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**Biochemical Characterization of Lectin Isolated from the Seeds of Shear  
 Butter Tree (*Vitellaria Paradoxa*)**

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**ABSTRACT**

The study on biochemical characterization of lectin isolated from the seeds of shear butter tree (*Vitellaria paradoxa*) was carried out. The plants materials were obtained fresh from Kaduna metropolis. Seeds extracts from different plant sources were assay for the presence of lectins. Haemagglutinating activity of the crude extract was determined. Blood samples of 5ml each were collected from the three healthy donors belonging to the A, B and O blood group within Kaduna metropolis for its specificity in agglutination in all human erythrocyte type. Biochemical characterization of *V. paradoxa* seed lectin was also determined. Hemagglutinating activity of lectin from *V. Paradoxa* seeds was monitored using human erythrocytes. *V. Paradoxa* showed no specificity for human erythrocytes of ABO blood groups. It was concluded that lectin was inhibited by D-glucose and fructose making it a super-lectin since it was able to recognize structurally two different sugars. Its maximum agglutinating activity was obtained at pH of 7.5 and temperature of 25°C. It is recommended that lectins need to be isolated, purified and characterized to ascertain any similarity or difference in their properties between them and that of the seed. The molecular analysis also needs to be carried out on the lectins from the various plant parts. It is only from the genes that it can be ascertained that the lectin from the seed, leaf and stem back of *V. paradoxa* are the same or differ. The isolated lectin can also be tested against micro-organisms to ascertain if it has any anti-microbial property.

**Keywords:** D-glucose, erythrocytes, extracts, fructose, Haemagglutination

**1.0 INTRODUCTION**

Lectins are glycoproteins that are characterized by their capability to attach carbohydrates such as mannose, galactose, lactose, *N*-acetyl glucosamine, *N*-acetyl galactosamine, fucose, and rhamnose with significant specificity (Rubeena *et al.*, 2019). They bind specifically and reversibly to different types of glycoproteins or carbohydrates (Sharon, 2007). Due to these advantages, some sensitive dielectric sensors based on Concanavalin A lectin were used for the specific identification of glycoproteins and carbohydrates (Jiang *et al.*, 2019).

Exploratory studies on their health benefits have been initiated, because lectins have important physiological roles, including insecticidal action, antibacterial activity and antifungal effects, antihuman immunodeficiency virus, antitumor activity and analgesic activity (Jiang *et al.*, 2019).

Plants are the main source of lectins that are found in different parts of plants such as seeds, leaves, bark, roots, tubers, and fruits (Macedo *et al.*, 2015). It has been known for a long time that lectins occur in legumes, where they can be a major food source for both humans and animals (Lajolo, and Genovese, 2002). However, lectin has an antinutritional factor (Vasconcelos and Oliveira, 2004). Its antinutritional properties are most likely caused by their impairment of the integrity of the intestinal epithelium, and thus also the absorption and utilization of the nutrients that are present in the legumes (Vasconcelos and Oliveira, 2004). So, lectins containing foods are frequently consumed cooked or otherwise processed to reduce the level of the antinutritional factors and improve the utilization efficiency of legumes (Shi *et al.*, 2018). In addition, the lectins that are thought to survive gastric digestion have been a minor allergen, such as soybean seeds

(Jiang *et al.*, 2019). Therefore, its relevance to food safety requires intensive research to determine the digestibility of lectins from legumes, especially regarding the consumption of high levels of unprocessed or undercooked beans (Jiang *et al.*, 2019). Thus, more and more legume lectins are being studied intensively. Lectin with a molecular mass of approximately 60 kDa and two different subunits was isolated from the ground bean (*Vigna sesquipedalis* cv. ground bean) (Jiang *et al.*, 2019).

Biochemical studies revealed that these lectins are much conserved glycoproteins with a high degree of homology (De Hoff *et al.*, 2009). Molina and Vicente (2000) suggested that these proteins, encoded by tandem genes, presumably arose through duplication and divergence of an ancestral gene. They are also part of a protein family involved in plant defense mechanisms. Although the physiological role of lectins is still a subject of controversy, they have often been involved as enzymes in some metabolic pathways. Patel (2014) described certain galactosidases and mannosidases as phytohaemagglutinins. Moreover, several lectins from plants can act as elicitors for the production of a phytoalexin, as found in *Pisum sativum* (Molina and Vicente 2000). Some are able to bind to other enzymes in order to modify their activity. DNA polymerase  $\alpha$ ,  $\beta$  and  $\gamma$  activities in human lymphocytes are stimulated by *Phaseolus vulgaris* lectin (Dhuna *et al.*, 2005). In other cases, they have been assayed as *in vitro* inhibitors of protein synthesis (Dhuna *et al.*, 2005). The isolation, purification, characterization of new plant lectins have been of interest (Latha *et al.*, 2006). The presence of lectin is mainly detected through the haemagglutinating activity assay which is performed with a serial dilution of the lectin before an incubation step with human or other animal erythrocytes (Santos *et al.*, 2009).

