Research Article

Phytochemical Analysis and Antibacterial Potential of Marine Algae against Human and Aquatic Pathogens

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ABSTRACT

The phytochemical analysis and antibacterial activity were evaluated for the marine algae *Turbinaria conoides* and *Sargassum wightii* from kilakarai coast, Tamil Nadu, India. Different solvents viz, aqueous, ethanol, methanol and acetone were used for algal extraction to envisage the phytochemical analysis and antibacterial activity against human pathogens, *Escherichia coli* (MTCC 2939), *Pseudomonas aeruginosa* (MTCC 2453) and *Staphylococcus aureus* (MTCC 9706) and aquatic pathogens, *Aeromonas hydrophila* (MTCC 1739) and *Vibrio vulnificus* (MTCC 1146). Phytochemical analysis of sixteen different chemical compounds was carried out. The maximum ten compounds were present in the ethanol extract of *S. wightii* and nine compounds were present in the *T. conoides*. In human pathogens the maximum activity (22.66±0.5) was recorded from the ethanol extract of *S.wightii* against *E. coli* and the aquatic pathogen the maximum activity (22.66±0.5) was observed from the ethanol extract of *S. wightii* against *V. vulnificus*. Of the four solvents tested, ethanol was determined to be the best solvent for isolation of antibacterial compounds from the tested marine algae followed by methanol, acetone and aqueous. The aim of our studies was to identify algal phytochemicals, which possess activities against human and aquatic pathogenic bacteria and could be an alternative to the commonly used antibiotics in pharmaceuticals and aquaculture industry.

Keywords: Algae, Antibacterial, Phytochemical, Ciprofloxacin, MHA, MIC, MTCC.

INTRODUCTION

Marine algae comprise one of the compassionate substances which integrated remedy and pharmacotherapy, while some of the isolated substances have bacteriostatic and bactericidal properties.[1] Bacterial infection causes a high rate of mortality within the human population and aquaculture organisms. Nowadays using antibiotics has increased considerably due to heavy infection and the pathogenic microorganisms resistant to drugs are common due to indiscriminate use of antibiotic. The antibacterial compounds derived from the marine plants consist of various groups of chemicals.[2] Most of the compounds of marine algae show anti-bacterial activities.[3,4]

Bioactive compounds are naturally present in the algae or seaweeds and are biologically significant and play a vital role in defending themselves against various pathogenic microbes with the aid of showing the antimicrobial activity through inhibition or killing mechanisms. The secretion of those compounds varies among the seaweeds. Some produce more and a few products in minimum quantity. Many of the seaweeds possess bioactive components which inhibit the growth of some of the gram positive and gram negative bacterial pathogens.[5, 6] The seaweed became placed to be greater effective against various human and aquatic pathogenic microorganisms.[7, 8]

Marine macroalgae are recognized to generate quite a number of compounds and a quantity of them had been shown to possess the biological activity of potential medicinal value that takes widely greater discovery of metabolites with biological activity from seaweeds has extended significantly. It is used for both human and animal health applications.[8, 9] Hence the search for novel bioactive substances from natural sources is very important and seaweeds are this sort of useful resource with immense potential, waiting to be tapped substantially for the gain of mankind.

MATERIALS AND METHODS

Seaweed material and extraction

Brown algae *Turbinaria conoides* and *Sargassum wightii,* were freshly collected from the Kilakarai region located between 9.23135° N, 78.7844° E Ramanathapuram District, Tamil Nadu, India. The collected samples were washed in running water for 10 min, transported to the laboratory and shade dried (35±3 °C) for 36 h. The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples (5 g) and packed in Soxhlet apparatus and extracted successively with aqueous, ethanol, methanol and acetone for 10 h (Hun et al., 1994). The crude extracts were weighed and deep frozen (-20 °C) until tested.

