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Extraction, Isolation and Characterization of Lectins from *Moringa oleifera* seeds

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To my family, both immediate and extended, especially my mother, Ms Wiiwo J for the unconditional love, inspiration and relentless support;

To my father, the late Hasunahi S, for being the best dad I could ever ask for;

To my friends and everyone that guided and supported me physically and spiritually;

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To my colleague Chaima B with whom I have shared the ups and downs during this research, and to her family, for being a family to me, and

To everyone that loves science,

This is dedicated to each one of you!!!

Phiona

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To my family, both immediate and extended, especially my mother for the unconditional love, inspiration and relentless support;

To my father for being the best dad I could ever ask for;

To my friends and everyone that guided and supported me physically and spiritually;

To all my teachers and educators who have inspired and helped me acquire knowledge;

To my colleague Phiona with whom I have shared the ups and downs during this research and

To everyone that loves science,

This is dedicated to each one of you!!!

Chaima

Abstract

The purpose of the research was to study the isolation, purification and characterization of lectins from mature *Moringa oleifera* seeds. The lectins were extracted from the seeds with phosphate-buffered saline (PBS) at pH 7.4, and partial purification was accomplished by ammonium sulfate precipitation and gel filtration using Sephadex G-50.

Agglutination using glutaraldehyde-treated rabbit erythrocytes and human blood types A, B, O was carried to determine the presence and specificity of the lectin. Subsequently, the *Moringa oleifera* seed lectins were classified as a complete non-specific lectin since it agglutinated all human blood types and glutaldehyde-treated rabbit erythrocytes.

The results of carbohydrate specificity showed that the lectin had complex sugar specificity to fetuin. The lectins were found to be thermally stable, and stable within the pH range of 3-6. It was also considered as a non metallolectin since its activity was not significantly affected by EDTA.

Keywords: Lectins, Extraction, Agglutination, Characterization, *Moringa oleifera,* Inhibition test.

Résumé

Le but de la recherche était d'étudier l'isolement, la purification et la caractérisation des lectines des graines mûres de *Moringa oleifera*. Les lectines ont été extraites des graines avec une solution saline tamponnée au phosphate (PBS) à pH 7,4 et la purification a été effectuée par précipitation au sulfate d'ammonium et filtration sur gel en utilisant Sephadex G-50.

Une teste d'agglutination utilisant des érythrocytes de lapin fixé par glutaraldéhyde et des groupes sanguins humains A, B, O a été effectué pour déterminer la présence et la spécificité de la lectine. Les lectines des graines de *Moringa oleifera* ont été classée comme des lectines complète non spécifique puisqu'elles agglutinaient tous les types de sang humain et les érythrocytes de lapin fixé par glutaraldéhyde.

Les résultats de la spécificité glucidique ont montré que les lectines ont une spécificité pour la fétuine. Les lectines sont révélées thermiquement stable et stable dans la gamme de pH de 3-6. Elles ont également été considérées comme des non metallo lectines car l'addition d'EDTA n'a pas un effet considérable sur l'activité.

Mots clés: Lectines, Extraction, Agglutination, Characterisation, *Moringa oleifera*,

Le test d'inhibition.

ملخص

الهدف من هذا البحث هو دراسة عزل و تنقية و خصائص الليكتين المستخلص من بذور *Moringa oleifera* الناضجة.

تم استخراج الليكتين من البذور بنقعه في محلول ملحي (فوسفات مخزنة مالحة) في درجة الحموضة 7,4 ، و تمت تنقيته بعد ترسيب كبريتات الامونيوم باستخدام الترشيح و هلام Sephadex G-50.

لتحديد وجود اللكتين و معرفة خصائصه تم تنفيذ اختبار التراص باستعمال كريات الدم الحمراء للارنب المعالج ب Glytaraldehyde و انواع الدم البشري O, B, A.

صنف اللكتين المستخلص من البذور من بين اللكتينات الكاملة نظرا لالتصاقه بجميع انواع الدم البشري و خلايا الدم الحمراء للارنب المعالج ب Glytaraldehyde.

اظهرت نتائج نوعية الكربوهيدرات ان اللقاح لديه خصوصية اتجاه السكر المعقد Fetuin ، وان خصائصه تتمثل في استقراره حراريا و استقراره ضمن نطاق درجة الحموضة من 3 الى 6. كما تم اعتباره مركب كامل لعدم تاثره ب EDTA.

الكلمات المفتاحية : اللكتينات ، استخلاص ، التراص ، الخصائص *Moringa oleifera* ، اختبار التثبيط .

ABBREVIATIONS

CMoL: Coagulant *M. oleifera* Lectin

Con A: *Concanavalin A*

CRD: Carbohydrate Recognition Domain

FAO: Food and Agriculture Organization

Fuc: Fucose

HIV: Human Immunodeficiency Virus

Gal: Galactose

GalNAc: *N*-acetyl-D-galactosamine

GlcNAc: *N*-acetyl glucosamine

Man: Mannose

MO: *Moringa oleifera*

NeuNAc: *N*-acetylneuraminic

PNA: Peanut lectin

RBC: Red Blood Cell

Rh: Rhesus

SHA: Specific Hemagglutinating Activity

WGA: Wheat Germ Agglutinin

WSMoL: Water-Soluble *M. oleifera* Lectin

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Introduction

Biomolecules essentially fulfill their function through continual recognition of and binding to other molecules. Biomolecular recognition is therefore a phenomenon of prominent importance. Proteins are a remarkable example in view of their capacity to change shape. Several decades ago, the author of a well-known treatise on proteins **(Creighton, 1983)** wrote that ... the biological function of proteins almost invariably depends on their direct physical interaction with other molecules. **(Pierre, 2014).**

Amongst these proteins, our interest is 'lectins', a special class of proteins widely distributed in nature, which selectively recognize and reversibly bind to carbohydrates and glycoconjugates through their binding sites. (**Santos *et al.*, 2014).** These proteins were discovered towards the end of the 19th century and were referred to as hemagglutinins due to their ability to agglutinate erythrocytes or phytoagglutinins because they were originally found in extracts of plants. **(Sharon and Lis., 2004).**

Lectins have been powerful tools in preparative and analytical purposes in biochemistry, cell biology, immunology, molecular biology, pharmacology and clinical chemistry. (**Maricel *et al.*, 2004).** They manifest a diversity of activities including anti-insect activities, antitumor, immunomodulatory, antimicrobial and HIV-1 reverse transcriptase inhibitor, which may find applications in many therapeutic areas. (**Rabia *et al.*, 2013).**

According to the Food and Agriculture Organization's **(FAO)** report, about 70–80 % of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases **(Ekor., 2014)**, and about 25 % of the synthesized drugs are manufactured from medicinal plants **(Pan *et al.*, 2013).**

Moringa oleifera Lam., a common marginal tropical tree in Africa, also known as 'the miracle tree' has become in a decade one of the new food and economic important plant resources. **(Atakpama *et al.*, 2014).** Various parts of the plant including roots, leaves, and seeds possess various medicinal as well as nutritional values**. (Stohs and Hartman., 2015).**

The broad application and variety of uses of lectins show the need to isolate lectins from local and cheap sources since lectins are very expensive. One of the

possible local sources of lectin is the seed of horseradish or malunggay (*Moringa pterygosperma syn. Moringa oleifera*). (**Maricel *et al*., 2004).**

This present work was done to study the presence of lectins in this plant, which has never been studied in Algeria in line with the extraction of lectins.

The main aim of the study is to evaluate the agglutinating activity of the lectins extracted using rabbit erythrocytes, purification of the extract, study the effect of thermal and pH treatment on the stability of this lectin, and detect its specificity using the inhibition test by sugars.

Brief review on Lectins

I. LECTINS

1. Definition

The word *lectin* has been derived from the Latin word "**legere**", which means "to select", by William Boyd **(Boyd and Shapleigh., 1954).** This term was generalized to embrace all sugar-specific agglutinins of non-immune origin, irrespective of source and blood type specificity **(Sharon and Lis., 1972).** Lectins have the ability to bind carbohydrates and the name "hemagglutinins" is used when the sugar specificity is unknown.

Lectin is defined as a carbohydrate-binding protein of non-immune origin that agglutinates cells or precipitates polysaccharides or glycoconjugates **(Goldstein *et al.*, 1980).** They can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes, without altering the properties of the carbohydrates. **(Rabia *et al.*, 2013).**

The ability to agglutinate cells distinguishes these proteins from other macromolecules able to bind carbohydrates. In addition, their non-immune origin differentiates them from anti-carbohydrate immunoglobulins that agglutinate cells. **(Santos *et al.*, 2014).**

More than a hundred of these molecules have been isolated from plants, viruses, bacteria, invertebrates and vertebrates, including mammals. Lectins are a component of traditional herbs such as dietary and medicinal plants. **(Jasminka, 2015).**

2. History of lectins

The occurrence in nature of erythrocyte-agglutinating proteins has been known since the turn of the 19th century. **(Sharon and Lis., 2004).**

The study of lectins began with the work of Hermann Stillmark (1888) who, for the first time, observed that seed extracts (*Ricinus communis*) could agglutinate red blood cells. After this pioneer study, several theses and papers were published**. (Renato *et al.*, 1991).** Subsequently, H. Hellin, also at Tartu, demonstrated the presence of a toxic hemagglutinin, abrin, in extracts of the jequirity bean (*Abrus precatorius*).

In 1919, James B. Sumner at Cornell University (Ithaca, New York), isolated from jack bean (*Canavalia ensiformis*) a crystalline protein that he named concanavalin A and in this way obtained a pure hemagglutinin for the first time.

However, nearly two decades passed before Sumner and Howell (1936) reported that *Concanavalin A* agglutinates cells such as erythrocytes and yeasts and also precipitates glycogen from solution. They further showed that hemagglutination by *Concanavalin A* was inhibited by sucrose, demonstrating for the first time the sugar specificity of lectins. **(Sharon and Lis., 2004). Table 01** shows some milestones of lectinology.

Table 01: History of lectins. **(Renato *et al*., 1991).**

Year	Scientists	Discovery
1884	Warden and Waddel/ Bruyllants and venneman.	Toxicity in *Abrus precatorius* seed extracts.
1886	Dixson.	Toxicity in Ricinus communis seeds extracts.
1888	Hermann Stillmark.	Hemagglutinating activity in *Ricinus communis.*
1890	P Erlich.	Use of abrin and ricin in immunological research.
1908	K Lansteiner and H Raubitsheck.	Different hemagglutinating properties in various seeds extracts.
1919	J B Sumner.	Crystallization of concanavalin A.
1936	J B Sumner. S F Howell.	Lectins demonstrated to bind sugar Concanavalin A precipitates glycogen for some human blood group antigens.
1940	W C Boyd, R M Reguera and K O Renkonen.	Specificity of some lectins for some human blood group antigens.
1954	W C Boyd and E Shyleigh.	The name lectin proposed instead of hemagglutinin.
1960	P C Nowell.	Lectin from *Phseolus vulgaris* found to be mitogenic to resting lymphocytes.
1960	J C Aub.	Lectins preferentiallt agglutinate malignant cells.

1974	G Ashwell and A G Morell.	First mammalian lectin identified: hepatocyte asialoglycoprotein receptor specific for terminal galactose in serum glycoprotiens.
1976	Y Reisner.	Peanut agglutinin discriminates cortical from medullary cells in mice.
1977	Ofek *et al.*	Role of bacteria lectins in infection.
1980	Pusztai.	Interaction of *Phaseolus vulgaris* lectins with intestinal wall.
1981	Reisner *et al.*	Use of lectins in bone marrowt transplantation.
1984	Yajko *et al.*	Combined use of lectin and enzyme in clinical identification of micro-organisms.
1987	Harban-Mendoza *et al.*	Control of root-knot nematodes by lectins.
1988	De Oliveira *et al.*	Lectin and pancreas hyperthrophy.
1989	Diaz *et al.*	Root lectin as a specificity determinant in the *Rhizobium*-legume symbiosis.
1990	Yamauchi and Minamikawa.	*Con A* expression in *Escherichia coli* cells.

3. Detection and specificity

The simplest way to detect a lectin is to examine its ability to agglutinate erythrocytes or to precipitate glycoconjugates using the hemagglutination test. **(Figure 01A). (Goldstein *et al.*, 1980).**

Lectins are, in most cases, di- or multivalent and able to interact with carbohydrates or glycoproteins in solution or linked to cell membranes and their binding sites interact with cells forming various reversible linkages. Because of this ability, lectins are easily detected through agglutination assays**. (Santos *et al.*, 2014).**

For a better characterization of the lectin however, it is essential to determine whether it is specifically inhibited by mono- or oligosaccharides. This specificity is

usually determined by the hapten inhibition techniques, comparing the sugars on the basis of the minimum concentration to inhibit hemagglutination or precipitation reactions as shown in **Figure 01B**. (**Renato *et al*., 1991).**

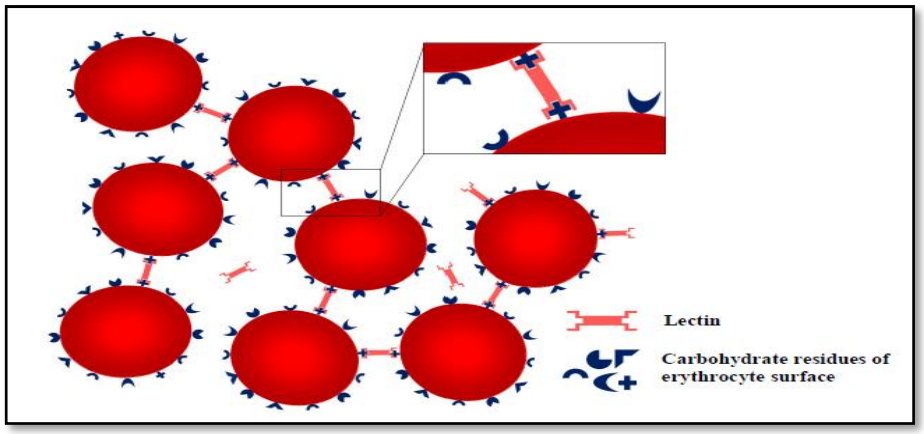


Figure 01A: Hemagglutination assay for lectin detection. **(Santos *et al*., 2014).**

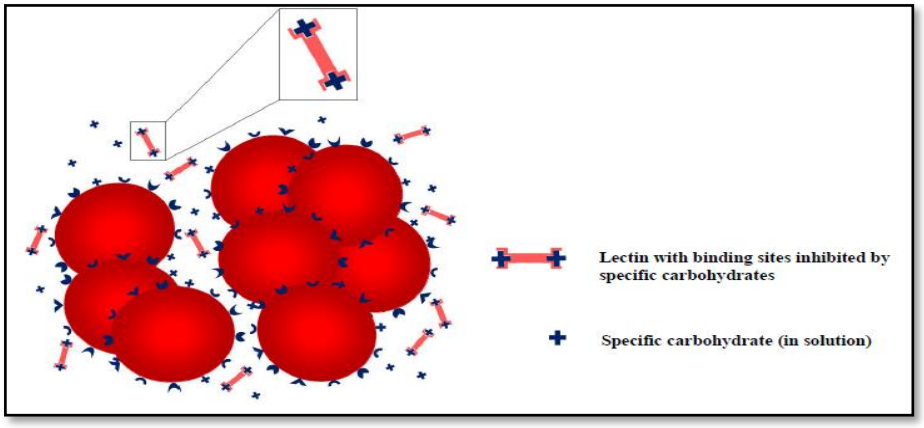


Figure 01B: Inhibition of hemagglutination to assure lectin presence and specificity with carbohydrate. **(Santos *et al*., 2014).**

Depending on carbohydrate specificity, major lectins are divided into mannose binding lectins, galactose/*N*-acetylgalactosamine binding lectins, *N*-acetylglucosamine binding lectins, *N*-acetylneuraminic acid binding lectins and fucose binding lectins as shown in **Figure 02.** (**Jasminka, 2015).**

