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STUDIES ON MARINE ALGAE FOR HAEMAGGLUTINIC ACTIVITY

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ABSTRACT

Lectins (agglutinins) are important in medical and immunological applications. Phytohaemagglutinins have been found useful in blood banking. Keeping in view of these facts, the marine algae found at Karachi coastal region have been screened for agglutinic activity by using human erythrocytes of A, B, AB and O group. Altogether 53 algal samples were collected and subjected to extraction, fractionation serial dilution and titre determinations. The total marine algae screened for haemagglutinic activity were 44 out of these 14, 13 and 17 belonged to Chlorophyta, Phaeophyta, and Rhodophyta respectively. Among these three groups the Rhodophyta showed the highest number of lytic activity. The green marine alga *Valoniopsis pachynema* showed a titre value between 2^2 and 2^3, which is statistically significant. In case of brown marine algae Colpomenia sinuosa was found to be active (titre 2^3), while *Dictyota dichotoma*, *D. indica* and *Iyengaria stellata*, furnished week titre value as 2^2. The red marine algae screened were 17, out of these 4 spp. showed significant activity (titre 2^3), and these are *Gelidium usmanghanii*, *Gracilaria foliifera*, *Hypnea pannosa* and *Hynea valentiae*. While *Scinaia fasciculais*, *Scinaia indica* and *Champia parvula* were found to be weak in their onset on human erythrocytes. The results obtained were quite in agreement with those reported in the literature.

Introduction

Lectins are specific receptor proteins present in plants (usually their seeds), in-vertebrate animals (snails, crabs etc.), and lower vertebrates (fish ova). Although many lectins have been reported but several of them found useful in blood banking. However relatively little work has been conducted on the lectins from marine algae, and that Rogers and Fish (1991) have reviewed marine algal lectins in detail. In an earlier study (Naqvi, Sheikh, Usmanghani, Shameel, Sheikh, 1992) screening of marine algae of Karachi for haemagglutinic activity was carriedout. This communications deals with lytic activity of 53 different marine algal samples.

Results and Discussion

Application and biological significance of marine algal agglutinins are diverse in nature. It is amply demonstrated that agglutinins are widely distributed in marine algae. Haemagglutinins have provided useful tools in immunology, cell biology, cancer research and other areas of research activities.

Agglutinins from seaweeds can be extracted with organic solvents. The activity of the extracts depends on the algal species. In general, the extracts in immiscible water solvents show weak activity in comparison with the extracts in miscible water solvents. Extraction into organic solvents appears not to be suitable for the screening of agglutinins in seaweeds, except ethanol, but it may be an important and useful previous step for the purification of the active principle. Agglutinins from seaweeds can be extracted by different ways: i) macerated with distilled water in a Waring Blendor; ii) dry powered seaweeds extracted with 0.85% sodium chloride solution; iii) fresh algae triturated in a mortar with acid-washed sand and 0.9% sodium chloride solution, and iv) homogenization with a hand-ultrahomogenizer with 0.85% sodium chloride solution. The possibilities for extracting agglutinins from seaweeds could be as under: Marine algae collected from different points of seacoast and were kept at -20°C until used. Extraction of agglutinins from seaweeds is carried out following a sequence of organic solvents with increasing polarity. The wet seaweeds (log) is minced in mortar with quartz-sand previously washed four times with 0.5N HCl, ten times with distilled water and then oven-dried at 180°C for 6h. Negative results were obtained when such sand was used for haemagglutinic activity in a control test. The macerated mixture was successively extracted with the different solvents in proportion 1:1 (w/v) in the following sequence : cyclohexane, benzene, dichloromethane, chloroform, diethylether, cthylacetate, acetone, ethanol and methanol. Each extract was dried in a rotary evaporator at 45°C after adding 5ml of PBS (Phosphate Buffered Saline, pH 7.2). The fraction obtained was filtered through Millipore filter (0.22 µm) and maintained at 4°C until used. In the same way, an extract in PBS is made as a control

