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Research Article

SCREENING OF FRESHWATER MICROALGAE SPECIES FOR OCCURRENCE OF LECTINS AND THEIR CARBOHYDRATE-BINDING SPECIFICITY

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ABSTRACT. Lectins are a group of proteins with specific carbohydrate-binding properties, gained importance as a potential bio-component for applications in therapeutic and biotechnology field. They are naturally present in various sources such as plants, algae, animals, bacteria, fungi, and viruses. Detection of multiple disease biomarkers which are present in the biological samples can be analyzed by using these proteins as a diagnostic tool. In the present study, forty different freshwater microalgae were isolated from various local water-bodies and characterized as *Chlorophyta* and *Ochrophyta* strains. Hemagglutination activity of algal extracts was conducted for all isolated strains to evaluate the presence of lectin content using both native and enzyme-treated erythrocytes of different animals such as sheep, goat, chicken, and humans. Further, the hemagglutination inhibition test was carried out by using various carbohydrates and glycoproteins. All the tested algal extracts exhibited hemagglutination activity for at least one source of erythrocytes. With sheep erythrocytes 60% of the total algae tested showed agglutination, 45% with chicken erythrocytes, 20% with goat erythrocytes, and 5% with human O-type erythrocytes. Among 40 isolates, 24 species exhibited higher hemagglutinating titer value with trypsin treated erythrocytes. Whereas, specific oligosaccharides and glycoproteins inhibited the hemagglutination activity of the algal extracts. Hence, obtained results suggest that native freshwater microalgae can be a good source of lectins with biological significance.

Keywords: *Microalgae, lectins, hemagglutination activity, carbohydrate-binding specificity*

INTRODUCTION

Freshwater microalgae are an essential group of freshwater bio-resources with many uses that beneficially effect human life. They produce a great variety of specific and potent bioactive compounds, including proteins, lipids, carbohydrates, pigments, enzymes, vitamins, and amino acids [1]. To date, many researchers focused on bioactive compounds of algal origin and their various pharmacological functions to develop new potent drugs as antimicrobial, anti-human immune deficiency virus (HIV), anti-cancer, and Alzheimer's therapeutics, etc. Among them, algal lectins are also gaining importance towards their utilization in various biological applications [2, 3, 4, 5]. They are non-immunoglobulin proteins which have the competence to bind specifically as well as reversibly to mono or oligosaccharides without altering their structure existing on the cell surfaces. This protein is also known as agglutinin or hemagglutinin since it agglutinates

the erythrocytes. They are involved in various cellular processes that depend on their specific identification of complex carbohydrates. They play a significant role in recognizing molecules in cell-cell or cell-matrix interactions and helpful in many biological functions. This property can be utilized for the purpose as a carbohydrate probe to detect the biomarkers present in the biological samples to diagnose various diseases [6]. Presently, few agglutinins of different origins have been used as diagnostic tools and as potent anti-cancer, anti-tumor, anti-inflammatory, and anti-viral agents [7, 8, 9]. In pharmaceutics, this protein is used for several biomedical purposes, including the fabrication of a cost-effective protein expression system, drug delivery, glycan profiling, and cancer diagnosis [10, 11, 12]. Therefore, their beneficial properties draw a great deal of consideration in various fields for a research study such as pharmaceutical, immunology, cell biology, membrane structure, and cancer research.

Agglutinin compound was first discovered by Stillmark in 1888 from Ricinus communis (Castor bean plant) and named as 'Ricin' [13]. In 1966, Boyd et al. [14] first reported the presence of this protein in marine algae. Since then, many research studies have been undertaken to survey the presence of agglutinin in various marine and freshwater algae, which include both micro and macroalgae. They have been reported that algal lectins differ from plant lectins in many characteristics. Due to their small structure and presence of disulfide linkages, algal lectins have better stability and binding specificity for complex carbohydrates and glycoconjugates [15]. The lectins from marine algae have been extensively recorded compared to freshwater algae. Moreover, characteristics of lectin vary in different algae, and also application of lectin likely depends on their functional attributes. The present study was mainly focused on the isolation and characterization of freshwater microalgae to screen potent strains in lectin production.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and procured from Himedia (India), Sigma Aldrich (US), and SD Fine (India).

