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EXTRACTION OF LECTIN FROM BANANA USING ACID SOLVENT

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A thesis

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School of Chemical and Bio-engineering

This is to certify that the thesis prepared by **Miss. Fatuma Abiyu** entitled "**Extraction of lectin from banana using acid solvent**" and submitted fulfillment of the requirements for the degree of Master of Science in Biochemical Engineering complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that thesis work titled "**Extraction of Lectin from Banana using Acid Solvent**" submitted for master degree at Addis Ababa University is my original work and has not previously been submitted for degree at this or any other universities, and that all resources of materials used for this thesis has been dully acknowledge/ referred.

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This thesis has been submitted for examination with my approval as a university advisor.

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DEDICATION

This thesis work is dedicated to my beloved parents especially my uncle, Mussa Ibrahim who have fully shouldered the burden of my families.

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ACRONYMS

KHB: - Krebs Hepes Buffer

HIV: - Human Immunodeficiency Viruse

MBL: - Mannose Binding Lectin

CRD: - carbohydrate recognition domain

gp: - Glycoprotein

rpm: - Revolution per minute

ABSTRACT

Banana is a major tropical fruit crop distributed in more than 120 countries with an annual production of 102 million tones. Banana Lectin is a lectin from the jacalin-related lectin family isolated from the fruit of the bananas (Musa acuminate). It exists as a homodimer which are two identical kda (kilodalton) sub unit. The objective of the thesis was extraction of lectin from banana for glycoconjugates. A number of literatures were reviewed for extraction of lectin.Lectin was extracted from ripen and unripen banana. The protein was highly stable and binds to a certain carbohydrate structure. These heterogeneous class of carbohydrate-binding proteins or glycoproteins of non-immune origin are capable of specific recognition of, and reversible binding to, carbohydrates without altering their covalent structure. The maximum amount of lectin (3.6 mg) was found from ripen banana that socked in neutral pH, at 25^{o}C and 24 hours. And it was checked its glycoconjugates activity using KHB kit and agglutination was observed on the wall of the plate. As socking time increased from 12-36 hours the amount of lectin decreased. Large amount of lectin was observed in neutral pH than in acidic and basic solutions. Besides as temperature increased from 25-30^{o}C, still amount of lectin decreased. This was due to the break-down of protein molecules in long socking time, acidic and basic solutions and high temperature.

***Key words**: Agglutination, Banana, Lectins*

CHAPTER ONE

1. INTRODUCTION

Banana is a major tropical fruit crop distributed in more than 120 countries with an annual production of 102 million tones. Banana belongs to the family Musaceae under the order of Zingiberales with two genera, Musa L. and Ensete Bruce. It is highly diversified throughout the world but has been reported to originate from Southeast Asia. The genus Musa consists of around 50 species while Ensete has nine species. On the basis of phenotypic traits and basic chromosome number, Musa has been divided into four sections namely, Eumusa, Rhodochlamys, Austra-limusa and Callimusa. Most edible bananas that have largely evolved from two wild species, M. acuminataColla and M. balbisianaColla belong to the section Eumusa [11]. Bananas are a great dietary source of potassium. ... A potassium-rich diet can help lower blood pressure, and people who eat plenty of potassium have up to a 27% lower risk of heart disease. Furthermore, bananas contain a decent amount of magnesium, which is also important for heart health.

Lectins are defined as proteins /glycoproteins possessing at least one non-catalytic domain which binds reversibly to a specific mono- or oligosaccharide [64]. They are carbohydrate-binding proteins which bind to glycoproteins, glycolipids, and also polysaccharides [16] which mediate various kinds of biological processes by binding to different sugar moiety [31]. Lectins are also called agglutinins because when it binds to cell surfaces results in agglutination reactions. They are highly diverse in their structure, molecular weight, composition, and number of sugar binding sites present per molecule [27]. They possess the ability to agglutinate erythrocytes with known carbohydrate specificity since they have at least one non catalytic domain that binds reversibly to specific monosaccharide or oligosaccharides.

BanLec (also BanLec-I or Banana lectin) is a lectin from the jacalin-related lectin family isolated from the fruit of the bananas Musa acuminata and Musa balbisiana. BanLec is one of the predominant proteins in the pulp of ripe bananas and has binding specificity for mannose and mannose-containing oligosaccharides.

Until about 15 years ago lectins were thought of as laboratory tools, useful for histochemistry and blood transfusion work. The fact that many common foods are rich sources of lectin was not considered by most biomedical scientists. In the last decade, however, there has been a flowering of knowledge about the interactions of lectins with body organs and tissues. Purified lectins are important in a clinical setting because they are used for blood typing. Some of the glycolipids and glycoproteins on an individual's red blood cells can be identified by lectins.

Lectin in plants is a defense against microorganisms, pests, and insects. They may also have evolved as a way for seeds to remain intact as they passed through animals' digestive systems, for later dispersal. Lectins are resistant to human digestion and they enter the blood unchanged. They are thought to play a role in immune function, cell growth, cell death, and body fat regulation. The contents in some parts of plants are higher e.g., 390 and 75 mg of the purified lectin was recovered from100g Remusatia vivipara tubers and Astragalusmongholicus respectively. Lectins are also found in seeds. The lectin content in non-legume plants is low,e.g., 3.3 mg lectin from 100 g Hibiscus mutabilis seeds. Lectins are found in abundance in legume seeds. Phaseolus vulgaris is an herbaceous annual plant grown worldwide for its edible beans, popular in both dry and green bean forms. The commercial production of beans is well distributed in world wide. There are different varieties, including Anasazi bean, black beans, cranberry bean, borlotti beans, pink beans, pinto beans, kidney beans, shell beans, white beans, yellow beans and French beans, etc. Lectins or hemagglutinins have been purified from different varieties of P. vulgaris. The lectin contents are low in some varieties and high in other varieties.

1.1. Statement of the Problem

The problems that enlightened to do this thesis work were categorized into three parts. Yields of lectin from other materials are usually extremely low, Environmental pollution caused by chemical insecticides and herbicides and Maximum amount of extracted lectin from banana.

In order to produce a large quantity of lectins, the first criterion is a high lectin content in the starting material. A large number of lectins or hemagglutinins have been purified from different organisms. As an example: - Lectins are found in different animals. However, the yields are usually extremely low mass purification of animal lectins necessitates bulk quantities of raw materials which make it not feasible. Yields of lectins from fresh mushrooms are low in fact, the water content in fresh mushrooms is very high. Hence, the lectin contents from plants are higher when compared to other sources of lectin.

Lectins have been suggested as one of the promising agents against insect pests and have been engineered successfully into a variety of crops including wheat, rice, tobacco, and potatoes. This approach could be used as a part of integrated pest management strategies and caveat pest attack. In general, it seems that large-scale implementation of transgenic insecticidal and herbicide-tolerant plants does not display considerable negative effects on the environment. Moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerable reduces the load of chemical insecticides and herbicides.

