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SCREENING OF MARINE ALGAE OF KARACHI FOR HAEMAGGLUTININ ACTIVITY

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ABSTRACT

Ethanol extracts of 66 species of marine benthic algae, collected from the coastal areas of Karachi, Pakistan, were tested for haemagglutination against human erythrocytes of blood groups A, B, AB and O. Out of 23, 16 and 27 species of green brown and red seaweeds only 12, 5 and 14 species respectively exhibited a positive test for the bioactivity of haemagglutinins i.e. the activity wits found in half of the investigated species of Chlorophyta and Rhodophyta but only in one third of Phaeophyta.

Introduction

The proteinaceous secondary metabolites, haemagglutinins or more specifically pbylohaemarglueiniaraadlectias cause the clumping or agglutination of red blood cells in *vit*ro (Fabregas *et al.,* 1984). The agglutination activity was known since the last century, when it was noticed that an extract of castor beans would agglutinate red blood cells of different animals. Although chemical (Us and Sharon, 1973; Sharon and Lis, 1977), immunological (Jeffe, 1977), nutritional (Jeffe, 1983), regulatory (Oppenheimer, 1977) and other aspects of phytohaemagglutinins from higher plants have been thoroughly reviewed, the published information about the quality of agglutinins from seaweeds is rather insignificant. Only *a few* reports have appeared on the preliminary screening of haemagglutinins in marine algae prior to this communication (Boyd *et al,* 1966; Blunden *et al,* 1975; Fabregas *et al.,* 1984-1986).

Haemagglutinins cause adverse effects like reduced growth, diarrhoea, decreased nutrient absorption and increased incidence of bacterial infections in humans. The haemagglutinins bind themselves with the cells in the intestinal wall and cause a nonspecific interference with the nutrient absorption. Haemagglutinins also impair the immune system, thus enhancing the susceptibility for bacterial infections (Cheeke and Shull, 1985). Therefore, keeping in view of their medical significance in immunohaematology and that some of the phytohaemagglutinins have been found useful in blood banking (Bird, 1977), it was thought worthwhile to survey the marine algae found at the seacoast of Karachi for haemagglutinic bioactivity.

Materials and Methods

Healthy specimens of different species of marine benthic algae were collected from Manora, Buleji and Paradise Point (the coastal areas of Karachi) during the months of October to April 1990-91. The seaweeds were thoroughly washed to remove the epiphytes, epizoons and attached debris. The algae were identified as per method describd by Shameel (1990), and the algal nomenclature used therein has been adopted in the present studies. Voucher specimens of each species are preserved in Seaweed Biology & Phycochemistry Lab., MAHO Biological Research Centre, University of Karachi.

The algal material was dried under shade and minced. The grinded material was soaked with 50% aqueous ethanol (EtOH) in a percolater for 15 days. The aqueous EtOH was filtered, and the extract was concentrated under reduced pressure below 30C 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 double dilutions (two-fold) of the extracts were prepared.

Phosphate buffer (pH 7) was prepared by mixing 35 ml of a solution of Na2HPOe.2H2O (26 g/I) and 65 ml of a solution of anhydrous Na2HPO4 (18 g/1). Samples of Rh^o human blood (blood groups A, B, AB and 0) were obtained from Husaini Blood Bank, Karachi. Erythrocytes (RBCs) were obtained by simple centrifugation of the blood and 2% RBC suspension was prepared in phosphate buffer.

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 small test tube (5 ml capacity) followed by incubation in a water bath at 25°C. Controls were also run simultaneously. 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 erythrocytes) indicated a positive reaction. Weak, moderate and strong agglutinations were determined on the basis of the extent of granular deposition.

