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ANTIBACTERIAL ACTIVITY, BIOCHEMICAL COMPOSITION AND PHYTOCHEMICAL SCREENING OF SEAWEEDS *SARGASSUM WIGHTII, CHAETOMORPHA CRASSA* AND *VALONIOPSIS PACHYNEMA*

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ABSTRACT

Now-a-days the importance of seaweeds has been increasing both for pharmaceutical industry and traditional users as they have biological activity. The present study was conducted to investigate the phytochemical and biochemical contents and antibacterial property of three different seaweeds such as *Chaetomorpha crassa*, *Valoniopsis pachynema* and *Sargassum wightii* collected from the coast of Mandapam, Tamil Nadu, India. Extracts of powdered seaweed were prepared using different polar and non-polar solvents (Distilled water, Ethanol and petroleum ether). The antibacterial activity of seaweeds was analyzed against human pathogenic bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using agar well diffusion method. The extracts showed the presence of phytochemical constituents like flavonoids, tannins, phenols, sterols and terpenoids. The seaweed extracts were analyzed for the presence of carbohydrates, proteins and lipids. The brown alga *Sargassum wightii* contains lesser amount of carbohydrates (2.6 mg) as compared to the *Chaetomorpha crassa* (3.15 mg) and *Valoniopsis pachynema* (5.4 mg). The results indicated that among the tested extracts for antibacterial activity, *Bacillus subtilis* and *Staphylococcus aureus* were susceptible to ethanol and petroleum ether extract, whereas all the four bacterial isolates were resistant and moderately susceptible to aqueous extract. The result obtained from the study points out that the active component present in ethanol extract could prove to be a great remedy for treating diseases. The infections caused by *Bacillus subtilis* can be treated with acetone extract of seaweeds.

KEYWORDS: Seaweeds; Biochemical; Phytochemical; Human Pathogens; Antibacterial activity.

1. INTRODUCTION

The coastal region of Tamil Nadu, South India produces a rich source of marine algae (Manivannan *et al*., 2010),[1] there they grow almost like grass in large areas, extending over hundreds of kilometers. Over 805 of world's plant and animal species (Jha and Xuzi-rong., 2004)[2] and with more than 1, 50,000, seaweeds are found in the intertidal zones and tropical waters of the ocean environment.

Seaweeds are popularly known as marine algae and are a primary source of natural products which are considered ecologically and biologically important component in the marine ecosystems (Vigneshpriya *et al*., 2019).[3] Seaweeds are primitive non- flowering plants without true root stem and leaves (Ashwin *et al*., 2014).[4] More than 10,000 species of algae have been reported from all over the world. There are more than 20,000 species of algae in the world; India. possesses 434 species of red algae, 194 species of brown seaweeds and 216 species of green seaweeds (NAAS, 2003) [5] They are renewable living sources of food, feed and fertilizer in many parts of the world. They contain different vitamins, minerals, proteins trace elements, iodine, bromine and bioactive substances (Bhaskar and Miyashitra., 2005) [6] including acids, lipids, steroids, fatty acids, phenolic compounds, antioxidants, carotenoids, peptides, sugar and alcohols etc. (McHugh, 2003; Duan *et al*., 2006; Kuda *et al*., 2007).[7],[8],[9]

Seaweeds are one of the important marine living resources could be termed as the futuristically promising plants and have been a source of food and medicine (Lavanya *et al*., 2011)[10] It can be classified into three broad groups based on pigmentation brown, red and green. They are referred to as Pheophyceae, Rhodophyceae and Chlorophyceae respectively. It provides a rich source of structurally diverse secondary

metabolites. Over 2,400 secondary metabolites have been isolated and described from the divisions of Rhodophyta, Phaeophyta and Chlorophyta many of which have been reported to have excellent biological activity (Faulkner, 2002)[11] The functions of these secondary metabolites are defense against herbivores, fouling organisms and pathogens; they also play role in reproduction, protection from UV radiation and as allelopathic agents (Aruna *et al*.,2010).[12] Marine algae are the most important source of non-animal sulfated polysaccharides. They have a wide range of biological activities like antibacterial, antifungal, antiviral, antitumor, antihypercholesterolemic, anticoagulant and antioxidant activities (Srivastava., 2010).[13] It also possesses bio-active components which inhibit the growth of some of the Gram-positive and Gram-negative bacterial pathogens. The algal extracts were used as a curative and preventive agent for various diseases such as antibiotics, anthelminthic, cough remedies, antihypertensive, antitumor and antidiarrhea (Kolanjinathan *et al*., 2009).[14]

Microbial diseases are the challenging threat to human population as well aquatic habitat. Human pathogenic bacteria have potential to cause the following diseases such as skin diseases, pneumonia, tetanus, typhoid fever, diphtheria, syphilis, meningitis and leprosy (Kandhasamy, 2008).[15] Preventing outbreaks or treating the disease with drugs or chemical tackles of these problems might create an issue. In aquaculture, diseases of microbial origin cause high mortality rates and lesions on fish skin with consequent economic losses worldwide (Toranzo., 2005).[16] Accordingly, seaweeds are considered as such a source of bioactive compounds offer defense against Gram-positive or Gram-negative bacteria (Lima-Filo et al., 2002).[17] The bactericidal agents found in algae include amino acids terpenoids, phlorotannin, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids (Watson and Cruz-Rivera, 2003).[18]

Microbes are abundant in environment and infection due to bacterial and fungal pathogens has become more common. Now-a-days clinical microbiologists are showing great interest in screening of seaweeds for antimicrobial activities and photochemical as potential of new therapeutics. Therefore, based on literature survey, marine algae have become recognized as potential source of antimicrobial substances in the development of a drug resistance in human pathogens. Keeping in view the importance of seaweeds and their usefulness, the current study was undertaken to investigate the antibacterial activity, biochemical composition and phytochemical screening of *Sargassum wightii* (brown alga), *Chaetomorpha crassa* and *Valoniopsis pachynema* (green algae) against clinical pathogens.

