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RESEARCH ARTICLE

Hemagglutinin activity of a few seaweeds of mandapam coast, tamil nadu

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ABSTRACT

The marine algae, namely, *Amphiroa fragilissima, Caulerpa scalpelliformis, Gracilaria edulis, Halimeda gracilis, Sargassum wightii,* and *Ulva reticulata* found in the Mandapam coastal region have been screened for agglutinin activity against human (A, B, AB, and O) erythrocytes and sheep, rabbit, and fish erythrocytes. Carbohydrate (0.98 mg/g) and nitrogen (0.14 mg/g) concentration was higher in *Halimeda gracilis*. Lipid (0.25 mg/g), protein (0.93 mg/g), and mannitol (0.069 mg/g) content was higher in *Sargassum wightii*. All 6 seaweed species showed positive agglutinin reactions against rabbit erythrocytes. *Halimeda gracilis* exhibited high hemagglutinin activity against fish and sheep erythrocytes. *Caulerpa scalpelliformis* and *Ulva reticulata* exhibited positive reaction against human A, B, AB, and O erythrocytes. The agglutinating activity of both the species was inhibited by simple sugars. Extracts of *Caulerpa scalpelliformis* showed cytotoxic activity against Hep 2 cell lines in vitro with a 50% inhibitory concentration (IC_{50}) of 250 µg/mL.

INTRODUCTION

Hemagglutination is a specific form of agglutination that involves red blood cells (RBCs). The proteinaceous secondary metabolites, hemagglutinins, cause the clumping or agglutination of RBCs *in vitro*. Lectins, agglutinins, or hemagglutinins are proteins that possess at least 1 noncatalytic domain that binds reversibly to a specific form of mono or oligosaccharides (Peumans and Van Damme 2000). Agglutinins are widely distributed in nature. These agglutinins bind to specific carbohydrates and produce unique effects such as aggregation of cells or the precipitation of glycoconjugates. Marine algal lectins are of lower molecular weight and used for drug targeting. More than 200 algal species have so far been reported to contain hemagglutinins. Marine algal agglutinins contain large amounts of acidic amino acids (aspartic acid and glutamic acid), hydroxyl amino acids (serine and threonine), and low amounts of basic amino acids (lysine, histidine, and arginine) (Hori *et al*. 1988). Hemagglutinating activity often varies between and within algal species. Recent findings evidenced that seaweeds contain antiviral, antibacterial, antifungal, and antitumor potential. Strong anti-HIV lectins have recently been isolated and characterized from a red alga (Mori *et al*. 2005). The increasing number of biological applications of lectins has stimulated research work on a large number of seaweed species, in which proteins with hemagglutinating activity have been detected. Some marine red algal lectins have tetrameric structures, e.g., *Gracilaria verrucosa* (Shiomi *et al*. 1981); trimeric, e.g., *Ptilota filicina* J. Agardh (Sampaio *et al.* 1998); and dimeric, e.g., *Palmaria palmata* (Kamiya *et al*. 1982) and *Vidalia obtusiloba* (Mertens *ex* C. Agardh) J. Agardh (Melo *et al*. 2004). Zandi *et al*. (2010) showed that 9.336 and 9.726 µg/µL of algal extract were the most effective concentrations against Jurkat and molt-4 cells, respectively. The water crude extract of the red alga *G. corticata* had significant anticancer activity, and it might be a good candidate for further investigations in order to develop a natural compound as an anticancer agent. The best antiproliferative activity was found with fucans F1.3v and F0.7v. However, F1.3v activity was much higher than that of F0.7v, inhibiting almost 100% of HeLa cell proliferation (Magalhaes *et al*. 2011). All algal glycolipids exhibited remarkable anticancer activities against both breast (MCF7) and liver human (HepG2) cancer cells, with IC_{50} values ranging from 0.47 to 2.89 µg/mL (El Baroty *et al*. 2011). F-fucoidan and U-fucoidan act on tumor cells and cause decreased vascularization or cell death. Fucoidan prevents tumors cells from being able to anchor themselves against the body (Decker 2011). This work presents the result of hemagglutination and anticancer activity of a few seaweeds of Mandapam district, Tamil Nadu, against human erythrocytes and rabbit, sheep, and fish erythrocytes.

