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In vitro bioactivity and phytochemical analysis of two marine macro-algae

Ravi Cyril*, Revathi Lakshmanan, Amuthavalli Thiyagarajan

Department of Zoology, Thiagarajar College, Madurai- 625 009, India

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ABSTRACT

Objective: To evaluate the *in vitro* bioactivity and phytochemical analysis of two marine macro-algae *viz.*, *Sargassum wightii* (*S. wightii*) and *Gracilaria verrucosa* (*G. verrucosa*).
Methods: The marine macro-algae were collected from Gulf of Mannar (latitude 8°55'–9°15' N and longitude 78°–79°16' E), India. The collected marine macro-algae were shade dried, powdered and extracted in Soxhlet apparatus with acetone, methanol, chloroform and petroleum ether separately. Antimicrobial assay was performed against nine bacterial pathogens (isolated from infected ornamental fish) and two fungal strains. The extracts were further tested for anti-hemolytic activity following standard protocol. The methanol extracts were also tested for its anti-cancer property against human AGS cell line.
Results: High antibacterial activity was displayed by the petroleum ether extract of *G. verrucosa* against *Micrococcus* sp. [(31.5 ± 6.5) mm]. Maximum [(21.5 ± 1.0) mm] anti-fungal activity was exerted by the methanol extracts of *S. wightii* and *G. verrucosa* against *Candida* sp. Acetone extract of *G. verrucosa* produced better anti-hemolytic activity (96.47% ± 0.31%) than other tested extracts. Methanol extract of both seaweeds failed to produce any anti-cancer activity against human AGS cell line. Quantitative phytochemical analysis revealed elevated levels of alkaloids (56 mg/g), saponins (44.5 mg/g), flavonoids (500 mg/g) and phenols (220 mg/g) in *G. verrucosa* when compared to *S. wightii*.
Conclusions: The tested macro-algae are good source of bioactive compounds, and *G. verrucosa* displayed better activity than *S. wightii* and could be explored further.

1. Introduction

Marine macro-algae also called as seaweeds are rich in minerals, polyunsaturated fatty acids and vitamins; hence it could potentially be exploited for both human and animal health applications[1,2]. Around 600 secondary metabolites have been so far identified from seaweeds[3] and compounds derived from them are associated with broad range of biological activities[4,5]. The emergence of antibiotic resistant microorganisms in aquaculture is a key obstacle to their extensive use. The residue of these chemicals in aquaculture products has also been reported as a serious concern for consumers[6]. The prevention and treatment of these infectious agents by applying natural products from marine organisms appears to be a possible alternative source[7].

The desirable physiological and morphological characteristics of erythrocytes made them an effective tool in drug testing and delivery[8,9]. Oxidative damage to the erythrocyte membrane leads to hemolysis and compounds having antioxidant properties have long term action on hemolysis[10]. Phytochemicals such as flavonoids, polyphenols and phlobatanins inhibited proliferation of cancer cells and influenced anti-inflammatory responses[11]. *Sargassum wightii* (family: Phaeophyceae) (*S. wightii*) is widely distributed in the southern coasts of India, harbors good amount of flavonoids and is an ideal target for medical and industrial applications[12]. *Sargassum* sp. contains various secondary metabolites with biological activities[13] and has also been used in the treatment of chronic bronchitis, hypertension, edema, goiter and tuberculosis of lymph nodes[14].

Gracilaria verrucosa (family: Rhodophyceae) (*G. verrucosa*) prevalent in Indian coast is known for its food value. The phycocolloids have a high growth rate owing to temperature tolerance and are rich in primary and secondary metabolites[15]. *Gracilaria* species have small esterification cell wall and phycocolloids, the main source of agar α-(1,4)-3,6-anhydro-L-galactose and β-(1,3)-D-galactose that are important for the industrial and biotechnological applications[16,17]. With this backdrop, the present study has been undertaken to compare the *in vitro* biological activity of solvent extracts of *S. wightii* and *G. verrucosa*.

*Corresponding author: Ravi Cyril, Department of Zoology, Thiagarajar College, Madurai- 625 009, India.
Tel: +91 9943192525
E-mail: ravi_cyril@yahoo.co.in
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2. Materials and methods

2.1. Sample collection and preparation

The marine macro-algae *S. wightii* and *G. verrucosa* were collected from Gulf of Mannar (latitude 8°55'–9°15' N and longitude 78°–79°16' E), India, during low tide with the help of local fishermen by hand picking and identified at Central Marine Fisheries Research Institute, Mandapam, India. The collected algae were washed with distilled water to remove the epiphytes and other marine organisms and brought to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water, shade dried and powdered in a domestic mixer.