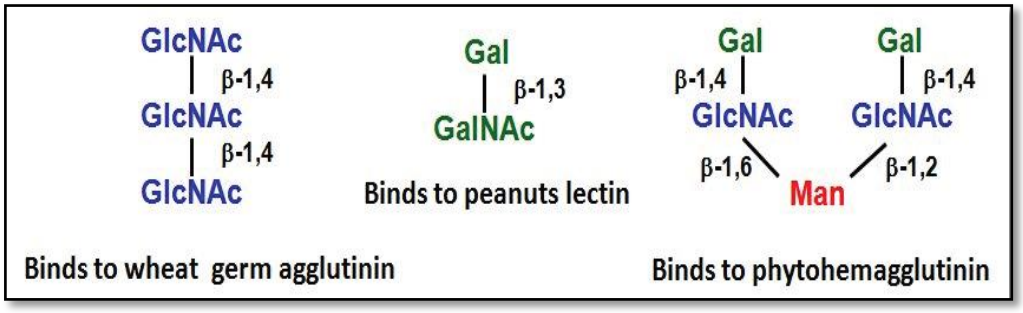


Figure 02: Binding Selectivities of Plant Lectins. The plant lectins wheat germ agglutinin **(WGA)**, peanut lectin **(PNA)** and phytohemagglutinin recognize different oligosaccharides. **(Jasminka, 2015).**

Table 02: Lectin specificity to monosaccharides**. (Sharon, 2007).**

Sugar	Lectins
Mannose **(Man)**	*Allium sativium ; Canavaliaensiformis ; Crocus sativus ; Diocleandiflora E.coli type 1 fimbriae ; ERGIC-53 . Galanthusnivalis ; MBLs of animals ; Pisumsativum.*
Fucose **(Fuc)**	*Aleuriaaurantia ; Anguilla Anguilla ; Lotus tetragonolobus ; Pseudomonas aeruginosalectin 2 ; Ulexeuropaeuslectin 1 ; Ulvalactuca ; Chromobateriumviolaceumlectin.*
Galactose **(Gal) /** *N*-acetylgalactosamine **(GalNAc)**	*Arachishypogaea ; Coprinuscinereus ; Entamoebahistolytica ; Erythina corallodendron ; Dolichosbiflorus ; Glycine max ; Griffoniasimplicfoglia lectin 1 ; helixpomatia ; HygrophorushypotheJus ; Phaseolus limensis*
N-acetylglucosamine **(GlcNAc)**	*Conglutinin ; Griffoniasimplicifoglialectin 2 ; Tachylectin-2 ; Triticum aestivum ; Ulexeuropaeuslectin 2; Psathyrellavelutina.*
N-acetylneuraminic **(NeuNAc)**	*Achatinafulica ; Cancer antennarius ; Hericiumarinaceum, Homarusamericanuslectin 1 ; Limaxflavus*

4. Structural characteristics

Specificity of lectins to carbohydrates, determined by three-dimensional structure of their binding sites, shows a conserved amino acid profile within families of lectins. **(Peumans and Van Damme., 1998).**

Lectins can have associated metal ions and interactions coordinated by water molecules and carbohydrates **(Sharon and Lis., 2002).** These proteins can present 2 to 12 sites of interaction, depending on the nature of the molecule and oligomerization state. **(Balzarini, 2006).**

Structural differences occur in lectins from the primary structure to the last degree of molecular organization; they may differ in amino acid sequence, change in the number of subunits, and in the nature of the polypeptides. Interactions between the subunits seem to play a dominant role in the stability of these proteins. **(Mitra *et al.*, 2002).**

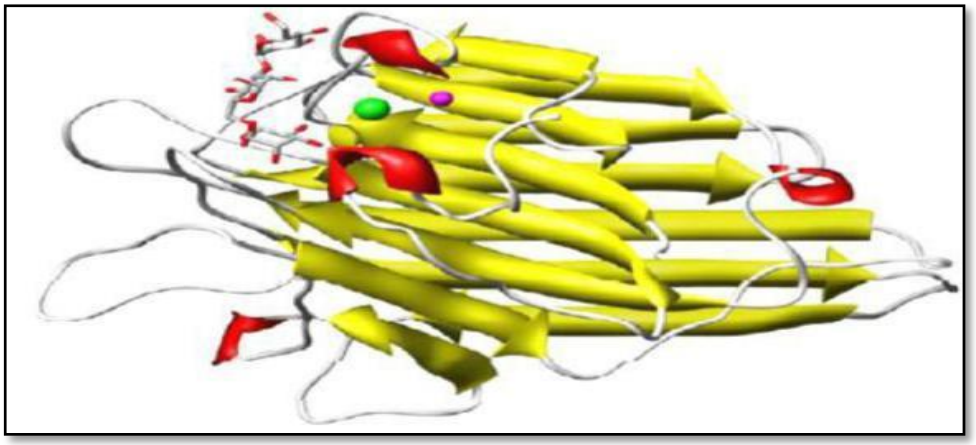


Figure 03: Graphic representation of a monomer of *Concanavaline A* from *Canavalia ensiformis* in complexe with the trimannosoide. **(Lenka, 2006).**

5. Production of lectins

Lectins are found in nature. A large number of lectins or hemagglutinins have been purified from different organisms.

a. Animal lectins

Lectins found in animals are most often found to aid in cell interactions. **(Berg *et al.*, 2002).** They seem to be involved in the mechanisms of endocytosis, intracellular translocation of glycoprotein, binding to glycoconjugates, apoptosis processes and

defense against microorganisms, regulating the processes of cell adhesion and migration as well as in binding of bacteria to epithelial cells.

In vertebrates, there are two classes of lectins, based on their location: **integral lectins** of membranes and **soluble lectins** present in intra and intercellular fluids (**Santos *et al*., 2014)**.

Gabius divided animal lectins into five main groups according to their structural characters. **(Gabius, 1997).**

Table 03: Animal lectins according to their structural characters. (**Gabius, 1997).**

Lectin	Characteristic
C-type lectin	➢ Depend on the presence of Calcium ions to bind carbohydrates. ➢ Conserved carbohydrate recognition domain **(CRD)**.
I-type lectins	➢ CRD similar to immunoglobulins.
Galectins/ S-type lectins	➢ Thiol dependent. ➢ CRD similar to B-galactosides.
Pentranxins	➢ Many subunits producing pentameric lectins.

b. Lectins from fungi, bacteria and viruses

Lectins in microorganisms appear to play several important roles, like the interaction of host cells, recognition in immunological processes, phagocytosis, and cell adhesion as shown in **Figure 04**. **(Ponchel and Irache., 1998).**

Fungal lectins, as in animals, are classified according to their structure, such as the lectin from *Coprinopsis cinerea,* related to galectins, a lectin class characterized by many conserved residues (**Wälti *et al*., 2008).**

Likewise, fungal lectins, as with plant lectins, can be classified according to their binding specificity: to mannose **(Francis *et al*., 2011),** arabinose **(Wang and Ng., 2005)**, acetyl-Dgalactosamine **(Chumkhunthod *et al*., 2006),** melibiose, xylose **(Zheng *et al*., 2007**), or lactose **(Liu *et al*., 2008).**

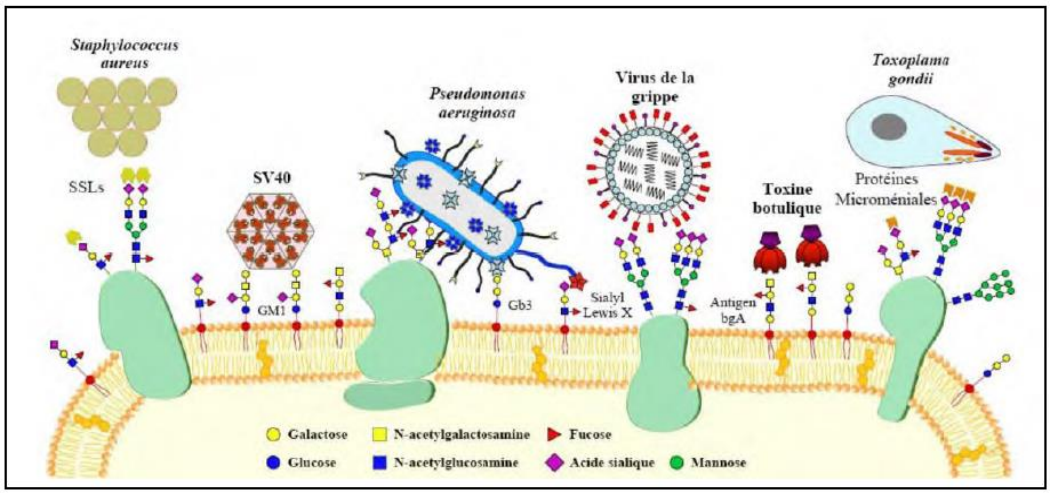


Figure 04: Roles of lectins in microorganisms. **(Imberty and Varrot, 2008).**

c. Plant lectins

Plants are a rich source of lectins and serve as the main source for isolation and analysis of these molecules. Lectins in plants play important roles such as acting as a reserve of nitrogen, as protein specific recognition factors and may also be involved in defense mechanisms against pathogenic microorganisms, insects and predators due to their toxicity. **(Ripoll *et al.*, 2003).**

Lectins are present in various plant tissues and have different biological properties.

Table 04: Biological activities of lectins from different plant tissues. **(Santos *et al.*, 2014).**

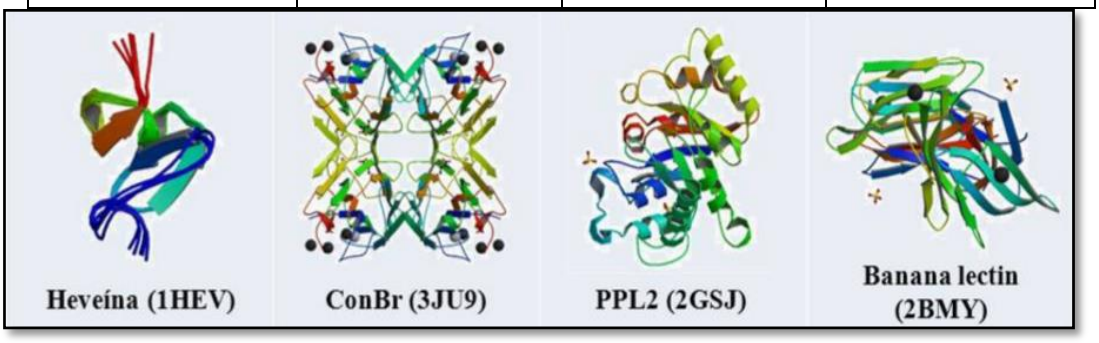
Tissues	Biological activities
Seeds	Anticoagulant and antiplatelet aggregating properties; coagulant, mitogenic, antibacterial, antifungal and antitumor activities.
Bark	Antifungal and insecticidal activities.
Heartwood	Termiticidal activity.
Stem	Antiviral and apoptosis-inducing activities.
Leaves	Antiviral, antibacterial and antifungal activities.
Fruits	Mitogenic, antiviral activities.

Roots	Antifungal and termiticidal activities.
Tubers	Insecticidal and antitumor activities.
Bulbs	Photolytic activity.
Rhizomes	Antiproliferative, immunostimulatory, antiviral, antifungal and antitumor and apoptosis-inducing activities.

There are four main, distinct classes of plant lectins based on their overall structure.

- **Merolectins**, with only one CRD, are small monovalent proteins with a single polypeptide and are incapable of precipitating glycoconjugates or agglutinating cells.
- **Hololectins** are lectins exclusively with CRDs, in which two or more of them are identical or very similar. This class includes all lectins that have multiple binding sites and are able to agglutinate cells or precipitate glycoconjugates.
- **Chimerolectins,** the result of protein fusion, contain one CRD and another unrelated domain acting independently. **(Kelany *et al.*, 2012).**
- **Superlectins** consist of molecules with two or more distinct CRDs. **(Van Damme *et al.*, 1998).**

Table 05: Structural classification of plant lectins. **(Van Damme *et al.*, 1998).**

Merolectins	Hololectins	Chimerolectins	Superlectins
			
Heveína (1HEV)	ConBr (3JU9)	PPL2 (2GSJ)	Banana lectin (2BMY)

Plant lectins can also be classified according to the specificity of their interactions with mannose/glucose **(Bari *et al.*, 2013)**, mannose/maltose, galactose/N-acetylgalactosamine, N-acetylglucosamine/ N-acetylglucosamine, galactose **(Wong *et al*, 2006),** mannose, fucose and sialic acid among others.

6. Biological properties and applications

Lectins stand out in biotechnology due to their many applications and other potential uses that are being evaluated daily and studied by researchers in lectinology. Lectins and their characteristic properties, mainly due to their ability to bind glycoconjugates, stand out as important tools in research covering various areas of science, especially in biochemistry, cellular and molecular biology, immunology, pharmacology, medicine and clinical analysis.

Lectins have a variety of effects on cells, such as agglutination, mitogenic stimulation, redistribution of cell surface components, modifying the activity of membrane enzymes, inhibition of bacterial and fungal growth, cell aggregation, toxicity, immunomodulation, among others. (**Santos *et al*., 2014).**

6.1. Lectins against microorganisms

Many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection **(Oppenheimer *et al*., 2008).** *Escherichia coli* (*E.coli*), for example, binds to host mannosides, while influenza virus binds to host sialic acids **(Mukhopadhyay *et al*., 2009).** Cytotoxic effects of lectins may be revealed by antitumoral and antiviral activities and also by deleterious effect on microorganisms **(Table 06)**. **(Rabia *et al*., 2013).**

Lectins, especially those with specificity to mannose or N-acetyl-glucosamine, have a remarkable anti-**HIV** (Human Immunodeficiency Virus) activity as shown in cell culture assays **(Molchanova *et al*., 2007).**

Table 06: Plant lectins with antimicrobial activity. **(Rabia *et al*., 2013).**

Plant (tissue)	Lectin specificity	Antimicrobial activity
Araucaria angustifolia (seed)	GlcNAc	*Clavibacter michiganensis, Xanthomonas axonopodis pv. Passiflorae.*
Talisia esculenta (seeds)	Man	*Colletotrichum lindemuthianum, F. oxysporum, S. cerevisiae*
Phaseolus coccineus (seeds)	Sialic acid	*Helminthosporium maydis, Gibberalla sanbinetti, R. solani,Sclerotinia sclerotiorum*

6.2. Lectins and insects

Lectins, beyond their effects on cells, also have the ability to act as insecticidal molecules against a variety of species. The mechanisms of insecticidal action of lectins are unknown, although entomotoxic activity seems to depend on the carbohydrate recognition property that they exhibit **(Paiva *et al*, 2012).** In general, lectins able to bind N-acetyl-D-glucosamine with affinity to chitin have insecticidal properties.

Arisaema helleborifolium lectin exhibited anti-insect activity towards the second instar larvae of B. cucurbitae **(Kaur *et al*., 2006).**

6.3. Lectins and cytochemistry/histochemistry

The glycan moieties covering cell surfaces are involved in many physiological and pathological processes related to cell. Disturbances in cell environment related to diseases frequently triggers changes in glycans such as fucosylation, abnormalities in glycan structures and uncommon glycans. **(Svarousky and Joshi., 2014).**

The ability of lectins to bind carbohydrates is useful in investigating the changes in the expression of glycans on cells in tissue markers. (**Roth., 2011).**

Generally, lectin histochemistry uses peroxidase-conjugated lectin followed by addition of diaminobenzidine and hydrogen peroxidase for visualization of binding.

