounds, which have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimitotic activities[2,3]. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides and it show anti-bacterial activities. Many metabolites isolated from marine algae have been shown to possess bioactive efforts[4].

Microorganism is the important component to create an infectious disease in human beings. So it is the right time to discover the new microbial drugs to cure the diseases against the human pathogens. Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world and it is useful to produce drugs for the infectious diseases has certain limitations due to changing patterns of resistance in pathogens and side effects they produced. These limitations rouse demand for improved pharmacokinetic properties which necessitate the continued research for new antimicrobial compounds for the development of drugs[5,6]. Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides[7]. Using organic solvents for antibacterial activity always provides a higher efficacy in extracting compounds[8]. The extraction of antimicrobials from the different species of seaweeds was solvent dependent. Methanol was a good solvent for extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species[9]. Screening of organic extracts from marine algae and other marine organisms is a common approach to identify compounds of biomedical importance. Hence, the present study was undertaken to assess and evaluate the antibacterial activity of selected marine green seaweeds of Chlorophyceae against the six strains of selected pathogenic bacteria.

2. Materials and methods

2.1. Collection of the algal material

The seaweeds belongs to the family Chlorophyceae like *Ulva lactuca* (*U. lactuca*), *Cheatomorpha linoides* (*C. linoides*) and *Helimeda macroloba* (*H. macroloba*) were collected in bulk quantity from coastal areas of Muttam, Kanyakumari district, Tamil Nadu, India during September 2012 to January 2013. Seaweed species exposed on sand and rocks were collected in sterile plastic bags and brought to the laboratory. Each species was washed thoroughly with running water to remove epiphytes, animal castings, attached debris and sand particles, and the final washings were done using distilled water and dried under shade. After that the samples were cut into small pieces with the help of chopper and powdered in a mixer grinder. The powdered samples were then stored in freezer for further study.

2.2. Preparation of seaweed extract

Approximately 5 g of the powdered materials were extracted successively with 250 mL of acetone, chloroform, ethanol and methanol by using Soxhlet apparatus for 8 h at room temperature not exceeding the boiling point of the solvents. The extracts were filtered by using Whattman No. 1 filter paper and then concentrated in vacuum at 40 °C by using hot air oven. The residues obtained were stored in a freezer at −20 °C until further tests. The seaweed extracts were further subjected for antimicrobial activity by agar disc diffusion method.

2.3. Bacterial strains

Gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and *Lactobacillus acidophilus* (*L. acidophilus*)] and Gram-negative bacteria [*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus mirabilis* (*P. mirabilis*)] stock cultures were obtained from the Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.

2.4. Antimicrobial activity

The antimicrobial properties were carried out by using agar disc diffusion method. About 6 mm of paper disc was prepared from Whatman No. 1 filter paper. The agar plate method was employed to find out the antibacterial activity against Gram-positive and Gram-negative bacteria. Muller-Hinton agar was used to preserve and incubate the bacterial strains at 37 °C for overnight. Then the diluted bacterial culture was placed on Muller-Hinton agar medium and spread throughout the plate by using sterile rod. The antibacterial activity was recorded by measuring the diameter zone of inhibition. Triplicates were maintained for each and every test.

3. Results

Different extracts of *U. lactuca*, *C. linoides* and *H. macroloba* were tested for their antimicrobial activity against six strains of Gram-positive, Gram-negative bacteria by agar disc diffusion method. The results of antimicrobial activity against tested pathogens were tabulated in Table 1.

Table 1

Antibacterial activity of selected seaweeds of Chlorophyceae against some human pathogens.

Extracts	Solvents	Zone of inhibition in mm					
		Gram +ve			Gram −ve		
		S.a	B.s	L.a	P.a	E.c	P.m
U. lactuca	Acetone	3	−	3	−	4	3
	Chloroform	−	−	2	−	−	−
	Ethanol	−	3	−	2	3	−
	Methanol	3	3	−	3	5	7
C. linoides	Acetone	3	3	3	−	3	3
	Chloroform	−	−	−	−	−	−
	Ethanol	2	3	−	−	3	−
	Methanol	2	3	−	4	6	−
H. macroloba	Acetone	3	3	3	3	5	−
	Chloroform	−	−	2	−	2	−
	Ethanol	3	3	−	3	3	−
	Methanol	−	3	−	5	3	−

S.a: *S. aureus*; B.s: *B. subtilis*; L.a: *L. acidophilus*; P.a: *P. aeruginosa*; E.c: *E. coli*; P.m: *P. mirabilis*.

Seaweed extracts in different solvents exhibited different antimicrobial activities with varying levels. Most of the seaweed extracts exhibited antibacterial activity against all the tested pathogens. The highest activity (7 mm) was observed in the extract of *U. lactuca* against *P. mirabilis* by using the methanol as a solvent and the lowest activity (2 mm) was obtained in the chloroform extract against *L. acidophilus* and ethanol extract against *P. aeruginosa*.

