Research Article

www.ijrap.net

EVALUATION OF HAEMAGGLUTINATION POTENTIALS OF LECTIN ISOLATED FROM ORDEAL TREE (*ERYTHROPHLEUM SUAVEOLENS*) STEM BARK

Ihuoma Onyeyirichi^{1*}, Inuwa Hajia Mario², Ibrahim Sani³, Okunola Joshua⁴, Nkeonye Ogechi⁵

¹Research officer I, Biochemistry Division, Basic Research Department, National Research Institute for Chemical Technology, Basawa, Zaria, Kaduna State, Nigeria

²Head, Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria

³Associate Professor, Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria

⁴Head, Petrochemical Division, National Research Institute for Chemical Technology, Basawa, Zaria, Kaduna State, Nigeria

⁵Research officer II, Biochemistry Division, Basic Research Department, National Research Institute for Chemical Technology, Basawa, Zaria, Kaduna State, Nigeria

Received on: 14/09/13 Revised on: 24/10/13 Accepted on: 11/11/13

*Corresponding author

E-mail: chiexotic86@gmail.com

DOI: 10.7897/2277-4343.04619

Published by Moksha Publishing House. Website www.mokshaph.com

All rights reserved.

ABSTRACT

Lectin from *Erythrophleum suaveolens* stem bark was partially purified and evaluated in terms of its haemagglutination activity. Ground, dried stem bark of *Erythrophleum suaveolens* was extracted by aqueous method and the protein extract was concentrated in 20-90 % saturation. The ammonium sulfate precipitate was subjected to gel filtration chromatography using Sephadex G-75 resin and fractions were tested for haemagglutination activity. The two fractions obtained with the highest hemmagglutinating activity were further subjected separately to ion-exchange chromatography using Sp-Sephadex C-50 resin. Isolated lectins were found to agglutinate chicken erythrocytes; fractions from gel filtration chromatography showed haemagglutinating activity of 0.1 HU/ μ L and 0.091 HU/ μ L, while the ion exchange fractions showed haemagglutinating activity of 0.05 HU/ μ L and all were stable at the temperature range of 30-60°C and pH range of 3-7. The carbohydrate content of the crude extract, Gel I, Gel II, Ion I and Ion II fractions were 0.04 %, 0.04 %, 0.03 %, 0.02 %, respectively. The crude and purified lectins inhibited agglutination in the presence of mannose and N-acetyl- glucoseamine sugars and showed five bands at positions 38kDa, 28kDa, 26kDa, 11kDa and 9kDa on a SDS-PAGE electrophoregram.

Keywords: Haemagglutinating activity, lectin, Bark, *Erythrophleum suaveolens*, ordeal tree, erythrocytes

INTRODUCTION

Plants are the richest and most convenient source of lectins¹. Plant lectin has been attracting much attention because of their ease of isolation and their usefulness as reagents for glycol-conjugates in solution and on cell surface¹. According to Sharon, lectins were discovered by their ability to agglutinate erythrocytes and hence the easiest and most convenient method of detecting lectin activity remains agglutination of a part of human, animal red blood cells or other cells. Generally lectins are particularly prevalent in leguminous plants, where they are localized in the cotyledons of the seeds and roots¹. Most lectins are basically non-enzymatic in action and non-immune in origin and bind to a carbohydrate moiety that is in solution or to one that is a part of protein/particulate body¹. Lectins have been found in diverse types of organisms, including animals²⁻⁴. In recent years, lectins have drawn large attention from researchers owing to their various biological activities such as antitumor⁵, mitogenic⁶, immunomodulatory^{2,3}, antifungal⁷, antiviral⁸ and anti-insect activities⁹. Lectins also have immense value in glycomics study, lectin microarray, has become a rapid and a simple tool of protein glycosylation, and lectin-modified micro-channels for mammalian cell capture and purification have been reported¹⁰. The isolation and characterization of non-leguminous lectins is of importance in order to study the

