Purification and Characterization of a Sialic Acid Specific Lectin Isolated from the Marine Crab *Grapsus albolineatus* (GaLec)

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Abstract

Lectins one of the defensive protein molecules have become the focus of intense interest for biologists and in particular for the research and applications in medicine. In this study, a lectin GaLec was purified from the marine crab Grapsus albolineatus by affinity chromatography using Fetuin-Sepharose 4B column. GaLec showed specific affinity for rat erythrocytes as evident from the hemagglutination assay. Physico chemical analysis of the GaLec demonstrated high hemagglutination activity ranging from pH 7.5 to 9 and temperature 0 to 40˚C. The GaLec was dependent on calcium, magnesium and manganese. It was reversibly sensitive to EDTA and trisodium citrate. Hemagglutination activity was inhibited by the sugar N-acetyl D-glucosamine and N-acetyl D-galactosamine and the glycoproteins fetuin and lactoferrin. Reduction in HA with disialylated fetuin confirms the sialic acid specificity of the lectin. The reduction of HAI following de-O-acetylation confirms the specificity of the lectin for O-acetyl sialic acid.

Keywords*: Grapsus albolineatus,* Fetuin, Sepharose, EDTA, Hemagglutination, Sialic acid

1. Introduction

Invertebrate lectins make an important contribution to innate immune protection and work along with epithelial barriers, cellular defence such as phagocytosis and pattern recognition receptors that trigger pro-inflammatory signalling cascades (Shibata et al. 1989). Lectins are glycoproteins that are characterized by their capability to bind to carbohydrates such as mannose, galactose, lactose, N-acetyl glucosamine, N-acetyl galactosamine, fucose and rhamnose with significant specificity. They differ from enzymes because their carbohydrate binding properties never change, and they are unlike antibodies, not induced as an immune response (Faheina-Martins et al. 2012). Lectins with specificity for different kinds of sialic acids and their glycosidic linkages serve as potential diagnostic tools (Mercy and Ravindranath 1992). Therefore, lectins that specifically recognize various sialic acids and their carbohydrate binding patterns can be used in investigations related to the cellular and tissue transport of carbohydrates, glycoproteins and calcium (Vasta 1992), cell adhesion (Devi et al. 2010), migration and apoptosis (Ponraj et al. 2016) and as cytolytic and cytotoxic factors (Armstrong et al. 1996). Purifying a lectin present in the hemolymph of decapod crustaceans is tedious as it has to be separated from abundant proteins such as hemocyanin, lipoproteins which tend to aggregate and stick to foreign surfaces as clotting factors and phenoloxidase (Stratakis et al. 1992). In affinity chromatography a potent inhibitor (sugar/glycoprotein) is immobilized on an insoluble matrix by coupling to agarose activated with Cyanogen bromide (Ravindranath et al. 1985).

Sialic acid specific lectins has wide applications in the field of immunology, cell biology, glycobiology, oncology and other areas of research activities and many reports predict their occurrence among arthropods and molluscs. Sialic acid specific lectins have been reported from crabs such as *Cancer antennarius* (Ravindarnath et al. 1985), *Scylla serrata* (Mercy and Ravindranath 1992), *Callinectus danae* (Moura et al. 2015), *Portunus trituberculatus* (Lu et al. 2017) and *Atergratis integerrimus* (Elayabharathi et al. 2019) and *Lamella lamellifrons* (Mary Mettilda Bai & Basil Rose 2020). Hence it was decided to purify the hemagglutinin or lectin of the marine crab *G. albolineatus* following affinity chromatography method using fetuin linked Sepharose 4B, based on the physico chemical characterization.

2. Materials and Methods

2.1 Animal Collection

The marine crab *Grapsus albolineatus* were collected from Kadiyapatanam (8.1262°N latitude and 77.3196°E longitude) and Muttom (37.6428°N latitude and 78.3924°E longitude) coasts, Kanyakumari, Tamilnadu, India.

2.2 Collection of hemolymph from crab

Hemolymph was drawn from both male and female healthy crabs. The clot and the cellular elements were removed by ultra-centrifugation at 30,000 rpm at 4°C. The serum was either used immediately or stored in freezer (-20°C).