Phytochemical analysis

The extracts from different solvents were tested for Steroids, Tannins, Terpenoids, Flavonoids, Saponins, Alkaloids, 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.[10, 11]

Antibacterial activity

Test microbes

The human pathogenic bacterial strains are *Escherichia coli* (MTCC 2939), *Pseudomonas aeruginosa* (MTCC 2453) and *Staphylococcus aureus* (MTCC 9706) and aquatic pathogens, *Aeromonas hydrophila* (MTCC 1739) and *Vibrio vulnificus* (MTCC 1146) were used for this experiment. The pathogenic bacteria were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTEC), Chandigarh, India. It was subcultured and used for the antibacterial assay.

Disc diffusion method

The antimicrobial activity of *Turbinaria conoides and Sargassum wightii* solvent extracts was assessed by the disc diffusion technique.[12] 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 extracts (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 °C, and the inhibition zones were measured in millimeter and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

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 108 CFU/mL of bacteria. Ciprofloxacin was used as positive control. The test tubes were incubated for 24 h at 37 °C. MIC was determined as the lowest concentration of extract showing OD of 600nm of spectrophotometer. All the data were statistically analyzed.

Statistical analysis

All the values were expressed as Mean ± Standard Deviation (SD). The statistical significance was evaluated by two-ways Analysis of Variance (ANOVA) using SPSS version 20 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Post-hoc analysis, Duncan.[13]

RESULTS AND DISCUSSION

Phytochemical analysis

In this study, the brown algae *T. conoides* and *S. wightii* were selected and analysis for the presence of phytochemical constituents. Secondary phytochemical compounds of Steroids, Tannins, Terpenoids, Flavonoids, Saponins, Alkaloids, Reducing sugar, Cardiac glycosides, Coumarins, Phlobatannins, Anthraquinones, Quinones, Glycosides, Phenols, Anthocyanin, Betacyanin was tested in four different solvents like Aqueous, Ethanol, Methanol and Acetone (Table 1). To compare two algae, the total maximum compounds were present in *S. wightii* than *T. conoides*. Of the four solvents, the maximum compounds were present in the ethanol extracts of *T. conoides* and *S. wightii*. The compounds cardiac glycoside, phenols and anthocyanin were present in four solvent extracts of *S. wightii* and phenols only present in four solvent extracts of *T. conoides*. The most important phytochemical assessments may be beneficial in the detection of bioactive principles and finally may additionally result in drug discovery and development. Further, those investigations help their quantitative estimation and qualitative separation of pharmacologically active chemicals.[14] Some phytochemicals made using algae have an anti-microbial interest and used for the enhancement of new anti-microbial drugs.[15]

The algae are amusing in secondary metabolites which consist of alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites, which might be of exquisite scientific price and have been broadly used within the drug and pharmaceutical industry.[16] The seaweed thus remains an essential source of bioactive natural substances.[17, 18]

Phytoconstituents along with phenol, flavonoids and tannin compounds are endorsed to be the maximum significant chemical additives of algal cells and may possibly a stimulating or inhibiting influence on microbial growth depending on their establishment and concentration.[19, 20]

Flavonoids contain biological activities with tumorostatic assets in addition to the inhibition of hepatic cholesterol biosynthesis.[21, 22] Flavonoids have produced extensive attention recently since their potential constructive effects on human health in aggressive diseases.[23] Phenolics that play a role within the renovation of the human body.[24] Steroids of plant foundation are known to be necessary for insecticidal, antimicrobial, antiparasitic and cardiotonic assets.[25] A few epidemiological kinds of researches revealed that algal phenolic compounds remaining in the diet are useful in treating coronary heart disease, anxiety, and osteoporosis stroke and different degenerative illness. Additionally, phenols have been reported to reveal pharmacological properties such as antibacterial, antiviral, antifungal, antiinflammatory or antitumoral and anticarcinogenic.[26] Tannins remained used therapeutically as antiviral, antibacterial, antiulcer and antioxidant alternatives. Quite some tannin comprising drugs are used in the treatment of piles, inflammation, burns and as astringent.[27] Some terpenes also display insecticidal properties.[28] The terpenoids from Dictyotaceae exhibit bioactivities along with inhibition of herbivores and antifungal, antibiotic, anti-inflammatory, cytotoxic, insecticidal and antiviral activities.[29] Alkaloids are normally created to antimicrobial properties contrary to both Gram-

positive and Gram-negative bacteria.[30, 31] Alkaloids are secondary metabolites well-known to be made by plants and are pharmaceutical prominence then they are used as drugs for the treatment of several diseases.[32]

