Partial Purification and Characterization of a lectin like protein from *Terminalia catappa* Seeds

Shaikh Fakeha Mohammed Rehan and Uzgare Ashish Sambhaji*
Department of Chemistry, Wilson College (Autonomous), Chowpatty Seaface Road, Mumbai- 400007, Maharashtra, INDIA
*ashish.uzgare@wilsoncollege.edu

Abstract

Lectins are proteins having the ability to specifically bind to selective carbohydrates or sugars located on the cell surface and molecules. This binding activity is highly specific, suggesting that each lectin protein is tailored to recognize and interact with a particular type of sugar molecule. The specificity of lectins plays a critical role in various biological processes including cell-to-cell recognition, communication and adhesion. By selectively binding to specific sugars, lectins help to facilitate the exchange of information between cells and molecules, enabling the proper execution of various physiological functions. The research aimed to investigate to partially purify and to characterize lectins from Terminalia catappa seeds. The process involved extracting the lectin from the seeds using physiological saline, partial purification by ammonium sulfate precipitation followed by dialysis.

The partially purified lectin extract was tested for multiple parameters. Hemagglutination assay revealed the highest lectin activity for human blood group B+ve, its sugar specificity towards galactose and lactose, loss of its hemagglutination activity by EDTA, enhanced hemagglutination by metal ions like Mg, Cr, Ca, Fe, etc. Effect of pH was found in the range of 4 to 11 and effect of temperature between 20 to 60^{0}C. The potential of the lectin for antimicrobial activity against E.coli and S. aureus was assessed by agar well diffusion method. Protein concentration was determined by Lowry's method, performed on a UV-Vis Spectrophotometer (Systronics model number 118).

Keywords: Lectins, *Terminalia catappa* seeds, Hemagglutination assay, Sugar specificity.

Introduction

Terminalia catappa is native to Southeast Asia also known as Indian almond. It grows best in moist tropical climates[22]. Plant lectin induced cytotoxicity, initiation of apoptosis and triggering of caspase cascade action leading to shrinkage and death of cancer cells are demonstrated in cell lines[21]. The other mechanism displayed by plant lectins includes polyamine sequestration, protein synthesis inhibition, reduced expression of telomerase activity and angiogenesis inhibition in tumor cells[40]. Lectins are proteins belonging to structurally diverse class that binds to carbohydrate moiety of the glyco-conjugates found on the animal cell surface. They occur ubiquitously in nature and are highly specific for sugar moieties.

During interaction, they form agglutinates with animal cells or precipitate as glyco-conjugates[38]. William Boyd discovered that the Latin word "legere," which means "to select," is the source of the English term "lectin"[29]. This generalized term was coined to incorporate all sugar-specific agglutinins of non-immune origin, irrespective of blood type specificity and source[32].

Lectins from plants are the most studied ones and many of them are isolated and characterized as hemagglutinins[7].The occurrence in nature of erythrocyte-agglutinating proteins has been known since the 19th century[33]. The Rhesus factor-based ABO blood group system consists of unique determinants and lectins bind to particular kinds of antigen found on red blood cells[1]. The simple procedure to detect a lectin is to probe its erythrocyte agglutination ability or to precipitate glycoconjugates using the Hemagglutination test[15].

The aim of this study is to partially purify and to characterize lectin like protein from seeds of *Terminalia catappa*. This study places particular emphasis on examining the protein's specificity toward various sugars, their effect of temperature, effect of EDTA, effect of metal ions and effect of pH, conducting a hemagglutination assay.

Material and Methods

Chemicals and reagents: All the chemicals used in this research work were of analytical grade. Solvents obtained from Himedia (India), SRL (India), Loba chemie (India), PCL (India) and Chemsworth (India) were of analytical grade. Human blood (RBCs) samples were collected from Blood Diagnostic Centre, Mumbai, Maharashtra, India.

Collection of sample: Natural samples of *Terminalia catappa* including seeds, leaves and fruits, were collected from Mumbai, Maharashtra, India and subsequently deposited at the Blatter Herbarium of St. Xavier's College (Autonomous) in Mumbai, Maharashtra, India, for identification and authentication.