2.2. Preparation of solvent extract

Standard protocol was followed for the extraction[18]. In Soxhlet apparatus, 15 g dried plant powder was extracted with acetone, methanol, chloroform and petroleum ether (100 mL) for 6 h. The extracts were filtered, concentrated and stored at 4 ˚C until further analysis.

2.3. Antibacterial assay

Bacterial strains were isolated from diseased ornamental fish (collected from aquarium, Kadachanethal, Madurai) following standard methodology and maintained on nutrient agar slants. The isolated strains were identified based on their morphological and biochemical characteristics as prescribed in Bergey's Manual of Determinative Bacteriology[19]. Antibacterial assay was performed by well diffusion method[20]. Following spread plate technique, a bacterial lawn was generated in Mueller-Hinton agar plates with 24 h broth culture of bacteria. Three wells (6 mm diameter each) were created in each plate with the help of a sterile well cutter. The extract and controls (respective solvents and gentamycin) were loaded into the wells and allowed to dry. The extracts were dissolved in their respective solvents (1 mg/mL) and five different concentrations (10 μL, 20 μL, 30 μL, 40 μL, 50 μL) were prepared and tested against each bacterium. The plates were incubated at 37 ˚C for 24–48 h and the zones of inhibition (if any) were measured.

2.4. Antifungal assay

Aspergillus niger and *Candida albicans* (*C. albicans*) (procured from the stock culture maintained at Thiagarajar College) were inoculated onto potato dextrose agar slants, incubated at 28 ˚C for 7 days and then stored at 4 ˚C. Spread potato dextrose agar plates were prepared with 2–3 days old broth culture of fungi. Three wells (6 mm diameter each) were made in each plate with the help of a sterile well cutter. The extract and controls (respective solvents and nystatin) were loaded into the wells and allowed to dry. The extracts were dissolved in their respective solvents (1 mg/mL) and from that, five different concentrations (10 μL, 20 μL, 30 μL, 40 μL, 50 μL) were prepared and tested against each fungal strain. After 72 h of incubation at 37 ˚C, the plates were observed for the presence of inhibition zones.

2.5. Anti-hemolytic assay

Anti-hemolytic activity was assessed as described by the standard method[21]. Venous blood samples were collected in ethylene diamine tetra acetic acid disodium salt vials from healthy human volunteers (25–30 years of age). Blood was diluted with saline (0.9% NaCl), centrifuged at 2000 r/min for 10 min and the separated erythrocytes were suspended in phosphate buffered saline (0.2 mol/L; pH 7.4). With 2 mL of the erythrocyte suspension, different concentrations of extract (100–500 μg/mL) were added and the final volume was made to 5 mL with phosphate buffered saline. The mixture was incubated for 5 min at room temperature and then 0.5 mL of H_2O_2 solution was added to induce oxidative degradation of the membrane lipids. In another set, quercetin (100–500 μg/mL) was taken as reference compound and treated in the similar way. Thereafter, the tubes were centrifuged at 2000 r/min for 10 min and the color intensity of the supernatant was measured spectrophotometrically at 540 nm (Systronics 2203). To achieve 100 percent hemolysis (control), 2 mL of distilled water was added to 2 mL of erythrocyte suspension. The relative hemolysis was calculated in comparison with the hemolysis in the control, which was taken as 100%. Experiments were performed in triplicate and inhibitory activity of different fractions on hemolysis was calculated and expressed as percent inhibition.

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of extract}}{\text{OD of control}} \times 100$$

2.6. Anti-cancer activity

Anti-cancer activity was carried out by MTT assay[22]. Desired number of human AGS cells were seeded in a 96 well plate and incubated for 24 h in incubator with 5% CO_2 at 37 ˚C. To obtain a dose response effect, each extract was diluted at 10 different concentrations (10 fold, 4 fold or half log dilution) in the cell line growth media. In parallel, the cells were treated with solvent control to assess its effect on cells. The existing media was removed and replaced with the media with various concentration of each extract and the cells were incubated for 48 h. The cell viability was detected by adding the MTT working solution by diluting the stock solution in growth media to the final concentration of 0.8 mg/mL. 100 μL of MTT working solution was added to each well and incubated for 4 h in CO_2 incubator. After incubation, the media was removed carefully without disturbing the formazan precipitate and dissolved in 100 μL of 100% dimethyl sulphoxide, incubated for 15 min in dark and the colorimetric estimation of formazan product was performed at 570 nm in a microplate reader.

