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Assessment of lectinic activity potentials in extracts of some tropical Euphorbiaceae

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Abstract

Lectins bind a variety of cells having cell surface glycoprotein or glycolipid, such as erythrocytes, leukemic cells, yeast and several types of bacteria. Several specificity groups have been identified such as mannose, galactose, N-acetyl glucoseamine, N-acetyl galactoseamine, L-fucose and N-acetyl neuraminic acid. The presence of two or more binding sites for each Lectin molecule allows the agglutination of many cell type. Sixteen (16) species of some tropical Euphorbiaceae plants were assessed for the presence of Lectins. The leaves of *Acalypha torta*, *Acalypha wiskesiana*, *Acalypha hispida*, *Codiaeum variegatum*, *Euphorbia milli*, *Euphorbia pulcherima*, *Jatropha curcas* and *Jatropha gossypifolia*; the seeds of *Croton tiglium*, *Ricinus communis* and *Tetracarpidium conophorum*; the stem of *Euphorbia tirucalli* and the tubers of *Manihot esculenta* (Cassava, vitamin A variety), *Manihot esculenta* (cassava, NR 8082 variety), *Manihot esculenta* (Cassava, TMX 419 variety), *Manihot esculenta* (Cassava, TMX 4(2) 1425 variety) were used for sourcing the Lectins. *A. torta*, *A. wiskesiana*, *C. variegatum*, *C. tiglium*, *E. milli*, *E. pulcherima*, *E. tirucalli*, *J. curcas*, *J. gossypifolia*, *R. communis* and *T. conophorum* agglutinated pooled washed human ABO cells in saline (direct haemagglutination) while *A. hispida* and the four varieties of *M. esculenta* showed no agglutination reaction. *E. pulcherima* showed specificity for B cells only while *E. tirucalli* showed specificity for O cells only, hence could be rightly indicated by referring to them as anti-B Ep and anti-H Et Lectins respectively (where Ep=*Euphorbia pulcherima* and Et=*Euphorbia tirucalli*). However *A. torta* and *T. conophorum* cross-reacted with pooled washed ABO cells in differing strengths and when standardized, showed that *A. torta* at a titre of 16 reacted specifically with O cells and *T. conophorum* at a titre of 128 reacted specifically with B cells. Based on this, these Lectins could be indicated as anti-H At and anti-B Tc respectively (Where At= *Acalypha torta* and Tc= *Tetracarpidium conophorum*). The protein content of the crude extracts of the sixteen (16) species were also assayed using Biuret protein assay method and the results revealed that there are no relation or association between the quantity of protein content and agglutination patterns of the extracts. This research has therefore succeeded in revealing presence of Lectinic properties in extracts of some tropical Euphorbiaceae.

Keywords: Lectin; Potential; Euphorbiaceae; *Acalypha torta*; glycoprotein

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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 found in some plants, particularly seeds and tubers such as cereal crops, potatoes and legumes [1]. Lectins are a complex group of proteins and/or glyco-proteins of non-immune origin possessing at least one non-catalytic domain which binds reversibly and specifically to monosaccharides, oligosaccharides and glyco-conjugates, and they are widely distributed in nature. Lectins exhibit quaternary structure: most are oligomeric, consisting of two to four polypeptide chains with one binding site per chain. Lectins mediate specific transient cell-cell adhesion. Lectins bind a variety of cells having cell surface glycoprotein or glycolipid, such as erythrocytes, leukemic cells, yeast and several types of bacteria. Several specificity groups have been identified such as mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, L-fucose and N-acetylneuraminic acid. The presence of two or more binding sites for each lectin molecule allows the agglutination of many cell type [2]. They associate through the formation of bonds such as hydrogen bond, metal coordinate bond, vanderwaal's and other hydrophobic interactions [3]. Some lectins require divalent cations such as calcium, magnesium and manganese to bind carbohydrates. Others require thiol groups being present instead. Most lectins have covalently linked carbohydrate. The sugar specificity of a lectin is defined by the carbohydrate for which it shows highest affinity. For instance, lectins interact with non-reducing glycosyl groups of polysaccharides and glycoproteins. Some can bind internal sugars or sugars at the reducing end. Some lectins with a small binding site can only recognize one particular monosaccharides; others with extended binding site bind preferentially to trisaccharides or tetrasaccharides [3]. Lectins are important in clinical setting because they are used for blood typing. This is due to the ability of lectins to identify and distinguish carbohydrate determinants on human blood cells. Lectins from *Dolichos biflorus* is used to identify cells belonging to A blood group. Lectins from *Griffonia simplicifolia* is used to identify the H antigen. Lectins can also be used to distinguish secretors from non-secretors [4].