Isolation of lectin from *Terminalia catappa* seeds: The extraction of lectin from *Terminalia catappa* seeds was done as per the method of Hiremath et al[17] with minor adjustments. Good quality *Terminalia catappa* seeds were allowed to soak in distilled water for 3 days at room temperature. The softened coat was peeled off and 30g seeds were ground in mortar and pestle and the powdered meal was

subjected to delipidation by adding 1:10 petroleum ether and by stirring with 4 changes every 30 mins. The delipidated powder was kept for extraction in physiological saline at 4ºC for 8 hours. The homogenate was centrifuged at 4000 rpm for 20-25 mins. Re-extraction was carried out and the combined supernatants were fractionally subjected for precipitation for 20%, 40%, 60% and 80% saturation respectively.

Purification of crude extract: The crude extract of *Terminalia catappa* seeds was partially purified using three steps:

1. Ammonium Sulphate precipitation was carried out as per the procedure of Hiremath et al[17] method.
2. Dialysis: The precipitated protein was further subjected to extensive dialysis using a dialysis tubing of molecular cut off 12-14 kDa as per the procedure of Haryanto et al[16]. The dialysis was carried out extensively as per the procedure of Ferreras et al[13]. The dialysed protein was centrifuged and the supernatant was stored at 4°C.

Estimation of Protein: The amount of protein in the extract was determined using the method of Lowry et al[27] by measuring the absorbance of light at 660 nm after reacting the samples with Folin-Ciocalteau reagent. A standard protein curve was plotted using bovine serum albumin (BSA) to calculate the protein concentration of each sample.

Hemagglutination assay: The hemagglutination assay was performed using 2 % suspension of normal human erythrocytes in saline and was carried out in a microtitre plate by using the two-fold serial dilution method of Jawade et al[19]. 100µl of the erythrocyte suspension was mixed with 100µl of serially diluted lectin. Agglutination activity was examined visually after incubation for 30 min at 37ºC. The highest dilution of the lectin that showed complete agglutination, is defined as the unit of hemagglutination activity (U) and termed as titer according to Dongre et al[11].

Sugar specificity: The inhibition of lectin-induced hemagglutination by various sugars was performed to investigate the sugar specificity. Mannose, glucose, galactose, dextrose, fructose and lactose sugar samples were prepared in saline. The sugar samples were mixed with an equal volume of a solution of serially diluted lectin. The mixture was allowed to stand for 30 min at 37ºC and then mixed with 2% human erythrocyte suspension. The known sugar which gives an agglutination with blood suspension and no agglutination of red blood cells in presence of lectin means that the tested lectin is specific to that sugar type[11].

Effect of EDTA on the Hemagglutination assay: The effect of EDTA on the Hemagglutination assay was done as per the procedure of Benseghir et al[6]. 100µl of EDTA at concentrations of 20mM, 30mM, 40mM and 50mM were added to 100µl of lectin solution that had been serially diluted. The mixture was then incubated at 37°C for 30 minutes. After this, a 2% suspension of erythrocytes was added. Two control tests were performed as positive control test where the hemagglutinating assay was tested without EDTA and a negative control test where no lectin was added.

Metal ion dependence: The effect on metal ion on Hemagglutination activity of lectin was studied as per the procedure of Sun et al[35]. The protein sample was pre-incubated at 37ºC for 30 mins with metal salts namely Mg^{+2} ($MgSO_4$), Cr^{+2} ($Cr_2(SO_4)_3$), Ca^{+2} ($CaCO_3$), Zn^{+2} ($ZnCl_2$), Fe^{+2} ($FeCl_2$) and Hg^{+2} ($HgSO_4$) followed by hemagglutination activity.

Effect of pH: The effect of pH was examined as per procedure of Jawade et al[19] by using buffer ranging from pH 1-12. pH 1-5 (0.2M Sodium acetate and Acetic acid buffer) and pH 6-12 (0.2M Disodium hydrogen phosphate and Sodium dihydrogen phosphate buffer) buffers were used. Equal volumes of serially diluted lectin sample and pH solutions were mixed and incubated at 37ºC for 30 mins and then hemagglutination assay with 2% erythrocytes was performed.

Effect of Temperature: The effect of temperature on the hemagglutination activity of the lectin was studied according to procedure of Jawade et al[19] by incubating the lectin solutions at various temperatures, ranging from 20°C to 100°C, for a duration of 30 mins followed by a hemagglutination test performed with a suspension of 2% erythrocytes.