*Vitellaria paradoxa* belongs to the family Sapotaceae and is divided into two sub-species: *nilotica* and *paradoxa*. The genus *Vitellaria* is considered by botanical authorities as monospecific (relating to or consisting of only one species) two subspecies are recognized ssp.

*paradoxa* and ssp. *nilotica*. The sub-species, *nilotica* is located primarily in Uganda and Sudan, with some occurrence in Ethiopia, while the sub-species, *paradoxa* is found in areas from the East African Republic to Senegal up to west Africa (Boffa, 2015). The ranges of the two do not overlap, although they have been found within 175 km of one another (Boffa, 2015). The difference between the two subspecies occurs primarily in the consistency of the butter content found within the nut (Boffa, 2015). It is commonly called Shea butter (English), Kareje (Fulfulde, Nigeria), Kadanya (Hausa, Nigeria) Koita (Gbagi, Nigeria). The size of the mature tree varies from 7 to 25 m. In cultivated fields the height is said to be typically 15-20 m. The leaves are simple, 10-25 cm long and with wavy margins.

Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections (El-Mahmood *et al.*, 2008). The bark is used to suppress cough and also to treat leprosy (El-Mahmood *et al.*, 2008). The plant according to Abubakar *et al.* (2013) is said to be used for the treatment of inflammation, rashes in children, dermatitis, chapping, irritation, ulcer and rheumatism. The paste of the root bark is taken orally to cure jaundice as well as applied topically to treat chronic sores and girth sores in horses (Abubakar *et al.*, 2013). Among other uses, the butter (fat extract) is also locally used in soap and pomade production and in the treatment of cough and minor bone dislocation (Ugese *et al.*, 2008). It is both a fruit tree and an oilseed crop. The plant is locally abundant in Nigeria in the derived savannah zones (Schreckenber, 2004). Shea butter oil is still considered second to palm oil as the most important source of cooking fat, particularly by West African rural dwellers (Ugese *et al.*, 2008). The aim of the study is to assess the biochemical characterization of lectin isolated from the seeds of shea butter tree (*Vitellaria paradoxa*). The objective of this study is to biochemically characterize the lectin isolated from the seeds of *Vitellaria Paradoxa*.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

The plants materials were obtained fresh from Kaduna metropolis which is located between latitudes 10°25'15"N and 10°36'08"N and longitudes 7°23'31"E and 7°29'33"E. Seeds extracts from different plant sources were assayed for the presence of lectins. The plants and their seeds were identified at the herbarium of Ahmadu Bello University, Zaria, Kaduna State. The plants voucher numbers have been recorded. The seeds

were listed below with their family and numbers in Table 1.

It was only one (*Vitellaria paradoxa* [shear butter tree]) out of the six plants that showed the presence of lectin. *Vitellaria paradoxa* belongs to the family Sapotaceae.

The seeds of the plant that showed agglutination were collected. The plant herbarium number has been recorded (NDABIOL/1619) in the Biological Sciences Department, Nigerian Defence Academy.

**Table 1: Details of Plants used for Preliminary Study**

Names of Plant	Family	Voucher number
<i>Isobertina tomentosa</i>	Caesalpinaceae	3114
<i>Strychnos innocua</i>	Loganiaceae	6831
<i>Landolphia owariensis</i>	Apocynaceae	016
<i>Uvaria chamae</i>	Annonaceae	2040
<i>Strychnos spinosa</i>	Loganiaceae	901
<i>Vitellaria paradoxa</i>	Sapotaceae	6954

### 2.2 Preparation of Seed Crude Extract

The seeds were finely ground by pounding it in a mortar. Twenty five grammes (25g) of the powdered seed was defatted using petroleum ether in the ratio 1:6 for 24 hours. The defatted sample was re-weighted before 50mM Phosphate Buffer (PB) pH 7.2 was added to the sample at a ratio of 1:6. The sample was kept in the refrigerator for 24 hours. The extract was obtained by filtering the soaked sample using a muslin cloth after the 24 hour period. The filtrate was clarified by centrifugation at 10,000rpm for 10 minutes. After centrifugation, the residue was discarded and the clarified crude was subjected to haemagglutination assay and protein determination (Welch *et al.*, 2020).