Preparation of ATCC 897 broth and agar media

According to lgcstandards-atcc.org, ATCC 897 broth and agar media were prepared using 50 ml/L of Beijerinck's solution (10 gm NH_4Cl, 0.4 gm $MgSO_4.7H_2O$, 0.2 gm $CaCl_2.2H_2O$, 1 L distilled water), 50 ml/L of Phosphate buffer (28.8 gm K_2HPO_4, 14.4 gm KH_2PO_4, 1 L distilled water), 1 ml/L of Hutner's trace elements (composition and preparation as shown in Table 1), 2 g/L of sodium acetate (pH 7.2). For the agar medium, 1.5% agar was used.

Isolation and identification of microalgae

Freshwater samples collected from various habitats in Bengaluru and Mysuru zones, Karnataka, India, were grown in a salt medium, namely ATCC 897 broth medium for 10 to 20 days at 25°C, 50-100 μEinsteins/m^2/s irradiance, and maintained under a 14:10 h light-dark photoperiod. After growth, a known volume of the sample was serially diluted using 0.9% saline medium, and requisite aliquots of appropriate serial dilutions of the sample were plated on to sterile pre-poured plates of ATCC 897 agar medium. These

inoculated plates were then incubated at the standard condition for 7 to 15 days. The isolated and purified cultures were maintained in broth as well as agar media and stored at 4°C for further study. Identification of microalgal cultures was carried out by conventional method, i.e., by observing microscopical structural characteristics. The algal cultures were sub-cultured at regular intervals of 30 days to maintain culture viability and purity.

Table 1. *Composition and preparation of Hutner's reagent*

Component	**Concentration (gm L^{-1})**	**Dissolved in known amount of water (ml)**
EDTA	50	250
$ZnSO_4.7H_2O$	22	100
H_3BO_3	11.4	200
$MnCl_2.4H_2O$	5.06	50
$FeSO_4.7H_2O$	4.99	50
$CoCl_2.6H_2O$	1.61	50
$CuSO_4.5H_2O$	1.57	50
$(NH_4)6Mo_7O_{24}.4H_2O$	1.1	50

Note: Above mentioned solutions were prepared separately and mixed together except EDTA solution. The solution mixture brought to boil condition this was followed by the addition of EDTA solution. The mixture turned green in color later allowed the reagent to cool to 70°C. Next, 85 mL of hot 20% KOH solution was added into the reagent mixture and made up to 1L by adding 115 mL of distilled water. Reagent was allowed to settle for one day until solution turn purple in color. Finally, it was filtered by using 2 layers of Whatman No.1 filter paper and stored at refrigerator condition for further use.

Preparation of crude protein extract from microalgal culture

Isolates of microalgae were cultured in ATCC 897 broth medium for 7-10 days, and the biomass was harvested by centrifugation. The resultant pellets were dried at 40°C for 24-48h and stored at -20°C. Dry biomass (5 gm) of microalgae was macerated in liquid nitrogen to obtain a fine powder and subjected to extraction of soluble protein at 1:3 (w/v) ratio in 0.1 M Tris-HCl buffer (pH 7.2), containing 0.15 M NaCl under constant stirring for 4 hrs at 4°C. Subsequently, the sample was centrifuged, and the resultant supernatant was collected for hemagglutination assay.

Hemagglutination activity assay

The blood specificity for microalga agglutinin was determined using native and enzyme-treated erythrocytes or red blood cells (RBCs) from different animals (sheep, chicken, and goat) as well as human O-type.

Preparation of erythrocytes

Blood samples of sheep, chicken, and goats were purchased from a local butcher shops, Bengaluru, while human blood was obtained from the registered blood bank, Bengaluru. One ml of each blood sample was centrifuged to separate erythrocytes, which were then washed thrice with 0.01M phosphate buffer saline PBS (pH 7.2 with 0.15 M NaCl) by centrifugation. The resultant packed cells were diluted with PBS to make a 2% (v/v) blood suspension and stored at 4ºC for further use.

Enzyme treated red blood cells (TRBCs) were prepared as follows: one-tenth volume of 0.5% (w/v) trypsin in PBS was added to 2% suspension of native erythrocytes and incubated at 37°C for 60 min. Later, the erythrocytes were washed four times with PBS, and a 2% suspension (v/v) of TRBCs in PBS was prepared [16].