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MATERIALS AND METHODS

Algal material

The following seaweeds *Amphiroa fragilissima* (Linnaeus) J. V. Lamouroux, *Caulerpa scalpelliformis* (R. Brown ex Turner) V.Agardh, *Gracilaria edulis* (S. G. Gmelin) P. C.

Silva, *Halimeda gracilis* Harvey ex J. Agardh, *Sargassum wightii* Greville, and *Ulva reticulata* Forsskal were collected from Mandapam coast, Tamil Nadu. After collection, the material was cleaned of any calcareous material, washed with tap water and then with distilled water to eliminate salt and salt particles, and finally stored at -20°C until use.

Determination of ash, lipid, carbohydrate, protein, nitrogen, mannitol, and alginate

The lipid (Bligh and Dyer 1959), carbohydrate (Hedge and Hofreiter 1962), protein (Lowry *et al*. 1951), nitrogen (Umbriet *et al*. 1974), and mannitol (Black *et al*. 1951a) was determined as per the standard protocol. Extraction of sodium alginate, calcium alginate, and alginic acid and determination of Rf value of mannuronic acid and glucuronic acid alginate using thin-layer chromatography (TLC) technique was determined according to (Haug *et al*. 1974).

Preparation of seaweed extract (Baqir *et al*., 1992)

Ten grams of each species was homogenized with 10 mL of phosphate-buffered saline (PBS, pH 7.2). It was centrifuged at 1000 rpm for 20 min. The supernatant was removed, filtered through a 0.45-µm Millipore filter, and kept at -20°C. The resultant solution was made up to 20 mL of volume and used as a test solution. The stock concentration of the seaweed extract was 1 g/mL.

Preparation of hemagglutinins (Baqir *et al*., 1992)

Dilutions were prepared in a 96-well microtiter plate: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 by adding 50 µL of phosphate buffer (pH 7.2) in each well. Then, 25 µL of algal extract was added into the 1:2 dilution and thereafter transferred 25 µL into the 1:4 dilution and 25 µL from the 1:4 to 1:8 and 25 µL from the 1:8 to 1:16 to obtain serial dilutions. For each algal extract, 10 dilutions for blood groups A, B, AB, and O and blood samples of sheep, rabbit, and fish were prepared.

Preparation of 2% suspension of erythrocytes (Baqir *et al*., 1992)

Human blood groups A, B, AB, and O were obtained from authorized blood banks in Madurai. Sheep, rabbit, and fish blood were collected from the market. Blood samples were maintained at 4°C in Alsever's solution. A 2% erythrocyte suspension in PBS (pH 7.2) was prepared from each type of blood group by centrifugation at 1000 *g* for 20 min and supernatant was removed. The blood was again centrifuged with PBS (pH 7.2). The supernatant was removed and the erythrocytes were obtained. About 2 mL of erythrocyte was added to 98 ml of phosphate buffer to obtain a 2% suspension of erythrocytes.

Assay of hemagglutinating activity (Baqir *et al*., 1992)

Hemagglutinating activity was investigated serially in different dilutions of each algal extract against all the 7 blood groups. Twenty-five microliters of 2% erythrocyte suspension, of the respective blood sample group, was added to all dilutions; shaken for few seconds; and then allowed to stand for 3 h. Control was also run simultaneously. An extract in PBS was made as control. Agglutination was observed after 3 h.

