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(RESEARCH ARTICLE)

Partial Purification, Complete and Incomplete Agglutinins Detection, Agglutination Properties and Standardization of Some Tropical Euphorbiaceae Plant Lectins

EGBOBE C.G.[1], ODIEGWU C.N.C.[2,*], OBI C.M.[2], ONWURAH O. W.[3], OKWELOGU I.S.[2] and EKEKWE E. N [2]

[1] Department of Subnational Support, Nigeria Centre for Disease Control (NCDC), Plot 801, Ebitu Ukiwe Street, Jabi, Abuja, Federal Capital Territory, Nigeria.

[2] Department of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University-Nnewi Campus, Anambra State, Nigeria.

[3] Department of Haematology & Blood Transfusion Science, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

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Abstract

Euphorbiaceae is a large family of flowering plants with numerous genera and species that occurs mainly in the tropics including the large variety that occurs in tropical Africa. Lectins are a heterogeneous class of carbohydrate-binding proteins of non-immune origin that specifically and reversibly recognize sugars or glycoconjugates without enzymatic modification. They play critical roles in cell–cell communication and molecular recognition, making them valuable in medical diagnostic applications. A total of Six (6) species of local Euphorbiaceae plants viz, *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, *Tetracarpidium conophorum*, and *Manihot esculenta* were collected, authenticated at Botany Department of Nnamdi Azikiwe University, Awka and their crude extracts obtained. Partial purifications were conducted on these extracts in two steps: Dialysis and Silica gel chromatography techniques. The crude and partially purified extracts were subjected to haemagglutination tests but the rest of the investigations viz, Direct and Indirect Agglutinins Detection, Agglutination Properties, and Standardization were performed using the partially purified extracts. Five (5) of the six (6) plants extracts except that of the *Manihot esculenta* agglutinated pooled washed human ABO cells. Agglutination properties of the lectins were determined by carrying out Protein electrophoresis in Barbitone buffer pH 8.6 on each of the extracts using human serum with distinct and characteristic globulin bands of Albumin (A), Alpha-1 (α_1), Alpha-2 (α_2), Beta (β) and Gamma (γ) as control. *A. torta* showed an albumin and slow gamma bands. *C. variegatum* and *E. milli* extracts gave no detectable bands. On the other hand, *R. communis* gave Gamma (γ), Alpha-1 (α_1), and Alpha-2 (α_2) globulin bands while *T. conophorum* exhibited Gamma (γ), Alpha-2 (α_2), and Beta (β) bands. The extract of *M. esculenta* gave no Gamma (γ) but only Alpha-1 (α_1) globulin band. Hence, these band patterns explain why the extracts with lectinic properties agglutinate human ABO cells while that of *M. esculenta* without lectinic activities does not. *A. torta, C. variegatum* and *T. conophorum* lectins were standardized because they cross reacted with the ABO cells in varying strengths. Lectins from *A. torta* designated anti-H At show specificity with O cells only at an appropriate dilution of 1 in 64 while lectins from *C. variegatum* designated Anti-A Cv and *T. conophorum* (Anti-B Tc) were found to be specific for A and B cells at titres of 16 and 128 respectively. This study has succeeded in demonstrating the partial purification, complete agglutinins detection, agglutination properties and standardization of some tropical Euphorbiaceae plants lectins, showing their potential usefulness in diagnostic and biotechnological applications.

Keywords: Euphorbiaceae plants; Agglutinins detection; Agglutination properties and Standardization of the lectins

* Corresponding author: ODIEGWU C.N.C

1. Introduction

Lectins are sugar-binding proteins of non-immune origin that binds carbohydrate reversibly and non-covalently without inducing any change in carbohydrate bond. Lectins are carbohydrate binding proteins that are found in most plants, particularly seeds and tubers such as cereal crops, potatoes and legumes (Singh et al., 2017).

A lectin is usually composed of two or more binding sites for carbohydrate units. The carbohydrate binding specificity of a certain lectin is determined by the amino acid residues that bind the carbohydrate. The binding specificities of lectins from plants have been well characterized. Some bacteria, for example, *Escherichia coli* contain lectins with which they are able to adhere to epithelia cells of gastrointestinal tract because this lectin recognize oligosaccharide units on the surface of target cells (Singh *et al*., 2017).

