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Screening of marine seaweeds for bioactive compound against fish pathogenic bacteria and active fraction analysed by gas chromatography-mass spectrometry

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the antibacterial activity of seaweeds *G. edulis*, *K. spicifera*, *S. wightii*, against *Vibrio*. The activity was assessed based on disc diffusion and MIC. Further, the active compound, *n*-hexadecanoic acid from *S. wightii* was purified and characterized based on GCMS.
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ABSTRACT

Objective: To isolate bioactive molecules from marine seaweeds and check the antimicrobial activity against the fish pathogenic bacteria.

Methods: Fresh marine seaweeds *Gracilaria edulis*, *Kappaphycus spicifera*, *Sargassum wightii* (*S. wightii*) were collected. Each seaweed was extracted with different solvents. In the study, test pathogens were collected from microbial type culture collection. Antibacterial activity was carried out by using disc diffusion method and minimum inhibition concentration (MIC) was calculated. Best seaweed was analysed by fourier transform infrared spectroscopy. The cured extract was separated by thin layer chromatography (TLC). Fraction was collected from TLC to check the antimicrobial activity. Best fraction was analysed by gas chromatography mass spectrometer (GCMS).

Results: Based on the disc diffusion method, *S. wightii* showed a better antimicrobial activity than other seaweed extracts. Based on the MIC, methanol extract of *S. wightii* showed lower MIC than other solvents. *S. wightii* were separated by TLC. In this TLC, plate showed a two fraction. These two fractions were separated in preparative TLC and checked for their antimicrobial activity. Fraction 2 showed best MIC value against the tested pathogen. Fraction 2 was analysed by GCMS. Based on the GCMS, fraction 2 contains *n*-hexadecanoic acid (59.44%).

Conclusions: From this present study, it can be concluded that *S. wightii* was potential sources of bioactive compounds.

KEYWORDS

Marine seaweeds, *Vibrio* sp. fish pathogenic, Thin layer chromatography, Gas chromatography-mass spectrometry

1. Introduction

Aquaculture fish production has increased significantly over the past few decades, which has led to intensive fish culture practices where stressors like overcrowding, transport, handling, grading and poor water quality are common[1]. It is widely demonstrated that farmed fish are more susceptible to disease agents due to the stressors posed by intensive rearing. Bacterial infection causes a high rate of mortality in human population and aquaculture organisms[2]. It is a primary pathogen of fishes, which causes a systemic infection leading to disease and death. The development of aquaculture has seen considerable economic losses due to pathogens of

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Vibrio sp[3].

Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria[4]. Decreased efficiency and resistance of pathogen to antibiotics have necessitated the development of new alternatives[5]. Moreover, the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut[6]. Due to the outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies and the researchers are now searching for new antibacterial agents[7].

Bioactive natural products are widely distributed in the plant kingdom, and extract from different plants as well as red, green and brown macro and micro algae can be used as natural products[8]. Marine algae represent an inexhaustible reservoir of raw materials used in pharmaceutical, medicine, food industries and cosmetics[9]. Marine algae serve as an important source of bioactive natural substances[10]. Special attention has been paid to antibacterial activities related to marine algae against several pathogens[11]. The extracts and active constituents of various marine algae have been shown to have antibacterial activity against Gram-positive and Gram-negative bacteria[12]. The antimicrobial compounds derived from the marine algae consist of a diverse group of chemical compounds[13].

The antimicrobial activity in seaweed extracts has been reported since 1917. Biological compounds extracted from some seaweed species, namely, Phaeophyceae, Rhodophyceae and Chlorophyceae, were proven to have potential medicinal activities such as, antibacterial, antiviral, antitumour, antifungal, antiprotozoa, and mosquito and larva control[14]. To date, only certain antibacterial activities of brown seaweed species have been studied in details [evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration]. Brown seaweeds like *Dictyota*[15] and *Sargassum*[16] have been studied and they showed promising antibacterial activity. Phenolic compounds which play a major role in antibacterial and antifungal activities are found abundantly in brown seaweeds when compared with the green and red seaweeds[17]. The objectives of this study were to collect seaweed and evaluate the bioactive compounds of marine seaweed by using different solvent extracts against the *Vibrio* sp. and partial purification and identification of compound by using gas chromatography-mass spectrometry (GCMS).

2. Materials and methods

2.1. Sample collection

Fresh marine seaweeds such as *Gracilaria edulis* (*G. edulis*), *Kappaphycus alvarezii* (*K. alvarezii*) and *Sargassum wightii* (*S. wightii*) were collected from Mandapam (Latitude 8°35′-9°25′ N; Longitude 78°08′-79°30′ E), Rameshwaram, south east coast of Tamil Nadu. Collected samples were washed with tap water in order to remove epiphytes and other marine organisms and then washed with distilled water and dried at 45 °C and ground.