2. MATERIALS AND METHODS

2.1 Study area

Mandapam, a small panchayath town in Rameswaram district, Tamil Nadu, India is a famous tourist attraction spot in Rameswaram. It is located at 98.28°N 79.12°E at an average elevation of 9 m (29 feet) with population of 15,799. Mandapam is the main land that links the island Rameswaram. Mandapam Beach, a quite beach with long speeches of sand is the gate way to too many small islands. The beach is the home to peaceful and scenery beauty with natural unique coral reef format on and marine vegetarian lying below the water surface. The main occupation of people living here is fishing.

2.2 Collection of seaweed

Seaweeds were collected during the lowest tide of chart datum from the seaweed infested locations. The macroalgae which infested exclusively on the intertidal rocky and other substratum was selected for the collection as to avoid other microalgal contamination. The live and healthy macroalgal sample was collected by handpicking method at a depth of 1-2 m.

2.3 Preservation of seaweed

Immediately after collection of seaweed from the Mandapam beach, the seaweed was washed in fresh seawater to avoid other algal contamination. To eliminate the epiphytes, extraneous matter, coarse sand and other calcareous impurities it is again washed with distilled water. The collected samples were transported to the laboratory in polythene bags under ice at 20℃ to avoid decomposition and loss of metabolites for identification and future reference.

2.4 Identification of seaweeds

The algal species collected were identified as *Sargassum wightii*, *Chaetomorpha crassa and Valoniopsis pachynema* with the help of seaweed taxonomist in Centre Salt and Marine Chemical Research Institute Mandapam camp, Tamil Nadu, India. Taxonomic classification of the algal species was made according to the developed and modified system of classification. The identification was based on (a) morphological, external and internal characteristics and (b) ecological distribution and habitat.

2.5 Preparation of seaweed powder

The seaweeds were washed thoroughly with tap water until unwanted impurities, adhering sand particles and extraneous matter like epiphytes and pebbles were removed. Followed by seaweeds were rinsed with sterile distilled water thrice to remove the extra adhered sand and dust materials. Later they were spread on filter paper and left few hours to absorb the excess water. Then the cleaned seaweeds were cut into small pieces, shade dried for two weeks and later the samples were made into coarse powder by grinding them in an electric mixer grinder and sieved through a mesh to get fine particles of size 150 µm. The powdered samples were stored in an airtight container until use.

2.6 Preparation of algal extract

The powdered seaweed, *Sargassum wightii*, *Chaetomorpha crassa* and *Valoniopsis pachynema* were subjected for extraction of the bioactive compounds. 10 g was weighted using an electric balance (Denver XS-210) and made into packet using Zerohaze filter paper (A Grade, SD"s). This powder was subjected to extraction (Harbourne, 1973)[19] with different solvents including ethanol, petroleum ether and distilled water with events of increasing polarity. The seaweed extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40 º C. The residue obtained was stored in a tightly closed glass vials in the refrigerator for further use. The aliquots were tested for the biochemical, phytochemical, antibacterial activities of algal species.

2.7 Selection and collection of bacterial strains

The seaweed extracts were tested against a panel of clinical isolates, the gram negative and positive pathogens such as *Pseudomonas aeruginosa* (Gram-negative), *Klebsiella pneumonia* (Gram-negative), *Bacillus subtilis* (Gram-positive) and *Staphylococcus aureus* (Gram-positive) obtained from PSG Institute of Medical science and research, Coimbatore, India. The bacterial stock cultures were maintained at 4 ℃. Test microorganisms were cultivated on nutrient broth agar at 37 ℃ for 18 h before inoculation for the assay broth culture, which contained bacteria, was added to medium that was prepared previously.

2.8 Antibacterial assay

The seaweed extracts were screened against selected gram-positive and gram-negative clinical pathogens. The antibacterial bioassay of the seaweed extract was carried out using the agar well diffusion method. At first, 6 mm holes were punched in nutrient agar medium (Hi Media Laboratories Pvt. Ltd) using a cork borer in nutrient agar plates inoculated with test microorganisms. Petri dishes were left 15 min until bacteria absorbed the medium. The seaweeds extracted with ethanol, distilled water and petroleum ether in with each solvent extraction of three seaweeds are poured in wells of each of the petri plates. To prevent drying all plates were covered with sterile plastic bags. The Petri plates were incubated under 37 ℃ for 24 h. After incubation the inhibition zones around the wells were measured on the underside of petri-dishes and expressed in nearest millimetres. The inhibition zones of three samples with each solvent were compared and the values were tabulated.

2.9 Biochemical analyses

The carbohydrate content was estimated by Anthrone method (Caroll., et al.1956).[20] Protein content was estimated by Bradford's method (Bradford et al., 1976).[21] The lipid content was estimated using chloroform-methanol mixture as described by Folch et al. (1957).[22]

2.10 Preliminary screening of phytochemicals

10 g powdered seaweeds were soaked in 100 ml of distilled water to obtain crude extract. The mixture then evaporated and filtered using sterilized 0.2 µm membrane syringe. A fraction of marine seaweeds *Sargassum wightii*, *Chaetomorpha crassa* and *Valoniopsis pachynema* was subjected to phytochemical analysis by adopting the standard qualitative procedures as reported by (Sadasivan and Manickam.,1996; Harborne., 1998).[23],[24] Natural chemical groups such as alkaloids, phenols, flavonoids, anthraquinones, tannins, saponins, quinones, sterols and terpenoids were probed.

3. RESULTS

3.1 Antibacterial activity of seaweeds

The extracts of seaweeds *Sargassum wightii*, *Chaetomorpha crassa* and *Valoniopsis pachynema* were tested for their antibacterial activity against four clinical bacterial isolates namely *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtills and Staphylococcus aureus*. Table 1 shows the antibacterial activity of the distilled water, ethanol and petroleum ether extracts of the seaweeds against the tested bacterial isolates. Figures 1-4 shows the zone of inhibition of seaweeds against the tested bacterial isolates.