In an earlier experiments (Naqvi, Sheikh, Usmanghani, Shameel, Sheikh 1992) carried out on marine algae of Karachi sea shore, the procedure was as follows: The algal material was dried under shade and minced. The grinded material was soaked with 50% aqueous ethanol (EtOH) in a percolater for a period of 15 days. The aqueous EtOH was filtered, and the extract was concentrated under reduced pressure below 30°C using a thin film rotary evaporator. The concentrated extract was lyophilized, thereafter completely dried and powdered. About 5 mg of the powdered extract was dissolved in 10 ml of distilled water and serial doubling dilutions of the extracts, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 were prepared. A phosphate buffer of pH 7.0 was prepared by mixing 35 ml of a

solution of $Na_2HPO_4.2H_2O$ (26 g/l and 65 ml of a solution of anhydrous Na_2HPO_4 (18 g/l). Samples of human blood of the blood groups A, B, AB and 0 were obtained all being Rh^+; these were centrifuged and erythrocytes obtained by reflecting the supernatant. About 2 ml of erythrocytes were added to 98 ml of phosphate buffer to obtain 2% suspension of erythrocytes.

Haemagglutinic activity was investigated serially in different dilutions of each seaweed extract against all the four blood groups. For this purpose 1 ml of each dilution was added to 1 ml of 2% suspension of erythrocytes in a sugar fermentation test tube of 5 ml capacity. Test tubes were then incubated in a water bath at 25°C. Controls were also run along with 2% suspension of erythrocytes in buffer alone. Smooth button formation due to settling of erythrocytes at the bottom showed negative activity, whereas a rough granular deposition in the tube due to agglutination of erythrocyte, indicated positive reaction. Low, medium and high agglutinations were determined on the basis of the amount of granular deposition at the bottom of the tube.

But this procedure adopted can be seriously criticized for many of the reasons, such as: i) Most workers use enzyme - modified erythrocytes for screening of lectins as this is a more sensitive system. Less anomalous results are obtained and a higher percentage of plants can be shown to contain lectins. ii) It is to be noted that 50% ethanol is used to prepare extracts. This would be expected to precipitate some of the proteins in the extracts and possibly some of the lectins. All research workers use aqueous extracts for the test.

In an earlier study (Naqvi, Sheikh, Usmanghani, Shameel, Sheikh, 1992) around 66 species of marine benthic algae, collected from Karachi seashore, were tested for haemagglutinic activity out of which only 31 exhibited positive activity. However, Blunden et al., 1972 cited that out of 100 British seaweeds only 19 species showed positive results for haemagglutination. Therefore, the frequencies of occurrence of haemagglutinins in British and Pakistani seaweeds are comparable and appear to be alike. Out of 23, 16 and 27 investigated species of green, brown and red seaweeds only 12, 5 and 14 species respectively furnished a positive test for haemagglutinins. The lot of positive results are somewhat puzzling, as higher dilutions of extract produce stronger haemagglutination. Although some degree of prozone phenomenon with algal lectins experienced is very rare. No endpoints of agglutination (titration values) have been determined which means that no comments on specificity can be made. Brown algae contain high concentrations of polyphenols which mimic lectin-mediated haemagglutination.

Among green seaweeds all the 9 investigated species of *Ulva* including *U. fasciata*

and *U. lactuca* showed negative response to agglutination. However, Blunden et al., 1972 observed positive activity in the case of *U. lactuca*, but Boyed et al., 1966 reported negative results with *U. fasciata* and *U. lactuca*; the results obtained confirm the latter findings. Negative observation in case of *Chaetomorpha* spp. and positive activities in *Codium* spp. displayed the identity of results with those of Blunden et al., 1972 inspite of different species from both the places. The tropical green seaweeds such as *Bryopsis pennata*, 4 species of *Codium*, 5 species of *Caulerpa, Decaisnella indica* (= *Udotea indica*) and *Halimeda tuna* responded positively with haemagglutination test. Several brown seaweeds screened against human blood groups depicted negative test including *Dictyopteris australis* and 4 species of Dictyota. These observations tally with the results of Blunden et al., 1972, but positive tests with *Colpomenia sinuosa* and *Padina tetrustromatica* were in contradiction with negative activity of *Colpomenia peregrina* and *Padina pavonia* respectively. Moreover, the tropical species such as *Lobophora variegata* (= *Zonaria variegata*), *Sargassum tenerrimum* and *Spatoglossum asperum* exhibited the positive haemagglutinic activity (Naqvi, Sheikh, Usmanghani, Shameel, Sheikh, 1992).