2.2. Preparation of seaweeds extract

Extracts of *S. wightii*, *G. edulis* and *K. alvarezii* were prepared based on the method of Fujiki *et al*[18]. Seaweed material was mixed with organic solvents such as methanol, ethyl acetate, acetone, chloroform and diethyl ether (1:50 w/v ratio). Each extraction was carried out in a Soxhlet apparatus for 24 h and after evaporation in vacuum extract was stored at -20 °C until use[19]. Seaweeds were also extracted with hot-water for the preparation of aqueous solutions.

2.3. Bacterial pathogens

Bacterial strains such as *Vibrio vulnificus* (*V. vulnificus*) [microbial type culture collection (MTCC) 1145], *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (MTCC 451) and *Vibrio harveyi* (*V. harveyi*) (MTCC 3438) used in this study were obtained from MTCC, Chandigarh, India. *Vibrio alginolyticus* (BRTR 07, GenBank Accession No KF758571) was isolated from fish *Mugil cephalus*. Colonies were selected from the bacterial stock and cultured on nutrient agar prepared with 50% of sea water and incubated at 28 °C for 24 to 48 h. After incubation, a pure colony of bacteria was selected for each tested organism prior to the antibacterial assays. Viability of all the strains were maintained on nutrient agar slants and stored at -20 °C for further use.

2.4. Antibacterial activity by disc diffusion method

Antibacterial activity of the three seaweeds' crude extracts were tested by disc diffusion method. Whatman No. 3 filter paper disc with 5 mm diameter was prepared and sterilized by autoclaving for 15 min at 121 °C. Sterile discs were impregnated with each seaweed extract at 50 mg/mL microgram concentration and allowed to dry. The bacterial pathogens with 1.2×10^7 CFU/mL concentrations were inoculated on Mueller-Hinton agar plates and spread using sterile cotton swab. The crude extract impregnated discs were placed over the Mueller-Hinton agar plates

inoculated with test pathogens. All the plates were incubated at 28 °C for 24 h. Paper discs treated with solvent alone were served as negative controls and assay was done in triplicates. Zones of inhibition were measured in millimetre in diameter[20].

2.5. The MIC

The MIC of the active extracts was determined by macro broth dilution method. The extracts were serially diluted two fold with nutrient broth to give concentrations of 10.00, 5.00, 2.50, 1.25, 0.625, 0.312, and 0.156 mg/mL. The 50 μL of each dilution was used to check the antibacterial activity against pathogens using the MIC method in triplicate. The tubes were inoculated with 100 μL of each bacterial suspension (5×10^6 CFU/mL). Sterile nutrient broth was used as negative control while different concentrations of 5.2, 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04, 0.02 and 0.01 μg/mL of tetracycline prepared by serial dilution were used as positive controls. The tubes were incubated at 27 °C for 24 h[21].

2.6. Partial purification of crude extract

2.6.1. Thin layer chromatography (TLC)

Based on the MIC, *S. wightii* was taken for the further study. The crude extract was purified by TLC using silica gel coated chromatography plates. To find out the best solvent system for good separation of crude compound, solvents such as chloroform, methanol were used in the following proportions: 9:1, 8:2, 6:4, 4:6, 2:8, and 1:9. Retention factor (R_f) value of the spot separated on the TLC plate was determined by adopting the following formula.

$$R_f \text{ value} = \frac{\text{Movement of solute from the origin}}{\text{Movement of solvent from the origin}}$$

2.6.2. Preparative TLC (PTLC)

Concentrated crude methanol extract of *S. wightii* was purified by TLC using chloroform: methanol as solvent systems. The crude extract was separated in a TLC plate with silica gel 60 G as stationary phase and chloroform: methanol mixture in the ratio of 8:2 as mobile phase. The eluted spots were representing various compounds. TLC resolved spots of methanol extract at various R_f values were scrapped from the TLC plate and the scrapped spots were dissolved in methanol, mixed well and centrifuged at 8000 r/min for 5 min. The supernatant (10 mg/mL) 50 μL of each fraction was used to check the antibacterial activity against pathogens using the MIC method in triplicate. Tetracycline was used as positive control. Experimental data represent mean±SD of each sample. The partially purified compound obtained from PTLC was tested for antibacterial activity against fish pathogenic organisms by MIC method as described earlier[22].

2.7. Analysis of compound

2.7.1. Fourier transform infrared (FTIR)

The purified active TLC fraction was analysed by FTIR. IR spectra were recorded in the 400-4000 cm^{-1} ranging with a resolution of 1 cm^{-1}. The room was kept at a controlled ambient temperature (25 °C) and relative humidity (30%)[23].