### 2.3 Haemagglutination Assay

Haemagglutinating activity of the crude extract was determined by two-serial fold dilution as described by Kuku and Eretan (2004). Fifty microliters (50ul) of the crude extract was

serially two-fold diluted using PBS in plastic multi-well microliter plates. 50ul of 5% erythrocyte suspension was added to each well. The blood samples used are those of human A, B O. The assay was carried out in a 96 well round bottom microliter plate. The first well of each row served as positive control to which 50microlitre of sample and 50 microliter of blood was added and the last well served as negative control containing 50microlitre of blood and 50microlitre of PBS solution. Between the positive and negative control each well contain blood, PBS and samples. First of all 50microlitre PBS was added to all the wells. Then 50microlitre of the crude extract was added to the first well and serially diluted until it gets to the negative control. Similar procedure was followed for the other sample. Finally 50microlitre of processed blood sample was added to each well. The plate was incubated for a period of one hour for agglutination reaction to take place, after which it was examined under the microscope. The activity was expressed as

haemagglutination titre which is the reciprocal of the last dilution showing visible agglutination of the red blood cell. Specific activity of the lectin was calculated by dividing the titer by the protein content, which is then expressed in mg per ml extract.

#### **2.4 Ammonium sulphate Precipitation**

The crude extract was subjected to 90% ammonium sulphate precipitation using Mojica and Merca (2006) nomogram. Ammonium Sulphate was added to the crude extract gradually while stirring it. It was then allowed to stand for 15 minutes, after which the mixture was centrifuged at 10,000rpm for 15 min. The precipitate was collected and dissolved in minimum amount of buffer and stored. Addition of ammonium sulphate, standing and centrifugation procedures were performed at 4°C to prevent protein denaturation.

#### **2.5 Dialysis**

The ammonium sulphate in the previous procedure was removed using membrane dialysis tubing. The tubing was pretreated by boiling it in 10mM Na<sub>2</sub>CO<sub>3</sub> and 10mM EDTA to open up the tube as described by Reed (2007). The tube was sealed at one end with a cotton string while the ammonium sulphate fraction was introduced through the other opening with the use of a pipette to ensure no formation of bubbles. After sealing the other end, the tube was placed in a beaker filled with Phosphate Buffer (PB) pH 7.2. The beaker was kept in the refrigerator with three changes before the next 24 hours. After 24 hours the desalted sample was collected by punching a hole at one end, allowing the liquid to flow into a container. The dialyzed sample was stored at 4°C for further use. The sample obtained from this step was tested for haemagglutinating activity as described above.

#### **2.6 Gel Filtration**

The sample that shows agglutination activity from the Ammonium Sulphate precipitated sample was applied directly to a Sephadex G-100 gel filtration column (3.5\*20cm) that has been previously equilibrated with 50mM phosphate buffer PB (pH 7.2). The sample was eluted with 50mM phosphate buffer pH 7.2 at flow rate of 20ml/hr. Thirty (30) fractions of 2ml each were collected. All the fractions were subjected to haemagglutination assay. All fractions that

exhibited agglutination were pooled together for further use.

#### **2.7 Effect of Temperature on Haemagglutinating Activity**

The effect of temperature on the agglutinating activity of the lectin was determined by carrying out assay at different temperatures according to the method described by Oliveira *et al.* (2002). Aliquots of the purified lectin were incubated in a water bath for 30 min at various temperatures (25-100°C). The solution was cooled on ice immediately and then allowed to warm up to room temperature. Haemagglutination assay was then carried out as previously described. Results were expressed as log of haemagglutination of control kept at 20°C for 30 min.