2.3 Hemagglutination assay

Hemagglutination assays were carried out as described by Ravindranath and Paulson (1987) to find out the presence of hemagglutinin and to know the erythrocyte specificity. The strategies for adsorption and elution steps, especially pH, temperature, calcium ion requirement and sugar/glycoprotein specificity were formulated based on previous reports (Rathika et al. 2022).

2.4 Chemicals

Purchased Polypropylene econo columns from Bio-Rad; Cyanogen bromide activated Sepharose 4B, sugars, glycoproteins, *Clostridium perfrigens* Neuraminidase (Type X) from Sigma, Bangalore and prestained molecular weight protein markers from Genei, Bangalore.

2.5 Buffers

Buffers used in this study were identified in the text by the following abbreviations: TBS (Tris Buffered Saline)- 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$; TBS-BSA (Tris Buffered Saline with Bovine Serum Albumin)- TBS, pH 7.5 with 0.05% BSA; HSB (High Salt Buffer)- 50 mM Tris HCl, pH 7.5, 1 M NaCl, 10 mM $CaCl_2$; EB (Elution Buffer)- 50 mM Tris-HCl, pH 7.5, 0.3 M NaCl, 10 mM disodium EDTA; Acetate buffer- 0.1 M sodium acetate, 0.5 M NaCl, pH 4; Coupling buffer- 0.1 M sodium bicarbonate, 0.5 M NaCl, pH 8.3; PBS (Phosphate Buffered Saline)- 75 mM NaCl, 75 mM Na_2HPO_4, pH 7.5; PBS-BSA (Phosphate Buffered Saline with Bovine Serum Albumin)- pH 7.5, with 0.05% BSA.

2.6 Purification of lectin

The lectin was purified by affinity chromatography by the method of Roche (1975). Clarified serum (20 ml) was applied to 3.5 ml of fetuin-Sepharose 4B econo column (Bio-Rad) previously equilibrated with TBS at 4°C. The effluent was collected at a rate of 0.6 ml/minute. The column was washed with HSB until the absorbency of the effluent at 280 nm was lesser than 0.002 and the effluent was tested for HA activity. The column was further washed with LSB at 4°C until the absorbency of the effluent was lesser than 0.002 at 280 nm and it was then washed with warm LSB (30±2°C) until the A_{280} of the effluent was lesser than 0.002. This step was necessary for obtaining homogenous lectin as it eluted the extra inert protein. All the buffers so far used containing the calcium required for lectin binding to fetuin-Sepharose 4B. The elution of lectin was done with EB that contained 10 mM disodium EDTA and collected as 1 ml fractions on ice in polypropylene tubes containing 10 µl of 10 mM calcium chloride at the rate of 0.3 ml/minute. The fractions were vortexed immediately after collection and kept on ice. Fractions containing lectin were pooled on the same day and dialysed against 10 mM $CaCl_2$ at 4°C for 30 minutes to 1 hour and the dialysate was then aliquoted, lyophilized (Lyodel-DPRG-1GH)) and stored at 20°C. The protein concentration of the lectin was estimated by Folin Ciocalteau Method (Lowry et al. 1951). The specific lectin activity was obtained by dividing the HA titer with the protein concentration of the sample (Suzuki and Nazori 1983; Wang et al. 2000).

2.7 Determining the molecular weight

Sodium dodecyl sulphate polyacrylamide 11% slab gel electrophoresis was performed according to Laemmli (1970). Samples were heated for 3 minutes at 100°C in sample buffer (25% 1 M Tris-HCl, pH 6.8), 4% SDS; 2% β-mercaptoethanol and 5% glycerol. Gels were fixed and stained with a solution containing 0.05% coomassie blue R-250, 7% acetic acid and 50% isopropyl alcohol and destained with a solution containing 50% ethanol and 7% acetic acid at room temperature (30±2°C).

2.8 pH and thermal stability

pH and thermal dependence of agglutinin was measured by pre-incubating the hemolymph at specific pH (5.5-11.5) and temperature (0˚C-100˚C) for 1 hour before adding erythrocyte suspension for hemagglutination assay.

