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Influence of environment on haemagglutinin activity and protein content of selected seaweeds from Gulf of Mannar Biosphere Reserve, India

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Abstract: Marine organisms are the potential source for bio chemical molecules. A variety of bio- active chemicals available among seaweeds, which is an important group of organisms in marine environment. The seaweeds available in the Gulf Of Mannar Biosphere Reserve (GOMBR), India, also showed intra and inter annual variations in their chemical composition. A total number of six seaweeds have been collected from the GOMBR in different seasons. Total protein content and haemagglutinin assays were carried over. The results showed the total protein content in greater variations during different seasons, with the maximum content during the summer season and the minimum content during the post monsoon season. Likewise, in the different blood groups, the haemagglutinin activity of the seaweed extracts showed pronounced variations, registering the minimum activity during the post monsoon season and the maximum activity during the summer season. Thus, the haemagglutinin activity of the seaweeds varied greatly corresponding to the protein content in different seasons. It suggests that protein content of the seaweeds is one of the important determining factors for the haemagglutinin activity. Further, it is concluded that the summer season is the best season for collecting seaweeds from GOMBR, for the extraction of protein and haemagglutinin.

Keywords: Seaweeds; Macro algae; Haemagglutinin; Protein; Seasons; Gulf of Mannar Biosphere Reserve

INTRODUCTION

Different algal species vary greatly in their biochemical constituents and detailed studies on the biochemistry of seaweeds have been conducted worldwide. Variations in biochemical constituents of seaweeds have been reported by several workers[1-5]. Investigations have also shown that biochemical constituents of the seaweeds are subjected to changes during different seasons, as the seaweeds are experiencing different environmental conditions in a year[6]. Seaweeds are renewable resources from marine environment. Certain seaweeds are having significant amount of lipids, proteins, vitamins and minerals[7-9], nutrient contents also vary with species[10, 11] season, temperature and geographical location [12, 13].Temperature also effect the growth and chemical content of *Palmyra palmate* [14] *Laminaria sp* [15] *Macrocystis sp* [16,17] and *Nereocystis sp* [18]. Such seasonal variations in biochemical constituents of seaweeds would definitely have influence on the haemagglutinin activity and therefore it is essential to understand the impact of different seasons on the haemagglutination activity of seaweeds and to find out the favourable season for large scale harvesting of suitable seaweed species for extraction of haemagglutinin. Hence, the present attempt is made.

MATERIAL AND METHODS

Collection Area: The Gulf of Mannar (where the seaweed samples were collected for the present investigation), lying along the east coast of India, is greatly influenced by the northeast monsoon which is prevalent during October – December of every year. Accordingly, four seasons can be categorized in a calendar year for this region viz. monsoon (October-Decembar), post monsoon (January-March), summer (April- June) and pre monsoon (July- September). Seaweeds, *Caulerpa racemosa, C. scalpelliformis, Sargassum wightii, Acanthophora spicifera, Gracilaria corticata* and *G. crassa* that showed broad spectrum of haemagglutinin activity were alone considered for studying the seasonal variations in haemagglutinin activity and protein content.

Protein estimation: Total protein content of the seaweed extracts was estimated as per the commonly used method [19] using Bovine Serum Albumin as standard. The protein content of the seaweed extract has been expressed as mg/ml of extract.

Haemagglutinin Test: Haemagglutinin tests were performed with 96 wells U bottom micro titer plates. Agglutinations were 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 with control of 0.85% NaCl solution with erythrocytes[20].Number of different blood groups that were agglutinated by a seaweed extract against the total number of blood groups tested was multiplied with 100 to find out the percent agglutination activity of seaweed extracts in the total blood samples (Table-3).

RESULTS AND DISCUSSION

Present study has revealed the occurrence of seasonal variations in protein content and haemagglutination activity of the different seaweed species tested **(Tables 1,2 and 3)**

Table 1: Protein content of seaweeds in different season

Seaweed species	Protein content (mg ml^{-1})			
	Post -Monsoon	Summer	Pre -monsoon Monsoon	
C. racemosa	0.62	1.25	0.76	0.8
C. scalpelliformis	0.68	0.98	0.71	0.79
S. wightii	0.43	0.87	0.44	0.71
A. spicifera	0.62	1.25	0.76	0.80
G. corticata	0.68	0.98	0.71	0.79
G. crassa	0.58	0.88	0.72	0.72

Table 2: Haemagglutinin activity (HU %) of seaweeds recorded in different seasons.

Seaweed	Post monsoon	Summer	Pre monsoon	Monsoon
C. racemosa	**44**	**77**	44	66
C.scalpelliformis	**11**	66	**22**	44
S. wightii	**11**	**77**	**22**	**55**
A. spicifera	**44**	100	**66**	**66**
G. corticata	**66**	100	**66**	**66**
G. crassa	**44**	100	**77**	**55**

Table 3: Haemagglutinin activity of Seaweed extract in different blood samples

Seaweed species	Activity (HU) in different blood samples								
	A	**B**	**AB**	**O**	**CB**	**SB**	**GB**	**RB**	**HB**
Post monsoon season									
C. racemosa	-	-	-	1:04	1:02	1:04	-	1:32	-
C. scalpelliformis	-	-	-	-	-	-	-	-	1:08
S. wightii	-	-	-	-	-	--	-	-	1:08
A. spicifera	-	-	-	1:04	-	1:04	-	1:16	1:08
G. corticata	1:08	1:04	-	1:04	1:04	1:04	-	-	1:08
G. crassa	1:04	-	-	-	1:04	-	-	1:08	1:04
Summer season									
C. racemosa	1:02	-	-	1:04	1:04	1:04	1:04	1:16	1:08
C. scalpelliformis	1:02	-	-	1:04	1:04	1:08	1:08	1:04	-
S. wightii	1:04	-	1:02	1:02	1:02	1:02	-	1:08	1:08
A. spicifera	1:08	1:08	1:04	1:04	1:08	1:08	1:04	1:32	1:16
G. corticata	1:04	1:04	1:04	1:08	1:16	1:08	1:08	1:16	1:32