Hemagglutination activity

Determination of hemagglutination activity was conducted in a 96-well microtiter V-plate, using a 2% suspension (v/v) of enzyme-treated and untreated erythrocytes [17]. In the next step, 50 µL of crude protein extract was placed in a microtiter plate containing 50 µL of PBS using two-fold serial dilution and then added with 50 µL of 2% (v/v) erythrocyte suspension. Hundred µl of PBS without algal extract was used as control. The plates were incubated for 1 hour at room temperature. Hemagglutination activity was observed visually when more than 50% of RBC in the well gets agglutinated, and it was tested as positive. The hemagglutination activity was expressed as a hemagglutination titer (HU). Hemagglutination titer value is defined as the reciprocal of the highest dilution showing positive hemagglutination activity.

Study of carbohydrate-binding specificity of lectin

The hemagglutination inhibition test was carried out to investigate the carbohydrate-binding specificity of screened lectin by using different carbohydrates such as monosaccharides, oligosaccharides, and glycoproteins [17]. In this study, the following sugars and glycoproteins used were D-glucose, D-galactose, D-maltose, D-mannose, D-fructose, sucrose, D-lactose, D-xylose, L-rhamnose, L-arabinose, D-ribose, D-cellobiose, L-stachyose, and D-raffinose as sugars, and yeast mannan and ovalbumin as glycoproteins. The concentration of each sugar was taken as 400 mM, whereas glycoprotein concentration was maintained as 2 mg ml^{-1}. This assay involves a 2-fold serial dilution of sugar solution. For this assay, the hemagglutinin solution exhibiting the hemagglutination titer eight was used.

Statistical analysis

All data were calculated and expressed as mean ±SD. Microsoft Excel 2010 (USA) was used for regression analysis.

RESULTS AND DISCUSSION

Lectin is considered as an important bioactive compound that plays a significant role in many biological processes [18]. The presence of lectins in freshwater microalgae has been recorded previously. Still, their number of active strains possessing the same found to be very limited compared with that of marine algae. In the present study, an attempt was made to screen for the lectin with strong hemagglutination activity from freshwater microalgae.

Isolation and identification of microalgae

In total, 40 different native freshwater microalgae were isolated from different habitats of Bengaluru and Mysuru, Karnataka state, India, and subjected for microscopic observation. Majority of the isolates belonged to the division *Chlorophyta* and following

genera such as *Scenedesmus, Chlorella, Coelastrella, Tetradesmus, Ettlia, Acutodesmus, Chlorococcum, Desmodesmus, Muriellopsis, Botryococcus, Neospongiococcum, Ankistrodesmus,* and *Dictyochloris,* whereas one of the strain belonged to *Ochrophyta* division, i.e. *Botrydiopsis.* The growth condition of microalgae was optimized. The salt medium, namely ATCC 897 broth medium, was found to be a suitable growth medium for all the screened strains maintained under 50-100 μEinsteins/m^2/s irradiance for 14:10 h light-dark photoperiod at 25°C and 120 rpm for 10 to 15 days.

Determination of hemagglutination activity

All the isolates were examined for hemagglutination activity with native as well as trypsin-treated erythrocytes of various animals such as sheep, chicken, goat, and humans to evaluate the presence of lectin. Results revealed that all the tested algal species exhibited the presence of agglutinin by hemagglutinating various animal and human erythrocytes. Agglutination of different erythrocytes by crude extracts varies significantly among algal species. Some of them were able to agglutinate more than one erythrocyte. Each isolate showed higher hemagglutination activity with trypsin treated erythrocytes than non-treated erythrocytes. Among 40 isolates, 60% of them showed positive for hemagglutination activity for sheep erythrocytes, 45% for chicken erythrocytes, 20% for goat erythrocytes, and 5% for human erythrocytes (Fig. 1).