minor structural differences that can lead to dramatic differences in biological properties and also to investigate the ubiquitous nature of the lectins¹¹. Considering the number of the lectins available in nature, the ease with which they could be prepared in the purified form, their amenability to chemical manipulation and the fact that they can be inhibited by simple sugars makes them attractive, therefore purification and characterization of lectins from variety of plant species interest researchers in the field of glycobiology¹¹. *Erythrophleum suaveolens* is a poisonous plant that belongs to the fabaceae family and is wide spread throughout tropical Africa. The bark extract has an excellent local anesthetic activity on the eyes and skin, although it causes irritation on the conjunctiva, trials using it as anesthetic in operations or tooth extractions were less successful, because symptoms of poisoning occurred in higher doses¹². From literature search, lectin has never been isolated or purified from *Erythrophleum suaveolens* hence, our decision to embark on this study. This study reports the purification and characterization the lectin from *Erythrophleum suaveolens* stem bark.

MATERIALS AND METHODS

The plant stem bark and leaves were collected from Kagarko, Kaduna State, Nigeria in the month of June, 2011 and identified to be *Erythrophleum suaveolens* with voucher number 242 at the Herbarium, Botany Unit,

Ahmadu Bello University, Zaria, Kaduna state. Erythrocyte samples were collected from a blend of chickens in Samaru slaughter house and used immediately. Sephadex G-75 and SP-sephadex C-50 were purchased from Sigma (USA) and Pharmacia (USA), respectively. Standard proteins were purchased from Gibco-BRL (Life Tech., USA). All other reagents used were of analytical grades.

Extraction and Concentration of Lectin

Powdered bark of *Erythrophleum suaveolens* bark (25 g) was suspended in 0.02M phosphate buffer saline (PBS) at pH 7.2 (1:20 w/v). The suspension was filtered through muslin cloth and then the filtrate was centrifuged at 8000 g for 15 minutes at 7°C^{13,14}. The crude extract was brought from 20 % - 90 % ammonium sulphate saturation¹⁵. The precipitate was collected by centrifugation at 8000 g for 15 minutes¹⁵ and re-dissolved in PBS at pH 7.2. The concentrated protein solution was subjected to further purification. Protein concentrations were determined according to Bradford¹⁶ using Bovine serum albumin as standard. Subsequently 2 % v/v chicken erythrocytes were prepared in 0.2M phosphate buffer saline (PBS) pH 7.2 as described by Oyedapo¹⁷. Haemagglutinating activity (HA) was determined as described by Calderón de la Barca¹⁸. Aliquots (100 µL) of phosphate buffer saline solution were added to each well of a 96-well microtiter plate, and a 100 µL of the sample was transferred to the first well. Serial dilutions (base 2) were prepared by transferring a 100 µL aliquot of a more concentrated sample to adjacent well containing diluents alone, it was mixed and dilution procedure continued. An aliquot (100 µL) of 2 % suspension of erythrocytes prepared from chicken erythrocytes was added to each well and the microtiter plate was maintained at room temperature. HA was determined visually after 1 hour and the results were expressed in haemagglutination units (HU), defined as the reciprocal of the maximum dilution presenting visible agglutination of red blood cells (that is, if the 1/4 titre represented the maximum dilution exhibiting agglutination and the volume of sample assayed was 100 µL, then the HA value would be 4 HU/100 µL). Negative control samples consisted of phosphate buffer saline solution and erythrocyte suspension only.