2.7.2. GCMS

The purified fractions were analyzed using an Agilent 6890 series high temperature GCMS, fitted with an auto injector. For GCMS analysis, a high temperature column (DB-5ht; 30 m×0.25 mm i.d.×0.25 μm film thickness) was used. By employing a high temperature column, we eliminated the need for derivatization of each sample. The injector and detector temperatures were set at 350 °C while the initial column temperature was set at 80 °C. A 2 μL sample volume was injected into the column and ran using split less mode. After 2 min, the oven temperature was raised to 150 °C at a ramp rate of 10 °C/min. The oven temperature was then raised to 250 °C at a ramp rate of 5 °C/min and finally the oven temperature was raised to 280 °C at a ramp rate of 20 °C/min and maintained at this temperature for 40 min. The helium carrier gas was programmed to maintain a constant flow rate of 1 mL/min. Identification of organic compound was matching their recorded spectra with the data bank mass spectra of NIST library V11 provided by the instruments software[24].

3. Results

3.1. Antibacterial activity of the seaweeds

In the antibacterial activity test, the methanol extract of *G. edulis* showed maximum zone of inhibition (32.66 mm) against *V. vulnificus* (Table 1). Acetone extract of *S. wightii* showed maximum zone of inhibition (26.33 mm) against *V. vulnificus*. *K. alvarezii* extract has not produced any zone of inhibition against *V. vulnificus*. The diethylether extract of *G. edulis* showed maximum zone of inhibition (24 mm) against *Vibrio anguillarum* (*V. anguillarum*). Chloroform extract showed a minimum zone of inhibition (21.33 mm). Acetone extract of *S. wightii* produced a maximum zone of inhibition (26 mm) against *V. anguillarum* (Table 1). Methanol and ethyl acetate extract of *K. alvarezii* showed a maximum zone of inhibition (23.3 mm) against *V. anguillarum*.

Diethylether extract of *G. edulis* showed maximum zone of inhibition against *V. parahaemolyticus* (23.33 mm). Methanol extract of *S. wightii* showed maximum zone of

Table 1
Antibacterial activity of seaweeds extracts against *Vibrio* sp (mm).

Vibrio sp.	Seaweeds	Ethyl acetate	Chloroform	Methanol	Diethyl ether	Acetone	Aqueous	Tetracycline (positive antibiotic)	Negative control
V. vulnificus	*G. edulis*	26.66±1.15	14.33±0.57	32.66±1.52	21.33±1.15	21.33±1.15	0	27.330±1.154	0
	S. wightii	22.66±0.57	21.66±0.57	24.00±1.00	23.66±1.15	26.33±0.57	0	27.33±1.15	0
	K. alvarezii	0	0	0	0	0	0	33.33±0.57	0
V. anguillarum	*G. edulis*	22.66±1.15	21.33±0.57	23.66±0.57	24.00±1.00	22.00±1.00	0	31.66±1.52	0
	S. wightii	23.33±0.57	24.33±0.57	24.66±1.15	25.00±1.00	26.00±0.57	0	29.00±1.00	0
	K. alvarezii	23.33±0.57	23.00±1.00	23.33±1.15	21.00±1.00	22.00±0.00	0	25.33±0.57	0
V. parahaemolyticus	*G. edulis*	21.66±0.57	22.66±0.57	16.66±0.57	23.33±0.57	23.00±1.00	0	27.66±0.57	0
	S. wightii	29.00±1.00	31.00±1.00	32.66±0.57	32.00±0.00	30.66±0.57	0	32.66±0.57	0
	K. alvarezii	15.33±1.15	23.66±0.57	25.00±1.00	23.00±1.00	17.00±1.00	0	29.00±1.00	0
V. harveyi	*G. edulis*	22.66±0.57	20.33±0.57	22.33±0.57	23.00±1.00	22.33±0.57	0	27.33±1.15	0
	S. wightii	24.66±0.57	24.33±0.57	22.00±1.00	22.33±0.57	23.66±0.57	0	28.66±0.57	0
	K. alvarezii	24.33±0.57	24.66±0.57	23.33±0.57	22.330±0.577	17.00±1.00	0	28.330±0.577	0

Data are expressed as mean±SD. Data are mean of triplicate determinations.

inhibition (32 mm) against *V. parahaemolyticus* (Table 1). Methanol extract of *K. alvarezii* showed maximum zone of inhibition against *V. parahaemolyticus*. Diethylether extract of *G. edulis* produced a maximum zone of inhibition against *V. harveyi* (23 mm). Ethyl acetate extract of *S. wightii* showed maximum zone of inhibition against *V. harveyi* (24.66 mm) (Table 1). Chloroform extract of *K. alvarezii* showed a maximum zone of inhibition (24.66 mm) against *V. harveyi*. No zone of inhibition was observed in aqueous extract of all the seaweeds against *Vibrio* sp.