The highest activity of *C. linoides* was observed in the methanol extract (6 mm) against *E. coli* and the lowest activity (2 mm) was seen in the ethanol and methanol extract against *S. aureus*, and all of the other pathogens were resistant to the chloroform extract. Regarding *H. macroloba*, the highest activity was observed in the methanol extract (5 mm) against *P. aeruginosa* and acetone extract (5 mm) against *E. coli*. The lowest activity was seen in chloroform extract (2 mm) against the *L. acidophilus* and *E. coli*. However in most of the seaweeds, methanol extract was found to be more effective followed by acetone extract. All the above said Gram-positive and Gram-negative bacterial strains were resistant to the chloroform extracts of all green seaweed varieties.

4. Discussion

The marine environment has great potential for the discovery of lead compounds that could be used against infectious diseases and parasites. Seaweeds are primitive non flowering plants without root, stem and leaves. They contain different vitamins, minerals, trace elements, protein, iodine, bromine and bioactive substances. Nowadays microorganisms playing a significant role to produce infections and making it global growing problems. So this is the right time to discover new drugs and antimicrobial compounds from seaweeds with diverse chemical structures and novel mechanisms of action for new and emerging infectious diseases[10]. The antibacterial activity of seaweeds may be varied due to many factors like the habitat, season of algal collection, different growth stages of plant, experimental methods, method of extraction and solvent used in extraction[11]. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction of seaweeds. Many workers have been employed different solvents for screening the antibacterial activity of seaweeds and made comparisons[12,13].

A high antimicrobial activity of seaweed extracts has been reported against both Gram-negative and Gram-positive bacteria[14]. Rajasulochana *et al*. have reported that the chloroform extract of *Enteromorpha compressa* and *Chaetomorpha antennina* had a moderate bactericidal and fungicidal activity against *S. aureus*, *B. subtills*, *E. coli*, *Proteus vulgaris* and two fungal species *Aspergillus niger* and *Candida albicans*[15]. Umamaheswari *et al*. observed the antibacterial activity of marine macro alga *Chaetomorpha aerea*[16], the maximum antibacterial potential was recorded from the ethanol extract against *P. aeruginosa*, and the minimum was noted in methanol extracts against *Micrococcus* sp. and *Salmonella typhii*. Karthikaidevi *et al*. reported the antibacterial activity of three species of marine macro algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* from the Gulf of Mannar coast[17]. The maximum activity was noted in the methanol, ethanol and acetone extracts against *Klebsiella pneumonia*, *S. aureus*, *Enterococci* sp., *P. aeruginos* and *Vibrio parahaemolyticus*. Thirumaran and Anantharama reported the antibacterial activity of marine macro alga *Caulerpa scalpeliformis* from Gulf of Mannar coast[18], the maximum activity was noted in methanol extracts against *Salmonella typhii*, *Micrococcus* sp. and *Shigella bodii*.

The selected seaweed extracts were most effective against Gram -ve strains (*P. aeruginosa* and *E. coli*) compared to Gram +ve strains (*B. subtilis* and *S. aureus*) in the disc diffusion antibacterial assay). These results are in agreement with observations on antibacterial activities of different medicinal plants as reported by previous workers[19,20]. The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane, which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside[21]. However, the Gram-positive bacteria do not possess such outer membrane and cell wall structures[22].

Microorganisms especially bacteria plays a considerable role to create a high rate of morbidity and mortality in human population and aquaculture organisms[11]. For example, the food borne diseases was caused by the bacterium *B. cereus*[23]. *E. coli*, *S. aureus* and *P. aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella typhi* causes diarrhoea and typhoid fever. *P. aeruginosa* is an important and prevalent pathogen among burned patients, and it is capable of causing life-threatening illness[10].

This research finding gives further scope to screen the chemical constituents of the extracts which will be very useful to combat the various diseases caused by pathogenic bacteria. The microbicidal activities observed in the crude methanolic extracts of the selected green algae from the southeast coast of India provide good evidence that algae maintain effective antimicrobial chemical resistance, and this antibacterial property is due to the presence of oleic (51.33%) and *n*-hexadecanoic acids (42%). From the present study, it can be concluded that the selected green alga for the study has a potential source of bioactive compounds. Finally we recommend that, seaweeds from the coastal areas are potential sources of bioactive compounds and should be investigated for natural antibiotics. These compounds can be utilized for the development of natural antibiotic against multidrug resistant bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

During the last few decades there had been an increasing interest in the study of marine seaweeds and their traditional use in different parts of the world. Documenting the indigenous knowledge through antimicrobial studies is important for the conservation and utilization of biological resources from marine.

Research frontiers

The present paper deals with the antimicrobial activity of the selected marine green algae *U. lactuca*, *C. linoides* and *H. macroloba* against three strains of Gram-positive bacteria and three strains of Gram-negative bacteria.

Related reports

In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimitotic activities.

Innovations and breakthroughs

The antimicrobial properties were carried out by using Muller Hinton agar disc diffusion method.

Applications

The higher inhibitory activity of seaweed extracts has been reported against both Gram-negative and Gram-positive bacteria, which is useful for further studies.

Peer review

The background information and methodology of the present paper was very clear. The selected seaweed extracts were most effective against Gram-negative strains (*P. aeruginosa* and *E. coli*) compared to Gram-positive strains (*B. subtilis* and *S. aureus*).

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