Among red seaweeds *Ahnfeltia plicate*; exhibited a positive while *Laurencia pinnatifida* and *Plocamium cartilagineum* a negative reaction, whereas Blunden et al., 1972 observed a negative activity in all these species from British Islands. The positive activities of *Gracilaria foliifera* and *Polysiphonia platycarpa* are in contradiction with the negative results shown by the British species of these genera. In other cases the results agreed with those of Blunden et al., 1972. Furthermore, the tropical species such as *Acanthophora spicifera, Botryocladia leptopoda, Ceturoceras clavulatum*, 2 species of *Hypnea, Sarconema filifonnis, Scinaia complanata* and *Solieria chordalis* also caused red blood cells to agglutinate (Naqvi, Sheikh, Usmanghani, Shameel, Sheikh, 1992).

In this study 53 marine benthic algal samples were tested randomly without consideration of their taxanomic consideration so as to check the authenticity of the results of haemagglutions. Presence of haemagglutinins are demonstrated in some of the marine algae. Agglutination results as positive or negative are given in Table 1 and 2, and agglutination titre of 44 species with human erythrocytes are summarized in Table 3, 4 and 5. Because the haemagglutinic activity on marine algae gave diversified results quite unexpected and some times do not tally with the results reported from same species by earlier research workers.

Out of total 53 marine algal samples screened for haemagglutinic activity 14 algae samples belong to green algae, 13 to brown algae, and 17 to red algae. However, many of these algal samples were duplicates as being collected during different dates and different time intervals. The duplicate samples are number 26 and 52, as well as 49 and 16 in case of marine algal species of Chlorophyta; whereas 5 and 23, 19 and 44, 31 and 38; 25, 28;

51, 13 and 32 are marine species of Phaeophyta origin. It is interesting to note that duplicate samples of *Valoniopsis pachynema* (26, 52) exhibit the identical titre values as 2^1, 2^2, 2^3, 2^3 for human erythrocytes of A, B, 0, AB for sample 26 and 2^2, 2^3, 2^3, 2^2 for sample 52. Again identical results are obtained for *Iyengana stellata* where marine algal sample 25 showed titre as -, 2^2, 2^2, 2^2; while the sample 28 gave values as 2^1, 2^2, 2^2, 2^1; but the third sample 51, furnished a deviating titre as 2^1, -, -, 2^2. As such the two samples of *Iyengana stellata* exhibiting identical values should be considered, and third sample 51 result may be discarded. The negative result for identical algal samples were obtained for *Padina tetrastromatica* (5, 23), *Spatoglossum variabile* (19, 44), *Sargassum tenemimum* (13, 32).

In case of green algae (Chlorophyta) only *Valoniopsis pachynema* showed a titre value between 2^2 and 2^3 which can be termed as significant. The weak agglutinic activity was obtained for *Caulerpa faridii* as 2^2, while the rests of the green marine algae exhibited no response for haemagglutination (Table 3).

The brown algae (Phaeophyta) provided with rather weak activity for *Dictyota dichotoma* as 2^2, *D. indica* as 2^2, *Iyengana stellata* as 2^2. Only spp. of brown algae which showed some promise as lytic is *Colpomenia sinuosa* 2^3. The rest of the species displayed negative results in case of brown algae screened. Here it can be concluded that the method adopted actually showed no prozone effect and the occurrence of polyphenols in brown algae should have given the strongly positive result. The brown algae screened for haemagglutinic activity (Table 4) has not been fully investigated except the chemical constituents of *Stoechospermum marginalum*.