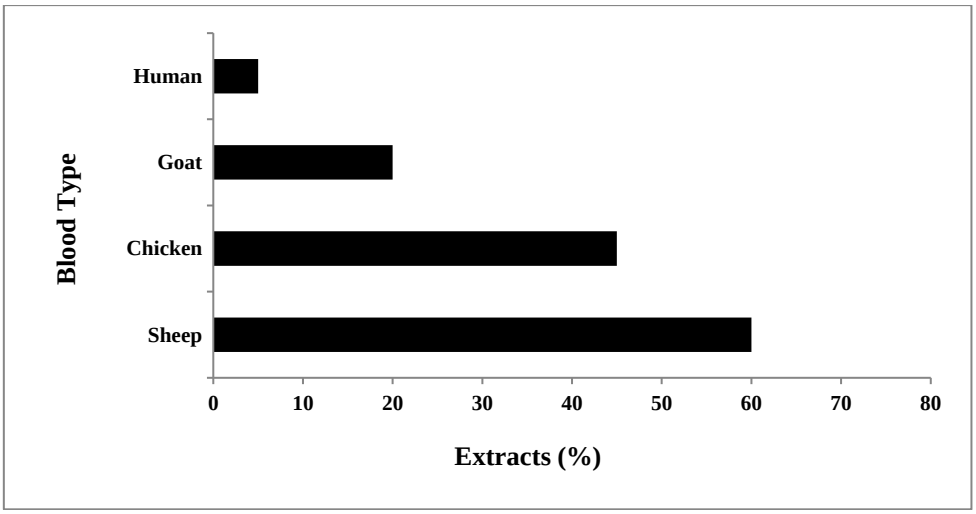

Fig. 1. *Percentage distribution of hemagglutination activity among the freshwater microalgae tested*

Strains such as *Coelastrella* sp., *Tetradesmus obliquus, Ettlia oleoabundans, Chlorella ovalis, Desmodesmus* sp., *Chlorella vulgaris, Chlorella lewinii, Scenedesmus bijuga, Chlorococcum* sp., *Scenedesmus* sp., *Chlorella* sp., *Botryococcus* sp., *Botrydiopsis* sp., and *Dictyochloris* sp., showed higher hemagglutinating titer value ranged from 8192 to 1024 HU. The remaining strains showed weak titer value for different animal erythrocytes, which ranged from 512 to 16 HU (Table 2).

Table 2. *Hemagglutination activity (HA) of algal extracts for different types of erythrocytes*

Strain Name	HA of algal extracts with erythrocytes[a]							
	Sheep		Chicken		Goat		Human O-type	
	N	T	N	T	N	T	N	T
Coelastrella sp.	1024	8192	16	32	-[b]	-	-	-
Tetradesmus obliquus	64	2048	-	-	-	-	4	16
Ettlia oleoabundans	128	1024	-	-	-	-	-	-
Chlorella sp.	128	4096	-	-	-	-	-	-
Chlorella sp.	-	-	32	128	-	-		
Chlorella sp.	128	4096	-	-	-	-	8	32
Chlorella zofigiensis	256	1024	-	-	-	-	-	-
Chlorella sp.	64	512	-	-	-	-	-	-
Muriellopsis sp.	-	-	-	-	128	1024	-	-
Scenedesmus armatus	128	2048	8	32	-	-	-	-
Chlorella ovalis	512	4096	-	-	-	-	-	-
Desmodesmus sp.	-	-	256	1024	-	-	-	-
Scenedesmus sp.	512	4096	4	16	-	-	-	-
Chlorella vulgaris	4	64	512	1024	-	-	-	-
Chlorella lewinii	16	128	256	4096	-	-	-	-
Scenedesmus bijuga	-	-	512	4096	-	-	-	-
Chlorella coloniales	-	-	-	-	256	2048	-	-
Scenedesmus sp.	32	512	-	-	-	-	-	-
Chlorella autotrophica	-	-	512	1024	-	-	-	-
Chlorella sp.	128	512	-	-	-	-	-	-
Chlorococcum sp.	64	2048	-	-	-	-	-	-
Scenedesmus sp.	4	16	128	1024	-	-	-	-
Muriellopsis sp.	-	-	-	-	256	1024	-	-
Chlorella minutissima	-	-	128	2048	-	-	-	-
Chlorella fusca	-	-	512	2048	-	-	-	-
Botryococcus sp.	128	1024	-	-	-	-	-	-
Chlorella saccharophila	64	128	-	-	-	-	-	-
Scenedesmus sp.	32	1024	8	16	-	-	-	-
Neospongiococcum sp.	128	512	-	-	-	-	-	-
Chlorella sp.	-	-	8	256	-	-	-	-
Botrydiopsis sp.	-	-	32	1024	-	-	-	-
Chlorella ellipsoidea	-	-	-	-	32	128	-	-
Desmodesmus sp.	8	16	-	-	64	2048	-	-
Dictyochloris sp.	-	-	-	-	4	512	-	-
Coelastrella sp.	-	-	-	-	256	1024	-	-
Ankistrodesmus sp.	-	-	18	256	-	-	-	-
Chlorella sp.	8	32	-	-	-	-	-	-
Desmodesmus sp.	-	-	16	512	-	-	-	-
Chlorella sp.	16	128	-	-	4	8	-	-
Ettlia sp.	32	256	4	8	-	-	-	-

[a] Hemagglutination activity was expressed as a titer, the reciprocal of the highest dilution showing positive hemagglutination.