From the table 1 it was observed that the zone of inhibition was found to be maximum in the ethanol extract of seaweed, *Sargassum wightii*. The ethanol extract was found to be more active against *Staphylococcus aureus* (16 mm) followed by *Bacillus subtilis* (15 mm), *Klebsiella pneumoniae* (12 mm) and *Pseudomonas aeruginosa* (12 mm). The aqueous extracts exhibited the zone of inhibition of 14 mm, 13 mm, 13 mm and 12 mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis* respectively. The petroleum ether extract showed the zone of inhibition of 13 mm, 12 mm, 12 mm and 10 mm respectively against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Table 1: Antibacterial activity of the seaweeds extract against the selected pathogenic bacteria.

SEAWEEDS	EXTRACTS	TEST ORGANISM (ZONE OF INHIBITION MM)			
		P. aeruginosa	***B. subtilis***	***K. pneumonia***	***S. aureus***
Sargassum wightii	Distilled Water	13	12	13	14
	Ethanol	12	15	12	16
	Petroleum ether	11	12	13	12
Chaetomorpha crassa	Distilled water	16	14	14	13
	Ethanol	17	13	15	14
	Petroleum ether	14	13	13	12
Valoniopsis pachynema	Distilled water	15	14	14	13
	Ethanol	13	15	14	11
	Petroleum ether	17	13	14	15

In *Chaetomorpha crassa* the ethanol extract was found to be more active against *Pseudomonas aeruginosa* (17 mm) followed by *Klebsiella pneumoniae* (15 mm), *Staphylococcus aureus* (14 mm) and *Bacillus subtilis* (13 mm). The aqueous extracts exhibited the zone of inhibition of 16 mm, 14 mm, 14 mm and 13 mm against *Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae,* and *Staphylococcus aureus* respectively. The petroleum ether extract showed the zone of inhibition of 14 mm, 13 mm, 13 mm and 12 mm respectively against *Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae,* and *Staphylococcus aureus* respectively.

In *Valoniopsis pachynema* petroleum ether was the best solvent showed high activity against *Pseudomonas aeruginosa* (17 mm) followed by *Staphylococcus aureus* (15 mm), *Klebsiella pneumoniae* (14 mm) and *Bacillus subtilis* (13 mm). The aqueous extracts exhibited the zone of inhibition of 15 mm, 14 mm, 14 mm and 13 mm against *Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. The ethanol extract showed the zone of inhibition of 15 mm, 14 mm, 13 mm and 11 mm respectively against *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively.Hence, *Bacillus subtilis* and *Pseudomonas aeruginosa* were susceptible to ethanol and petroleum ether extract, whereas all the four bacterial isolates were resistant and moderately susceptible to aqueous extract.

The result obtained from the study points out that the active component present in ethanol extract could prove to be a great remedy for treating diseases. The infections caused by *Bacillus subtilis* can be treated with acetone extract of seaweeds.

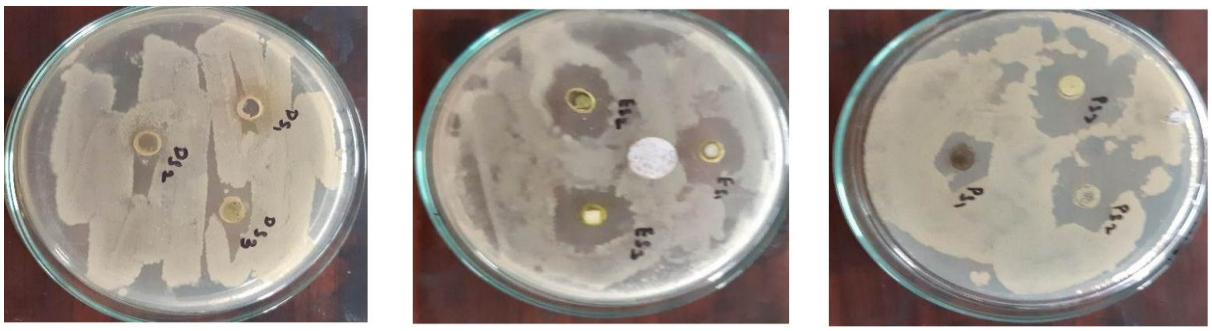


Fig. 1: Antibacterial activity of seaweed extracts against *bacillus subtilis.*

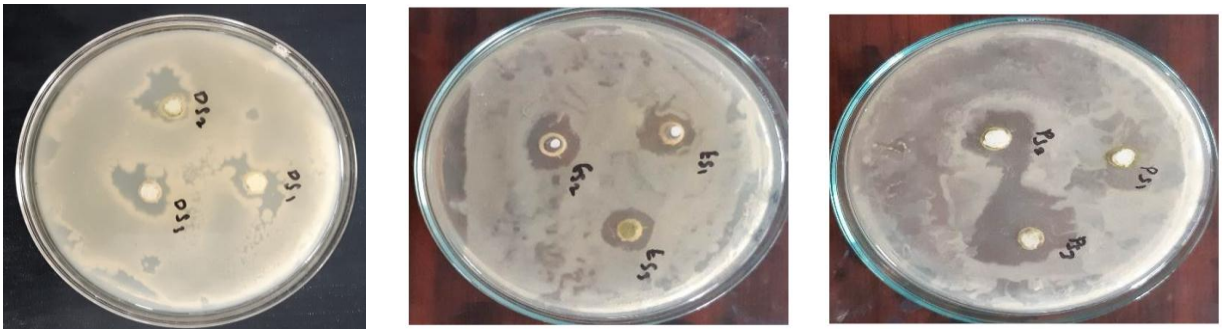


Fig. 2: Antibacterial activity of seaweed extract against *pseudomonas aeruginosa.*