In this study it appears that red algae (Rhodophyta) have shown a good promise as it has shown a very significant agglutinic activity for many of the species. Out of 17 marine red algae ermined four algae furnished a significantly positive results and these algae are *Gelidium usmanghanii* (a new species established by Prof. Dr. M. Shamed), *Gracilaria foliifera*, *Hypnea pannosa*, and *H. valentiae*, the titre was 2^3 for all these species. The red algae which gave weak haemagglutinic activity (titre 2^1-2^2) are *Champia parvula*, *Scinaia fascicularis*, and *S. indica*. The rest of the red algae screened for lysis exhibited negative response (Table 5).

These results are in general agreement with other reports about the occurrence and activity of agglutinins with human erythrocytes (Rogers et al, 1988, Rogers et al., 1989, Okamoto et al., 1990, Fabregas et al., 1988, Hori et al., 1987, Fabregas et al., 1989, Rogers et al., 1982, Rogers et al., 1979, Rogers et al., 1979a, Rogers, 1984, Fletcher et al, 1989, Blundell et al, 1972, Naqvi et al., 1992). Maximum agglutinating was found in the case of marine algae belonging to Rhodophyta if comparative account of Chlorophyta, Phaeophyta and Rhodophyta is chalked out.

The marine algae with significant titre (2^3), therefore, forms a good material for the isolation and characteri[i]zation of algal haemagglutinins. Because the active molecule having agglutinins effect may be useful in deciphering the biological functions.

Experimental

Algal specimens

Specimens of different species of marine benthic algae were collected from the different coastal areas of Karachi during the month of October to April during 1992-93. The seaweeds were thoroughly washed to remove the epiphytes, epizootic and attached debris. The algae were identified after Shamed (1992), and algal nomenclature used in that check-list has been adopted in this communication. The marine algal specimens were air dried under shade and kept at room temperature until used. The list of marine algae tested is given in Table 1 and 2.

Preparation of extract

Samples (10 g) of each species were ground in a mortar with quartz sand (1:1, w/w) which had been previously washed four times with 05 N HCI, ten times with distilled water, again washed with 5% HU and finally washed ten times with distilled water, and then oven drived at 180°C for six hours. The algal-sand mixture was homogenised with 10 ml of phosphate buffered saline (PBS) pH 7.2 and centrifuged at 1000 g for 20 minutes. The supernatant was removed, filtered through a 0.22-pm Millipore filter and kept at -20°C until testing shortly afterward.

Negative results were obtained when such sand was used for haemagglutinic activity in the control test. The resultant solution was made up to a 20 ml of volume and used as a test solution.

Preparation of serial dilution

For the haemagglutinating activity prepared serial dilutions for each algae against each blood group were utilized. Dilutions prepared in a 5 ml capacity test tubes i.e. 1:2, 1:4, 1:8, 1:16 and 1:32 by add[i]ng 1 ml previously prepared phosphate buffered saline pH 7.2 in each test tube then added 05 ml algal extract into 1:2 dilution mixed and transferred 0.5 ml into 1:4 mixture and 05 ml from 1:4 to 1:8 and 05 ml from 1:8 into 1:16 to obtain serial dilutions. For each algal extract twenty dilutions for blood groups A, B, O, AB were prepared.

Preparation of 2% suspension of erythrocytes

All groups of human blood A, B, 0 and AB were obtained from the Liaquat National Hospital, Karachi. Blood samples were maintained at 4°C in Alsevier's solution. A 2%

erythrocytes suspension in phosphate buffer solution (pH 7.2) was prepared from each type of blood group by centrifugation at 1000 g for 20 minutes and supernatant was removed. Again the blood was centrifuged with phosphate buffer saline pH of 72. The supernatant was removed and erythrocytes obtained.

Assay of Haemagglutinating activity

Haemagglutinating activity was investigated serially in different dilutions of each algal extract against all the four blood groups. The 05 ml 2% erythrocytes suspension of "A" group was added in all dilutions shaken, for few seconds and then allowed to stand for incubation in a water bath at 25°C for 30 minutes; control was also run simultaneously. After that test tubes were further left to stand at room temperature for 24 horus, agglutination was observed.

