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ORIGINAL ARTICLE

Chemical Characterization and Antimicrobial Activity of the crude Extract of the Seaweed *Caulerpa* sp.

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ABSTRACT
Seaweeds are multicellular marine algae and are known to have the ability to produce bioactive metabolites. Therefore, an attempt was made to prepare crude extract from a green macro alga Caulerpa and then to study the antimicrobial activity of these extracts. Standard paper disc diffusion method was used for the antimicrobial assay. Overall results were highly encouraging as antibacterial activity was exhibited against all the test microorganisms. The results therefore confirmed that the marine algae have the potential of producing chemicals with antimicrobial activity.
Keywords: *Algae, Seaweeds, Caulerpa sp., bioactive metabolites, antibacterial activity, Thin Layer Chromatography, FTIIR*

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INTRODUCTION

Seaweeds are important members of algal group of the plant kingdom. They are multicellular benthic algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient and chemical composition [1]. Apart from their use as a food and as feed, seaweeds have many important commercial applications. Marine algae have been exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan, not for health aspects. Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics [2]. Seaweeds also find their use in the drug discovery arena.

Seaweeds are reported to be a potential source of antimicrobial agents. Extract of the seaweed Sargassum spp exhibited a dose-dependent free radical scavenging action against DPPH radical and hydroxyl radical and antimicrobial activity. In addition, inhibition of lipid peroxidation and glutathione-S-transferase activities were also observed [3]. Cox et al 2010 have reported strong antimicrobial and antioxidant activity from the extracts prepared from the six edible seaweed species found in Ireland [1]. Manivannan et al 2011 reported promising activity of the extracts prepared from brown seaweeds against human bacterial as well as fungal pathogens [2]. Extracts of green seaweeds have been shown to have potent activity oral pathogens by Sujatha et al. [4].

*Caulerpa, the a*lgae under present invstigation is a macroalgae that falls into the *Chlorophyta* Phylum (green algae group). It grows in various shades of bright green colors, as well as different forms and shapes - some growing tall, others growing as mats.

These green macro algae only use their runners and roots for anchoring themselves in place, deriving their nourishment (nitrates & phosphates) from the water by means of absorption through their blades or fronds. *Caulerpa* sp. can have either fine cylindrical or elongated needle-shaped blades or thickened blades and branchlets with a thicker rhizome. They are classified in Division: Chlorophyta; Class : Ulvophyceae; Order : Bryopsidales and Family: Caulerpaceae.

Oweing to the abundant occurrence of this species along coastal line of Konkan region of Maharashtra, the present study was undertaken to prepare crude extracts from this green seaweed and evaluate their antimicrobial activity.

MATERIALS AND METHODS

Collection of seaweed

Macroalgae *Caulerpa* sp. was collected from the intertidal region of Ratnagiri located along the west coast of India (16^0C 59.5'N, 73^0C 16.5'E) in March 2015. Seaweed samples were transported to the laboratory in a cold sterile condition and then transferred to 4^0C.

Seaweed samples were collected and cleaned with seawater and then with freshwater to remove debris as well as epiphytes. Seaweed was dried at room temperature and powdered for further extraction.

Preparation of extract

100 grams of powdered seaweed sample was extracted by using methanol:Chloroform (1:1) solver mixture. Seaweed powder was soaked in the solvent and kept for overnight. The extraction procedure was repeated for three time. Extract was further concentrated by using rotary evaporator. The dried (solvent free) extract was stored in the refrigerator for further use.

Thin layer chromatography

Thin layer chromatography (TLC) is an invaluable method used in chemistry and biochemistry for the separation and analysis of a wide variety of molecular mixtures. TLC methods can be used to separate mixtures of inorganic ions, organic molecules and bio organic compounds such as pigments, lipids, amino acids, nucleotides and sugars.

The TLC plate used was made up of aluminum sheet on to which silica gel was used as an absorbent. The dried crude extract of seaweed was weighed (approximately 10 mg) and dissolved in 50µl of methanol. Sample of 2-3µl was applied on the TLC plate with the help of capillary tube. The plate was kept in a petroleum ether/ethyl acetate system (50:50 vol/vol) and butanol/acetic acid/water solvent system. After development, the solvent was evaporated, and the dried plates were kept under UV, iodine chamber and were sprayed with 5% H_2SO_4 and ninhydrin for detection of various well separated bands. Results of all different conditions were compared.