3.2. The MIC

The lowest concentration of the extract showing no visible growth after incubation was taken as MIC of particular extract against the respective pathogen. Ethyl acetate extract of *G. edulis* showed low MIC value of 6.25 mg/mL against *V. harveyi*. Chloroform extract of *G. edulis* showed the lowest MIC value against *V. parahaemolyticus* (3.12 mg/mL). Methanol extract of *G. edulis* showed the lowest MIC value against *V. vulnificus* and *V. anguillarum* (12.5 mg/mL) (Table 2). Diethylether extract of *G. edulis* showed the lowest MIC value against *V. harveyi* (3.12 mg/mL). Acetone extract of *G. edulis* showed the lowest MIC value against *V. vulnificus*, *V. parahaemolyticus* and *V. anguillarum* (6.25 mg/mL) followed by *V. harveyi* (12.5 mg/mL).

Ethyl acetate extract of *S. wightii* showed the lowest MIC value against *V. vulnificus* and *V. anguillarum* (3.12 mg/mL). Chloroform extract of *S. wightii* showed the lowest MIC value against *V. vulnificus*, *V. parahaemolyticus* and *V. harveyi* (6.25 mg/mL). Methanol extract of *S. wightii* showed the lowest MIC value against all the four *Vibrio* sp. (*V. vulnificus*, *V. anguillarum*, *V. parahaemolyticus* and *V. harveyi*–3.12 mg/mL). Diethylether extract of *S. wightii* showed the lowest MIC value against *V. vulnificus* and *V. harveyi* (3.12 mg/mL) followed by *V. anguillarum* and *V. parahaemolyticus* (6.25 mg/mL). Acetone extract of *S. wightii* showed the lowest MIC value against *V. anguillarum* and *V. harveyi* (3.12 mg/mL) (Table 2).

Table 2
Minimal inhibitory concentration of seaweeds.

Seaweeds	Solvent	*V. vulnificus*	*V. anguillarum*	*V. parahaemolyticus*	*V. harveyi*
G. edulis 100 mg/mL	Ethyl acetate	12.500	25.000	12.500	6.250
	Chloroform	6.250	6.250	3.120	12.500
	Methanol	12.500	12.500	50.000	6.250
	Diethyl ether	6.250	25.000	25.000	3.120
	Acetone	6.250	6.250	6.250	12.500
S. wightii 100 mg/mL	Ethyl acetate	3.120	3.120	25.000	6.250
	Chloroform	6.250	25.000	6.250	6.250
	Methanol	3.120	3.120	3.125	3.120
	Diethyl ether	3.120	6.250	6.250	3.120
	Acetone	6.250	3.120	12.500	3.120
K. spicifera 100 mg/mL	Ethyl acetate	0.000	25.000	1.250	12.500
	Chloroform	0.000	6.250	6.250	50.000
	Methanol	0.000	6.250	6.250	6.250
	Diethyl ether	0.000	3.120	3.120	25.000
	Acetone	0.000	3.120	3.120	3.120
Antibiotic mg/mL	Tetracycline	0.064	0.032	0.016	0.032

Data are expressed as mean±SD. Data are mean of triplicate determinations.

Ethyl acetate extract of *K. alvarezii* showed the lowest MIC value against *V. harveyi* and *V. parahaemolyticus* (12.5 mg/mL). Chloroform extract of *K. alvarezii* showed the lowest MIC value against *V. anguillarum* and *V. parahaemolyticus* (6.25 mg/mL). Methanol extract of *K. alvarezii* showed the lowest MIC value against *V. anguillarum*, *V. parahaemolyticus* and *V. harveyi* (6.25 mg/mL) (Table 2). Diethylether extract of *K. alvarezii* showed the lowest MIC value against *V. anguillarum* and *V. parahaemolyticus* (3.12 mg/mL). Acetone extract of *K. alvarezii* showed the lowest MIC value against *V. anguillarum*, *V. parahaemolyticus* and *V. harveyi* (3.12 mg/mL). Based on the MIC, methanol extract of *S. wightii* showed a better result when compared with other seaweed extracts. It was taken for further studies.