2.7. Phytochemical analysis

The extracts of *S. wightii* and *G. verrucosa* were subjected to qualitative phytochemical analysis following standard protocol[23]. Quantification of total alkaloids, flavanoids, phenols and saponins was also performed.

2.8. Statistical analysis

All statistical analysis was performed using the SPSS software package, version 11.5. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Antibacterial activity

Nine bacterial pathogens were isolated from diseased ornamental fish and identified based on the biochemical characteristics (Table 1). The bacterial pathogens were subjected to antibacterial assay

Table 1

Biochemical characterization of the bacterial pathogens isolated from infected ornamental fish.

Biochemical tests	Strain1	Strain2	Strain3	Strain4	Strain5	Strain6	Strain7	Strain8	Strain9
Gram staining	+ve, Cocci	+ve, Spherical shaped bacteria	+ve, Rod shaped bacteria	-ve, Rod	-ve, Rod	+ve, Cocci	-ve, Rod	-ve, Rod	-ve, Rod
Starch hydrolysis	+	+	+	+	+	-	+	+	+
Casein hydrolysis	-	-	+	-	-	-	-	+	-
Gelatin hydrolysis	-	-	+	-	-	-	-	-	-
Carbohydrate fermentation	+	+	-	-	+	-	+	+	-
Catalase test	-	+	+	+	+	+	+	+	-
Indole test	-	-	-	-	+	-	-	+	-
Methyl red test	+	+	-	-	+	-	+	-	-
Voges-Proskauer	-	+	+	-	-	-	-	+	-
Citrate utilization	-	-	-	-	-	-	+	+	-
H_2S production	-	-	-	-	+	-	+	+	-
Urease test	-	+	-	-	-	-	-	-	+
Triple sugar iron test	-	-	Alkaline slant, acid butt	-	-	-	Alkaline slant, acid butt	Alkaline slant, acid butt	Alkaline slant, acid butt
Name of the bacteria	*Streptococcus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Shigella* sp.	*E. coli*	*Micrococcus* sp.	*Aeromonas* sp.	*Proteus* sp.	*Vibrio* sp.

+: Positive result; -: Negative result; *E. coli*: *Escherichia coli*.

against the solvent extracts of *S. wightii* and *G. verrucosa* and the obtained results are presented in Tables 2 and 3. All the tested extracts displayed antibacterial activity and the concentration of the extracts was directly proportional to the growth inhibition of bacteria. Maximum zone of inhibition [(31.5 ± 6.5) mm] was exerted by the petroleum ether extract of *G. verrucosa* against *Micrococcus* species.

3.2. Antifungal activity

The extracts of *S. wightii* and *G. verrucosa* were tested against *Aspergillus niger* and *C. albicans* and the results are depicted in Figure 1. All the extracts inhibited the growth of both fungi and it was concentration dependent *i.e.*, as the concentration increased, the inhibitory effect also increased. Maximum inhibition zone was exercised by the acetone extract of *S. wightii* [(13.5 ± 1.5) mm] and *G. verrucosa* against *C. albicans* [(21.5 ± 1.0) mm] followed by chloroform extract of *S. wightii* against *Aspergillus* sp. [(20.5 ± 0.5) mm].

3.3. Anti-hemolytic activity

Anti-hemolytic activity of the solvent extracts of *S. wightii* as well as *G. verrucosa* was studied and the results are presented in Table 4. The percentage of inhibition was in the following order: acetone > control > chloroform > methanol > petroleum ether. Acetone extract (96.47 ± 0.31) of *G. verrucosa* showed high level of inhibitory action on H_2O_2 mediated hemolysis. Lysis was found to be reasonably increased with an increase in concentration. Although results were statistically insignificant when compared to control, significant difference was observed ($P < 0.05$) among the treatment.