2.9 Cations and EDTA

Cations and EDTA treatment to study divalent cations (Ca^{2+}, Mg^{2+} and Mn^{2+}) dependence of Hemagglutinin, HA assays were performed in TBS (pH 7.5) without and with these ions at varying concentrations. To study the effect of calcium chelators (EDTA and trisodium citrate) on the agglutinin, the hemolymph was pre-incubated at different concentrations (0 to 100 mM) of EDTA and trisodium citrate for 1 hour before adding erythrocyte suspension for HA assay

2.10 Hemagglutination Inhibition Assay

The Hemagglutination inhibition (HAI) assay was carried out with known concentration of glycoproteins and sugars by following the procedure of Ravindranath et al. (1985) to know the carbohydrate specificity of the agglutinin.

2.11 Sialic acid specificity of the purified lectin

To find out whether the lectin is sialic acid specific or not, HA and HAI assays were performed with sialidase treated erythrocytes and glycoproteins respectively.

2.12 Sialidase treatment of erythrocytes and sialoglycoproteins

To study the sialic acid/neuraminic acid specificity, hemagglutination assay was performed with asialo erythrocytes (Ravindranath et al. 1988; Mercy and Ravindranath 1993). A reaction mixture (total 5 ml) containing 10% washed erythrocytes in PBS-BSA (pH 7.5) and 140 mU (milli units) neuraminidase of *Clostridium perferigens* (Type X sigma) was incubated for 4 hours at room temperature. The treated cells were washed with PBS-BSA three times and pelleted by low speed centrifugation. Finally they were washed in TBS-BSA (pH 7.5) and used for HA activity analysis.

Asialofetuin was prepared by incubating 2 mg of glycoproteins with 0.1 unit of *Clostridium perfringens* sialidase (Type X, Sigma) in 400 µl of 5 mM acetate buffer, pH 5.5 for 3 hours at 37°C. As a control, fetuin was treated similarly without sialidase treatment. HAI assay was performed with purified lectins for treated and untreated glycoprotein against 1.5% of rat erythrocytes.

2.13 O-acetyl specificity

De-O-acetylation of silaic acids in the glycoprotein fetuin was performed following the procedures of Sarris and Palade (1979) and Schauer (1982). A solution of 750 µl of glycoprotein fetuin (5 mg/ml) was added to 250 µl of 0.04 N of NaOH, vortexed and incubated on ice for 45 minutes and neutralized with 1 ml of 0.01 N HCl. For control, untreated fetuin was reconstituted in TBS. The HAI assay was performed using base treated and untreated fetuin against purified lectin with 1.5% suspension of rat erythrocytes.

3. Results

3.1 Purification of lectin affinity chromatography

Lectin from the hemolymph of the marine crab *G. albolineatus* was purified by affinity chromatography on fetuin coupled Sepharose 4B. Major portion of the protein is removed from the column when washing with high salt buffer and low salt buffer. The purification fold of lectin from hemolymph by affinity chromatography resulted in 170 fold increase in specific activity (Table -1).

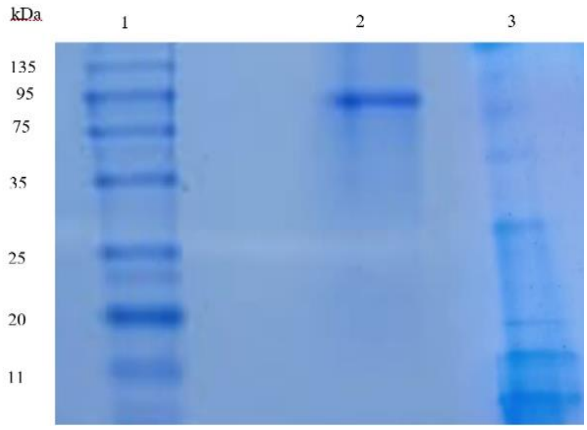
Table 1: Purification of lectin from the marine crab *G. albolineatus*

Sample	**Volume (ml)**	**Protein**	**Total activity (HA units)**	**Specific activity (HA units/mg)**	**Purification fold**
Crude hemolymph	30	3203.1	2.45×10^{6}	767.25	1
Clarified serum	20	1423.6	4.04×10^{5}	287.72	37.5
Purified lectin using Fetuin Sepharose affinity column	15	4.71	6.14×10^{5}	1.3×10^{5}	170.01

3.2 Electrophoresis analysis

The purified lectin (GaLec) was analysed using SDS-PAGE electrophoresis. GaLec showed a single protein band with a molecular weight of 95 kDa (Fig 1).