Hemagglutination is the process by which red blood cells (erythrocytes) clump together due to the action of specific agents such as viruses, antibodies, or lectins. In the case of lectins, hemagglutination occurs because of their ability to recognize and bind specific carbohydrate structures on the surface of red blood cells. The mechanisms include Specific Binding where Lectins interact with glycoconjugates (glycoproteins and glycolipids) present on the erythrocyte membrane, Cross-Linking which is due to multivalence, lectins can bind multiple erythrocytes at once and Agglutination in which the cross-linking results in visible clumping or lattice formation, termed hemagglutination (Chenze *et al*., 2024).

Thus, this research was designed to extract/isolate, partially purify, detect agglutinins, determine agglutination properties and standardize some tropical Euphorbiaceae plant lectins.

The specific objectives include: 1. Extract and isolate lectins from some tropical Euphorbiaceae plants using method of Odiegwu *et al*. (2011). 2. Partially purify the crude lectin extract from some tropical Euphorbiaceae plants by means of ammonium sulphate precipitation, dialysis and column chromatography methods. 3. Detect the presence of agglutinins in the isolated lectins extract from some tropical Euphorbiaceae plants. 4. Determine the agglutination properties and thus characterize lectins from some tropical Euphorbiaceae plants using protein electrophoresis. 5. Standardize the lectins that react with different agglutinating strengths with ABO Red blood cells. 6. Explore the commercial viability of the isolated lectins from some tropical Euphorbiaceae plants.

2. Materials and Methods

The leaves of *Acalypha torta* (Muel), *Euphorbia milli* (Crown of thorns), *Codiaeum variegatum* (variegated croton); ten (10) seeds of *Ricinus comminus* (Castor oil plant), *Tetracarpidium conophorum* (African walnut) and two (2) tubers of *Manihot esculenta* (Cassava, Vitamin A variety) were used for this study.

2.1. Partial Purification of the crude extracts

The crude extracts were purified employing: A. Ammonium Sulphate Precipitation. B. Dialysis and C. Silica gel column Chromatography purification methods and were carried out based on the following principles:

2.1.1. Ammonium sulphate precipitation method

This is a simple and effective means of fractionating proteins as at high salt concentrations the natural tendency of proteins not to aggregate is overcome, since the surface charges are neutralized. Charge neutralization means that proteins tend to bind together, form large complexes and hence are easy to precipitate out by mild centrifugation.

2.1.2. Dialysis method

Dialysis is a classic laboratory technique that relies on selective diffusion of molecules across a semi-permeable membrane to separate molecules based on size.

2.1.3. Silica gel column chromatography method

The crude extracts of the tropical Euphorbiaceace plants extracts were partially purified using Silica gel as the stationary phase and Phosphate buffer as the mobile phase.

2.2. Method of sample analysis

Agglutination tests were carried out on the crude and partially purified extracts using Haemagglutintion assay. The separately pooled washed human ABO red blood cells were washed four times in saline, and 5% suspension of the cells were made and used for the haemagglutination tests.

2.3. Indirect Haemagglutination tests for detection of Incomplete agglutinins on the extract that did not react in saline (Direct Haemagglutination test) using 30% Bovine serum albumin

The extract negative in saline was further tested for incomplete agglutinins by indirect haemagglutination. Equal volumes of the extract and 5% pooled RBCs were incubated at 37 °C for 1 h, after which one drop of 30% bovine serum albumin was layered into the tube and incubated for an additional 30 min at 37 °C. Agglutination was then assessed macroscopically and microscopically.

2.4. Indirect Haemagglutination tests for the detection of incomplete agglutinins on the extract that did not react in saline (Direct Haemagglutination test) using Anti Human Globulin (AHG) Serum.

The extract negative in saline was also tested for incomplete agglutinins using the anti-human globulin (AHG) method. Equal volumes of 5% pooled, washed human ABO Rh(D)+ red blood cells and the partially purified extract that did not react in saline nor in the 30% Bovine serum albumin serological conditions were mixed and incubated at 37 °C for 1 h. The mixtures were washed three times and resuspended as a 10% cell suspension. Two drops of AHG serum were added, centrifuged briefly at 1500 g, and examined for agglutination both macroscopically and microscopically.