3.4. Anti-cancer activity

The outcome of the MTT assay of the methanol extract of *S. wightii* and *G. verrucosa* against human AGS cell lines is depicted in Figure 2. Viable cells in the assay revealed that the extracts did not

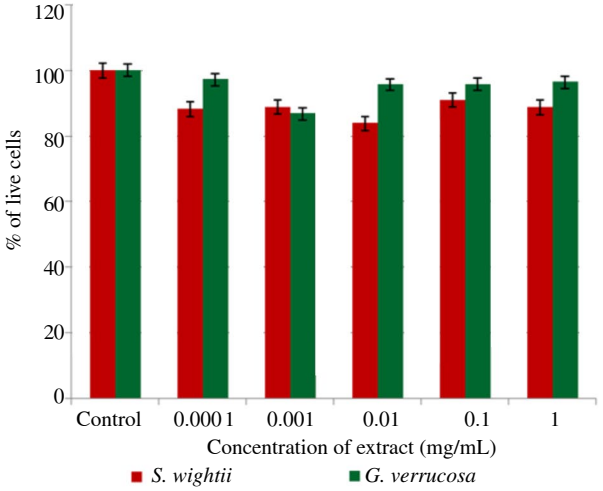

Figure 2. Anti-cancer activity of methanolic extract of marine macro-algae.

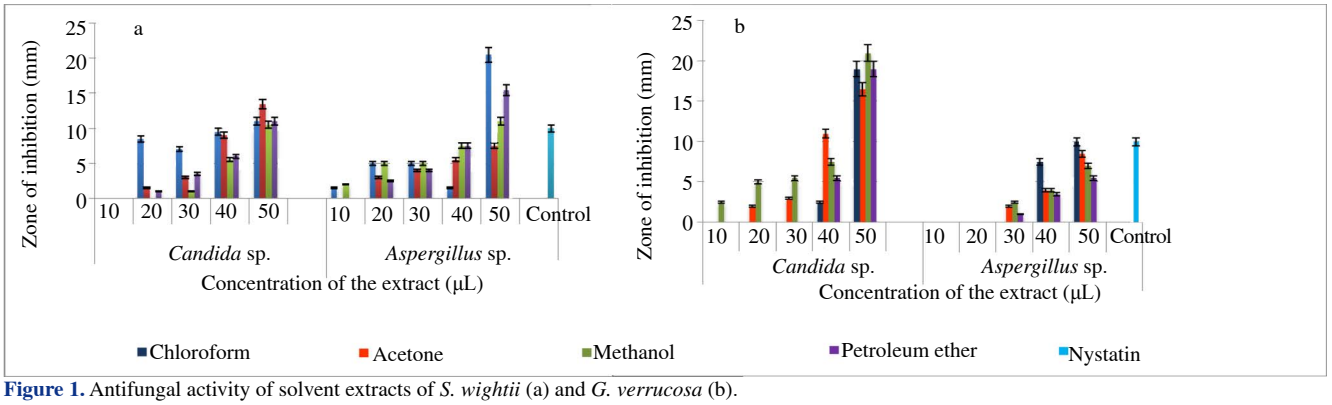

Figure 1. Antifungal activity of solvent extracts of *S. wightii* (a) and *G. verrucosa* (b).

have a recordable effect even at higher concentrations. Control wells showed 100% viable cells. Results revealed that the methanol extract of *S. wightii* and *G. verrucosa* had no anti-cancer activity against AGS cell line.

Table 2

Zone of inhibition (mm) exerted by the solvent extracts of *S. wightii* against the bacterial pathogens isolated from infected ornamental fish.

