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The green microalgae *Tetradesmus obliquus* (*Scenedesmus acutus*) as lectin source in the recognition of ABO blood type: purification and characterization

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Abstract
Lectins have potential applications in agriculture, medicine, and biotechnological research and have become an important tool in the identification of different blood groups. Lectins can be obtained from different sources, such as microalgae. *Tetradesmus obliquus* is a green microalga that produces bioactives with high added value. A novel lectin was purified from microalgae *T. obliquus* by ion exchange and gel filtration chromatographies and characterized by molecular mass determination; carbohydrate contents; recognition of the rabbit, bovine, horse, and ABO blood type; inhibition by carbohydrate and glycoprotein; optimum pH and temperature; and their stability. Lectin was purified with a purification factor of 4.2-fold and yield of 3.13 in a two-step using ion exchange and gel filtration. It agglutinated human type O and rabbit erythrocytes but not bovine, horse, human erythrocytes types A1, A2, B, and AB. Lectin showed optimum pH at 7.0–7.5 and stability for 90 min, while the optimum temperature of 20–50 °C showed fairly good thermostability. The lectin is a glycoprotein with 5.37% of carbohydrate, an apparent molecular mass of 78 kDa as determined by gel filtration, and the activity was decreasing in the presence of ions and inhibited only by glycoprotein azoalbumin, azocasein, and albumin, characteristics that indicate the lectin from *T. obliquus* as unique among microalgal lectins.

Keywords ABO type · Biochemical characterization · Chlorophyceae · Hemagglutinins · Isolation · Protein

Introduction

Lectins or hemagglutinins are carbohydrate-binding proteins/glycoproteins reversibly of non-immune origin with multiple potential applications in agriculture, medicine, and biotechnological research. It has been well documented that lectins play important roles in cellular and molecular recognition and have been exploited as carriers of chemotherapeutic agents, biomarkers of specific microorganisms, potential diagnostic reagents, and anticancer drugs, antiviral, antimicrobial, antiparasitic, anti-inflammatory, and larvicidal activity (Coelho et al. 2017).

In addition, lectins seem comparable to antibodies in their sensitivity and this property is useful in clinical fields. It shows a highly specific reaction to different sugar heads that are present on the erythrocyte surface membrane, bound to either glycoprotein or glycolipids, specifying ABO or MN blood groups. Recently, a limited number of lectins have been used in the recognition of blood groups. In the ABO blood group especially, lectins from *Dolichos biflorus*, *Vicia cracca*, *Phaseolus limensis*, *Otala lactea*, and *Crotalaria striata* potentially recognize blood group A substance (Khan et al. 2002), but only lectins from *Dolichos biflorus* are considered Anti-A1 commercially.

Lectins are widespread in distribution and have been isolated from viruses, bacteria, fungi, insects, plants, algae, and vertebrates. Lectins purified from different species or even the same species may show differences in structure, molecular size, and carbohydrate specificity, resulting in different biological properties.

Plant lectins have been intensively studied. However, lectins extracted from photosynthetic microorganisms are less known. In the literature, there are reports of lectins extracted from microalgae such as *Nostoc*, *Microcystis*, *Oscillatoria*,

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Scytonema, *Chlorella*, *Scenedesmus*, *Desmodesmus*, and *Chlorococcum*. Although considerable progress has been made in understanding the distribution and biochemical characteristics of microalgal lectins, much less is known about algal lectins than those derived from plants. This means that the utilization of algal lectins is not as well advanced as lectins from other sources.

Tetradesmus obliquus is a green microalga (previous name = *Scenedesmus acutus* (Wynne and Hallan 2016)). This group of microalgae (Chlorophyceae, Scenedesmaceae) may be a promising source of lectins as it has been shown to produce a huge variety of bioactive products with potential commercial values, such as lipids, carbohydrates, proteins, vitamins, amino acids, and beta-carotene that are used as feedstock for energy production, feed additives, food, cosmetics, and medicine (Ishaq et al. 2016). In addition, microalgal bioprocesses have been highlighted because they do not compete with food crops as requiring arable land and can use waste as nutrients for their development.