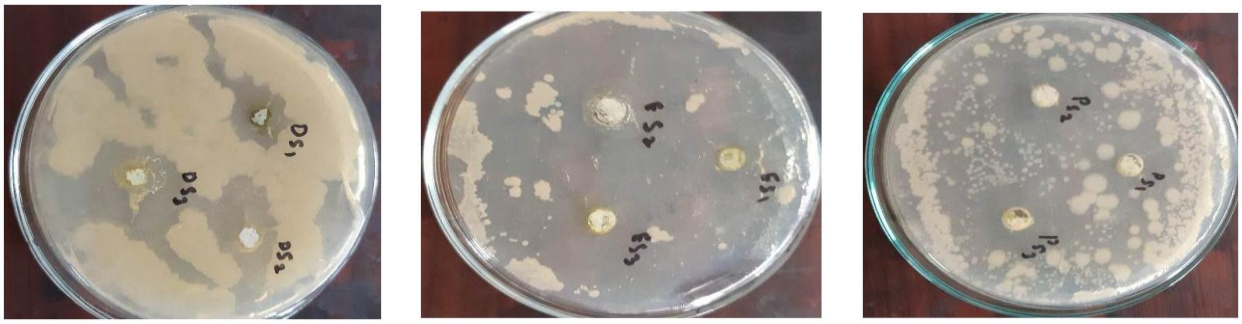


Fig. 3: Antibacterial activity of seaweed extracts against *staphylococcus aureus.*

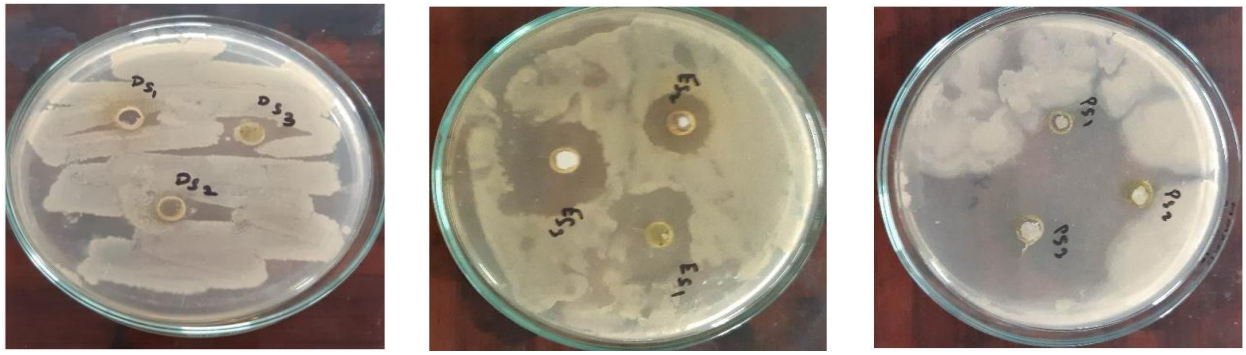


Fig. 4: Antibacterial activity of seaweed extracts against *klebsiella pneumonia.*

3.2 Phytochemical analyses of seaweeds

Seaweeds contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Tonthubthimthong *et al*., 2001).[25] Phytochemicals are known to possess antioxidant (Wang *et al*., 2009)[26] and antimicrobial properties (Khan and Wassilew, 1987)[27] Due to these properties, they are largely used for medicinal purpose. In the present study qualitative phytochemical analysis of various extracts of seaweeds *Sargassum wightii*, *Chaetomorpha crassa*, *Valoniopsis pachynema* was carried out and results are tabulated in Table 2.

Table 2: Qualitative analyses of phytochemicals in the aqueous extract of seaweeds.

S. No.	Phytochemical	*Sargassum wightii*	*Chaetomorpha crassa*	*Valoniopsis pachynema*
1.	Saponin	-	+	+
2.	Phenol	+	-	+
3.	Alkaloid	-	+	+
4.	Protein	+	-	-
5.	Tannin	+	+	-
6.	Flavonoid	-	+	+
7.	Anthraquinone	+	-	-
8.	Terpenoid	+	+	+

"+" - Presence "-"- Absence

The result obtained in the qualitative analyses of *Sargassum wightii* indicates the presence of phenols, protein, tannins, anthraquinones and terpenoids in the aqueous extracts. It was observed that saponin, alkaloid and flavonoid were absent. In *Chaetomorpha crassa*, saponin, alkaloid, tannin, flavonoid and terpenoid was present and phenol, protein and anthraquinones was absent. In *Valoniopsis pachynema*, saponin, phenol, alkaloid, flavonoid and terpenoid were present and protein, tannin and anthraquinones was absent.

The phytochemical analysis showed the aqueous extract of seaweeds has at least five phytochemicals, which makes it to have the antibacterial property against the pathogens.

3.3 Biochemical analyses of seaweeds

The seaweed extracts were analyzed for the presence of carbohydrates, proteins and lipids. The amount of the carbohydrates, proteins and lipids are shown in fig. 5. The brown alga *Sargassum wightii* contains lesser amount of carbohydrates (2.6 mg) as compared to the *Chaetomorpha crassa* (3.15 mg) and *Valoniopsis pachynema* (5.4 mg).

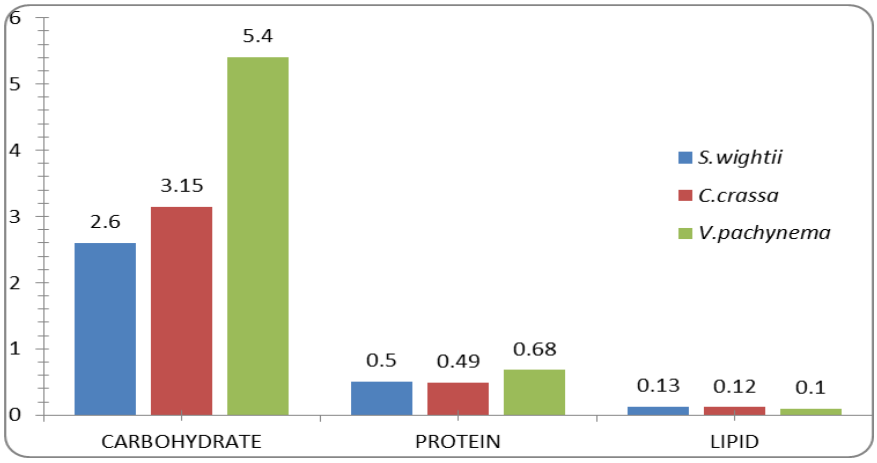


Fig. 5: Biochemical composition of seaweeds.