Antimicrobial activity: The antibacterial activity of the partially purified lectin against the bacterial pathogens, including E. *coli* and *S. aureus*, was assessed using the agar well diffusion method as per the procedure of Dongre et al[11]. Wells measuring 8mm in depth were made on the agar using sterile cork borer. 100µl of partially purified lectin was added to the wells. The plates were incubated at 37°C for 24hrs.

Results and Discussion

The lectin was partially purified from *Terminalia catappa* seeds, by using ammonium sulphate precipitation between 20-80% followed by dialysis and centrifugation. The protein concentration of *Terminalia catappa* seeds lectin extract was found to be 1mg/ml by using Lowry's method of protein estimation. Hemagglutination assay was performed on human blood groups A, B and O using partially purified lectins extracted from *Terminalia catappa* seeds. These lectins showed positive agglutination activity against the ABO blood group system as summarized in table 1.

Negative hemagglutination activity is shown by a button formation and positive hemagglutination activity is inferred by observing mat formation at the bottom of the well. Blood group B+ showed the highest hemagglutination assay upto a dilution 1/512, Blood group O+ showed hemagglutination activity upto a dilution 1/32. Blood group A+ showed

hemagglutination activity up to the dilution of 1/8 as mentioned in figure 2.

Figure 1: *Terminalia catappa* seeds

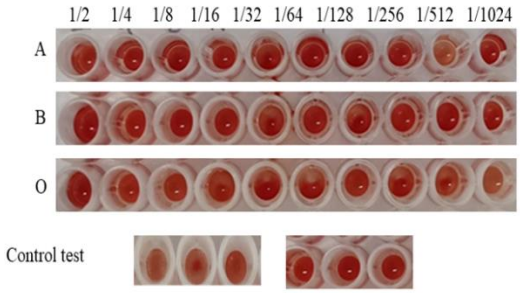

Figure 2: Hemagglutination activity of *Terminalia catappa* seeds lectin with 2% erythrocytes.