In the present study, a lectin from *T. obliquus* was purified, characterized, and evaluated for its potential in the agglutination of the difference in blood group.

Materials and methods

Microorganisms and culture conditions

Tetradesmus obliquus (A5F5402) was isolated from Açude of Apipucos (Recife, Pernambuco, Brazil, coordinates 8° 1′ 13.08″ S; 34° 55′ 56.51″ W) and cultivated in 250-mL Erlenmeyer flasks each containing 50 mL BG-11 medium at 30 ± 1 °C, continuous light of 52 ± 4 μmol photons m^{-2} s^{-1}, under constant aeration for 15 days, when it reached the end of the logarithmic growth phase. Then, cells were removed by filtration, dried, and used for further analysis.

Lectin extraction

Dried biomass was homogenized at a concentration of 100 mg mL^{-1} in Tris-HCl-buffered saline (0.2 M, pH 7.2), using a magnetic homogenizer for 9 h at 4 °C (Chu et al. 2006). The homogenate was centrifuged at 10000×*g* for 8 min at 4 °C. The supernatant was the crude extract of *T. obliquus*.

Precipitation step

Crude extract was precipitated by adding ammonium sulfate slowly to the clarified cell extract with gentle stirring at 4 °C until the solution was saturated at a concentration of 0–60% and 60–80% (w/v). The protein precipitated by centrifugation (8000×*g* for 30 min) was dissolved in 10 mM Tris-HCl buffer (pH 7.5) and dialysed against the same buffer for 18 h at 4 °C. After dialysis, hemagglutinating activity and protein content of each fraction were estimated.

Purification and molecular mass determination by size-exclusion chromatography of *Tetradesmus obliquus* lectin

Tetradesmus obliquus lectin was purified using two-step chromatography using ion exchange and gel filtration. Supernatant (crude extract) of *T. obliquus* was loaded on to anion-exchange chromatography using DEAE Sephadex A-50 column (1.6 cm × 10 cm) pre-equilibrated with 20 mM Tris-HCl buffer pH 7.2. The unabsorbed fraction was eluted with the equilibration buffer. Elution was carried out at a flow rate of 1 mL min^{-1}, and the absorbance was monitored at 280 nm. The peak was pooled, concentrated, and was used to determinate protein total and hemagglutinating activity.

Fractions exhibiting hemagglutinating activity were pooled, concentrated, and injected into on FPLC-Superdex G-75 HR10/300GL fast flow column (1.0 cm × 80 cm) (AKTA avant 25, Sweden) pre-equilibrated with 10 mM Tris-HCl buffer (pH 7.0). The elution rate was 0.5 mL min^{-1}, and the absorbance was determined at 280 nm. Peaks were used to determinate protein total and hemagglutination activity. Peak with hemagglutination activity was reloaded in the same conditions to obtain lectin purified. Weight determination was achieved using Gel Filtration Markers Kit for protein molecular weights 6500–66,000 Da from Sigma-Aldrich.

Hemagglutinating activity assay

Hemagglutinating activity was measured with erythrocytes treated with glutaraldehyde using the method of Correa and Coelho (1995). Twofold serial dilutions of the sample (50 μL) were mixed with 2.5% (v/v) erythrocyte buffer solution (50 μL) at 25 °C in a 96-well microtiter plate. Saline was used as the blank control, and the results were recorded after incubation at 25 °C for 45 min. Lectin activity was determined by its ability to agglutinate erythrocytes. Hemagglutination was recorded visually, and mat formation indicated the presence of lectin activity, while button formation at the bottom of cavity showed the absence of lectin activity. Serial twofold dilutions of the sample were used to determine the hemagglutinating titer (U).

