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Antibacterial Activity and Phytochemical Analysis of Selected Seaweeds from Mandapam Coast, India

[1]Seenivasan. R*, [1]Rekha. M, [1]Indu. H, and [2]Geetha.S
[1]Division of Environmental Biotechnology, School of Bio sciences and Technology, VIT University, Vellore-632 014, Tamil Nadu,India.
[2]Department of Plant Biology and Biotechnology, Arignar Anna Govt.Arts College for Women, Walajapet-632 513 Tamilnadu, India.

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ABSTRACT

Seaweeds are marine macroscopic algae which form an important component of marine living organisms.The antibacterial activity of three species of seaweeds *Codiumadhaerens* Anderson (green algae) *Sargassum wightii* Greville (brown algae) ,*Acanthophora spicifera* (Vahl.) Boergs (red algae) from intertidal region of the Mandapam coastal water were analysed against human pathogenic bacteria like *Staphylococcus aureus, Vibrio cholerae, Shigelladysentriae, Shigellabodii, Salmonella paratyphi, Pseudomonas aeuroginosa* and *Klebsiella pneumoniae*. The present study was also carried out to investigate the phytochemical constituents like alkaloids, flavanoids, phenols, proteins and free amino acids, saponins, sterols, terpenoids and Sugars in all samples and coumarin and glycosides, quinones and tannin, estimation of biochemical composition (protein, sugar, lipid), photosynthetic pigments like chlorophyll, carotenoid and mineral composition. The results indicated that the maximum protein content (6.396±0.97%) was recorded in the brown alga *S. wightii*. The maximum carbohydrate content (6.29±0.063%) was recorded in the red alga *A. spicifera*. The maximum lipid content (1.213±0.02%) was recorded in green alga *C.adharens*. The highest total phenol (216.65±17.38) and flavanoid (379.99±21.813) was in the brown seaweed *S. wightii*. The maximum chlorophyll 'a' (0.347±0.051), total chlorophyll (0.438±0.061) and carotenoid (0.670±0.225) were recorded in the brown seaweed *S. wightii* where as chlorophyll 'b' (0.107±0.016) was highest in *C. adharens*. Among the 14 minerals analyzed most of them were highest in the red alga *A. spicifera.* Among the three seaweeds screened for their antibacterial activity the brown alga *S. wihgtii* is more superior to the red alga *A. spicefera* and green alga *C.adharens* in controlling the growth of most of the pathogens tested. The highest zone of inhibition (13mm) was recorded in methanol extract of the red alga against *Vibrio cholerae*.

INTRODUCTION

From the time immemorial the macroscopic marine algae have been closely associated with human life and are being exhaustively used in numerous ways as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids (Levering *et al*., 1969; Chapman, 1970). Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants. They also contain protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature.. The phytochemicals from marine algae are extensively used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper mostly as gelling, stabilizing and thickening agents. Seaweeds or marine macroalgae are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world.

* *Corresponding Author*

In addition to vitamins and minerals, seaweeds are also potentially good sources of proteins, polysaccharides and fibres (Lahaye, 1991 and Darcy-Vrillon, 1993). Indian seaweeds are of great food value and certain seaweeds contain 16 to 30% protein on dry weight and have all essential amino acids which are not available in vegetable food materials. The highest protein content is recorded for red seaweeds such as *Porphyra tenera* and *Palmaria palmata* which contain, respectively, up to 47 and 35% proteins expressed as dry weight (Fujiwara-Arasaki *et al*., 1984; Morgan *et al*., 1980). Brown seaweeds, with the exception of *Undaria pinnatifida*, are known to contain proteins forming 5 to 10% of dry weight. Green algae belonging to the genus *Ulva* contain 18 to 26% proteins (Bruni and Stancher, 1973; Nisizawa *et al*., 1987).

Seaweeds are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman *et al*., 2003). Extracts of marine algae were reported to exhibit antibacterial activity (Siddhanta *et al*., 1997, Mahasneh *et al*., 1995, Sachithananthan and Sivapalan 1975).

Several workers have reported that the seaweed extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of seaweeds have been studied for their antibacterial activity both in India and abroad (Pratt *et al*., 1951; Chester and Stott, 1956; Burkholder *et al*., 1960; Allen and Dawson, 1960; Nunez and Sanabria, 1975). Sitakara Rao and Tipnis (1964) found high protein content in three species of *Ulva.* Dhargalkar *et al*., (1980) have estimated the protein, carbohydrate and organic carbon content in 43 marine algal species collected from different marine stations along the Maharashtra coast and observed more protein and carbohydrate content in Chlorophycean and Rhodophycean then Phaeophycean algae. Total lipid, sterol and chlorophyll content of *Enteromorpha intestinalis, Caulerpa taxifolia, Gelidiella acerosa, Gracilaria corticata* and *Padina gymnospora* were estimated (Parekh *et al*., 1983). Parekh *et al*., (1985) investigated biochemical composition of *Enteromorpha flexuosa* of Gujarat coast. Reeta Jeyasankar *et al.,* (1990) have also estimated protein, lipid and carbohydrate contents of 23 species of green algae collected from the Mandapam area of the Tamilnadu coast. Selvaraj and Sivakumar (1998) studied biochemical composition of three species of *Sargassum* from Pamban coast.

Shoba *et al*., (2001) studied the biochemical composition of algae along the southern Kerala coast with special reference to fiber content. Seasonal variation in growth and biochemical constitutions such as protein, carbohydrate and lipid in *Hypnea valentiae, Acanthophora spicifera, Laurencia papillosa, Enteromorpha compressa, Ulva lactuca* and *Caulerpa racemosa* were observed for one year from Mandapam coast (Kaliaperumal*et. al* ., 2002). Recently Hannah Vasanthi and Rajamanikam (2003) studied variations in the chemical constituents of the marine red alga *Hypnea valentiae* from Tuticorin and Mandapam coast. Dinesh *et al*., (2007) studied the nutritive properties of 20 species of seaweeds from Gulf of Mannar. Padma Sridhar *et al*., (1984) screened the antibacterial activity of extracts of marine algae representing Chlorophyta and Rhododphyta collected from Vishakapatnam Coast against two pathogens and also tested their ability to inactivate the enzyme penicllinase under *in vitro*. Padmakumar and Ayyakkannu (1986) revealed the antimicrobial activity of marine algae collected from Porto Novo and Pondicherry waters, against 6 bacterial and 2 fungal pathogens. Rao and Parekh (1982) showed that crude extracts of seaweeds are active only against gram positive bacteria. Extracts of marine algae were reported to exhibit antibacterial activity (Siddhanta *et al*., 1997; Mahasneh *et al*., 1995). Vanitha *et al*., (2003) reported the antibacterial action of nine seaweeds collected from Kanyakumari coast against human upper respiratory tract pathogens which include both Gram positive and Gram negative bacteria.

