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Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*

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ABSTRACT

The present study investigates phytochemical screening and antibacterial efficacy of various solvent extracts of marine algae such as *Sargassum swartzii* against some selected human and fish pathogenic bacteria. The brown seaweed *Sargassum swartzii* was collected from Kudankulam, Tirunelveli district, Tamil Nadu, India. Soxhlet extraction method was used to get the extracts of different solvents like aqueous, ethanol, methanol, acetone and were tested for their qualitative phytochemical screening using common standard tests. Quantitative phytochemical analysis such as total phenolic content and total flavonoid content of *S. swartzii* and antibacterial activity against human pathogens viz., *Escherichia coli, Pseudomonas aeruginosa* and *Stephylococcus aureus* and fish pathogens viz., *Aeromonas hydrophila* and *Vibrio vulnificus* bacteria using disc diffusion methods. Preliminary phytochemical screening of sixteen different chemical compounds were carried out. The maximum biochemical compounds were present in the ethanol extract and the minimum compounds were present in aqueous extract. The ethanol extract was significantly higher in phenolic content (15.35 ±2.61 mg of GAE/g) and the methanol extract was significantly higher in flavonoides content (26.92±7.14 mg of QE/g). In human pathogen the highest antibacterial activity was present in *Pseudomonas aeruginosa* (18.00±0.00mm) and the fish pathogen was significantly higher in *Aeromonas hydrophila.* The present study showed that the ethanol extract of marine brown seaweed *Sargassum swartzii* contains bio active constituents with highest antibacterial activity against human and fish pathogen.

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***Keywords*:** *Sargassum swartzii*, Total phenol, Total flavonoid, Antibacterial activity, *Aeromonas hydrophila, Pseudomonas aeruginosa*

1. INTRODUCTION

In the face of the extraordinary progress in the field of human medicine, infectious diseases caused by bacteria, virus, fungi and parasites are still a main threat to public health and worldwide economies. They are caused by several types of infection such as drug resistant diseases, frequently connecting bacteria and many emerging pathogens [1, 2].

Regularly use artificial drugs to prevent or control the infectious disease caused by microbes. Normal use of these drugs leads to increase of resistance by the microbes against the drugs [3-5]. It is not only resistant but also the cost of synthetic chemicals leads to search for alternate medicine such as environmental resources [6]. One of the natural resource is algae, which are known to show potential novel bioactive material [7].

Marine algae is known to produce a variety of compounds and some of them have been shown to possess biological activity of potential medicinal value the discovery of metabolites with biological activities from seaweeds has increased significantly. Marine macro algae or seaweeds are one of nature's the major biologically active resources as they possess a wealth of bioactive compounds. It is exploited for both human and animal health applications.

Seaweeds have caused an emerging interest in the biomedical area, mainly due to their contents of bioactive substances which show great potential as anti-inflammatory, antimicrobial, antiviral, and antitumor drugs [8, 9]. Indeed, several species of algae have been found to be the sources of polysaccharides and glycoproteins with immune – stimulant, antitumor, and antiviral activity [9, 10]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventing properties. They are not mandatory for humans to consume. However, it is proven that these chemicals protect plants as well as humans eating them [11]. Seaweeds also contain a range of unique phytochemicals not present in terrestrial plants. As such, edible seaweeds may be the only relevant dietary source of some of these factors. This capacity is endowed by the presence of sulfated polysaccharides, polyphenolic compounds and antioxidant enzymes [12]. Smith, [9] reported seaweeds and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Recently, secondary metabolites known as phytochemicals have been extensively investigated as a source of medicinal agents [13]. These phytochemicals plays an important role in antimicrobial activity and used as a treatment for many microbial infections [14-15]. In the present study, different solvents like aqueous, ethanol, methanol, and acetone were used to extract the seaweed *Sargassum swartzii*. The seaweed extract was characterized by various analytical methods, phytochemical, qualitative, quantitative analysis and their antibacterial efficacy of human and fish pathogens.

2. MATERIAL AND METHODS

2. 1. Plant material and extraction

The seaweed was collected from Kudankulam (8.1798° N, 77.7051° E), Tirunelveli district, Tamil Nadu, India. *Sargassum swartzii* was taxonomically identified and the Voucher specimen is stored in the department of marine science, Bharthidasan University,

Tiruchirappalli, Tamil Nadu, India. The collected marine algae was cleaned to remove the epiphytes and irrelevant matter. The samples are washed 3 to 4 times with seawater and then fresh water. The samples were once again rinsed with sterile distilled water and the seaweed material was dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with organic solvents with increasing order of polarity i.e. aqueous, ethanol, methanol and acetone respectively. The extract was exposed to an evaporator at 50^{o}C. The crude extract was then tested for their phytochemical and antibacterial activity against selected human and fish pathogens.