2.5. Standardization of the partially purified extracts that cross reacted in different strengths with ABO cells.

Extracts that cross-reacted with ABO red blood cells in different agglutinating strengths were standardized by serial tube dilution as described by Odiegwu et al. (2011). Eleven tubes were set up for each extract against groups A, B, and O cells. Serial three-drop dilutions of the extracts were prepared in saline across the tubes, followed by addition of an equal volume of 5% pooled, washed RBC suspension of the respective blood groups. After incubation at room temperature for 1 h, agglutination was assessed macroscopically and microscopically. The highest dilution showing definite agglutination was recorded as the titre.

2.6. Determination of Agglutination properties of the partially purified Haemagglutinating Euphorbiaceae plants lectins and the non-Haemagglutinating extract by protein electrophoresis using Human serum as control.

These Euphorbiaceae plants extracts were applied on cellulose acetate paper and subjected to electrophoretic mobility using Shandon electrophoretic tank in Barbitone buffer of alkaline pH (pH 8.6) at constant voltage of 150 V for 30 minutes and using Human serum that has distinct and characteristic globulin bands of Albumin (A), Alpha -1 (α_1), Alpha-2 (α_2), Beta (β) and Gamma (γ) as control. The extracts migration bands patterns on the cellulose acetate paper were then cleared with acetic acid and stained with 0.2% ponceau S. stain and observed (Odiegwu and Ukaejiofo, 2010).

3. Results and Discussion

The results of the analysis obtained in this research are as illustrated below in Tables 1,2,3,4,5,6,7 and Figures 1, 2,3, 4. Tables 1 to 4, represent the Haemagglutination pattern of the crude and partially purified lectins with the ABO red blood cells. Tables 5 to 7, represent the standardization of the extracts that cross reacted with the ABO red blood cells in different agglutinating strengths. Figure 1 is the image of the haemagglutination pattern of the partially purified lectin with the ABO red cells, Figure 2 shows the image of the haemagglutination negative control, Figure 3 shows the image of the haemagglutination pattern of the non-reacting *Manihot esculenta* with the ABO red blood cells. Figure 4 shows the protein electrophoresis results of the partially purified extracts using human serum as control.

Table 1 Haemagglutination Assay for Detection of Lectinic Activity and Complete Agglutinins in crude Euphorbiaceae Plant Extracts.

		REACTIVITY			**AVIDITY**		
Euphorbiaceace species	Local names	A-cells	B-cells	O-cells	A-cells	B-cells	O-cells
Acalypha torta	Muel	++++	+++++	clump	Less than 1min	Less than 1min	Less than 1min
Codiaeum variegatum	Variegated croton	++	+	+	5mins	5mins	5 mins
Euphorbia milii	Crown of thorns	++	++	++	5mins	5mins	5mins
Manihot esculenta	Cassava	_	_	_	_	_	_
Ricinus comminus	Castor oil plant	++	++	++	7mins	7mins	7 mins
Tetracarpidium conophorum	African walnut	Clump	Very strong clump	clump	2 secs	2secs	2 secs

Table 2 Haemagglutination Assay for Detection of Lectinic Activity and Complete Agglutinins in Partially Purified Euphorbiaceae Plant Extracts

		REACTIVITY			**AVIDITY**		
Euphorbiaceace species	**Local names**	**A-cells**	**B-cells**	**O-cells**	**A-cells**	**B-cells**	**O-cells**
Acalypha torta	Muel	++++	+++++	clump	Less than 1min	Less than 1min	Less than 1min
Codiaeum variegatum	Variegated croton	++	+	+	5mins	5mins	5 mins
Euphorbia milii	Crown of thorns	++	++	++	5mins	5mins	5mins
Manihot esculenta	Cassava	_	_	_	_	_	_
Ricinus comminus	Castor oil plant	++	++	++	7mins	7mins	7 mins
Tetracarpidium conophorum	African walnut	Clump	Very strong clump	clump	2 secs	2secs	2 secs

Table 3 Indirect Haemagglutination tests for detection of Incomplete agglutinins in the extract that did not react in saline (Direct Haemagglutination test) using 30% Bovine serum albumin

Plant specie	A cells	B cells	O cells
Manihot esculenta	_	_	_

Table 4 Indirect Haemagglutination tests for the detection of incomplete agglutinins in the extract that did not react in saline (Direct Haemagglutination test) nor in Bovine serum albumin using Anti Human Globulin (AHG) Serum.