Kandhasamy and Arunachalam (2008) found out the *in vitro* antibacterial property of seaweeds viz. *Caulerpa racemosa, Ulva lactuca, Gracilaria folifera, Hypnea musciformis, Sargassum teneerimum, S. myriocystem* and *Padina tetrastomatica* collected from Koodankullam village, Tirunelveli, Tamilnadu against gram negative and gram positive pathogenic bacteria. Some commonly occurring marine algae *Caulerpa scalpelliformis, Ulva lactuca, Pandina tetrastromatica, Stoecchospermum marginatum* and *Acanthophora spicifera* have been collected from the coast of Tuticorin, Tamilnadu and evaluated for antifungal and antibacterial activity by using four solvents such as petroleum ether, chloroform, methonal and benzene by Jothibai margret *et al*., (2008). Arulsenthil *et al*., (2008) found out the antibacterial activity of the methanol, diethyl ether, acetone and dichloromethane extracts of *Padina boergesnii* collected from Tuticorin Coast against 10 human pathogenic bacteria. The present study was carried out with three marine seaweeds *Codium adhaerens* Anderson (green algae) belonging to the family Codiaceae, *Sargassum wightii* Greville (brown algae) belonging to the family Sargassaceae and *Acanthophora* ***spicifera*** (Vahl.) Boergs (red algae) belonging to the family Rhodomelaceae. The present study was undertaken with objectives (i) To screen the phytochemical constituents present in the three seaweed (ii) To estimate the biochemical composition and photosynthetic pigments of three seaweeds (iii) To estimate the total phenol and flavanoids content in the seaweeds (iv) To analyze mineral composition of the seaweeds (v) To evaluate the antibacterial activity of three seaweeds against seven Human pathogens.

MATERIALS AND METHODS

Sample collection

Fresh plants of *Codium adharens, Sargassum wightii* and *Acanthophora spicifera* were collected from the intertidal region of the Mandapam coastal water and immediately brought to the laboratory in plastic bags containing water to prevent evaporation. Then the plants were washed thoroughly with tap

water to remove extraneous materials. Samples were dried in oven at 37˚ C, till constant weight and obtained and ground in an electric mixer (Lima-Filho *et al*., 2002). The powdered samples were than stored in refrigerator.

Preliminary Phytochemical screening

The dried, powdered samples were subjected to qualitative tests for the identification of phytochemical constituents according to standard procedures (Harborne, 1973; Brindha *et al*.1981; Lala 1993).

Estimation of Protein

The protein was estimated by Biurette method (Raymont *et. al* ., 1964). To 5 mg of dried powdered sample, 1ml of distilled water followed by 4ml of biurette reagent were added and incubated for 30 minutes in the room temperature. Then the mixture was centrifuged for 10 minutes at 4000rpm. The supernatant solution was collected and the optical density was measured in a Spectrophotometer at 540 nm.

Estimation of Lipid

The lipid was estimated by using chloroform methanol mixture as described by Folch *et al.* (1956). 10 mg of dried powder sample taken in a test tube, 5 ml of chloroform- methanol (2:1) mixture was added. The mixture was incubated at room temperature for 24hrs after closing the mouth of the test tube with aluminium foil. After the incubation, the mixture was filtered using a filter paper. The filtrate was collected in a 10 ml pre-weighed beaker, which was kept on a hot plate. The chloroform-methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample.

Estimation of Total sugar

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5 ml was taken and 5 ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm.

Estimation of Phenol

Total phenolic assay was determined by using Folin-Ciocalteu assay (Sadasivam and Manickam, 1992). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot (1ml) of extract or standard solution of caffic acid is added to 250ml of flask containing 9ml of distilled water. A reagent blank using double distilled water was prepared .1ml of folin-ciocalteu phenol reagent was added to mixture and shaken. After 5 minutes 10ml of 7% sodium bi carbonate was added. The solution was diluted to 25ml with distilled water and mixed. After incubation for 90 minutes at room temperature. The absorbancy is determined by 750nm with UV spectrophotometer. Total phenolic content is expressed as mg caffic acid equivalents mg/100 gm dry weight samples were analyzed in duplicates.

Estimation of Flavonoids

Total flavonoid content was measured by the Aluminum chloride calorimetric assay (Zhishen et al., 1999). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot 1ml of extracts or standard solution of catechin (20, 40, 60, 80 &100mg/l) was added to 10ml volumetric flask containing 4ml of double distilled water. To the flask was added 0.3ml 5% sodium nitrate, After 5 min., 0.3ml 10% aluminum chloride was added. At sixth minute, 2ml of 1M NaOh was added and the total volume was made upto 10ml with double distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/100g dry mass samples were analyzed in duplicates.

Estimation of Chlorophyll

The amount of chlorophyll present in the leaves was estimated by the method of Arnon (1949). 500 mg of leaf tissue was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well and the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was stored. The pellet was re-extracted by repeated washing with 5 ml of 80 % acetone till it became colourless. All the extracts were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was determined by using the following formula

$$\text{Chlorophyll 'a' (mg/g.fr.wt.)} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/g.fr.wt.)} = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophyll (mg/g.fr.wt.)} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times W} \times V$$

Where, A = Absorbance at respective wave length = Volume of extract (ml), W = Fresh weight of the sample (g)

Estimation of Carotenoid

The amount of Carotenoid was estimated by the method of Kirk and Allen, 1965.The same chlorophyll extract was measured at 480 nm in spectrophotometer to estimate the Carotenoid content.