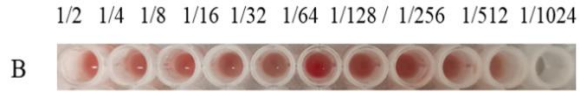

Figure 3: Hemagglutination activity of blood group B in saline extract

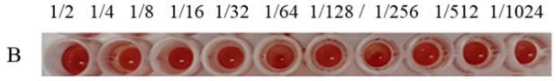

Figure 4: Hemagglutination activity of blood group B after dialysis

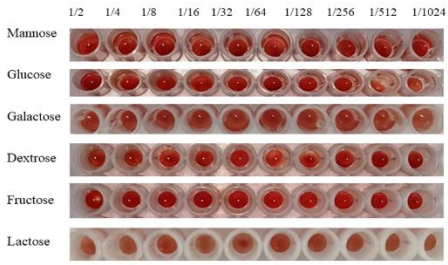

Figure 5: Sugar specificity of *Terminalia catappa* lectin

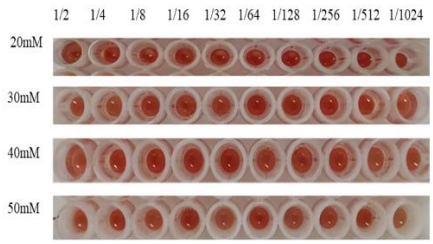

Figure 6: Hemagglutination activity on EDTA

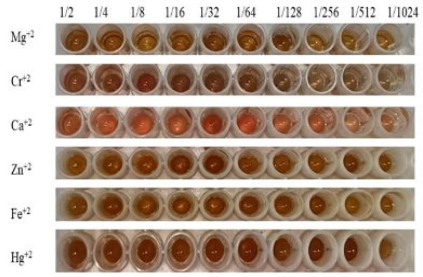

Figure 7: Effect on metal ions

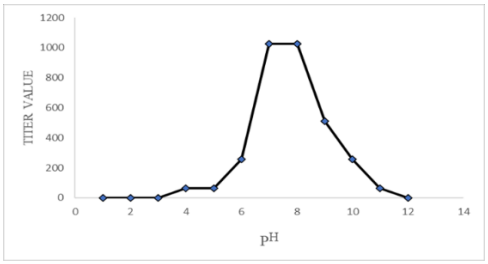

Figure 8: Effect of pH on the hemagglutination activity

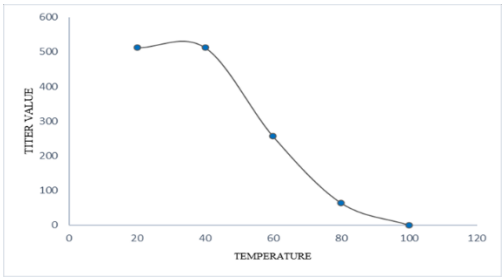

Figure 9: Effect of temperature on the hemagglutination activity

Hemagglutination activity results in Table 1 revealed that the human blood group B+ shows strong agglutination compared to O+ and A+**.** Blood group B+ showed a significant increase in hemagglutination activity after dialysis. In saline extract, hemagglutination activity was observed up to a dilution of 1/64. However, after dialysis, the hemagglutination activity increased substantially, reaching up to a dilution of 1/512. This is clearly depicted in table 2 and further illustrated in figures 3 and 4. The results of our study are in agreement with other studies from *moringa oleifera seed* lectin studied by Benseghir et al[6].

Lectin from leaves of *Euphorbia tithymaloides* (L.) showed hemagglutination activity in human blood group O+[19] and *Phaseolus vulgaris* (Red Kidney Bean) lectins showed hemagglutination activity in human blood group A+[11]. *Chlorella sorokiniana* lectin *(CSL)* showed hemagglutination activity towards chicken erythrocytes and no activity was found with human RBCs of A, B and O blood groups[4].

Sugar specificity: The hemagglutination activity of *Terminalia catappa* seed lectin was inhibited by galactose and lactose, thereby confirming the sugar moiety to be galactose and lactose. However, hemagglutination activity was observed in presence of mannose, glucose, dextrose and

fructose as summarized in table 2 and as shown in figure 5, thereby ruling out their presence as sugar moiety. These observations are in line with the studies on lectin reported in many instances, for example, hemagglutination activity of the purified red kidney beans lectin was not inhibited by mostsimple sugars such as glucose, galactose, fructose, sucrose and dextrose. However, it was successfully inhibited by xylose and maltose[11].

Lectin from Loach Skin Mucus showed that the hemagglutination activity was inhibited by glucose, arabinose, galactose, xylose, lactose, mannitol, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and L-fucose[35]. The indian borage plant *(Plectranthus amboinicus)* lectin binds specifically to galactose, dextrose, sucrose and raffinose, which proved to be potent inhibitor of its hemagglutination activity. Dextrose is the most effective inhibitor of indian borage plant *(Plectranthus amboinicus)* lectin[3].

Effect of EDTA: There was no hemagglutination activity visible in different concentrations (20mM, 30mM, 40mM and 50mM) of EDTA. In figure 6, the absence of hemagglutination activity in the presence of EDTA suggests that *Terminalia catappa* seed lectin might be a metalloprotein, with metal ions affecting its activity.

Effect of metal ions: Enhanced hemagglutination activity of lectin is visible in the presence of metal ions like Mg^{+2}, Cr^{+2}, Ca^{+2}, Zn^{+2}, Fe^{+2} and inhibited presence of Hg^{+2} ion as seen in figure 7, indicating that *Terminalia catappa* seed lectin may be a metalloprotein.

Table 1
Hemagglutination assay of lectins of *Terminalia catappa* seeds with Human erythrocytes

Human Blood group	A	B	O
Agglutination	+	+++	++

Table 2
Purification profile of *Terminalia catappa* seed lectin

	Total Hemagglutination Activity (HU)
Hemagglutination activity of blood group B in saline	1/64
After Dialysis Hemagglutination activity of blood group B	1/512

Table 3
Sugar specificity test of *Terminalia catappa* seed lectin

Sugar	**Hemagglutination activity**
Mannose	+
Glucose	+
Galactose	-
Dextrose	+
Fructose	+
Lactose	-

Table 4
Hemagglutination activity at different pH

pH	**Hemagglutination activity**
1	-
2	-
3	-
4	+
5	+
6	+
7	++
8	++
9	+
10	+
11	+
12	+