It has been reported that the maximum amount of extracted lectin from Banana was 3.8mg/100 g [65]. This amount is very small comparing to its advantage. As a result of these this thesis aims to produce lectin from banana and optimize it with its optimum conditions.

1.2. Objective of the Study

1.2.1. General Objective

The general objective of this study was to extract lectin from banana, purify it by using open column chromatography method and to test its optimum conditions.

1.2.2. Specific Objective

- To extract lectin from banana by using acetic acid
- To purify it by using chromatography method
- To optimize extraction parameters of lectin
- To quantify lectin concentration using spectrophotometer
- To see its activity by using KHB kit.

1.3. Significance of the Study

Lectins have become the focus of intense interest for biologists and in particular for the research and applications in agriculture and medicine. These proteins with unique characteristics have found use in diverse fields of biology and as more lectins are being isolated and their role in nature elucidated, they continue to occupy an important place in agricultural and therapeutic areas of research including anti insect activity, anti-tumor, antimicrobial and HIV- 1 reverse transcriptase inhibitor, which may find applications in many therapeutic areas. Plant lectins are also powerful tools for the isolations and fractionation of glycoconjugates and for the study of oligosaccharides and glycoproteins for different applications. In order to introduce the science behind extraction of lectin from banana for different applications like as a drug, for instance, HIV has been and still is a disease that is concerning the world as it affects human without distinguishing sex, age or wealth, So it may use as indicators for researchers who have done in area of HIV to extract drug for HIV inhibition in banana.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. History of Lectin

Lectins are carbohydrate binding proteins other than immunoglobulins that display no enzymatic activity towards the recognized sugars. Lectins have been retrieved in almost all forms of life, and animal lectins, though fulfilling a variety of functions, have been implicated in defense against pathogens, immune regulation and prevention of autoimmunity [22]. In marine invertebrates, lectins have been thought to participate in immune response by inducing bacterial agglutination or by acting as opsonins to enhanced phagocytosis by coelomocytes.

An extensive discussion regarding the physiological significance of lectins in both vertebrates and invertebrates has been reported [22]. In the case of invertebrate humoral lectins, it has been postulated more than twenty years ago that one of their physiological functions is distinction of self from non-self in cooperation with cellular and humoral defense processes. A large number of lectins has been isolated and characterized from deuterostomian and protostomian invertebrates, such as tunicates, insects, crustaceans and molluscs [6]. As far as the presence of lectins in echinoderms is concerned, lectins have been found in the sea urchins Anthocidaris crassispina and Paracentrotus lividus. In the latter species, the reported authors described the occurrence of a natural C-type lectin with a molecular weight (MW) of more than 200 kDa in the coelomic fluid. In the present study, a low MW lectin was purified from the coelomic fluid of the sea urchin P. lividusby ion-exchange chromatography on DEAE Sephadex. This molecule has a MW of 13 kDa and displays a Ca2+ independent hemagglutinating activity.

Numerous plant lectins have been characterized with respect to their molecular structure and biochemical and carbohydratebinding properties; but by far the majority have been isolated from seeds or vegetative tissues of plants belonging to the class Magnoliatae (also called dicotyledons). Only those isolated from a few Gramineae species such as wheat (Triticum vulgare), barley (Hordeum vulgare), rye (Secale cereale), couch grass (Agropyrum repens), and rice (Oryza sativa) were obtained from plant species belonging to the class Liliatae (also called

monocotyledons). To find out whether lectins also occur among other species of the latter class, and if so, whether they are related to, or at least comparable with those isolated from dicotyledonous species, we looked for the presence of hemagglutinins in a number of species belonging to the family Liliaceae. Here we describe the isolation and partial characterization of a lectin from tubers of meadow saffron (Colchicum autumnale), a typical Liliaceae species.

In the modern view, proteins specifically recognizing and reversibly binding to certain carbohydrate glycoconjugate structures without change in covalent structure of the recognized glycosyl ligands are considered to be lectins. This definition most adequately accounts for the properties and characteristics of this group of proteins that are known in modern glycobiology. Lectins can be isolated from nearly all types of living organisms from viruses to humans, although those of higher plants still form the most numerous group of lectins. Lectins represent a large heterogeneous class of molecules with different properties and varying physiological functions [60].

Several immunological reactions, such as agglutination of cells (including erythrocytes) and precipitation of glycoproteins and polysaccharides, are typical of lectins. Some lectins cause selective agglutination of malignant tumor cells; this indicates that tumor and normal cells have different surface structure. Lectins are accepted as the most informative molecular probes allowing identification of glycoconjugates of cells and tissues and study of their dynamics under physiological and pathological conditions.

Being incorporated in the structure of tissues of animals, plants, and bacteria, lectins participate in regulation of their metabolism as well as in protection from some environmental agents. However, when isolated from living objects, lectins can be the valuable biochemical reagents used in experimental cyto-chemistry, diagnostics of some diseases, and in biotechnological processes of isolation of complex carbohydrate-containing substances. In recent years there have been attempts to use lectins as drugs.

These facts dictate the study of lectins by researchers working in various branches of biology, first and foremost in biochemistry, cytology, microbiology, plant physiology, biotechnology, and pharmacology. In spite of the fact that the first lectin was isolated more than a hundred years ago, intensive study of lectins began only in the last 30 years. Marine organisms are relatively

new sources of lectins. However, studies of the physiological role of these compounds as well as works with the use of endogenous and exogenous lectins in various living systems are now rather active. Lectins of marine invertebrates are of particular interest. They have been found in more than 300 species, and their carbohydrate specificity and molecular structures are defined.

Lectins of annelid worms and sponges, the most primitive representatives of this group, have molecular mass not more than 40 kDa and usually are monomeric or dimeric proteins. As taxonometric or anatomic complexity of marine invertebrates rises, heterogeneity of lectins also rises: complex multimeric forms are organized in quaternary structures. Even lectins of shellfish and echinoderms demonstrate wide heterogeneity, being multimeric as well as monomeric and dimeric proteins. Lectins of arthropods – organisms with active modus vivendi, are multimers with molecular mass 450 kDa and higher. Comparative analysis of plant lectins demonstrates an analogous tendency: unlike lectins of terrestrial plants, which have subunit structure and specificity to mono- and oligosaccharides, lectins of an older group of plants marine algae, have the lower molecular masses, are usually monomeric, and do not exhibit affinity to monosaccharide residues. It is interesting that analogously to lectins of higher plants and v ertebrates, lectins of invertebrates have higher affinity to β-anomeric forms of carbohydrates than to α-anomeric forms [33].