Partial purification of *Erythrophleum suaveolens* Lectin

1 ml of the ammonium sulphate precipitate (mg/ml) was loaded on Sephadex G-75 column (9 x 2 cm³), previously equilibrated with phosphate buffer, pH 7.2, fractions of 5 ml were collected, elution was monitored at 280 nm and the fractions were assayed for haemagglutination activity. Ion exchange chromatography was carried out as described by Knight¹⁹ using Sp-Sephadex C-50 cation exchanger. The column was pre-equilibrated with 0.02M Phosphate buffer pH 7.2. The fractions that showed haemagglutination activity from gel filtration chromatography were loaded separately on the column and was eluted with NaCl gradient (0.01- 0.3M) at a flow

rate of 4 ml / 5 minutes. A total of 25 fractions of 5mls each were collected and assayed for haemagglutination activity and total protein. SDS-PAGE of the purified lectins were carried out as described by Laemmli²⁰. The gel was silver stained by the method of Vari and Bell²¹.

Characterization of *Erythrophleum suaveolens* Lectins Effect of Temperature on *Erythrophleum suaveolens* Lectin

The effect of temperature on lectin activity was determined as described by Sampaio²². Aliquots of the purified lectin were incubated in a water bath for 30 minutes at temperatures from 30°C-100°C and then cooled on ice and assayed for haemagglutination activity. The haemagglutination activity of the control that was kept at 20°C for 30 minutes was used as reference.

Effect of pH on Haemagglutination Activity Using Different Buffers

The effect of pH on the haemagglutinating activity was determined by carrying out the haemagglutinating assay of the lectins using the following buffers at different pH values; 0.2M glycine-NaOH buffer, pH 10-12; 0.2M citrate-phosphate buffer, pH 3-7, and 0.2M borate buffer, pH 7.5-8.5 and 9.0-9.5. The control values were the haemagglutination titre of the lectin using 0.2M Phosphate buffer saline, pH 7.2.

Sugar Inhibition Test

The inhibitory effects of various sugars on the haemagglutinating activity of chicken erythrocyte by the isolated lectins were determined as described by Kuku²³. For each sugar, 50 µL each of 1 M and 0.5 M were placed in separate microtitre plates wells. This was followed by the addition of 50 µL of the lectins to each well. Then 50 µL of 2 % (v/v) erythrocyte suspension was added to each mixture, mixed and monitored for agglutination after 1 hour. The sugars used for this experiment were: N-acetyl glucosamine, glucose, maltose, lactose, galactose, arabinose, raffinose, maltose, mannose and xylose.

Carbohydrate Content of *Erythrophleum suaveolens* Lectin

The carbohydrate content was determined by phenol-sulphuric method of Dubois²⁴ using D-glucose as standard.

RESULTS AND DISCUSSION

Saline extract of *Erythrophleum suaveolens* lectin agglutinated chicken erythrocytes. This haemagglutinating activity was inhibited by both N- acetyl-glucoseamine and D- mannose of all the sugars tested. (Table 1)

The 20-90 % ammonium sulphate fractionation of the bark extract when subjected to gel filtration on Sephadex G-75 (Figure 1) gave two major protein peaks, which showed haemagglutinating activity. The sample of the active peaks from gel filtration chromatography was further purified on Sp-sehadex C-50 Ion exchange chromatography independently.

Table 1: Summary of Purification Steps of Lectins from the Stem Bark of *Erythrophleum suaveolens*

Steps	Total protein (mg)	Total activity (HU)	Specific Activity (Unit/mg)	Purification fold	% yield
Crude extract	22,380	550	0.03	1	100
NH ₄ SO ₄	3176	90	0.03	1	16.36
Gel I	125.95	7	0.06	2	1.27
Gel II	145.6	8	0.05	1.67	1.46
Ion I	1.06	2.5	2.36	78.67	0.46
Ion II	1.5	2.5	1.67	55.67	0.46

Data for 25 g of *Erythrophleum suaveolens* stem bark. Volume of the crude extract used was 500 ml.

HU- One hemagglutination unit (HU) is defined as the reciprocal of the maximum dilution still causing a visible agglutination. Gel I and Gel II are the different fractions from gel filtration chromatography. Ion I and Ion II are the different fractions from ion exchange chromatography.