Observation of agglutination

Smooth button formation due to settling of erythrocytes at the bottom showed negative activity, whereas a rough granular deposition in the tube due to agglutination of erythrocyte, indicated positive reaction. Low, medium and high agglutinations were determined on the basis of the amount of granular deposition at the bottom of the tube. The activity was expressed as titre, the reciprocal of two fold dilution giving positive haemagglutination.

Experimental working

Earlier, our group carried out studies on marine algal lectins from Karachi. The details of which are as follows:

The algal material was dried under shade and minced. The grinded material was soaked with 50% aqueous ethanol (EtOH) in a percolator for a period of 15 days. The aqueous EtOH was filtered, and the extract was concentrated under reduced pressure below 30°C using a thin film rotary evaporator. The concentrated extract was lyophilized, thereafter completely dried and powdered. About 5 mg of the powdered extract was dissolved in 10 ml of distilled water and serial doubling dilutions of the extracts, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 were prepared.

A phosphate buffer of pH 7 was prepared by mixing 35 ml of a solution of Nat HPO_4. $2H_2O$ (26 g/1) and 65 ml of a solution of anhydrous Na_2HPO_4 (18 g/1). Samples of human blood of the blood groups A, B, AB and 0 were obtained from Husaini Blood Bank, Karachi, all being Rh^+, they were centrifuged and erythrocytes obtained by rejecting the supernatant. About 2 ml of erythrocytes was added to 98 ml of phosphate buffer to obtain 25% suspension of erythrocytes.

Haemagglutinic activity was investigated serially in different dilutions of each seaweed extract against all the four blood groups. For this purpose 1 ml of each dilution was added to 1 ml of 2% suspension of erythrocytes in a sugar fermentation test tube of 5 ml capacity. Test tubes were also run along with 2% suspension of erythrocytes in buffer alone. Smooth button formation due to settling of erythrocytes at the bottom showed negative activity, whereas a rough granular deposition in the tube due to agglutination of erythrocyte, indicated positive reaction. Low, medium and high agglutinations were determined on the basis of the amount of granular deposition at the bottom of the tube.

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Table 1
Haemagglutinic activity of marine algae

Swaweeds		"AB"					"O"				
"Algae"		1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32
1.	*Furcellaria lumbricalis*	-	-	-	-	-	-	-	-	-	-
2.	*Hypnea valentiae*	+	+	+	+	+	+	+	+	+	-
3.	*Gracilaria foliifera*	+	+	+	+	+	+	+	+	+	+
4.	*Ulva reticulata*	-	-	-	-	-	-	-	-	-	-
5.	*Padina tetrastromatica*	-	-	-	-	-	-	-	-	-	-
6.	*Stokeyia indica*	-	-	-	-	-	-	-	-	-	-
7.	*Laurencia obtusa*	-	-	-	-	-	-	-	-	-	-
8.	*Enteromorpha intestinalis*	-	-	-	-	-	-	-	-	-	-
9.	*Caulerpa taxifolia*	-	-	-	-	-	-	-	-	-	-
10.	*Sarconema furcellatum*	-	-	-	-	-	-	-	-	-	-
11.	*Laurencia platyclada*	-	-	-	-	-	-	-	-	-	-
12.	*Gelidium usmanghanii*	+	+	+	+	+	+	+	+	+	+
13.	*Sargassum tenerrimum*	-	-	-	-	-	-	-	-	-	-
14.	*Chaetrmorpha antenina*	-	-	-	-	-	-	-	-	-	-
15.	*Hypnea pannosa*	+	+	+	+	+	+	+	+	-	+
16.	*Caulerpa scalpelliformis*	-	-	-	-	-	-	-	-	-	-
17.	*Enteromorpha prolifera*	-	-	-	-	-	-	-	-	-	-
18.	*Laurencia pinnatifida*	-	-	-	-	-	-	-	-	-	-
19.	*Spatoglossum variable*	-	-	-	-	-	-	-	-	-	-
20.	*Bryopsis pennata*	-	-	-	-	-	-	-	-	-	-
21(a).	*Scinaia indica*	+	+	+	+	+	+	+	+	+	+
22(b).	*Dictyota indica*	+	+	+	+	+	+	+	+	+	+
23(c).	*Padina tetrastromtica*	+	+	+	+	+	+	+	+	+	+
24(d).	*Caulerpa faridii*	+	+	+	-	-	+	+	+	+	+
25(e).	*Iyengaria stellata*	-	-	-	-	-	+	+	+	+	+
26(f).	*Valoniopsis pachynema*	+	+	+	+	+	+	+	+	+	+
27(g).	*Colpomenia sinuosa*	+	+	+	+	+	+	+	+	+	+
28(h).	*Iyengaria stellata*	+	+	+	+	+	+	+	+	+	-
29(i).	*Champia parvula*	+	-	+	+	+	+	+	+	+	+
30(j).	*Jania adherens*	-	-	-	-	-	-	-	-	-	-
31(k).	*Stoechospermum marginatum*	-	-	-	-	-	-	-	-	-	--
32(l).	*Sargassum tenerimum*	+	-	-	-	+	+	-	+	-	-
33(su).	*Codium dwarkense*	+	-	-	-	-	-	-	-	-	-