As the organization level of the corresponding taxon of animals rises, the level of lectins changes from constitutive to facultative, which is more advantageous in the physiological sense, and the ability for secretion of lectins in hemolymph is achiev ed. Thus, most lectins isolated from sponges are components of their cell membranes; this is related with the absence of structured inner medium in the organism and distinct tissue differentiation [46].

In more complex organisms lectins are also either components of cell membranes or are synthesized and then secreted by cells of coelomic or hemal fluids (coelomicinv ertebrates), and also by other organs and tissues.

In spite of the fact that some lectins of invertebrates are essentially homologous to lectins isolated from vertebrates, by now such an important class of proteins cannot be completely classified in families because for most the amino acid sequence is not yet established. Usually there are attempts to classify them as lectins of higher animals. Ogawa and coworkers suggest

classification of lectins of marine invertebrates into Ca^{2+} dependent lectins (C-lectins), F-type lectins, and rhamnose-binding lectins (RBL) [41].

Thus, a number of isolated lectins of sea hydrobionts belong to the C-lectin family, but in contrast to many of the C-type lectins of vertebrates having several different functional domains, most of the C-type lectins of invertebrates include only the carbohydrate-recognizing domain (CRD). Dependence of activity on the presence of $Ca^{2+}$is the main feature of lectins of this type [32].

Lectins belonging to the galectins, a family of evolutionally conservative lectins having affinity to β-galactosides and widespread in mammals, have been isolated from marine invertebrates in recent years. The number of lectins with established primary and threedimensional structures has rapidly increased in the past few years. It is interesting that similarity in the tertiary structures of lectins isolated from various organisms was noted in spite of the absence of any homology in their amino acid sequences [60].

So, lectins isolated from marine hydrobionts belong to the significantly different class of proteins not only from the viewpoint of structure, but also of functional aspects including their unique carbohydrate specificity. The literature data and results of our research on lectins isolated from some kinds of marine hydrobionts, such as clams, ascidians, worms, sponges, and algae are presented in this review because in recent years the authors have been isolated lectins from these sources and studied their structures and functions [60].

The binding nature of Lectins is similar to antibodies, forming an irreversible covalent bond. An example of a high molecular weight polysaccharide which conveys a protective effect is arabinogalactan, found in a variety of foods and herbal medicines. This class of molecules has been shown to occupy the binding sites of various micro-organismsm preventing them from attaching to cellular surfaces and making it easier for the immune system to eliminate them. Lictins play a crucial role in diverse biological processes, particularly in host defense mechanisms, inflammation, and metastasis. Owing to their binding specificities, lectins are employed in a number of biochemical and clinical research areas. Lectins are known to cause a number of biological effects including lymphocyte proliferation (blastogenesis) and the induction

of cytokine production as well as having the ability to inhibit specific antibody stimulated T cell activity [15].

Since lectins distinguish themselves from all other plant proteins by their specific carbohydrate-binding activity, one can reasonably assume that their physiological role involves their sugar-binding properties. In principle, any lectin-mediated reaction or process relies on the specific binding of the lectin to a glycoconjugate receptor (irrespective of whether this receptor is located within or outside the plant). Therefore, the search for the physiological role of plant lectins was always intimately linked to the search for their natural receptors. In the case of lectins, receptors can be defined as glycoconjugates that possess a carbohydrate moiety with a structure complementary to that of the binding site of the lectin. This implies that glycoconjugates of different nature (e.g. glycoproteins, glycolipids, and polysaccharides) but with identical (or structurally similar) carbohydrates can act as receptors for the same lectin [67].

Many plant species contain carbohydrate-binding proteins, which are commonly referred to as either lectins or agglutinins. Generally speaking, lectins are proteins that bind reversibly to specific mono- or oligosaccharides. Since the initial discovery of a hemagglutinating factor in castor bean extracts by Stillmark in 1888, several hundred of these proteins have been isolated and characterized in some detail with respect to their carbohydrate-binding specificity, molecular structure, and biochemical properties. Lectins from different plant species often differ with respect to their molecular structure and specificity. It is important, therefore, to realize that all plant lectins are artificially classified together solely on the basis of their ability to recognize and bind carbohydrates [67].

2.2. Sources of lectin

2.2.1. Lectins of Clams

Phylum Mollusca is one of the most numerous and important groups of the animal realm: there are more than 200,000 species found in terrestrial, freshwater, and marine environments. Study of lectins from mollusks is of significant interest because of data on their antitumor, antiviral, and antimicrobial activity. However, among lectins of Mollusca phylum, those isolated from species having economic or medical value such as mussels or oysters are of maximal interest. Most lectins that are either associated or not associated with cell membrane of hemocytes and

often perform protective function in animals have been isolated from hemolymph of clams. Lectins have also been detected in plasma and various organs and tissues: digestive gland, gonads, and soft tissues. Lectins from plasma are usually multimeric protein complexes with several sites for binding to ligands. Lectins of clams are specific to various carbohydrates. Specificity to Gal, GalNAc, Glc, Lac, Fuc, and also to sialic acids and glycoproteins of mucin type is typical of lectins of these marine invertebrates. Such wide range of specificities of lectins of the Bivalvia family can be due to the presence of multiple isoforms of lectins specific for a particular monosaccharide or a group of monosaccharides with similar structures. For example, a heterogeneous sialospecific lectin composed of three iso-lectins has been found in hemolymph of the mussel Modiolus modiolus.

Two of these lectins, modiolin H and modiolin E, agglutinate human and horse erythrocytes, respectively, but only for modiolin E Ca^{2+} is required for manifestation of activity. Different subunits seem to be capable of binding to several monosaccharides with different affinity. Such organization can extend the range of ligands bound by them. Moreover, lectins of clams consisting of identical subunits are able to react with various monosaccharides. Lectin of the oyster Pinctada fucata is an example; with maximal efficiency it binds to Gal, and it also exhibits specificity to other carbohydrates (Glc, Man, Fuc, GalN, GalNAc, ManNAc, NeuNAc) and agglutinates various kinds of erythrocytes. Lectins isolated from the clams T egillarca granosa and Ostrea chilensis also demonstrate multi-specificity [8].

Most lectins isolated from clams are of C-type. Most often lectins of this type, e.g. those isolated from the clams Tridacna maxima, T. derasa, T. crocea, T. gigas,Crassostrea virginica, Pinctada fucata martensii, and T egillarca granosa are high molecular weight multisubunit proteins with molecular masses in the range 123-500 kDa. Experimental data indicate that the specificity to Gal and GalNAc as well as ability for agglutination of erythrocytes of various types is typical of the C-type lectins of clams. However, low molecular weight lectins also occur among the C-type lectins. For example, a Ca^{2+} dependent lectin (chilectin) with molecular mass 12 kDa has been isolated from hemolymph of the oyster Ostrea chilensis. Analysis of the primary structure of codakin, a lectin from the clam Codakia orbicularis (molecular mass 14 kDa), showed partial homology with various C-type lectins of higher animals. The amino acid sequence of codakin contains a tripeptide fragment EPN (Glu-Pro-Asn) often found in Man-binding lectins [17].