Results and Discussion

Around 66 species of marine benthic algae, collected from Karachi seashore, were tested for haemagglutinic activity, out of which only 31 exhibited positive activity (Table 1). However, accord[i]ng to Blunden *et al.* (1975) only 19% species of the British seaweeds showed positive results for haemagglutination. Therefore, the frequency of haemagglutination in Pakistani seaweeds appears to be more than double as compared with that of the British seaweeds. Out of 23, 16 and 27 investigated species of green, brown and red seaweeds of Pakistan only 12, 5 and 14 species respectively furnished a positive test for haemagglufinins. However, Fabregas *a at* (1986) observed a positive activity in 28 species of Spanish brown seaweeds.

Table 1

Haemagglutinic activities of the extracts of Karachi seaweeds against human RBCs (- = no agglutination; + = weak, + + = moderate and + + + = high agglutinations).

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
CHLOROPHYTA												
Ulvales												
Ulva anandii Amjad et Shameel	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. bifrons Ardré	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. fasciata Delile	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. fenestrata Post. et Rupr.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. lactuca Linn.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. reticulata Forsst.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. rigida C. Ag.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. sorensenii Chapm.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
U. taeniata (Setch.) Setch. et Gardn.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Cladophorales												
Chaetomorpha antennina (Bory) Kütz.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
C. spiralis Okam.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
Sphonocladales												
Valoniopsis pachynema (Mart.) Børg.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Bryopsidales												
Bryopsis pennata Lamour.	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)
Codiales												
Codium coronatum Setch.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
C. flabellatum Silva et Nizam.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
C. iyengarii Børg.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Caulerpales												
Caulerpa faridii Nizam.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
C. racemosa (Forssk.) J. Ag.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
C. scalpelliformis (R. Brown) C. Ag.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
C. sertularioides (S.G. Gael.) Howe	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)
C. taxifolia (Vahl) C. Ag.	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)	- (++)	+ (+++)	++ (+++)
Decaisnella indica (A. et E.S. Gepp) Farghaly	+ (++)	+ (+++)	++ (+++)	+ (++)	+ (+++)	++ (+++)	+ (++)	+ (+++)	++ (+++)	+ (++)	+ (+++)	++ (+++)
Halimeda tuna (Ellis et Soland.) Lamour.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
PHAEOPHYTA												
Dictyotales												
Dictyopteris australis (Sond.) Askenasy	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Dictyota dichotoma (Huds.) Lamour.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
D. divaricata Lamour.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
D. hauckiana Nizam.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
D. indica Sond. ex Kütz.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Lobophora variegata (Lamour.) Womers.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Padina tetrastromatica Hauck	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Spatoglossum asperum J. Ag.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
S. variabile Fig. et De Notar.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Stoechospermum marginatum (C. Ag.) Kütz.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Scytosiphonales												
Colpomenia sinuosa (Mart. ex Roth) Derb. et Sol.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Hydroclathrus clathratus (C. Ag.) Howe	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
Iyengaria stellata (Børg.) Børg.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Jolyna laminarioides Guimaraes *in* Guim. *et al.*	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Fucales												
Sargassum tenerrimum J. Ag.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Stokeyia indica Thivy et Doshi	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
RHODOPHYTA												
Bonnemaisoniales												
Scinaia complanata (Coll.) Cotton	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
S. fascicularis (Børg.) Huisman	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
S. hatei Børg.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Gelidiales												
Gelidium pusillum (Stackh.) Le Jol.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
G. usmanghanii Afaq. *et* Shameel	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Cryptonemiales												
Halymenia porphyriaeformis (Børg.) Parkins.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Corallinales												
Jania adherens Lamour.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Gigartinales												
Cystoclonium purpureum (Huds.) Batt.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
Gracilaria foliifera (Forssk.) Børg.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Hypnea pannosa J. Ag.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
H. valentiae (Turn.) Mont.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Plocamium cartilagineum (L.) Dixon	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
P. telfairiae (W. Hook. et Harv.) Harv. ex Kütz.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Sarconema filiforme (Sond.) Kylin	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Solieria chordalis (C. Ag.) J. Ag.	+ (++)	++ (++)	++ (++)	+ (++)	++ (++)	++ (++)	+ (++)	++ (++)	++ (++)	+ (++)	++ (++)	++ (++)
Ahnfeltiales												
Ahnfeltia plicata (Huds.) Fries	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Rhodymeniales												
Botryocladia leptopoda (J. Ag.) Kylin	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Champia parvula (C. Ag.) Harv.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
C. salicornioides Harv.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Ceramiales												
Acanthophora spicifera (Vahl) Børg.	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)	+ (+)	+ (++)	+ (++)