2. 2. Preliminary Phytochemical Analysis

The extracts from different solvents were tested for Steroids, Tannins, Terpenoids, Flavonoides, Saponins, Alkaloides, Reducing sugar, Cardiac glycosides, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Phenols, Anthocyanin, Betacyanin. Phytochemical screening of the extract was carried out according to the standard methods[16,17].

2. 3. Quantitative Phytochemical Analysis

2. 3. 1. Total phenol content

Total phenolic content of seaweed was estimated using Singleton and Rossi, [18] standard protocol with some modifications. Foline Ciocalteu reagent (0.5 mL) was added to 0.1 mL of the seaweed extract prior to the addition of 7 mL of distilled water. The mixture was incubated in the dark for 5 min at room temperature. To this mixture, 1.5 mL of sodium carbonate solution was added and incubated for 2 h at room temperature. The absorbance of the blue color was read at 765 nm using UV-Spectrophotometer. Total phenolic content of the extract was calculated as Gallic acid equivalents (mg GAE/g of extract).

2. 3. 2. Total flavonoid content

The total flavonoid content of seaweed extract was determined by Liu *et al*. [19] method with slight modifications. Seaweed extract (0.5 mL) was added to 2.5 mL of distilled water, followed by addition of sodium nitrite (0.150 mL, 5%). This solution mixture was incubated for 6 min at room temperature, then aluminum chloride (10%, 0.3 mL) was added and allowed to stand for 5 min. Finally, 1 mL of 1 M sodium hydroxide was added and made up the volume to 5 mL with distilled water. The absorbance was read at 510 nm using UV-Spectrophotometer. Total flavonoid content was expressed as milligram of quercitin equivalents (mg QE/g of extract).

2. 4. Test microorganisms

The human pathogen bacterial strains used for this experiment were *Escherichia coli, Pseudomonas aeruginosa,* and *Stephylococcus aureus* and fish pathogens *Aeromonas hydrophila* and *Vibrio vulnificus* were used for this experiment. The human pathogenic bacteria were obtained from the Laboratory of microbiology, KAP Viswanathan Government Medical College. Tiruchirappalli, Tamil Nadu, India. Fish pathogens were obtained from microbial type culture collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India.

Mueller-Hinton broth (MHB) was obtained from Hi-Media while solvents used were of HPLC grade.

2. 4. 1. Disc diffusion method

The antimicrobial activity of *Sargassum swartzii,* different solvent extract was assessed, by the disc diffusion technique [20]. Mueller Hinton agar (MHA) plates were prepared and individually swabbed with pathogenic bacteria. The sterile discs (6mm) were placed over the surface of the agar plates. Seaweed extract (1 mg/mL) was added on the discs at various concentrations (50, 100, 250 and 500 µg/mL). A disc containing standard concentrations of the antibiotic Ciprofloxacin (20 µg/disc) was used as positive control. The agar plates were incubated for 24 h at 37 ºC, and the inhibition zones were measured in millimeter and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

2. 4. 2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was carried out according to the methods of National Committee for Clinical Laboratory Standards (NCCLS). The seaweed extracts were selected for the solvents aqueous, ethanol, methanol and acetone. The initial test concentration of extract was 1 mg/mL. Each tube containing 2 mL of broth was inoculated with 5µl of bacterial suspension containing 10^8 CFU/mL of bacteria. Ciprofloxacin was used as positive control. The test tubes were incubated for 24 h at 37 ºC. MIC was determined as the lowest concentration of extract showing OD of 600nm of spectrophotometer. All the data were statistically analyzed.

2. 5. Statistical Analysis

All the values were expressed as Mean ± Standard Deviation (SD). The statistical significance was evaluated by two-way Analysis of Variance (ANOVA) using SPSS version 20 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Post-hoc analysis, Duncan [21].

3. RESULTS

3. 1. Preliminary phytochemical analysis

Preliminary phytochemical screening of sixteen different chemical compounds (Steroids, Tannins, Terpenoids, Flavonoides, Saponins, Alkaloides, Reducing sugar, Cardiac glycosides, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Phenols, Anthocyanin, Betacyanin) was tested in four different solvents (Aqueous, Ethanol, Methanol and Acetone) of species *Sargassum swartzii* (Table 1).