Purification of lectin

Twenty-five grams of the seaweeds were ground and was defatted by soaking in petroleum ether for 24 h at room temperature. The defatted seaweed was stirred well on a magnetic stirrer with 250 mL of PBS overnight at 4°C. The extract was filtered and spun for 30 min at 10,000 *g*. The supernatant was collected and ammonium sulphate was added to the sample with constant stirring to a concentration of 40% saturation and kept overnight. The precipitate was removed by centrifugation at 20,000 *g* for 30 min. The clear supernatant was then adjusted to 60% saturation by further addition of ammonium sulphate and kept overnight. The precipitate thus formed was collected by centrifugation and dissolved in 20 mL of PBS. Any precipitate formed was removed by centrifugation, and the clear supernatant was collected. Thus, the lectin was purified according to Laija *et al*. (2010).

Hemagglutination inhibition assay (Sampaio *et al.*, 1998)

Inhibition studies were performed using 2 seaweed species lectin extracts: *Caulerpa scalpelliformis and Ulva reticulata*. The carbohydrate-binding specificity of the purified lectin was assessed by the ability of a series of sugars: arabinose, fructose, glucose, galactose, and lactose. The inhibition assay was carried out by 2-fold serial dilution of sugar (0.1 M) solutions in 0.15 M NaCl. Twenty-five microliters of sugar and equal volume of lectin solution was added to each well and the mixture was allowed to interact for 1 h at room temperature. A 2% suspension of human erythrocytes of 4 different blood groups was added to each well to a final volume of 100 µL, and this mixture was incubated at 37°C for 30 minutes followed by another 30 minutes interval at room temperature. The lowest concentration of a specific sugar or glycoprotein that inhibited hemagglutination (minimum inhibitory concentration [MIC]) was recorded.

Preparation of seaweed extracts for anticancer activity

Seaweeds were rinsed with distilled water. One gram of dried *Caulerpa scalpelliformis* was homogenized in 100 mL of cold double-distilled water. Then, it was filtered in Whatman paper No. 1. The clarified crude seaweed extract was sterilized by Millipore filter with 0.45-µm pore size and was stored at -20°C, until the date of use.

Stock concentration of *Caulerpa scalpelliformis*

Ten milligrams of seaweed extract was dissolved in 10 mL of serum-free Eagle's minimal essential medium (MEM) giving a concentration of 1 mg/mL. The stock was prepared fresh and filtered through 0.45-µm filter before each assay. Working concentrations of extract ranged from 1 mg/mL to 7.8125 mg/mL.

Preparation of working stock of 1 mg /mL

To 4.5 mL Minimum Essential Medium, 0.5 mL of stock was added to give a working concentration of 1 mg/mL. Extract

concentration was prepared from the working stock in MEM without fetal calf serum (FCS). Required volume of test sample for each concentration was prepared. A 48-h monolayer culture of Hep 2 cells at a concentration of 1 lakh/well was seeded in 24-well titer plates. The plate was microscopically examined for confluent monolayer, turbidity, and toxicity if the cells became confluent. The growth medium (MEM) was removed using a micropipette. The monolayer of cells was washed twice with MEM without FCS to remove the dead cells and excess FCS. To the washed cell sheet, 1 mL of medium (without FCS) containing defined concentration of the seaweed extract was added to the respective wells. Each dilution of the extract ranged from 1:1 to 1:64, and they were added to the respective wells of the 24-well microtiter plates. To the cell control wells, 1 mL MEM (without FCS) was added. The plates were incubated at 37°C in 5% CO_2 environment and observed for cytotoxicity using an inverted microscope.

MTT assay

MTT assay is called as 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide. The medium was removed from the wells carefully for the MTT assay. Each well was washed with MEM (without FCS) 2–3 times, and 200 µL of 5 mg/mL MTT concentration was added and incubated for 6–7 h in 5% CO_2 incubator for observation of cytotoxicity. After incubation, 1 mL of dimethylsulfoxide (DMSO) was added in each well and mixed by a pipette and left for 45 s. If any viable cells were present, addition of formazan crystals and solubilizing reagent (DMSO) showed a purple color formation. The suspension is transferred into the cuvette of a spectrophotometer and optical density (OD) values were read at 595 nm with DMSO as a blank.