Name of the pathogen	Conc. of the extract (µL)	Methanol extract	Chloroform extract	Acetone extract	Petroleum ether
Streptococcus sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	1.0 ± 0.5	NZ	3.5 ± 0.5	NZ
	40	6.0 ± 1.0	5.5 ± 0.5	6.5 ± 1.5	2.5 ± 0.5
	50	6.5 ± 0.5	7.0 ± 1.0	10.0 ± 1.0	13.5 ± 1.5
	Control	10.0 ± 2.0	12.5 ± 2.5	14.0 ± 1.0	15.0 ± 1.0
				3.5 ± 0.5	
Staphylococcus sp.	10	NZ	NZ	NZ	NZ
	20	4.0 ± 1.0	2.0 ± 1.1	4.5 ± 1.5	NZ
	30	4.5 ± 0.5	8.0 ± 4.6	4.0 ± 1.0	3.0 ± 1.0
	40	8.0 ± 1.0	6.5 ± 1.5	6.0 ± 3.4	9.0 ± 1.0
	50	8.5 ± 0.5	10.5 ± 1.5	13.5 ± 1.5	10.0 ± 5.7
	Control	11.5 ± 1.5	15.0 ± 2.0	18.5 ± 1.5	14.0 ± 2.0
Bacillus sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	NZ	3.5 ± 1.5	NZ	NZ
	40	NZ	6.0 ± 1.0	NZ	5.0 ± 2.0
	50	5.0 ± 3.0	11.0 ± 1.0	NZ	9.5 ± 0.5
	Control	11.0 ± 1.0	17.0 ± 1.0	1.2 ± 0.5	9.5 ± 1.5
Shigella sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	NZ	NZ	3.5 ± 0.5	4.0 ± 1.0
	40	NZ	NZ	7.0 ± 2.0	5.5 ± 1.5
	50	2.5 ± 0.5	4.0 ± 2.0	10.0 ± 1.0	9.5 ± 0.5
	Control	11.0 ± 1.0	8.5 ± 1.5	14.0 ± 2.0	13.5 ± 1.5
E. coli	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	6.0 ± 1.0	6.5 ± 2.5	7.0 ± 1.0	NZ
	40	6.5 ± 1.5	7.5 ± 0.5	8.5 ± 1.5	NZ
	50	10.5 ± 1.5	14.5 ± 0.5	7.5 ± 0.5	2.0 ± 1.0
	Control	18.5 ± 1.5	14.5 ± 1.5	11.0 ± 2.0	16.5 ± 1.5
Micrococcus sp.	10	NZ	NZ	NZ	3.0 ± 2.0
	20	7.0 ± 1.0	6.5 ± 0.5	6.5 ± 2.5	6.0 ± 1.0
	30	8.0 ± 1.0	6.0 ± 4.0	7.0 ± 2.0	8.5 ± 1.5
	40	10.5 ± 0.5	9.0 ± 1.0	9.5 ± 0.5	10.0 ± 2.0
	50	15.5 ± 0.5	12.5 ± 0.5	9.5 ± 1.5	14.5 ± 1.5
	Control	18.0 ± 1.0	17.5 ± 1.5	10.0 ± 3.0	18.5 ± 1.5
Aeromonas sp.	10	NZ	NZ	NZ	3.0 ± 1.0
	20	NZ	4.0 ± 1.0	6.5 ± 1.5	8.5 ± 0.5
	30	NZ	8.0 ± 2.0	8.0 ± 2.0	11.5 ± 1.5
	40	8.5 ± 0.5	11.0 ± 1.0	7.5 ± 0.5	9.5 ± 2.5
	50	9.5 ± 0.5	13.5 ± 1.5	11.5 ± 1.5	12.5 ± 1.5
	Control	10.5 ± 1.5	16.0 ± 2.0	23.5 ± 1.5	22.5 ± 1.5
Proteus sp.	10	NZ	NZ	NZ	3.0 ± 1.0
	20	NZ	4.0 ± 1.0	6.5 ± 1.5	8.5 ± 0.5
	30	NZ	8.0 ± 2.0	8.0 ± 2.0	11.5 ± 1.5
	40	8.5 ± 0.5	11.0 ± 1.0	7.5 ± 0.5	9.5 ± 2.5
	50	9.5 ± 0.5	13.5 ± 1.5	11.5 ± 1.5	12.5 ± 1.5
	Control	10.5 ± 1.5	16.0 ± 2.0	23.5 ± 1.5	22.5 ± 1.5
Vibrio sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	NZ	6.0 ± 2.0	2.5 ± 0.5	NZ
	40	NZ	7.0 ± 1.0	8.5 ± 0.5	5.5 ± 1.5
	50	9.0 ± 2.0	6.5 ± 1.5	9.5 ± 1.5	9.0 ± 1.0
	Control	12.5 ± 3.5	13.5 ± 2.5	14.0 ± 1.0	24.0 ± 2.0

NZ: No zone of inhibition; Conc.: Concentration.