Plant specie	A cells	B cells	O cells
Manihot esculenta	_	_	_

Table 5 Results of standardization of *Acalypha torta* lectin

Blood group	Neat	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
A cells	++++	+++	+	_	_	_	_	_	_	_	_
B cells	+++++	++++	++	+	_	_	_	_	_	_	_
O cells	Clump	+++++	+++	++	+	+	+	_	_	_	_

Titre = 64 for O cells

Table 6 Results of standardization of the *Codiaeum variegatum* lectin

Blood group	Neat	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
A cells	++	+	+	+	+	_	_	_	_	_	_
B cells	+	+	+	_	_	_	_	_	_	_	_
O cells	+	+	+	_	_	_	_	_	_	_	_

Titre = 16 for A cells

Table 7 Results of standardization of the *Tetracarpidium conophorum* lectin

Blood group	Neat	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
A cells	++++	+++	++	++	+	+	+	_	_	_	_
B cells	clump	+++++	++++	++++	+++	++	++	+	_	_	_
O cells	++++	+++	++	++	+	+	+	_	_	_	_

Titre = 128 for B cells

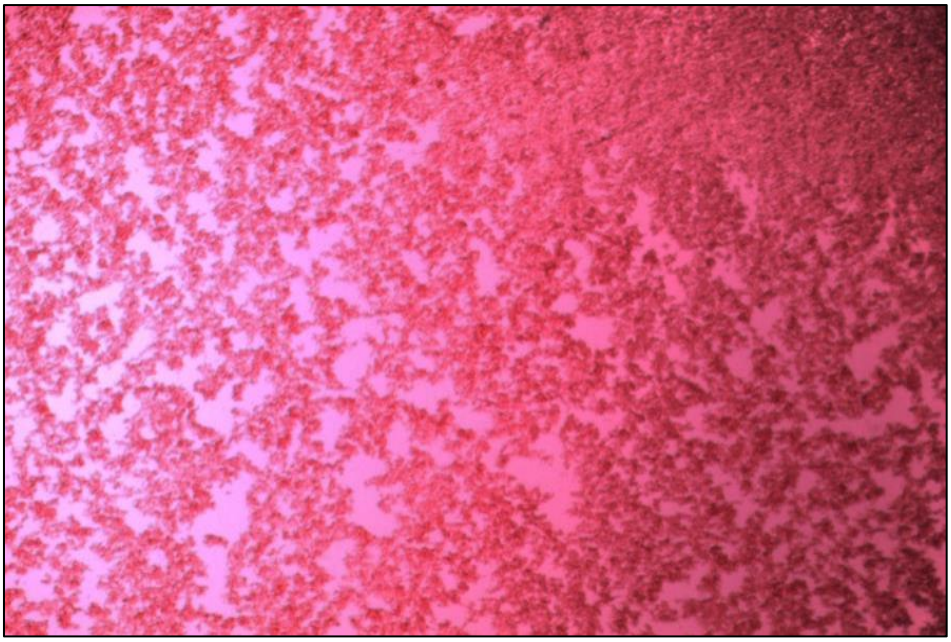


Figure 1 10x Photomicrograph haemagglutination pattern of the Euphorbiaceae plants lectins with human ABO cells

Figure 2 10x Photomicrograph of phosphate buffered saline with human ABO cells haemagglutination pattern negative control