Table 5
Hemagglutination activity at different temperature

Temperature in ºC	20ºC	40ºC	60ºC	80ºC	100ºC
Hemagglutination activity	++	++	+	+	-

Researchers on lectins have reported significant effect of metal ions on lectin activity. *Tetradesmus obliquus* lectin[34], which showed hemagglutination activity was inhibited by Fe^{+2}, Ca^{+2}, Mg^{+2}, Zn^{+2} and Cu^{+2}. Loach skin mucus lectin lost its hemagglutination activity after dialysis against EDTA solution and regained activity when incubated with Ca^{+2}, suggesting it as a metalloprotein where Ca^{+2} plays a crucial role in its function and structure[35].

Moringa oleifera lectin is not a metaloprotein, hence it does not need metals for its activity[6]. The hemagglutination activity of *Chlorella sorokiniana* lectin (CSL) was not inhibited by Ca^{+2} ions but was slightly inhibited by Mg^{+2} ions compared to the initial activity[4]. Lectin from seeds of *Erythrina speciosa* is Ca^{+2} and Mn^{+2} dependent metaloprotein[24].

Effect of pH: There is no hemagglutination activity observed in the pH range 1-3 and at 12 whereas it is visible in the pH range 4-11 with maximum hemagglutination activity observed at pH 7 and 8 as summarized in table 4. Hence it can be inferred that *Terminalia catappa* seed lectin is stable in the pH range 4-11 as can be seen in the graph of pH vs titer value as in figure 8.

According to literature reviews, similar result were obtained in the lectin from the leaves of *Euphorbia tithymaloides* (L.) (pH 4-11)[19], *Bryothamnion triquetrum* (pH 4–11)[14], *Phaseolus vulgaris* (Red Kidney Bean) lectin (pH 3-10)[11] and *Moringa oleifera* lectin (pH 3-6)[6].

Effect of Temperature: Hemagglutination activity is observed between the temperature range 20°C- 40°C. The graph of temperature vs titer value indicates that as the temperature changes above and below this range, lectin activity gradually decreases and is totally absent at 100°C. From the figure 9, we can deduce that *Terminalia catappa* seeds contain lectin which has a high stability between 20°C and 40°C as shown by the peaks in the curve.

As the temperature exceeds 60°C, activity and stability gradually decrease and cease at 100°C. Similar results were observed in the seed lectins of *Phaseolus vulgaris* (Red Kidney Bean) [11], *Erythrina speciosa* seed lectin[24] and leaf lectin of *Euphorbia tithymaloides* (L.)[19], while *Moringa oleifera* lectin[6] and *Chlorella sorokiniana* lectin (CSL)[4] showed hemagglutination activity up to 70ºC.

Antimicrobial activity: Preliminary study of the partially purified *Terminalia catappa* lectin showed inhibitory effect on the growth of *E.coli* and *S. aureus.* Further detail study in this area will be interesting to study. Studies by El-Araby et al[12] on the lectins from Egyptian cultivars (fava bean, lentil and pea) showed antibacterial activity against *Staphylococcus aureus*, *Streptococcus mutants*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* but not against *Escherichia coli*. *Phaseolus vulgaris* (Red Kidney Bean*)* lectin showed antibacterial activity against *Escherichia colli*, *Pseudomonas*, *Klebsiella* and *S.aureus*, while the lectin had no antibacterial activity against *S. typhi*[11].

Conclusion

In this work lectin isolated from seeds of *Terminalia catappa* was partially purified by using ammonium sulphate precipitation method between 20-80% saturation and dialysis. Protein concentration was found to be 1mg/ml using Lowry's method of protein estimation. Partially purified *Terminalia catappa* seeds lectin exhibited strong agglutination with human erythrocytes with the titer against the blood group B+ as compared to O+ and A+**.** For blood group B+, hemagglutination activity was observed up to a dilution of 1/64 in saline extract. After dialysis, the activity increased and was detectable up to a dilution of 1/512. Sugar specificity of isolated lectin is towards galactose and lactose.