N – Native erythrocytes, T – Trypsin treated erythrocytes, [b] (-) No detectable activity.

From the results of this screening, we observed that all 40 crude algal extracts exhibited hemagglutination activity with wide variation in their ability to agglutinate

different erythrocytes. These strains were active against at least one type of animal erythrocytes tested. Among them, only two isolates, i.e., *Tetradesmus obliquus* and *Chlorella* sp. were capable of agglutinating human erythrocytes along with animal erythrocytes. The majority of algal species reacted strongly against sheep and chicken erythrocytes. This study was reliable with other previous reports on the occurrence and activity of hemagglutinins of *Chlorophyta* species against sheep and chicken erythrocytes [19, 20]. Similar such studies have been reported that animal erythrocytes are more suitable for lectin detection in marine algae than human erythrocytes [20, 21, 22, 23, 24]. Some of the reports have been highlighted that freshwater microalgae such as *Chlorella* sp., *Chlamydomonas* sp., *Coelastrum* sp., *Selenastrum* sp., *Monoraphidium* sp., *Tetradesmus obliquus*, *Scenedesmus quadricauda*, and *Scenedesmus ecornis* have the efficiency to agglutinate human A, B, O-type and rabbit erythrocytes [19, 25, 26]. The present study was slightly consistent with these reports only in the case of *Tetradesmus obliquus* and *Chlorella* sp. Both strains exhibited very negligible hemagglutination activity towards human O-type erythrocytes, but higher activity with sheep erythrocytes. Therefore, we can say that there is a range of affinity with the algal species against different erythrocytes.

Research studies have reported the effective extraction of algal agglutinin by using different extraction solutions, including Tris-HCl buffer, PBS, aqueous ethanol, acetone, and NaCl. In one of the study showed higher hemagglutination activities ranging from 2^3 to 2^{12} HU among freshwater algal species such as *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Selenastrum*, *Monoraphidium*, and *Coelastrum*, where 20% ethanol was used to extract algal agglutinin [19]. A similar type of phenomenon was observed among marine *Chlorophyta* species such as *Chlorella ellipsoidea* and *Ankistrodesmus*, which exhibited hemagglutination titer value ranged from 2^6 to 2^{11} HU [25]. In our study, we used liquid nitrogen with 0.1 M Tris-HCl buffer (pH 7.2) containing 0.15 M NaCl, which was found to be more effective in extracting the agglutinins from microalgal cells and showed higher hemagglutination titer value. This suggests that differences in hemagglutination titer value depend on the solvent or method of algal extraction. Moreover, hemagglutination activity of algae could enhance with enzyme-treated erythrocytes than native erythrocytes [21, 27]. It has been proved from many studies that pretreatment of erythrocytes with enzymes, such as trypsin, papain, albumin, and pronase, can improve evidently the detection of agglutinins in algal extracts [7, 19, 28]. Similarly, in the present study, trypsin treated erythrocytes exhibited an increase in hemagglutination titer value than non-treated erythrocytes.

Study of carbohydrate-binding specificity of lectin

The algal extracts showing strong hemagglutination activity were further examined for carbohydrate-binding specificity. Different carbohydrates such as monosaccharides, oligosaccharides, and glycoproteins were used. Most of the strains exhibited carbohydrate-binding specificity to more than two sugars. Carbohydrates such as D-galactose, sucrose, D-lactose, D-raffinose, L-stachyose, yeast mannan, and ovalbumin exhibited as inhibitors of the hemagglutinating activities of *Coelastrella* sp., *Tetradesmus obliquus*, *Ettlia oleoabundans*, *Chlorella ovalis*, *Chlorococcum* sp., *Desmodesmus* sp., *Chlorella vulgaris*, *Chlorella lewinii*, and *Scenedesmus bijuga*, on sheep, chicken and goat erythrocytes. However, other monosaccharides such as D-glucose, D-fructose, and D-maltose also inhibited the hemagglutination activity of few algal extracts belonging to the genera of *Coelastrella*, *Ettlia oleoabundans*, *Scenedesmus*, and *Chlorella*. The