An example is *Parkia pendula* lectin conjugated to horseradish peroxidase was evaluated as a histochemical marker for characterization of meningothelial tumor tissue. **(Beltrao *et al*., 2003).**

6.4. Lectins and anti-tumor activity

Tumour cell surfaces vary in composition of glycoconjugates in comparison to normal cells**. (Eckhardt and Goldstein.,1983).** Evidence is now emerging that lectins are dynamic contributors to tumor cell recognition (surface markers), cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, augmentation of host immune defense, cytotoxicity, and apoptosis. **(Hamid *et al*., 2013).**

(Liu *et al*., 2009) reported that *ConA* , a typical legume lectin with a mannose/glucose-binding specificity, was reported to induce apoptosis in murine macrophage PU5-1.8 cells through clustering of mitochondria and release of cytochrome *c*.

Brief review on ABO blood system

II. ABO SYSTEM

1. History

It was not until the year 1900, when **Karl Landsteiner** at the University of Vienna, discovered why some blood transfusions were successful while others could be deadly.

Landsteiner discovered the ABO blood group system by mixing the red cells and serum of each of his staff. He demonstrated that the serum of some people agglutinated the red cells of other. From these early experiments, he identified three types, called A, B and C (C was later to be re-named O for the German "Ohne", meaning "without", or "Zero", "null" in English). The fourth less frequent blood group AB was discovered a year later. **(Dariush *et al*., 2013).**

After this discovery, gradually from 1927, other blood groups were also discovered and reported including the Rhesus **(Rh)** blood group that was discovered by **Landsteiner** together with his American colleague **Alexander Wiener** in **1940, 1941.**

2. The ABO system

ABO remains the most important in transfusion and transplantation among all the blood group systems**. (Sano *et al*., 2012).** The blood type is defined by oligosaccharide structures, which are specific to the antigens, thus, blood group antigens are secondary gene products, while the primary gene products are various glycosyltransferase enzymes that attach the sugar molecules to the oligosaccharide chain. **(Ewald** and **Susan., 2016).** The gene that determines human ABO blood type is located on chromosome 9 (9q34.1) and is called ABO glycosyltransferase. **(Ranadhir *et al*., 2014).**

The system comprises complex carbohydrate structures that are biosynthesized by the A and B transferases encoded by the *A* and *B* genes, respectively**. (Schenkel, 2000).** He also recognized that the sera of individuals lacking one or both antigens usually contain anti-A and/or anti-B is agglutinins. In the course of further investigations many subgroups and phenotypes have been detected which are defined both by quantitative differences in antigen content as well as by a characteristic tissue distribution of the antigens. **(Brunner, 2000).**

Table 07: Antigens and antibodies of the ABO system. **(Bailly *et al*., 2015).**

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

Agglutination of red cells arises because one individual's serum contains naturally occurring antibodies against ABO antigens that are missing from that individual's own red cells, but which are present on the cells of another: e.g. group A individuals have Anti-B that agglutinates group B cells. **(Figure 05) (Anatole and Marcela., 1996).**

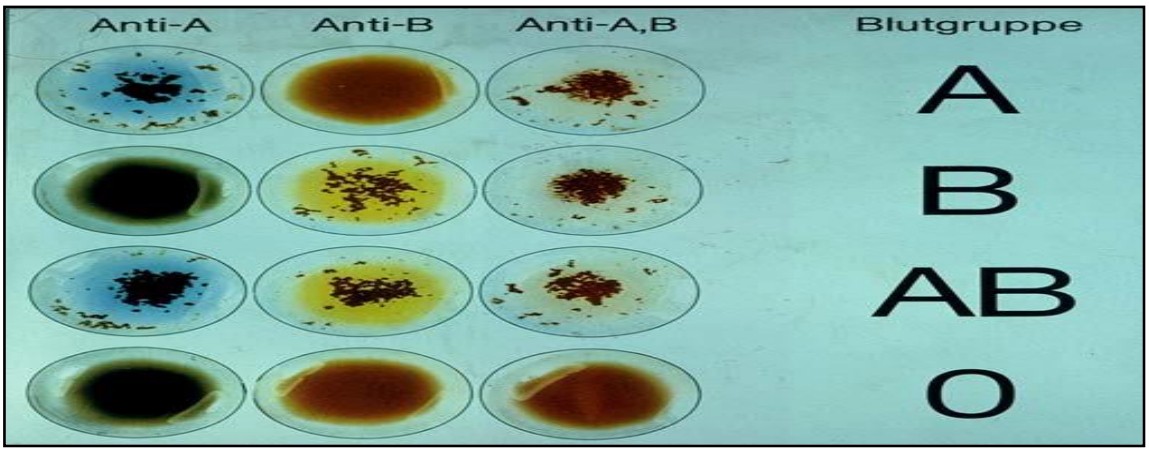


Figure 05: Agglutination tests of ABO blood group system. **(Stelling, 2008).**

3. The Rhesus system

Rhesus system is the second most important blood group system after ABO. **(Westhoff., 2004).** Red blood cell surface **(RBCs)** of an individual may or may not have an Rh factor or immunogenic D-antigen. Accordingly, the status is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). In contrast to the ABO system, anti-Rh antibodies are, normally, not present in the blood of

individuals with D-negative RBCs, unless the circulatory system of these individuals has been exposed to D-positive RBCs**. (Ranadhir *et al.*, 2015).**

4. Antigen Structures of blood groups of the ABO system

ABO blood types are determined by a cell surface marker that identifies this as belonging to self or to that individual. These cell surface markers are characterized by a protein or lipid that has an extension of a particular arrangement of sugar. **Figure 06** shows the arrangement of sugars that determine each of the A, B, and O blood types**. (Goldsby *et al.*, 2000).**

It should be noted that each is identical except that types A and B have an additional sugar; N-acetylgalactosamine for A, and Galactose for B. **(Daniel, 2008).**

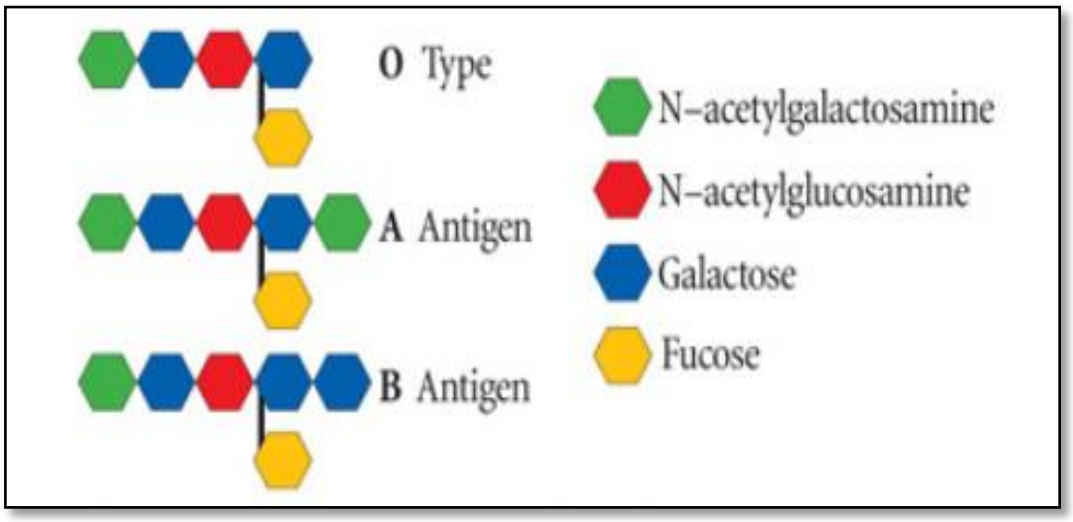


Figure 06: ABO antigen specificity. **(Daniel, 2008).**

5. Plant lectins and their specificity in ABO system

The presence of carbohydrates on the outer surface of the cell membranes of erythrocytes **(Figure 06)** facilitates the blood group detection by lectins specific to the carbohydrates.

Table 08: Plant lectins and their specificity in ABO system. **(Paolo and Richard., 1992).**

Lectin from (common name)	**Blood type**	**Monosaccharide recognized**

Griffonia simplicifolia ***(GS-I)***	A,B	α -D-Gal, α -D-GalNAc
Griffonia simplicifolia ***(GS-IB$_4$)***	B	α -D-Gal
Griffonia simplicifolia ***(GS-IA4)***	A	α -D-GalNAc
Helix pomatia ***(HPA)***	A	α -D-GalNAc
Ptilota plumose	B	α -D-Gal
Anguilla anguilla	O	α –L-Fuc
Ulex europaeus ***(UEA-I)***	O	α –L-Fuc
Laburnum alpinum ***(LA-I)***	O	(GlcNAc) 2-3
Laburnum alpinum ***(LA-II)***	O	Gal

Brief review on Moringa oleifera

III. *MORINGA OLEIFERA*

1. Generalities

Moringa oleifera Lam. (Moringaceae) is a medium-sized tree autochthonous from northeastern India and widely distributed worldwide throughout the tropics and subtropics **(Teixeira *et al.*, 2012).** The distribution of *M. oleifera* in the world is outlined in **Figure 07**.

It is recognized as a vibrant and affordable source of phytochemicals, having potential applications in medicines, functional food preparations, water purification, and biodiesel production. **(Ramesh *et al.*, 2016).**

Due to its rich source of certain macro and micro nutrients which are of great importance, *Moringa oleifera* is referred to as a **'Miracle tree'. (Adewumi and Samson., 2016).**

It is in fact said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach **(Lakshmipriya, 2016).**

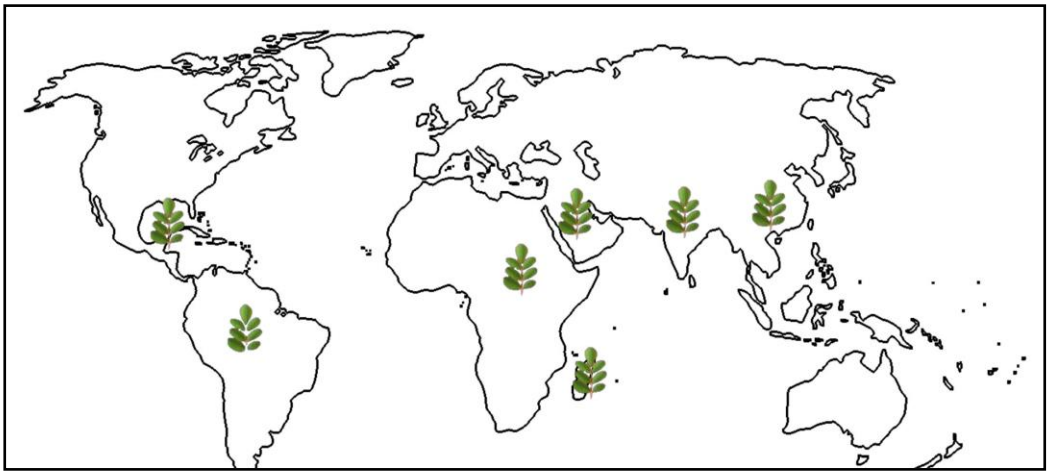


Figure 07: The distribution of *Moringa oleifera* in the World.
(www.outline-world-map.com).

2. Description of *Moringa oleifera* plant

Moringa oleifera Lam is a tropical deciduous perennial fast growing plant dicotyledonous tree which can reach 7-12 m of height, sometimes even 15 m **(Larissa *et al.*, 2013).**

The stem is brittle with a corky, whitish-gray bark, with drooping branches, pale green and bipinnate or more commonly tripinnate leaves (30–60 cm long) with opposite, ovate leaflets **(Pandey *et al.*, 2011).**

The *Moringa* tree grows best in the temperature range of 25–35°C, under direct sunlight, at an altitude of 500 m, and in slightly acidic to alkaline soil (pH 5.0–9.0); although it can tolerate excess temperature, up to 48°C, frost in winter, altitude, and a wide variety of soil conditions. **(Ramesh *et al*, 2016).**

Photo 01: A tree of *Moringa oleifera.* **(Alessandro *et al.*, 2015).**

Photo 02: Aspects of *Moringa Oleifera*, A: tree, B: seedpods, C: flowers, D: seeds. **(Lakshmipriya, 2016).**

3. Taxonomic classification

Table 09: Classification of *Moringa oleifera.* **(Garima *et al.*, 2011).**

Kingdom	**Plantae**
Sub kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	*Moringa*
Species	*Oleifera*

4. Properties of *Moringa oleifera*

Every part of the plant (leaves, seeds, roots, flowers, bark, fruits and pods) is a rich storehouse of a number of nutrients such as proteins, fibre and minerals that play an important role in nutrition. (**Lakshmipriya *et al.*, 2016).**

Several extractions also tested positive for the presence of phytochemicals like unique glucosinates, flavonoids, and phenolic acids, carotenoids, tocopherols and polyunsaturated fatty acids.

These phytochemicals of *M. oleifera* have shown antidyslipidemic, anthelmintic, antihyperglycemic, anti-inflammatory, antimicrobial, antioxidant, antiproliferative, anti-ulcer, antiurolithiatic, and hepatoprotective properties. **(Garima *et al.*, 2011).**

Table 10: Bioactive compounds in *M. oleifera* seeds.

Compounds	**References**
Alkaloids	
Moringine	**(Kirtikar *et al.*, 1975).**
Flavonoids	
Catechin	**(Govardhan *et al.*, 2013).**
Epicatechin	**(Govardhan *et al.*, 2013).**
Quercetin	**(Govardhan *et al.*, 2013).**
Kaempferol	**(Singh *et al.*, 2009).**
Phenolic acids	
Gallic acid	**(Singh *et al.*, 2009, Govardhan *et al.*, 2013).**
P-Coumaric acid	**(Govardhan *et al.*, 2013).**
Ferulic acid	**(Govardhan *et al.*, 2013).**
Caffeic acid	**(Govardhan *et al.*, 2013).**
Protocatechuic acid	**(Govardhan *et al.*, 2013).**
Cinnamic acid	**(Govardhan *et al.*, 2013).**
Ellagic acid	**(Singh *et al.*, 2009).**
Glycosides	
4-(2/3/40-O-acetyl-α-L-rhamnopyranosyloxy)benzyl glucosinolate	**(Maldini *et al.*, 2014).**

Shelled *M. oleifera* seeds have the property to decontaminate arsenic from water and can be used as domestic and environment-friendly safe technology **(Kumari *et al.*, 2006)** due to their coagulant properties.

Two lectins deemed coagulant *M. oleifera* lectin **(cMoL)** and water-soluble *M. oleifera* lectin **(WSMoL)** are among the coagulant proteins present in the seeds **(Santos *et al.*, 2009; Ferreira *et al.*, 2011).**

M. oleifera seeds contain 33–41 % (w/w) oil, known as ‘‘ben oil’’, because of the contents of behenic acid (C22, docosanoic acid, &7 % w/w), which possesses significant resistance to oxidative degradation **(Rashid *et al.*, 2008).**

Material and Methods

MATERIALS AND METHODS

1. Plant material

Mature *M. oleifera* (Family: Moringaceae) seeds were brought from the gardener of Algeria. We used the seeds to extract lectins.

Photo 03: *Moringa oleifera* seeds.

2. Methods

A. Preparation of *M. oleifera*

- **Drying***:* The seeds of *Moringa oleifera* were dried at room temperature for 7 days.
- **Grinding:** The dried seeds were then ground using a mortar and pestle till a powder was obtained.

Photo 04*:* The powder obtained from *M.oleifera* seeds.

B. Extraction of lectin from *M. oleifera*

Moringa oleifera seed powder was treated with phosphate buffered saline (PBS, 10mM, pH 7.4) at a ratio of 1:10(w/v). The extraction of proteins was continued by stirring the mixture for 24 hours under refrigeration.

The extract was filtered, using filter paper, then centrifuged at 6300xg for 30 minutes. The residue was disposed of while the supernatant was recovered and used as the crude lectin source.

