ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Screening of Indian marine macro algae (Seaweeds) for haemagglutinin activity

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Manuscript Info

Manuscript History:

Received: 11 February 2014
Final Accepted: 25 March 2014
Published Online: April 2014

Key words:

Haemagglutinin, agglutinin, chlorophyceae, phaeophyceae, rhodophyceae.

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Abstract

Haemagglutinin (Lectins) are the compounds agglutinate (Clumping) the erythrocytes of the blood cells. Screening of 21 Indian seaweeds from chlorophyceae (green algae), phaeophyceae (brown algae) rhodophyceae (red algae) were collected from the Gulf of Mannar Biosphere Reserve. Haemagglutinin activities in 9 different blood samples were screened. However all the seaweed extract were not active on all blood samples there was the specificity differ greatly in the native and pappine treated erythrocytes. Majority of the seaweeds screened are a potential source of haemagglutinin as there extracts agglutinate one of the blood groups tested.

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1) 1. Introduction

Usefulness of haemagglutinin in various fields of biological research such as cytology, cell biology, immunology and cancer research has caused a growing interest in the discovery and isolation of agglutinins (lectins) from various new biological sources. As a result, a great number of living organisms have been reported to have haemagglutinins (lectins). However, in comparison, there are only limited studies on algal haemagglutinins, inspite of the availability of large number of species and amount of marine macroalgae (seaweeds) (Ainouz et al., 1995), as compared to higher plants. Therefore, in the present study, seaweeds were screened for the haemagglutinin activity.

2) 2. Materials and methods

2.1. Collection of seaweeds

A total number of 21 seaweeds (Table 1) belonging to green algae (8 species), brown algae (4species) and red algae (9 species) were collected from the Mandapam coast, Manali island and Hare island of the Gulf of Mannar Biosphere Reserve, in September 1999.

2.2. Preparation of seaweed extracts

The seaweeds selected for the study (Table 1) were handpicked and thoroughly washed in the field itself with the native seawater so as to remove epiphytes. The cleaned seaweeds were transported to the laboratory under frozen condition and stored at -20^{o}C. Extraction was performed by grinding the frozen seaweed to a fine powder in liquid nitrogen (Sampio et al., 1998b). The powder was stirred with 70% (v/v) aqueous ethanol (powder to solvent ratio of 1:5) at 4^{o}C for 30 minutes and then filtered through Whatman No.4 paper under vacuum. The filtrate was re-extracted under the same condition and filtered again. This step was repeated thrice. The material was then extracted with 0.05M Tris-HCl, pH 8.0, 0.15 N NaCl, 0.01M $CaCl_2$ Tris buffered saline (TBS) 1:2 w/v, stirred for 18 hours at 4^{o}C and centrifuged at 15000 Xg for 30 min at 4^{o} C. After centrifugation, the pellet was resuspended in TBS, dialyzed against distilled water and then in TBS. The dialyzed samples were lyophilized for further use.

2.3. Preparation of erythrocytes

Red blood cells of human A, B, O and AB groups (obtained from the blood bank of Rajah Muthaiah Medical College, Annamalai University) chicken, goat and sheep (obtained from venous puncture of healthy animals) and horse (obtained from Tamil Nadu Veterinary and Animal Science University, Chennai) were used for haemagglutinin tests. Blood samples were collected in preheparinised tubes and washed three times with 10 volumes of 0.85% NaCl, centrifuged and a 2% erythrocyte suspension was prepared from each type of blood. Saline containing papain (0.01mg/ml) was added to the packed cells to give a 2% suspension and incubated at 20^{o}C for one hour . The suspension was washed 6 times with cold 0.85% NaCl. After centrifuging at 2000 Xg for 5 minutes for three times, the enzyme (papain) treated cells were resuspended in 0.85% NaCl to give 2% ready erythrocyte suspension for haemagglutination assays (Ainouz and Sampaio, 1991; Ainouz et al., 1992) .

Haemagglutinin tests were performed in both native (without enzyme) and papain (enzyme) treated erythrocytes.

2.4. Haemagglutination test

Aliquots of 25 μL of the seaweed extracts were used to get serial two fold dilutions with NaCl (0.85%). Equal volumes of erythrocyte suspensions were added to each well of a micro-titer plate (96 Wells, U bottom), gently shaken and incubated at 37^{o}C for 30 min. The mixture was left for 30 min at room temperature (28^{o}C) and the agglutination was observed visibly. The agglutination titer has been expressed as the reciprocal of the highest dilutions showing positive results. The assays were carried out in duplicates. A control was also maintained with 0.85% NaCl solution with erythrocytes (Ravindranath and Paulson, 1987).