Table continue ...

Swaweeds	"AB"					"O"				
" Algae "	1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32
34(su). *Codium iyengarii*	+	+	+	-	-	+	+	-	+	-
35(su). *Codium flabellatum*	+	+	-	-	-	+	-	-	-	-
36(su). *Caulerpa racemosa*	+	-	-	-	-	+	+	-	-	-
37(su). *Caulerpa chemnitiza*	+	-	-	-	-	-	+	-	-	+
38(su). *Stoechospermum marganatium*	-	-	-	-	-	-	-	-	-	-
39(su). *Dictyota dichotoma*	-	-	-	-	-	+	+	+	+	+
40(su). *Galaxaura oblongata*	-	-	-	-	-	-	-	+	-	+
41(su). *Hinksia mitchelliae*	+	+	+	+	+	+	+	+	+	+
42(su). *Scinaia fascicularis*	+	+	+	+	+	+	+	+	+	+
43(su). *Ulva fasciata*	+	-	-	-	-	+	-	-	-	-
44(su). *Spatoglossum variabile*	+	+	+	-	-	+	-	+	-	-
45(su). *Ahnfeltia plicata*	-	-	-	-	-	-	-	-	-	-
46(su). *Plocamium cocceineum*	-	-	-	-	-	-	-	+	+	-
47(su). *Dictyopeteris australis*	-	-	-	-	-	-	-	+	+	-
48(su). *Sargassum ilicifolium*	-	-	-	-	-	-	-	+	-	+
49(su). *Caulerpa scalpelliformis*	-	-	-	-	-	+	+	+	+	+
50(su). *Spatoglossum asperium*	-	-	-	-	-	+	+	+	+	+
51(su). *Iyengaria stellata*	-	-	-	-	-	+	+	+	+	+
52(su). *Valoniopsis pachynema*	+	+	+	+	+	+	+	+	+	+
53(su). *Acanthophora spicifera*	-	-	-	-	-	+	+	+	+	+