Table 2: Sugar Inhibition of Haemagglutination activity of *Erythrophleum suaveolens* stem bark lectin

Agglutination Titre	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁸	2 ⁹	2 ¹⁰	2 ¹¹	2 ¹²
Control	+	+	+	+	+	+	+	-	-	-	-	-
Glactose	+	+	+	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	-	-	-	-	-	-
Fructose	+	+	+	+	-	-	-	-	-	-	-	-
N-acetyl-Glucosamine	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	-	-	-	-	-	-	-	-	-
Raffinose	+	+	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	-	-	-	-	-	-	-	-
Xylose	+	+	+	+	-	-	-	-	-	-	-	-
Mannose	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	-	-	-	-	-	-	-	-

Each experiment consisted of 100 µl of serially diluted extract in a microtitre plate well. 100 µl of 0.5 M sugar solution and 50 µl of 2 % suspension of erythrocytes (chicken) were added to each well.

Table 3: Sugar Inhibition of Haemagglutination Activity of *Erythrophleum suaveolens* stem bark extract

Agglutination Titre	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁸	2 ⁹	2 ¹⁰	2 ¹¹	2 ¹²
Control	+	+	+	+	+	+	+	-	-	-	-	-
Glactose	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	-	-	-	-	-	-
Fructose	+	+	+	+	+	+	-	-	-	-	-	-
N-acetyl-Glucosamine	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	-	-	-	-	-	-	-	-	-
Raffinose	+	+	+	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	-	-	-	-	-	-	-	-
Xylose	+	+	+	-	-	-	-	-	-	-	-	-
Mannose	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	-	-	-	-	-	-	-	-	-

Each experiment consisted of 100 µl of serially diluted extract in a microtitre plate well. 100 µl of 1M sugar solution and 50 µl of 2 % suspension of erythrocytes (chicken) was added to each well.

Figure 1: Elution Profile and Haemagglutination Activity of 20-90 % Ammonium Sulphate Fractions of *Erythrophleum suaveolens* Lectin from Sephadex G-75 Gel Filtration Column

Figure 2: Elution Profile of Pooled Ion Exchange Chromatographic Column Fraction I of *Erythrophleum suaveolens* Lectin from Sp-Sephedex C-50

Figure 3: Elution Profile of Pooled Ion Exchange Chromatographic Column Fraction II of *Erythrophleum suaveolens* Lectin from Sp-Sephedex C-50

Figure 4: Effect of Temperature on the Haemagglutinating Activity of *Erythrophleum suaveolens* stem bark lectin

Figure 6: Effect of pH on the Haemagglutinating Activity of *Erythrophleum suaveolens* stem bark lectin

Plate 1: Electrophoregram of Proteins from the Various Fractions

Lane 1- Molecular Markers, Lane 2- Crude Extract (38KDa), Lane 3- NH_4SO_4 Concentrate (36KDa), Lane 4 - Gel I (28KDa), Lane 5- Gel II (26KDa), Lane 6- Ion I (11KDa), Lane 7- Ion II (9KDa).

The elution profile of fraction 14 (peak I) is represented in (Figure 2). A single peak was obtained with haemagglutination activity of 0.5 HU/ml. While the elution profile of fraction 38 (peak II) is represented in (Figure 3). A single peak was also obtained with haemagglutination activity of 0.5 HU/ml.