3.5. Phytochemical analysis

The preliminary phytochemical analysis revealed the presence of alkaloids, amino acids, carbohydrates, cardiac glycosides, essential oil, flavonoids, pholabatanins, reducing sugar, tannins, terpenoids, saponins, phenolic compounds and xanthoprotein in the extract of *S. wightii*. Alkaloids, amino acids, essential oil, flavonoids, reducing sugar, saponins, phenolic compounds and terpenoids were detected in the extract of *G. verrucosa* and the results are presented in Table 5. High amount of alkaloids (56 mg/g), saponins (44.5 mg/g), flavonoids (500 mg/g) and phenols (220 mg/g) were detected in *G. verrucosa* (Figure 3).

Table 3

Inhibition zone (mm) produced by the extract of *G. verrucosa* against the pathogens isolated from fish.

Name of the pathogen	Conc. of the extract (µL)	Methanol extract	Chloroform extract	Acetone extract	Petroleum ether
Streptococcus sp.	10	NZ	NZ	2.5 ± 1.5	3.5 ± 1.5
	20	NZ	NZ	5.0 ± 2.0	4.0 ± 1.0
	30	2.5 ± 1.5	3.5 ± 1.5	6.0 ± 1.0	5.5 ± 2.5
	40	6.0 ± 1.0	7.0 ± 1.0	7.0 ± 2.0	8.0 ± 1.0
	50	9.0 ± 1.0	16.5 ± 1.5	10.5 ± 1.5	12.0 ± 1.0
	Control	16.5 ± 1.5	18.5 ± 1.5	13.5 ± 1.5	12.0 ± 2.0
Staphylococcus sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ
	40	5.5 ± 0.5	3.5 ± 1.5	NZ	NZ
	50	15.5 ± 1.5	13.5 ± 1.5	7.5 ± 1.5	5.5 ± 2.5
	Control	19.5 ± 2.5	21.5 ± 1.5	15.0 ± 3.0	17.0 ± 3.0
Bacillus sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	3.5 ± 1.5	NZ	1.5 ± 0.5	4.5 ± 0.5
	40	4.5 ± 1.5	2.5 ± 1.5	9.5 ± 1.5	7.0 ± 3.0
	50	5.5 ± 2.5	10.0 ± 2.0	12.0 ± 2.0	9.0 ± 1.0
	Control	7.5 ± 2.5	10.5 ± 1.5	14.5 ± 5.5	10.5 ± 0.5
Shigella sp.	10	5.5 ± 2.5	NZ	NZ	NZ
	20	5.5 ± 2.5	NZ	NZ	NZ
	30	7.0 ± 2.0	2.0 ± 1.0	NZ	NZ
	40	9.5 ± 2.5	8.5 ± 3.5	5.5 ± 2.5	3.5 ± 1.5
	50	14.5 ± 3.5	9.5 ± 0.5	9.0 ± 1.0	7.5 ± 2.5
	Control	24.0 ± 1.0	10.0 ± 2.0	11.5 ± 1.5	8.5 ± 1.5
E. coli	10	NZ	NZ	NZ	NZ
	20	7.0 ± 2.0	3.0 ± 2.0	NZ	5.0 ± 1.0
	30	13.5 ± 1.5	5.0 ± 3.0	NZ	4.5 ± 0.5
	40	20.5 ± 0.5	6.0 ± 2.0	NZ	6.0 ± 2.0
	50	25.0 ± 1.5	10.0 ± 3.0	2.0 ± 1.0	11.0 ± 1.0
	Control	25.5 ± 1.5	18.5 ± 0.5	8.5 ± 0.5	17.5 ± 2.5
Micrococcus sp.	10	9.5 ± 2.5	10.5 ± 1.5	7.0 ± 2.0	9.0 ± 1.0
	20	11.0 ± 1.0	11.5 ± 0.5	10.0 ± 2.0	11.5 ± 1.5
	30	12.5 ± 1.5	10.5 ± 1.5	14.5 ± 0.5	10.5 ± 1.5
	40	13.5 ± 2.5	13.5 ± 1.5	14.0 ± 1.0	16.5 ± 1.5
	50	28.5 ± 1.5	19.0 ± 1.0	26.0 ± 4.0	31.5 ± 6.5
	Control	29.5 ± 0.5	25.5 ± 4.5	30.0 ± 2.0	36.5 ± 7.5
Aeromonas sp.	10	6.0 ± 2.0	NZ	6.0 ± 2.0	2.5 ± 1.5
	20	7.0 ± 1.0	2.5 ± 1.5	6.5 ± 1.5	10.0 ± 2.0
	30	9.5 ± 0.5	6.5 ± 1.5	9.5 ± 2.5	10.0 ± 2.0
	40	10.0 ± 2.0	9.0 ± 1.0	11.0 ± 1.0	12.0 ± 2.0
	50	12.0 ± 2.0	11.5 ± 1.5	16.0 ± 2.0	12.5 ± 2.5
	Control	22.0 ± 3.0	16.5 ± 1.5	23.5 ± 1.5	16.5 ± 2.5
Proteus sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ
	40	NZ	NZ	5.5 ± 2.5	NZ
	50	NZ	4.0 ± 1.0	6.5 ± 1.5	3.0 ± 1.0
	Control	2.5 ± 0.5	5.0 ± 1.5	13.0 ± 2.0	9.5 ± 0.5
Vibrio sp.	10	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ
	30	5.5 ± 1.5	1.5 ± 0.5	NZ	NZ
	40	12.5 ± 3.5	6.0 ± 1.0	1.5 ± 0.5	2.5 ± 1.5
	50	20.5 ± 1.5	15.5 ± 1.5	13.5 ± 1.5	10.5 ± 1.5
	Control	21.5 ± 1.5	22.0 ± 2.0	20.5 ± 0.5	19.5 ± 0.5