Cell viability (%) = Mean OD/Control OD × 100

RESULTS AND DISCUSSION

Lectins sometimes referred to as hemagglutinins or agglutinins are glycoproteins with an ability to agglutinate RBCs. Lectins with hemagglutinating properties occur in a variety of red, green, and brown algae. The results of the research study on hemagglutinin activity of selected seaweeds of Mandapam coast are discussed. The ash content was highest in *Caulerpa scalpelliformis* whereas as there was no significant difference in the ash content of other 5 seaweed species. El-Sarraf and Shaarawy (1994) observed that the ash content was 53.0 mg g^{-1} for *Ulva fasciata* and 350.0 mg g^{-1} for *Corallina mediterranea*. The low ash content showed that there was little non-combustible residue such as minerals in the seaweed samples. The low percentage of ash also indicated that the samples were not contaminated by any calcareous organisms. Ratana-arpon and Chirapart (2006) showed that the ash content in *Caulerpa lentillifera* (24.21%) was higher than that found in *Ulva reticulata* (17.58%). The carbohydrate content was highest in *Halimeda gracilis* (0.98 mg/g of seaweed), whereas in the other seaweeds, there was only a marginal variation in the carbohydrate content. Awad *et al*. (2009) showed that the carbohydrate content of the brown algae *Padina pavonica* and *Hydroclathrus clathratus* were 19.20% and 25.60%, respectively. High carbohydrate (24–44%) content was reported in seaweeds in Indian shores. Algae are rich in polysaccharides and their cell walls are composed of stable polysaccharides of a very high molecular weight (Percival and McDowell, 1967). Algal carbohydrates are considered as important groups of cell constituents for storage material and energy, and they are produced as a function of environmental conditions.

The amount of lipid varied from 19 to 26 mg/g of seaweed. The lipid content was highest in *Ulva reticulata.* The total lipid content in *Caulerpa lentillifera* and *Ulva reticulata* was 0.75–0.86%, which were in accordance with 0.7–1.05% in red and brown algae (Sanchez-Machado *et al*. 2004). The lipid content of *P. gymnospora* was 1.4% ± 0.30%. Rives *et al*. (2010) showed that the lipid content in marine algae from the North West Mexican Pacific coast ranged from 0.4% to 1.47%. Total nitrogen content was higher in *Halimeda gracilis* and *Caulerpa scalpelliformis* and was lowest in *Amphiroa fragilissima.* Nitrogen content was high (0.96%) in *Pterocladiella capillacea*; intermediate (0.42–0.58%) in *Ulva australis*, *Lobophora variegata*, *Amphiroa curras*, and *Delia pulehu*; and low (0.16–0.26%) in the remaining species. Nitrogen content was high in New Zealand seaweeds: 0.79–1.03% for *Enteromorpha intestinalis* and *Pterocladia lucida* and ranged from 0.20–0.63% in other species (Taylor and Steinberg 2005). *Caulerpa scalpelliformis* and *Ulva reticulata* have a greater percentage of protein content. The protein content was the lowest in *Halimeda gracilis*. The difference in the protein content can be attributed to the age of the algae in different samples since nitrogen levels usually decline with increasing maturity (El-Sarraf and El-Shaarawy 1994). High protein values were noted in *Corallina mediterranea* and *Ulva fasciata* ranging from 68.0 to 34.39 mg g^{-1}. Mannitol content was greater in *Ulva reticulata* and *Caulerpa scalpelliformis* and minimum in *Gracilaria edulis.* Mannitol content ranged from 1.04% to 2.52% dry weight in *Padina pavonica* and *Hydroclathrus clathratus* (Awad *et al*. 2009).