3.3. TLC and PTLC

In the TLC, 8:2 ratio of chloroform: methanol mixture separated the compound in the TLC. TLC plated showed a

2 separated fraction (Figure 1). R_f value of the separated spots were calculated as 0.87 and 0.75. Methanol extract of *S. wightii* cured extract were run in the PTLC plate. Methanol and chloroform proportions at 8:2 ration solvent showed 2 spot in the PTLC plate. The PTLC fraction 1 and 2 were checked for antimicrobial activity against the tested organisms (Table 3). The PTLC purified second fractions showed lowest MIC value to all the tested microorganisms after 24 h of incubation. The PTLC second fraction was analysed by FTIR and GCMS.

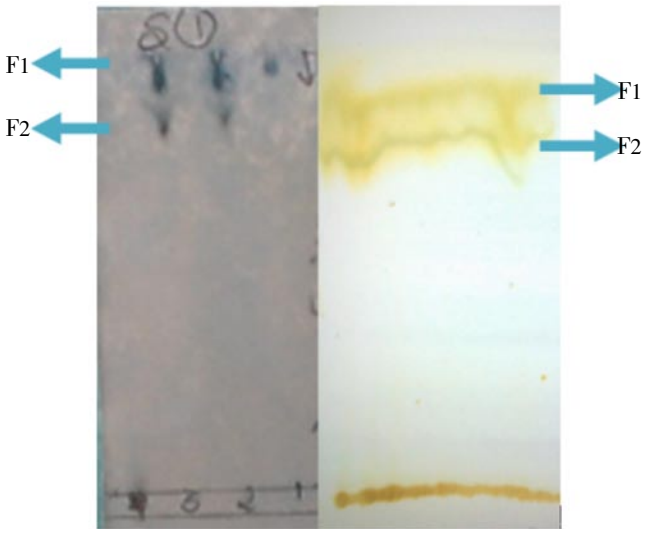

Figure 1. Methanol extract of *S. wightii* separated by TLC and PTLC. a. Methanol extract of *S. wightii*, b. TLC plate, c. PTLC (Fraction F1 and fraction F2).

Table 3

Minimal inhibitory concentration of *S. wightii* PTLC fraction 1 (F1) and 2 (F2).

Seaweeds	*V. vulnificus*		*V. anguillarum*		*V. parahaemolyticus*		*V. harveyi*	
	F1	F2	F1	F2	F1	F2	F1	F2
S. wightii (mg/mL)	–	0.16	–	0.32	–	0.16	0.16	0.16
	0.16	0.32	–	0.16	–	0.16	–	0.32
	–	0.16	–	0.32	–	0.32	–	0.08
	–	0.32	0.16	0.32	0.16	0.32	–	0.08
	0.16	0.32	0.16		–	0.16	–	0.32
Antibiotic tetracycline (mg/mL)	0.064		0.032		0.016		0.032	

Data are expressed as mean±SD. Data are mean of triplicate determinations.

3.4. FTIR analysis

The TLC 2nd fraction was analysed by FTIR (Figure 2). The FTIR spectrum indicated several intense characteristic bands related with functional groups presented in the TLC fraction of *S. wightii*. FTIR frequency ranging of 594.08 cm^{-1} intensities was medium (C–C stretch), indicating the presence of chloro compound; 673.16 cm^{-1} intensities was medium (CH bending), indicating the presence of arenes; 1 267.23 cm^{-1} intensities was medium (C–C–C), indicating the presence of aldehydes and ketones; 1 396.46 cm^{-1} intensities was medium (CH_3), indicating the presence of alkanes; 2 353.16 cm^{-1} intensities was strong (P–H r Si–H), indicating the presence of phosphine or silane; 2 931.83 cm^{-1} intensities was strong (CH_3), indicating the presence of alkanes; and 3 452.21 cm^{-1} intensities was strong (–N=C=N–), indicating presence of the carbodiimides (Table 4).