3) 3. Results

The results show that all the eight green algae, three brown algae out of four and eight red algae out of nine were able to agglutinate at least one of the nine erythrocytes tested. It was observed that one red and one brown algae did not show agglutination of either untreated erythrocytes (Table 2) or enzyme treated erythrocytes (Table 3).

Sensitivity of the blood cells to agglutination differed in both enzyme treated and native blood cells. In most of the blood groups, enzyme (papain) treated blood cells were more sensitive to agglutination than untreated. In chick blood, 66.6% of seaweed extracts were active in treated cells against 42.8% in untreated; 23.8% against 19.0% in human blood group AB, 52.3% against 42.8% in human blood group O, 61.9% against 57.1% in sheep blood, 85.7% against 28.5% in rabbit blood and 76.1% against 9.5% in horse blood. There was no impact of enzyme treatment on human blood groups A and B. But, there was a negative impact on the goat blood that had resulted in lesser sensitivity i.e. 14.2% of seaweed extracts were active in treated blood against 19% of untreated goat blood.

Table 1. List of seaweed species collected and their places of occurrence

Sl. No.	Species	Group	Occurrence
1	Bryopsis plumosa (Huds.) Ag.	Green algae	Mandapam coast
2	Caulerpa latevirens Areschoug	Green algae	Mandapam coast
3	C. microphysa (Weber–van Bosse) J. Feldmann	Green algae	Manoli island
4	C.racemosa (Forssk.) Weber–van Bosse	Green algae	Mandapam coast
5	C.scalpelliformis (R.Br.) Weber–van Bosse	Green algae	Hare island
6	Enteromorpha compressa (Linn.) Grev.	Green algae	Mandapam coast
7	Halimeda macroloba Decaisne	Green algae	Mandapam coast
8	Ulva lactuca Linn.	Green algae	Manoli island
9	Dictyota bartayresiana Lamour.	Brown algae	Manoli island
10	Padina tetrastromatica Hauck.	Brown algae	Mandapam coast
11	Sargassum longifolium (Turner) G. Agardh	Brown algae	Mandapam coast
12	S. wightii Greville	Brown algae	Mandapam coast
13	Acanthophora spicifera (Vahl.) Boergesen	Red algae	Mandapam coast
14	Centroceras clavulatum (C.Ag.) Mont.	Red algae	Hare island
15	Champia globulifera Boergesen	Red algae	Mandapam coast
16	Cheilosporum spectabile Harvey	Red algae	Hare island
17	Gracilaria corticata J.Ag.	Red algae	Mandapam coast
18	G. crassa Harvey	Red algae	Manoli island
19	G. edulis (S.Gmelin) P.Silva	Red algae	Mandapam coast
20	Hypnea valentiae (Turn.) Mont.	Red algae	Mandapam coast
21	Laurencia poiteaui (Lamouroux) Howe	Red algae	Manoli island

Herbarium specimens of the seaweeds were prepared as per the procedure outlined by Dawson (1966) and were identified up to species level by referring to standard literature (Edwards, 1970; Umamaheswara Rao, 1970, 1987).

Table 2. Agglutination activity of seaweed extracts on native erythrocytes of chick(CB), Human A, B, AB, O, sheep (SB), goat (GB), rabbit (RB) and horse (HB).

Sl.No.	Seaweed species	CB	Human blood				SB	GB	RB	HB
			A	B	AB	O				
1	Bryopsis plumose	+		+			+		+	
2	Caulerpa racemosa			+		+	+		+	
3	C. microphysa					+	+		+	
4	C. scalpelliformis	+	+							
5	C. lateverens	+				+	+			
6	Enteromorpha compressa	+	+							
7	Halimeda macroloba					+				
8	Ulva lactuca	+		+		+	+			
9	Dictyota bartayresiana									
10	Padina tetrastromatica									
11	Sargassum longifolium	+								
12	S. wightii		+				+			
13	Acanthophora spicifera	+		+	+		+	+		+
14	Centroceros clavulatum	+		+	+	+			+	
15	Champia globulifera									
16	Cheilosporum spectabilis									
17	Gracilaria corticata	+	+	+	+	+	+			
18	G. crassa		+	+	+	+	+	+		+
19	G. edulis						+			
20	Hypnea valentiae						+	+	+	
21	Laurentia porteaui					+	+	+	+	

+ denotes agglutination activity

Table 3. Agglutination activity of seaweed extracts on enzyme (papain) treated erythrocytes of chick (CB), human A, B, AB, O, sheep (SB), goat (GB), rabbit (RB) and horse (HB).