Antibacterial activity and Minimum Inhibitory Concentration

The antibacterial activity of *T. conoides* and *S. wightii* extracts on three human pathogens were presented in Table 2. Of the two algae the highest zone of inhibitory activity was found in ethanol extract of *T. conoides* against *P. aeruginosa* (19.66±0.5) and ethanol extract of *S. wightii* against *E. coli* (22.66±0.5) at 500µg concentration. Of the two algae screened for their antibacterial activity in the present investigation *S. wightii* was more superior to *T. conoides* against the human pathogens in controlling their growth. The result of two ways ANOVA reveals that there is a significant difference ($p<0.05$) in antibacterial activity of all four extracts of two algae. Among the four solvents aqueous, ethanol, methanol and acetone extracts inhibited the growth of all the pathogens tested, of which ethanol extracts are more active.

The antibacterial activity of *T. conoides* and *S. wightii* extracts on two aquatic pathogens were presented in Table 3. Of the two algae the highest zone of inhibitory activity was found in methanol extract of *T. conoides* against *V. vulnificus* (21.66±0.5) and ethanol extract of *S. wightii* against *V. vulnificus* (22.66±0.5) at 500µg concentration. Of the two algae screened for their antibacterial activity in the present investigation *S. wightii* was more superior to *T. conoides* against the aquatic pathogens in controlling their growth. The result of two ways ANOVA reveals that there is a significant difference ($p<0.05$) in antibacterial activity of all four extracts of two algae. Among the four solvents aqueous, ethanol, methanol and acetone extracts inhibited the growth of aquatic pathogens tested, of which ethanol extracts are more active.

The minimum inhibitory concentration of *T. conoides* (Table 4), when compare to all the five selected pathogens the lowest concentration of 10 µg/ml of acetone extract showed the highest inhibitory activity (56.07 %) against *P. aeruginosa* and MIC of *S. wightii* (Table 5), the lowest concentration of 10 µg/ml of methanol extract showed the highest inhibitory activity (43.62%) against *A. hydrophila*.

The capability of marine algae to produce secondary metabolites of potential interest has been comprehensively documented.[6] According to earlier reports anti-bacterial activity depends on algal species, the efficiency of the extraction method, and the resistance of the tested bacteria.[33] Data obtained in the present study indicated that, ethanol was the most effective solvent for the extraction of the bioactive compounds followed by methanol. Furthermore, *S. wightii* were the most effective marine algae against tested bacterial species followed by *E. coli*, *P. aeruginosa*, *S. aureus*, *A. hydrophila* and *V. vulnificus*. These results are in agreement with that earlier reports.[34] Ethanol extraction has been reported to result in extracts with higher antibacterial activity than petroleum ether, while few other reports indicated chloroform as better extractant than ethanol and petroleum ether.[35] From the results of the present study it is clear that, organic solvents always have higher efficiency in extracting anti-bacterial compounds compared to water as extractant and ethanol as a solvent proved to be best suited for the extraction of the antibacterial constituents from the algae.