Carotenoid: µg/g.fr.wt. = ΔA.480 + (0.114 X ΔA. 663) – (0.638 X ΔA. 645) Where, ΔA = Absorbance at respective wave length

Estimation of Minerals (AOAC, 1995)

The oven dried plant material was accurately weighed in 0.2 g quantity in a dry conical flask and 10 ml of diacid mixture (2:5 of Nitric acid and Perchloric acid) were added. The contents

of conical flask were allowed to stand for a few hours for cold digestion. The conical flasks were then kept on a hot plate and the contents were digested by increasing the temperature. The digestion was continued till the content becomes colorless. The digested material was filtered through Whatman No.40 filter paper by repeatedly washing the conical flask with a small volume of Distilled water. The filtrate collected was made up to a suitable volume. The filtrate thus obtained was suitably diluted and fed into ICP - Perkin Elmer Mayer Optical Emission Spectrophotometer, Optima 2100 DV. The Co, Mg, Cr, Al, Ni, Cd, Cu, Mn, Fe, Zn, Pb were analyzed. Na, K and Ca were analyzed in Flame Photometer.

Extraction

Powdered samples were soaked in the organic solvents with the increasing order of polarity viz., hexane, chloroform and methanol (1:4 w/v), and extracted for ten days at room temperature, and the extracts were collected and concentrated. The concentrates were reconstituted with their respective extractants (5 mg ml^{-1}).

Bacterial strains used

The bacterial strains *Staphylococcus aureus, Vibrio cholerae, Shigella dysentriae, Shigella bodii, Salmonella paratyphi, Pseudomonas aeuroginosa* and *Klebsiella pneumoniae* used for the present study were received from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu, India.

Antibacterial assay

Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes (Norris and Fenical 1985). Briefly, sterile filter paper discs, 6 mm in diameter (Whatman # 1), were loaded with 20 µl of the different extracts and air dried. Discs containing solvents alone were used as controls. The discs were placed on Muller Hinton agar plates inoculated with each of the previously mentioned microorganisms. Plates were incubated for 24 h at 35°C and the inhibition zones measured around the discs (mm diameter).

RESULTS

Phytochemical screening

The important phytochemical viz. alkaloids, glycosides, coumarins, flavanoids, phenols, proteins and free amino acids, quinones, saponins, sterols, teropinoids and sugars were screened for their presence and presented in **Table-1**.

Table. 1: Preliminary phytochemical screening of the samples.

Tests	*C.adharens*	*S. wightii*	*A. spicifera*
Alkaloids	+	+	+
Coumarin	-	-	-
Flavanoids	+	+	+
Glycosides	-	-	-
Phenol	+	+	+
Protein and Aminoacid	+	+	+
Quinone	-	-	-
Saponin	+	+	-
Sterols	+	+	+
Sugar	+	+	+
Tannin	-	-	-
Terpenoids	+	+	+

Note: + (present), - (absent)

Biochemical analysis

Protein, carbohydrate and lipid content and total phenol and flavanoid content of some selected species of seaweeds are presented in Table-2. The total % of phenol and flavanoid of seaweeds is presented in Fig-2. Protein content in the seaweeds varied from 5.219±0.53 to 6.396±0.97%.

The maximum protein content (6.396±0.97%) was recorded in the brown alga *S. wightii* and the green alga *C.adharens recorded* the minimum content (5.219±0.53 %). Carbohydrate content of seaweeds varied from 5.12±0.126 to 6.29±0.063 %. The maximum carbohydrate content (6.29±0.063%) was recorded in the red alga *A. spicifera* and the minimum (5.12±0.126 %) was recorded in the green alga *C. adharens***.** Lipid content of seaweeds varied from 1.014±0.03 to 1.213±0.02 %. The maximum lipid content (1.213±0.02 %) was recorded in green alga *Codium adharens* and the brown seaweed *S. wightii* recorded the minimum (1.014±0.03 %). The total phenol and flavanoid content ranged between 50.66±9.69 to 216.65 ±17.38 and 216.29±16.68 to 379.99±21.81 respectively (**Table-2**). The highest total phenol (216.65±17.38) and flavanoid (379.99±21.813) was in the brown seaweed *S. wightii*. The mean total protein, lipid and sugar percentage of seaweeds is shown in **Fig-1.**

Table. 2: Biochemical composition of three Seaweed species.

Parameters	*C. adharens*	*S. wightii*	*A. spicefera*
Protein (%)	5.219±0.53	6.396±0.97	5.291±.373
Sugar (%)	5.116±0.13	6.146±0.0851	6.294±0.063
Lipid (%)	1.213±0.02	1.014±0.03	1.114±0.02
*Phenol	50.66±9.691	216.65±17.38	136.47±7.941
**Flavonoids	329.47±33.99	379.99±21.813	216.29±16.682

Note:*mg/caffic acid equivalence ; ** mg/catechin equivalence.

Photosynthetic pigments

The photosynthetic pigments like chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content were estimated and presented in Table-3. The maximum chlorophyll 'a' (0.347±0.051) and total chlorophyll (0.438±0.061) and carotenoid (0.670±0.225) was recorded in the brown seaweed *S. wightii* where as chlorophyll 'b' (0.107±0.016) was highest in *C. adharens.* The mean photosynthetic pigment present is shown in Fig-3.

Table. 3: Photosynthetic pigments of three Seaweed species (mg/g fr.wt.)

Parameters	*C.adharens*	*S. wightii*	*A. spicifera*
Chlorophyll a	0.347±0.051	0.215±0.041	0.094±0.020
Chlorophyll b	0.091±0.010	0.107±0.016	0.036±0.004
Total chlorophyll	0.438±0.061	0.322±0.055	0.130±0.023
Carotenoids	0.385±0.145	0.670±0.225	0.314±0.046