#### **2.8 Effect of pH on the Haemagglutination Activity**

The effect of pH on the activity of the lectin was determined by pre-incubating the lectin samples in the following buffers at the various pH following the method of Kuku and Eretan (2004). The buffers used are 50mM sodium phosphate/acetic acid buffer at pH (4- 5), 50mM sodium phosphate/ HCL pH (6- 7), 50mM Tris-HCl buffer pH (8-9), and 50mM glycine/NaOH buffer at pH (10-11). Ten microlitre (10ul) of the purified seed lectin was subjected to different pH level by adding 30ul of buffers at different Ph, 10ul of the blood sample was then added afterwards. After which the solutions were neutralized to pH 7.2. Then they were assayed for haemagglutinating activity. The control value was the haemagglutination titre of the lectin in PBS, pH 7.2.

#### **2.9 Inhibition of Haemagglutination Activity by Various Sugars**

The haemagglutination inhibition test to investigate inhibition of lectin-induced haemagglutination by various carbohydrates was performed in a manner similar to the haemagglutination test as described by Kuku *et al.* (2004). The experiment consisted of serial two-fold dilutions of sugar samples prepared in phosphate buffer saline in a 96 well microtitre plate. All the dilutions were mixed with an equal volume (50 µl) of the lectin solution of known

haemagglutination units. The mixture were allowed to stand for 1 hr at room temperature and then mixed with 50 µl of a glutaraldehyde fixed 5% rabbit erythrocytes suspension. The haemagglutination titres obtained was compared with a non-sugar containing blank. In this study, the sugars used are: glucose, galactose, maltose, fructose, sucrose, melibiose, rhamnase and arabinose. The minimum concentration of the sugar in the reaction mixture which completely inhibited haemagglutination units of the lectin sample was obtained.

### 2.10 Carbohydrate Content Determination

Total neutral sugar was determined by the phenol/sulphuric acid method (Liu *et al.*, 2016) with D-glucose serving as the standard. A standard curve was constructed using glucose at 10, 20,40,60,80 and 100ug/ml concentrations. 50microlitre of the lectin sample was pipetted into test tubes to which 1ml of 5% phenol was added followed by addition of 5ml 96% sulphuric acid. The tubes were then allowed to stand for 10minutes before transfer into a water bath set to 30°C for 20minutes. The test tubes were read using a spectrophotometer at 490nm using distilled water as the blank.

### 2.11 Blood Collection and Treatment

Blood samples of 5ml each were collected from the three healthy donors belonging to the A, B and O blood group within Kaduna metropolis. The blood groups were confirmed by subjecting them to a blood group test. The blood samples were

collected in a bottle containing ethylene diamine tetra acetate (EDTA) to prevent the blood from coagulating.

Five millilitre (5ml) of each of the blood samples were mixed with 5ml of Phosphate Buffer Saline (PBS) pH 7.2 in a centrifuge tube. The mixtures were subjected to centrifugation at 1500rpm for 5minutes at room temperature. The supernatant were removed and discarded using a Pasteur pipette. More 5ml of PBS was added, centrifuged again and the supernatant discarded. The process (dilution with PBS and centrifugation) was repeated several times until the supernatant became clear. This process of diluting and centrifugation of the blood sample was to get rid of all other component of the blood leaving behind only the red blood cells. The compacted red blood cells were then diluted with PBS until the volume reaches 100 ml making 5% blood suspension. The prepared blood samples were stored in the refrigerator.

### 2.12 Data Analysis

The data were presented in using charts using Microsoft Office Excel (2007).

## 3.0 RESULTS

### 3.1 Preliminary Screening of Seeds Extract for the presence of Lectin

The result of the pilot study on the ten seeds assayed for the presence of lectin. Only *Vitellaria paradoxa* out of the ten seed extracts screened for lectin shows agglutination with red blood cells. This is the only test available to show the presence of lectin.

**Table 2: Plant Extracts Screened for the Presence of Lectin**

Names of Plant Species Screened	Presence of Lectin
1. <i>Isoberlina tomentosa</i>	LNP
2. <i>Strychnos innocua</i>	LNP
3. <i>Landolphia owariensis</i>	LNP
4. <i>Uvaria chamae</i>	LNP
5. <i>Strychnos spinosa</i>	LNP
6. <i>Vitellaria paradoxum</i>	LP

KEY: LNP= Lectin not present. LP=Lectin present

### 3.2 Sugar Inhibition Test

All the sugar tested did not inhibit the agglutination activity except glucose and fructose (Table 3) with minimum inhibitory concentration of 12.5mM.