Table 1: Phytochemical analysis of *Turbinaria conoides* and *Sargassum wightii* extracts

S. No	Phytochemical compounds	*T. conoides*				*S. wightii*			
		Solvents				Solvents			
		Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol	Acetone
1	Steroids	-	+	-	-	-	-	-	-
2	Tannins	-	-	-	-	+	-	+	-
3	Terpenoids	+	+	+	-	+	+	+	-
4	Flavonoids	-	-	+	-	-	+	+	+
5	Saponins	+	+	+	-	+	-	-	+
6	Alkaloids	-	+	-	+	-	+	-	-
7	Reducing sugar	+	-	-	-	+	+	+	-
8	Cardiac glycosides	-	-	-	-	+	+	+	+
9	Coumarins	+	+	+	+	-	-	-	+
10	Phlobatannins	+	-	-	-	-	+	-	-
11	Anthraquinones	+	-	+	-	-	+	-	-
12	Quinones	-	+	+	-	-	+	+	+
13	Glycosides	-	+	+	-	-	-	-	-
14	Phenols	+	+	+	+	+	+	+	+
15	Anthocyanin	-	-	-	-	+	+	+	+
16	Betacyanin	-	+	+	+	-	-	-	-

+ Present -Absent

Table 2: Antibacterial activity of *Turbinaria conoides* and *Sargassum wightii* extracts against human pathogens (Mean ± SD, n= 3)

Solvents	Concentration (µg/ml)	Zone of Inhibition (mm)					
		T. conoides			*S. wightii*		
		E. coli	*P. aeruginosa*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. aureus*
Aqueous	50	6±0.0	7.33±0.5	9.33±0.5	6±0.0	6±0.0	6±0.0
	100	6±0.0	9.66±0.5	11.00±1.0	6±0.0	6±0.0	6±0.0
	250	7.33±0.5	11.33±0.5	13.33±0.5	8.66±0.5	8.33±0.5	6±0.0
	500	8.00±1.0	13.33±0.5	14.66±0.5	9.66±0.5	11.33±0.5	6±0.0
	C	25.33±0.5	21.33±1.1	20.66±0.5	22.66±0.5	21.00±0.00	22.33±0.5
Ethanol	50	10.33±0.5	8.66±0.5	6±0.0	7.33±0.5	6±0.0	6±0.0
	100	11.33±0.5	15.66±0.5	11.66±0.5	7.66±0.5	6±0.0	6±0.0
	250	15.33±0.5	16.66±0.5	13.66±0.5	12.66±0.5	8.33±0.5	8.33±0.5
	500	16.33±0.5	19.66±0.5	17.66±0.5	22.66±0.5	10.66±0.5	11.66±0.5
	C	21.33±0.5	23.66±0.5	21.66±0.5	24.66±0.5	23.33±1.5	21.00±1.0
Methanol	50	8.66±0.5	10.66±0.5	8.66±0.5	10.00±0.0	6±0.0	6±0.0
	100	9.66±0.5	12.33±0.5	12.00±0.0	11.66±0.5	6±0.0	6±0.0
	250	12.33±0.5	15.66±0.5	14.66±0.5	8.66±0.5	9.66±0.5	6±0.0
	500	16.66±0.5	17.66±0.5	18.33±0.5	9.33±1.1	12.33±0.5	12.00±1.0
	C	22.66±0.5	21.00±1.0	22.00±1.0	22.33±1.1	23.33±1.5	23.00±1.0
Acetone	50	7.66±0.5	8.66±0.5	9.66±0.5	10.33±0.5	6±0.0	6±0.0
	100	11.33±0.5	10.66±0.5	11.66±0.5	8.33±1.1	6±0.0	6±0.0
	250	11.66±0.5	11.66±0.5	14.00±0.5	8.00±1.0	6±0.0	10.33±0.5
	500	16.00±1.0	14.66±0.5	15.66±0.5	10.00±1.0	11.00±0.0	11.33±.5
	C	22.00±1.0	22.66±0.5	21.33±0.5	22.66±1.5	24.33±0.5	27.33±.5

*C: Ciprofloxacin, ANOVA (P<0.05)

Table 3: Antibacterial activity of *Turbinaria conoides* and *Sargassum wightii* extracts against aquatic pathogens (Mean ± SD, n= 3)