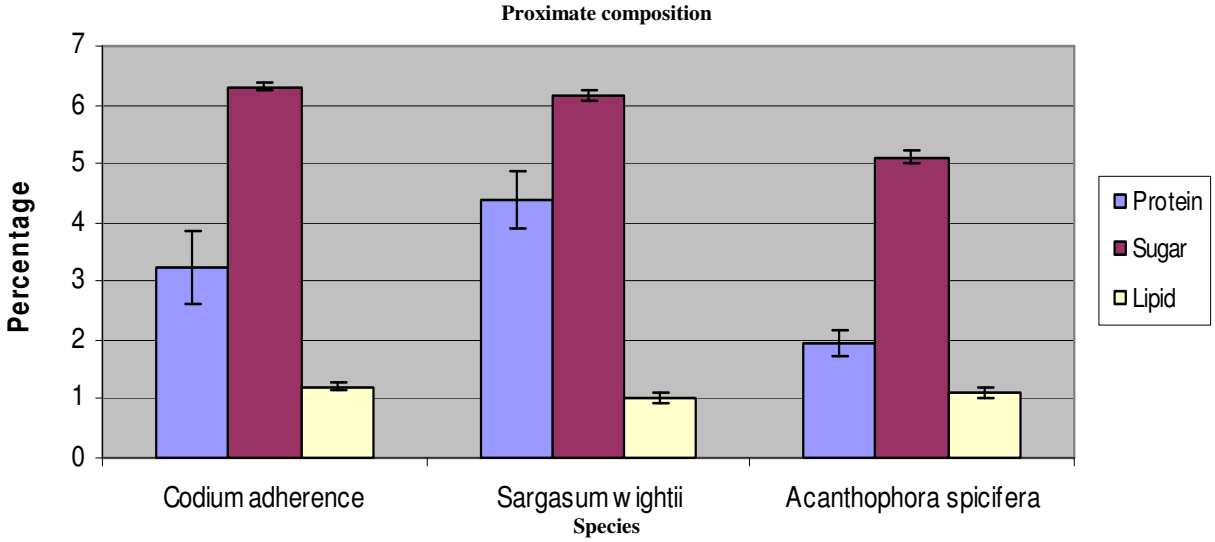


Fig. 1. Mean total protein, lipid and Sugar percentage of seaweeds.

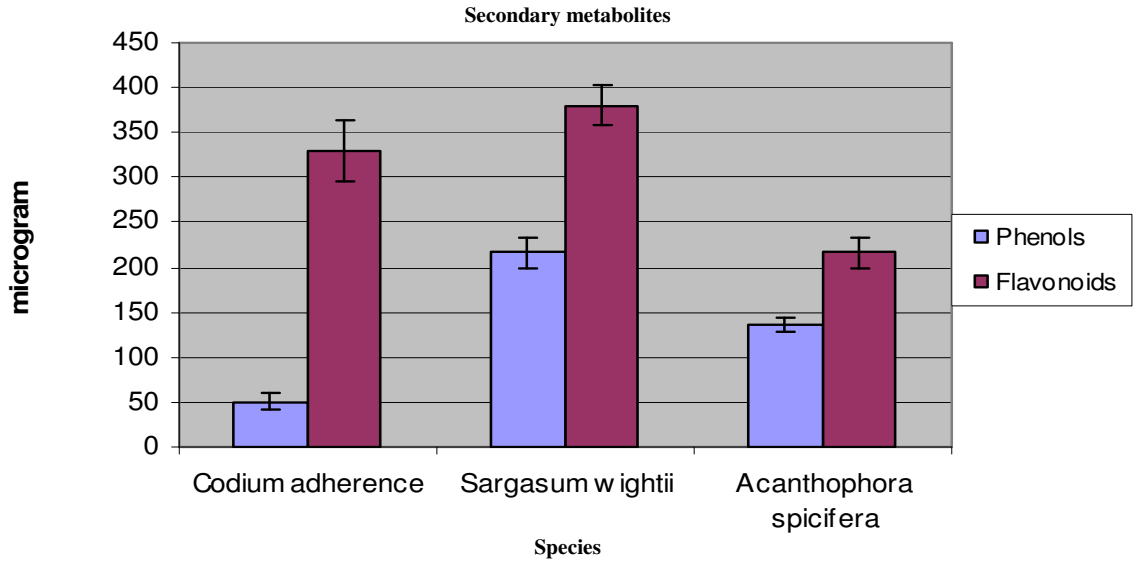


Fig. 2: Mean total phenol and flavanoid of seaweeds.

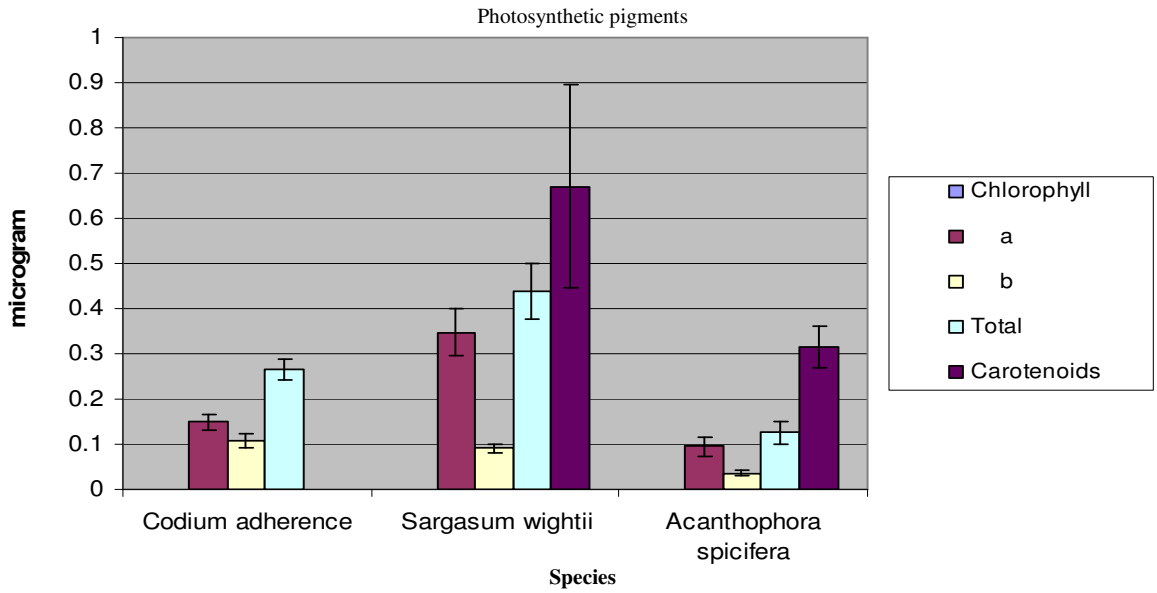


Fig. 3: Mean photosynthetic pigments of seaweeds.