Sl.No.	Seaweed species	CB	Human blood				SB	GB	RB	HB
			A	B	AB	O				
1	Bryopsis plumose	+	+			+	+		+	
2	Caulerpa racemosa	+		+		+	+		+	+
3	C. microphysa					+			+	
4	C scalpelliformis	+	+	+	+		+		+	+
5	C lateverens	+					+		+	+
6	Enteromorpha compressa						+		+	+
7	Halimeda macroloba	+					+		+	+
8	Ulva lactuca	+		+	+	+	+		+	+
9	Dictyotabartayresiana									
10	Padina tetrastromatica					+			+	+
11	Sargassum longifolium					+			+	+
12	s.wightii	+							+	+
13	Acanthophora spicifera	+	+	+	+	+	+	+	+	+
14	Centroceros clavulatum	+		+		+			+	
15	Champia globulifera	+								+
16	Cheilosporum spectabilis									
17	Gracilaria corticata	+	+	+	+	+	+	+	+	+
18	G. crassa	+	+	+	+	+	+	+	+	+
19	G. edulis						+		+	+
20	Hypnea valentiae	+					+		+	+
21	Laurentia porteaui	+					+		+	+

+ denotes agglutination activity

Further, among the different blood samples tested, the sensitivity of enzyme treated rabbit and horse bloods showed a broader spectrum of agglutination percentage (85.7% and 76.1%) of seaweed extracts than the human blood samples.

3.1. Green weeds (Chlorophyceae)

Among the extracts of the eight species of green weeds tested with nine different blood samples, all the seaweed species showed agglutination activity at least in one of the blood samples. However, the agglutination activity of seaweed extracts differed in native erythrocytes and papain (enzyme) treated erythrocytes. In native erythrocytes, extracts of B. plumosa, C. racemosa and U. lactuca were active in 44% of blood samples followed by C. microphysa and C. latevirens (each 33%), C. scalpelliformis and E. compressa (each 22%) and H. macroloba (11%). In enzyme treated erythrocytes, extract of C. scalpelliformis and U. lactuca agglutinated 77% of blood samples followed by C. racemosa (66%), B. plumosa (55%), C. laterverens and H. macroloba (44% each), E. compressa (33%) and C. microphysa (22%) (Table 4).

3.2. Brown weeds (Phaeophyceae)

Among the extracts of the four species of brown weeds tested, all the species, except Dictyota bartayresiana, showed agglutination activity atleast in one of the blood samples tested. In native erythrocytes, P. tetrastromatica did not agglutinate any of the blood groups. However 33% of blood cells were agglutinated in the enzyme treated erythrocytes. Extract of S. longifolium agglutinated 11% of native erythrocytes and 33% of enzyme treated erythrocytes. But, S. wightii agglutinated a higher percent of native blood cells (22%) than S. longifolium but both the species agglutinated the same percent of enzyme treated erythrocytes (33%). Percentage of agglutinated blood groups (both native and enzyme treated) in different brown seaweed extracts is given in Table 5.

3.3. Red weeds (Rhodophyceae)

Among the extracts of the nine species of red weeds tested, except C.spectabilis, all the other species showed agglutination activity in atleast one of the blood samples (both native and enzyme treated) tested. In native erythrocytes, G. crassa agglutinated 77% of the blood cells followed by A. spicifera ,G. corticata (each 66%), C. clavulatum (55%), L. porteaui (44%), H. valentiae (33%) and G. edulis (11%). In enzyme treated erythrocytes, extracts of A. specifera, G. corticata and G. crassa were active against all the blood groups (100%). Extracts of C. clavulatum, H. valentiae and L. porteii were active in 44% blood cells while G. edulis extract showed activity in 33% and C.globulifera extract showed activity in 22% of all the blood cells (Table 6).

In general, in native erythrocytes, sheep blood was agglutinated by a broad spectrum of red weed extracts than other blood groups whereas in enzyme (papain) treated erythrocytes, blood cells of rabbit, horse and chick were agglutinated by a broader spectrum of red weed extracts, in addition to sheep blood.

Table 4. Percentage of different erythrocytes agglutinated by green weeds

Sl. No.	Seaweed species	Percentage of agglutinated erythrocytes	
		in native	in treated
1	Bryopsis plumose	44	55
2	Caulerpa racemosa	44	66
3	C. microphysa	33	22
4	C scalpelliformis	22	77
5	C lateverens	33	44
6	Enteromorpha compressa	22	33
7	Halimeda macroloba	11	44
8	Ulva lactuca	44	77

Table 5. Percentage of different erythrocytes agglutinated by brown weeds

Sl. No.	Seaweed species	Percentage of agglutinated erythrocytes	
		In native	in treated
1	Dictyota bartayresiana	0	0
2	Padina tetrastromatica	0	33
3	Sargassum longifolium	11	33
4	S. wightii	22	33