The electrophoregram of the crude and purified proteins is represented in Plate 1. The molecular makers are represented in lane 1. The crude lectin showed one band with molecular weight of 38KDa as represented on lane 2. The NH_4SO_4 concentrated protein also showed a single band with molecular weight of 36KDa as represented on lane 3. Gel fractions I and II both showed single bands each with molecular weights of 28KDa, and 26KDa (shown on lanes 4 and 5) respectively. Ion fractions I and II also showed single bands each with molecular weights

of 11KDa and 9KDa (shown on lanes 6 and 7) respectively. Evaluation of *Myracrodruon urundeuva* bark lectin and *Myracrodruon urundeuva* hardwood lectin by electrophoresis showed single polypeptide bands in PAGE as reported by Roberto *et al*²⁵. The detection of single band only in PAGE for basic proteins revealed that both lectins have positive net charge and that the established purification protocols yielded homogeneous lectins²⁵. Inhibition occurred with mannose and N-acetylglucoseamine at both 0.5M and 1M concentrations respectively. This inhibition indicates that the sugar-lectin interaction was stronger than the erythrocyte-lectin attraction and consequently formed a distinct button of the erythrocytes at the bottom of the well. Therefore, *Erythrophleum suaveolens* lectin is mannose and N-

acetyl-glucoseamine sugar specific. Bourne *et al*²⁶ reported that carbohydrate binding specificity was exhibited by jacalin, the seed lectin from *Artocarpus integrifolia* towards galactose and mannose. Structural analysis demonstrated that this may be due to the large size of the carbohydrate-binding site of jacalin that enables it to accommodate monosaccharides with different hydroxyl conformations²⁷. The saccharide binding specificities of lectins can be utilized in the purification, characterization and sequencing of polysaccharides, polypeptides and glycoproteins¹⁵. D-Mannosamine and N-acetyl-D-glucosamine was reported to inhibit agglutination of promastigotes²⁸. This inhibition indicates that the lectin contains numerous binding sites that were compatible with a wide range of monosaccharides receptors. Non-inhibition of agglutination by some of the sugar molecules indicates that erythrocyte-lectin interaction may be due to affinity for lectin substitution at certain positions of the sugar moiety which determines their specificity²⁷. It could also be as a result of the non specificity of lectin binding sites to the individual sugar anomers²⁹. The erythrocytes-lectin binding may not be affected by the size and shape of sugar residues²⁹. The carbohydrate containing the sugar or its derivatives may cross react with the lectin²⁹. *Erythrophleum suaveolens* is stable up to 60°C³⁰, but when heated above this temperature there is a rapid loss of activity and at 90°C activity was completely lost (Figure 4).

Hevea brasiliensis lectin was reported, to have a stable haemagglutinating activity at 60°C²⁸ while *Ptilota filicina* lectin was reported to be stable at 50°C²². Stability over a wide temperature range is typical of plant lectins and can be attributed to their role in plant defense mechanisms against environmental stress³¹. The stability of lectins from *Erythrophleum suaveolens*, similar to other plant lectins was probably dependent upon several structural characteristics. The compact globular structures of lectins due to high number of hydrophobic interactions, hydrogen bonds and disulphide bridges (intra and inter chains), molecular aggregation and glycosylation in general results in high structural stability of native lectins^{32,33}. *Erythrophleum suaveolens* are glycoproteins in nature because they gave orange yellow color in the presence of phenol-sulfuric acid with different carbohydrate contents which is in accordance with the report of Khan *et al*³⁴ on *Chorchorus olitorius* lectins which contained 1.3 %, 1.2 % and 0.8 % neutral sugars, Syed *et al*³⁵ also reported the formation of orange yellow color in the presence of phenol-sulfuric acid by rice-bran lectin with sugar and neutral content of 4 % and 5.4 % Kuku *et al*²³ reported that a purplish pink color was obtained by staining *Kalanchoe crenata* lectin with Schiff's reagent. Purified *Erythrophleum suaveolens* lectin had a stable haemagglutination activity within the pH range of 3-7. The activity, however, fell off fairly rapidly thereafter, with essentially all activity lost after pH 9.2 as represented in Figure 5. It is possible that changes in the ionization state with an increase in pH may have led to a weaker binding of the metal ions, which were apparently required for the maintenance of the structure that was required for maximal activity²³. The pH dependence,

which is observed in virtually all enzyme reactions, is a consequence of the protein composition²³.