Fig 1 SDS-PAGE of purified lectin from the hemolymph of the crab *G. albolineatus*



3.3 Hemagglutination assay

The hemolymph lectin of the marine crab *G. albolineatus* agglutinated a wide variety of mammalian erythrocytes with varying HA titer: rat > goat = mice > ox = rabbit = buffalo > guinea pig = pig = human B = human O. Maximum HA titer (2048) was observed with rat erythrocytes (Table 2).

Table 2: HA titer of GaLec

Erythrocytes (n=5)	HA titer	
	Native	Purified
Rat	2048	2048
Goat	8	8
Mice	16	16
Rabbit	2	2
Buffalo	2	2
Pig	0	0
Human B	2	2
Human O	0	0

3.4 Effect of pH and temperature

Hemagglutination activity of the hemolymph lectin was sensitive to pH and temperature. The HA activity was maximum between pH 7.5-9 which gradually decreased with either increased acidity or alkalinity. Maximum HA was observed between temperature 0˚-40˚C. When the sample was heated above 70˚C, a complete loss of hemagglutination was observed (Table - 3).

Table 3: Effect of pH and temperature on GaLec

pH (n=10)	HA titer		Temperature (˚C) (n=10)	HA titer	
	Native	Purified		Native	Purified
5.5	256	256	0	2048	2048
6	512	512	10	2048	2048
6.5	512	512	20	2048	2048
7	2048	2048	30	2048	2048
7.5	2048	2048	40	2048	2048
8	2048	2048	50	1024	1024
8.5	2048	2048	60	512	512
9	2048	2048	70	128	128
9.5	1024	1024	80	0	0
10	512	512	90	0	0
10.5	512	512	100	0	0
11	256	256			

3.5 Effect of cations and chelators on EDTA

HA titer value was altered with the different concentrations of divalent cations like Ca^{2+}, Mn^{2+} and Mg^{2+}. 10 mM concentration of Ca^{2+}, Mn^{2+} and Mg^{2+} was found to be the optimum concentration. Presence of EDTA had a significant effect on the HA activity of the hemolymph lectin (GaLec). There was no reduction in HA up to 1 mM and a great reduction was observed at 10 mM and complete loss at 20 mM of disodium EDTA (Table – 4 & 5).

Table 4: Effect of cations on GaLec

Concentration of cations (Mm) (n=10)	HA titer					
	Ca^{2+}		Mg^{2+}		Mn^{2+}	
	native	Purified	native	Purified	native	Purified
0	1024	1024	1024	1024	1024	1024
0.01	1024	1024	1024	1024	1024	1024
0.1	1024	1024	1024	1024	1024	1024
1	1024	1024	1024	1024	1024	1024
10	**2048**	**2048**	**2048**	**2048**	**2048**	**2048**
20	1024	1024	1024	1024	1024	1024
30	1024	1024	1024	1024	512	512
40	1024	1024	512	512	512	512
50	512	512	256	256	128	128
100	256	256	128	128	128	128

Table 5: Effect of calcium chelators on GaLec

Concentration of calcium chelators (mM) (n=10)	HA titer					
	Disodium EDTA		Tetra sodium EDTA		Trisodium citrate	
	Native	Purified	Native	Purified	Native	Purified
0	1024	1024	1024	1024	1024	1024
0.01	2048	2048	2048	2048	1024	1024
0.1	2048	2048	2048	2048	1024	1024
1	2048	2048	2048	2048	1024	1024
10	512	512	2048	2048	2048	2048
20	0	0	512	512	1024	1024
30	0	0	512	512	256	256
40	0	0	256	256	128	128
50	0	0	0	0	0	0
100	0	0	0	0	0	0

3.6 Hemagglutination Inhibition Assay

Sugar binding specificity of hemolymph lectin was examined by hemagglutination inhibition tests using carbohydrates and glycoproteins. Agglutinability was inhibited by sugars like GlcNAc and GalNAc. Of the various glycoprotein tested hemagglutinability was inhibited by fetuin and lactoferrin (Table 6 & 7).