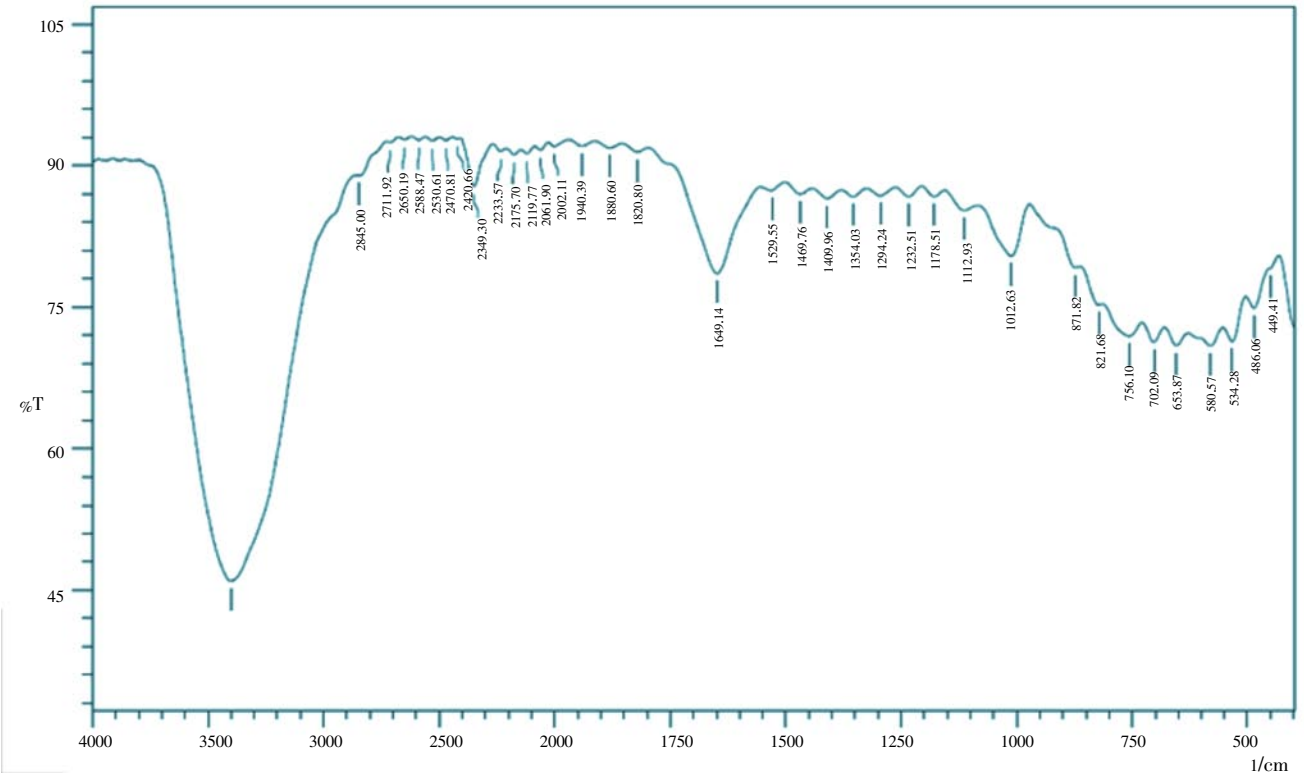

Figure 2. FTIR-PTLC fraction 2 of *S. wightii*.

Table 4

Major peak values of the FTIR methanol extract of *S. wightii*.

Frequency ranges (cm^{-1})	Intensities	Assignment and remarks	Group or functional class
594.08	Medium	C- C stretch	Chloro compound
673.16	Medium	CH bending	Arenes
1109.07	Medium	C-C -C bending	Aldehydes & ketones
1267.23	Medium	C-C-C bending	Aldehydes & ketones
1396.46	Medium	CH_3	Alkanes
1635.64	Strong	C=O stretch	Aldehyde ketone
2353.16	Strong	P-H r Si-H	Phosphine or silane
2931.83	Strong	CH_3	Alkanes
3452.21	Strong	-N=C=N-	Carbodiimides

3.5. GCMS analysis

In TLC, the active compound separated from *S. wightii* fraction was analysed by GCMS. The GCMS data of *S. wightii* active fraction are showed in Figure 3 and the relative percentage of identified compounds is summarized in Table 5. *n*-Hexadecanoic acid (59.44%) was followed by tetradecanoic acid (7.98%), 3-eicosene, (E) (7.18412%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (4.7968%), dibutyl phthalate (3.30%), 9-eicosene, (E) (2.19%), 3-eicosene (3.10%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.83%), hexadecanoic acid, methyl ester (1.42%), 1-(3-isopropylidene-5,5-dimethyl-bicyclo [2.1.0]pent-2-yl)-ethanone (1.06%), *n*-decanoic acid (0.81%), 2-dodecene, (Z) (0.34%), 6-methyl-6-(5-methyl-furan-2-yl)-hept-3-en-2-one (0.22%), cyclohexanol, 2,4-dimethyl (0.15%) and pentanoic acid, 2-(aminooxy) (0.08%).

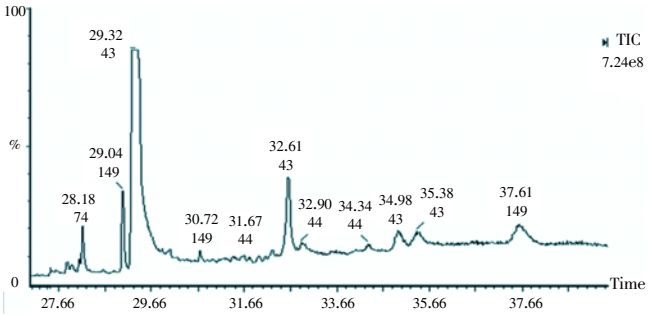

Figure 3. GCMS-PTLC fraction 2 of *S. wightii*.