The protein content was high *in V. pachynema* (0.68 mg) followed by *C. crassa* (0.49 mg) and *S. wightii* (0.5 mg). The lipid content was almost same in all the three samples (0.1 mg). The results of biochemical composition revealed that carbohydrate, protein content was high in *Valoniopsis pachynema* as compared to the *Chaetomorpha crassa* and *Sargassum wightii*.

3.4 SEM (Scanning electron microscopy)

The surface feature and morphological characteristics of the seaweeds was studied using SEM (10 X magnification) (Fig. 6-8). The study was useful in determining the particle shape and porous structure of the seaweeds. From the micrograph it was evident that pores within the seaweed particles are assorted, the surface was rough and irregular in shape with high heterogeneity that might contribute to the availability of secondary metabolites.

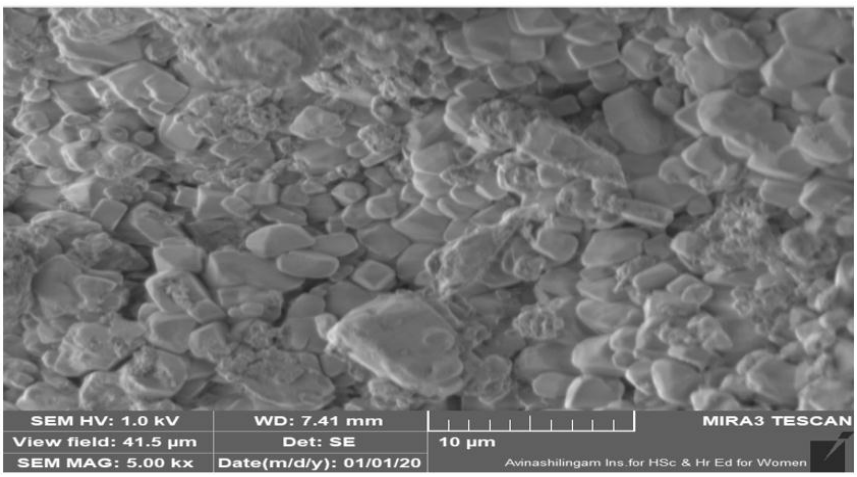


Fig. 6: SEM micrograph of *sargassum wightii* (10 µm).

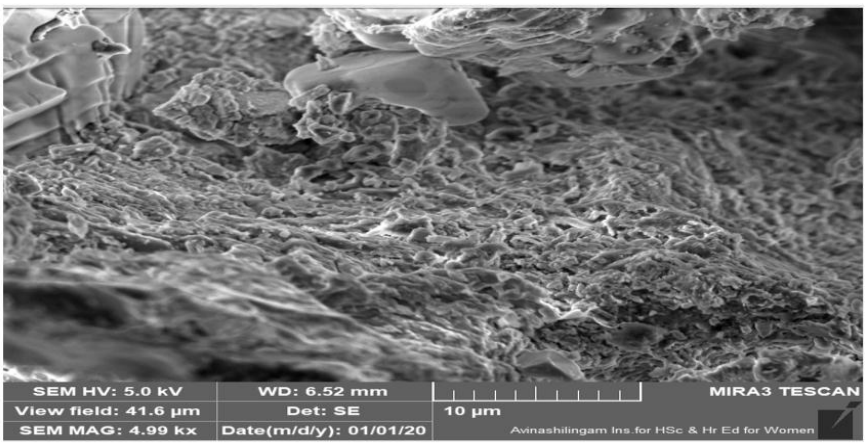


Fig. 7: SEM micrograph of *chaetomorpha crassa* (10 µm).

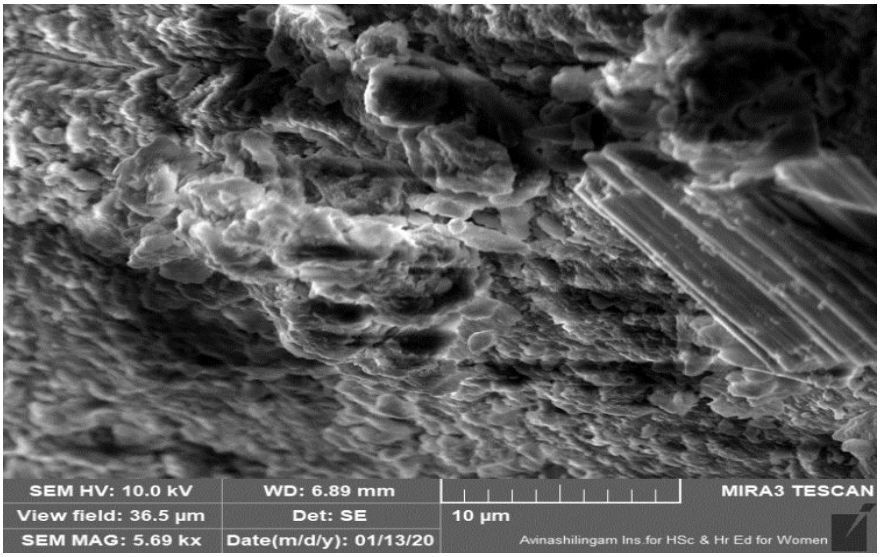


Fig. 8: SEM Micrograph of *Valoniopsis pachynema* (10 µm).