Table 2
Haemagglutinic activity of marine algae

Swaweeds		"AB"					"O"				
"Algae"		1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32
1.	*Furcellaria lumbricalis*	-	-	-	-	-	-	-	-	-	-
2.	*Hypnea valentiae*	+	+	+	+	+		+	+	+	+
3.	*Gracilaria foliifera*	+	+	+	+	+	+	+	+	+	+
4.	*Ulva reticulata*	-	-	-	-	-	-	-	-	-	-
5.	*Padina tetrastromatica*	-	-	-	-	-	-	-	-	-	-
6.	*Stokeyia indica*	-	-	-	-	-	-	-	-	-	-
7.	*Laurencia obtusa*	+	+	+	+	+	+	+	+	+	+
8.	*Enteromorpha intestinalis*	-	-	-	-	-	-	-	-	-	-
9.	*Caulerpa taxifolia*	-	-	-	-	-	-	-	-	-	-
10.	*Sarconema furcellatum*	+	+	+	+	+	+	+	+	+	+
11.	*Laurencia platyclada*	+	+	+	+	+	+	+	+	+	+
12.	*Gelidium usmanghanii*	+	+	+	+	+	+	+	+	+	+
13.	*Sargassum tenerrimum*	-	-	-	-	+	+	+	+	+	+
14.	*Chaetrmorpha antenina*	+	+	+	+	+	+	+	+	+	+
15.	*Hypnea pannosa*	+	+	+	+	+	+	+	+	+	+
16.	*Caulerpa scalpelliformis*	+	+	+	+	+	+	+	+	+	+
17.	*Enteromorpha prolifera*	-	-	-	-	-	-	-	-	-	-
18.	*Laurencia pinnatifida*	+	+	+	+	+	+	+	+	+	+
19.	*Spatoglossum variable*	-	-	-	-	-	-	-	-	-	-
20.	*Bryopsis pennata*	+	+	+	+	+	+	+	+	+	+
21(a).	*Scinaia indica*	+	+	-	-	+	+	+	+	+	+
22(b).	*Dictyota indica*	-	-	-	-	-	+	+	+	+	+
23(c).	*Padina tetrastromtica*	+	-	-	-	-	+	+	+	+	+
24(d).	*Caulerpa faridii*	-	-	-	-	-	+	+	+	+	+
25(e).	*Iyengaria stellata*	-	-	-	-	-	+	+	+	+	+
26(f).	*Valoniopsis pachynema*	+	+	+	-	-	+	+	+	+	+
27(g).	*Colpomenia sinuosa*	+	+	-	-	-	+	+	+	+	+
28(h).	*Iyengaria stellata*	-	+	-	-	-	+	+	+	+	+
29(i).	*Champia parvula*	-	+	-	-	-	+	+	+	+	+
30(j).	*Jania adherens*	+	+	-	-	-	+	+	+	+	+
31(k).	*Stoechospermum marginatum*	-	-	-	-	-	-	-	-	-	-

Table continue ...

Swaweeds	"AB"					"O"				
" Algae "	1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32
32(l). *Sargassum tenerimum*	+	-	-	-	+	+	-	+	-	-
33(su). *Codium dwarkense*	+	-	-	-	-	+	+	+	+	+
34(su). *Codium iyengarii*	-	-	-	-	-	+	+	+	+	+
35(su). *Codium flabellatum*	+	-	-	-	-	+	+	+	+	+
36(su). *Caulerpa racemosa*	-	-	-	-	-	-	-	-	-	-
37(su). *Caulerpa chemnitiza*	+	-	-	-	-	-	+	-	-	+
38(su). *Stoechospermum marganatium*	-	-	-	-	-	-	-	-	-	-
39(su). *Dictyota dichotoma*	-	-	-	-	-	+	+	+	+	+
40(su). *Galaxaura oblongata*	-	-	-	-	-	-	-	-	-	-
41(su). *Hinksia mitchelliae*	-	-	-	-	-	-	-	-	-	-
42(su). *Scinaia fascicularis*	-	-	-	-	+	+	+	+	+	+
43(su). *Ulva fasciata*	+	-	-	-	-	+	+	+	+	+
44(su). *Spatoglossum variabile*	-	-	-	-	-	+	+	+	+	+
45(su). *Ahnfeltia plicata*	-	-	-	-	-	+	+	+	+	+
46(su). *Plocamium cocceineum*	+	+	+	+	-	+	+	+	+	+
47(su). *Dictyopeteris australis*	-	-	-	-	-	-	-	-	-	-
48(su). *Sargassum ilicifolium*	-	-	-	-	-	-	-	-	-	-
49(su). *Caulerpa scalpelliformis*	+	-	+	-	-	+	+	+	+	+
50(su). *Spatoglossum asperium*	-	-	-	-	-	-	-	-	-	-
51(su). *Iyengaria stellata*	+	+	+	-	-	-	-	-	-	-
52(su). *Valoniopsis pachynema*	+	+	+	+	+	+	+	+	+	+
53(su). *Acanthophora spicifera*	-	-	-	-	-	-	-	-	-	-