Table 6: HAI titer of the GaLec by sugars

Sugars	HAI titer		Minimum concentrations required for inhibition (mM)	Relative Inhibitory Potency (%)
	Native	Purified		
D-Galactosamine	4	4	50	6.25
D-Glucose-6-Phosphate	4	4	50	6.25
α-lactose	8	4	50	6.25
Trehalose	8	8	25	12.5
D-Mannosamine	8	8	25	12.5
N-acetyl neuraminic acid	16	16	12.5	25
N-acetyl-D-Glucosamine	64	64	3.12	100
N-acetyl-D-Galactosamine	64	64	3.12	100

Table 7: HAI titer of the GaLec by glycoprotein

Glycoproteins	HAI titer		Sialic acid		Minimum concentrations required for inhibition (µg/ml)	Relative inhibitory Potency
	Native	Purified	Nature	Linkage		
BSM	2	2	NeuAc/NeuGc	α 2-6 GalNAc	2500	6.25
PSM	2	2	NeuAc	α 2-3 Gal β 1-3 GalNAc	2500	6.25
Transferrin	2	2	NeuGc	α, 2-3 GaL 1-4 GluNAc β1	2500	6.25
Apotransferrin	4	4	NeuGc	-	1250	12.5
Thyroglobulin	8	8	NeuGc	α 2-6 Gal	625	25
Lactoferrin	16	16	NeuAc/NeuGc	-	312.5	50
Fetuin	32	32	NeuGc	α 2-3 Gal	62.5	100

3.7 Sialic acid specificity of the purified lectin

64 fold reduction in the HA titer of the lectin was noticed with the sialidase tested rat erythrocytes. This inferred the sialic acid specificity of the lectin.

Table 8: HA/HAI titer of GaLec with sialidase treated and untreated erythrocyte/glycoprotein

Treatment (n=3)		HA/HAI titer
Rat erythrocytes	Untreated	2048
	Treated	32
Glycoprotein fetuin	Untreated	32
	Treated	16

3.8 De-O-acetylation

De-O-acetylation of fetuin declines the hemagglutination inhibition activity. It reveals the O-acetyl specific nature of the lectin (Table 9).

Table 9: HAI titer of GaLec with De-O-acetylated fetuin

Treatment (n=3)	HAI titer
Untreated	32
Treated	16

4. Discussion

The hemolymph lectin of the marine crab *Grapsus albolineatus* (GaLec) was purified by affinity chromatography. The hemagglutinability of the lectin showed the greater affinity for rat erythrocytes may be due to the presence of sialic acid residues in the appropriate position that could be easily recognised by the *G. albolineatus* hemolymph lectin. The glycocalyx of rat erythrocytes contain NeuGc/NeuAc/4(9)-O-acetylated sialic acids, NeuAc α 2-3 Galβ1, GalNAc β1-4 Gal (3-2α NeuGc) β1-4 Glcβ1 in addition to NeuGc α, 2-3 Galβ1-4 GluNAcβ1 residues. These results also suggest that the RBC types agglutinated by a lectin from the marine crab *G. albolineatus* probably share a common surface receptor but with a quantitative difference in HA sites.

The HA activity of the hemolymph lectin GaLec was optimum between pH 7.5-9 and it was sensitive to low or high pH. Basic neutral pH represents the natural environment of animals. The polymeric hemocyanin molecules are in equilibrium with agglutinin molecules and with rise in pH at and above 7.5 the hemocyanin molecules may dissociate and release the agglutinin. These could be the cause for the increase in HA activity at and above pH 7.5 which remained stable upto pH 9. Hemagglutination was stable between temperatures 0˚- 40˚C, but the activity was completely abolished beyond 80˚C. The loss of hemagglutinability with increasing temperature is evidently due to heat induced denaturation of lectin. These denaturation may weaken the interaction between lectin and the carbohydrate ligand leading to attenuated agglutinating activity (Qadir et al. 2013). HA titer value was altered with the different concentrations of divalent cations like Ca^{2+}, Mg^{2+} and Mn^{2+}. 10 mM concentration of Ca^{2+}, Mg^{2+} and Mn^{2+} was found to be the optimum concentration. Very low and high concentrations of divalent cations are significant in stabilizing the primary structure of hemagglutinins (Anderson & Good 1975).