Seaweeds	Blood group A			Blood group B			Blood group AB			Blood group O		
	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)	1:2 (1:16)	1:4 (1:32)	1:8 (1:64)
Centroceras clavulatum (C. Ag.) Mont.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)
Membranoptera murrayi Børg.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Laurencia filiformis (C. Ag.) Mont.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
L. glomerata (Kütz.) Kütz.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
L. obtusa (Huds.) Lamour.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
L. pinnatifida (Huds.) Lamour.	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Polysiphonia platycarpa Børg.	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)	+ (+++)	++ (+++)	++ (+++)

Among green seaweeds all the 9 investigated species of *Ulva* including U. *fasciata* and *U. lacluca* showed negative response to agglutination. However, Blunden *et al* (1975) observed positive activity in the case of *U. lactuca*, but Boyd *et al* (1966) reported negative results with *U.* fasciata and *U. lactuca*, our results confirm

the later's findings. Negative observation in the case of *Chaetomorpha spp.* and positive activities in *Codium* spp. displayed the identity of results with those of Blunders *et al.* (1975) 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 the haemagglutination test.

Several brown seaweeds screened against human blood groups depicted negative test includ[i]ng *Dictyopteris australlis* and 4 species of *Dictyota*. These observations are analogous to the results of Blunden *et al.* (1975), but positive tests with *Colpomenia sinuosa* and *Padina tetrastromatica* were in contradiction to the negative activity of *Colpmenia peregrina* and *Padina pavonia* respectively. The filamentous brown algae i.e. species of *Ectocarpus, Giffordia* and *Hincksia,* which exhibit haemagglutination (Fabregas *et al,* 1986), could not be investigated due to their non-availability in sufficient quantities. Moreover, the tropical species such as *Lobophora variegata* (= *Zonaria variegata*), *Sargarsum tenerrimum* and *Spatoglossum asperum* exhibited the positive haemagglutinic activity.

Among the red seaweeds *Ahnfellia plicata* exhibited a positive while *Laurencia pinnatifida* and *Plocamium cartilagineum* a negative reaction, whereas Blunden *et al*. (1975) observed a negative activity in all these species from British Islands. However, Fabregas *et al* (1984) detected a positive activity in *L. pinnadfida.* The positive activities of Gracilana fohffera and Polyrfphonia platycarpa are in contradiction to the negative results shown by the British species of these genera. However, Fabregas *et al* (1984, 1985) observed a positive activity in these genera from the north-western coast of Spain. In other cases our results agreed with those of Blunden *et al* (1975). Furthermore, the tropical species such as *Acanthophora spicier., Botryocladia leptopoda, Cendoceras clavutum* 2 species of Hypnea, *Sarconemo filiformis, Scinaia cornplanata* and *Solieria chordalis* also agglutinated the RBCs.

If a comparative account of haemagglutinin test for different species of Chlorophyta, Phaeophyta and Rhodophyta is chalked out then half of the species belonging to Chlorophyta and Rhodophyta exhibited the activity, while the seaweeds of Phaeophyta showed the activity in only one third of the investigated species. However, 80% of the brown seaweeds investigated from the north -western coast of Spain (Fabregas *et al,* 1986) exhibited the haemagglutinic activity, while only 20% of the red seaweeds showed a positive activity (Fabregas *et al*., 1984, 1985). Broadly speaking our results are analogous with the findings of Blunden *et al.* (1975), a few deviations therein could be the result of differences in the extraction technique as well as the environmental and habitat variations between British and Pakistani seaweeds.

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