Lectins are ubiquitous proteins and have been isolated from viruses, fungi, bacteria, invertebrates, unicellular organisms, animals and plants [5]. Lectins have become the focus of intense interest for biologists and in particular for the research and applications in agriculture and medicine. These proteins with unique characteristics have found use in diverse fields of biology and as more lectins are being isolated and their role in nature elucidated, they continue to occupy an important place in agricultural and therapeutic areas of research. The role of lectins include endocytosis and intracellular transport of vector glycoprotein mechanisms [6], induction of apoptosis in tumoral cells [7], blocking of HIV infection [8], regulation of bacterial cell adhesion and migration [9] and control of protein levels in the blood [10]. Lectins are composed of four important sites which accounts for their activities, they include; Metal binding sites, hydrophobic sites, glycosylation sites, carbohydrates binding sites [11]. The specificity of the binding of lectins suggests that there are endogenous saccharides receptors in the tissue from which they are derived or glyco-conjugates with which the lectin is specialized to interact with. Application of lectins can be seen in blood typing, anti-tumor activities, anti-insect activity, anti-viral activity, in biochemical warfare, as mitogens and chemotherapeutic agents.

Lectins have been found in many plant groups including Euphorbiaceae which is a large family of flowering plants with 300 genera and around 7500 species. Most spurge are herbs, but some, especially in the tropics are shrubs or trees. This family occurs mainly in the tropics with majority of the species in the Indo-Malaysian region and tropical America. A large variety occurs in tropical Africa but they are not as abundant or varied as in these two other tropical regions. A number of plants of this spurge family are of considerable economic importance. Prominent plants include cassava (*Manihot esculenta*), Castor oil plant (*Ricinus communis*), Barbados nut (*Jatropha curcas*), and the Para rubber tree (*Hevea brasiliensis*). Many are grown as ornamental plants such as poinsettia (*Euphorbia pulcherima*). In medicine, some species of Euphorbiaceae have proved effective against genital herpes [12].

The ABO blood group system is the clinically most important blood group system which was discovered by an Austrian Scientist Karl Landsteiner in 1900. Landsteiner described the A, B and O blood groups and showed the presence of one of the two antigens either A or B or neither on the red cells of each individual. He also showed that the serum from such individual contained different antibodies, example, blood group A contained antibody B, blood group B contained antibody A, blood group O contained both A and B antibodies [4]. In 1902, two of Landsteiner's students Alfred Von Decastello and Adriano Struli discovered the fourth blood type, the AB blood group which contains both A and B antigens on the red cells with no antibody in the serum. The ABO antigens are detectable early in foetal life long before birth but at birth they are fully developed although they are more weakly reacting than adult cells. The reaction between the antigen on the red cells and the corresponding antibody in serum is generally observed in the form of definite agglutination of these red cells [4].

The ABO antigens are the most immunogenic of all the blood groups and thus remain of prime importance in transfusion science. The H antigen is an essential precursor to the ABO blood group antigens. The H locus is located on chromosome

19 and it contains 3 exons that span more than 5kb of genomic DNA. It encodes a fucosyl transferase that produces the H antigen on the Red blood cells. The H antigen is a carbohydrate sequence with carbohydrate linked to protein (with a minor fraction attached to a ceramide moiety). It consists of a chain of B-D-galactose, B-D-N-acetyl glucosamine, B-D-galactose and 2-linked α -L-fucose, the chain being attached to the protein or ceramide. The ABO locus is located on chromosome 9. These antigens are always found on the red cells and body fluid of secretors and may be mostly glycosphingolipids. The antigens are detectable early in fetal life long before birth but at birth they are fully developed although they are more weakly reacting than adult cells. The blood group terminal sugars are added by specific transferases [4].