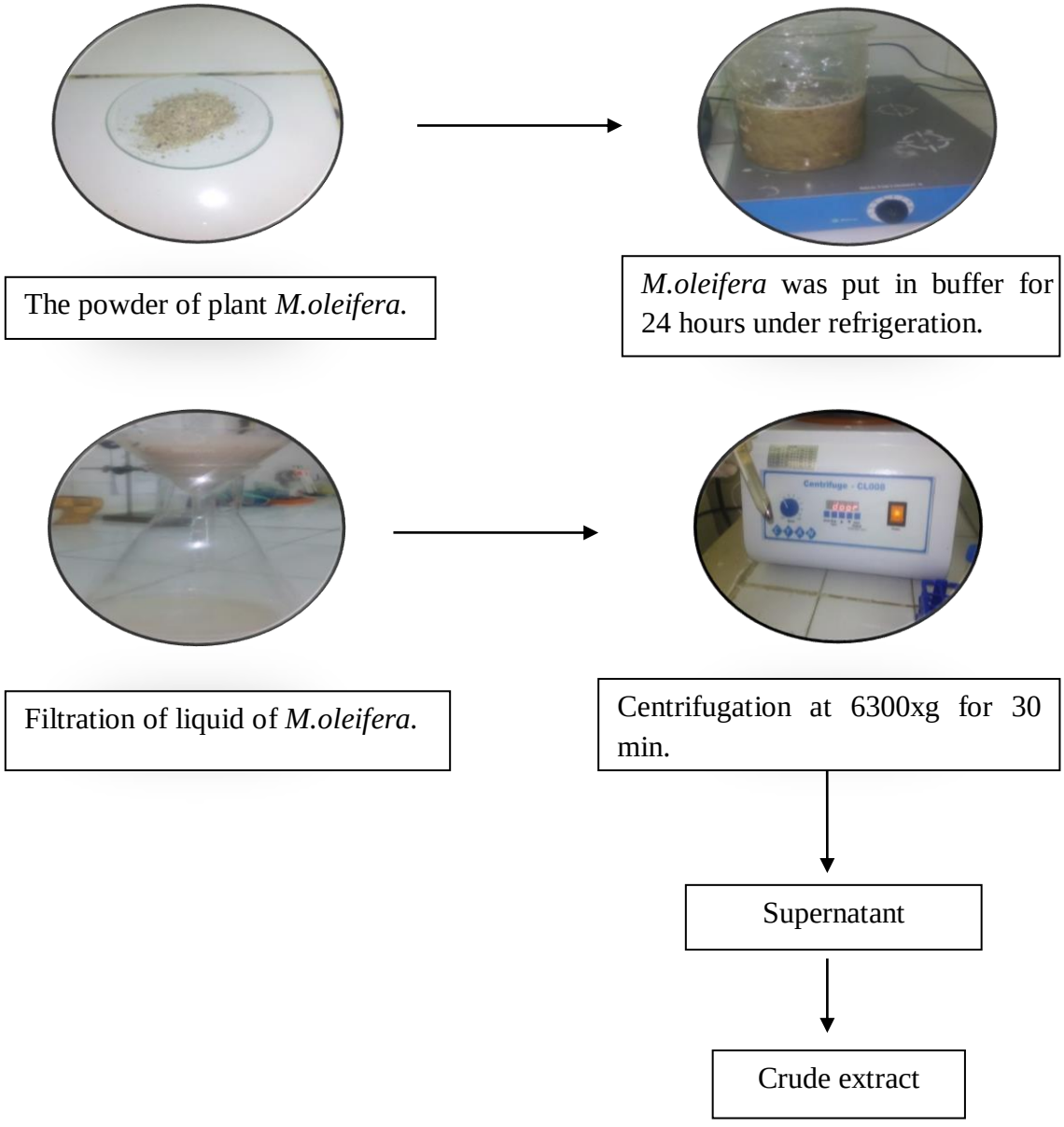


Figure 08: Diagram showing the extraction procedures of lectins from *Moringa oleifera* seeds.

C. Preparation and treatment of rabbit erythrocytes

This was carried out according to **Kumar and Rao., 1986**. Rabbit blood was obtained from the laboratory-animal facility at the University of the Mentouri Brothers, Constantine 1. It was collected in a 4 ml heparin tube. The mixture was centrifuged at 3000xg for 10 min at 4°C, and the residue obtained is washed 3 times with NaCl solution 0.9%, followed by a centrifugation after each washing.

This precipitate was treated by dissolving it in a solution of 1% glutaraldehyde, centrifuged and washed two times.

D. Testing for Agglutinating activity

Hemagglutinating activity **(HA)** was assessed in microtiter plates according to **(Correia and Coelho., 1995)**. 50µl of the extract is added to 50µl of glutaraldehyde treated rabbit blood. The plate was allowed to stand for 30 minutes and was observed for agglutinating activity with naked eye as a gelatinous form.

- A control test was performed by adding 50µl of erythrocytes to 50µl of PBS.

E. Limit of the agglutinating activity

This test was performed to determine the highest dilution that causes agglutination, hence the agglutinating strength of the lectin. . HA, the reciprocal of the highest dilution of the sample promoting full agglutination of erythrocytes, was defined as one hemagglutination unit **(Chumkhunthod *et al*., 2006).**

It was performed according to the method of serial double dilution described by **Eretan O., 2004.**

50µl of PBS was put in each well of one horizontal line of the microtitre plate, then 50µl of the crude extract was added to the first well, and was serially diluted two-fold along the line. 50µl of fixed erythrocytes was added to each well. The agglutination activity was observed after incubation of the plate for 1 hour at room temperature.

1. Further Purification

a. Salting-out by Ammonium sulfate

Protein solubility is affected by ions. At a very high ionic strength, protein solubility decreases as ionic strength increases in the process known as 'salting-out'. **(Duong and Gabelli., 2014).**

The crude extract was precipitated using ammonium sulfate. The amount of $(NH4)_2SO_4$ to be added was based on the monogram table by **Cooper (1977)**. Ammonium sulfate was added to the crude PBS extract in two fractions 0-50%, and 50-80% over a 30min period with constant stirring at 4°C. The precipitate was separated by centrifugation at 8000xg for 20 min at 4°C and was kept at 0°C while the supernatant was further saturated with $(NH4)_2SO_4$ to completely precipitate any remaining protein in solution.

Likewise, the precipitate was collected by refrigerated centrifugation. It was dissolved in minimum amount of PBS (pH 7.4).

- The precipitates are then tested for agglutinating activity to obtain the fraction with the maximum protein precipitate.
- The same procedure as above is followed to test the limit of the agglutinating activity, only changing the crude PBS extract with the precipitate.

b. Dialysis

This step is done to permit desalting of the precipitate. The precipitated protein (0-50%), the most active fraction, is submitted to dialysis (membrane of exclusion limit 12kDa) against 1litre of PBS (0.01M, pH 7.4). The reaction takes place over night under agitation at 4°C.

c. Gel filtration chromatography

The fraction after dialysis was subjected to molecular sieving through the equilibrated Sephadex G-50 column eluted with PBS (pH 7.4). 5mL of the sample was loaded to the column and a 2 mL fraction of the eluted sample was collected at a flow rate of 0.1ml/sec by gravity. The eluted fractions were monitored for spectrophotometric absorbance at 280nm.

The fractions that showed a peak in the optic density were subjected to an agglutination test.

2. Protein determination

The protein contents of content of the lectin samples obtained during the purification process was determined according to the method of Bradford et al (1951) using bovine serum albumin as standard.

A volume range of the standard (0, 10, 20, 30, 40, 50, 60, 70, 80, 100 µl) is prepared in glass test tubes from a solution of BSA 1mg/ml, each with three repetitions. These volumes are then adjusted to 100µl with distilled water.

100µl of PBS, the extract and of each of the fractions are also prepared. 4ml of the Bradford solution is added to each of the test tubes, and left for 10 minutes for reaction. The absorbance of each is measured at 595nM against the empty solution.

3. Characterization of the lectin

a) Inhibition test

This test permits us to know the potential ligands (sugars and glycoproteins) of the lectin. The active fraction (0-50%) is diluted with PBS, pH 7, 4. (To 100µl of the fraction is added 3100µl of the buffer.)

Different carbohydrates and glycoproteins were prepared with a concentration of 400mM with PBS.

25µl of PBS was deposited in each well. 25µl of the ligand was added to the first well, and then a series of double dilutions in the next 2 wells. 25µl of the diluted active fraction was added to each well and incubated for 30 minutes at room temperature.

After incubation, 50µl of rabbit erythrocytes are added to the wells and incubated for 1hour at room temperature and the agglutinating activity was observed with a naked eye.

b) Effect of temperature on the stability of the protein

The crude lectin extract was subjected to various temperatures, 20°C, 30°C, 40°C, 50°C, 60°C and 70°C, 80°C, 90°C, 100°C for 30 min.

2ml of the extract was incubated at various temperatures, 30°c, 40°c, 50°c, 60°c, 70°c, 80°c, 90°c and 100°c in a water bath for 30 min, and then cooled at room temperature.

- The heat-treated lectin was tested for agglutination using treated rabbit blood.

Photo 05: The crude lectin extract at different temperature.

c) Effect of pH on the agglutinating activity

The effect of pH on the agglutinating activity of the dialyzed fraction (30-50%) is carried out on a range pH 2-10. **(2, 3, 4, 5, 6, 8, 10)**.

2ml of the fraction is placed in a dialysis membrane and submerged in a beaker containing the buffer solution of the specific pH medium. The reaction takes place under agitation, at 4°C for 24 hours. It is then neutralized in 300ml of PBS, (10mM, pH 7.4) for 4 hours at 4°C.

The limit of the agglutination is then tested as in the previous tests.

d) The agglutination test with ABO system

The test was carried out to deduce the specificity of the blood group extract; it was carried out on the human red blood cell antigens belonging to the ABO blood group system using the erythrocytes of the different blood group (A, B and O).

e) Effect of EDTA on the agglutinating activity

50µl of EDTA at different concentrations (20mM, 30mM, 40mM, and 50mM) was added to 50µl of the 0-50% fraction and incubated for one hour to allow reaction to take place.

The four mixtures were tested for agglutinating activity, (50µl of PBS in all wells, 50µl of the mixture in the first well, then a series of double dilution in the rest of the wells, and then 50µl of glutaraldehyde treated rabbit erythrocytes.

Two control tests were performed, a positive control test, where the agglutinating activity is tested using the fraction without EDTA and a negative control test in which we do not add any lectin containing fraction.

Results and Discussion

RESULTS AND DISCUSSION

1. Agglutination test of the crude extract

Agglutination is defined as the formation of clumps of cells or particles and lectins have the ability to agglutinate erythrocytes.

Most lectins are able to interact with red blood cells via to the interaction between membrane carbohydrates. If the erythrocyte was placed in a well, the natural sedimentation leads to a deposit of the red cells at the bottom of the well of the microtiter plate. The addition of the crude extract allows the formation of a network between erythrocytes and lectins. These interactions form a homogeneous gelatinous suspension; this corresponds to the phenomenon of agglutination.

Table 11: Agglutination of the crude extract of *Moringa oleifera* seeds.

Plant	**Agglutination test**
Moringa oleifera (seeds)	+++

+++: Presence of agglutination.

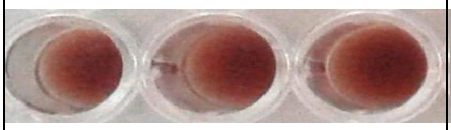
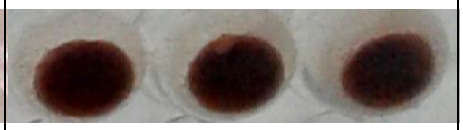
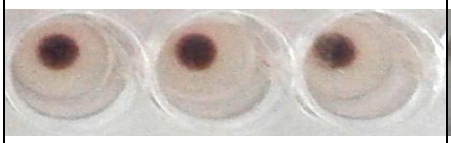
Test	Front view	Back view
Agglutination with *MO* extract		
Control test		

Photo 06: Agglutination test of crude lectin source.

✓ A positive result is observed as a layer covering the bottom of the well of the microtiter plate, while a negative result is indicated by precipitation of the erythrocytes at the bottom of the wells and a red point is observed.

✓ This confirms the presence of lectins in the saline crude extract and their ability to agglutinate glutaraldehyde-treated rabbit erythrocytes.

✓ **Lubag (1988)** and **Pahm (1990)** previously reported the presence of agglutination factors in the seeds.

2. Limit of agglutination test

Obtaining the positive result was a go-ahead to test for the limit of this positive agglutination test. Agglutination activity is expressed as the highest dilution ratio for which agglutination was observed.

Table 12: limit agglutination of extract *M.oleifera* seeds.

Titer value / Extract	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
M.oleifera	+++	+++	+++	+++	+++	++	++	+	-	-	-	-

+++: Very strong agglutination, ++: Strong agglutination, +: Low agglutination, - : absence of agglutination.

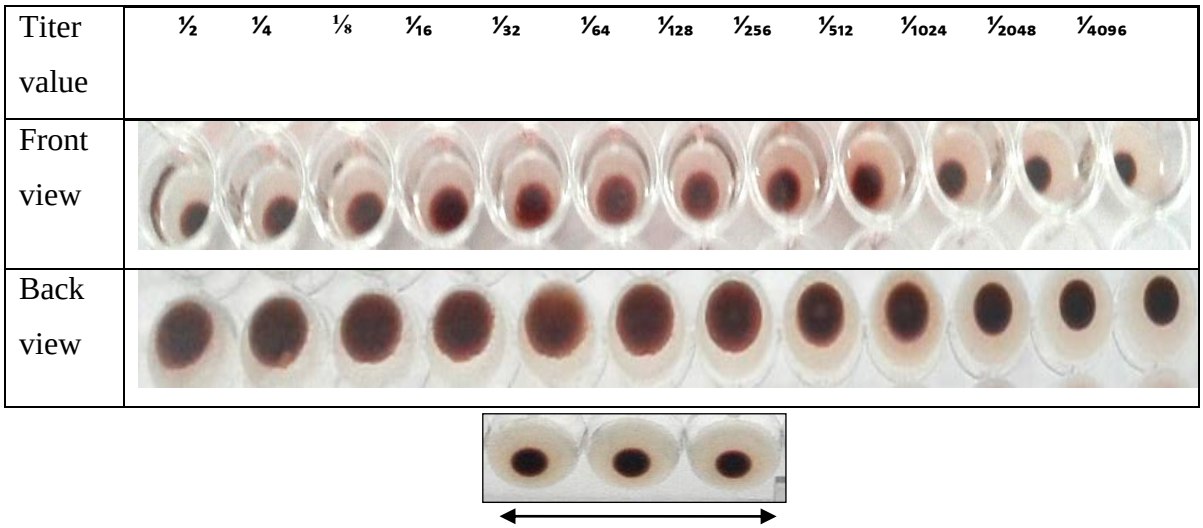


Photo 07: Limit of the agglutination test of the crude extract.

✓ These results indicated the diminution of the agglutination activity with increased dilution along the microtiter plate and agglutination stops at the titer value of 1/128.

3. Purification of the crude lectin extract

a) Salting-out

Lectins were separated from other substances co-extracted by PBS in the crude extract by precipitating it with ammonium sulfate. Ammonium sulfate was chosen as precipitating agent in this study due to its high solubility in water and produces high ionic strength. Increase in ionic strength decreases the protein solubility. **(Scopes, 1994).**

The crude extract was subjected to different levels of $(NH_4)_2SO_4$ to be partially purified. Agglutination was observed by the two dissolved precipitates collected at 0-50% and 50-80% saturation.

The photo below show that the 0-50% $(NH_4)_2SO_4$ precipitate exhibited much more agglutination even at the highest dilution more than the 50-80% $(NH_4)_2SO_4$ precipitate.