The maximum biochemical compounds were present in the ethanol extract and the minimum compounds were present in aqueous extract. When comparing to other biochemical compound, phenol and coumarins were present in all the selected solvents followed by flavonoides and terpenoides present in three solvents. Anthocyanin and Betacyanin were not present in selected solvent.

Table 1. Preliminary phytochemical screening of brown seaweed extract of *Sargassum swartzii*

S. No	Phytochemicals	*Sargassum swartzii*			
		Aqueous	**Ethanol**	**Methanol**	**Acetone**
1	Steroids	-	+	+	-
2	Tannins	-	+	-	-
3	Terpenoids	-	+	+	+
4	Flavonoides	-	+	+	+
5	Saponins	-	+	-	+
6	Alkaloides	+	-	-	-
7	Reducing sugar	-	+	-	+
8	Cardiac glycosides	-	-	+	-
9	Coumarins	+	+	+	+
10	Phlobatannins	-	+	-	-
11	Anthraquinones	-	+	+	-
12	Quinones	+	-	+	-
13	Glycosides	-	+	-	-
14	Phenols	+	+	+	+
15	Anthocyanin	-	-	-	-
16	Betacyanin	-	-	-	+

Note: +, Present; -, Absent

3. 2. Quantitative phytochemical analysis

3. 2. 1. Total phenol content

The total phenolics content of the *S. swartzii* was found to be 9.79±1.00, 10.73±3.21, 11.06±3.22 and 15.35±2.61 mg GAE/g in the Aqueous, Acetone, Methanol and Ethanol extract respectively (Figure 1). The highest amount of phenolic content was found in the ethanol extract (15.35±2.61 mg GAE/g). While the lowest was in Aqueous extract (9.79±1.00 mg GAE/g).

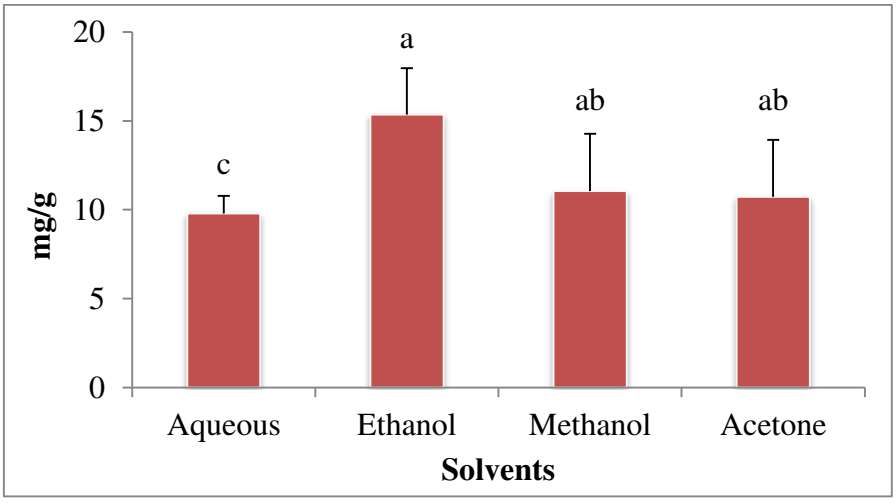

Figure 1. Quantitative phytochemical screening of Total Phenol of *Sargassum swartzii*

3. 2. 2. Total flavonoid content

The flavonoids naturally originate biological activity in seaweeds. The amount of flavonoids content of the *S. swartzii* was found to be higher in the methanol extract (26.92± 7.143 mg QE/g) when followed by acetone (16.55±1.111 mg QE/g) and ethanol (5.44±1.11 mg QE/g) and aqueous respectively (Figure 2).