Table 5

List of fragmented compounds for the *S. wightii* by GCMS.

Peak name	Molecular weight	Formula	Retention time	Peak area	Peak area (%)
Pentanoic acid, 2-(aminooxy)-	133	$C_5H_{11}NO_3$	27.60	85111	0.0827
6-Methyl-6-(5-methyl-furan-2-yl)-hept-3-en-2-one	206	$C_{13}H_{18}O_2$	27.84	231980	0.2254
Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	28.18	1464364	1.4229
Dibutyl phthalate	278	$C_{16}H_{22}O_4$	29.04	3405158	3.3087
n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	29.32	61175412	59.4433
3-Eicosene, (E)	280	$C_{20}H_{40}$	32.61	7393428	7.1841
9-Eicosene, (E)	280	$C_{20}H_{40}$	35.38	2258347	2.1944
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	278	$C_{16}H_{22}O_4$	37.61	4936591	4.7968

4. Discussion

Salama *et al*. reported that different solvents had the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent[25]. In this study phytoconstituents extracted from three seaweeds using different solvents exhibited different spectrum of activities against *Vibrio* sp. Rangaiaha *et al*. also reported that seaweed extracts in different solvents exhibited different antimicrobial activities[26]. The high and low effect of organic extract against microorganisms could be related to the presence of different bioactive metabolites[27,28]. In the present study, *S. wightii* had a good antimicrobial activity against *Vibrio* sp.

Kandhasamy and Arunachalam reported that extracts prepared from seaweed with methanol showed the best activity[2]. In the present study, methanol extract of seaweeds showed a good antibacterial activity against the *Vibrio* sp. Manilal *et al*.[29] and Rangaiah *et al*.[26] reported that methanol extraction yielded higher antimicrobial activity than other solvents.

Wefky and Ghobrial[30] and Fareed and Khairy[31] reported that acetone was the suitable solvent for the extraction of bioactive compounds from seaweeds. In the present study, acetone extract of *S. wightii* showed maximum zone of inhibition against *V. vulnificus* and *V. anguillarum* as well. Osman *et al*. reported that acetone was the best solvent for extraction of the bioactive compounds. Meanwhile, it gave the highest antimicrobial activity against the selected pathogens[32].

Bibiana *et al*. reported that the maximum activity of diethyl ether extract of *S. wightii* and *K. alvarezii* was against tested pathogens[33]. In this study, diethylether extract of *G. edulis* showed maximum zone of inhibition against *V. parahaemolyticus* and *V. harveyi*.

Kanjana *et al*. reported that plant materials could be classified as antimicrobial agents based on MIC values of their extracts[19]. Extracts with MIC values at less than 100 mg/mL are classed as strong inhibitors, at 100-500 mg/mL as moderate inhibitors, at 500-1 000 mg/mL as weak inhibitors and at more than 1 000 mg/mL as inactive inhibitors. According to this classification, the low MIC values are found in *G. edulis*, *S. wightii* and *K. alvarezii*, which is the indication of seaweed extracts' efficacy against the tested organisms. Saxena *et al*. documented MIC varying from 12.5 to 1 000 μg/mL when testing different concentrations of *Rhusglaba* extracts on both Gram-negative and Gram-positive bacteria[34].

Neveen *et al*. reported that MICs (μg/mL) of ethyl acetate extracts for *Anabaena variabilis* and *Anabaena circinalis* were against *Aeromonas* species[35]. In our present study, ethyl acetate extract of seaweeds showed better MIC value against the *Vibrio* sp. Chloroform extracts might have higher solubility for more of active antimicrobial phytoconstituents, consequently displaying the highest relative antimicrobial activity[36]. Chloroform extract of *G. edulis*, *S. wightii* and *K. alvarezii* showed the lowest MIC value against *Vibrio* pathogen.

Methanol extract of *G. edulis* showed a MIC value against *V. vulnificus* and *V. anguillarum*. *S. wightii* showed MIC value against all the *Vibrio* spp. (*V. vulnificus*, *V. anguillarum*, *V. parahaemolyticus* and *V. harveyi*) and *K. alvarezii* showed the lowest MIC value against *V. anguillarum*, *V. parahaemolyticus* and *V. harveyi* (0.625 mg/mL). The methanolic extract of *Ecklonia cava* and *Ecklonia kurome* showed MIC value against *Staphylococcus epidermidis* was 2.5 mg/mL and *Staphylococcus latiuscula* was 0.63 mg/mL[37].