Table 6. Percentage of different erythrocytes agglutinated by red weeds

Sl. No.	Seaweed species	Percentage of agglutinated erythrocytes	
		In native	in treated
1	Acanthophora spicifera	66	100
2	Centroceros clavulatum	55	44
3	Champia globulifera	0	22
4	Cheilosporum spectabilis	0	0
5	Gracilaria corticata	66	100
6	G. crassa	77	100
7	G. edulis	11	33
8	Hypnea valentiae	33	44
9	Laurencia porteaui	44	44

Sl. No.	Seaweed species	Blood group
1	B. plumose	Human blood B
2	C. laetiverens	Sheep
3	C. microphysa	Sheep
4	E. compressa	Human blood a, Chick
5	H. macroloba	Human blood O
6	S. longifolium	Chick
7	S. wightii	Sheep
8	C. clavulatum	Human blood AB
9	H. valentiae	Goat
10	L. poiteaui	Human blood O, Goat

4) 4. Discussion

In the Indian scenario, present investigation is the only study on screening of Indian seaweeds for haemagglutinin activity. Altogether twenty one seaweeds collected from the Gulf of Mannar Biosphere Reserve were screened for haemagglutinin activity in nine different bloods viz. chick blood, human blood groups A, B, AB and O, sheep blood, goat blood, rabbit blood and horse blood. However, all the seaweed extracts were not active on all the blood samples as there was specificity in agglutination. This specificity also differed greatly in the native and papain (enzyme) treated erythrocytes.

Ainouz et al. (1992) stated that the treatment with the enzyme, papain, was more effective in the determination of human blood cell agglutination. This holds good in the present study where agglutination was found in more number of enzyme treated blood samples than the native blood cells, with the extracts of different seaweed species. However, it was observed in the present study that the enzyme treatment did not show uniform action in the agglutination process as it was dependent on the blood group and the seaweed extract used. This would explain why some of the seaweed extracts that could agglutinate the native blood cells, negatively influenced the enzyme treated blood cells causing no agglutination after the enzyme treatment.

The seaweed extracts that showed agglutination in the native blood cells but not in the enzyme treated blood cells are given below.

Presence of agglutinin in seaweeds has been well documented throughout the world by several authors (Rogers and Hori, 1993; Rogers et al., 1977, 1980, 1982, 1986, 1991, 1993, 1994; Lesniak et al., 1982; Fish, 1989; Sampaio et al., 1996a,b, 1998a,b, 1999; Fabregas et al., 1985, 1988, 1992; Benevides et al., 1996, 1998, 1999). However, there is no complete study on the agglutinin of the marine algal flora of India. Of the twenty one seaweed species screened in the present study, nine species (C. alpelliformis, C. macroloba, S. longifolium, S. wightii, C. spectabilis, G. corticata, G. crassa, H. valentiae, and L. porteaui) were found to contain agglutinins (lectins) for the first time.

In other countries, extracts of Ulva lactuca (Sampaio et al., 1998b), Caulerpa recemosa and Acanthophora spicifera (Ainouz et al., 1992) and Bryopsis plumosa (Rogers et al., 1980) were reported to agglutinate blood cells. B. plumosa extract, which was previously reported to agglutinate human blood groups A, B and O, did not agglutinate blood group A in the present study. Likewise, the extract of A. spicifera which showed agglutination activity with native blood cells of chick and goat in the present study showed no activity in the enzyme treated blood cells in an earlier study (Ainouz et al., 1992). Further, no activity was observed in the untreated rabbit blood in the present study. Extract of C. recemosa was not reported earlier to show any agglutination activity on any of the blood cells. On the contrary, in the present investigation, C. recemosa was found to show activity in 44% of the native blood and 66% in the enzyme treated blood. Agglutinin activity of the extract of B. plumosa in human blood groups B and O has been confirmed in the present study, as observed in a previous investigation (Rogers et al., 1980). But there was no activity in the blood group A, contrary to the previous study. Thus, agglutination activity of the different seaweed extracts could not be specified in different blood groups tested.

The present study has thus revealed the fact that a great majority of the seaweeds examined are a potential source of haemagglutinins, as their extracts agglutinated atleast one of the blood groups tested. So, there is much scope to explore the possibilities of utilizing the commonly available renewable natural resources, the seaweeds, for large scale extraction of haemagglutinin. Further, the present study suggests that the seaweed extracts possess potential haemagglutinins and a variety of erythrocytes should be used in the screening process for confirmation, as stated by Hori et al. (1988). Broad spectrum activity of extracts of some seaweeds (C. recemosa, C.scalpelliformis, U. lactuca, A. spicifera, G. corticata and G. crassa) and their sensitivity to sheep and human blood group O (native), rabbit, horse and chick blood cells (enzyme treated) remain to be confirmed.

The authors thank the authorities of CAS in Marine Biology, Annamalai University for providing facilities and encouragement. One of them R.M thanks the University Grants Commission, New Delhi, for granting UGC-teacher fellowship.

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