NZ: No zone of inhibition; Conc.: Concentration.

Table 4

Anti-hemolytic activity of the extracts of *S. wightii* and *G. verrucosa.*

Name of the seaweed	Concentration of the extract (mL)	Control (quercetin)	Chloroform extract	Acetone extract	Methanol extract	Petroleum ether extract
S. wightii	0.2	90.33 ± 4.72	50.00 ± 2.54	33.33 ± 8.38	43.93 ± 3.59	50.16 ± 3.11
	0.4	92.56 ± 3.00	84.77 ± 0.47	82.60 ± 0.96	85.71 ± 0.82	85.10 ± 0.81
	0.6	91.41 ± 6.31	85.77 ± 0.34	85.27 ± 0.47	87.33 ± 0.89	86.38 ± 0.47
	0.8	92.12 ± 2.35	92.99 ± 0.56	90.83 ± 0.92	91.45 ± 0.35	90.58 ± 0.69
	1.0	96.39 ± 2.02	96.07 ± 1.01	93.03 ± 0.45	93.66 ± 0.47	93.33 ± 0.56
G. verrucosa	0.2	90.33 ± 4.72	37.66 ± 4.49	55.83 ± 1.69	49.83 ± 4.36	42.16 ± 3.06
	0.4	92.56 ± 3.00	83.88 ± 2.64	88.11 ± 1.03	85.83 ± 0.40	85.33 ± 0.75
	0.6	91.41 ± 6.31	85.16 ± 1.08	90.21 ± 0.82	88.60 ± 1.03	87.33 ± 1.08
	0.8	92.12 ± 2.35	89.66 ± 0.69	93.49 ± 0.37	93.20 ± 0.72	92.12 ± 0.17
	1.0	96.39 ± 2.02	92.36 ± 0.36	96.47 ± 0.31	96.03 ± 0.54	94.70 ± 0.49

Significant difference was observed ($P < 0.05$). Results were expressed as mean ± SD.

Table 5

Qualitative phytochemical analysis of *S. wightii* and *G. verrucosa.*

Phytochemicals	Marine macroalgae	
	S. wightii	*G. verrucosa*
Alkaloids	+	+
Amino acid	+	+
Aromatic acid	-	-
Carbohydrates	+	-
Cardiac glycosides	+	-
Essential oil	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Pholabatanins	+	-
Reducing sugar	+	+
Saponins	+	+
Steroids	-	-
Tannis	+	-
Terpenoids	+	+
Xantho protein	+	-

+: Present; -: Absent.