Hemagglutinin activity of selected seaweeds against fish, sheep, rabbit, and human erythrocytes

Hemagglutinating activity was investigated serially in different dilutions of each seaweed extract against all the 4 human blood groups and using fish, rabbit, and sheep erythrocytes. Controls were also run along with 2% suspension of respective erythrocytes in buffer alone. Smooth button formation due to settling of erythrocytes at the bottom showed negative activity whereas rough granular deposition in the well due to agglutination of erythrocytes, indicated positive reactions. Low, medium, and high agglutination were determined on the basis of the amount of granular deposition at the bottom of the well. Seaweeds *Gracilaria edulis* and *Halimeda gracilis* depicted positive reactions against fish erythrocytes, whereas *Amphiroa fragilissima*, *Caulerpa scalpelliformis*, *Sargassum wightii,* and *Ulva reticulata* showed negative reactions (Table 2). But for rabbit erythrocytes all the 6 different seaweeds showed positive reactions (Table 3). High hemagglutinating activity was detected only for *Halimeda gracilis* for sheep erythrocytes but *Gracilaria edulis* and *Amphiroa fragilissima* exhibited negative reaction (Table 4). For human O erythrocytes, hemagglutinating activity was high in *Caulerpa scalpelliformis*, *Ulva reticulata*, and *Amphiroa fragilissima,*

and for *Halimeda gracilis* and *Sargassum wightii* medium hemagglutinating activity was observed. For human erythrocytes from at least one of the sources, but rabbit and human erythrocytes appeared to be the most suitable for the erythrocytes AB there was high hemagglutinating activity in the seaweeds *Caulerpa scalpelliformis*, *Ulva reticulata*, and *Gracilaria edulis*. Other 2 seaweeds *Halimeda gracilis* and *Sargassum wightii* showed medium hemagglutinating activity. Only *Amphiroa fragilissima* showed a negative reaction. Agglutinating activity often varies between and within the seaweed species assayed. Most of the extracts agglutinated detection of agglutination reaction but less active against fish and sheep erythrocytes. Only higher dilutions exhibited agglutination activity. Different specificities for antigens or carbohydrates receptors on the erythrocytes may account for the variation in the agglutinating activity (Chun *et al*. 2006). Highest agglutinin activity was found with rabbit erythrocytes, which correlate with the report of (Fabregas *et al*. 1986)

Table 1. Total ash, carbohydrate, lipid, nitrogen, protein and mannitol content present in seaweeds

	Ash	Carbohydrate	Lipid nitrogen	Nitrogen	Protein	Mannitol
Amphiroa fragilissima	0.075	0.90	0.02	0.05	0.90	0.06
Gracillaria edulis	0.07	0.89	0.015	0.10	0.82	0.04
Sargassum wightii	0.075	0.87	0.025	0.07	0.93	0.06
Halimeda gracilis	0.07	0.98	0.018	0.14	0.80	0.05
Ulva reticulata	0.075	0.90	0.027	0.09	1.10	0.13
Caulerpa scalpelliformis	0.08	0.92	0.024	0.13	1.23	0.11

Table 2. Haemagglutinic activity of seaweeds against fish erythrocytes

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	-	-	-	-	-	-	-	-	-	-
Gracilaria edulis	-	-	-	-	+++	+++	+++	+++	+++	+++
Sargassum wightii	-	-	-	-	-	-	-	-	-	-
Halimeda gracilis	-	-	-	++	++	++	++	++	+++	+++
Ulva reticulata	-	-	-	-	-	-	-	-	-	-
Caulerpa scalpelliformis	-	-	-	-	-	-	-	-	-	-

Table 3. Haemagglutinic activity of seaweeds against rabbit erythrocytes

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Gracilaria edulis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sargassum wightii	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Halimeda gracilis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Ulva reticulata	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Caulerpa scalpelliformis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 4. Haemagglutinic activity of seaweeds against sheep erythrocytes