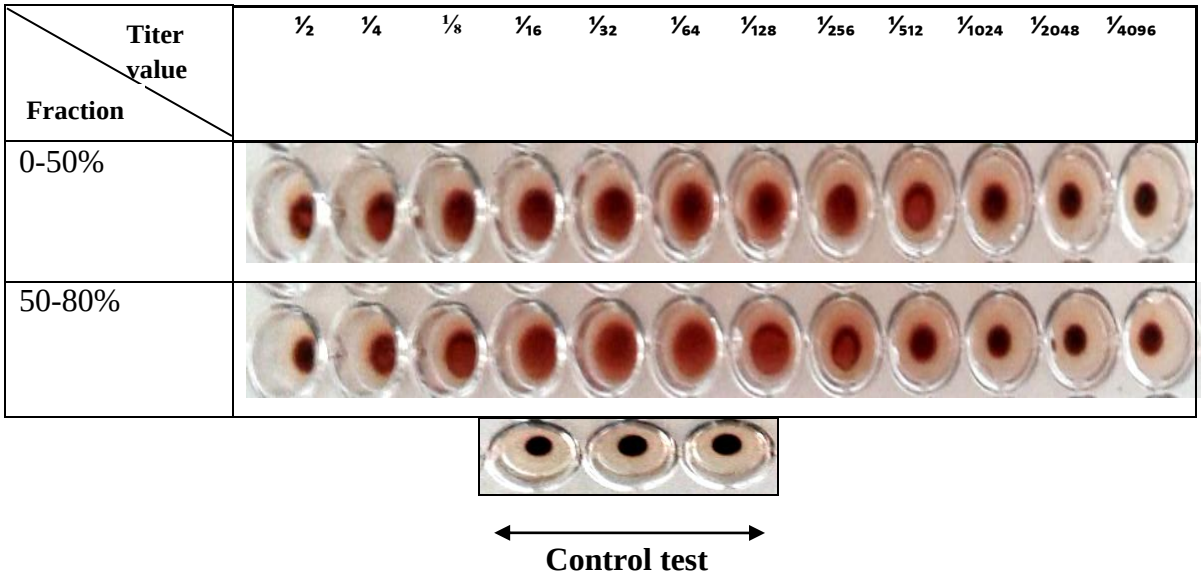


Photo 08: Limit of the agglutination test of extract *M.oleifera* seeds after precipitation.

✓ The two fractions indicated no agglutination in the ½ titer. This is due to the high concentration of the lectins which instead of agglutination causes the rupture of the erythrocytes by osmosis.

✓ In the 0-50% fraction, agglutination is observed up to the $1/_{512}$ dilution while in the 50-80% fraction, the agglutination stops at the $1/_{256}$ dilution titer.

Conclusion: The 0-50% fraction contains the highest concentration of the protein hence the most active to use for the rest of the tests.

b) Gel Chromatography

Gel filtration using Sephadex G-50 can resolve proteins with molecular weights between 1.5 to 30KDa. **(Ciaran *et al*., 2011).**

The figure below shows the elution profile of the dialysed 0-50% ammonium sulfate fraction. Two peaks were visible after reading the absorbance of the 55 fractions collected (2ml per tube) and the fractions **20**, **21**, **22**, **29** and **30** which fall on the first and second peak showed agglutinating activity.

The seeds of Moringa oleifera may be containing two different lectins.

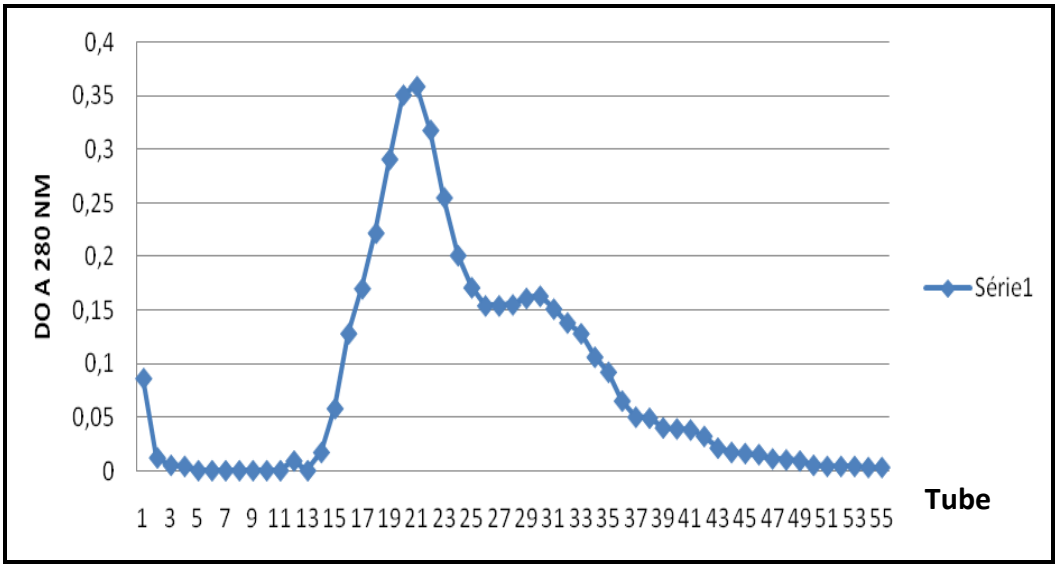


Figure 09: Elution profile of the 0-50% ammonium sulfate precipitate on Sephadex G-50 column.

- **Agglutination activity of purified fractions**

When tested for the agglutinating activity we find;

✓ The first peak **(F20**, **21** and **22)** exhibited the maximum lectin activity.

✓ While the second peak **(F29**, **30**, **31** and **32)** showed a decreased lectin activity.

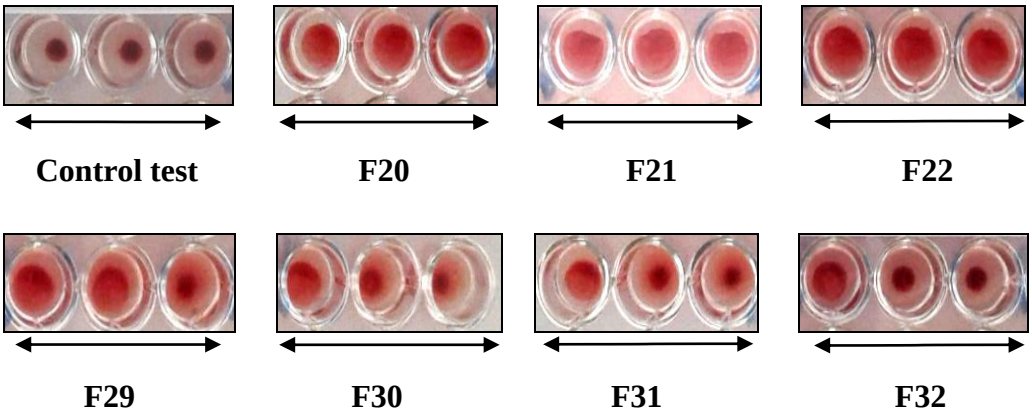


Photo 09: The agglutination test of different fractions.

The fractions with the highest agglutinating activity **(20**, **21**, **22**, **29**, and **30)** were subjected to an agglutination test limit and the results are shown below;

Titer value / Fraction	½	¼	⅛	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
20												
21												
22												
29												
30												

Photo 10: Limit of the agglutination test of different fraction 20, 21, 22, 29, 30.

✓ **F20** showed the highest agglutinating activity up to a dilution of **1/64**, followed by **F21** and **F22** both having an agglutination activity up to the **1/32** dilution well, and then **F29** and **F30** which only showed an agglutinating activity up to **¼**.

4. Protein determination

Basing on the **Bradford** protein assay standard curve produced using BSA and its linear regression equation; we were able to determine the quantity of proteins in each fraction (the crude extract, the 0-50% fraction and the fraction after chromatography).

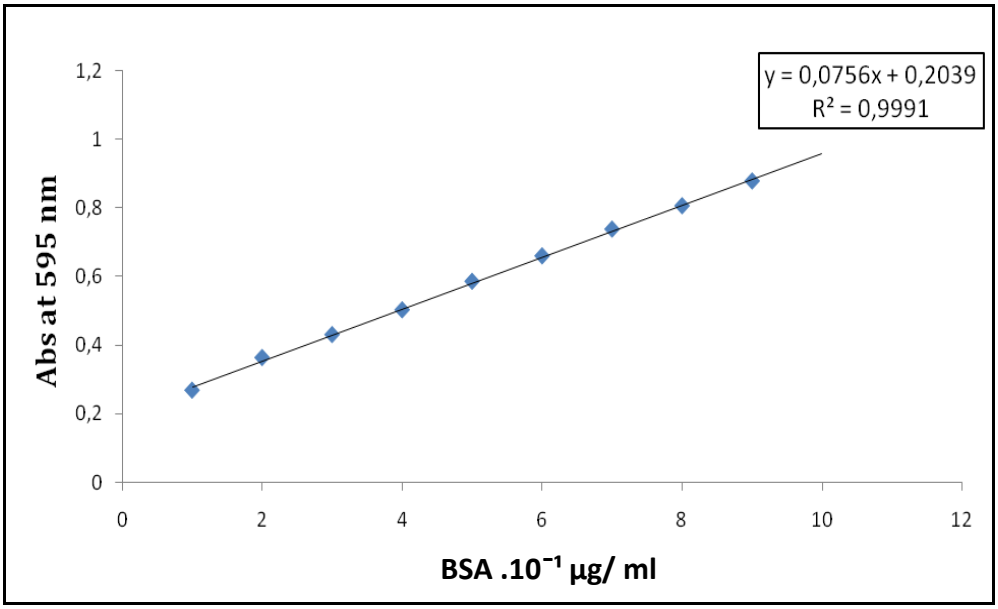


Figure 10: Bradford assay standard curve.

Table 13: Protein content of different fractions of *Moringa oleifera* seeds.

Fraction	Absorbance	Protein content (µg/ml) x10
Crude extract	0.787	77.87
0-50% fraction	0.827	83.2
Fraction after chromatography	0.529	43.47

Table 14: Purification profile of *Moringa oleifera* seed lection.

Fraction	**Total volume** (ml)	**Total protein** (mg)	**Total activity** (HU)	**SHA** (HU/mg)	**Purification fold**	**Yield %**
Crude extract[1]	500	38.935	128	3.288	1	100
0-50% $(NH_4)_2SO_4$ fraction	50	4.16	512	123.08	37.433	400
G-50 Gel filtration	4	0.1739	64	368.028	111.931	50

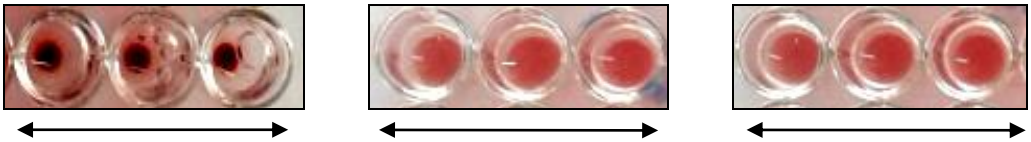
[1], 50g of seed powder were used, HU is the minimal concentration of protein required to cause visible agglutination of a 4% glutaldehyde treated rabbit erythrocytes.

✓ The protein was purified by 111.931 fold and with 50% yield.

5. Characterisation of the lectin

a. The inhibition test

The inhibition test was performed with certain saccharides to determine the specificity of the carbohydrate extracts. Inhibition would occur if the sugar-lectin interaction is stronger than the erythrocyte-lectin attraction and would consequently form a distinct point of the erythrocyte at the bottom of the well.



Fetuin | Other Sugars | **Control test**

Photo 11: Inhibition test.

✓ The agglutinating activity of the lectin was only affected by fetuin, a type O glycoprotein while the other sugars showed no inhibition effect and showed the same result as the control test in which no ligand was added.

✓ The non-inhibition of agglutination by the simple sugar molecules may be due to the fact that the lectin binding sites are non-specific for the sugar anomers. **(Lacsamana and Merca., 1994).**

Table 15: Inhibition test of *Moringa oleifera* seed lectin.

Sugar	**Agglutinating activity**	**Sugar**	**Agglutinating activity**	**Sugar**	**Agglutinating activity**
Glucose	-	Ovalbumie	-	Glucosamine HCl	-
Mannitol	-	Rhamnose	-	Saccharose	-
Fructose	-	Maltose	-	Mucine	ND
Galactose	-	D-Sorbitol	-	Arabinose	-
Xylitol	-	Fetuin	+	Fucopyrannoside	-
Fucose	-	Inuline	-	Mannose	-

-: presence of agglutinating activity, +: inhibition of the agglutinating activity, ND: No defined.

b. Inhibition limit of Fetuin

The minimuim concentration of fetuin causes inhibition of agglutination of lectins from *Moringa oleifera*, is shown in the following table:

Table 16: Inhibition limit agglutination of fetuin with extracts *M.oleifera* seeds.

Titer value / Fraction	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
Fetuin	+++	+++	++	+	-	-	-	-	-	-	-	-

+: inhibition of agglutinating activity, - : presence of the agglutinating activity

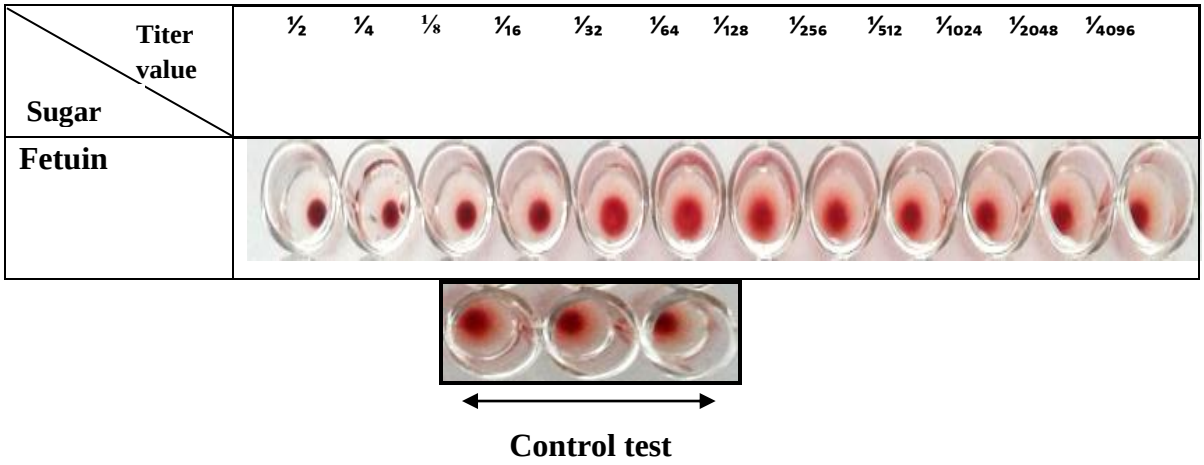


Photo 12: Inhibition limit test of fetuin with *M.oleifera*.

✓ The inhibition limit test of fetuin showed that it inhibits the hemagglutinating activity of the lectin up to the

✓ The inhition of agglutination by fetuin shows the similarity between the lectin in this study and the *Moringa* lectin **(MoL)** isolated by **Katre *et al*, 2008**.

✓ The non-inhibition of agglutination by fructose differeciates the lectin in this study from the Water-soluble *Moringa* lectin which is specific to fructose. **(Ferreira *et al*., 2011).**

c. Effect of temperature

The stability of the lectins with heat was investigated by subjecting it to different temperatures for a period of 30 minutes. Heat treatment of the lectin in this study revealed that it is a thermo stable protein.