The absence of hemagglutination activity in the presence of EDTA suggests that *Terminalia catappa* seed lectin might be a metalloprotein. Enhanced hemagglutination activity of *Terminalia catappa* seed lectin is visible in various metals such as Mg^{+2}, Cr^{+2}, Ca^{+2}, Zn^{+2}, Fe^{+2} and not visible in Hg^{+2}. These observations indicate that *Terminalia catappa* seed lectin is a metalloprotein that responds positively towards above mentioned metal ions for its functionality. This lectin showed optimum activity in the pH range 7-8 and temperature range 20-40°C. Highly acidic and basic pH inhibit the activity of *Terminalia catappa* seed lectins whereas hemagglutination activity starts minimizing at 60ºC and loses their activity at 100ºC.

Preliminary study indicated the presence of antimicrobial activity against *E.coli* and *S.aureus* which needs to be studied in detail. It is evident from reviews and descriptions of the literature that lectin is important in the field of medicinal, molecular and cellular biology. Lectins have vast applications such as in the treatment of different illnesses, as a possible medicinal agent and play a role as biomarkers. Lectins have gained immense importance in various studies these days. In order to have lectins for the food and medicine of the future, these plants should be investigated through experimental research.

Acknowledgement

The authors wish to acknowledge the Principal and the Management of John Wilson Education Society's Wilson College for providing the laboratory facilities to carry out the proposed research work. The support extended by the Head of the Department of Chemistry and all the teaching and support staff is duly acknowledged.

We very fondly dedicate this paper to Late Dr. D. Vardhan, Ex Professor, Ruia College, Mumbai. Vardhan Sir had been a vital force in motivating young teachers like me to take up research and attend conferences.

References

1. Ajit C.G. and Kanjaksha G., Use of lectins in immunohematology, *Asian Journal of Transfusion Science*, **10(1)**, 12-21 **(2016)**

2. Arekal R., Kumar M., Keerthana H., Dharmaraj M., Muralidhar S., Priya R. and Janakiraman S., Isolation and Characterization of Bacteriocin-Producing Lactic Acid Bacteria from Fermented Foods, *Journal of Food Science and Technology*, **59(9)**, 2826-2834 **(2023)**

3. Arekal R.N., Kumar M.S., Keerthana H., Kumar M., Muralidhar S., Priya R. and Janakiraman S., Purification and characterization of lectin from Indian borage leaves (Plectranthus amboinicus), *Journal of Biomedical and Pharmaceutical Sciences*, **12(1)**, 1-10 **(2023)**

4. Arekal R.N., Savitha J., Kumar A., Gunashree B.S. and Divyashree M.S., Structural and functional analysis of a novel galactose-binding lectin derived from Chlorella sorokiniana MW769776, *Journal of Biomolecular Structure and Dynamics*, **41(12)**, 5246-5262 **(2023)**

5. Bashir H., Khan T., Masood A. and Hamid R., Isolation, purification and characterization of a lectin from a local Kashmiri variety of soybean (Glycine max), *Journal of Bio Sciences*, **22(1)**, 1-8 **(2010)**

6. Benseghir C. and Namulodi P., Extraction, isolation and characterization of lectins from Moringa oleifera seeds, Master's dissertation, University of Mentouri Brothers, Constantine 1, Faculty of Nature and Life Sciences **(2017)**

7. Cagliari R., Kremer F.S. and Pinto L.D.S., Bauhinia lectins: Biochemical properties and biotechnological applications, *International Journal of Biological Macromolecules*, **119**, 811-820 **(2018)**

8. Cavada B.S., Bari A.U., Pinto-Junior V.R., Lossio C.F., Silva M.T.L., Souza L.A.G., Oliveira M.V., Correia S.E.G., Vital A.P.M.S., Lima L.D., Osterne V.J.S. and Nascimento K.S., Purification and partial characterization of a new lectin from Parkia panurensis Benth. Ex H.C. Hopkins seeds (Leguminosae family; Mimosoideae subfamily): Evaluation of its biological effects, *Journal of Applied Biotechnology*, **8(2)**, 1-13 **(2020)**

9. De Oliveira A.S., Lóssio 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. and Do Nascimento K.S., Detection, purification and characterization of a lectin from freshwater green algae Spirogyra spp., *Journal of Phycology and Algal Research*, **3(1)**, 1-9 **(2016)**