Protein concentration

The concentration of total protein was measured by the method of Smith et al. (1985), using bovine serum albumin as the standard.

Erythrocytes sample

Erythrocytes were obtained from the blood samples of rabbit, bovine, horse, and human (A subgroup 1, A subgroup 2, B, AB, and O). Blood was mixed with 1/10 volume of 3.7% Na-citrate and centrifuged at 8000×*g* for 20 min and stored until use.

The recognition of subgroup A1 was performed using the tube technique (Fresenius HemoCare Brasil Ltda, Itapecerica da Serra, São Paulo, Brasil. 2017). A 2–3% suspension of red blood cells (erythrocytes) was washed 3 times in PBS solution and added Anti-A1 Lectin reagent (*Dolichos biflorus*) (Lorne Laboratories, UK) in a test tube as the positive control.

Carbohydrate analysis

The carbohydrate content of the lectin was analyzed by the phenol–sulfuric acid method in microplate format according to Masuko et al. (2005). The results were expressed as percentage of carbohydrate to protein determination (% of glucose per mg of protein). The standard curve was obtained using 50–450 nM of glucose.

Hemagglutination inhibition by sugars and glycoprotein

Saccharides with a high affinity toward the lectin could block the binding site of lectin from red blood cell glycoproteins/glycolipids, inhibiting hemagglutination. The sugar inhibition assay was performed in a manner analogous to the hemagglutination test. Serial twofold dilutions of 25 μL of various sugar (20 mM) or glycoprotein (1.0%) samples were prepared in PBS in a 96-well U-shaped microtiter plate. Afterward, 25 μL of lectin sample with 2048 hemagglutination units was added to the solution mixture. The plate was incubated in room temperature for 40 min followed by the addition of 50 μL of 2.5% erythrocytes rabbit suspension to each well. The number of wells which showed hemagglutination was counted and compared with the control in which PBS was added instead of sugar or glycoprotein solution. Sugars tested included rhamnose, arabinose, fructose, glucose, sucrose, galactose, and raffinose, and glycoproteins were lactoferrin, azoalbumin, azocasein, lysozyme, and albumin. The residual activity was determined under the standard assay conditions and expressed as percentage relative activity compared with control samples.

Effect of ion on hemagglutinating activity

The purified lectin was pre-incubated at room temperature for 30 min with 10 mM of various ions as $ZnSO_4$, $ZnCl_2$, $FeCl_3$, $FeCl_2$, $MnSO_4$, $CaCl_2$, $CuSO_4$, $MgSO_4$, $Na_2S_2O_3$, and $K_2S_2O_5$. Then, 2.5% erythrocytes rabbit systems (v/v) were added, and the samples were incubated in a water bath at 37 °C, for 120 min. After incubation, hemagglutinating activity was assayed as described previously.

Effect of pH and temperature on the hemagglutinating activity

The influence of pH on hemagglutinating activity was determined by incubating purified lectin at 25 °C for 30 min in 10 mM buffers of different pH: citrate phosphate (pH 4.0–7.5) and Tris-HCl (pH 7.0–9.0), glycine-NaOH (pH 10.0). To determine pH stability, the purified lectin (50 μL) was incubated with buffers (50 μL) at 25 °C at various pH and then samples were neutralized, and activity was assayed after 30, 60, and 90 min to elucidate the pH stability of lectin at varied pH. The residual activity was determined under the standard assay conditions.

To determine the optimum temperature, hemagglutinating activity was measured at temperatures ranging from 20 to 100 °C. The thermal stability was examined with pre-incubation of purified lectin at determined temperatures after 30, 60, and 90 min of incubation and assayed for hemagglutination under standard assay conditions. Hemagglutination activity at any given temperature was expressed as percentage relative activity compared with control samples.