High temperature is a powerful denaturating agent leading to protein unfolding through breaking of hydrogen bonds that maintain protein structure. **(Daggett and Levitt., 1992).**

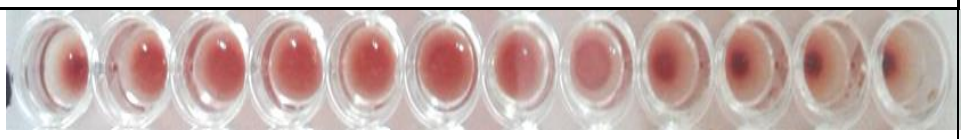
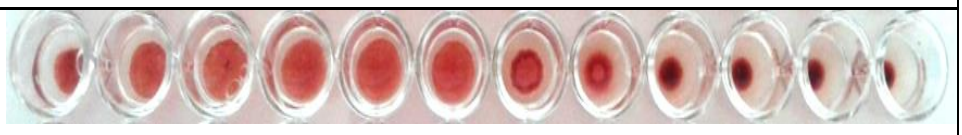
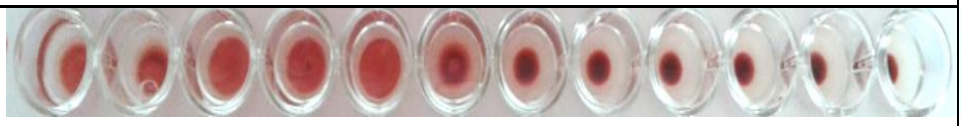
Thermostability is a common feature of protein from MO seeds. MOCP isolated by **(Gassenschmidt *et al*., 1995)** remained active after 5h heat treatment at 95°C.

Table 17: Effect of temperature on the agglutination test of *Moringa oleifera*.

T°C	30	40	50	60	70	80	90	100
HU	+++	+++	++	+	++	+	+	+

+++: very high agglutinating activity, ++: high agglutinating activity, +: low agglutinating activity,

HU: hemagglutinating unit.

Titer value / **T°C**	½ ¼ ⅛ 1/16 1/32 1/64 1/128 1/256 1/512 1/1024 1/2048 1/4096
30	
40	
50	
60	
70	

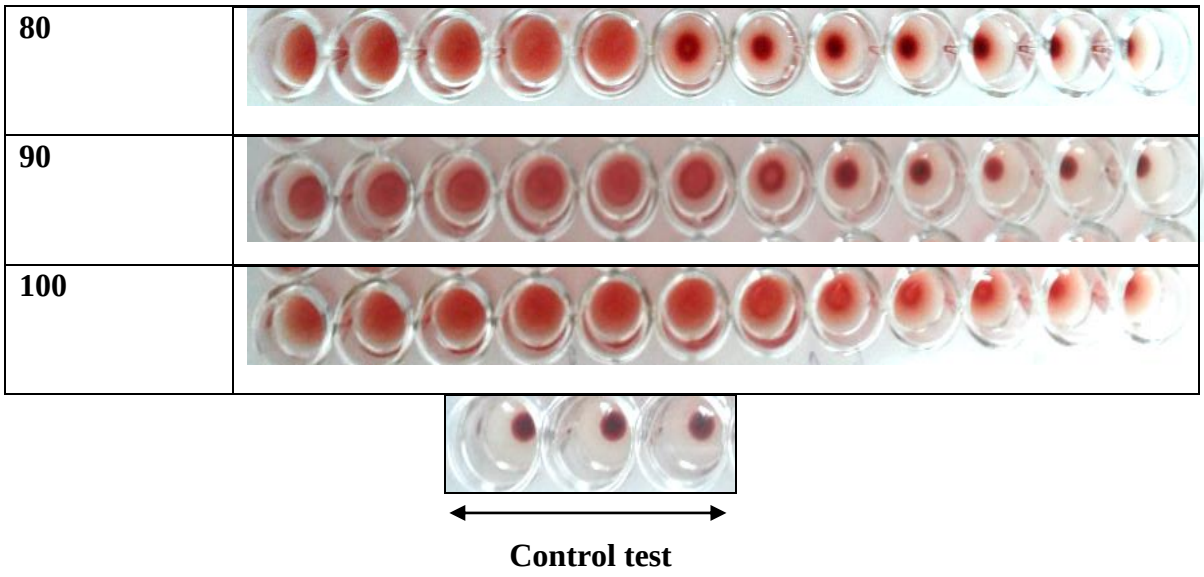


Photo 13: Effect of temperature on the agglutination activity of *Moringa oleifera*.

✓ The highest agglutinating activity of the lectin is observed at 30 and 40°C, and an increase in the temperature relatively decreased the activity, but didn't completely eliminate it. No significant agglutinating activity difference was observed at temperatures higher than 40°C.

✓ Thermal stability of the lectin isolate indicates that the extraction of lectin from Moringa Oleifera seeds can be done at room temperature.

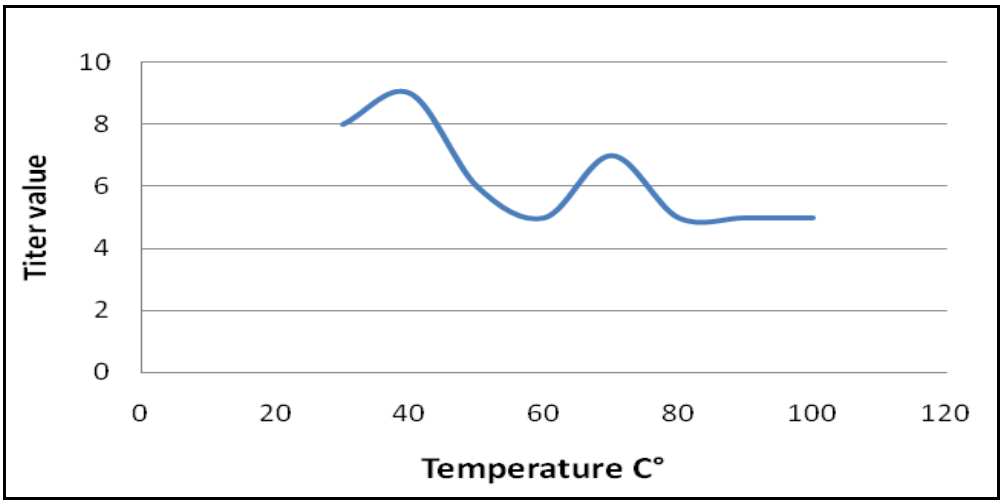


Figure 11: Effect of temperature on the agglutinating activity.

✓ From the figure above, we can deduce that our *Moringa oleifera* seeds contain two lectins which have a high activity at 40°C and 70°C as shown by the peaks in the curve.

d. Effect of pH on the agglutinating activity

The lectin shows remarkable pH stability, its activity being unaffected throughout the entire range of pH from 3 to 10.This is in contrast to lectin from *Parkia javanica* beans which is stable in pH 7–10. **(Utarabhand and Akkayanont., 1995).**

Table 18: Effect of pH on the agglutination test of *Moringa oleifera.*

PH	2	3	4	5	6	8	10
HU	+	+++	+	++	+++	+	+

+++: very high agglutinating activity, ++: high agglutinating activity, +: low agglutinating activity, HU: hemagglutinating unit.

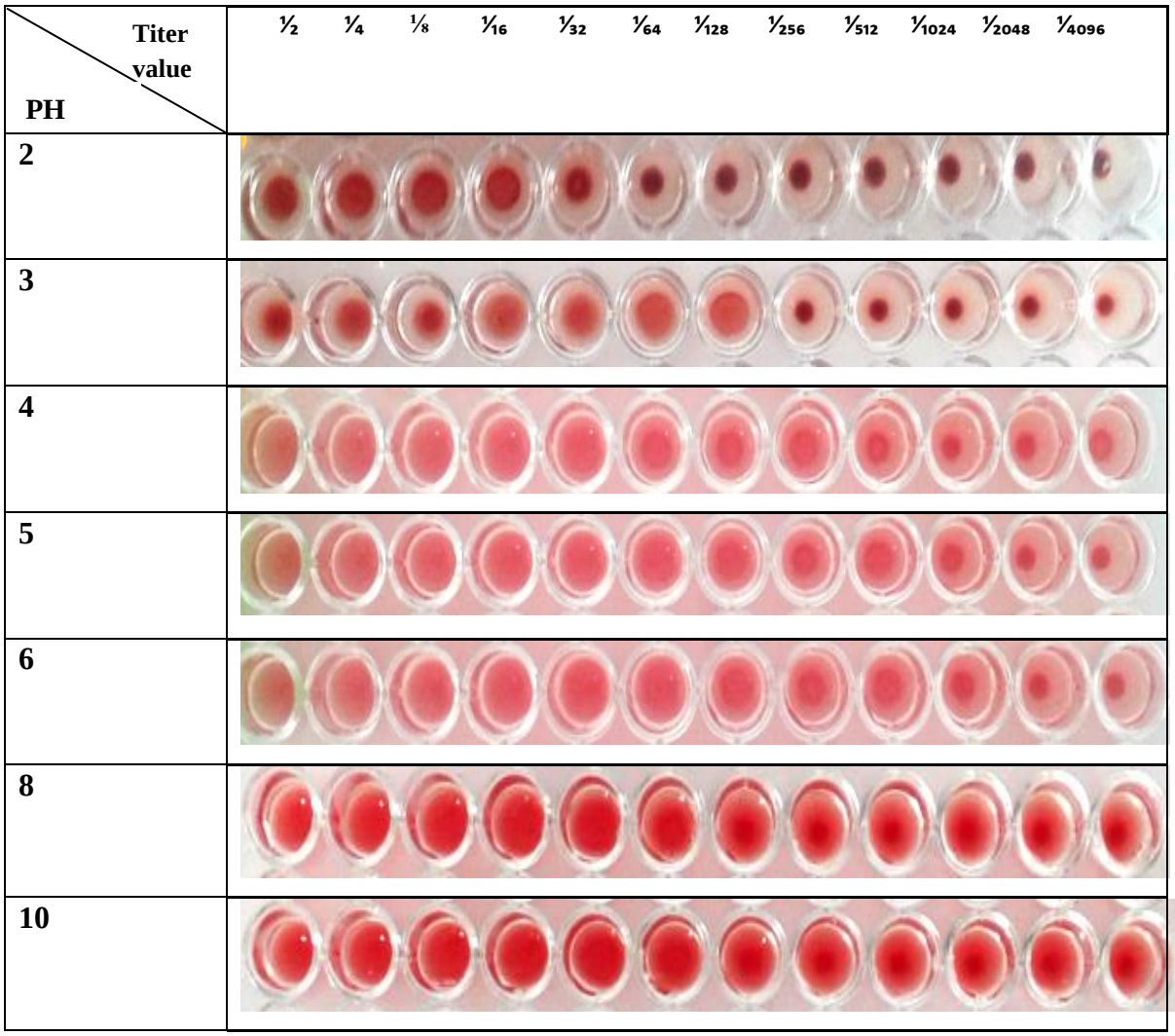


Photo 14: Effect of pH on the agglutination test of *Moringa oleifera.*

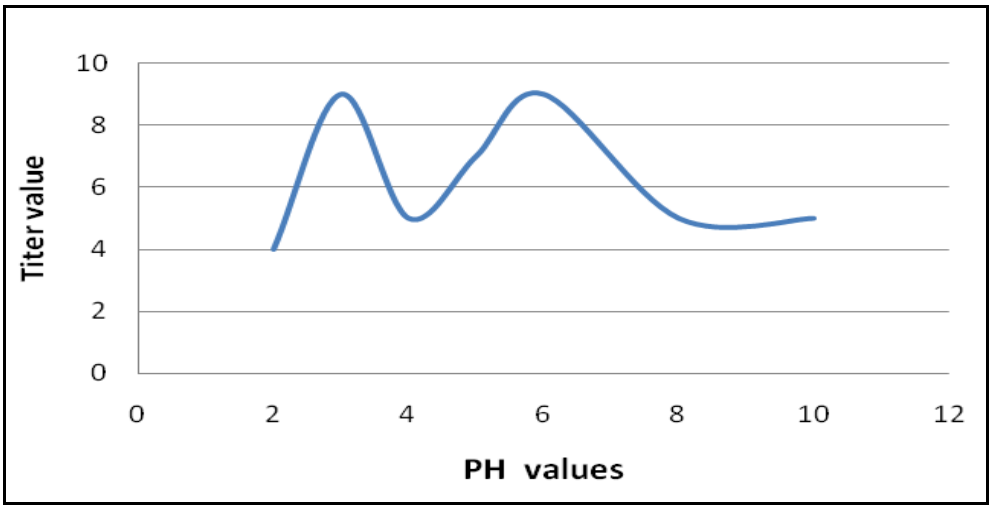


Figure 12: Effect of pH on the agglutinating activity.

✓ From the figure above, we can deduce that the lectin activity is stable and has two isoforms at pH 3 and 6.

e. Agglutination test with the ABO blood system

The human blood types have different sugar moieties on the surface of the cell as shown in **(Figure 06)**. Agglutination occurs when the lectin interacts with these sugar moieties.

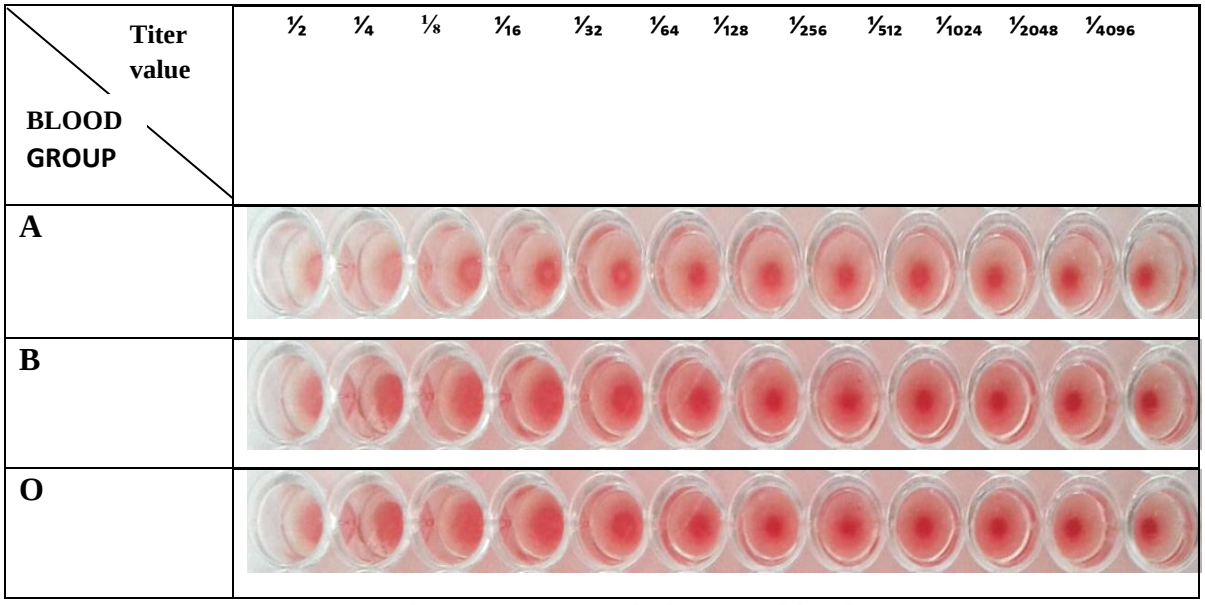


Photo 15: Agglutination test with the ABO blood system.

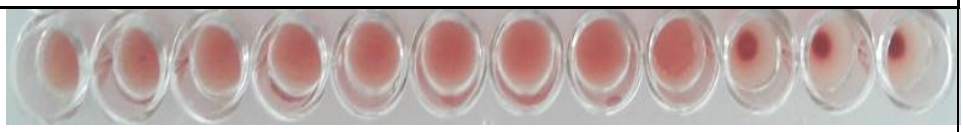
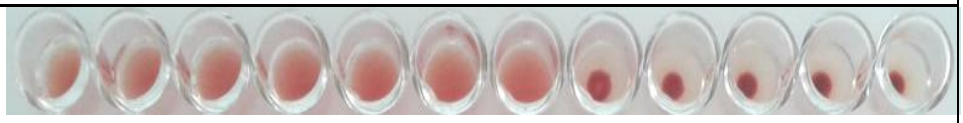
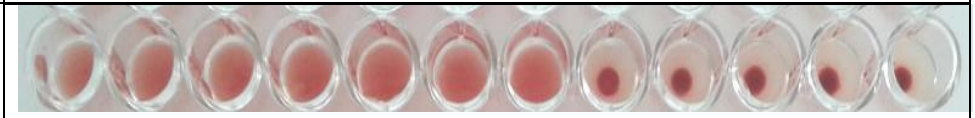
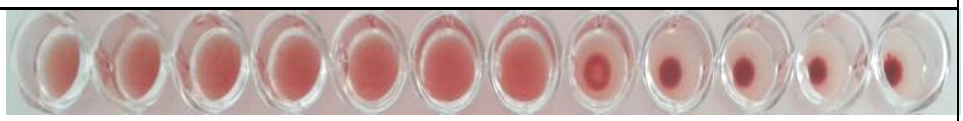
✓ The agglutinating activity was observed in the 3 blood groups, but was significant in blood groups B and O, which showed an activity up to the 1/32 titer, while blood group A showed a weak agglutinating activity up to the ¼ titer well.