The tropics include all the areas of the earth where the sun reaches a point directly overhead at least once during the solar year. The tropics refer to a region of the earth by the equator. It is limited in latitude to the Tropic of Cancer in the Northern Hemisphere at 23.4378°N, and the Tropic of Capricorn in the Southern Hemisphere at 23.4378°S. The tropics can also be called the tropical zone and the Torrid Zone. The tropics are associated with the three most significant imaginary lines of the earth viz: the equator, the tropic of cancer and the tropic of capricorn. The tropics are distinguished from other climatic and biomatic regions of Earth, which are the middle latitudes and the polar region on either side of the equatorial zone. The tropics comprise 40% of the Earth's surface area and contain 36% of the Earth's landmass [13]. Many tropical areas have two major seasons- dry and wet or rainy season. The wet season is the time of the year when most of the average annual rainfall in the region falls [14].

Many families of plants have been studied and found to possess lectinic properties with many useful end applications. However, little or no study of the plants belonging to the Euphorbiaceae family has been carried out in our environment which is a typical example of tropical region. The basic aim of this research therefore was to assess some local Euphorbiaceae plants for the presence of lectins by extracting the seeds, leaves, stems and tubers of some Euphorbiaceae plants family and determine those with lectinic properties by carrying out direct and indirect haemagglutination test on them using fresh pooled washed groups A,B and O Red blood cells.

The specific objectives of this research therefore are:

- To extract the seeds, leaves, stems, and tubers of some tropical Euphorbiaceae plants and determine those with lectinic activities by carrying out direct and indirect haemagglutination tests on them.
- To standardize the extracts that showed different strengths of agglutination reactions with ABO cells with a view to determining the dilution at which the crude extracts can be diluted for profitable Blood Group Serology use.
- To determine the protein contents of the crude extracts with a view to finding out if there is association or relation between degrees of protein content with lectinic properties.
- To explore the viability of commercial production of the extracts with lectinic potentials by deducing the extracts that are at least capable of showing specificity with an ABO cell.

2. Material and methods

The seeds, leaves, stems, and tubers of the Sixteen (16) species of the Euphorbiaceae plant family were collected from different farmlands and gardens in Awka and Nnewi and its environs and other parts of Nigeria. These seeds, leaves, stems and tubers were professionally authenticated by an experienced taxonomist at the Department of Botany Nnamdi Azikiwe University, Awka, Anambra State, Nigeria after initial identification at the Department of Botany, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.

The Blood samples used for the haemagglutination tests were collected from the Blood bank of Nnamdi Azikiwe University Teaching Hospital Nnewi, and JENIC Clinical &Molecular Laboratories, Ifite-Awka, Anambra state, Nigeria.

2.1. Method of Sample Analysis

2.1.1. Extraction of Euphorbiaceae Plants Seeds, Leaves, Stems, and Tubers

Dry leaves of *Acalypha torta*, *Acalypha wiskesiana*, *Acalypha hispida*, *Codiaeum variegatum*, *Euphorbia milii*, *Euphorbia pulcherima*, *Jatropha curcas* and *Jatropha gossypifolia*, seeds of *Croton tiglium*, *Ricinus communis* and *Tetracarpidium conophorum*, stem of *Euphorbia tirucalli* and the tubers of *Manihot esculenta* (Cassava, vitamin A variety), *Manihot esculenta* (cassava, NR 8082 variety), *Manihot esculenta* (Cassava, TMX 419 variety), *Manihot esculenta* (Cassava, TMX 4(2) 1425 variety) of the species of the Euphorbiaceae plants were crushed into powder using pestle and mortar weighed using Mettler balance and extracted according to the method of Odiegwu et al., 2011 [15].

2.1.2. Protein Assay of the Crude Extracts Using Biuret Protein Assay Method

This was carried out by mixing 0.02ml of the standard and 0.02ml of each of the crude extracts with 1ml of the Biuret reagent and were allowed to stand for Ten (10) minutes at room temperature and the absorbance were read spectrophotometrically at 546nm.

2.1.3. Method of Determining the Lectin Potentials of the Crude Extracts.

Two (2) haemagglutination techniques were employed in carrying out this viz, tile and tube methods.

Tile Method

- A drop of the 5% suspension of the pooled washed A, B and O cells were placed on a dried white tile.
- A drop each of the crude Euphorbiaceae extracts were placed in each of the 5% suspension of the pooled washed A, B and O cells.
- These were mixed and rocked back and forth.
- Presence of agglutination were checked both macroscopically and microscopically using x10 objective with the condenser iris sufficiently closed.