In hemagglutination inhibition assay the specificity of the lectin towards carbohydrates is mainly N-acetyl-D-Galactosamine and N-acetyl-D-Glucosamine and the glycoprotein fetuin and lactoferrin. The N-glycosidically linked units of fetuin includes a single polypeptide chain to which three heteropolysaccaride units made up of sialic acid, galactose, GluNAc and mannose are attached through asparagine residues (3:3:3:5) (Baennziger and Fiete 1979). The heteropolysaccaride units also include the presence of two residues of α 2-3 and one residue of α 2-6 sialic acids. Fetuin thus contains sialic acid α 2-3 and sialic acid α 2-6 in a 2:1 ratio. The distal end of glycan chains are linked via α 2-3 linkage to Gal and GalNAc to sialic acid (Tsuji et al. 1996), α 2-3 linkage to GalNAc (Murali Krishna et al. 1992) and α 2-6 linkage to GlcNAc (Green et al. 1995). Lactoferrin consists of single polypeptide chain, with two glycans attached to it through N-glycosidic linkages. They possess N-acetyl neuraminic acid, mannose, fucose, N-acetyl glucosamine and N-acetyl Galactosamine (Coderville et al. 1992).

A sialic acid specific lectin from the marine crab *Grapsus albolineatus* was purified by affinity chromatography using fetuin coupled Sepharose 4B column. The purified lectin was designated as GaLec. The marine crab *G. albolineatus* hemolymph lectin (GaLec) resulted in 170 fold increase in specific activity. The physico-chemical characterization of the hemolymph lectin was more or less same as the native (Rathika et al. 2022). The purified lectin was homogenous lectin with a single band at 95 kDa. Homogenous lectin with different molecular weight were also reported in the arthropods (Nowak and Barondes 1975, Ponraj et al. 2016,

Mary Mettilda Bai and Basil Rose 2020). Sialidase treatment of rat erythrocytes and sialoglycoproteins resulted in the depletion in the affinity of the lectin to desialylated erythrocytes and glycoproteins, confirming its sialic acid specificity. Also de-O-acetylation of glycoprotein reduced the inhibitory ability of the glycoprotein fetuin tremendously. Hence it could be suggested that the inhibitory potency of fetuin may be due to O-acetyl group (Wasik et al. 2017). Thus it confirms the O-acetyl sialic acid specificity of the lectin isolated from the crab *G. albolineatus* and could be used to identify malignant cells as that of the lectin isolated from the crab *Cancer antennarius* (Ravindranath et al. 1985).

5. Conclusion

The hemolymph lectin (GaLec) was purified from the marine crab *Grapsus albolineatus.* The results presented here provide evidence of the purified lectin GaLec which possesses carbohydrate binding site for specific sugars GlcNAc and GalNAc and glycoproteins fetuin and lactoferrin. The study of invertebrate lectins, a host defence system may lead to useful applications, such as discovery of unique molecules of pharmacological importance and the development of strategies for control of harmful microorganisms, insects and parasites. Isolation of an O-acetyl sialic specific lectin is of prime importance as it can be applied in lectin targeted therapy against cancer cells. Moreover, this lectin can be conjugated to nanomaterials that are biocompatible and applied as nano based drug delivery system to treat microbial disease and cancer and the invitro studies are in progress.

Acknowledgement

The authors are grateful to the management of Holy Cross College (Autonomous), Nagercoil for providing all facilities to carry out the research in the Research centre of the Department of Zoology.

6. Reference

[1] R.S. Anderson and R.A. Good, “Naturally occurring hemagglutinin in the tunicate, Halocynthia phyriformis”, Biological Bulletin vol. 148, (1975), pp. 357-367.

[2] P.B. Armstrong, S. Swarnakar, S. Srimal, S. Misquith, E.A Hahn, R.T. Aimes, RT and J.P Quigley, “A cytolytic function for a sialic acid binding lectin that is a member of the pentraxin family of proteins”, The Journal of Biological Chemistry, vol. 271, (1996), pp. 14717-14721.

[3] J.U. Baenziger and D. Fiete, “Structure of the complex oligosaccharides of fetuin”, Journal of Biological Chemistry, vol. 254, no. 3, (1979), pp. 789-795.