FTIR spectroscopy

5 mg of dried crude extract was taken and crushed to a fine powder using motor and pestle. Kalium bromide was added and evenly mixed with the sample to obtain a homogenized fine powder. The sample was then placed in the moulds and pressed using mechanical strength for 20-30 sec using a clean alcohol sterilized spatula. The sample pellet with the mould was then placed on the sample pan and was ready for analysis. The scanning was done at frequency wavelength 400-4000 cm^{-1} with resolution of $4cm^{-1}$. The scanning results were displayed in % transmission absorbance.

Antimicrobial assay of crude extracts of seaweed

Antimicrobial assay was done for crude extracts of seaweed. A Kirby-Bauer method recommended by the Clinical and Laboratory Standards Institute (CSLI) is usually used for antimicrobial susceptibility testing (5). The test cultures were revived from the glycerol stock using appropriate growth mediums. The test cultures included human clinical pathogens. The glycerol stock was added to sterile Muller Hinton Broth and was incubated on rotary shaker incubator at 28 ± 2°C for 24-48h.

Preparation of discs with extracts: Whatman's filter paper No. 1 was used for preparing the disc. It was punched to obtain a single disc of size 6 mm in diameter. The discs were packed in aluminium foils and sterilized in autoclave at 121°C, 150 psi for 15 min. The crude extract was used for antimicrobial testing. 25 mg of the dried extract of seaweed was weighed and dissolved in 50 µl of methanol separately. 5µl of the extract was applied on the sterile disc.

Muller Hinton Agar plates were used for test cultures. The plates were divided into grids and were numbered according to the extract number. 200 µl of the test organism was spread on the plate and discs loaded with the extract were placed in their corresponding grids with the help of alcohol sterilized forceps. Streptomycin (25 µg) was used as the standard antibiotic. The plates were incubated at 37°C for 24 h. After incubation, plates were observed for zone of inhibition. The inhibition zones shown by the extracts against the test pathogenic cultures were measured in mm. Final results were calculated as total diameter of inhibition zone minus diameter of disc 6mm.

RESULTS

Seaweed crude extract

From 100 grams seaweed powder 6 grams of crude extract was obtained

Thin layer chromatography

Chromatography is a method for separating the components of a mixture by differential adsorption between a stationary phase and a mobile (moving) phase. In this experiment, in the crude extract of seaweed total two bands were visible in solvent system 10% petroleum ether in ethyl acetate (Fig. 1). These results were useful further for the separation of active principles. Interestingly, both compounds were UV visible.

Fig. 1 Separation of components of Thin Layer Chromatography

FTIR spectroscopy:

In the crude extract of seaweed a medium sharp peak was observed at 3325.28 cm^{-1} wave number at 36-39% transmission absorbance indicating the presence of aliphatic secondary amine group, whereas weaker peak was also seen 2632.83 cm^{-1} frequency wavelength at 45-48% transmission absorbance depicting that it is aldehyde. A strong peak was seen at 1516.05 cm^{-1} frequency wavelength at 39-42% transmission resulting in nitro group (N-O) (Table 1; Fig. 2).

Table 1 FTIR analysis of seaweed extract

Sr. No.	Sample specific groups wavenumber (cm^{-1})	Peak Intensity	Absorption frequency region (cm^{-1})	Bond	Functional group	% Transmission
1	3325.28	Strong, broad,	3330-3250	N-H	Aliphatic secondary amine	36-39%
2	2632.83	Weak	2600-2550	C-H	Aldehyde	45-48%
3	2129.41	Weak, broad	2260-2190	CΞC	Alkyne	75-78%
3	1683.86	Strong, broad	1680	C=O	Secondary or tertiary amide	30-33%
4	1516.05	Strong, broad	1550-1500	N-O	Nitro compound	39-42%
5	1454.33	Medium, broad	1465	C-H	Alkane	36-39%