10. Djabayan-Djibeyana P., Carpenter B., Medina-Ramírez G., Andueza-Leal F., León-Leal A., Djabayan-Russo A., Jaramillo-Abril D., Valarezo-García C. and Araujo-Baptista L., Cold steeping infusion: A novel lectin extraction technique for the isolation, purification and partial characterization of lectins from the green Venezuelan marine alga Caulerpa serrulata, *Journal of Applied Phycology*, **31(6)**, 1269-1281 **(2018)**

11. Dongre P., Shaikh N., Gangwal P. and Pawar K., Isolation, partial purification and application of lectin isolated from Phaseolus vulgaris (red kidney bean), *Journal of Applied Biotechnology*, **6(1)**, 1-6 **(2019)**

12. El Araby M.M., El Shatoury E.H., Soliman M.M. and Shaaban H.F., Characterization and antimicrobial activity of lectins purified from three Egyptian leguminous seeds, *AMB Express*, **10**, 90 **(2020)**

13. Ferreras J.M., Clemencia M.C.M., Hizon-Fradejas A., Uy L.Y. and Torio M.A., Isolation, purification and characterization of proteins in "Señorita" banana (Musa acuminata (AAA) 'Señorita') pulp with bioactive peptides exhibiting antihypertensive and antioxidant activities, *Applied Sciences*, **11(5)**, 2190 **(2021)**

14. Fontenelle T.P.C., Lima G.C., Mesquita J.X., Lopes J.L.D.S., de Brito T.V., Vieira Junior F.D.C., Sales A.B., Aragao K.S., Souza M.H.L.P., Barbosa A.L.D.R. and Freitas A.L.P., Lectin obtained from the red seaweed Bryothamnion triquetrum: Secondary structure and anti-inflammatory activity in mice, *International Journal of Biological Macromolecules*, **112**, 1122–1130 **(2018)**

15. Goldstein I.J., Hughes R.C., Monsigny M., Osawa T. and Sharon N., What should be called a lectin?, *Nature*, **285**, 66 **(1980)**

16. Haryanto H. and Kamilah S.N., Partial characterization of protein extracted from Terminalia catappa seed behaving as lectin that is capable of mouse sperm agglutination, AIP Conference Proceedings, **1844**, 030002 **(2017)**

17. Hiremath K., Hegde P., Sharma M. and Inamdar S.R., A modified method for purification of N-acetylgalactosamine-specific lectin from Butea monosperma seeds and its effect on human hepatocellular carcinoma cell growth, *Journal of Plant Biochemistry and Biotechnology*, **28(1)**, 1-10 **(2019)**

18. Janporn S., Ho C.T., Chavasit V., Pan M.H., Chittrakorn S. and Ruttarattanamongkol K., Physicochemical properties of Terminalia catappa seed oil as a novel dietary lipid source, *Journal of Food and Drug Analysis*, **23(2)**, 201-209 **(2015)**

19. Jawade A.A., Pingle S.K., Tumane R.G., Sharma A.S., Ramteke A.S. and Jain R.K., Isolation and characterization of lectin from the leaves of Euphorbia tithymaloides (L.), *International Journal of Biological Macromolecules*, **136**, 1126-1134 **(2016)**

20. Jiang B., Wang X., Wang L., Lv X., Li D., Liu C. and Feng Z., Two-step isolation, purification and characterization of Lectin from Zihua Snap Bean (Phaseolus vulgaris) Seeds, *Polymers (Basel)*, **11(5)**, 785 **(2019)**

21. Jiang Q.L., Zhang S., Tian M., Zhang S.Y., Xie T., Chen D.Y., Chen Y.J., He J., Liu J., Ouyang L. and Jiang X., Plant lectins, from ancient sugar-binding proteins to emerging anti-cancer drugs in apoptosis and autophagy, *Cell Proliferation*, **48(1)**, 17–28 **(2015)**

22. Jony M.F.A. and Kumar R.B.R., A comprehensive review on the pharmacological activity of Terminalia catappa (Combretaceae) – An update, *Journal of Pharmaceutical Sciences and Research*, **11(3)**, 1578-1586 **(2013)**

23. Kim J.Y., Lee J.Y., Kim J.H. and Park Y.K., Inhibitory effect of lectin on lipoxygenase activity and lipid accumulation in adipocytes, *Journal of Agricultural and Food Chemistry*, **65(31)**, 7344-7351 **(2017)**