### 3.3 Effect of pH on *Vitellaria paradoxa* Seed Lectin.

Agglutination was observed within the pH range of 7.5 to 9 with the highest obtained at pH 7.5. Activity decreases above and below this pH range (Fig. 1).

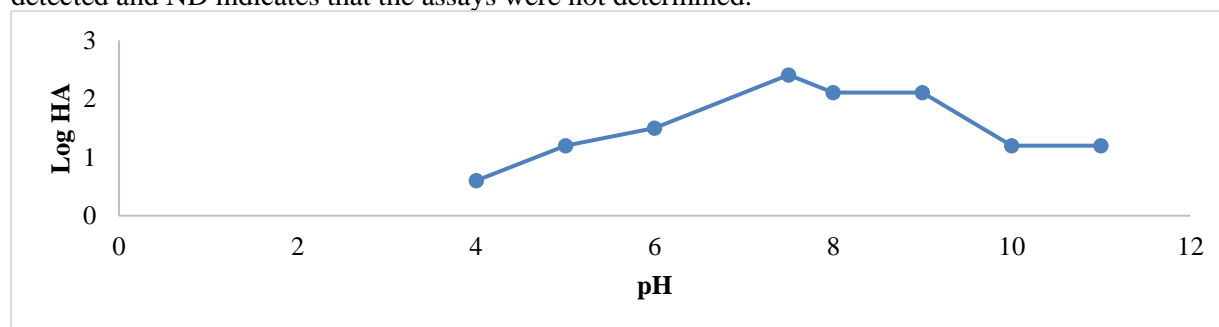
### 3.4 Effect of Temperature on the HA of *Vitellaria paradoxa* Seed Lectin

Maximum agglutination was observed at 25°C and 30°C. Activity was still obtained up to 60°C above which the activity declined and was completely loss at 70°C (Fig. 2).

**Table 3: Inhibition of Haemagglutinating Activity of the Seed Lectin of *Vitellaria paradoxa* by Carbohydrates**

Inhibitors	Minimum Inhibitory Concentration
D-glucose	12.5mM
L-rhamnose	N
Lactose	ND
Sucrose	N
D-fructose	12.5mM
Melibiose	N
Arabinose	N
Maltose	N
Mannose	ND
Galactose	N
Trehelose	ND
Rabinose	ND

Carbohydrates (0.2 M) were used. N indicates that no inhibition of Haemagglutinating Activity (HA) was detected and ND indicates that the assays were not determined.



**Fig. 1: Effect of pH on Haemagglutinating Activity of *Vitellaria paradoxa* Seed Lectin**

The buffers used were 0.5M sodium phosphate/acetic acid buffer, pH (4- 5); 0.5M sodium phosphate/HCL buffer, pH (6-7); 0.5M Tris-HCl buffer, pH (8-9); and 0.5M glycine/NAOH buffer, pH (10-11).

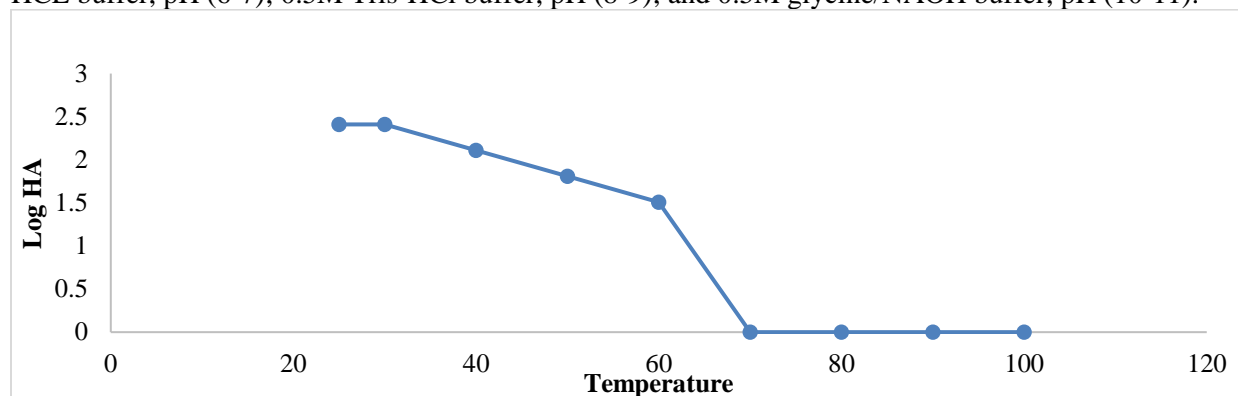


Fig. 2: Effect of Temperature on the Haemagglutinating Activity of *Vitellaria paradoxa* Seed Lectin

### 3.5 Carbohydrate Content Estimation

There was no sugar detected in the lectin after the test was carried out. The absorbance read negative value for the three times it was carried out.