Tube Method

- Two (2) drops of 5% suspension of the pooled washed A, B and O cells were placed into different tubes labeled A, B and O respectively.
- Two (2) drops of each of the crude extracts were placed in each of the labeled tubes and mixed well.
- The set up was incubated at room temperature for 1 hour.
- The above steps were repeated for all the crude extracts.
- Presence of agglutination were observed both macroscopically and microscopically using x10 objective with the iris condenser sufficiently closed.

2.1.4. Further Testing

All the crude extracts that did not produce visible agglutination with the Red cell suspended in saline, were tested using 30% Bovine albumin and Anti-human globulin (AHG) technique as follows:

Indirect Haemagglutination Test Using 30% Bovine Serum Albumin (BSA)

- One (1) volume of each of the crude extracts was mixed with one volume of 5% suspension of washed pooled ABO cells in a tube.
- The tube was incubated at 37°C for 1hour.
- A drop of 30% Bovine albumin was layered gently into the tube.
- The tube was incubated further at 37°C for 30 minutes.
- The tube was then examined for agglutination macroscopically and microscopically.
- The above procedures were repeated for each of the crude extracts.

Indirect Haemagglutination Test Using Anti-Human Globulin (AHG) Serum

- Three (3) volumes of 5% suspension of red cells of washed pooled groups A, B and O Rhesus 'D' positive cells was added to three (3) volumes of the crude extracts in a tube.
- The tube was incubated at 37°C for 1 hour.
- The content of the tube was washed three times and 10% suspension was made.
- Two drops of AHG was added to the tube and the tube was spun for a few seconds at 1500g.
- The tube was then examined for agglutination both macroscopically and microscopically.
- The above procedure were repeated for each crude extracts under investigation.

Standardization of Extracts that Cross Reacted with ABO Cells in Different Strength.

2.1.5. Procedure

- Eleven (11) test tubes labeled A were set up for test and control.
- From tube two, three (3) drops of normal saline was added to each tube using a Pasteur pipette.
- Three (3) drops of the extract was then added to tube 1 and 2, tapped to mix and three (3) drops from tube 2 were transferred to tube 3.

- The contents of tube 3 were mixed and three (3) drops were transferred to tube 4. This was continued up to tube eleven (11) and three (3) drops from tube eleven (11) were discarded.
- The above set ups were repeated in two sets of Eleven (11) test tubes labeled B and O respectively.
- Three (3) drops each of 5% cell suspension of the pooled, washed A, B and O cells was placed into the tubes labeled A, B and O respectively.
- The contents of the tubes were mixed properly by tapping the tubes and left to stand at room temperature for one hour.
- The tubes were checked for agglutination macroscopically and microscopically by streaking the cells on slide which was viewed with a microscope using x10 objective with the condenser iris sufficiently closed.
- The last tube that showed definite agglutination in each of the crude extracts against A, B and O cells was noted and taken as the Titre (Odiegwu *et al.*, 2011).

3. Results

Table 1 shows the direct agglutination test using the Sixteen (16) Crude extract against 5% saline suspension of pooled washed A, B and O Red Blood Cells.

Table 1 Direct agglutination test using the crude extracts

Euphorbiaceae Plant species	Local names	A-cells	B-cells	O-cells	A-cells	B-cells	O-cells
<i>Acalypha torta</i>	Muel	++++	+++++	clump	Less than 1min	Less than 1min	Less than 1min
<i>Acalypha hispida</i>	Chenille plant	-	-	-	-	-	-
<i>Acalypha wiskesiana</i>	Fire dragon plant	++	-	+	5mins	-	5 mins
<i>Codiaeum variegatum</i>	Variegated croton	++	+	+	5mins	5mins	5 mins
<i>Croton tiglium</i>	Purging croton	+++	+++	+++	5mins	5 mins	5 mins
<i>Euphorbia tirucalli</i>	Indian tree spurge	-	-	+	-	-	7mins
<i>Euphorbia milii</i>	Crown of thorns	++	++	++	5mins	5mins	5mins
<i>Euphorbia pulcherima</i>	Poinsettia	-	+	-	-	7 mins	-
<i>Jatropha curcas</i>	Physic nut	++	++	++	5 mins	5 mins	5 mins
<i>Jatropha gossypifolia</i>	Wild cassava	+	++	-	5mins	5 mins	-
<i>Manihot esculenta</i>	Cassava (vitamin A)	-	-	-	-	-	-
<i>Manihot esculenta</i>	Cassava(NR 8082)	-	-	-	-	-	-
<i>Manihot esculenta</i>	Cassava(TM419)	-	-	-	-	-	-
<i>Manihot esculenta</i>	Cassava(TM4(2) 1425)	-	-	-	-	-	-
<i>Ricinus communis</i>	Castor oil plant	++	++	++	7mins	7mins	7 mins
<i>Tetracarpidium conphorum</i>	African walnut	clump	Very strong clump	clump	2 secs	2secs	2 secs