minimum concentrations (i.e., 50, 100, and 200 mM) of the above-mentioned sugars and glycoproteins concentration (1 and 0.5 mg ml^{-1}) showed complete inhibition of eight hemagglutinating units of algal lectins. However, few sugars showed negative result for inhibition of hemagglutination activity, which included L-rhamnose, L-arabinose, D-mannose, D-xylose, D-ribose, and D-cellobiose (Table 3).

Table 3. *Hemagglutination-inhibition test of microalgae agglutinins with sugars and glycoproteins*

Sugars (mM), Glycoprotein (mg ml^{-1})	**[a] Algal Extracts**														
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**
D(+) glucose	-[b]	-	100	-	-	-	-	-	-	-	50	-	-	-	-
D(+) galactose	200	100	-	-	100	200	-	-	-	-	100	-	-	200	-
D(+) fructose	100	-	-	-	-	-	100	100	-	-	50	-	-	-	-
D(+) lactose	-	-	-	-	-	-	-	-	-	100	-	-	-	-	-
D(+) Maltose	-	-	50	-	-	-	-	200	100	-	-	-	-	-	200
Sucrose	200	-	-	200	-	-	-	200	200	-	200	-	200	-	-
D(+) xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+) raffinose	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-
L(+) arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+) mannose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L(+) stachyose	-	-	-	-	-	-	200	-	-	-	-	-	100	-	-
Yeast mannan	1	0.5	1	-	-	-	-	-	-	0.5	-	-	-	-	-
Ovalbumin	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-
L(+) rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+) ribose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D(+) cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] A. *Coelastrella* sp., B. *Tetradesmus obliquus*, C. *Ettlia oleoabundans*, D. *Chlorella* sp., E. *Chlorella ovalis*, F. *Desmodesmus* sp., G. *Chlorella vulgaris*, H. *Chlorella lewinii*, I. *Scenedesmus bijuga*, J. *Chlorococcum* sp., K. *Scenedesmus* sp., L. *Botryococcus* sp., M. *Botrydiopsis* sp., N. *Coelastrella* sp., O. *Dictyochloris* sp.
[b] (-) No detectable activity

In the case of carbohydrate-binding specificity, it is generalized that *Chlorophyta* species of freshwater and marine algae have a broad affinity for complex carbohydrates rather than monosaccharides [20, 26]. However, few of them have established that the hemagglutination activity of green algal extracts has inhibited by monosaccharides [25, 29, 30, 31, 32, 33]. Our study demonstrated that few strains belonging to *Coelastrella*, *Ettlia oleoabundans*, *Scenedesmus*, and *Chlorella* genera exhibited affinity towards monosaccharides. As well, other strains agglutinin activity was inhibited by complex carbohydrates. Therefore, the profile of hemagglutination-inhibition by carbohydrates is diverse, most probably depending upon the origin of algal species. This suggests that the

variability in cellular carbohydrates arrangement could be the reason for the difference in carbohydrate-binding specificity in each algal species [25].

This study is promising for our future work because of the fact that the study of lectin properties from many freshwater microalgae such as *Coelastrella*, *Ettlia oleoabundans*, *Botrydiopsis*, and *Dictyochloris* has not been done extensively when compared with the isolates belonging to the genera *Scenedesmus* and *Chlorella*. Since our isolates have exhibited higher hemagglutinating titer value with more carbohydrate-binding specificity, they can be further exploited to identify an ideal lectin for biotechnological applications such as a diagnostic tool to detect cancer biomarkers, carrier of chemotherapeutic agents, taxonomic marker of specific microorganisms, and glycan profiling, etc., which has to be, however ascertained by further work.

CONCLUSION

In conclusion, we report that the lectin crude extracts from microalgae belonged to the *Chlorophyta* and *Ochrophyta* division were able to hemagglutinate more than one type of trypsin treated animal erythrocytes rather than human erythrocytes. These algal lectins exhibited a strong affinity towards different types of carbohydrates. Hence, the outcome of this study conclusively established that microalgal cultures with high lectin producing characteristics are present in diversified habitats. They need to be well characterized and exploited commercially for biotechnological applications.