Diethylether extract of *G. edulis* showed MIC value against *V. harveyi* (0.312 mg/mL), *S. wightii* showed MIC value against *V. vulnificus* and *V. harveyi* (0.312 mg/mL) and *K. alvarezii* showed MIC value against *V. anguillarum*, and *V. parahaemolyticus* (0.312 mg/mL). Xavier *et al*. reported that acetone extract of *S. wightii* showed better MIC value than other solvents[38]. The present study suggests that acetone extract has a good antimicrobial activity against the pathogens. In our present study, it also supported that acetone extract of *G. edulis*, *S. wightii* and *K. alvarezii* showed moderate MIC values when compared with other solvents.

Zubia *et al*. suggested that the great variation observed in the potential antimicrobial components in seaweeds could be due to the external environmental factors such as herbivory, light, depth, salinity and nutrients of their growing environment[39]. All of these factors could act on the spatiotemporal regulation on metabolic expression of the active compounds leading to marked qualitative and quantitative variations among their similar species at a smaller scale than different species. Thus, this might be some of the reasons that led to the higher bacteriostatic activity in *Sargassum polycystum*.

However, crude seaweeds extracts are mixed with many compounds and their active portion may be very low. FTIR major peak showed that it has phenol, aldehyde and ketone groups as a major compound in the seaweeds. Phenolic compounds exhibit good antioxidant and antimicrobial activities[40,41]. Further investigations should focus on attempts to purify active compounds and to elucidate their chemical structure. The most active extract led non cytotoxicity to fish. The extracts from *S. wightii* could be a source of antibacterial compounds with potential use in aquaculture, in order to control fish infections and as fish feed component.

A wide range of lipophilic antibacterial compounds have been isolated from gastropod molluscs, including polyunsaturated fatty acids and alkaloids[42]. Polat and Ozogul reported that palmitic acid (hexadecanoic acid) and oleic acid were the major fatty acids found in the seaweeds that they examined[43]. Fatty acids and sterols content determined in red alga *Chondrus crispus* showed that the main fatty acids were palmitic, palmitoleic, oleic, arachidonic and eicosapentanoic acids[44]. Besides halogenated compounds, fatty acids have been identified as antimicrobial substances in algae[45]. Bansemir *et al*. reported that *Corallium rubrum* contained several fatty acids with antimicrobial activities[14].

It is well known that fatty acids are a vital constituent of both terrestrial and marine plants[46]. Antimicrobial properties of fatty acids were reported as early as 1960; synthesis of fatty acids in seaweed is controlled by both biotic and abiotic factors[47]. Evidence supporting bioactivity of fatty acids was earlier demonstrated in certain microalgae and mangrove plants[48].

The remarkable difference between our results and the results obtained in previous studies may be due to several factors. One of the main reasons is the seasonal variation of the seaweeds and another important reason could be due to difference in the extraction procedure to recover the active metabolites and differences in assay methods that would result in different susceptibilities of the target strains. The volatile compounds observed from Mandapam coastal area were the credible evidence that algae maintain effective antimicrobial chemical defences. From the present study, it can be concluded that *S. wightii* are potential sources of bioactive compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Aquaculture is an important sector to produce fishes for human consumption. But, the vibriosis in fishes cause severe mortality and make heavy loss to the aquatic farm owner. Since seaweed is an important source of bioactive natural products, it has been used as an antimicrobial agent to prevent vibriosis in aquatic farm reared fishes.

Research frontiers

The present research work shows the screening of antibacterial activity of seaweeds *G. edulis*, *K. spicifera*, *S. wightii*, against *Vibrio*. Among them, the best one was purified and characterized based on the MIC result.

Related reports

The antimicrobial activity in seaweed extracts has been reported since 1917. Biological compounds extracted from some seaweed species, namely, Phaeophyceae, Rhodophyceae and Chlorophyceae, were proven to have potential medicinal activities such as, antibacterial, antiviral, antitumour, antifungal, antiprotozoa, and mosquito and larva control.

Innovations and breakthroughs

In this present study, author identified that *n*-hexadecanoic acid of *S. wightii* showed significant activity against *Vibrio* sp.

Applications

From the literature survey, it has been found that seaweeds have potent medicinal applications. This scientific study support and suggest the use of *n*-hexadecanoic acid produced by *S. wightii* as an antibacterial agent to prevent *Vibrio* infection in fishes.

Peer review

This is a valuable research work in which authors have demonstrated the antibacterial activity of seaweeds *G. edulis*, *K. spicifera*, *S. wightii*, against *Vibrio*. The activity was assessed based on disc diffusion and MIC. Further, the active compound, *n*-hexadecanoic acid from *S. wightii* was purified and characterized based on GCMS.

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