Key :(-) No agglutination (+) Weak agglutination (++) Mild agglutination (+++) Moderate agglutination (++++) Strong agglutination (+++++) Clump agglutination.

Table 2 illustrates the indirect haemagglutination (30% BSA) test using the Crude extracts of the Five (5) species that did not react in table 1.

Table 2 Indirect haemagglutination test using 30% bovine serum albumin for the extracts that did not react in table 1

Plant species	A cells	B cells	O cells
<i>Acalypha hispida</i>	-	-	-
<i>Manihot esculenta</i> (vitamin A)	-	-	-
<i>Manihot esculenta</i> (NR 8082)	-	-	-
<i>Manihot esculenta</i> (TMX 419)	-	-	-
<i>Manihot esculenta</i> (TMX 4(2) 1425)	-	-	-

Key: No agglutination (-)

Table 3 depicts the indirect haemagglutination (AHG) test using the Crude extracts of the Five (5) species that did not react in table 1.

Table 3 Indirect haemagglutination test using anti-human globulin for the extracts that did not react in table 1

Plant species	A cells	B cells	O cells
<i>Acalypha hispida</i>	-	-	-
<i>Manihot esculenta</i> (vitamin A)	-	-	-
<i>Manihot esculenta</i> (NR 8082)	-	-	-
<i>Manihot esculenta</i> (TMX 4(2) 1425)	-	-	-

Key: No agglutination (-)

Table 4 Portrays the standardization of *Tetracarpidium conophorum* (Tc). This Lectin was standardized because it cross reacted with pooled washed ABO cells in differing strengths and was therefore standardized to find out the dilution at which it is specific to an ABO cell. From the table it can be deduced that at 1/128 dilution, the Lectin reacted specifically with B cells only and hence its Titre was found to be 128.

Table 4 Standardization of *Tetracarpidium conophorum* (Tc) 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

Table 5 Standardization of *Acalypha torta* (At) 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=16 for O cells

Table 5 Reveals that the *Acalypha torta* (At) Lectin also reacted specifically with O cells only at 1/16 dilution and hence the final Titre was found to be 16.

Table 6 demonstrates the protein concentration contents of the Crude extract using the Biuret Protein Assay Method.

The crude extracts of Eleven (11) species (69%) showed visible agglutination reactions of varying degrees of avidity, while those of the other Five (5) species (31%) did not give any reaction with the direct and indirect agglutination tests. The standardization results of the *Tetracarpidium conophorum* and *Acalypha torta* Lectin conducted show that they reacted specifically with B and O cells at Titres of 128 and 16 respectively. The protein assay results also show that each Crude extract gave different concentration of protein content and have no relation or association with the agglutination patterns of the extracts. The results are as illustrated below.

Table 6 Protein assay of the crude extracts using Biuret Protein assay method

Test Sample	Protein Content (g/l)
Protein standard	60
<i>Acalypha torta</i>	25.0
<i>Acalypha hispida</i>	15.0
<i>Acalypha wikesisana</i>	15.0
<i>Codiaeum variegatum</i>	20.8
<i>Croton tiglium</i>	9.0
<i>Euphorbia pulcherima</i>	13.8
<i>Euphorbia milii</i>	15.3
<i>Euphorbia tirucalli</i>	8.3
<i>Jatropha curcas</i>	13.5
<i>Jatropha gossypifolia</i>	24.4
<i>Manihot esculenta</i> (vitamin A)	10.8
<i>Manihot esculenta</i> (NR 8082)	8.1
<i>Manihot esculenta</i> (TMX 419)	9.2
<i>Manihot esculenta</i> (TMX 4(2) 1425)	8.1
<i>Ricinus communis</i>	21.8
<i>Tetracarpidium conophorum</i>	14.4