Protein molecule and the active center were capable of reacting with H⁺ or OH⁻²³. Any pH change is therefore associated with a change in the ionization state of the molecule, which in turn, determines the binding forces between enzyme and substrate³⁶. It is also possible that the increase in OH⁻ ions causes a change in the ionization state of the lectin, thereby affecting the binding forces between the lectin and the erythrocyte membrane that eventually lead to loss of activity. The lectin of *Ptilota filicina* was also stable in the pH range of 4-9, retaining 50 % of its activity at pH 3 and 10, and 25 % of its activity at pH 11-12²³. The lectin from *Parkia javanica* beans was stable in the pH range of 7-10³⁶. It was also reported that, more acidic or basic pH decreases both the stability and activity of lectins³⁷.

CONCLUSION

In this work, lectins were successfully isolated, partially purified and partially characterized from *Erythrophleum suaveolens*.

REFERENCES

- Sharon N, Lis H. History of lectins: From Hemagglutinins to Biological Recognition Molecules. *Glycobiology* 2004; 14: 53–62. <http://dx.doi.org/10.1093/glycob/cwh122> PMID:15229195
- Engel M, Bachmann M, Schroeder HC, Rinkevich B, Kljajic Z, Uhlenbruck G, *et al*. A Novel Galactose and Arabinose Specific Lectin from the Sponge *Pellina semitubulosa*. Isolation, Characterization and Immunological Properties. *Biochimie* 1992; 74: 527–37. [http://dx.doi.org/10.1016/0300-9084\(92\)90150-D](http://dx.doi.org/10.1016/0300-9084(92)90150-D)
- Terada T, Watanabe Y, Tateno H, Naganuma T, Ogawa T, Muramoto K, *et al*. Structural, characterization of a rhamnose-binding glycoprotein (lectin) from Spanish mackerel (*Scomberomorus niphonius*) eggs. *Biochim Biophys Acta* 2007; 1770: 617–29. <http://dx.doi.org/10.1016/j.bbagen.2006.11.003> PMID:17184920
- Ng TB, Lam YW, Woo NYS. The Immunostimulatory Activity and Stability of Grass Carp (*Ctenopharyngodon idellus*) roe Lectin. *Vet Immunol Immunopathol* 2003; 94: 105–12. [http://dx.doi.org/10.1016/S0165-2427\(03\)00067-9](http://dx.doi.org/10.1016/S0165-2427(03)00067-9)
- Abdullaev FI, De Mejia EG. Antitumor effect of plant lectins. *Nat Toxins* 1997; 5: 157–63. <http://dx.doi.org/10.1002/19970504NT6> PMID:9407559
- Cheung Allen HK, Wong Jack H, Ng TB. *Musa acuminata* (Del Monte banana) Lectin is a Fructose-Binding Lectin with Cytokine-Inducing Activity. *Phytomedicine* 2009; 16: 594–600. <http://dx.doi.org/10.1016/j.phymed.2008.12.016> PMID:19195858
- Liu QH, Wang HX, Ng TB. First report of a xylose-specific lectin with potent hemagglutinating, anti proliferative and anti-mitogenic activities from a wild ascomycete mushroom. *Biochem Biophys Res Commun* 2006; 345: 1573–8. PMID:16730651
- Hsu HC, Hsu CI, Lin RH, Kao CL, Lin JY. Fip-vvo, a new fungal immunomodulatory protein isolated from *Volvariella volvacea*. *Biochem J* 1997; 323: 557–65. PMID:9163352 PMCID:PMC1218355
- Macedo ML, Damico DC, Freire MG, Toyama MH, Marangoni S, Novello JC. Purification and characterization of an N-acetylglucosamine-binding Lectin from *Koeleria paniculata* seeds and its effect on the Larval Development of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and *Anagasta kuehniella* (Lepidoptera: Pyralidae). *J Agric Food Chem* 2003; 51: 2980–6. <http://dx.doi.org/10.1021/jf034013i> PMID:12720380
- Zheng T, Yu HM, Alexander CM, Beebe DJ, Smith LM. Lectin-modified micro channels for mammalian cell capture and purification. *Biomed Micro devices* 2007; 9: 611-7. <http://dx.doi.org/10.1007/s10544-007-9074-2> PMID:17516171
- Laija SN, Mahesh S, Smitha LS, Remani P. Isolation and Partial Characterization of Two Plant Lectins. *J Biol. Sci* 2010; 2(4): 232-237.