Results

Tetradesmus obliquus extract agglutinated rabbit erythrocytes obtained titer higher than 2^{24} (Table 1), and it was used for purification steps. A lectin from *T. obliquus* was purified by two chromatographic steps, namely ion exchange chromatography with a DEAE-Sephadex column and gel filtration with Superdex G-75, which revealed strong agglutination activity (Table 1). The first purification step was ammonium sulfate precipitation. The crude extract obtained from *T. obliquus* was subjected to precipitation in ammonium sulfate in two fractions of saturation, 0–60% and 60–80%, in order to determine which precipitated fraction has the highest hemagglutinating activity. The 60–80% saturation fraction had high hemagglutinating activity (Table 1) and was selected for purification by chromatography. However, the 0–60% fraction also had significant protein concentration with low hemagglutinating activity.

The precipitate obtained by 60–80% ammonium sulfate saturation was desalted by dialysis and then applied to an anion-exchange chromatography column. The precipitate gave a single active peak in ion exchange chromatography on DEAE Sephadex (Fig. 1) with a titer of 40,960 and specific activity of 49.3 titer g^{-1}. The active

Table 1 Summary of purification of *Tetradesmus obliquus* lectin

Step	Titer total	Protein (mg mL^{-1})	Specific activity (titer g^{-1})	Yield (%)[c]	Fold purification[d]
Crude	16,777,216.0[a]	1.020	_[b]	_[b]	_[b]
Precipitate 0–60%	24,567.0	0.400	10.2	_[b]	_[b]
Precipitate 60–80%	131,072.0	0.630	52.4	100	1.00
Ion exchange	40,960.0	0.083	49.3	31.3	0.94
First step of gel filtration	12,288.0	0.043	47.6	9.38	0.91
Second step of gel filtration	4096.0	0.018	222.6	3.13	4.20

[a] Activity higher than 1,677,216 ($>2^{24}$)

[b] It was not possible to calculate

[c] Yield is calculated on the basis of titer total in precipitate 60–80%

[d] Purification factor is calculated on the basis of specific activity in precipitate 60–80%

peak was further separated into two peaks in molecular exclusion chromatography with a Sephadex G-50 column. The first peak (fraction I) exhibited strong hemagglutination activity (47.6 titer g^{-1}) and molecular mass estimated at 78 kDa, whereas the second peak (fraction II) showed weak activity (Fig. 2a). Only the first peak was reloaded in the same conditions to observe the purity protein. As shown in Fig. 2b, only one peak was observed, showing that the lectin was purified with purification factor of 4.2-fold and overall yield of 3.13%. A summary of the lectin purification process is shown in Table 1. In addition, carbohydrate analysis using the phenol–sulfuric acid assay revealed that lectin was a glycoprotein with carbohydrate content of 5.37%.

Hemagglutination activity of purified lectin was tested with human A1, A2, B, AB, and O-type erythrocytes. Table 2 summarizes the results of the hemagglutination titers. Among the erythrocytes analyzed (human blood group A1, A2, B, AB, and O), lectin from *T. obliquus* exhibited a preference only for human blood group O and rabbit erythrocytes and no hemagglutinating activity was observed with bovine and horse erythrocytes.

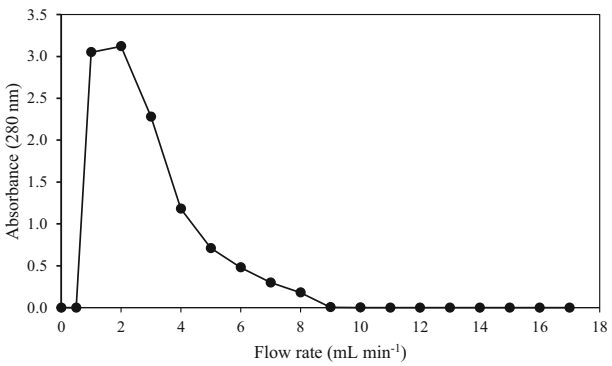

Fig. 1 Elution pattern of the *Tetradesmus obliquus* lectin during anion-exchange chromatography on DEAE-Sephadex