The variation observed in the different seaweeds studied in the present study was presented in **Table-4**. Amontg the14minerals analyzed the concentrations are K(900µgg^{-1}), Ca (800 µg g^{-1}),Mg (9490µg g^{-1}), Mn (106 µg g^{-1}) were higher in the green alga, Cd (3.2µg g^{-1}) was high in the brown alga and Na (710 µg g^{-1}), Co (2.1 µg g^{-1}), Cr (31.3 µg g^{-1}), Cu (48.5 µg g^{-1}),Fe (2602µg g^{-1}), Zn (97.4 µg g^{-1}) Al (3462µg g^{-1}), Ni (6.4 µg g^{-1}) and Pb (9µg g^{-1}) were found to highest in the red alga.

Table. 4: Mineral composition of seaweeds (µg g^{-1}).

Minerals	*C.adharens*	*S. wihgtii*	*A. spicifera*
Na	500	690	**710**
K	**900**	180	580
Ca	**800**	80	250
Co	2	1.3	**2.1**
Cr	20.7	9.2	**31.3**
Cu	48.4	39	**48.5**
Cd	1.1	**3.2**	1.9
Fe	2549	252.1	**2602**
Mg	**9490**	5908	8154
Mn	**106**	21.3	77.7
Zn	96.2	73.6	**97.4**
Al	349.5	179.2	**3462**
Ni	5.8	2.1	**6.4**
Pb	8.7	5.6	**9**

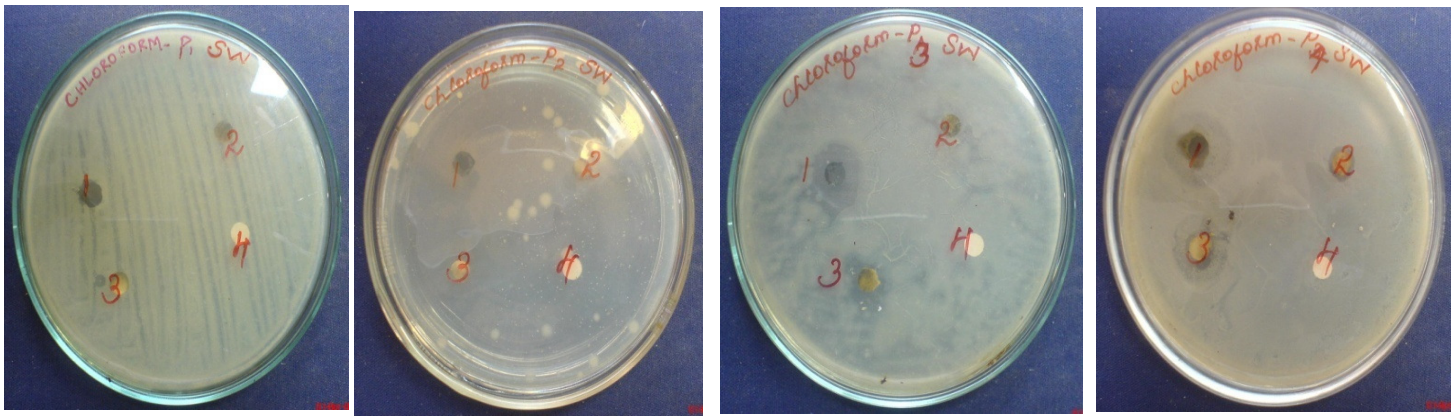
Antibacterial activity

The antibacterial activity of *C. adharens, S. wihgtii* and *A. spicifera* extracts on seven human pathogens were presented in **Table-5**. Of the three Seaweeds screened for their antibacterial activity in the present investigation the brown alga *S. wihgtii* is more superior to the red alga *A. spicifera* and green alga *C.* in controlling the growth of most of the pathogens tested. Among which the brown alga *S. wihgtii* produced some what better results than the red alga *A. spicefera*, but both of them are not much active against *Shigella dysenteriae*. Among the three solvents tested, methanol and chloroform extracts of seaweeds exhibit better activity. Hexane extract of all the three Seaweeds did not effective against *Shigella bodii*. The highest zone of inhibition (13mm) was recorded in methanol extract of the red alga against *Vibrio cholerae* followed by (11mm) in methanol extract of the brown alga against the same pathogen and the chloroform extract of red alga against same pathogen. The zone of inhibition of different pathogenic bacteria is depicted in **Fig 4, 5, 6**.

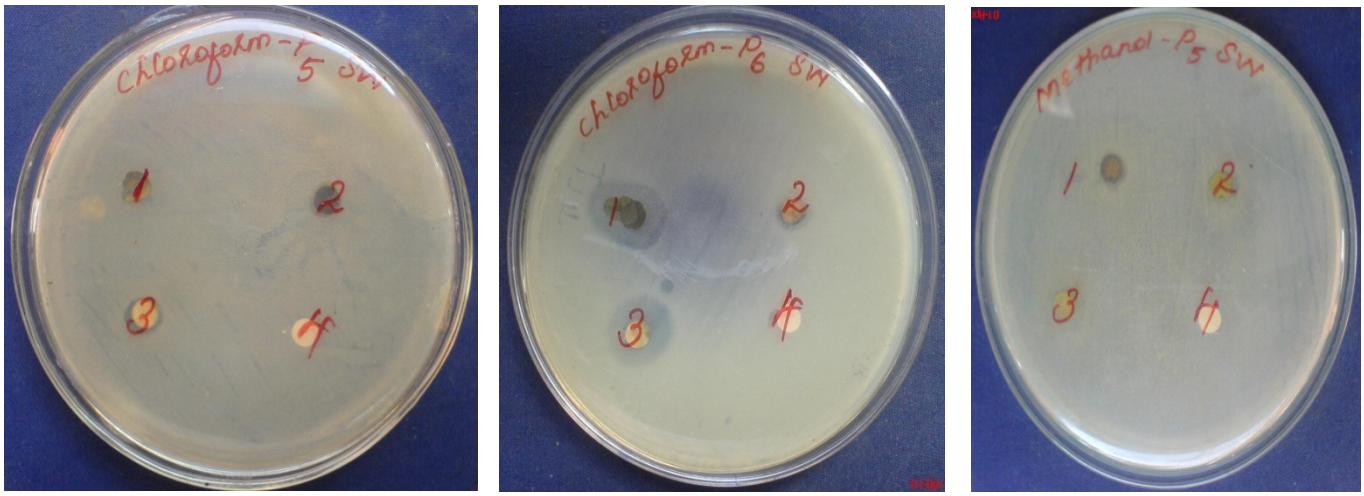
Table. 5: Antibacterial activity of three Seaweed species.