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	-	-	-	-	-	-	-	-	-	-
Gracilaria edulis	-	-	-	-	-	-	-	-	-	-
Sargassum wightii	+	+	+	-	-	-	-	-	-	-
Halimeda gracilis	+++	-	-	++	++	++	+++	+++	+++	+++
Ulva reticulata	+	+	+	+	+	+	+	+	+	+
Caulerpa scalpelliformis	+	+	+	+	+	+	+	+	+	+

Table 5. Haemagglutinic activity of seaweeds against human erythrocytes (A blood group)

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	+	+	+	+	+	+	+	+	+	+
Gracilaria edulis	-	-	-	-	-	-	-	-	-	-
Sargassum wightii	-	-	-	+++	+++	+++	+++	+++	+++	+++
Halimeda gracilis	-	-	-	-	-	-	-	-	-	-
Ulva reticulata	++	+++	+++	+++	-	+++	+++	+++	+++	+++
Caulerpa scalpelliformis	-	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 6. Haemagglutinic activity of seaweeds against human erythrocytes (B blood group)

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	-	-	-	-	-	-	-	-	-	-
Gracilaria edulis	-	-	-	-	-	-	-	-	-	-
Sargassum wightii	-	-	-	-	-	-	-	-	-	-
Halimeda gracilis	-	-	-	-	-	-	-	-	-	-
Ulva reticulata	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
Caulerpa scalpelliformis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

showing maximum titer for *Fucus serratus*, *Laminaria saccharina*, and *Himanthalia elongata*. Percentage of

Table 7. Haemagglutinic activity of seaweeds against human erythrocytes (AB blood group)

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	-	-	-	-	-	-	-	-	-	-
Gracilaria edulis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sargassum wightii	++	++	++	++	++	++	++	++	++	++
Halimeda gracilis	++	++	++	++	++	++	++	++	++	++
Ulva reticulata	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Caulerpa scalpelliformis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 8. Haemagglutinic activity of seaweeds against human erythrocytes (O blood group)

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Amphiroa fragilissima	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Gracilaria edulis	-	-	-	-	-	-	-	-	-	-
Sargassum wightii	++	++	++	++	++	++	++	++	++	++
Halimeda gracilis	++	++	++	++	++	++	++	++	++	++
Ulva reticulata	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Caulerpa scalpelliformis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

[a]- -No agglutination [b]+ -Weak agglutination
[c]++ -Moderate agglutination [d]+++ -High agglutination

Table 9. Inhibition of human erythrocyte agglutinic activity with sugars

Sugars	*Caulerpa scalpelliformis*				*Ulva reticulata*			
	A	B	AB	O	A	B	AB	O
Arabinose	0.58±0.28	0.58±0.28	0.60±0.42	0.60±0.09	0.08±0.02	0.24±0.24	0.22±0.02	0.28±0.23
Fructose	0.60±0.23	0.22±0.02	0.32±0.22	0.22±0.02	0.23±0.02	0.24±0.22	0.28±0.05	0.06±0.03
Galactose	0.60±0.23	0.22±0.02	1.125±0.32	1.125±0.62	0.79±0.56	0.84±0.48	0.79±0.56	0.84±0.48
Glucose	0.42±0.24	0.37±0.16	0.07±0.06	0.23±0.08	0.07±0.06	0.39±0.28	0.28±0.23	0.10±0.06
Lactose	0.10±0.06	0.84±0.48	0.10±0.06	0.60±0.42	0.10±0.06	0.10±0.06	0.10±0.06	0.28±0.24

Table 10 MTT assay of extracts of *Caulerpa scalpelliformis*

S.no	Concentration (µg/ml)	Dilutions	Cell viability
1	1000	Neat	26.31
2	500	1:1	42.10
3	250	1:2	54.38
4	125	1:4	59.64
5	62.5	1:8	70.17
6	31.25	1:16	84.21
7	15.625	1:32	91.22
8	7.8125	1:64	94.73
9	Cell control	-	100

hemagglutinin activity against sheep erythrocytes was about 40%, whereas Fabregas *et al*. (1986) showed 71% hemagglutinin activity for sheep erythrocytes using different seaweeds.