Hemagglutination activity of purified *T. obliquus* lectin was not inhibited by any of the monosaccharides, sucrose disaccharide, lysozyme, and lactoferrin glycoprotein, whereas it was inhibited by azoalbumin, azocasein, and albumin glycoproteins (Table 3). *Tetradesmus obliquus* lectin showed optimal activity at pH 7.0–7.5 and temperature between 20 and 50 °C (Figs. 3 and 4). Thermal stability was determined by incubating lectin at 25–100 °C in a water bath for 0–90 min. The lectin was not affected by exposure to a temperature of 50 °C for 90 min but declined rapidly, reaching 6.25% of the

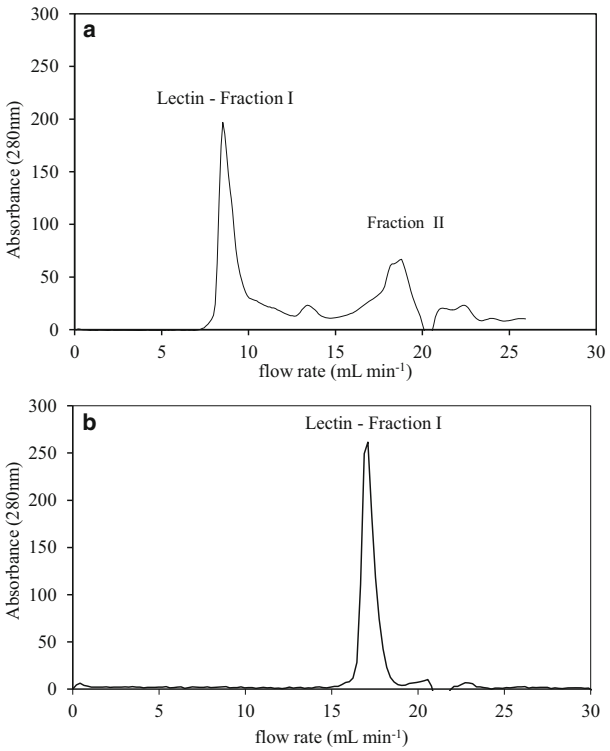

Fig. 2 **a** Elution pattern of the lectin on size-exclusion chromatography of *Tetradesmus obliquus* lectin on a FPLC-Sephadex G-50 Column (first step). Fraction I showed hemagglutination titer. **b** Elution pattern of fraction I reload on a FPLC-Sephadex G-50 Column (second step)

Table 2 Blood group specificities of purified *Tetradesmus obliquus* lectin

Blood group specificity	Hemagglutinating activity
A1	0.00
A2	0.00
B	0.00
AB	0.00
O	2048
Rabbit	2048
Bovine	0.00
Horse	0.00

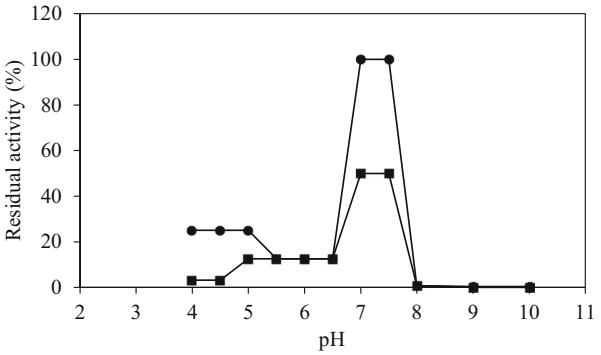

Fig. 3 Effect of pH (●) and stability for 90 min (■) on lectin purified from *Tetradesmus obliquus*

control value, when heated at 60 °C for 30 min. The hemagglutinating activity was totally abolished when the lectin was heated at 70 °C for 30 min. Lectin exhibited thermostability and retained 100% activity at 50 °C even after 60 min, and maintained activity of 50% of the control value for 90 min, while activity lectin has been shown to be lost at temperatures higher than 70 °C.