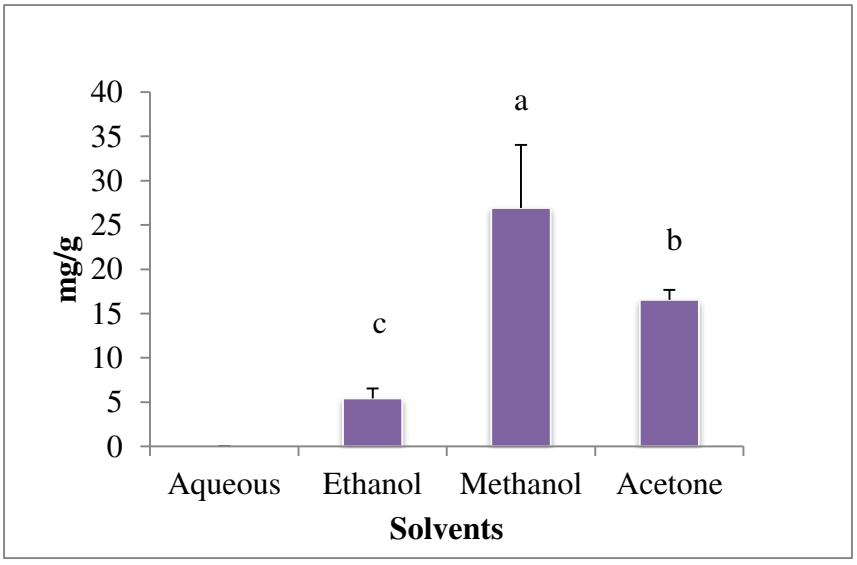

Figure 2. Quantitative phytochemical screening of Total Flavonoides of *Sargassum swartzii*

3. 3. Antibacterial activity

The antibacterial activity of seaweed *S. swartzii* in four different extracts against five different bacterial strains, three human pathogens and two fish pathogens are shown in Table

2. In human pathogens, two are gram negative and one is gram positive. When comparing gram positive and negative, the gram negative bacteria has more inhibition activity against the selected solvents. In fish pathogens, the species *A. hydrophila* had more inhibition activity than *V. vulnificus* in selected solvents. All the four solvents at 500 µg/mL of concentration produced a maximum zone of inhibition against selected bacterial strains followed by 500 > 250 > 100 > 50. In the present work, among the tested four different solvents, methanol residue is the most effective antibacterial activity than other solvents in human pathogenic bacteria. And ethanol extract is a higher antibacterial activity than other solvents in fish pathogenic bacteria. When comparing the five bacterial pathogens *A. hydrophila* exhibit the highest inhibition activity in our selected seaweed materials and the lowest activity was present in the bacteria *S. aureus*.

3. 4. Minimum Inhibitory Concentrations (MIC)

The extracts that showed any antibacterial activity in this assay were subjected to the minimum inhibitory concentration assessment and the results are presented in Table 3. The MIC of seaweed were determined by serial broth dilution method and *S. swartzii* showed the inhibitory range of (34.05 -94.29). The highest percentage of inhibitory concentrations was found in ethanol extract (94.29 %), while the lowest percentage was in Aqueous extract.

Table 2. Antibacterial activity of seaweed *Sargassum swartzii* extract against human and fish pathogens

Solvents	Concentration (µg/mL)	Bacterial Species				
		E. coli	*P. aeruginosa*	*S. aureus*	*A. hydrophila*	*V. vulnificus*
Aqueous	50	8.00 ± 0.00^{cD}	7.66 ± 0.57^{cC}	8.00 ± 0.00^{cE}	NA	NA
	100	9.00 ± 0.00^{cD}	8.66 ± 0.57^{cC}	8.33 ± 0.57^{cE}	9.66 ± 0.57^{cA}	8.66 ± 0.67^{cB}
	250	10.66 ± 0.57^{cD}	$9.66\pm0 .57^{cC}$	8.66 ± 0.57^{cE}	12.66 ± 0.57^{cA}	9.45 ± 0.57^{cB}
	500	14.33 ± 0.57^{cD}	12.33 ± 0.57^{cC}	11.00 ± 1.00^{cE}	12.00 ± 0.00^{cA}	10.00 ± 0.00^{cB}
	C	21.33 ± 0.57^{cD}	19.00 ± 0.00^{cC}	20.00 ± 0.00^{cE}	23.33 ± 0.57^{cA}	23.33 ± 0.57^{cB}
Ethanol	50	NA	8.00 ± 0.00^{aC}	NA	8.66 ± 0.57^{aA}	14.66 ± 0.57^{aB}
	100	7.66 ± 0.57^{aD}	11.00 ± 1.00^{aC}	7.66 ± 0.57^{aE}	15.66 ± 0.57^{aA}	18.33 ± 0.57^{aB}
	250	11.33 ± 0.57^{aD}	11.33 ± 0.57^{aC}	7.66 ± 0.57^{aE}	18.66 ± 0.57^{aA}	19.66 ± 0.57^{aB}
	500	15.33 ± 0.57^{aD}	13.66 ± 0.57^{aC}	10.33 ± 0.57^{aE}	21.66 ± 0.57^{aA}	21.33 ± 0.57^{aB}
	C	20.33 ± 0.57^{aD}	20.00 ± 2.00^{aC}	20.00 ± 0.00^{aE}	24.66 ± 0.57^{aA}	24.66 ± 0.57^{aB}
Methanol	50	7.66 ± 0.57^{bD}	8.66 ± 0.57^{bC}	NA	8.66 ± 0.57^{bA}	8.66 ± 0.57^{bB}
	100	10.33 ± 0.57^{bD}	10.33 ± 0.57^{bC}	8.33 ± 0.57^{bE}	12.33 ± 0.57^{bA}	9.33 ± 0.57^{bB}
	250	11.33 ± 0.57^{bD}	15.00 ± 1.00^{bC}	12.33 ± 0.57^{bE}	14.33 ± 0.57^{bA}	11.66 ± 0.57^{bB}