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Conflict of Interest. "The authors declared that there is no conflict of interest."

Authorship Contributions. Concept: A.R., J.S., Design: A.R., Data Collection or Processing: A.R., Analysis or Interpretation: A.R., Literature Search: A.R., Writing: A.R., J.S.,

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REFERENCES

[1] Cardoso, K. H. M., Guaratini, T., Barros, M. P., Falcao, V. R., Tonon, A. P., Lopes, N. P., Campos, S., Torres, M. A., Souza, A. O., Colepicolo, P., Pinto, E. (2007): Metabolities from algae with economical impact. Comparative Biochemical Physiology 146: 66-78.

[2] Lam, S. K., Ng, T. B. (2011): Lectins: production and practical applications. Applied Microbiology Biotechnology 89: 45–55.

[3] Swamy, M. L. A. (2012): Marine algal sources for treating bacterial diseases. Advance in Food Nutrition and Research 64: 71–84.

[4] Nascimento, K. S., Cunha, A. I., Nascimento, K. S. (2011): An overview of lectins purification stratergies. Journal of Molecular Recognition 25: 527-541.

[5] Teixeira, E. H., Arruda, F. V. S., Nascimento, K. S. (2012): Biological applications of plants and algae lectins: An overview. In: Chang CF, ed. Carbohydrates-comprehensive studies on glycobiology and glycotechnology. Croatia: In Tech: 533–558.
[6] Sharon, N., Lis, H. (2003): Lectins, Second edn. Kluwer, Dordrecht.
[7] Kawakubo, A., Makino, H., Ohnishi, J., Hirohara, H., Hori, K. (1997): The marine red alga *Eucheuma serra* J. Agardh, a high yielding source of two isolectins. Journal of Applied Phycology 9: 331–338.
[8] Pinto, V. P. T., Debray, H., Dus, D., Teixeira, E. H., Oliveira, T. M., Carneiro, V. A., Teixeira, A. H., Filho, G. C., Nagano, C. S., Nascimento, K. S., Sampaio, A. H., Cavada, B. H. (2009): Lectins from the red marine algal species *Bryothamnion seaforthii* and *Bryothamnion triquetrum* as tools to differentiate human colon carcinoma cells. Advance Pharmacology of Science 4: 1-10.
[9] Singh, R. S., Thakur, S. R., Bansal, P. (2013): Algal lectins as promising biomolecules for biomedical research. Critical Reviews in Microbiology Early Online: 1–12.
[10] Singh, R. S., Tiwary, A. K., Kennedy, J. F. (1999): Lectins: Sources, activities and applications. Critical Reviews in Biotechnology 19: 145–178.
[11] Nascimento, K. S., Nagano, C. S., Nunes, E. V., Rodrigues, G. V., Calvete, J. J., Saker, S., Sampaio, W. R. L., Farias, S. A. H. (2006): Isolation and characterization of a new agglutinin from the red marine alga *Hypnea cervicornis*. J Agardh Biochem Cell Biol 84: 49–54.
[12] Coelho, L. C. B. B., Silva, P. M. S., Lima, V. L. M., Pontual, E. V., Paiva, P. M. G., Napoleao, T. H., Correia, M. T. S. (2017): Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications. Evidence Based Complement Alternative Medicine 1594074: 1–22.
[13] Franz, H. (1988): The ricin story. Advance Lectin Research 1: 10-25.
[14] Boyd, W. C., Almodovar, L. R., Boyd, L. G. (1966): Agglutinins in marine algae for human erythrocytes. Transfusion 6: 82–83.
[15] Rogers, D. J., Hori, K. (1993): Marine algal lectins: new developments. Hydrobiologia 260/261: 589–593.
[16] de Oliveira, A. S., Lossio, C. F., Rangel, A. J., Martins M, G, Q., do Nascimento, F. E. P., de Andrade M. L. L., Cavada, B. S., Lacerda**,** S.R., do Nascimento, K. S. (2017): Detection, purification and characterization of a lectin from freshwater green algae *spirogyra* spp. An Academic Bras Cienc 89: 2113-2117.
[17] Awoyinka, O. A., Dada, O. O. (2011): Partial Purification and Characterization of Lectin from the Seeds of *Cissus poplunea*. European Journal of Medicinal Plants 1: 130-139.
[18] Ganapati, G. B., Kartika, N. S., Nagaraja, N. N., Vivek, V. N., Lingaraju, S., Ramesh, S. B., Shashikala, R. I., Suguna, K., Bale, M. S. (2010): Purification, characterization and molecular cloning of a monocot mannose-binding lectin from *Remusatia vivipara* with nematicidal activity. Glycoconjugates Journal 27: 309–320.
[19] Praseptiangga, D. (2015): Algal lectins and their potential uses. Squalen Bull Mar Fish Post harvest Biotech 10: 89-98.
[20] Chu, C. Y., Liao, W. R., Huang, R., Lin, L. P. (2004): Haemagglutinating and antibiotic activities of freshwater microalga. World Journal of Microbiology and Biotechnology 20: 817–825.
[21] Hung, L. D., Ly, B. M., Trang, V. T. D., Ngoc, N. T. D., Hoa, L. T., Trinh, P. T. H. (2012): A new screening for hemagglutinins from Vietnamese marine macroalgae. Journal of Applied Phycology 24: 227–235.
[22] Hori, K., Oiwa, C., Miyazawa, K., Ito, K. (1988): Evidence for wide distribution of agglutinins in marine algae. Botany Marine 31:133–138.
[23] Chiles, T. C., Bird, K. T. (1989): A comparative study of the animal erythrocyte agglutinins from marine algae. Comprehensive Biochemical Physiology 94B: 107–111.
[24] Hori, K., Miyazawa, K., Ito, K. (1990): Some common properties of lectins from marine algae. Hydrobiologia 204/205: 561–566.