2.2.2. Lectin of Ascidians

Hemagglutinating activity has been discovered in hemolymph of many ascidians, which are considered as a key group in phylogenesis of chordates. Hemocytes are responsible for synthesis of lectins of hemolymph or at least are the main place of their synthesis, since lectins initially have been membrane receptors of hemocytes. A large number of lectins binding to a wide variety of carbohydrates including α1-6Gal, β1-4Gal, GalNAc, GlcNAc, ManN, Fuc, lactose, melibiose, raffinose, mannans, sialoconjugates, and bacterial LPS have been isolated from ascidians. Many researchers rationalize the wide variety of ligand specificities of lectins of hemolymph by the fact that lectins of ascidians have protective function in the animals, and for manifestation of this function molecular forms (structural classes and isoforms) should be variable. Protective function also results in a wide variety in molecular masses of lectins of ascidians [45].

High molecular weight lectins with masses 90-600 kDa in the native state have been isolated from the ascidians Clavelina picta, Styela plicata, Botrylloides leachii, and Halocynthia roretzi. Low molecular weight lectins with masses 14-63 kDa have been isolated from H. pyriformis, C. picta, Didemnum candidum, D. ternatanu. Polyandrocarpa misakiensis, Botryllus schlosseri, S. plicata, and Ascidia malaca. Both high and low molecular mass lectins are mainly subunit proteins, although monomers are also described. Most of the lectins are Ca^{2+} dependent. The presence of several lectins with overlapping carbohydrate specificity is a characteristic feature of some species of ascidians [36].

In spite of the lack of structural information that would allow assignment of lectins of ascidians to one of the known classes, some demonstrate significant structural homology with lectins isolated from vertebrates. For example, a number of the natural or inducible galectins have been found in plasma or on the surface of hemocytes. Gal-binding humoral opsonin with the properties of galectin has been found in the supernatant of lysate of hemocytes from the colonial ascidian B. schlosseri , and humoral opsonin of C-type has been isolated from hemolymph of the ascidian Styela clava. Cytoplasmic lectins, Ca^{2+} independent and β-galactoside-specific, with properties typical of galectins, have been isolated from the ascidian Ciona intestinalis. Several lectins that can be ascribed to the class of collectins or ficollectins playing an important role in

the innate immunity of the organism hav e been isolated from ascidians. Collectins or ficollectins have similar structures containing collagen-like and carbohydratebinding domains. In the case of collectins, the CRD is specific for Glc/Man or Gal derivatives, and ficollectins have a fibrinogen-like domain at the C-end; this domain can interact with various carbohydrates, most often with GlcNAc. Thus, two Gal-specific metal-dependent lectins with molecular masses 43 and 90 kDa have been isolated from the ascidian S. plicata and characterized.

Determination of amino acid content and amino acid sequence shows that these lectins are similar to collectins of higher animals. Similar lectins have been isolated also from other ascidians. The ascidian H. roretzi closely related to S. plicata has four ficollin-like genes encoding fibrinogen-like domains and a collagen-like sequence. A number of genes similar to genes of ficollins and collectins have been found in the genome of the ascidian C. intestinalis. Recently, a new member of the RBL family was isolated from the colonial ascidian B. schlosseriand characterized. This lectin is shown to play an important role in immunity of the ascidian [36].

Two Gal-specific lectins, DCL-I and DCL-II, were isolated from plasma of the ascidian Didemnum candidum, and their physicochemical properties, amino acid content, and the N-terminal amino acid sequence were studied. Both lectins have subunit structure, similar molecular mass, pH optimum, and thermal stability and are metal-independent proteins. However, it appears that in spite of the similar physicochemical characteristics, these lectins differ in amino acid content and have serologic difference. It was also shown that DCL-I exhibits mitogenic activity towards mouse thymocytes and splenocytes [14].

Two lectins were isolated from the colonial ascidian Didemnum ternatanum: a GlcNAc-specific lectin (DTL) and a GlcNAc/GalNAc-specific lectin (DTL-A). Both are Ca2+ independent, have rather low molecular mass (9 and 14 kDa, respectively), but DTL in the native state exists as a trimeric structure, while DTL-A forms high molecular weight aggregates. Beside this, DTL and DTL-A have different N-terminal amino acid sequences and differ in binding to erythrocytes. DTL-A agglutinates both trypsinized and non-trypsinized erythrocytes, while DTL only trypsinized ones. In contrast to all other known GlcNAc-specific lectins, DTL has a less extended carbohydrate-binding site. The hydroxyl groups at C-3 and C-4 and also the acetamide group of GlcNAc participate in binding. Similar results were obtained for DCL-I from the

ascidian D. candidum. This lectin also has a small CRD that interacts only with the hydroxyl groups at C-2, C-3, and C-4 of Gal residues. In contrast to DTL, DTL-A binds not only to GlcNAc, but also to GalNAc, BSM, asialoBSM, heparin, and dextran sulfate. DTL-A binding to structurally different ligands suggests multi-specificity of this lectin. The data on inhibition of DTL-A binding to BSM and heparin indicate that this lectin probably contains two independent sites: GlcNAc/GalNAc-binding site and an additional one responsible for interaction with heparin and dextran sulfate. A number of heparin-binding lectins have been isolated from higher animals, including humans, whereas only several such lectins have been found in marine invertebrates, and only one similar lectin has been isolated from ascidians [8].

2.2.3. Lectins of Sea Worms

Only limited data on lectins of sea worms is now available as compared to the data on lectins of other sea invertebrates. Isolation, physicochemical properties, and in some cases biological activity and primary structure of such lectins have been described. Amphitritin, a Ca^{2+}independent GlcNAc-specific lectin with molecular mass 30 kDa was the first hemagglutinin isolated from a sea worm Amphitrite ornata. Later a number of lectins recognizing Gal, GalNAc, and GlcNAc were isolated from other sea worms (polychaetes, echiurids, sipunculids)and characterized. All these lectins agglutinate human erythrocytes and in some cases also rat, sheep, and rabbit erythrocytes, the maximal activity being exhibited against the latter [66].