[4] B. Coderville, G. Strecker, J.M. Wieruszeki and F.G. Johannes, “Heterogenity of bovine lacto transferrin glycans. Characterization of α-NeuAc-(2→6)-β-D-GalNAc-(1→4)-β-D-GluNAc substituted N-linked glycans”, Carbohydrate Research, vol. 236, (1992), pp. 145-164.

[5] R.V. Devi, M.R. Basil-Rose and P.D. Mercy Prospect for lectins in arthropod”, Italian Journal of Zoology, vol. 77, (2010), pp. 254-260.

[6] T. Elayabharathi, V.J Mary and M.M. Bai, "Characterization of a novel O-acetyl sialic acid specific lectin from the hemolymph of the marine crab, *Atergatis integerrimus* (Lamarck 1818)", Fish and Shellfish Immunology, vol. 106, (2019), no. 25, pp. 1131-1138.

[7] G.V. Faheina-Martins, A.L. da Silveira, B.C. Cavalcanti, M.V. Ramos, M.O. Moraes, C. Pessoa and D.A. Araujo, "Antiproliferative effects of lectins from Canavalia ensiformis and Canavalia brasiliensis in human leukemia cell lines" Toxicol In Vitro, vol. 26, (2012) pp. 1161–1169.

[8] P.J. Green, C.T. Yuen, R.A. Childs, W. Chai, M. Miyasaka, R. Lemoine, A. Lubineau, B.Smith, H. Ueno, K.C. Nicolaou and T. Feizi, "Further studies of the binding specificity of the leukocyte adhesion molecule, L-selectin towards sulphated oligosaccharides suggestion of a link between the selectin and the integrin mediated lymphocyte adhesion systems", Glycobiology, vol. 5, (1995), pp. 29-38.

[9] R.M. Moura, A.A. Melo, Carneiro, R.F. Rorigus, P. Delatorre, K.S. Nascimento, S. Saker-Sampaio, C.S. Nagano, B.S. Cavada, and H. Sampaioa, "Hemagglutinating/Hemolytic activities in extracts of marine invertebrates from the Brazilian coast an isolation of two lectins from the marine sponge *Cliona varians* and the sea cucumber *Holothuria grisea"*, Annals of the Brazilian Academy of Sciences, vol. 87, no. 2, (2015), pp. 973-984.

[10] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, "Protein measurement with folin phenol reagent", The Journal of Biological Chemistry, vol. 193, no. 1, (1951), pp. 265-275.

[11] J. Lu, Z. Yu, C. Mu, R. Li, W. Song, and C. Wang, "Characterization and functional analysis of novel C-type lectin from the swimming crab *Portunus trituberculatus*", Fish and Shellfish Immunology, vol. 64, (2017), pp. 185-192.

[12] S. Mary Mettilda Bai and M.R. Basil Rose "Purification and Characterization of hemolymph lectin of freshwater crab, Lamella lamellifrons (Alcock, 1909)", Annals of Tropical Medicine and Public Health, vol. 23, no. 7, (2020), pp.1174-1187.

[13] G. Muralikrishna, G. Reuter, J. Peter-Katalinic, H. Egge, F.G. Hanish, H.C. Siebert and R. Schauer, "Identification of a new ganglioside from the starfish Asterias rubens", Carbohyrates, vol. 236, (1992), pp. 321-326.

[14] P.D. Mercy and M.H. Ravindranath, "Purification and Characterization of N-glycolyl neuraminic acid specific lectin from *Scylla serrate*", Experientia, vol.48, no.5, (1993), pp. 498-500.

[15] P.D. Mercy and M.H. Ravindranath, "An agglutinin with unique specificity for N-glycolyl sialic acid residues of thyroglobulin in the hemolymph of a marine crab *Scylla serrata*", European Journal of Biochemistry, vol.215, no.3, (1992), pp. 697-704.

[16] T.P. Nowak and S.H. Barondes, "Agglutinin from *Limulus Polyphemus*. Purification with formalinized horse erythrocytes as the affinity adsorbent", Biochemica et Biophysica Acta, vol. 393, no. 1, (1975), pp. 115-123.