Human pathogens	Seaweeds	Inhibition Zone (mm) Organic solvents		
		Hexane	Chloroform	Methanol
1 *Staphylococcus aureus*	CA	T	2	6
	SW	4	T	8
	AS	4	T	6
	Control	NA	NA	NA
2 *Vibrio cholerae*	CA	T	9	6
	SW	3	6	11
	AS	4	10	**13**
	Control	NA	NA	NA
3 *Shigella dysenteriae*	CA	T	5	2
	SW	6	3	T
	AS	4	4	T
	Control	NA	NA	NA
4 *Salmonella paratyphi*	CA	T	7	9
	SW	8	T	8
	AS	4	5	8
	Control	NA	NA	NA
5 *Shigella bodii*	CA	NA	T	4
	SW	NA	6	8
	AS	NA	2	T
	Control	NA	NA	NA
6 *Pseudomonas aeroginosa*	CA	T	4	6
	SW	T	T	9
	AS	2	6	8
	Control	NA	NA	NA
7 *Klebsiella pneumoniae*	CA	2	6	4
	SW	4	**2**	T
	AS	4	4	**NA**
	Control	NA	NA	NA

Note: CA- *Codium adharens* , **SW-***Sargassum wightii* and **AS-***Acanthopora spicefera*, **NA- no activity, T- trace**

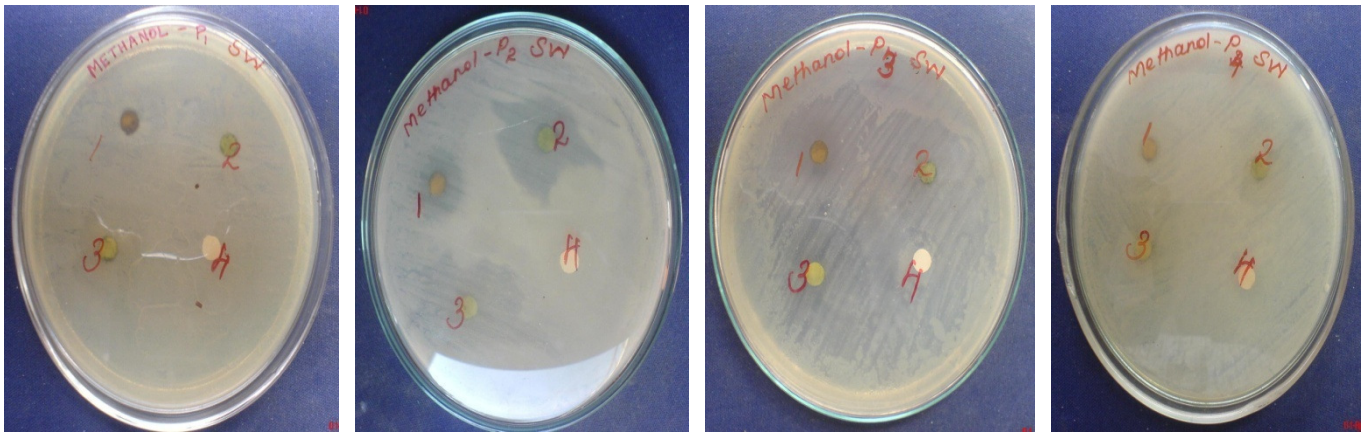


P1 P2 P3 P4

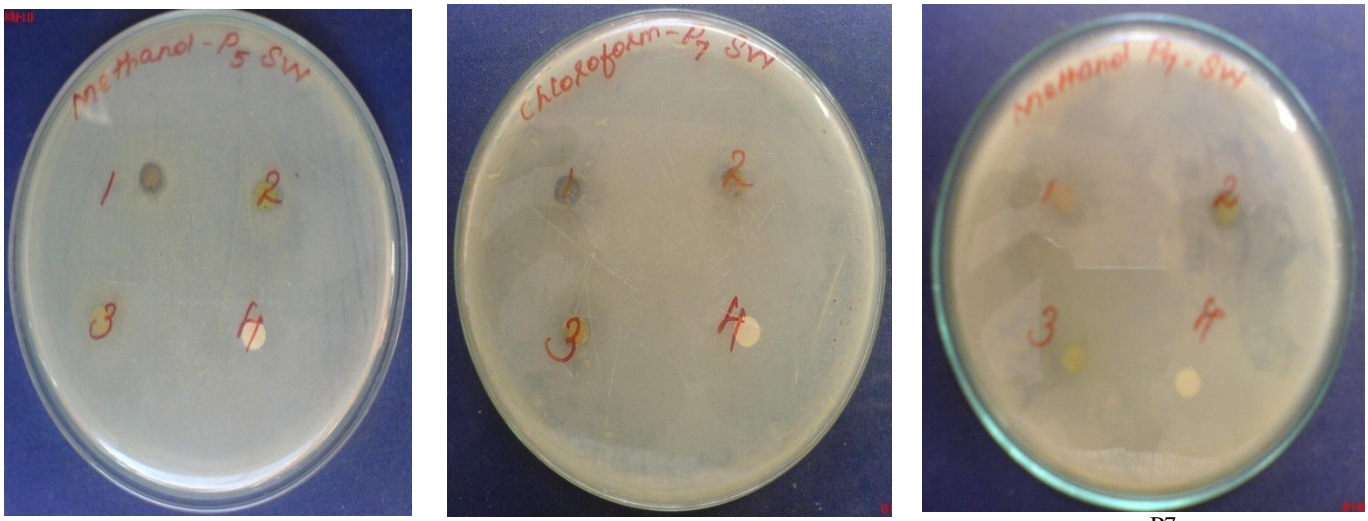


P5 P6 P7

Fig. 4 : Antibacterial activity of chloroform extract of seaweeds.



P1 P2 P3 P4



P5 P7

Fig. 5 : Antibacterial activity of methanol extract of seaweeds.