Solvents	Concentration (µg/ml)	Zone of Inhibition (mm)			
		T. conoides		*S. wightii*	
		A. hydrophila	*V. vulnificus*	*A. hydrophila*	*V. vulnificus*
Aqueous	50	6±0.0	6±0.0	6±0.0	6±0.0
	100	6±0.0	6±0.0	10.33±0.5	6±0.0
	250	7.33±0.5	7.00±0.0	12.00±0.5	7.00±0.0
	500	11.33±0.5	7.00±0.00	14.66±0.5	9.33±0.5
	C	25.00±1.0	21.00±1.0	23.33±1.5	19.33±0.5
Ethanol	50	8.66±0.5	6±0.0	10.33±0.5	12.66±0.5
	100	9.33±0.5	9.66±0.5	11.66±0.5	17.33±0.5
	250	11.66±0.5	12.33±0.5	14.66±0.5	19.66±0.5
	500	12.33±0.5	14.66±0.5	18.33±0.5	22.66±0.5
	C	28.00±1.0	24.00±1.0	24.66±0.5	21.33±1.1
Methanol	50	9.66±0.5	10.33±0.5	8.33±0.5	11.66±0.5
	100	11.66±0.5	12.33±0.5	10.33±0.5	12.33±1.1
	250	14.33±0.5	14.66±0.5	14.33±0.5	15.33±0.5
	500	17.66±0.5	21.66±0.5	15.00±0.0	18.33±0.5
	C	22.00±1.0	24.33±0.5	21.00±1.0	24.33±0.5
Acetone	50	10.66±0.5	6±0.0	8.00±0.0	8.00±0.5
	100	12.33±0.5	9.33±0.5	11.33±0.5	11.66±2.5
	250	14.33±0.5	9.66±0.5	14.66±0.5	12.66±2.5
	500	17.66±0.5	15.33±0.5	20.66±0.5	14.00±2.0
	C	29.00±1.0	26.00±1.0	24.33±0.5	22.33±2.5

*C: Ciprofloxacin, ANOVA (P<0.05)

Table 4: MIC of *Turbinaria conoides* extracts against human and aquatic pathogens

Bacterial species	Solvents								Ciprofloxacin % of Inhibition
	Aqueous		Ethanol		Methanol		Acetone		
	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	
E. coli	100	53.84	50	84.13	50	95.46	50	59.13	93.20
	80	53.02	40	78.50	40	61.53	40	57.96	
	60	52.60	30	68.88	30	60.64	30	52.19	
	40	51.16	20	55.21	20	53.84	20	52.54	
	20	45.74	10	50.54	10	52.19	10	51.16	
P. aeruginosa	50	57.62	50	75.11	50	70.80	50	61.41	97.74
	40	57.42	40	58.26	40	65.78	40	58.77	
	30	56.65	30	56.20	30	63.02	30	58.39	
	20	54.01	20	53.69	20	62.05	20	57.36	
	10	53.89	10	52.28	10	54.40	10	56.07	
S. aureus	50	60.89	100	75.71	50	67.92	50	63.57	96.54
	40	58.21	80	60.51	40	66.77	40	62.93	
	30	55.33	60	59.10	30	66.32	30	61.78	
	20	54.82	40	52.58	20	63.76	20	54.95	
	10	52.26	20	45.36	10	52.77	10	54.31	
A. hydrophila	250	62.71	50	62.29	50	65.01	100	70.15	95.83
	150	59.21	40	54.74	40	64.83	80	63.92	
	100	57.94	30	53.95	30	61.32	60	62.77	
	50	56.07	20	53.17	20	54.86	40	59.63	
	25	55.52	10	52.74	10	54.56	20	53.11	
V. vulnificus	250	62.57	100	99.16	50	64.63	100	94.01	96.78
	150	57.62	80	86.55	40	63.66	80	92.54	
	100	56.72	60	84.11	30	58.90	60	80.00	
	50	55.94	40	66.94	20	55.17	40	70.73	
	25	54.53	20	48.55	10	53.69	20	53.24	