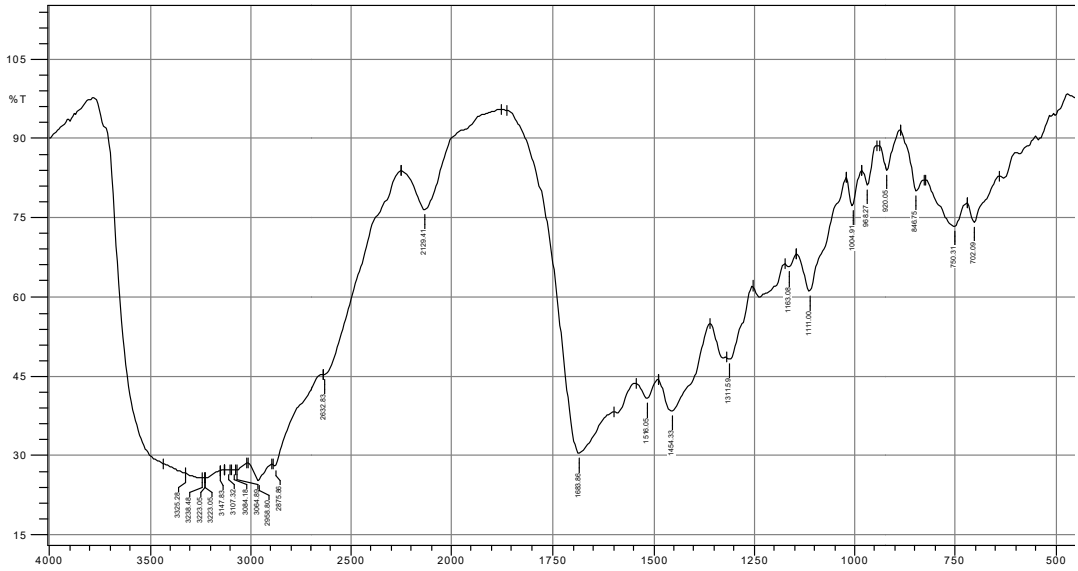

Fig. 2 FT-IR Spectrum of the seaweed crude extract

Antimicrobial activity:

The crude extract of Seaweed showed antibacterial activity against almost all the test bacterial isolates (Table 2). Maximum zone of inhibition of 12 mm was found against Salmonella sp. (Fig. 3) Followed by a zone of inhibition of 7 mm against E. coli. The crude extract also inhibited the growth of Pseudomonas aeruginosa. Least activity was seen against S. aureus and *Vibrio parahaemolyticus* (Table 2).

Table 2 Antimicrobial activity of seaweed crude extract against clinical pathogens
Number shows diameter of zone of inhibition.

Test bacterial isolates	Seaweed crude extract		Streptomycin (25µg)
	25µg	100µg	
Escherichia coli	2	7	11
Staphylococcus aureus	-	3	8
Salmonella sp.	4	12	10
Pseudomonas aeruginosa	2	6	7
Vibrio parahaemolyticus	-	2	7

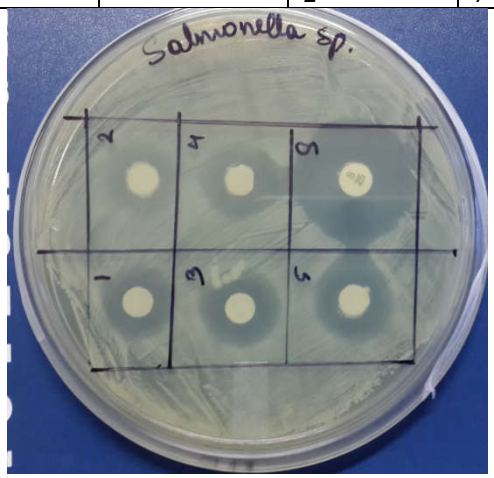

Fig. 3 Antimicrobial activities of seaweed extract against Salmonella spTwo concentrations with three replicates were tested. Figure shows zone of inhibition.

DISCUSSION

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [1, 6]. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [7, 8, 9]. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective mechanisms and compounds [10].

Approximately 841 species of marine algae are reportedly available [11]. These vast varieties of seaweeds were found to possess useful untapped biochemical compounds, which might be a potential source of drug leads in the future [12].

As indicated in this paper, the crude extract of Seaweed was found to be active against almost all test bacterial isolates. The antimicrobial activity of the extract was especially very strong against *E. coli* and *Salmonella* sp. The crude extract of seaweed showed two major UV visible compounds on TLC plate and in FTIR spectroscopy it showed the presence of alkane, amines and nitro functions groups. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from seaweeds. Further studies are warranted to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

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