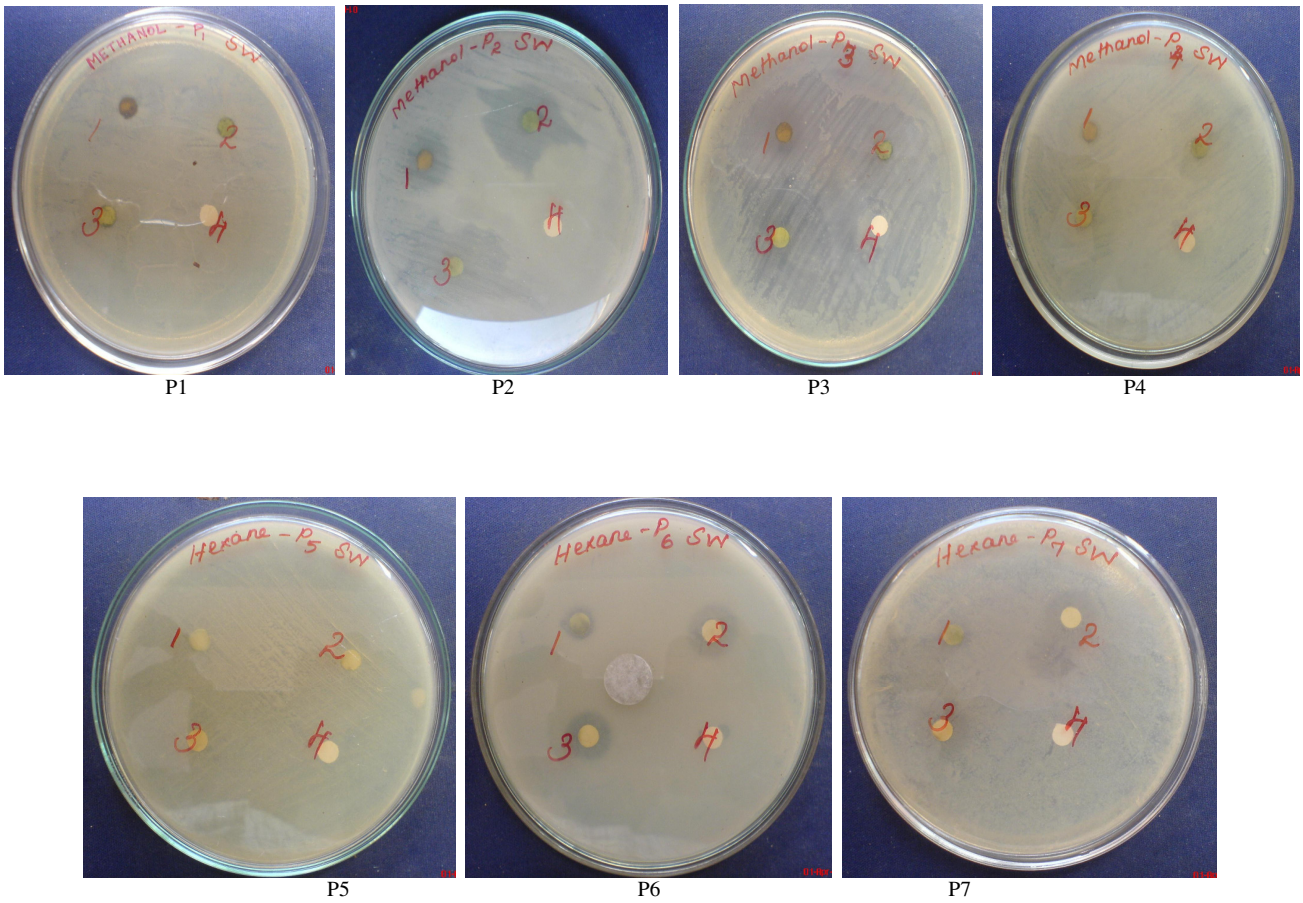


Fig. 6 : Antibacterial activity of hexane extract of seaweeds.
1- *S. wightii*, **2**- *C. adharens*, **3**- *A. spicefera*, **4.** Control
P1- *S. aureus*, P2- *V. cholerae*, P3- *S. dysenteriae*, P4- *S. paratyphi*, P5- *S. bodii*
P6-*P. aeroginosa*, P7- *K. pneumoniae*

DISCUSSION

Seaweeds are primitive non flowering plants without root, stem and leaves. They contain different vitamins, minerals, trace elements, protein, iodine, bromine and bioactive substances. Many polysaccharides are recovered from seaweeds. The most important of them are agar, alginic acid, laminarin, fucoidin, galactans, carrageenan, xylan and mannans. The phytochemical screening of seaweeds showed the presence of alkaloids, flavanoids, phenol, protein and aminoacid, sretols, sugar and terpenoids in all seaweed samples tested. Coumarin, glycosides, quinone and tannin were absent in the all the three plant samples and saponin is absent in the red algae.

In the present study the maximum protein content was recorded in the brown alga *S. wightii* and the minimum in the green alga *C. adharens.* Similarly Dinesh *et al*., (2007) recorded highest protein content in brown alga *Tubinaria ornata* from Gulf of Mannar region and Anitha *et al*., (2008) recorded maximum protein in the brown alga *Turbinaria conoides* and minimum in *Gracilaria corticata* from the same Mandapam coast. These reports are supportive to the present study that the Phaeophycean members showed highest protein content than the Rhodophycean and Chlorophycean members. There are some other reports which recorded maximum protein other than the Phaeophycean members. Selvi *et al*., (1999) reported more protein content in red alga *Hypnea valentiae*. Mairh *et al*., (1983) reported 22.22% of crude protein in *Ulva fasciata*.

Dhargalkar *et al*., (1980) from the Maharashtra coast and Shoba *et al*., (1988) Kovalam coast noted maximum value of carbohydrate content in Rhodophycean members than in Phaeophycean and Chlorophycean members. In the present study the Rhodophycean members showed high carbohydrate content than chlorophycean and phaeophycean members. The high content of carbohydrate in red algae is might be due to higher phycocolloid content in their cell walls (Dhargalkar *et al.,* 1980). The maximum lipid content was recorded in green alga *C. adharens* and the brown seaweed *Sargassum wightii* recorded the

minimum in this investigation. Similarly Muthuraman and Ranganathan (2004) recorded highest lipid content in green alga *U. fasciata*.