Table 5: MIC of *Sargassum wightii* extracts against human and aquatic pathogens

Bacterial species	Solvents								Ciprofloxacin % of Inhibition
	Aqueous		Ethanol		Methanol		Acetone		
	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	Con. (mg/ml)	% of Inhibition	
E. coli	100	42.65	50	44.64	50	43.26	50	39.83	93.20
	80	36.53	40	40.86	40	41.00	40	37.50	
	60	33.03	30	35.98	30	40.10	30	36.19	
	40	32.48	20	35.98	20	38.94	20	33.44	
	20	32.55	10	37.50	10	36.33	10	32.00	
P. aeruginosa	250	39.83	250	73.63	250	72.05	500	59.76	97.74
	200	37.11	200	59.12	200	69.66	400	53.91	
	150	37.56	150	50.64	150	58.49	300	50.40	
	100	29.23	100	41.38	100	47.59	200	39.12	
	50	16.84	50	37.79	50	38.83	100	32.87	
S. aureus	250	-	250	89.44	500	87.42	250	61.24	96.54
	200	-	200	81.49	400	76.46	200	54.92	
	150	-	150	64.18	300	67.36	150	52.31	
	100	-	100	52.88	200	60.37	100	49.49	
	50	-	50	34.12	100	50.27	50	25.82	
A. hydrophila	100	43.44	50	51.78	50	51.66	50	44.47	95.83
	80	43.26	40	45.43	40	48.88	40	43.98	
	60	41.81	30	43.86	30	47.49	30	43.08	
	40	41.26	20	40.54	20	47.79	20	42.35	
	20	39.69	10	40.42	10	43.62	10	41.69	
V. vulnificus	50	46.36	50	51.89	50	51.76	50	46.49	96.78
	40	40.12	40	48.16	40	45.14	40	44.37	
	30	39.35	30	47.52	30	43.34	30	43.34	
	20	38.90	20	43.40	20	43.08	20	42.12	
	10	38.39	10	42.44	10	40.12	10	40.90	

The variations in antibacterial interest can be due to the method of extraction and location of samples that had been collected Kandhasamy and Arunachalam.[36] All extracts showed higher inhibition of antibacterial activity, consistent with Febles *et al*.[37] suggested that methanol extract yielded better antimicrobial activities for seaweed. In a distinct observe by Bansemir *et al*.[38] dichloromethane extract of *Dictyota dichotoma* did not show any inhibition against *Aeromonas hydrophila* which turned into in comparison with the prevailing have a look at in which ethanol extracts shows efficaciously inhibited antibacterial activity against *Aeromonas hydrophila*.

Seaweed has been defended in the direction of a capability source of antibacterial compounds towards both Gram-negative and Gram-positive pathogenic microorganism.[39] Taskin *et al*.[40] said that ethanol extract of eight seaweed species belonging to brown, red and green algae exhibited huge spectrum activity of both antibacterial and antifungal activities. Agreeing to previous reports anti-bacterial activity depends on seaweed species, the efficiency of the extraction method and the resistance of the pathogenic bacteria.[39, 41] Numerous researches achieved within the extraction of seaweeds using chloroform and ethyl acetate also good quality antibacterial activity.[35, 42]

As in advance instances, marine algal extracts had been used for treatments of common infectious diseases; remedies with plants having an antibacterial interest are a potential useful alternate in aquaculture.[43] A sum of works has been sporting out on crude and purified compounds attained from seaweeds for assessing their bioactive ability. Brown seaweeds are known to encompass more bioactive components than either green or red seaweeds.[44] In the present investigation the brown seaweeds *T. conoides* and *S. wightii* was observed as a prolific antibacterial activity.

CONCLUSION

The results of the present investigation on selected species of marine algae indicated scope for deriving biologically active compounds which are effective in inhibiting the growth of the pathogenic bacteria for human and aquatic. There is great scope for further investigations toward drug development.

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