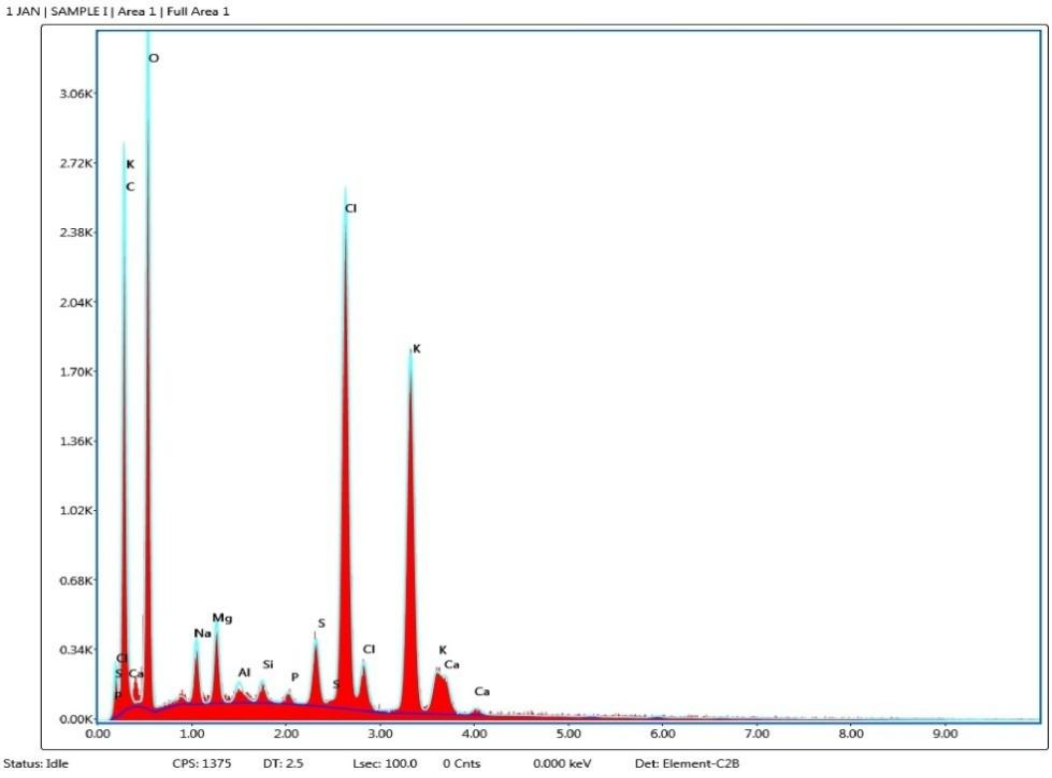
3.5 EDX (Energy dispersive X- ray spectroscopy)

In this study various elements were identified from seaweeds by EDX spectrum (Fig. 9-11). The EDX analysis was able to qualitatively identify chemical elements present on the surface of the algae biomass. This analysis was conducted at several regions on the macroalgae biomass. This analysis on different parts of the biomass allowed the verification of compositional heterogeneity. The regions presented different concentrations of chemical elements.

From the micrographs of *Sargassum wightii* after the extraction process with an organic solvent showed the morphological change, presented lamellar morphology with oval-shaped material aggregated in the algae biomass as shown in Fig. 9. In addition to the chemical element Si, others including C, O, Na, Mg, Al, S, P, Cl, K and Ca were detected.

From the micrograph of *Chaetomorpha crassa*, the following elements were observed: carbon, oxygen, sodium, magnesium, aluminum, silicon, sulfur, chlorine, calcium, potassium and iron (Fig. 10).

From the micrograph of *Valoniopsis pachynema*, the following elements were observed: C, O, Na, Mg, Al, Si, Au, Mo, Cl, K and Ca (Fig.11).



Element	Weight %	Atomic %	Error %
C K	26.36	40.28	10.57
O K	34.04	39.05	10.11
Na K	1.71	1.37	11.14
Mg K	1.59	1.20	7.53
Al K	0.34	0.23	18.77
Si K	0.37	0.24	14.33
P K	0.25	0.15	21.42
S K	1.61	0.92	7.89
Cl K	15.56	8.06	2.55
K K	16.20	7.60	3.00
Ca K	1.97	0.90	9.73

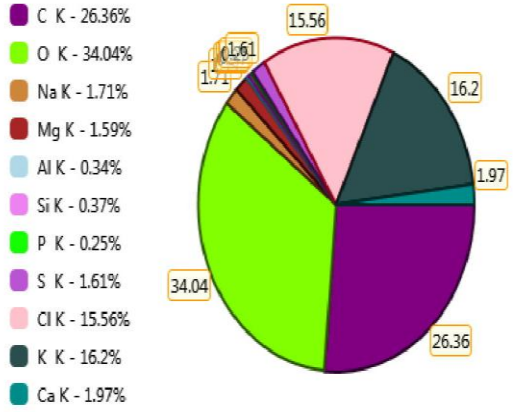
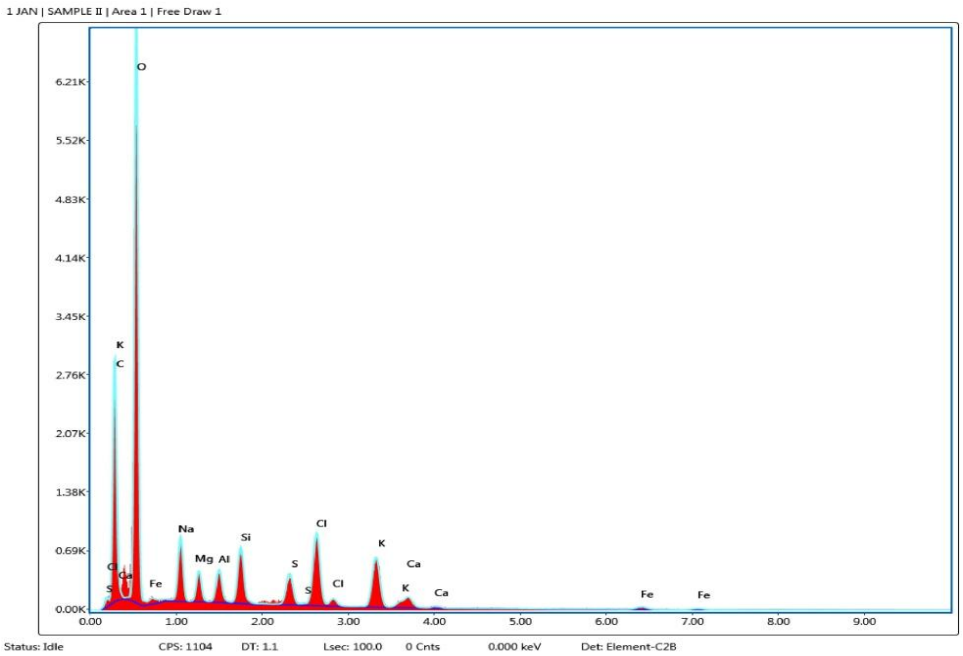