✓ The lectin is therefore considered to be non-blood group type specific. This is similar to cMoL isolated by **Santos *et al.*, 2009** and the *Crataeva tapia* bark lectin isolated by **Araujo *et al*., 2012.**

✓ Non-blood type specificity of the lectin may be due to the presence of multiple binding sites where it can recognise all the determinants for each blood type. **(Aragones and Merca., 1998).**

f. Effect of EDTA on the agglutinating activity

The addition of EDTA to the lectin extract showed no significant difference in its agglutinating activity despite the concentration of the EDTA.

Titer value / **EDTA** (mM)	½	¼	⅛	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
0												
20												
30												
40												

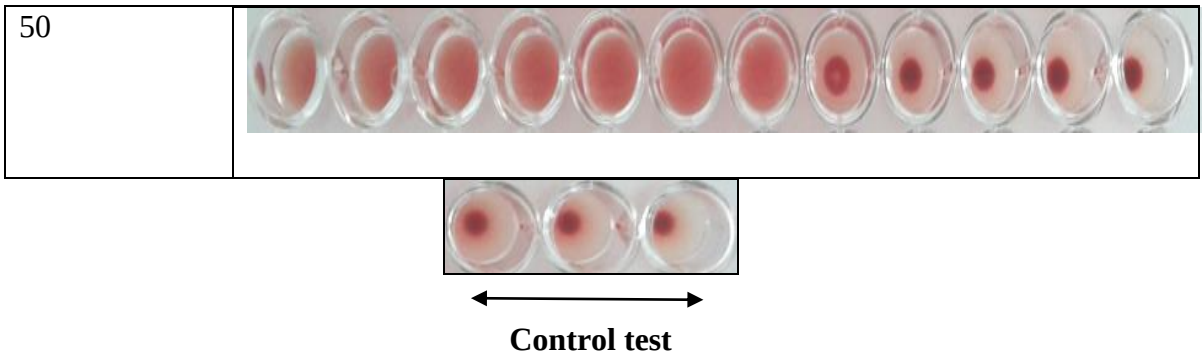


Photo 16: Effect of EDTA on the agglutinating activity.

✓ The activity of the lectin decreased by a factor of 4 on addition of EDTA at any concentration. The positive control test (0 EDTA) showed an agglutinating activity up to the 1/512 titer value, while on addition of EDTA, at all concentrations (20, 30, 40,50), the agglutinating activity stopped at 1/128.

✓ From these results, we can deduce that the lectin is not a metaloprotein, hence it doesnot need metals for its activity.

Conclusion and perspective

The key for efficient detection treatment and healing of pathological condition is the biorecognition event.

Lectins have become a very popular class of biorecognition molecules due to their ability to recognize carbohydrates and glycoconjugates in cells, tissues sections, and biological fluids. They are isolated from distinct sources such as virus, bacteria, fungi, algae, animals, and plants. **(Luana *et al.*, 2017).**

In this study we extracted, isolated, purified and characterized phytolectins from mature *Moringa oleifera* seeds. Extraction with PBS solution isolated two lectins from the whole seed powder. Salting-out of the crude extract with ammonium sulfate showed maximum precipitation at 0-50% $(NH4)_2SO_4$ saturation and resulted in a 37.433 purification fold. Further purification was achieved by gel filtration on Sephadex G-50 column registering a 111.931 purification fold and 50% yield.

All fractions (crude extract, 0-50%, and chromatogratography) showed a positive result of agglutinating activity with glutaldehyde-treated rabbit erythrocytes. Furthermore, the ability to agglutinate all human blood types indicates that the lectin is a non blood type specific.

✓ When subjected to the inhibition test; the 0-50% $(NH4)_2SO_4$ precipitate fraction showed specificity to fetuin.

✓ The two lectins were found to be thermostable, with optimal temperatures of 40 and 70°C. They were more active in a pH range of 3-6 where it exhibited maximum agglutination.

✓ The lectins can be considered as non metalloproteins since they can agglutinate rabbit erythrocytes in presence of EDTA, a chelating agent.

Even though the study of lectins in *Moringa oleifera* seeds has already been investigated, the lectins isolated in this study exhibit properties that are not particular to the lectins in literature.

Perspectives;

This study stimulates more investigation on the following aspects on the lectins from *Moringa oleifera* seeds,

- Purification of the lectins by affinity chromatography.
- Determination of the molecular weight of the lectins by SDS-PAGE.

- The role of *Moringa oleifera* seed lectins in the purification of water.
- Anti-microbiological roles of the lectins.
- Immunomodulatory and anti-cancer activity tests of the lectins.

Literature cited

- **Adewumi. T., Samson. A., (2016).** *Moringa oleifera* as a Food Fortifiant: Recent Trends and Prospects. *Journal of the Saudi Society of Agricultural Sciences.*

- **Balzarini. J., (2006).** Inhibition of VIH Entry by Charbohydrate-Binding Proteins. *Antiviral Research*. Vol 71, p 237.

- **Bailly. P., Chiaroni. J., Roubinet. F., (2015).** Les Groups Sanguins Erythrocytaires. *John Libbey Eurotext*. Paris.

- **Bari. A. U., Silva. H. C., Silva. M. T. L., Júnior. F. N. P., Cajazeiras. J. B., Sampaio. A. H., Leal. R. B., Teixeira. E. H., Rocha. B. A. M., Nascimento. K. S. Nagano. C. S., Cavada. B. S., (2013).** Purification and Partial Characterization of a New Mannose/Glucose-Specific Lectin from *Dialium guineense* Willd Seeds that Exhibits Toxic Efect. *Journal of Molecular Recognition*. Vol 26, p 351.

- **Beltr˜ao. E. I. C., Medeiros. P. L., Rodrigues. O. G., (2003).** *Parkia pendula* Lectin as Histochemistry Marker for Meningothelial Tumour. *European Journal of Histochemistry*. Vol 47, p 139-142.

- **Berg. J. M., Tymoczko. J. L., Stryer. L., (2002).** Lectins Are Specific Carbohydrate-Binding Proteins. *In Biochemistry*. New York, USA.

- **Bradford. M. M., (1976).** A Rapid and Sensitive Method for the Quantization of Microgram Quantities of Protein utilizing the Principle of Protein-Dye Binding. Anal. *Biochemisrty.* Vol 72, p 248-254.

- **Christiansen. M. N., Chik. J., Lee. L., Anugraham. M., Abrahams. J. L., Packer. N. H., (2014).** Cell Surface Protein Glycosylation in Cancer. *Proteomics.* Vol 14, p 525-546.

- **Chumark. P., Khunawat. P., Sanvarinda. Y., (2008).** The in Vitro and ex Vivo Antioxidant Properties, Hypolipidaemic and Antiatherosclerotic Activities of Water Extract of *Moringa oleifera* Lam Leaves. *Ethnopharmacol*. Vol 116, p 439-446.

- **Chumkhunthod. P., Rodtong. S., Lambert. S. J., Fordham-Skelton. A. P., Rizkallah. P. J., Wilkinson. M. C., Reynolds. C. D., (2006).** Purification and Characterization of an N-acetyl-D-galactosamine-Specific Lectin from The Edible

Mushroom *Schizophyllum commune*. *Biochimica Biophysica Acta*. Vol 1760, p326-332.

- **Ciaràn. O., Philip. M. C., Brendan. F. O., (2011).** Gel Filtration Chromatography. *Methods in Molecular Biology*. Vol 681, p 25-33.
- **Correia. M. T. S., Coelho. L. C. B. B., (1995).** Purification of a Glucose / Mannose Specific Lectin Isoform 1, from Seeds of *Cratylia mollis Mart* (*Camaratu* bean). *Applied Biochemistry and Biotechnology*. Vol 55, p 261-273.
- **Daggett. V., Michael. L., (1992).** Molecular Dynamics Simulations of Helix Denaturation. *Journal of Molecular Biology*. Vol 223, p 1121-1138.
- **Daniel. C., (2008).** ABO Blood and Human Origins. *Institute for Creation Research*.
- **Da Silva. L. C. N. L., Filho. C. M. B., Paula. R. A., Coelho. L. C. B. B., Da Silva. M. V., Correia. M. T. D. S., (2014).** *Cratylia mollis* Lectin: A Versatile Tool for Biomedical Studies. *Current Bioactive Compounds*. Vol 10, p 44-54.
- **De Mejía. E. G., Prisecaru. V. I., (2005).** Lectins as Bioactive Plant Proteins: A Potential in Cancer Treatment. *Critical Reviews in Food Science and Nutrition*. Vol 45, p 425-445.
- **Eckhardt. A. E., Goldstein. I. J., (1983).** Occurrence of Alpha-D-Galactosyl Containing Glycoprotein's on Ehrlich Tumor Cell Membranes. *Biochemistry*. Vol 22, p 5280-5289.
- **Ekor. M., (2014).** The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. *Front Pharmacology*.
- **Ewald. D. R., Sumner. S. C., (2016).** Blood Type Biochemistry and Human Disease. *Wiley Interdisciplinary Reviews Systems Biology and Medicine*. Vol 8(6), p 517-535.
- **Ferreira. R. S. T. H., Napolea. A. F. S., Santos. R. A., Sa´. M. G., Carneiro-da-Cunha. M. M. C., Morais. R. A., Silva-Lucca. M. L. V., Oliva. L. C. B. B. Coelho., Paiva. P. M. G., (2011).** Coagulant and Antibacterial Activities of The

Water-Soluble Seed Lectin from *Moringa oleifera*. *Letters in Applied Microbiology*. Vol 53, p186-192.

- **Francis. F., Jaber. K., Colinet. F., Portetelle. D., Haubruge. E., (2011).** Purification of a New Fungal Mannose-Specific Lectin from *Penicillium chrysogenum* and its Aphicidal Properties. *Fungal Biology*. Vol 115, p1093-1099.

- **Fu. L. L., Zhao. X., Xu. H. L., Wen. X., Wang. S. Y., Liu. B., Bao. J. K., Wei. Y. Q., (2012).** Identification of MicroRNA-Regulated Autophagic Pathways in Plant Lectin-Induced Cancer Cell Death. *Cell Proliferation*. Vol *45*, p 477-485.

- **Fuglie. L. J., (2005).** The *Moringa* Tree: A local solution to malnutrition Church World Service in Senegal.

- **Gabius. H. J., (1997).** Animal Lectins. *European Journal of Biochemistry*. Vol 243, p 543.

- **García-Gasca. T., García-Cruz. M., Hernandez-Rivera. E., López-Matínez. J., Castañeda-Cuevas. A. L., Yllescas-Gasca. L., Rodríguez-Méndez. A. J., Mendiola-Olaya. E., Castro-Guillén. J. L., Blanco-Labra. A., (2012).** Effects of Tepary Bean (*Phaseolus acutifolius*) Protease Inhibitor and Semipure Lectin Fractions on Cancer Cells. *Nutrition Cancer*. Vol 64, p 1269-1278.

- **Gassencschimdt., Jany. K. D., Tauscher. B., Niebergall. H., (1995).** Isolation and Characterization of a Flocculating Protein from *Moringa oleifera* Seeds. *Biochimica Biophysica Acta*. Vol 1243, p 477- 481.

- **Gorelik. E., Galili. U., Raz. A., (2001).** On The Role of Cell Surface Carbohydrates and Their Binding Proteins (Lectins) in Tumor Metastasis. *Cancer Metastasis Reviews*. Vol 20, p 245-277.

- **Govardhan. S. R. S., Negi. P. S., Radha. C., (2013).** Phenolic Composition, Antioxidant and Antimicrobial Activities of Free and Bound Phenolic Extracts of *Moringa oleifera* Seed Flour. *Journal of Functional Foods.* Vol 5, p1883-1891.

- **Hassan. M. A. A., Rouf. R., Tiralongo. E., May. T. W., Tiralongo. J., (2015).** Mushroom Lectins: Specificity, Structure and Bioactivity Relevant to Human Disease. *International Journal of Molecular Sciences*. Vol 16, p 7802-7838.

- **Julien. S., Videira. P. A., Delannoy. P., (2012).** Sialyl-Tn in Cancer: (How) Did we miss The Target?. *Biomolecules*. Vol 2, p 435-466.

- **Kansal SK. K. A., (2014).** Potential of *Moringa oleifera* for The Treatment of Water and Waste Water. *Chemical Reviews*. Vol 114(9), p 4993-5010.

- **Karoline. S. A., (2008).** Etudes Structure-Fonction de Lectines (DiscI et DiscII) de *Dictyostelium discoideum. Ecole Doctorale Chimie et Sciences du Vivant.*

- **Katre. U. V., Suresh. C. G., Khan. M. I., Gaikwad. S. M., (2008).** Structure-Activity Relationship of a Hemagglutinin from *Moringa oleifera* Seed. *International Journal of Macromolecular Biology*. Vol 43, p 203-207.

- **Kim. M., Rao. M. V., Tweardy. D. J., Prakash. M., Galili. U., Gorelik. E., (1993).** Lectin-induced Apoptosis of Tumour Cells. *Glycobiology*. Vol 3, p 447-453.

- **Lacsamana. M. S., Merca. F. E., (1994).** Isolation and Characterization of a Lectin from The Visceral Region of The Golden Snail, *Pomacea sp*. *Philipp Journal of Science*. Vol 123, p 77-97.

- **Lakshmipriya. G., Kruthi. D., Devarai. S. K., (2016).** *Moringa oleifera*: A Review on Nutritive Importance and its Medicinal Application. *Food Science and Human Wellness*. Vol 5, p 49-56.

- **Lam. S. K., Ng. T. B., (2011).** Lectins: Production and Practical Applications. *Applied Microbiology and Biotechnology*. Vol 89, p 45-55.

- **Lehrach. H., Kessler. T., Ogilvie. L., Schütte. M., Wierling. C., (2017).** Modelling Molecular Mechanisms of Cancer Pathogenesis: Virtual Patients, Real Opportunities. In Mechanisms of Molecular Carcinogenesis. *International Publishing: Cham, Switzerland*. Vol 2, p 359-374.

- **Leite. Y. F. M. M., Silva. L. M. C. M., Amorim. R. C. N., Freire. E. A., Jorge, D. M. M., Grangeiro. T. B., Benevides. N. M. B., (2005).** Purification of a Lectin from The Marine Red Alga *Gracilaria ornate* and Its Effect on The Development of The Cowpea Weevil Callosobruchus Maculates (*Coleoptera: Bruchidae*). *Biochimaca Biophysica Acta*. Vol 1724, p 137-145.

- **Li. C., Simeone. D. M., Brenner. D. E., Anderson. M. A., Shedden. K. A., Ruffin. M. T., Lubman, D. M., (2009).** Pancreatic Cancer Serum Detection using a Lectin/Glyco-Antibody Array Method. *Journal of Proteome Research*. Vol 8, p 483-492.

- **Liu. B., Cheng. Y., Zhang. B., Bian. H. J., Bao. J. K., (2009).** Molecular Mechanisms of *Polygonatum cyrtonema* Lectin-induced Apoptosis and Autophagy in Cancer Cells. *College of life Science*s. Vol 5, p 253-255.