Table 3
Hemagglutinic activity of green marine algae

Chlorophyta	Human erythrocytes			
	A	B	O	AB
Enteromorpha intestinalis (L). Nees (8)	-	-	-	-
Enteromorpha prolifera (Miill) J. Ag. (17)	-	-	-	-
Ulva fasciate Delile (43)	-	-	-	-
Ulva reticulata Forssk (4)	-	-	-	-
Chaetomorpha antennina (Bory) Kirtz (14)	-	-	-	-
Valoniopsis pachynema (Mart) Borg. (26, 52)	2^1	2^2	2^3	2^3
	2^2	2^3	2^3	2^2
Codium dwarkense Borg. (33)	-	2^2	-	-
Codium flabellatum Silva et Nizam (35)	-	2^2	-	-
Codium iyengarii Borg. (34)	-	2^2	-	-
Caulerpa cheminitzia (Esper) Lamour (37)	-	-	-	-
Caulerpa faridii Nizam (24)	-	2^2	2^2	2^2
Caulerpa racemosa (Forssk) J. Ag. (36)	-	-	-	-
Caulerpa scalpelliformis (Brown) C. Ag. (49, 16)	2^1	-	-	2^2
	-	-	-	-
Caulerpa taxifolia (Vahl) C. Ag. (9)	-	2^2	-	-

Table 4
Hemagglutinic activity of brown marine algae

Chlorophyta	Human erythrocytes			
	A	B	O	AB
Hincksia mitchelliae (Harv) Siva (41)	-	-	-	-
Dictyopteris australis (Sond) Asken (47)	-	-	-	2^1
Dictyota dichotoma (Huds) Lamour (39)	-	2^2	2^2	2^3
Dictyota indica sond ex Kutz (22)	-	2^3	2^3	2^2
Padina tetrastromatica Hauck (5, 23)	-	-	-	-
	-	-	2^2	-
Spatoglossum asperum J. Ag (50)	-	-	-	2^2
Spatoglossum variable Fig. et Notar (19, 44)	-	-	-	-
	-	-	-	-
Stoechospermum marginatum (C. Ag.) Kutz (31, 38)	-	-	-	-
Colpomenia sinuosa (Roth) Derb et. sol. (27)	2^1	2^3	2^3	2^3
Iyengaria stellata (Borg) Borg. (25, 28, 51)	-	2^2	2^2	2^2
	2^1	2^2	2^2	2^1
	2^1	-	-	2^2
Stokeyia indica Thivy et Doshi (6)	-	-	-	-
Sargassum ilicifolium (Turn) C. Ag. (48)	-	-	-	2^1
Sargassum tenerrimum T. Ag. (13, 32)	-	-	-	-
	-	-	-	-

Table 5
Hemagglutinic activity of red marine algae

Chlorophyta	Human erythrocytes			
	A	B	O	AB
Galaxaura oblongata (Soland) Lamour (40)	-	-	-	-
Scinaia fascicularis (42)	2	2^2	2^2	2^1
Scinaia indica (21)	2^2	2^2	2^2	2^3
Gelidium usmanghani, Shameel (12)	2^3	2^3	2^3	2^3
Jania adherens Lamour (30)	-	-	-	-
Gracilaria folufera (Forssk) Borg. (3)	2^3	2^3	2^3	2^3
Plocamium cocceinum (46)	-	-	-	-
Furcellaria lumbricalis (Huds) Lamour (1)	-	-	-	-
Sarconema furcellatum Zanard (10)	-	-	-	-
Hypnea pannosa (15)	2^3	2^3	2^3	2^3
Hypnea valentiae (Turn) Mont. (2)	2^3	2^3	2^3	2^2
Ahnfeltia plicata (Huds) Fries (45)	-	-	-	-
Champia parvula (C. Ag.) Harv. (29)	2^1	2^2	2^2	2^2
Acanthophora spicifera (vahl) Borg. (53)	-	-	-	-
Laurencia obtusa (Huds) Lamour (7)	2^2	2^2	-	-
Laurencia pinnatifida (Huds) Lamour (18)	2^2	2^2	-	-
Laurencia platyclada Borg. (11)	2^2	2^2	-	-