24. Konozy E.H.E., Bernardes E.S., Rosa C., Faca V., Greene L.J. and Ward R.J., Isolation, purification and physicochemical characterization of a D-galactose-binding lectin from seeds of Erythrina speciose, *Archives of Biochemistry and Biophysics*, **416(1)**, 941-951 **(2002)**

25. Krishnaveni M., *In vitro* antioxidant activity of *Terminalia catappa* nuts, *Asian Journal of Pharmaceutical and Clinical Research*, **7(4)**, 36-39 **(2014)**

26. Kurokawa T., Tsuda M. and Sugino Y., Purification and characterization of a lectin from *Vestaria floribunda* seeds, *Journal of Biochemistry*, **69(3)**, 341-347 **(1976)**

27. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry*, **193(1)**, 265-275 **(1951)**

28. Odiegwu C.N.C., Ukaejiofo E.O., Tothill I.E., Chianella I. and Okey-Onyesolu C.F., Purification and characterization of lectin isolated from Nigerian Achatina achatina snail, *GSC Advanced Research and Reviews*, **5(3)**, 1-9 **(2020)**

29. Oyd W.C. and Shapleigh E., Specific precipitating activity of plant agglutinins (lectins), *Science*, **119(3096)**, 419 **(1954)**

30. Patil M.B. and Deshpande K.V., Isolation and characterization of lectin from leaves of Dregea volubilis, *International Journal of Advanced Research in Biotechnology*, **3(1)**, 1-7 **(2015)**

31. Pohleven J. and Kos J., Affinity Chromatography of Lectins, In Affinity Chromatography: Principles and Applications, 157-176 **(2012)**

32. Sharon N. and Lis H., Lectins: Cell-agglutinating and sugar-specific proteins, *Science*, **177(4053)**, 949-959 **(1972)**

33. Sharon N. and Lis H., History of Lectins: from Hemagglutinins to Biological Recognition Molecules, *Glycobiology*, **14**, 53-62 **(2004)**

34. Silva A.J., Cavalcanti V.L.R., Porto A.L.F., Gama W.A., Brandao-Costa R.M.P. and Bezerra R.P., The green microalgae Tetradesmus obliquus (Scenedesmus acutus) as lectin source to recognition of ABO blood type: Purification and characterization, *Journal of Applied Phycology*, **32(1)**, 103–110 **(2020)**

35. Sun P., Ren Y., Zheng J. and Hu A., Purification and characterization of a new lectin from loach skin mucus, *Journal of Food Science and Technology*, **55(4)**, 1080-1087 **(2019)**

36. Tamboli F.A., More H.N., Bhandugare S.S., Patil A.S., Jadhav N.R. and Killedar S.G., Estimation of total carbohydrate content by the phenol-sulfuric acid method from Eichhornia crassipes (Mart.) Solms, *International Journal of Pharmaceutical Sciences and Research*, **10(3)**, 1-6 **(2020**)

37. Toda M., Jimbo M., Muramoto K., Sakai R. and Kamiya H., Isolation and characterization of a D-galactose-binding lectin from the acorn barnacle Balanus rostratus, *Journal of Shellfish Research*, **64(4)**, 638-642 **(1998)**

38. Van Damme E.J.M., Peumans W.J., Pusztai A. and Bardocz S., Properties and biomedical applications of plant lectins, In van der Meer A.P.J.M. and Kalk J.T.K., eds., Handbook of plant lectins, John Wiley & Sons, 1-23 **(1998)**

39. Weerawatanakorn M., Janporn S., Ho C.T. and Chavasit V., Terminalia catappa Linn. Seeds as a new food source, *Songklanakarin Journal of Science and Technology*, **37(5)**, 507-514 **(2015)**

40. Yau T., Dan X., Ng C.C. and Ng T.B., Lectins with potential for anti-cancer therapy, *Molecules (Basel, Switzerland)*, **20(3)**, 3791–3810 **(2015)**

41. Zuo Z., Fan H., Wang X., Zhou W. and Li L., Purification and characterization of a novel plant lectin from Pinellia ternata with antineoplastic activity, *Journal of Plant Physiology*, **248**, 125-133 **(2012)**.

(Received 09th August 2024, accepted 12th September 2024)