The presence of ions affected hemagglutination activity caused by lectin from *T. obliquus*. All evaluated ions reduced hemagglutinating activity by more than 90%, and the strongest inhibition was with $CaCl_2$ which completely inhibited hemagglutinating activity (Table 4).

Discussion

Hemagglutination activity from *T. obliquus* extract was higher than that of other microalgal extracts. Among eighteen species of *Chlorella*, sixteen species of *Chlamydomonas*, three species of *Spirulina*, two species of *Scenedesmus*, and one species each of *Synechococcus*, *Selenastrum*, *Monoraphidium*, *Coelastrum*, and *Eutetramorus*, *Scenedesmus* species obtained high value of 2^8 using 20% ethanol and acetone (1:10, w/v) to extract preparation (Chu et al. 2004). This difference of titer may be due to the solvent and/or method of extraction used to obtain the extract with hemagglutinating activity. Singh and Walia (2018) related that *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Chlorococcum infusiformis*, *Desmodesmus subspicatus*, and *Desmodesmus dimorphus* have cell surface–bound lectin activity. Thus, extraction using magnetic stirring of the Tris-HCl buffer pH 7.0 may have provided high values of hemagglutinating activity in *T. obliquus* extracts.

In addition, high hemagglutinating activity in the *T. obliquus* extract can be too due to the presence of polyphenols which causes false hemagglutination activity. Previous studies have shown that *Scenedesmus* spp. contain high concentrations of polyphenols which mimic lectin-mediated hemagglutination (Shetty and Sibi 2015; Strejckova et al. 2016; Hamed et al. 2017). In this context, the purification of protein with hemagglutinating activity becomes necessary to indicate the presence of lectin in the *T. obliquus* extracts.

Table 3 Effect of carbohydrates and glycoprotein on lectin purified (initial hemagglutination, 2048 U)

	Residual activity (%)
Carbohydrate (20 mM)	
L-rhamnose	100
L-Arabinose	100
D-fructose	100
D-sucrose	100
D-glucose	100
D-galactose	100
D-raffinose	100
Glycoprotein (1.0%)	
Lactoferrin	100
Azoalbumin	0.00
Azocasein	0.00
Lysozyme	100
Albumin	0.00

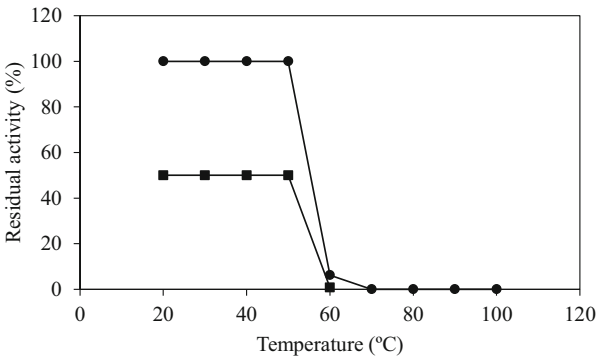

Fig. 4 Effect of temperature (●) and stability for 90 min (■) on lectin purified from *Tetradesmus obliquus*

Table 4 Effect of metal ions on purified lectin (initial hemagglutination activity, 2048 U)

Ions	Residual activity (%)
$ZnSO_4$	0.59
$ZnCl_2$	0.59
$FeCl_3$	6.25
$FeCl_2$	2.64
$MnSO_4$	0.29
$CaCl_2$	0.00
$CuSO_4$	4.15
$MgSO_4$	0.59
$Na_2S_2O_3$	2.54
$K_2S_2O_5$	2.05