Treatment of erythrocytes by trypsin resulted in their enhanced agglutination by lectins. Most lectins are active in the pH range 6.0-9.0, and the presence of bivalent metal cations were not required, although metal-dependent lectins are also found, e.g. a thermally stable lectin from the coelomic liquid of Sipunculus nudus. Molecular masses of the isolated lectins vary within the range 12-36 kDa, some of the lectins are monomers, and in several cases they appear to be dimeric or even tetrameric proteins; this suggests the presence of disulfide bonds between cysteine residues in the lectin molecule. The destruction of oligomeric structure by addition of β-mercaptoethanol can result in complete loss of hemagglutinating activity, as shown for the lectin from the coelomic liquid of S. nudus. Most of isolated lectins are glycoproteins; carbohydrate content varies within the range 2-13%, and the amino acid composition is characterized by increased content of acidic amino acids [36].

Hemagglutinating activity of lectins is inhibited by simple sugars: Gal, GalNAc, GlcNAc and their derivatives, Gal-containing oligosaccharides (lactose, melibiose), and in some cases by glycoproteins: mucin, fetuin, and their desialized derivatives and also by N-glycans. The effect of the various substituents and also their steric position in hapten molecules has been studied; introduction of para-nitrophenyl aglycon and also the methyl group at C-1 in α- and β-positions can result in decrease as well as increase in the inhibiting properties of a ligand. Thus, lectin from coelomic liquid of S. nudus demonstrates significant specificity to α-anomers (Me-α-D-Galp), while others, e.g. lectins from Chaetopterus variopedatus, the echiroid worm Urechis unicinctus, and two annelid worms Neanthes japonica and Marphysa sanguine to β-anomers. Lectins from Perinereis nuntia and Serpula vermicularis demonstrate specificity to both α- and β-anomers of Gal and GlcNAc, respectively. It is experimentally proved that some lectins of sea worms, e.g. U. unicinctus, N. japonica, M. sanguine, P. nuntia, are bound to endogenous ligands in the animal body, and that is why lectins are extracted in the presence of a hapten [66].

A Ca2+ independent Gal-specific lectin (30 kDa) named CVL has been isolated from the sea worm Chaetopterus variopedatus and characterized in our laboratory. CVL exhibits specificity to β-D-Galp. It was also shown that mucin, fetuin, and their desialized derivatives are efficient inhibitors of hemagglutination. Desialization of fetuin is accompanied by a significant increase in its inhibiting activity, while the desialized mucin exhibits the same activity as the initial glycoprotein. The binding properties of the lectin do not depend on the presence of bivalent cations.

Two new Ca^{2+}independent lectins were isolated, SVL-1 and SVL-2, from the sea worm Serpula vermicularis. Both these lectins are oligomers: two subunits are bound by disulfide bonds and organized in a tetramer. Such structural organization of lectins is rather rare, though similar tetrameric structures have been described for lectins from the tropical sponges Aplysina archeri and A. lawnosa. It was shown that hemagglutinating activity of SVL-1 is inhibited by the branched mannans and glycoproteins with O-bound carbohydrate chains such as mucins, while SVL-2 is a GlcNAc-specific lectin. As shown by solid-phase lectin–enzyme analysis, N-glycans with GlcNAc terminal residues ovomucoid and ovalbumin are efficient inhibitors among glycoproteins [18].

2.2.4. Lectins of Sponges

Hemagglutinins in sponges were first found in. Then lectins from various species of these invertebrates were isolated and rather well characterized from the following species: Aaptos papillata, Geodia cydonium, Axinella polypoides, Aplysina archeri and A. lawnosa, Aphrocallistes vastus, Haliclona cratera, Suberites domuncula, Halichondria panacea, H. okadai, Cinachyrella alloclada and Desmapsama anchorata. Most of these studies were done in Germany, Japan, and Brazil [41].

Studies of 48 species of sponges from various regions of the world's oceans have shown that lectins are constituents of 42% of sponge species. Isolated and characterized lectins of sponges usually are low molecular weight proteins, agglutinate human erythrocytes of ABO groups, and exhibit specificity to Gal, Lac, and GalNAc. Some species contain several lectins with different or equal specificity; in the latter case they differ in amino acid composition and isoelectric point.

There are fewer lectins exhibiting specificity to other monosaccharides and glycoproteins. Three lectins (I, II, and III) with different molecular mass, isoelectric point, amino acid composition, and carbohydrate specificity have been isolated from the sponge A. papillata. Lectins II and III have maximal affinity to GalNAc, but their activity is also inhibited by GlcNAc and sialic acid. Lectin I has maximal affinity to ligands containing terminal GlcNAc residues. Hemagglutination caused by a lectin from the sponge H. panacea is inhibited by fetuin, GalA, GlcA, and Fuc. Mucin-binding lectin CAL inhibiting growth of E. coli K1 has been isolated from the sponge Craniella australiensis. For most lectins of sponges, the presence of metal cations is not required for exhibiting activity. Clustering of sequences of lectins from various species with subsequent comparison in pairs has shown that lectins of sponges could be precursors of the S-type lectins of vertebrates [41].

2.2.5. Lectins of Red Algae

Among marine hydrobionts, algae along with marine invertebrates attract attention of researchers as potential sources of lectins. Accessibility of algae and their possible cultivation in large volumes is accompanied by complexity of their protein isolation caused by the presence of pigments in large amounts; that is why a few lectins have been isolated from marine plants as compared with terrestrial ones, and only a few lectins have been completely characterized. The presence of lectins in 24 algae species from the seacoast of Puerto Rico was first reported by

Boyd in 1966. Most lectins have been isolated from red algae such as Cystoclonium purpureum, Palmaria palmata, Eucheuma serra, Enantiocladia duperreyi, Amansia multifida, Gracilaria verrucosa, G. ornata, G. cornea, and Hypnea cervicornis.

Algal lectins differ from plant lectins in certain properties. Lectins of red algae mainly have low molecular masses, and their isoelectric points are within the range 4.0-6.0. Most of them, e.g. lectins from the red algae Carpopeltis flabellata, Solieria robusta, Bryothamnion seaforthii, B.triquetrum, Pterocladiella capillacea, G. ornata, and G. cornea are monomeric proteins with high content of acidic amino acids and do not require the presence of bivalent cations for exhibiting their biological activity. These lectins do not bind to monosaccharides, and their activity is inhibited by complex oligosaccharides and/or glycoproteins. However, it is known that some lectins from red algae are tetramers (G. verrucosa, trimers Ptilota filicina, P. serrata, and P. gunneri or dimers Palmaria palmata and Vidalia obtusiloba). Their hemagglutinating activity can be metal-dependent and inhibited by simple sugars and in some cases by glycoproteins. A lectin from P. gunneri is not inhibited by glycoproteins [36].

Lectin TCL was isolated from the red alga Tichocarpus crinitus; this is a monomeric metal-independent glycoprotein. TCL activity is inhibited by glycoproteins mucin and fetuin, but it is not inhibited by simple monosaccharides. Specificity, molecular organization, and physical and chemical properties such as thermal stability, pH dependence, and pI of TCL are similar to those of other lectins from red algae, e.g. Carpopeltis flabellate, S. robusta, G. bursa-pastoris, G. ornata, and G. cornea. In spite of the progress attained in biochemical characterization of lectins from marine algae, additional information is required for further understanding of theirproperties, structure, and biological functions [34].