High hemagglutinin activity by *Caulerpa scalpelliformis* and *Ulva reticulata* can be correlated to a higher concentration of soluble protein. This is in confirmation with the study of Benividas *et al*. (1999) in *Gelidium pusillum*. The tropical green seaweeds from Mandapam coast responded positively with the hemagglutinating test against human erythrocytes. This corresponds to the observations of a positive test for the bioactivity of hemagglutinins with different green seaweeds (Baqir *et al*. 1992). The brown seaweed *Sargassum wightii* screened against human blood groups depicted negative test except for A erythrocytes. A few deviations could be the result of the differences in the extraction technique as well as the environmental and habitat variations between Indian Mandapam coast seaweeds and British, Pakistan, and Vietnamese seaweeds.

Hemagglutination inhibition assay

Some peculiar properties of marine algal hemagglutinins such as preferential agglutination, no affinity for monosaccharides, and relatively low molecular occurrence of monomeric forms are demanding further studies in order to explain the actual physiological functions of lectins. Hemagglutination inhibition studies of *Caulerpa scalpelliformis* and *Ulva reticulata* were carried out using sugars against human erythrocytes. The agglutinating activity of both the species was inhibited by simple sugars. The minimum inhibitory concentration that inhibited hemagglutination for *Ulva reticulata* ranged from 0.070 to 0.140 mg/mL, –A blood group, 0.40 to 0.56 mg/mL – B blood group, 0.070 to 0.140 mg/mL-AB blood group, and 0.070 to 0.56 mg/mL-O blood group (Table 9). The MIC that inhibited hemagglutination for *Caulerpa scalpelliformis* ranged from 0.07 to 0.56 mg/mL, A blood group; 0.07 to 1.125 mg/mL, B blood group; 0.035 to 1.125 mg/mL, AB blood group; and 0.035 to 1.125 mg/mL, O blood group (Table 9). It is generally considered that algal agglutinins have specificity for monosaccharides. This consideration is supported by the fact that agglutinin activity of many purified agglutinin was inhibited by monosaccharides. Activity present

in *Caulerpa cupressoides* exhibited inhibition by simple sugars arabinose, galactose, lactose, raffinose, and fructose (Souza *et al*. 2007). The result of the present study is in contradiction with Dinh *et al*. (2009) who observed that hemagglutination activities of algal species were not inhibited by any of the monosaccharides examined, except the extracts of *Codium arabicum* and *Gracilaria eucheumoides*.

Antitumor activity of *Caulerpa scalpelliformis* against Hep 2 cells

Algae have gained special interest owing to their biological properties. There are many reports on the immunomodulating and antitumor activities of algae. Extracts of *Caulerpa scalpelliformis* showed cytotoxic activity against Hep 2 cells *in vitro* with a cytotoxic dose$_{50}$ of 250 mg/mL. Caulerpin a common metabolite is found to be present in the genus *Caulerpa*, which show cytotoxic activity (Ortega and Echevarrieta 2007). Raniello *et al*. (2007) confirmed the presence of secondary metabolites such as Caulerpenyne, which inhibit cytotoxic, antiviral, antiproliferative, and apoptotic effects. Similar findings were also documented by Wang *et al*. (2008 b) against HL-60 and MCF-7 cell lines. The antiproliferative activity of extracts on the HeLa cell line had 70–90% reduction of the non-cultured *U. flabellum extracts* IC_{50}, whereas extracts obtained after 30 days of culture increased IC_{50} by approximately 78–185% (Moo-Puc *et al*. 2011). The present study showed that *Caulerpa scalpelliformis* can be a potential target for producing various drugs for the prevention and treatment of cancer (Table 10).

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