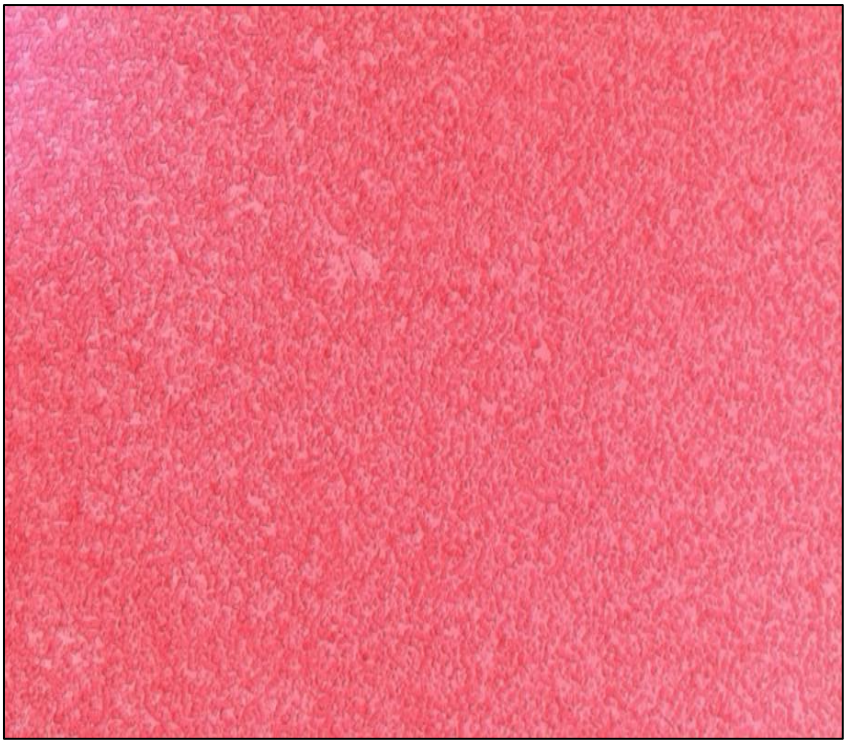
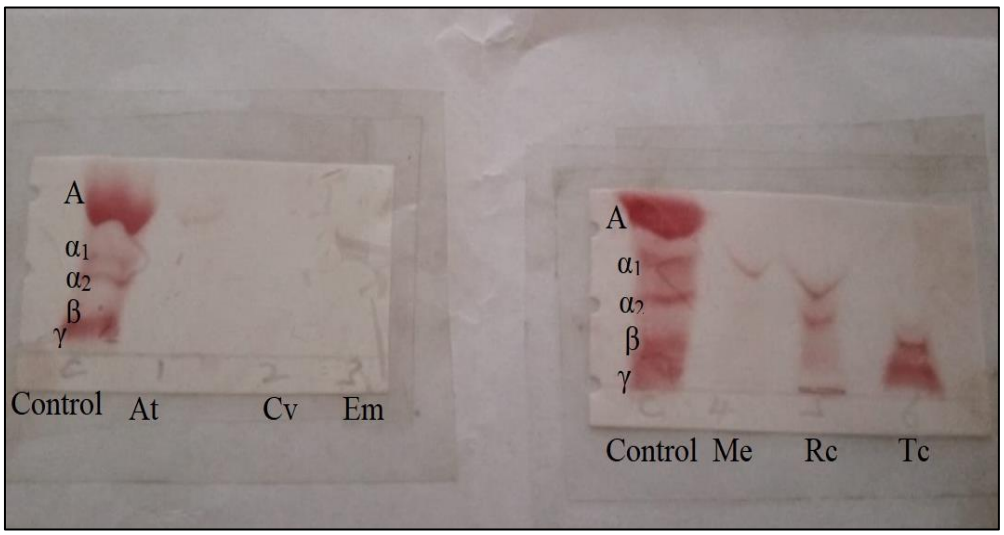


Figure 3 10x Photomicrograph of non-haemagglutinating *M. esculenta* extract with human ABO cells



Key: A = Albumin, α_1 = Alpha 1, α_2 = Alpha 2, β = Beta globulin and γ = Gamma; C= Control, At = *Acalypha torta,* Em = *Euphorbia milli*, Cv = *Codiaeum variegatum,* Rc = *Ricinus comminus,* Tc = *Tetracarpidium conophorum* lectins and Me = *Manihot esculenta.*

Figure 4 The migration bands patterns showing the characterization of the partially purified *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum, Ricinus comminus, Tetracarpidium conophorum* lectins and *Manihot esculenta* extract by protein electrophoresis using human serum as control

Protein electrophoresis of globulin bands migration patterns, showing the agglutination properties of the partially purified *Acalypha torta* (At), *Euphorbia milli* (Em), *Codiaeum variegatum* (Cv), *Ricinus comminus* (Rc), *Tetracarpidium conophorum* (Tc) lectins and *Manihot esculenta* (Me) extract by protein electrophoresis using human serum as control.