4. Discussion

Lectins are sugar binding proteins of non-immune origin that reversibly bind oligosaccharides of glycoconjugates without enzymatically modifying them. They agglutinate Red blood cells because they recognize and bind specific carbohydrates (saccharides) found on cell surfaces including Red blood cells. The ability of plant Lectins to detect subtle variation in carbohydrate structures found on molecules, cells and organisms has made them a paradigm for protein-carbohydrate recognition. This research was informed by interesting findings on Lectins conducted in developed world and elsewhere as illustrated below:

Yao et al., (2008), examined and established the ability of the legume Lectin, FRIL (Fltz receptor interacting Lectin), extracted from *Dolichos* specie to preserve neural progenitor cells in suspension culture *in vitro*. They discovered that FRIL could preserve neural progenitor cells *in vitro* by inhibiting both cell proliferation and differentiation [16].

Another interesting finding is that of legume Lectin, which is one of the most well studied families of plants proteins that display a degree of carbohydrate specificity. But the lack of frame work to explain their carbohydrate binding specificities has precluded a rational approach to alter their ligand binding activity in a meaningful manner. However,

the work carried out by Sharma and Surolia sought to provide answers to this problem [17]. Their study reported an extensive analysis of sequences and structures of several legume lectins and showed that despite the hyper variability of their common regions, they exhibit a significant pattern of uniformity within.

Also, *Triticum vulgare* lectin or Wheat germ agglutinin (WGA) isolated from *Triticum vulgare* (Wheat germ) target the delivery of drug which promotes bioavailability of biologically active substance especially for poorly absorbed compounds and allows the reduction of drug toxicity and interchangeable target potential. Therefore the study of Shen et al., (2011) on Wheat germ agglutinin (WGA), was shown to have potential as a carrier for drug delivery [18].

Mistletoe lectins also have cytotoxic effect on tumor cells by inducing cytokine production by monocytes, which play an important role in cancer growth inhibition [19].

This study was therefore carried out to add to the already rich knowledge base for plant lectins. Sixteen (16) plant species of the Euphorbiaceae family were used for the study. While the seeds of some were used, the leaves, stems and tubers of others respectively were extracted and the extracts were subsequently tested with pooled washed ABO cells to investigate their potentials for lectinic activities. The lectinic activity of each of crude extracts was established based on their ability to agglutinate cells of ABO blood group. Eleven (11) species (69%) of the sixteen species used for the study showed lectinic activities while the other five (5) species (31%) did not react in any of the three (3) used serological conditions. The crude extracts of *Acalypha torta*, *Acalypha wiskesiana*, *Codiaeum variegatum*, *Croton tiglium*, *Euphorbia milii*, *Euphorbia tirucalli*, *Euphorbia pulcherima*, *Jatropha curcas*, *Jatropha gossypifolia*, *Ricinus communis* and *Tetracarpidium conophorum* showed lectinic activities in that they reacted with pooled washed ABO cells in saline room temperature (direct haemagglutination test) while the remaining five (5) species: *Acalypha hispida*, *Manihot esculenta* (vitamin A), *Manihot esculenta* (NR 8082), *Manihot esculenta* (TMX 419), *Manihot esculenta* (TMX 4 (2) 1425) did not show any agglutination reaction with pooled washed ABO cells in saline room temperature, 30% Bovine serum albumin (BSA) and Anti-human globulin (AHG) serum serological conditions (indirect haemagglutination tests). Two (2) out of the Eleven (11) species that showed lectinic activities in direct haemagglutination test gave specific reaction (reacted with only one cell type); *Euphorbia pulcherima* reacted with B cells while *Euphorbia tirucalli* reacted with O cells. Thus, they can rightly be indicated by referring to them as anti-BE_p and anti-HE_t respectively (where E_p= *Euphorbia pulcherima* and E_t= *Euphorbia tirucalli*). The other nine species cross reacted with more than one cell type; they are *Acalypha torta*, *Acalypha wiskesiana*, *Codiaeum variegatum*, *Croton tiglium*, *Euphorbia milii*, *Jatropha curcas*, *Jatropha gossypifolia*, *Ricinus communis*, *Tetracarpidium conophorum*. The extracts which cross-reacted in different agglutinating strengths were standardized using the serial doubling dilution method to determine the titre at which each of them would react specifically with a particular cell type [4]; [20]; [15]). The crude extracts were diluted from 1 in 1(neat) to 1 in 1024 and incubated at room temperature. Each of the dilutions were tested with A, B and O cells suspension and each of the tubes from neat to 1 in 1024 was checked for agglutination macroscopically and microscopically. The last tube that showed definite agglutination for each of the seed extracts against A, B and O cells indicate the Titre.