2.3. Biological Activity

Functions of lectins in nature are variable and based on their ability to recognize and bind carbohydrate fragments of glycoconjugates either in solution or on a cell surface. Lectins are involved in various physiological processes, functioning as cell-surface receptors, participating in intercellular interactions in the course of cell development and differentiation, and fulfilling a protective function as participants of the innate immunity. Interactions between humoral factors, hemocytes, and target cells resulting in phagocytosis and encapsulation of xenomaterial play a key role in recognition of nonself tissues and cells. The agglutinizing and opsonizing plasma

factors are most often lectins. Lectins in plasma of clams are major proteins, that is, their relative concentration is rather high. They selectively agglutinate gram-positive and gram-negative microorganisms associated with marine invertebrates in nature. Agglutination allows immobilization of a pathogen and prev ents its spread and penetration into other tissues. Binding and phagocytosis of a microorganism by a hemocyte is defined by the presence of carbohydrate structures on the bacterial surface and lectins on the hemocyte surface [34].

Acting as opsonins, humoral lectins can bind to the microorganism surface by one binding site and to the hemocyte surface by another binding site, thus forming a bridge between a phagocyte and a bacterium. The role of specific lectins in the parasite–host system is noted. Certain lines of mollusks, producing large amounts of lectins that agglutinate sporecysts of parasites, appear to be resistant to infection. This fact suggests that lectins are bioregulators in systems of symbiosis of animal organism with microorganisms and parasites of invertebrates. While studying antibacterial activity of lectins of marine hydrobionts in experiments in vitro, it has been shown that they can exhibit inhibitory as well stimulatory effects on growth of gram-positive and gram-negative bacteria. This depends on specificity of each lectin and its ability to bind to carbohydrate chains on the surface of the bacterial cell. In recent years, many papers on estimation of expression of lectin mRNA in response to bacterial invasion have been published. The lectins are supposed to be involved in recognition and removal of bacterial pathogens from the mollusk organism [60].

The interaction of lectins were studied CGL, DTL, DTL-A, and TCL with some representatives of gram-positive (Bacillus subtilis, Salinibacterium amurskyense, Staphylococcus aureus) and gram-negative (Arenibacter troitsensis, Chryseobacterium scophthalmum, Escherichia coli, Pseudomonas aeruginosa) bacteria and also fungi (Candida albicans). As shown by agglutination, solid-phase lectin-enzyme analysis, and turbidimetry, concentration-dependent binding is observed for all lectins. This binding is destroyed by specific inhibitors, and thus the carbohydrate–protein nature of the interaction is supported. This indicates the presence of carbohydrate structures specific to these lectins on the surface of bacteria [22].

Binding to specific carbohydrate receptors on a cell surface, lectins activate these receptors and thus initiate a signal cascade. If the cells are involved in the immunity system, binding to lectin can induce a specific cell response, including induction of secretion of cytokines. For certain

lectins, cytokine-inducing activity has been shown in experiments with human and murine immunecompetent cells. As shown in experiments with human peripheral blood cells, lectins CGL, DTL, DTL-A, SVL-1, SVL-2, and TCL isolated by us enhance synthesis of anti-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ. Moreover, CGL and TCL decrease overexpression of TNF-α and IL-6 on stimulation of cells by LPS, thus exhibiting immune modulating properties. MTL, a lectin from a mussel, also stimulates the induced and spontaneous production of anti-inflammatory cytokines TNF-α and IFN-γ. In a case of induction of cells by LPS, it was observed some decrease in the level of anti-inflammatory cytokine IL-4, especially at low concentration of lectin; this indicates that lectin exhibits immune modulating activity. In the last six years several papers on cytokine-like molecules of invertebrates, comparative immunology, and evolution of cytokines have been published. In these papers the terms "putativ e cytokine", "cytokine-like", etc. have been used for the first time. The data suggest that lectins are factors stimulating production of cytokine analogs in mollusks. One more property of lectins has been used for many years while studying cell proliferation. Some lectins are mitogens, activating lymphocytes and inducing their division. It has been shown that such activity is first typical ofphytolectins such as concanavalin A (Con A) and phytohemagglutinin. Some lectins therewith selectively affectonly T- or B-lymphocytes, while others stimulate proliferation of both groups of lymphocytes. The produced effect depends on concentrations used, valence, and/or molecule dimensions. Lectin often causes a cytotoxic effect on lymphocytes on increasing above the optimal concentration. Several new lectins exhibiting mitogenic activity have been isolated from marine hydrobionts during the last 20 years. This activity is mainly typical of lectins isolated from spongesand marine algae [46].

As for lectins isolated in our laboratory, only TCL and SVL-1 stimulate growth of lymphocytes; however, they appeared to be significantly weaker activators than the well-known phytomitogen Con A. It should be noted that for exhibiting mitogenic activity, significantly higher concentrations of lectins from marine hydrobionts are required than those of phytolectins.

Today much attention is given to lectins of marine hydrobionts, first of all algae, exhibiting anti-HIV activity. In contrast to the antiviral preparations used in medicine, aimed at suppression of the life cycle of HIV, lectins prevent penetration and further propagation of virus, binding to carbohydrate chains of glycoproteins of its envelope and blocking association of virion with

receptor on cell membrane. In this case the highly mannose-containing N-bound oligosaccharide chains act as ligands for the lectin. A lectin exhibiting affinity to Glc, Man, and GlcNAc and a high anti-HIV activity was isolated from the red alga Griffithsia spp. in 2005. Further studies have demonstrated that this lectin, named griffithsin, is active against atypical pneumonia and other pathogenic coronaviruses, hepatitis C virus, and Japanese encephalitis virus. This lectin does not exhibit mitogenic activity towards human mononuclear cells and does not affect production levels of the main cytokines, so why further development of antiviral preparations based on griffithsin is perspective [35].

Lectin KAA-2 exhibiting antiviral activity against a wide variety of influenza strains including swine influenza virus H1N1 has been isolated from another red alga, Kappaphycus alvarezii. Lectin BCA isolated from the green alga Boodlea coacta is also active against influenza viruses of A and B types and HIV. Lectins isolated by us also demonstrate antiviral activity. As shown by in vitro experiments, CGL (mussel), DTL and DTL-A (ascidian), and SL V-1 and SL V-2 (sea worm) lectins exhibit antiviral activity against HIV, this activity being increasing in the following series: CGL, DTL-A, SVL-2, DTL. Lectin CVL from a sea worm inhibits the cytopathic effect induced by HIV-1. Probably lectins block viral proteins gp120 or gp41, which are glycoproteins with highly-mannose and hybrid types of carbohydrate chains, and thus hinder binding [35].