Fig. 9: EDX of *Sargassum wightii.*



Element	Weight %	Atomic %	Error %
C K	24.59	34.43	9.87
O K	48.74	51.24	8.84
Na K	4.64	3.39	8.97
Mg K	1.61	1.12	9.14
Al K	1.61	1.01	7.56
Si K	2.62	1.57	5.60
S K	2.02	1.06	7.06
Cl K	5.83	2.76	3.87
K K	5.70	2.45	4.21
Ca K	1.47	0.62	11.18
Fe K	1.16	0.35	18.55

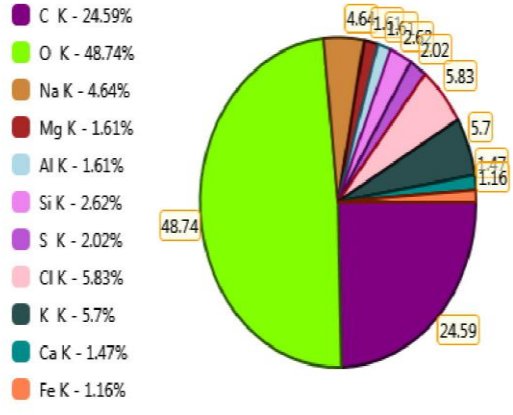
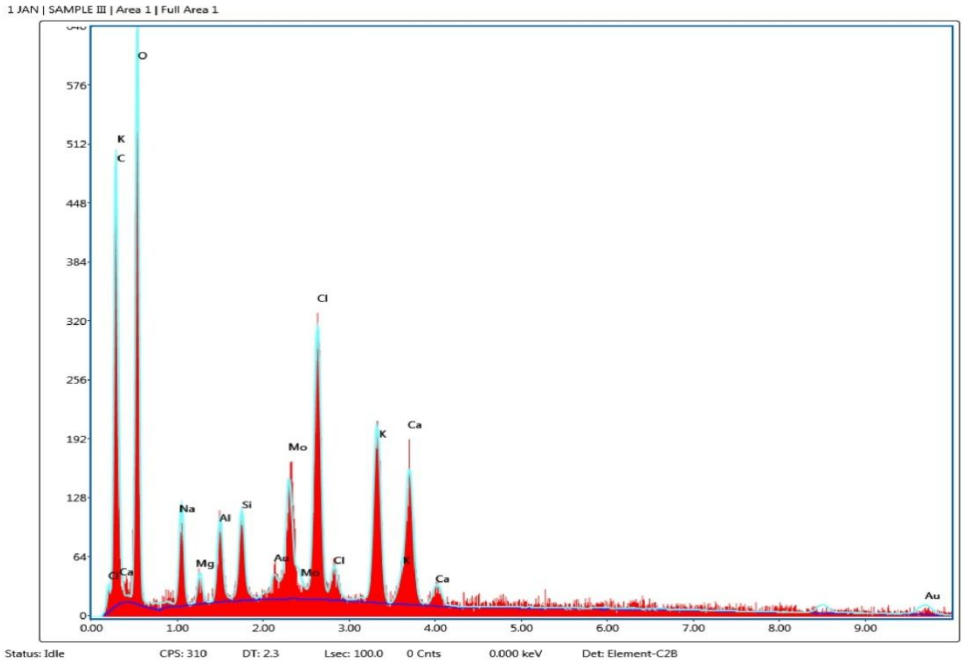


Fig. 10: EDX of *Chaetomorpha crassa.*



Element	Weight %	Atomic %	Error %
C K	21.39	35.40	12.51
O K	33.64	41.78	11.47
Na K	3.29	2.85	13.02
Mg K	0.71	0.58	20.85
Al K	1.82	1.34	10.62
Si K	1.86	1.32	10.28
Au M	1.27	0.13	27.36
Mo L	7.05	1.46	10.00
Cl K	10.45	5.85	5.53
K K	9.35	4.75	6.86
Ca K	9.16	4.54	7.57

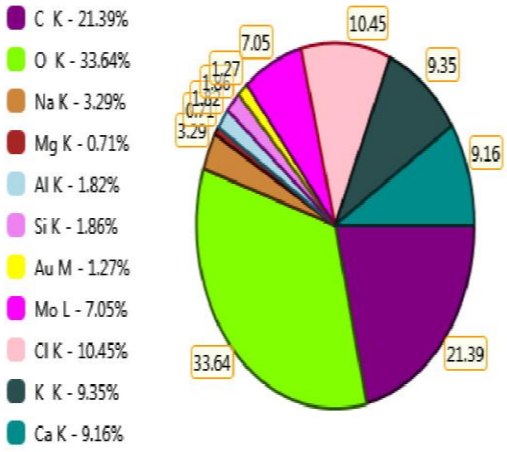


Fig. 11: EDX of *Valoniopsis pachynema.*

4. DISCUSSION

Seaweeds comprise different vitamins, minerals, trace elements, proteins, iodine, bromine and bioactive substances. Thus, seaweeds are admirable sources of medicine. Seaweeds provide a rich source of structurally diverse secondary metabolites. These secondary metabolites offer defense against pathogens.

4.1 Antibacterial activity of seaweed

The main purpose of the study was to evaluate the capability of seaweeds, *Sargassum wightii*, *Chaetomorpha crassa* and *Valoniopsis pachynema* from Mandapam cost, to inhibit the growth of some clinical pathogenic species. In the present study the brown seaweed shows the maximum antibacterial activity against pathogenic bacteria. In *Chaetomorpha crassa*, the green algae ethanol extract shows the maximum antibacterial activity against bacterial pathogens. *Valoniopsis pachynema*, green algae petroleum ether, ethanol and distilled water showed highest antibacterial activity against *Pseudomonas aeruginosa*. The ethanol extract also showed highest activity in *S. aureus*. The activity of *S. aureus* is also inhibited highly by the petroleum ether extract.