Acalypha torta produced Titre of 4, 8 and 16 against A, B and O cells respectively with varying avidities. This implies that at titre of 16, *Acalypha torta* can be used as Anti-HA_t diagnostic reagent (A_t= *Acalypha torta*). *Tetracarpidium conophorum* produced a titre of 64, 128, 64 against A, B and O cells respectively with varying degrees of avidity, which implies that at titre of 128 *Tetracarpidium conophorum* can react specifically with B cells, thus at dilution of 128 it can be used as Anti-BT_c diagnostic reagent (where T_c= *Tetracarpidium conophorum*). Thus, these two species of Euphorbiaceae lectins can be standardized and used as anti-sera diagnostic reagents and are hence viable for commercial production.

From the protein assay carried out on the crude extracts of the sixteen (16) species of the Euphorbiaceae plants, it was observed that each of the species have a different protein concentration with *Acalypha torta* having the highest protein concentration of 25.0g/l while *Manihot esculenta* (TMX 4(2) 1425) and *Manihotesculenta* (NR 8082) have the lowest protein concentration of 8.1g/l. Therefore, the protein concentrations do not have any association or relation with their agglutination pattern. Thus, the various degrees of protein contents of the crude extracts do not influence their agglutination strength or pattern.

These isolated Euphorbiaceae lectins aside from being used for blood grouping can also find use in other areas such as for induction of mitosis [21]; [22], for bacterial pathogen identification [23]; [24], in biochemical warfare [25], as markers in cancer research [26] etc. as lectins have been shown to have numerous end uses applications.

5. Conclusion

The analysis obtained from this research show that:

- Eleven (11) out of the Sixteen (16) species of the Euphorbiaceae plants showed lectinic activities potentials.
- Five (5) out of the Sixteen (16) species of the Euphorbiaceae plants showed no lectinic potentials.
- *Euphorbia pulcherima* and *Euphorbia tirucalli* reacted specially with B and O cells and can be indicated as Anti-B_Ep and Anti-HE_i lectins respectively.
- *Acalypha torta* and *Tetracarpidium conophorum* could similarly be indicated as Anti-HA_t and Anti-B_Tc respectively.
- The agglutination pattern of the species are not necessarily influenced by their protein concentration since there is no association or relation between the protein concentration and lectinic properties.

Recommendations

Based on the research findings, the following recommendations are advocated:

- Purification of the plant extracts in other to further substantiate their lectinic potentials, facilitate their specificity and deduce their other Medical and Industrial applications.
- The Molecular weight and specific sugar moiety of these isolated Lectins should be deduced with a view to increasing the knowledge about these newly discovered Lectins.
- Immunological assays, Binding tests with parasites, Polymerase chain reaction (PCR) analysis etc. should be carried out on these Euphorbiaceae lectins with a view to determining their other end uses applications.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest amongst the authors as all the authors contributed in one way or the other in conducting the research and in writing the manuscript which was eventually articulated and submitted for publication by the corresponding author.

Statement of ethical approval

This research is in full compliance with ethical standards and moreover, neither human nor animal subjects were used while conducting this research.

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Author's short biography



Dr. Odiegwu Chukwujekwu Nwabueze C. was born in Enugu, Enugu State, Nigeria and hails from Abacha in Idemili North Local Government Area of Anambra State, Nigeria. He had Academic tutelage of over three decades, studying under numerous Teachers, Lecturers and Professors at Ogbete River Primary School, Enugu; Sacred Heart Seminary, Nsude-Enugu; St. John Cross Seminary, Nsukka; National Grammar School, Nike-Enugu; University of Nigeria, Nsukka; University of Nigeria, Enugu Campus cum University of Nigeria Teaching Hospital, Enugu all in Enugu State, Nigeria; Cranfield University, Cranfield, and Staffordshire University, Stoke both in England, United Kingdom (UK).