### 4.0 DISCUSSION

In this study, preliminary screenings of six different seed extracts were carried out for the presence of lectin out of which only one showed agglutination (*Vitellaria paradoxa*). This is not surprising since most of the plants screened are not of the legume family and Aranda-Olmedo and Rubio (2020) reported that in plants, leguminous seeds are a particularly rich source of lectins, while only a few have been isolated from other families. *Vitellaria paradoxa* seed lectin is a lectin obtained from *V. paradoxa* seeds which belongs to the Sapotaceae family. Much lectin from the Sapotaceae species has not been described. The present study indicates the presence of lectin in the seeds of *V. paradoxa*. Haemagglutinating activity was detected in the seed extract. This is similar to *Moringa oleifera* in which lectin was isolated in the extract of the seeds (Andre´a *et al.*, 2009). The haemagglutination titre of the extracts was measured. Haemagglutination titre is the highest dilution giving a visible agglutination. Agglutination was observed in all the various erythrocytes tested against all the plant seed extract. Therefore, the lectin from the seed of *V. paradoxa* is not specific in its agglutination since it agglutinated all human erythrocyte type A, B O. This is similar to the lectin isolated from *Archidendron jiringa* Nielsen which shows haemagglutinating activity against human blood

group (A, B, AB and O) (Sarinya *et al.*, 2010). Similarly, the previous studies of lectin from Egyptian *Pisum sativum* seeds, *Bauhinia monandra* (Coelho *et al.*, 2000) and the extracts of *Kalanchoe crenata* leaves, which agglutinate all human blood groups (Kuku *et al.*, 2004). So also the lectin isolated by Oliveira *et al.* (2014) from *Artocarpus incisa* which agglutinates human erythrocytes of the ABO system.

The *V. paradoxa* lectin may then be classified into the category of lectins that agglutinate erythrocytes of all human blood groups alike, which are usually referred to as non-specific lectins (Kuku *et al.*, 2009). Therefore the lectin from *V. paradoxa* agglutinated red blood cells non-specifically which is typical of many lectins (Kuku *et al.*, 2009). *V. paradoxa* seed lectin is therefore not a blood group specific lectin and it is multivalent lectins since only the multivalent lectins can agglutinate blood groups tested for haemagglutination.

Lectins generally show specificity to sugars which is determined by the monosaccharides that inhibits their haemagglutinating activity or their precipitation with glycoconjugates. They can therefore be classified according to their carbohydrate binding specificity. D-fructose and D-glucose with minimum inhibitory concentration (MIC) of 12.5 mM for both sugars inhibited the lectin activity. Sugar inhibitory studies carried out in this study is similar to that observed in the lectin from the seeds of *Cissus poplunea* towards human erythrocytes which was inhibited by D

fructose and D-glucose (Awoyinka *et al.*, 2011). In general sugar inhibition is in agreement with those found for the numerous marine algal lectins, such as *Cystoclonium purpureum* (Huds.) Batters (Awoyinka and Dada, 2011), *Solieria chordalis* (C. Agardh) J. Agardh (Awoyinka and Dada, 2011), *Gracilaria bursa-pastoris* (Gmelin) Silva (Awoyinka and Dada, 2011), *Solieria filiformis* (Abreu *et al.*, 2018) were one or two sugars inhibited the activity of the lectins. The haemagglutination activity of *Phaseolus vulgaris* was inhibited by N-glycans from glycoproteins (Valadezvega *et al.*, 2011). Agglutinating activity of *Arachis hypogaea* lectin was inhibited by lactose, raffinose, melibiose and D-galactose when reacted with human O blood group treated with neuraminidase (Jieet *et al.*, 2011). The haemagglutinating activity of *Erythrina senegalensis* lectin was inhibited by galactosyl moieties with lactose being the most potent inhibitor with MIC (minimal inhibitory concentration) of 0.195mM (Kuku *et al.*, 2012). Extracts of plants from *Moringa Oleifera* were inhibited by azocasein and asialofetuin (Andre´a *et al.*, 2009). So also the haemagglutinating activity of the lectin from *Dioscorea bulbifera*, towards human erythrocytes was sensitive to inhibition by D-arabinose and D-dulcitol with minimum inhibitory concentration of 0.781 mM and 6.25 mM respectively (Kuku *et al.*, 2014). In a similar way the lectin from *Bauhinia variegata* seeds was inhibited by D-galactose and its derivatives, especially lactose (Luciano *et al.*, 2008). The lectin interaction with carbohydrates are performed through a combination of hydrogen bonds between sugar hydroxyl groups and protein main-chain, as well as side-chain groups, water-mediated contacts, van der Waals packing of the hydrophobic sugar ring face against an aromatic residue, and hydrophobic interactions (Sol *et al.*, 2007). *V. paradoxa* seed lectin is therefore a super-lectins since it can recognize structurally different sugars.