Seaweed has been proven to be a potential source of antibacterial compounds towards both gram-negative and gram-positive pathogenic bacteria (Kolajinathan *et al*. 2009).[28] Taskin *et al*. (2007)[29] reported that ethanolic extract of eight seaweed species belonging to Chlorophyta, Phaeophyta and Rhodophyta exhibited broad spectrum activity of both antibacterial and antifungal activities. In this study, the brown seaweed was found to be more active than the red and green seaweeds. The results were similar with the study by Lavanya and Veerappan (2011)[30] which reported that

the brown seaweed extracts showed higher activity than the red seaweed extracts. Nagayama *et al*. (2002)[31] suggested that the strong antibacterial activities from brown seaweed may be due to the compounds such as phlorotannins, eckol and eckol related-compounds that have strong bactericidal activity.

4.2 Phytochemical screening

The result of the phytochemical screening revealed the presence of high number of phytochemical compounds in seaweeds studied. Terpenoid was present in all the three seaweeds. *Sargassum wightii* contains phenol, protein, tannin and anthraquinone as compared with the *Chaetomorpha crassa* and *Valoniopsis pachynema*. Other phytochemicals such as saponin, alkaloid and flavonoid are absent. The green macroalgae *Chaetomorpha crassa* was detected for saponin, alkaloid, tannin flavonoid and anthraquinone whereas phenol, protein and anthraquinone are absent. In the present investigation *Valoniopsis pachynema* contain saponin, phenol, alkaloid flavonoid and terpenoids and other shows to be negative.

High values of total phenols and flavonoids for brown seaweed *Sargassum wightii* have been shown by Sreenivasan *et al*., (2012).[32] The extracts of green macroalgae *Chaetomorpha crassa*, the flavonoid compounds are very important element for the plant to survive from their environment. This compound regulates plant growth, inhibit or kill many bacterial stains, major viral enzymes and destroy some pathogens (Stirk *et al*., 2007).[33] Saponins were used as an anti-inflammatory agent in a dietary product (Mittler *et al*., 2002).[34] Tannins are used as antioxidant, antiviral and antibacterial agents. Steroids were used for its antimicrobial, anti-parasitic, cardio tonic properties (M. Gazali*et al*., 2019).[35] *Valoniopsis pachynema* exhibit the presence of terpenoids, flavonoids, alkaloid, phenol and saponins which could induce the antimicrobial properties against gram-positive and gram-negative bacteria. Moreover, it is rich in phenolic compounds (Devaraj *et al.*, 2016).[36]

4.3 Biochemical analyses of seaweed

The result of the phytochemical and antibacterial analysis revealed the presence of high amount of biochemical compounds, which were studied in three species of seaweed. From the study, maximum carbohydrate was recorded in *Chaetomorpha crassa* belonging to Chlorophyceae and some seaweed with high carbohydrate contents are Rhodophyceae. Similarly, investigation of Paise and Sabale (2010)[37] revealed that the maximum carbohydrate was recorded in *Sargassum*, a brown alga and a minimum was found in *Gracilaria*, a Rhodophyceae. Chakraborthy and Santra (2008)[38] recorded higher carbohydrate in green seaweeds like *Ulva lactuca* (35.27%) and *Encephalitozoon intestinalis* (30.58%). Similar kind of result that green seaweed has high carbohydrate content than red and brown seaweeds was reported by Kaliperumal *et al*. (2004)[39] which is corroborated with the present investigation.

In the present study highest protein content was encountered in *Valoniopsis pachynema* a green alga and brown alga *Sargassum wightii*. Similarly, Dinesh *et al*. (2007)[40] reported the high protein content in brown alga *T. ornate* from Gulf of Mannar Region and Anitha *et al*. (2008)[41] recorded the maximum protein in the brown alga *T. conoides* and minimum in *G. corticate* from the Mandapam coast.

4.4 Sem with edx

The SEM images made possible the visualization of the morphology of the seaweeds. The results are in accordance with Ingrid *et* al. (2019)[42] reported that the sample from *Dictyota menstrualis* in natura revealed dense morphology while the sample after extraction with dichloromethane presented lamellar morphology.

Marine macroalgae (seaweeds) can be rich in Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Ni, Co, Cr, Cd and carbohydrates, where these elements vary among different species, and many of these elements have important nutritional value (El-Said and El-Sikaily, 2013).[43] Carbon and oxygen could be found in different substances in the seaweed, for example, in the polysaccharides stored in the algae biomass as mannitol and laminarin (Davis *et al*., 2003).[44] The alginate is found in the amorphous matrix or as extracellular material of the biomass from the brown algae. The study of Costa et al. (2016)[45] reported that through SEM-EDX results of *Sargassum filipendula* seaweed showed the presence of diatoms shells composed by Si, Na, Mg, Al, S, K, Ca and Fe.

Presence of metals can be explained by the ability of polysaccharides to "sequester" these elements. Andrade and other researchers (2010) concluded in their study that *P. gymnospora* seaweed synthesizes more polysaccharides as a defense mechanism (Andrade *et al*., 2010).[46]

5. CONCLUSION

Marine environment represents resources comprising of wide range of resources for synthesizing new drugs to compact many major diseases. So, recently many researchers have screened marine macroalgae (seaweeds) for their antibacterial, antifungal, antioxidant, antitumor and immune modulator properties and are used for development of new drug across worldwide using these treasures under the deep sea. This is one of the prime reasons for selecting seaweeds for our study which were widely distributed in the Mandapam region, Rameswaram coast, Tamil Nadu. The exploration of seaweed associated compounds promise to deliver novel bio-actives with potential pharmaceutical applications. Seaweeds provide unique and novel bioactive metabolites of unprecedented structure, with antibacterial, antifungal, antiviral, anti-inflammatory

anticancer and antiangiogenic activities. These might provide highest drug candidates for pharmaceutical as well as agricultural and industrial applications. Hence the prevention and treatment of infectious diseases and causative organisms by applying products from marine algae appears as a possible alternative.

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