4. Discussion

This study investigated the partial purification, agglutinins detection, agglutination properties, and standardization of six tropical Euphorbiaceae plant extracts. Haemagglutination assays confirmed lectinic activity in *Acalypha torta, Euphorbia milli, Codiaeum variegatum, Ricinus communis,* and *Tetracarpidium conophorum,* whereas *Manihot esculenta* showed no activity. The ability of these extracts to agglutinate pooled human ABO erythrocytes indicates the presence of carbohydrate-binding domains capable of recognizing specific sugar moieties on red cell membranes, consistent with previous reports (Kabir et al., 2020; Odiegwu et al., 2020). The positive reactions in saline suggest the presence of complete agglutinins, analogous to IgM-type Immunoglobulins which can directly bridge erythrocytes due to their multivalent binding sites (Pusztai et al., 2004).

Standardization revealed distinct binding preferences among the lectins. *T. conophorum* lectin (Anti-B Tc) showed highest reactivity with blood group B at titre of 128, indicating strong affinity for galactose residues; *A. torta* lectin (Anti-H At) reacted preferentially with group O red blood cells at titre of 64, implying specificity for H antigen; and *C. variegatum* lectin (Anti-A Cv) reacted specifically with group A red blood cells at titre of 16, reflecting affinity for N-acetylgalactosamine. These findings agree with the established carbohydrate determinants of ABO antigens and highlight the diagnostic potential of these lectins for serological typing of blood groups (Odiegwu & Ukaejiofo, 2010). *R. communis* could not be standardized because it cross reacted with the ABO red blood cells in the same agglutinating strength, but however, its valuable usefulness could be exploited in other areas of lectins applications.

Electrophoretic characterization to deduce the agglutination properties of the extracts revealed γ-globulin bands in *R. communis* and *T. conophorum,* correlating with their strong haemagglutination profiles. In contrast, *M. esculenta* lacked γ-globulin fractions, consistent with its lack of lectinic activity. The similarity of protein banding patterns between *A. torta, R. communis,* and *T. conophorum* and human serum proteins suggests functional mimicry, enabling their efficient binding to erythrocyte carbohydrate moieties (Hassan et al., 2015; Khan et al., 2023).

Collectively, these findings demonstrate that tropical Euphorbiaceae lectins exhibit diverse and specific haemagglutination properties, with *A. torta, C. variegatum,* and *T. conophorum* showing promise as candidate reagents for blood group typing and identification studies. This work therefore expands existing knowledge on Euphorbiaceae lectins and provides insights for their applications in diagnostics and biotechnology.

5. Conclusion

The study demonstrates that lectins from selected Euphorbiaceae plants possess distinct haemagglutination and agglutinin properties with varying affinities for ABO blood group antigens. *Acalypha torta, Codiaeum variegatum,* and *Tetracarpidium conophorum* exhibited strong and specific reactivity, underscoring their potential as alternative reagents for blood group typing, serological studies and identification studies. The lack of correlation between protein concentration and agglutination strength highlights the importance of structural specificity over protein abundance in lectin function. Overall, these findings provide new insights into the diagnostic and biotechnological potential of tropical Euphorbiaceae lectins.

Recommendation

Further studies should focus on detailed structural characterization of Euphorbiaceae plant lectins to determine their carbohydrate-binding specificities, molecular weights, specific sugar basis of agglutination. Enhanced standardization and stability testing are necessary to validate their reliability as diagnostic reagents for blood group typing and identification studies. Finally, safety and toxicity assessments are recommended to evaluate their suitability for clinical and biotechnological applications including testing the lectins in the various areas of Medical Sciences such as in lymphocyte blastogenesis/mitogenic stimulation of lymphocytes, detection of malignant/cancerous cells, the effects of the lectins in Haemopoiesis and haemostasis as well as in Histopathology, Chemical pathology etc.

Compliance with ethical standards

Disclosure of conflict of interest

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Statement of ethical approval

Ethical approval for this research in compliance with International standards as obtainable in Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, was sought and obtained before embarking on this research.

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