[17] T. Ponraj, M. Paulpandi, R. Vivek, K. Vimala and S. Kannan, "Protein regulation and Apoptotic induction in human breast carcinoma cells (MCF-7) through lectin from *G. beauts*" International Journal of Biological Macromolecules, vol. 95, (2016), pp. 1235-1245.

[18] S. Qadir, J.H. Wani, S. Rafiq, S.A. Ganie, A. Masood and R. Hanrid, "Evalutation of antimicrobial activity of a lectin isolated and purified from *Indigofera heterantha*", Advances in Bioscience and Biotechnology, vol. 4, no. 12, (2013), pp. 999- 1006.

[19] R.K. Rathika, S. Mary Mettilda Bai, J. Vinoliya Josephine Mary, T. Citarasu and F. Vargila, "Physico-chemical characterization of Hemolymph hemagglutinin of the marine crab *Grapsus albolineatus"*, International Journal of Pharmaceutical Science Review and Research, vol. 7, no. 2, (2022), pp. 153-165.

[20] A.C. Roche, R. Schauer and M. Monsigny, "Protein-sugar interactions, purification by affinity chromatography of Limulin, An N-Acyl-Neuraminidyl binding protein", FEBS Letters, vol. 57, No. 3, (1975), pp. 245-249.

[21] M.H. Ravindranath and J.C. Paulson, "O-acetylsialic acid specific lectin from the crab *Cancer antennarius*", Methods in Enzymology, vol. 138, (1987), pp. 520-527.

[22] M.H. Ravindranath, H.H. Higa, E.L. Cooper and J.C. Paulson, "Purification and Characterization of an O-acetyl sialic acid specific lectin from the marine crab *Cancer antennarius*", Journal of Biology. Chem. Vol. 260, (1985), pp. 8850-8856.

[23] E. Stratakis, G. Fragkiadakis and E. Carpeli-Moustaizi, "Isoaltion an characterization of a non sex-specific lipoprotein from hemolymph of freshwater crab, Potamon potamios", Journal of Biological Chemistry Hoppe Seyler, vol. 373, no. 8, (1992), pp. 665-674.

[24] M. Shibata, T. Ohkubo, H. Takahashi and R. Inoki, "Modified formalin test: Cahracteristic biphasic pain response", Pain, vol. 38, no. 8, (1989), pp. 347-352.

[25] T. Suzuki and S. Natori, "Identification of a protein having hemagglutinating activity in the hemolymph of the silk worm *Bombyx mori"*, Journal of Biochemistry, vol. 93, (1983), pp. 583-590.

[26] A.H. Sarris and G.E. Palade, "The sialoglycoproteins of murine erythrocyte ghosts", Journal of Biology Chemistry, vol. 254, (1979), pp. 6724-6731.

[27] R. Schauer, "Chemistry, metabolism and biological functions of sialic acids', Adv. Carbohydrate", ChemBiochem, vol. 40, (1982), pp. 131-234.

[28] S. Tsuji, A.K. Datta and J.C. Paulson, "Systematic nomenclature of sialyltransferase", Glycobiology, vol. 6, (1996), pp. v-vii.

[29] L. Vazquez, F. Masso, P.Rosas, L.F. Montano and E. Zenteno, "Purification and characterization of a lectin from Macrobrachium rosenbergii (Crustacea, Decapoda) hemolymph", Comparative Biochemistry and Physiology Part B: Comparative Biochemistry vol. 105, no. 3, (1993), pp. 617-623.

[30] G.R. Vasta, "Invertebrate lectins: distribution, synthesis, molecular biology and function, In: the glycoconjugates: composition, structure and function", (Allen, HJ & EC Kisailus, eds), Marcel Dekker, New York, (1992), pp. 593-634.

[31] H. Wang, J. Gao and T.B. Ng, "A new lectin with highly potent antihepatoma and antisarcoma activities from the Oyster mushroom *Pleurotus ostreatus*", Biochemical and Biophysical Research Communications, vol. 275, no. 3, (2000), pp. 810-816.

[32] B.R. Wasik, K.N. Barnard, R.J. Ossiboff, Z. Khedri, K.H. Feng, H. Yu, X. Chen, D.R. Perez, A. Varki and C.R. Parrish, "Distribution of O-acetylated sialic acids among target host tissues for influenza virus", Host Microbe Biology, vol, no. 5, (2017), pp. e00379-e00416.