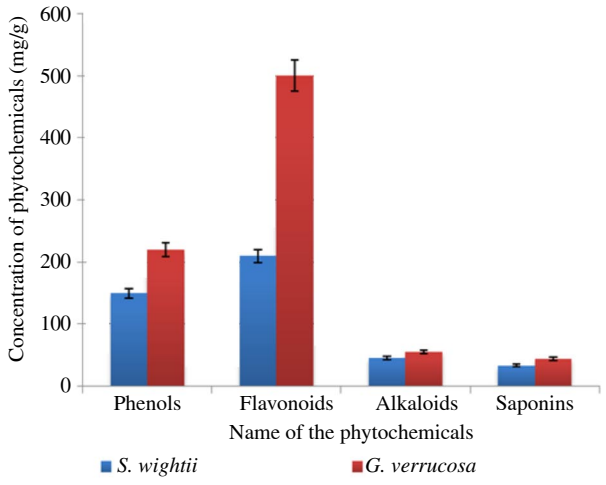

Figure 3. Quantitative phytochemical analysis in the extract of macro-algae.

4. Discussion

Secondary metabolites derived from marine macro-algae have been associated with a broad range of biological activities such as antibacterial, antiviral, antifungal, antifouling and anti-inflammatory effects as well as cytotoxic and antimitotic activities[24]. In the present study, the extract of *G. verrucosa* exhibited higher anti-microbial activity than *S. wightii*, and was much prominent in the petroleum ether extract against *Micrococcus* sp. [(31.5 ± 6.5) mm]. The extracts of *G. verrucosa* were effective against Gram negative (*Pseudomonas aeruginosa* and *E. coli*) than Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*)[25]. The present results are also in agreement with the observations of Prasad *et al.*[26]. The results of the present study revealed that, bacteria showed varied sensitivity to different solvent extracts. The petroleum ether extract was the most active and exerted broader zone of inhibition against most of the tested pathogens. The secondary metabolites in the seaweeds along with halogenating compounds are responsible for antimicrobial activities[4]. The anti-microbial action of seaweeds would also be attributed to the season, time of collection, growth condition of seaweed and solvents used for extraction[27] genetic or biochemical composition of pathogenic bacteria[28]. Furthermore, the solvent polarity, concentration and physiochemical properties also modulate the inhibitory action[29] which has been observed in the present study also. As the concentration of the extract increased, the inhibition percentage also increased[30]. Besides target sites modification, enzymatic inactivation and insufficient concentration of the bioactive compounds also lead to pathogen resistance[31], hence certain extracts did not exhibit any significant activity.

Binding of flavonoids to the red blood cell membranes significantly inhibits lipid peroxidation, protects them from free radical mediated oxidative hemolysis and enhances their integrity against lysis[20]. In the present study, inhibition of H_2O_2 mediated hemolysis by the extracts of both the seaweeds indicates the presence of radical scavenging phytochemicals especially, flavonols, flavonoids and tannins. Anticancer activity is one of the most important activities perceived in marine algae. Methanol extracts of both *S. wightii* and *G. verrucosa* showed no significant inhibition against human AGS cell line. This could possibly be due to the insolubility of certain bioactive compounds in the solvent[32]. *G. verrucosa* contain prostaglandin A_2 which seems to be responsible for gastrointestinal disorder, known as "ogonori" poisoning in Japan[4]. The antitumour activity of ethanol extract of *Sargassum* sp. against cell lines such as MCF-7 (breast cancer) and Hep G2 (liver cancer) through cell shrinkage, cell membrane blebbing and formation of apoptotic bodies[33]. Hence, these seaweeds should be tested for cytotoxicity against other cancer cell lines.

A number of studies have been reported on the phytochemistry of seaweeds across the world[31]. The bioactive metabolite from *Amphiro anceps* exhibited antagonistic activities against two chicken meat associated pathogens[34]. Normally seaweeds possess alkaloids, glycosides, saponins, steroids, phenols and tannins[35] with the relative active secondary metabolites which have been extensively used in pharmaceutical industry[36] which has been observed in the present study also. Comparatively, *G. verrucosa* had maximum amount of flavonoids, phenols, alkaloids and saponins. The phytochemical investigation revealed the presence of secondary metabolites with varied degree, which was directly coincided with the previous

evidence on the secondary metabolites and various *in vitro* bioassays were carried out to establish their bioactivities.

It has been concluded that the solvent extracts of the tested marine macro-algae *S. wightii* and *G. verrucosa* possessed significant bioactivity and were rich in bioactive compounds that could be explored further and incorporated into human and animal health applications. The day of designing and developing a new drug from both the seaweeds is not so far.

Conflict of interest statement

We declare that we have no conflict of interest.

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