	500	14.66±0.57^{bD}	18.00±0.00^{bC}	15.66±0.57^{bE}	20.33±0.57^{bA}	15.00±1.00^{bB}
	C	20.33±0.57^{bD}	21.33±0.57^{bC}	20.00±0.00^{bE}	22.33±0.57^{bA}	24.66±0.57^{bB}
Acetone	50	NA	8.33±0.57^{bC}	NA	8.66±0.57^{bA}	10.66±0.57^{bB}
	100	8.66±0.57^{bD}	8.66±0.57^{bC}	10.33±0.57^{bE}	9.66±0.57^{bA}	12.00±0.00^{bB}
	250	14.33±0.57^{bD}	12.66±0.57^{bC}	11.66±0.57^{bE}	17.66±0.57^{bA}	14.66±0.57^{bB}
	500	17.00±1.00^{bD}	13.66±0.57^{bC}	14.33±0.57^{bE}	19.66±0.57^{bA}	18.33±0.57^{bB}
	C	20.33±0.57^{bD}	20.33±0.57^{bC}	20.75±0.95^{bE}	24.66±0.57^{bA}	24.33±0.57^{bB}

*__NA__: Not Available, __C__: Ciprofloxacin
ANOVA (P<0.05)
Duncan test: Identical lower case superscripts denote similar values vertically
Identical upper case superscripts denote similar values horizontally

Table 3. MIC value of *Sargassum swartzii* extracts against the human and fish pathogens

Bacteria species	Aqueous		Ethanol		Methanol		Acetone		Ciprofloxacin % of Inhibition
	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	
E. coli	50	44.71	100	94.29	50	62.50	100	53.02	93.20
	40	40.31	80	89.21	40	55.70	80	47.59	
	30	39.62	60	55.15	30	52.06	60	46.08	
	20	38.32	40	49.10	20	50.41	40	44.50	
	10	34.20	20	34.54	10	49.58	20	41.82	
P. aeruginosa	50	61.28	50	57.04	50	65.91	50	48.68	97.74
	40	58.19	40	43.72	40	60.45	40	48.36	
	30	52.28	30	42.89	30	53.44	30	47.45	
	20	49.00	20	42.37	20	54.34	20	46.30	
	10	43.60	10	41.28	10	52.54	10	43.34	

S. aureus	50	57.25	100	89.20	100	69.13	100	60.63	96.54
	40	58.46	80	68.24	80	62.68	80	45.75	
	30	52.58	60	63.83	60	56.99	60	39.55	
	20	49.32	40	61.27	40	55.52	40	35.46	
	10	43.96	20	43.45	20	51.62	20	34.05	
A. hydrophila	100	58.54	50	63.98	50	61.20	50	51.72	95.83
	80	55.83	40	55.34	40	59.57	40	49.06	
	60	55.58	30	54.01	30	56.55	30	48.70	
	40	55.34	20	51.72	20	51.90	20	45.61	
	20	55.16	10	38.73	10	50.21	10	42.41	
V. vulnificus	100	61.73	50	87.45	50	71.57	50	51.44	96.78
	80	56.33	40	66.04	40	68.93	40	51.38	
	60	54.46	30	58.39	30	68.87	30	50.67	
	40	49.64	20	53.95	20	63.47	20	50.54	
	20	48.03	10	49.19	10	53.82	10	48.36	

4. DISCUSSION

The present study was to evaluate the phytochemistry and antibacterial activity of shade dried generally powdered seaweed *Sargassum swartzii* with a variety of solvents such as aqueous, ethanol, methanol and acetone.