12. Neuwinger HD. African Ethno botany: Poisons and Drugs. Chapman and Hall, London, United Kingdom; 1996. p. 941.
13. Cristina S, Angelita DC, Custódio DS, Flávia C, Almeida M, Celeste MP. Partial purification of leaf lectin from *Manihot esculenta* and screening its fungicidal activity. Journal of Agricultural Biotechnology and Sustainable Development 2010; 2(8): 136-141.
14. Maria CS, Angelita DC, Custódio DS, Flávia CA, Celeste MP. Partial purification of leaf lectin from *Manihot esculenta* and screening its fungicidal activity. Journal of Agricultural Biotechnology and Sustainable Development 2010; 2(8): 136-141.
15. Cooper TG. The Tools of Biochemistry. Wiley-Interscience, New York; 1942.
16. Bradford MM. A rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. Anal. Biochem 1976; 72: 248-254. [http://dx.doi.org/10.1016/0003-2697\(76\)90527-3](http://dx.doi.org/10.1016/0003-2697(76)90527-3)
17. Oyedapo OO, Ekundayo O, Koenig WA. Volatile leaf Oil Constituents of *Lantana camara*, L. from Nigeria. Flav. and frag. J. 2004; 18: 384-386.
18. Calderón De La Barca AM, Ochoa JL and Valencia ME. Effect of the Extraction of a Hemagglutinin on the Nutritive Value of *Amaranthus leocarpus* Seeds. J. Food Sci 1985; 50: 1700-1702. <http://dx.doi.org/10.1111/j.1365-2621.1985.tb10569.x>
19. Knight CS. Improved Techniques with Advanced Ion Exchange Cellulose. Advan. Chrom 1967; 4: 61-63.
20. Laemmli UK. Cleavage of Structural Proteins during the Assembly of the Bacteriophage T4. Nature 1973; 227: 680-685. <http://dx.doi.org/10.1038/227680a0>
21. Vari F, Bell K. A simplified Silver Diammine Method for the Staining of Nucleic Acids in Polyacrylamide Gels. Electro 1996; 17(1): 20-5.
22. Sampaio AH, Rogers DJ, Barwell CJ. A Galactose-specific Lectin from the Red Marine Alga *Ptilota filicina*. Phytochem 1998; 48: 765-769. [http://dx.doi.org/10.1016/S0031-9422\(97\)00966-7](http://dx.doi.org/10.1016/S0031-9422(97)00966-7)
23. Kuku A, Eretan OB. Purification and Partial Characterization of a Lectin from the fresh leaves of *Kalanchoe crenata* And J Biochem. and Mol. Biol 2004; 37(2): 229- 233. <http://dx.doi.org/10.5483/BMBRep.2004.37.2.229>
24. Dubois M, Gilles KA, Hamilton JK, Rebers PA and Smith F. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem 1956; 28: 350-356. <http://dx.doi.org/10.1021/ac60111a017>
25. Roberto AS, Nataly DS, Clécia S, Thiago HN, Francis SG, Benildo SC, et al. Larvicidal Activity of Lectins from *Myracrodruon urundeuva* on *Aedes aegypti*. Comparative Biochem and Physio, Part C 2009; 149: 300–306.
26. Bourne Y, Mazurier J, Legrand, D, Rouge P, Montreuil J, Spik G, Cambillau C. Structures of a Legume Lectin Complexed with the Human Lactotransfer in 2 Fragment and with an Isolated Bi antennary Glycopeptide: Role of the Fucose Moiety. Structure 1994; 2: 209–219. [http://dx.doi.org/10.1016/S0969-2126\(00\)00022-8](http://dx.doi.org/10.1016/S0969-2126(00)00022-8)