2.4. Practical Applications of Lectin

2.4.1. Anti-insect Activity of Lectins

The epithelial cells along the digestive tract of phytophagous insects are directly exposed to the contents of the diet and, therefore, are possible target sites for plant defense proteins. Since glycoproteins are major constituents of these membranes, the luminal side of the gut is literally covered with potential binding sites for dietary lectins. One can easily imagine that when the binding of a lectin to a glycoprotein receptor provokes a local or systemic deleterious effect the insect may be repelled, retarded its growth, or even killed [67].

Lectins have been suggested as one of the promising agents against insect pests and have been engineered successfully into a variety of crops including wheat, rice, tobacco, and potatoes. This

approach could be used as a part of integrated pest management strategies and caveat pest attack. In general, it seems that large-scale implementation of transgenic insecticidal and herbicide-tolerant plants does not display considerable negative effects on the environment. Moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerable reduces the load of chemical insecticides and herbicides.

2.4.2. Antifungal Activity of Lectins

Since plant lectins cannot bind to glycoconjugates on the fungal membranes or penetrate the cytoplasm of the cells because of the presence of a thick and rigid cell wall, a direct interference with the growth and development of these organisms (i.e. through an alteration of the structure and/or permeability of the membrane or a disturbance of the normal intracellular processes) seems unlikely. However, indirect effects based on the binding of lectins to carbohydrates exposed on the surface of the fungal cell wall are possible [67].

Despite the large numbers of lectins and hemagglutinins that have been purified, only a few of them manifested antifungal activity. The expression of *Gastrodiaelatalectins* in the vascular cells of roots and stems was strongly induced by the fungus *Trichodermaviride*, indicating that lectin is an important defense protein in plants. Plant lectins can neither bind to glycoconjugates on the fungal membranes nor penetrate the cytoplasm owing to the cell wall barrier. It is not likely lectins directly inhibit fungal growth by modifying fungal membrane structure and/or permeability. However, there may be indirect effects produced by the binding of lectins to carbohydrates on the fungal cell wall surface. Chitinase-free chitin-binding stingin nettle (Urticadioicalectin) impeded fungal growth. Cell wall synthesis was interrupted beause of attenuated chitin synthesis and/or deposition. The effects of nettle lectin on fungal cell wall and hyphal morphology suggest that the nettle lectin regulates endomycorrhizal colonization of the rhizomes. Several other plant lectins inhibit fungal growth. The first group includes small chitin-binding merolectins with one chitin-binding domain, e.g., hevein from rubber tree latex and chitin-binding polypeptide from Amaranthuscaudatus seeds. The only plant lectins that can be considered as fungicidal proteins are chimerolectins belonging to the class I chitinases. However, the antifungal activity of these proteins is ascribed to their catalytic domain.

2.4.3. Lectins-antitumor Drugs

Some plant lectins were previously used as simple tumor recognition tools to differentiate malignant tumors from benign and the degree of glycosylation associated with metastasis. In the recent years, they have been developed for utilization as sophisticated microarray for better recognizing malignant tumors in diagnosis and prognosis of cancer. On the other hand, these lectins have been well-known to possess antitumor activities, via targeting programmed cell death (PCD), which is a cell-intrinsic mechanism for eliminating harmful cells and maintaining homeostasis, including apoptosis and autophagy [29].

2.4.4. Lectins-antiviral Drug

Plant lectins have been reported to inhibit viral infection, replication, or systemic spread. Although this lack of evidence does not preclude a possible antiviral effect of plant lectins, it seems logical in view of the absence of glycans on plant viruses. It is worth mentioning in this context that several plan lectins are potent inhibitors in vitro of animal and human viruses, which have glycoproteins in their virions. Some plant lectins may have an indirect antiviral role. For instance, the presence of insecticidal lectins may prevent and/or reduce the spread of insect-transmitted viral disease [67]. Many studies have shown that the envelope proteins gp120 and gp41 of HIV-1 are highly glycosylated consisting of N-linked carbohydrates. The glycosylation provides a formidable barrier for the development of a strong antibody response but at the same time provides a potential site of attack by the innate immune system through the MBL [26].

The surfaces of retroviruses such as human immunodeficiency virus (HIV) and many other enveloped viruses are covered by virally-encoded glycoproteins. Glycoproteins gp120 and gp41 present on the HIV envelope are heavily glycosylated, with glycans estimated to contribute almost 50% of the molecular weight of gp120. Agents that specifically and strongly with the glycans may disturb interactions between the proteins of the viral envelope and thecells of the host. Sugar-binding proteins can crosslinkglycans on the viral surface and prevent further interactions with the co-receptors. Unlike the majority of current antiviral therapeutics that acts through inhibition of the viral life cycle, lectins can prevent penetration of the host cells by the viruses.

Monocot mannose-binding lectins, e.g. *Liliaceae, Amyryllidaceae*, and *Orchidaceae*lectins, exhibit anti-retroviral activity. That is possible for applications in crop protection field. Pleurotusostreatuslectin (POL) has also been tested for its ability to inhibit HIV-1 reverse transcriptase. No antiviral activity has been displayed but instead POL exerted a powerful antitumour activity [15]. Plant lectins have been isolated from bark, cladodes, flowers, leaves, rhizomes, roots and seeds. Alternatively, plant recombinant lectins have been expressed in heterologous systems. Plant lectins can be glycosylated molecules and staining on polyacryamide gel specific for glycoprotein can easily reveal the presence of glycan in the lectin structure; carbohydrate moiety characterization can be performed after lectintryptic digestion in gel followed by enzymatic deglycosylation and mass spectrometric analysis. The compact globular structures, molecular aggregation and glycosylation in general result in high structural stability of lectins [42].

The D-mannose-specific lectin from Gerardiasavaglia was firstly reported to prevent infection of H9 cells with human immunodeficiency virus (HIV)-1. Furthermore, the lectin inhibited syncytium formation in the HTLV-IIIB/H9-Jurkat cell system and HIV-1/human lymphocyte system by reacting with the oligosaccharide side chains of the HIV-1 gp120 envelop molecule (high-mannose oligosaccharides). A year later, the lectinsconcanavalin A, wheat germ agglutinin, Lens culinaris agglutinin, Viciafaba agglutinin, Pisumsativum agglutinin and phytohaem (erythro) agglutinin were found to bind to gp120. They were able to inhibit fusion of HIV-infected cells with CD4 cells by a carbohydrate-specific interaction with the HIV-infected cells. Plant lectins displayed anti-coronaviral activity, especially mannose-binding lectins, in severe acute respiratory syndrome coronavirus. They interfered viral attachment in early stage of replication cycle and suppressed the growth by interacting at the end of the infectious virus cycle (Banana (Musa acuminate) lectin inhibited HIV replication.