The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive metabolites and compounds of interest in the pharmaceutical industry [22, 23].

The qualitative phytochemical analysis of *Sargassum swartzii* is presented in Table – 1. The majority of the bioactive compounds like Steroids, Tannins, Terpenoids, Flavonoides, Alkaloides, Saponins, Cardiac glycosides, Reducing sugar, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Betacyanin and Phenols are established. Algae is a natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumor, antioxidants and anti-inflammatories [24]. A large number of algal extract products have been found to have antimicrobial activity [25, 26].

Phytoconstituents such as phenol, flavonoids and tannin compounds are recommended to be the most important chemical components of algal cells and could have an activating or inhibiting effect on microbial growth depending on their establishment and concentration [27-32]. The phenolic contents in the *S. swartzi* (Figure 1) were significantly different between different extracts ($P<0.05$). The ethanolic extract showed higher phenolic content. Matanjum *et*

al. [33] observed the similar result that the brown seaweeds contained higher phenolic content than the red and greed seaweeds. Duan *et al*. [30] observed higher phenolic content (73.7 GE/g) in the ethyl acetate soluble fraction of red alga *P. urceolata.* However, Kuda *et al*. [34] observed the phenolic content of 0.18 mg catechin equivalents/g in the ethanolic extract of brown seaweed, *P. kuromo*.

Flavonoids contain a large group of naturally occurring compounds generally distributed in the plant kingdom and some of these compounds have been reported to contain various and strong biological activities with antioxidative tissue protective and tumoristatic effects as well as the inhibition of hepatic cholesterol biosynthesis [33, 35-37]. In the present results, total flavonoid content was highest in methanol extracts (Figure 2).

Secondary metabolites are phenolics that play a role in the maintenance of the human body [38]. The presence of phytoconstituents, such as phenols, flavonoids and tannin seaweeds indicates the option of antioxidant activity and this activity will help in preventing a number of diseases through free-radical scavenging activity [39].

The ability of marine algae to create the potential of secondary metabolites [40, 41]. Bacterial diseases cause high speed of mortality in human population and aquaculture industries [42]. For an example, *Bacillus subtilis* is responsible for causing food borne gastroenteritis. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella* sp. cause diarrhea and typhoid fever [43]. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness [42, 44]. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems.

According to previous reports anti-bacterial activity depends on algal species, the efficiency of the extraction technique and the resistance of the pathogenic bacteria [45, 46]. The present study indicated that, ethanol was the most effective solvent for the extraction of the bioactive compounds followed by methanol. The brown algae *S. swartzii* showed antibacterial activity against selected gram positive and gram negative human and fish pathogenic bacteria maximum activity were recorded in the fish pathogen *A. hydrophila* in ethanol extract and followed by human pathogen *P. aeruginosa* in methanol extract when compared to the other solvent. Some other studies performed in the extraction of seaweeds using chloroform and ethyl acetate also good quality antibacterial activity [31, 47, 48].

Since previous times, marine plant extracts have been used for treatments of common infectious diseases, treatments with plants having antibacterial activity are a potential beneficial alternative in aquaculture [49]. A number of works have been undertaken on crude and purified compounds obtained from seaweeds for evaluating their bioactive potential. Brown seaweeds are known to contain more bioactive components than either green or red seaweeds [50].

Hanniffy and Kraan, [51] described that the macro algal species, *Ulva, Porphyra* and *Palmararia palmata* showed a strong antibiotic activity against fish and human pathogens. Bansemir *et al*. [52] discussed that dichloromethane, methanol and water extracts of twenty six species of cultivated seaweeds were screened for their antibacterial activities against five fish pathogenic strains. Previously reported crude extracts from the seaweeds, *Gracilaria edulis*, *Calorpha peltada* and *Hydroclothres sp*. were screened for their antibacterial activity against six fish pathogens [45]. Lavanya and Veerappan, [53] discussed the extracts of six seaweed samples that were screened for antibacterial activity against fish and human pathogens. In our study results showed that seaweeds all extracts have shown moderate activity against all pathogens.

5. CONCLUSIONS

It can be concluded from the present findings that the different extract of *S. swartzi* collected from the Tirunelveli costal region, that ethanol and methanol extract have considerable amount of phenol and flavonoid content and potential antimicrobial activity against human and fish pathogens.

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Conflict of interest

We declare that we have no conflict of interest.

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