[25] Dalton, S. R., Longley, R. E., Bird, K. T. (1995): Hemagglutinins and immunomitogens from marine algae. Journal of Marine Biotechnology 2: 149-155.
[26] Liao, W. R., Huang, R. (2000): Agglutination of human and animal erythrocytes in marine unicellular algae. Journal of Indian Microbiology and Biotechnology 24: 262–266.
[27] Silva, A. J., Cavalcanti, V. L. R., Porto, A. L. F., Gama, W. A., Brandao-Costa, R. M. P., Bezerra, R. P. (2019): The green microalgae *Tetradesmus obliquus* (*Scenedesmus acutus*) as lectin source in the recognition of ABO blood type: purification and characterization. Journal of Applied Phycology 4: 1-10.
[28] Hori, K., Miyazawa, K., Ito, K. (1986): Preliminary characterization of agglutinins from seven marine algal species. Bulletin in Japanese Society of Science Fish 52: 323–331.
[29] De Souza, B. W. S., Teixeira, D. I. A., Andrade, F. K., Melo, M. R. S., Munoz, A. M., Freitas, A. L. P. (2007): A Survey of Antarctic Algae for agglutinins. Oecol Bras 11: 122-130.
[30] Bird, K. T., Chiles, T. C., Longley, R. E., Kendrick, A. F., Kinkema, M. D. (1993): Agglutinins from marine macroalgae of the Southeastern United States. Journal of Applied Phycology 5: 212–213.
[31] Alvarez-Hernandez, S., De Lara-Isassi, G., Arreguin-Espinoza, R., Arreguin, B., Hernandez-Santoyo, A., Rodriguez-Romero, A. (1999): Isolation and partial characterization of giraffine, a lectin from the Mexican endemic alga *Codium giraffa* Silva. Botany Marine 42: 573–580.
[32] Freitas, A. L. P., Teixeira, D. I. A., Costa, F. H. F., Farias, W. R. L., Lobato, A. S. C., Sampaio, A. H., Benevides, N. M. B. (1997): A new survey of Brazilian marine algae for agglutinins. Journal of Applied Phycology 9: 495-501.
[33] Ambrosio, A., Sanz, L., Sanchez, E., Wolfenstein-Todel, C., Calvete, J. (2003): Isolation of two novel mannanand L-fucose-binding lectins from the green alga *Enteromorpha prolifera:* biochemical characterization of EPL-2. Archieve Biochemistry Biophysics 415: 245–250.
[34] Wang, S., Zhong, F. D., Zhang, Y. J., Wu, Z. J., Lin, Q. Y., Xie, L. H. (2004): Molecular characterization of a new lectin from the marine alga *Ulva pertusa*. Acta Biochim Biophysics Sin 36: 111–117.