Isolation of lectins can be achieved by a combination of different purification steps. Acids (e.g., acetic acid; Naeem et al. 2007), organic solvent (e.g., acetone; Medeiros et al. 2010) or salt (e.g., ammonium sulfate) can be used to precipitate lectins. Precipitation with ammonium sulfate at the 60–70% concentration is the most widely used. For example, Raja et al. (2011) used 60% to precipitate *Aegle marmelos* fruit lectin, Abreu et al. (2018) used 70% to precipitate *Solieria filiformis* lectin (SfL), and Yi et al. (2003) used 75% to precipitate *Chlorella pyrenoidosa* lectin. This is in accordance with the present work that had higher hemagglutinating activity in the 60–80% fraction (Table 1). On the other hand, lower fractions of ammonium sulfate (0–60%) can also precipitate protein with weak hemagglutinating activity (Table 1), as observed for the lectin from the cyanobacterium *Oscillatoria agardhii* that was precipitated with 20–60% of ammonium sulfate (Sato et al. 2000).

Lectin from *T. obliquus* was purified using anion-exchange DEAE-Sephadex and gel filtration chromatography. Other microalgal lectins have been purified using conventional chromatographic approaches (Sato et al. 2000; Melo et al. 2004). The majority of reports on lectin activity from other microalgae and cyanobacteria used a multistep purification protocol. Two-step purification of the lectin demonstrates the efficiency of the procedure.

Most of the lectins isolated from algae, microalgae, and cyanobacteria are low molecular weight proteins, with values among 3.5 to 57 kDa. Therefore, the native molecular mass estimated for *T. obliquus* lectin by gel filtration of 78 kDa was considered high when compared with lectins isolated from most species of microalgae such as the green algae *Spirogyra* spp. (56 kDa; Oliveira et al. 2017) and *Chlorella pyrenoidosa* (15 kDa; Yi et al. 2003) and the cyanobacteria *Microcystis aeruginosa* (57 kDa; Yamaguchi et al. 1998), *Microcystis viridis* (12.2 kDa; Yamaguchi et al. 1999), *Nostoc* (11 kDa); *Oscillatoria agardhii* (13 kDa; Sato et al. 2000), and *Scytonema varium* (9.7 kDa; Bokesch et al. 2003).

Tetradesmus obliquus lectin is a glycoprotein with sugar contents (5.37%) lower than other isolated from some other algae and cyanobacteria, e.g., *Oscillatoria agardhii* (16%; Sato et al. 2000), *Tichocarpus crinitus* (6.9%; Molchanova et al. 2009), and *Eucheuma* sp. (8–10%; Kawakubo et al. 1999), and higher than *Meristiella echinocarpa* lectin (2.0%; Chaves et al. 2018), *Gracilaria ornata* lectin (2.9%; Leite et al. 2005), *Ulva pertusa* lectin (1.2%; Wang et al. 2004).

Lectin from *T. obliquus* was specific for human O-type erythrocytes. Human blood O type possesses a lower amount of sialic acids when compared with other human blood types (Bulai et al. 2003), and rabbit erythrocytes have previously been shown to have a low content of sialic acid on the surface when compared with horse and bovine erythrocytes (Ish et al. 1993, Angel and Burness 1977; Munksgaard et al. 2014). So, lectin from *T. obliquus* recognizes sialic acid–poor erythrocytes. Similar results have been observed by hemagglutinins from the Chlorophyceae *Ankistrodesmus* sp. (titer = 2^{25}) and *Chlorella ellipsoidea* (titer = 2^3) (Liao and Huang 2000). Chu et al. (2004) reported that *Scenedesmus quadricauda* and *Scenedesmus ecornis* extract agglutinated O more strongly that A or B human blood type and lectin from *C. pyrenoidosa* agglutinated erythrocytes from rabbit, sheep, and pigeon but not from duck, chicken, and human A, B, or O blood cell groups with the highest activity in rabbit erythrocytes (Hung et al. 2012). This study is consistent with other reports on the activity of microalgae hemagglutinins in O human and rabbit erythrocytes (Liao and Huang 2000; Chu et al. 2004; Hung et al. 2012).