In the present study the highest total phenol and flavanoid was in the brown seaweed *S. wightii*. Marry & Vimalabai (2003) screened four brown seaweeds from Tuticorin coast for their phenol content and reported highest value in *Padina tetrastromatica*. Pedersen (1964) reported that the phenol content increased with the increasing age of the tissue and with increasing salinity. The highest total chlorophyll was recorded in the green alga *C. adherence* and minimum in the red alga *A. spicifera.* Similarly Muthuraman and Ranganathan (2004) reported maximum chlorophyll in the green alga *Caulerpa scalpelliformis* among the 12 seaweeds tested which include phaeophycean and Rhodophycean member also. The chlorophyll content of *S. wightii* is more than the red alga *A. spicifera*, this also in conformity with the results of Jeyasankar et al., (1990) . The highest carotenoid content was recorded n the brown seaweed *S. wightii,* similarly Muthuraman and Ranganathan (2004) reported maximum carotenoid content in the brown seaweed *S. wightii*.

Among the 14 minerals analyzed K, Ca, Mg, Mn were higher in the green alga, Cd was high in the brown alga and Na, Co, Cr, Cu ,Fe, Zn, Al, Ni and Pb were found to highest in the red alga. Vimalabai and Mary (2003) reported higher level of Fe in *Acanthophora spicifera* and *G. corticata* and Mn is also high in *G. corticata* of Tuticorin coast. The mineral fraction of some seaweed even account for up to 40% of dry matter, however in some cases the mineral content of seaweeds is recorded even higher than that of land plants and animal products (Ito and Hori, 1989). Seaweeds are known as an excellent source of minerals, especially sodium and iodine, due to their high polysaccharide content which could also imply a high level of soluble and insoluble dietary fiber (Lahaye, 1991).

The seaweeds tested in the present study for their antibacterial property, includes green, brown and red algae. There are a number of reports regarding the medicinal important of seaweeds belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae from the corners of the world. Several scientists from India and abroad (Bukholder *et al*., 1960; Martinez Nadal *et al*., 1963; Bhakuni and Silva, 1974; Glombitza, 1970; Hornsey and Hide, 1974; Sreenivasa Rao and Parekh, 1982; Naqvi *et al*., 1981; Sreenivasa Rao *et al*., 1982;) have made consistent efforts to detect the antibacterial activity from seaweeds.

Of the three seaweeds screened in the present study for their antibacterial activity the brown alga *S. wihgtii* is more superior to the red alga *A. spicifera* and green alga *C.* in controlling the growth of most of the pathogens tested. Among which the brown alga *S. wihgtii* produced somewhat better results. Caccamese *et al*. (1985) has reported that the brown algal extracts showed higher activity than the extracts of red algae. The earlier investigations (Burkholder *et al*., 1960; De compos – Takaki *et al*., 1988) showed higher antibacterial activity in the species of Phaeophyta and Rhodophyta. Pesando and Caram (1984) and Reichelt and Borowitzka (1984) have reported that extracts from brown algae show higher degrees of antibacterial activity rather than extracts obtained from red and green algae.

A similar distribution of activity has been reported for algae from Atlantic coasts (Biard *et al*., 1980). Similarly selvi and Selvaraj (2000) noted higher activity in brown algae. The less inhibitory effect was observed by the green alga *C. adharens* in the present study. Antibacterial activity of nine species of seaweeds belonging to brown, red and green algae revealed that red and brown seaweeds had greater antibacterial activity than the green algae (Padmini Sreenivasa Rao *et al*, 1991).

Martinez–Nadal *et al*, (1963) mentioned that benzene and diethyl ether were the suitable solvents for extracting of antibiotic principles. Hornsey and Hide, (1974) used acetone as a solvent for extracting antimicrobial compounds from British marine algae. In the present investigation, methanol and chloroform extracts of Phaeophyceae and Rhodophyceae are every active against several bacterial pathogens tested. Shelat (1979) found methanol and dimethyl sulphaxide extracts of Sargassum sp. were active against Gram positive bacteria. Though the degree of antibiotic property depends upon the suitable solvent used for the extraction, it could also depend upon the condition or state of the sample along with the season in which that alga was collected. Besides, there are several factors such as the age of the plant, duration of storage, temperature, preparation of the media and pH which could indirectly affect the degree of antibiotic activity (Rao, 1995). It is known that many marine algae produce antimicrobial substances (Fenical, 1975) and their production varies with season (Ragan and Glombitza, 1986). The extracts of *Padina boergesenii* and *Hypnea valentiae* were shown to inhibit the action by Naja nigricollis venom when inoculated into mice (Vasanthi *et al*., 2003). Arul Senthil *et al*., 2008 reported that the crude diethyl ether extract of the seaweed *P. boergesenii* showed wide spectrum antibacterial activity against 10 human pathogenic bacteria. They also stated that dichloromethane extract showed wide spectrum activity which is comparatively less than that of diethyl ether extract. The variation of antibacterial activity of extracts might be due to distribution of antimicrobial substances, which varied from species to species as suggested by Lustigman and Brown (1991).

CONCLUSION

The phytochemical screening of seaweeds showed the presence of alkaloids, flavanoids, phenol, protein and aminoacids, sterols, sugar and terpenoids in all seaweed samples tested. coumarin, glycosides, quinone and tannin were absent in the all the three plant samples and saponin is absent in the red alga. Among the three seaweeds screened for their antibacterial activity the brown alga *S. wihgtii* is more superior to the red alga *A. spicefera* and green alga *C.* in controlling the growth of most of the pathogens tested. The highest zone of inhibition (13mm) was recorded in methanol extract of the red alga against *Vibrio cholerae*. The present investigation brings out adequate data on the phytochemical constitute biochemical composition, mineral composition, photosynthetic pigments and antibacterial potential of three seaweed extract for the synthesis novel antibiotics. Thus

these seaweeds could be collected and utilized effectively in product preparation for the beneficial of mankind. Further research studies are being carried out on the other species of seaweeds from the same habitat in order to provide complete data on the nutritive and antimicrobial potential of these plants.

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Conflict of interests

The authors declares there are no conflict of interests.

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