- **Liu. C., Zhao. X., Xu. X. C., Li. L. R., Liu. Y. H., Zhong. S. D., Bao. J. K., (2008).** Hemagglutinating Activity and Conformation of a Lactose-Binding Lectin from Mushroom *Agrocybe cylindracea*. *International journal of Biological Macromolecules*. Vol 42, p 138-144.

- **Liu. Z. B., Hou. Y. F., Min-Dong. D. G. H., Wu. J., Shen. Z. Z., Shao. Z. M., (2009).** PA-MSHA Inhibits Proliferation and Induces Apoptosis through The Up-Regulation and Activation of Caspases in The Human Breast Cancer Cell Lines. Journal Cell Biochemistry. Vol 108, p 195-206.

- **Luciana. A. L., Franco. A. R., Rute. A. P., Thiago. H. N., Patrícia. M. G. P., Luana. C. B., (2017).** Cytotoxicity of The Coagulant *Moringa oleifera* Lectin (cMoL) to B16-F10 Melanoma Mells. *Toxicology in Vitro*. Vol 44, p 94-99.

- **Marie-José. S., (2008).** Systeme ABO.

- **Mariaterese. M., Salwa. A. M., Fausta. N., Paola. M., Giacomo. L. P., Marzia. F., Gina. R. D. N., Mario. C., Giorgio. P., (2014).** *Moringa oleifera*: Study of Phenolics and Glucosinolates by Mass Spectrometry. *Journal of Mass Spectrometry*.

- **Mitra. N., Srinivas. V. R., Ramya. T. N. C., Ahmad. N., Reddy. G. B., Surolia. A., (2002).** Conformational Stability of Legume Lectins Reflects Their Different. *Biochemistry*. Vol 41, p 9256.

- **Mody. R., Joshi. S., Chaney. W., (1995).** Use of Lectins as Diagnostic and Therapeutic Tools for Cancer. *Journal of Pharmacology and Toxicology Methods.* Vol 33, p 1-10.

- **Molchanova. V., Chikalovets. I., Chernikov. O., Belogortseva. N., Li. W., Wang. J. H., Yang. D. Y. O., Zheng. Y. T., Lukyanov. P., (2007).** A β-Galactose-Specific

Lectin isolated from The Marine Worm *Chaetopterus variopedatus* possesses Anti HIV-1 Activity. *Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology*. Vol 145, p 184.

- **Paiva. P. M. G., Pontual. E. V., Napoleão. T. H., Coelho. L. C. B. B., (2012).** Larvae: Morphology, Biology and Life Cycle. *Nova Science Publishers*. Inc., New York, p 37.

- **Pandey. A., Pradheep. K., Gupta. R., (2011).** Drumstick Tree (*Moringa oleifera Lam.*): A Multipurpose Potential Species in India. *Genetic Resources Crop Evolution*. Vol 58, p 453-460.

- **Pan. S. Y., Zhou. S. F., Gao. S. H., (2013).** New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. *Evidence-Based Complementary and Alternative Medicine Complement*. p 25.

- **Paolo. F. Z., Richard. D. C., (1992).** Lectins and Their Uses as Biotechnological Tools. *Department of Biochemistry and The Complex Carbohydrate Research Center*. The University of Georgia. Athens, USA.

- **Peumans. W. J., Van Damme. E. J. M., (1998).** Plant lectin: Versatile Proteins with Important Perspectives in Biotechnology. Biotechnology and Genetic Engineering. Vol 15, p 199-228.

- **Pierre. B., Bizzari. A. R., Cannistraro. R., (2014).** Biomolecular Recognition: The Current Challenge. *Dynamic Force Spectroscopy and Biomolecular Recognition, CRC Press - Boca Raton*. p1-50.

- **Ponchel. G., Irache. J. M., (1998).** Specific and Non-Specific Bioadhesive Particulate Systems for Oral Delivery to The Gastrointestinal Tract. *Advences Drug Deliver Reviews*. Vol 34, p 191.

- **Rabia. H., Akbar. M., Ishfak. H. W., Shaista. R., (2013).** Lectins: Proteins with Diverse Applications. *Journal of Applied Pharmaceutical Science*. Vol 3 (4), p 93-103.

- **Ramesh. K. S., Iyyakkannu. S., Young-Soo. K., (2016).** Phytochemicals of *Moringa oleifera*: A Review of Their Nutritional, Therapeutic and Industrial Significance. *Biotechnology*. Vol 6, p 203.

- **Ranadhir. M., Nitasha. M., Girija. P. R., (2014).** Blood Groups Systems. *Indian Journal Anaesth*. Vol 58, p 524-8.

- **Renato. A. M., Iracema. L. A., José Tadeu. A. O., Benildo. S. C., (1991).** Plant Lectins, Chemical and Biological Aspects. Mem. Instit Oswaldo Cruz, Rio de Janeiro. Vol 86, p 211-218.

- **Rie. S., Tamiko. N., Keiko. T., Rieko. K., Yoshihiko. K., Junichi. T., Haruo. T., Toshihiro. Y., Kazuto. I., Takayuki. M., Akihiko. Y., Kazumi. I., Kenichi. O., Makoto. U., (2012).** Expression of ABO Blood-Group Genes is Dependent Upon an Erythroid Cell-Specific Regulatory Element That is Deleted in Persons with The Bm Phenotype. *Transfusion Medicine*. Vol 119, N 22.

- **Ripoll. C., Favery. B., Lecomte. P., Van Damme. E., Peumans. W., ABAD. P., Jouanin. L., (2003).** Evaluation of The Ability of Lectin from Snowdrop (*Galanthus nivalis*) to Protect Plants against Root-Knot Nematodes. *Plant science*. Vol 164, p 517-523.

- **Roth. J., (2011).** Lectins for Histochemical Demonstration of Glycans. *Histochemistry and Cell Biology*. Vol 136, p 117-130.

- **Saini. R. K., (2015).** Studies on Enhancement of Carotenoids, Folic acid, Iron and Their Bioavailability in *Moringa oleifera* and In vitro Propagation. University of Mysore, Mysore.

- **Santos. A. F. S., Luz. A. L., Argolo. A. C. C., Teixeira. J. A., Paiva. P. M. G., Coelho. L. C. B. B., (2009).** Isolation of A Seed Coagulant *Moringa oleifera* Lectin. *Process Biochemistry*. Vol 44, p 504-508.

- **Santos. A. F. S., Luz. L. A., Pontual. E. V., Napoleão. T. H., Paiva. P. M. G., Coelho. L. C. B. B., (2015).** *Moringa oleifera*: Resource Management and Multiuse Life Tree. *Advance Research*. Vol 4, p 388-402.

- **Santos. A. F. S., Napole˜ao. T. H., Bezerra. R. F., (2013).** Strategies to Obtain Lectins from Distinct Sources. *In Advances in Medicine and Biology*. Vol 63, p 33-60.

- **Santos. A. F. S., Silva. M. D. C., Napoleão. T. H., Paiva. P. M. G., Correia. M. T. S., Coelho. L. C. B. B., (2014).** Lectins: Function, Structure, Biological Properties and Potential Applications. *Current Topics in Peptide and Protein Research*. Vol 15.

- **Schenkel-Brunner. H., (2000).** Human Blood Groups. *Springer-Verlag Wien*.

- **Scopes. R. K., (1994).** Protein Purification: Principles and Practice. *Springer-Verlag New York.*

- **Sharon. N., (1993).** Lectin-Carbohydrate Complexes of Plants and Animals: An Atomic View. *Trends Biochemistry Science*. Vol 18, p 221-226.

- **Sharon. N., Lis. H., (2002).** How Proteins bind carbohydrates: Lessons from legume lectins. *Journal of Agricultural and Food Chemistry*. Vol 50, p 6586-6591.

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

- **Silva. F. D. O., Santos. P. D. N., Figueirôa. E. O., De Melo. C. M., Neves. J. K. D. A. L., Arruda. F. V. S., Cajazeiras. J. B., Do Nascimiento. K. S., Texeira. E. H., Cavada, B. S., (2014).** Antiproliferative Effect of *Canavalia brasiliensis* Lectin on B16F10 Cells. *Research in Veterinary Science*. Vol 96, p 276-282.

- **Singh. B. N., Singh. B. R., Singh. R. L., Prakash. D., Dhakarey. R., Upadhyay. G., Singh. H. B., (2009).** Oxidative DNA damage Protective Activity, Antioxidant and Anti-quorum Senseing Potentials of *Moringa oleifera*. *Food and Chemical Toxicology*. Vol 47, p 1109-1116.

- **Stohs. S. J., Hartman. M. J., (2015).** Review of The Safety and Efficacy of Moringa oleifera. *Phytotherapy Research.* Vol 29, p796-804.

- **Svarovsky. S. A., Joshi. L., (2014).** Cancer Glycan Biomarkers and Their Detection-Past, Present and Future. *Analytical Methods*. Vol 6, p 3918-3936.

- **Szöke. T., Kayser. K., Trojan. I., Kayser. G., Furak. J., Tiszlavicz. L., Baumhäkel. J. D., Gabius. H. J., (2007).** *European Journal Cardio-Thoracic*. Vol 31, p 783.

- **Teixeira. E. M. B., Carvalho. M. R. B., Neves. V. A., Silva. M. A., Arantes-Pereira. L., (2014).** Chemical Characteristics and Fractionation of Proteins from *Moringa oleifera* Lam Leaves. *Food Chemistry*. Vol 147, p 51-54.

- **Varki. A., (1993).** Biological Roles of Oligosaccharides: All of The Theories are Correct. *Glycobiology*. Vol 3, p 97-130.

- **Wälti. M. A., Walser. P. J., Thore. S., Grünler. A., Bednar. M., Künzler. M., Aebi. M., (2008).** Structural Basis for Chitotetraose Coordination by CGL3, a Novel Galectin-Related Protein from *Coprinopsis cinerea*. *Journal of Molecular Biology*. Vol 379, p 146.

- **Wang. H., Ng. T. B., (2005).** Isolation of Trichogin, an Antifungal Protein from Fresh Fruiting Bodies of The Edible Mushroom *Tricholoma giganteum*. *Biochemical and Biophysical Research Communications*. Vol 337, p 621-628.

- **Wong. J. H., Wong. C. C. T., Ng. T. B., (2006).** Purification and Characterization of a Galactose-Specific Lectin with Mitogenic Activity from *Pinto Beans*. *Biochimica Biophysica Acta*. Vol 1760, p 808-813.

- **Zheng. S., Li. C., Ng. T. B., Wang. H. X., (2007)**. Lectin with Mitogenic Activity from The Edible Wild Mushroom *Boletus edulis*. *Process Biochemistry*. Vol 42, p 1620-1624.

Annex

Annex 01: Preparation of Buffer

- Preparation of buffer (phosphate DI _ SODIQUE = PBS).

Products	Quantity
Na_2HPO_4	1,44g
$NaHPO_4$	0,28g
Kcl	0,2g
NaCl	8g
DISTILED WATER	1L

Annex 02: Sample Preparation in iron Exchange Chromatography.

	Final percent saturation to be obtained																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Starting percent saturation	Amount of ammonium sulphate to add (grams) per liter of solution at +20 °C																
0	113	144	176	208	242	277	314	351	390	430	472	516	561	608	657	708	761
5	85	115	146	179	212	246	282	319	358	397	439	481	526	572	621	671	723
10	57	86	117	149	182	216	251	287	325	364	405	447	491	537	584	634	685
15	28	58	88	119	151	185	219	255	293	331	371	413	456	501	548	596	647
20	0	29	59	89	121	154	188	223	260	298	337	378	421	465	511	559	609
25		0	29	60	91	123	157	191	228	265	304	344	386	429	475	522	571
30			0	30	61	92	125	160	195	232	270	309	351	393	438	485	533
35				0	30	62	94	128	163	199	236	275	316	358	402	447	495
40					0	31	63	96	130	166	202	241	281	322	365	410	457
45						0	31	64	98	132	169	206	245	286	329	373	419
50							0	32	65	99	135	172	210	250	292	335	381
55								0	33	66	101	138	175	215	256	298	343
60									0	33	67	103	140	179	219	261	305
65										0	34	69	105	143	183	224	267
70											0	34	70	107	146	186	228
75												0	35	72	110	149	190
80													0	36	73	112	152
85														0	37	75	114
90															0	37	76
95																0	38

Annex 03:

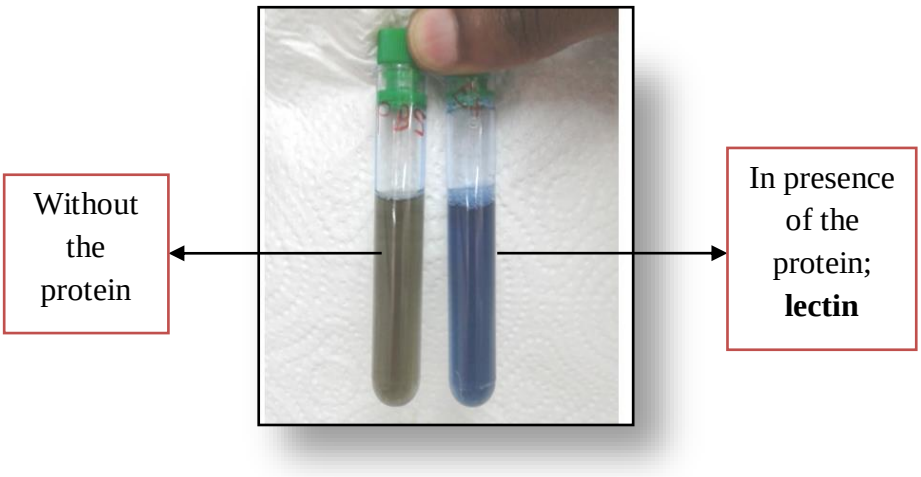
- **SHA** (Specific Hemagglutinating Activity) = **Total activity / Total protein**
- **Purification fold = SHA of purified fraction / SHA of crude extract.**
- **Yield=** Original total activity that is found present in the purified fraction. **Total activity of purified fraction / Total activity of crude extract.**

Annex 04: Preparation of Sugar

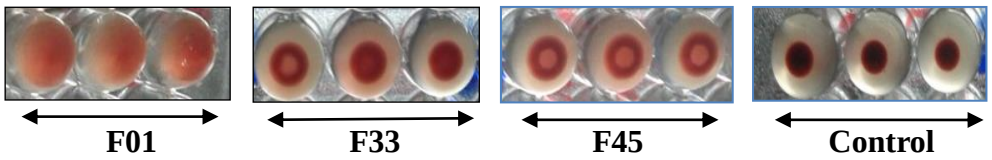
- Preparation of sugar 400mM.

Sugar/glycoprotéins	PBS(buffer)
400 mM	2 ml

Annex 05: Eye observation of color change of Bradford's solution in presence of lectins



Annex 06: Agglutination Test after Anion Exchange Chromatography



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EXTRACTION, ISOLATION AND CHARACTERIZATION OF LECTINS FROM *MORINGA OLEIFERA* SEEDS

Abstract

The purpose of the research was to study the isolation, purification and characterization of lectins from mature *Moringa oleifera* seeds. The lectins were extracted from the seeds with phosphate-buffered saline (PBS) at pH 7.4, and partial purification was accomplished by ammonium sulfate precipitation and gel filtration using Sephadex G-50.

Agglutination using glutaraldehyde-treated rabbit erythrocytes and human blood types A, B, O was carried to determine the presence and specificity of the lectin. Subsequently, the *Moringa oleifera* seed lectins were classified as a complete non-specific lectin since it agglutinated all human blood types and glutaldehyde-treated rabbit erythrocytes.

The results of carbohydrate specificity showed that the lectins had complex sugar specificity to fetuin. The lectins were found to be thermally stable, and stable within the pH range of 3-6. It was also considered as a non metallolectin since its activity was not significantly affected by EDTA.

Keywords: Lectins, Extraction, Agglutination, Characterization, *Moringa oleifera*, Inhibition test.

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