Following the completion of his National Youth Service Corps (N.Y.S.C.) with the Medical Centre (Sick Bay) of the Nigeria Naval Ship Urhiapele (Naval Base), Ogorode, Sapele, Delta State, Nigeria in 1996, he delved into Diagnostic Medicine Practice. Consequently, he became the Director of Jenic Associates, a conglomerate of Clinical Diagnostic Laboratories, Research, Pharmacy and Clinical Services.

Dr. C.N.C. Odiegwu's passion for Academics prompted him to enrol for Postgraduate studies at University of Nigeria, Enugu Campus culminating in his obtaining Master of Science (M.Sc.) Degree in Haematology and Blood Transfusion Science in May, 2006. In August, 2008, he embarked on Doctor of Philosophy (Ph.D) Degree in Haematology and Blood Transfusion Science of the University of Nigeria, Nsukka. In September, 2013 his Ph.D Programme was near completion and needed Bench Work Research to complete, but owing to lack of certain Equipment, Facilities, Materials, Expertise locally, he had to undergo Overseas Ph.D Bench Work Research at Department of Advanced Diagnostics & Biosensors, Cranfield University, Cranfield, Bedfordshire, England, UK following the Award of Research Grant to him by Tertiary Education Trust Fund (TETFund) of Nigeria. To the Glory of God, despite his chequered experiences, he conducted the Overseas Bench Work Research and successfully defended and obtained the Ph.D Degree in July, 2014. Consequent upon the advised need to study Molecular Biology since it is central to success in his areas of Research, he enrolled with self-funds for Master of Science (M.Sc.) Degree in Molecular Biology with Staffordshire University, Stoke, England, UK and commenced studies in September, 2019. Owing to CoVID-19 Pandemic he couldn't finish the Programme as scheduled but however, is set to complete and successfully obtain the Staffordshire University, England, UK M.Sc. Degree in Molecular Biology in November, 2022.

In recognition of his giant strides in Academics, Nnamdi Azikiwe University (Unizik), Awka, Anambra State, Nigeria had to appoint him Haematology & Blood Transfusion Science Lecturer II to Department of Medical Laboratory Science, College of Health Sciences, Unizik, and Nnewi Campus on 14th May, 2008. He was promoted to Lecturer I by the University on 1st October, 2011, to Senior Lecturer on 1st October, 2015 and Associate Professor on 1st October, 2019. His meritorious Academic conducts and Teachings prompted the Enugu State University of Science & Technology (ESUT), Agbani, and Enugu State, Nigeria to appoint him Adjunct Senior Lecturer in Haematology & Blood Transfusion Science to Department of Medical Laboratory Science, College of Medicine, and Park Lane, Enugu Campus on 2nd June, 2017. Similarly, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria was pleased to appoint him Sabbatical Senior Lecturer in Haematology & Blood Transfusion Science and Charter Head of Department (HOD) for the establishment of New Department of Medical Laboratory Science in the University in May, 2021.

Dr. Odiegwu C.N.C. is a Member of many Academic, Professional, Pious and Humanitarian bodies including: Member Academic Staff Union of Universities (ASUU); Member International Research and Development Institute (IRDI); Member Association of Medical laboratory Scientists of Nigeria (AMLSN) and is the Charter Chairman of the Unizik Chapter of the Association; A devout Catholic and Member League of Sacred Heart of Jesus; A Rotarian since October, 2000 and was Installed the 18th President of the Rotary Club of Enugu-South in 2002-2003 Rotary year. His devotion to the goals of Rotary prompted the then District Governor to appoint him District Governor's Special Representative (DGSR) for the formation of Rotary Club of Awka-Nnamdi Azikiwe University in 2009 and he was Installed the Charter President of the Club 2009-2011 Rotary year; Member of Igwe Cabinet and Nze na Ozo Society of Abacha and was Bestowed with Chieftaincy Title of Ezeamama of Abacha by Igwe of Abacha since August, 2018.

As a prolific Academic, Dr. Odiegwu has many Research Publications in both Local and International Journals to his credit. Currently, his Research interest is focused on Purification, Characterisation and Applications of Tropical Lectins, including on Molecular Haemato-Oncology. He is happily married to his lovely wife – Ugochinyerem (a Statistician) and they are blessed with Chibudom, Tobeolise and Adaolise. To God is the Glory!