27. Wu Albert M, Sugiiand Shunji. Coding and classification of D-galactose, N-acetyl-D-galatosamine and β -D-Galp-[1- 3(4)]- β -D-GlcPNAc, specificities of applied lectins. Carbh. Resources 1991; 213: 127-43.
28. Svobodova M, Volf P, Killick Kendrick R. Agglutination of *Leishmania* promastigotes by midgut lectins from various species of *phlebotomine* sandflies. Ann Top Med parastol 1996; 90(3): 329-36.
29. Lacsamana MS, Merca FE. Isolation and Charaterization of Lectin from the Visceral region of the Golden Snail, *pomacea* sp. Philipp. J Sciences 1994; 123: 77-97.
30. Wittsuwannakul R, Wittsuwannakul D, Sakulborirug C. A lectin from the bark of the rubber tree (*Hevea brasiliensis*). Phytochem 1998; 47: 183-187. [http://dx.doi.org/10.1016/S0031-9422\(97\)00329-4](http://dx.doi.org/10.1016/S0031-9422(97)00329-4)
31. Peumans WJ, Van Damme EJ. Lectins as plant defense proteins. Plant Physio 1995; 109: 347–352. <http://dx.doi.org/10.1104/pp.109.2.347>
32. Moreno FB, Oliveira TM, Martil DE, Viçoti MM, Bezerra GA, Abrego JR, Cavada BS, Azevedo WF. Identification of a new quaternary association for legume lectins. J. Struct. Bio 2008; 161: 133–143. <http://dx.doi.org/10.1016/j.jsb.2007.10.002> PMID:1806 8379
33. Kawsar SM, Fujii Y, Matsumoto R, Ichikawa T, Tateno H, Hirabayashi J, et al. Isolation, purification, characterization and glycan-binding profile of a D-galactoside specific lectin from the marine sponge, *Halichondria okadai*. Comparative Biochem Physio B 2008; 150: 349–357. <http://dx.doi.org/10.1016/j.cbpb.2008.04.004> PMID:18534886
34. Khan MH, Mijanur TM, Rahman MS, Shahanaz K, Pervin F, Absar N. Purification and Characterization of Lectins from Jute (*Chorchorus olitorius*) Leaves. Journal of the Chinese Chemical Society 2008; 55: 1171-1177.
35. Syed RK, Syed EH, Nurul A. Re-investigation on the Purification and Characterization of Rice-bran Lectin. Pakistan Journal of Biological Sciences 2001; 4(2): 197-200. <http://dx.doi.org/10.3923/pjbs.2001.197.200>
36. Adolph L, Lorenz R. Enzyme Diagnosis in Diseases of the Heart, Liver and Pancreas, John Wiley and Sons Incorporated. New York, USA; 1982.
37. Utarabhand P, Akkayanont P. Purification of a lectin from *Parkia javanica* beans. Phytochem 1995; 38: 281-285. [http://dx.doi.org/10.1016/0031-9422\(94\)00550-D](http://dx.doi.org/10.1016/0031-9422(94)00550-D)

Cite this article as:

Ihuoma Onyeyirichi, Inuwa Hajia Mario, Ibrahim Sani, Okunola Joshua, Nkeonye Ogechi. Evaluation of haemagglutination potentials of lectin isolated from ordeal tree (*Erythrophleum suaveolens*) stem bark. Int. J. Res. Ayurveda Pharm. 2013;4(6):869-875 <http://dx.doi.org/10.7897/2277-4343.04619>

Source of support: Nil, Conflict of interest: None Declared