The haemagglutinativg activity of *V. paradoxa* seeds lectin was found to be stable at optimum pH ranges of pH 7.5 – 9 with the highest agglutination obtained at pH 7.5. Its capacity and activity was reduced below pH 7.5 and above pH 9. More acidic or basic pH affects its activity. This behavior is similar to that of other lectins as

reported by Carneiro *et al.* (2017) where lectin from *Parkia javanic* depended on pH with its optimal value at pH 7 and stable between pH 7 - 10. Also, more acidic or basic pH decreased both the stability and activity. The lectin of *P. filicinawas* 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 11-12 (Carneiro *et al.*, 2017). Lectin from *Salvia bogotensis* seeds has its maximum pH stability at pH 7.0 and 8.0, and declined to zero at below pH 2.0 and above 12.5 (Vega and Pérez, 2006). *Cissus poplunea* lectin was stable at pH range of 6-8 and 10-11 (Awoyinka *et al.*, 2011). The activity of *Momardica charantia* lectin was maximum (100%) at the optima of pH range 5-8 (Awoyinka *et al.*, 2011).

The relatively lower reduction of activity, at increased basic pH values may be due to some degree of base-induced denaturation. It is possible that changes in the ionization state with an increase in pH may lead to a weaker binding of the metal ions, which are apparently required for the maintenance of the structure that is said to be required for maximal activity. 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 substrate (Kuku *et al.*, 2005). It is also possible that the increase in OH<sup>-</sup> ions caused a change in the ionization state of the lectin, thereby affecting the binding forces between the lectin and the erythrocyte membrane that eventually led to a loss of activity (Kuku *et al.*, 2005).

The lectin from *V. paradoxa* seeds was found to be heat stable from 25°C to 60°C. The lectin highest agglutinating activity was obtained at 25°C to 30°C but reduced considerably as the temperature increases up to 60°C. Its agglutinating activity was completely lost at 70 °C. In comparison lectin extracted from *C. annuum* was found to be stable between 20°C to 30°C (Alexander *et al.*, 2004; Kuku *et al.*, 2009). So also the haemagglutinating activity in some plant lectin and marine algae (Leite *et al.*, 2005) were affected only by exposure to a temperature of 70°C. However, there are certain reported lectin whose activity were not affected by increase in temperature such as lectin from *Ganaderma*

*caperise* which retain its activity when exposed to temperature at and above 70°C for 60 minutes (Ngai and Ngi, 2007). In addition, *K. crenata* lectin is heat-stable up to 90°C (Kuku *et al.*, 200305). Lectins from *Artocarpus integrifolia* and *Canavalia gladiata* were both found to be stable up to 100°C (Laija *et al.*, 2010). The lectin from *Moringa oleifera* was thermostable at 100°C (Andre'a *et al.*, 2009). The difference in all these values may be supported by Oliveira *et al.* (2002) who reported that heat stability of lectin differs from lectin to lectins. Kuku *et al.* (2009) also reported that lectins are known to be heat labile their activity can be decreased by heat treatment.

## 5.0 CONCLUSION

The lectin was inhibited by D-glucose and fructose making it a super-lectin since it was able

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to recognize structurally two different sugars. Its maximum agglutinating activity was obtained at pH of 7.5 and temperature of 25°C. A lot of research has been carried out on *V. paradoxa* plant parts (Shear butter tree) with much attention paid to the fruit pulp, the stem bark, the seed etc. Since the nut contain a high percentage of fat which is extracted for use it is assumed not to have a second benefit but it sure do contain lectin in addition to its high fat content.

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