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G. crassa	1:04	1:04	1:02	1:04	1:08	1:04	1:04	1:64	1:32
Pre monsoon season									
C. racemosa	-	1:04	-	1:02	-	1:04	-	1:04	-
C. scalpelliformis	1:02	-	-	-	1:04	-	-	-	-
S. wightii	1:02	-	-	-	-	1:04	-	-	-
A. spicifera	-	1:02	1:04	-	1:04	1:04	1:08	-	1:08
G. corticata	1:02	1:04	1:02	1:04	1:04	1:04	-	-	-
G. crassa	1:02	1:04	1:02	1:08	-	1:04	1:08	-	1:32
Monsoon season									
C. racemosa	-	-	1;2	1:04	1:02	1:04	-	1:04	1:04
C. scalpelliformis	-	-	-	-	1:04	1:02	1:04	1:08	-
S. wightii	-	-	1:02	1:04	1:02	-	1:02	--	1:04
A. spicifera	1:04	1:04	-	1:02	1:02	-	-	1:04	1:08
G. corticata	-	1:04	1:02	1:04	1:04	-	1:04	-	1:08
G. crassa	-	-	1:02	-	1:04	1:04	-	1:08	1:08

A ,B ,AB and O-Human Blood; CB- Chiick blood; SB- Sheep Blood; GB- Goat blood; RB-Rabbit blood; HB- Horse blood

Protein content: Table 1 shows the temporal variations in the protein content of the different seaweeds studied, during the different seasons. Protein content ranged from 0. 45 to 0.89 mg ml^{-1} in *C. recemosa*, 0.42 to 0.65 mg ml^{-1} in *C. scalpelliformis*, 0.44 to 0.87 mg ml^{-1} in *Sargassum wightii* , 0.62 to 1.25 mg ml^{-1} in *Acanthophora spicifera*, 0.71 to 0.98 mg ml^{-1} in *Gracilaria corticata* and 0.43 to 1.15 mg ml^{-1} in *G. crassa*. In general, in all the seaweeds, the protein content was high during the summer season, Low protein content was noticed during the post monsoon season.

Haemagglutinin activity: Haemagglutination activity (%) of the extracts (Table-2) of *C. racemosa* collected during the summer season showed positive activity (77%) with more number of blood samples followed by monsoon (66%), post monsoon and pre monsoon (44%) seasons. Extract of *C. scalpelliformis* exhibited activity with more number of blood samples (66%) during the summer season and with less number of samples (11%) during the post monsoon season. *S. wightii*, *A. spicifera* and *G. crassa* also showed similar pattern of activity during the different seasons. Extract of *G. corticata* was active in more number of blood samples during the summer season (100%) and showed lesser activity during the post monsoon, pre monsoon and monsoon seasons (66%). It is interesting to note that 100% agglutination activity was seen in all blood samples tested with the extracts of *A. spicifera*, *G. corticata* and *G. crassa*, collected during the summer season.

Thus, in general, the haemagglutinin activity of different seaweed extracts tested was more during the summer season and less during the post monsoon season.

DISCUSSION

Protein content of the seaweed extracts was high during the summer season in all the seaweeds tested (Table 1), though the protein content differed in the different species. Such variations in the protein content of different species could be related to their physiological and morphological variations as opined by Benevides *et al*.[21]. The present finding lends support to the observations of Ganesan and Kannan[6] in their study on the seasonal variations of biochemical constituents of some economic seaweeds of the Gulf

of Mannar have concluded that the protein content of the seaweeds is subjected to seasonal fluctuations registering the maximum protein content during the summer season when the nutrient concentrations were high and the environmental conditions were favourable.

Haemagglutinin activity of the seaweed extracts also showed distinct variations among the different seaweed species and seasons, studied presently. In general, haemagglutinin activity of the extracts of seaweed samples collected during the summer season was observed to be more in the broader-spectrum blood samples, as compared to other seasons. Corresponding to this, protein content of the extracts was also more during the summer season suggesting that protein content of the seaweeds is one of the important determining factors for the haemagglutinin activity. The haemagglutinin unit (HU) of the algal extracts varied from 1:2 to 1:64 during the different seasons in one and the same seaweed species as well as in different algal species (Table 3). This would indicate that the varying amounts of seaweed proteins recorded during the different seasons would have some influence on haemagglutinin activity.

CONCLUSION

It should also be noted that there was no agglutination activity in the extracts of any of the seaweeds collected during the post monsoon season with goat blood and during the pre-monsoon season with human blood group B, though significant amount of protein was present in the extracts of the seaweed samples. This finding indicates that it would be best to collect the seaweeds during the suitable season for the extraction of haemagglutinin. This would help a great deal when naturally occurring seaweed sources are considered for large-scale extraction of haemagglutinin. From the present study, it is suggested that the summer season is the best season for collection of seaweeds (from GOMBR, India) for haemagglutinin extraction, as also opined by Benevides *et al.*[21] for extraction of haemagglutinin from the seaweeds of Brazil.

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