Lectin from *T. obliquus* did not agglutinate subtypes A1 or A2 erythrocytes. This can be justified by the presence of some glycoproteins present on the surface of erythrocytes that promote inhibition of the binding of lectin to carbohydrates on the surface of the blood cell. In the literature, few lectins differentiate subgroups A1 and A2. *Dolichos biflorus* lectin is the best known in routine blood group serology by recognized only A1 erythrocytes. This lectin is widely used in blood banks for subgrouping. Lectin from *Vicia cracca*, *Phaseolus limensi*, and *Crotalaria striata* has also been shown to be potent in anti-A activity (Renkonen 1948; Khang et al. 1990; Khan et al. 2002).

Only azoalbumin, azocasein, and albumin glycoproteins inhibited hemagglutination activity of purified *T. obliquus* lectin. This suggests that *T. obliquus* lectin probably has complex carbohydrate specificity. These properties are also characteristic of many marine macroalgal lectins, such as the Chlorophyta (Hung et al. 2012) and of lectin from the cyanobacterium *Oscillatoria* (Sato et al. 2000).

Lectin purified from *T. obliquus* showed optimal activity at pH 7.0–7.5 and was stable after 90 min, losing activity outside of this range. The change in ionization state of a molecule affected by the pH determines the binding forces between the lectin and erythrocyte membrane, leading to a loss of

activity. Extremes of pH can alter the lectin native structure due to the changes in the ionization state of amino acid residues, which can lead to the denaturation of the lectin. Previously, a lectin from the red algas *Acrocystis nana* and *Tichocarpus crinitus* has also been reported to retain its maximum hemagglutinating activity within a pH range of 6.0–7.0 and 7.0–8.0, respectively (Molchanova et al. 2009; Anam et al. 2017).

Tetradesmus obliquus lectin showed optimal temperature of 20–50 °C and was stable at 50 °C for 90 min. Similar results were observed in lectins with high molecular mass such as red marine alga *Vidalia obtusiloba* lectin (Melo et al. 2004). On the other hand, *Chlorella pyrenoidosa* lectin (14–15 kDa) was tolerant of high temperature and its activity could be maintained even when the lectin solution was heated to 90 °C for 10 min (Yi et al. 2003). Normally, lectins with low molecular mass are more thermostable due to the three-dimensional structure of the protein and the presence of cysteines forming disulfide bonds (Nagano et al. 2002; Nascimento et al. 2006).

The addition of ions influenced hemagglutinating activity. As seen in many algal lectins, most of the lectins are non-dependent on divalent cations (Alvarez-Hernández et al. 1999; Chaves et al. 2018), for example, the lectins from the algae *Amansia multifida* (Costa et al. 1999), *Bryothamnion triquetrum* (Calvete et al. 2000), *Hypnea musciformis* (Nagano et al. 2002), *Tichocarpus crinitus* (Molchanova et al. 2009), and *Codium giraffa* (Alvarez-Hernández et al. 1999). On the other hand, the hemagglutinating activities of the red marine algae *Vidalia obtusiloba* (Melo et al. 2004) and *Pterocladiella capillacea* (Oliveira et al. 2002) were totally restored by the addition of the divalent cations (10 mM $CaCl_2$, $MgCl_2$). In contrast to the great majority of algal lectins, hemagglutinating activity exhibited by the purified lectin of *T. obliquus* was inhibited by ions and the most potent inhibitory substance was $CaCl_2$ (10 mM), unlike many agglutinins (lectin) from other sources. This suggests that *T. obliquus* may provide a valuable source for unique agglutinins.

In conclusion, we report purification and characterization of a novel type of lectin isolated from the alga *T. obliquus* that is easily mass-cultured in the laboratory, making it easy to supply the useful lectin. It has unique characteristics as high molecular weight, inhibition by different ions, specificity for poor-sialic acid erythrocytes, and complex carbohydrate, and does not agglutinate bovine, horse, and human A, B, and AB erythrocytes. Thus, this lectin could be considered as an alternative for applications in several areas of biotechnology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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