The treatment of AIDS with lectins was further investigated. Different lectins have different anti-HIVmechanisms. More recently, lectin from the polychaete marine worm Chaetopterusvariopendatus inhibited cytopathic effect induced by HIV-1 and the production of viral p24 antigen. The sea worm (Serpulavermicularis) lectin suppressed the production of viral p24 antigen and cytopathic effect induced by HIV-1.

Antiviral activity of a number of lectins that bind high-mannose carbohydrates has been described in the past. Examples of such lectins include jacalin, concanavalin A, Urticadiociaagglutinin, myrianthusholstiilectin, P. tetragonolobuslectin, and Narcissus pseudonarcissuslecin. Lectin binds to high mannose carbohydrate structures, including those found on viruses containing glycosylated envelope proteins such as human immunodeficiency virus typ-1 (HIV-1). Therefore, we hypothesized that BanLec might inhibit HIV-1 through binding of the glycosylated HIV-1 envelope protein, gp120.

2.4.5. The Antibacterial Activity of Lectins

The human collectin, mannose binding lectin (MBL) is one of the important components of innate immunity. It provides first line of defense by its ability to bind sugar residues on the bacterial surface through its carbohydrate recognition domain and activates the complement pathway leading to lysis of bacteria independent of antibody. Three point mutations in exon 1 of the MBL gene impair the expression of functional protein leading to a MBL deficient state, which leads to recurrent infections. Once identified, this state could be treated by MBL replacement therapy. MBL isolated from pooled human plasma has been used in phase 1 clinical trials for replacement therapy [26].

The cell wall of bacteria not only precludes any interaction between the glycoconjugates on their membrane and carbohydrate binding proteins but also prevents these proteins from penetrating the cytoplasm. Therefore, plant lectins cannot alter the structure and/or permeability of the membrane or disturb the normal intracellular processes of invading microbes. Therefore, if lectins play a role in the plant's defense against bacteria, it must be through an indirect mechanism that is based on interactions with cell wall carbohydrates or extracellular glycans. It has been suggested, for instance, that the potato lectin (which is as a cell wall protien) immobilized a virulent strains of pseudomonas solanacearumin the cell wall virulent strains were not recognized by the lectin, escaped attachment to the cell wall, and therefore were able to multiply and spread over the plant. Unfortunately, the presumed antibacterial activity of the potato lectin was inferred from in vitro experiments with lectin preparations of unknown purity. Consequently, the results have to be interpreted with care [67].

2.4.6. HIV Infection

In 2007, 33.2 million people were living with HIV, with 205 million new infections and 2.1 million deaths. The global prevalence of HIV is increasing due to ongoing acquisition of HIV, despite preventative measures, and longer survival time. Mortality is decreasing, largely owing to improved management, particularly with the introduction of highly active antiretroviral therapy (HAART), but only 24% of sufferers worldwide have access to antiretroviral therapy. The efficacy of treatment, even when available, is limited by the development of viral resistance and drug related side effects, and prevention and treatment of HIV remains a significant challenge [58].

Researchers at the University of Michigan have used molecular modifications to separate the antiviral and mitogenic functions of Banlec, a lectin that they have previously identified as a potent inhibitor of HIV replication that markedly reduces the replications of a range of HIV 1 isolates. Testing of the mutants revealed that the modification reduced potential mitogenic activity while maintaining potent anti-HIV activity, with potent IC50 values in the nanomolar range [12].

2.5. Techniques of extraction

2.5.1. Extraction in common buffers with overnight incubation

One gram of samples was homogenized with 2ml of buffer with varying pH range. Buffers used were acetate, Tris- HCL, borate citrate carbonate phosphate, glycine- HCL glycine- NaOH, PBS (phosphate buffer saline) and TBS (Tris buffer saline). Extraction was done at varying buffer concentration from 10mM to 1M. The overnight incubated samples were centrifuged at 10000 rpm for 10 min at 4°C and the clear supernatant was analyzed in 15% SDS-PAGE.

2.5.2. Extraction in salt with overnight incubation

1g of sample was homogenized in mortar and pestle with 2ml of 1M ammonium sulphate and incubated in 20oC for overnight. Clear supernatant was obtained after centrifuging it at 8000 rpm for 10min at 4oC and the supernatant was analyzed in 15% SDS-PAGE.

2.5.3. Extraction in buffer with PMSF

2ml of 0.1M potassium phosphate buffer pH 7 was used to homogenize 1g of sample in mortar and pestle. 0.1mM PMSF was used in extraction buffer. Clear supernatant was obtained after centrifuging it at 10000 rpm for 20min at 4oC and the supernatant was analyzed in 15% SDS-PAGE.

2.5.4. Extraction in buffer with salt precipitation

1g of sample was homogenized with 2ml of Tris buffer with different concentration starting from 10mM to 100mM at pH-7.5. After incubating the sample for six hours at 4oC it was centrifuged, at 8000 rpm for 10min. The clear supernatant was collected and added with a pinch of calcium chloride and the same centrifugation has followed to remove any precipitation. Resulted supernatant was analyzed in 15% SDS-PAGE.

CHAPTER THREE

3. MATERIALS AND METHODS

These studies were carried out in the bio engineering laboratory, school of chemical and bioengineering, Addis Ababa institute of technology, Addis Ababa University. The project work had three phases. The first phase was extraction, purification and detection of lectin activity. In the process of extraction and purification of lectin there was determination of the optimum activity of lectin under different conditions. After all the optimum temperature, pH and soaking time was determined.

3.1.Chemical and Materials

Banana, acetic acid, sodium hydro phosphate, sodium chloride, imidazole, sodium hydro oxide, sephadex are some of the chemicals and materials used for this thesis work. And these chemicals were bought from chemical suppliers (atomic educational materials supply PLC micron and Neway import-export and commissions agent). The equipment used for this study were beakers, flasks, mixer, mortar and pestle, refrigerator, oven, pH meter, centrifuge, open column chromatography, test tube, spectrophotometer, pipette and KHB kit.

3.1.1. Lyses Buffer Preparation

It was prepared by using chemicals like sodium hydro phosphate (NaH_2PO_4), sodium chloride (NaCl), imidazole for disinfectants 0.69g , 1.754g, 0.068 respectively and sodium hydro oxide(NaOH) in order to adjust the pH. Because it contains salt this lyses buffer was used to regulate the acidity and osmolarity of the column.

3.1.2. Wash Buffer preparation

Wash buffer was used to wash the salt residues from the column and it was prepared by using 5ml of phosphate buffer which is PH 7, 1.754g of sodium chloride, sodium hydro oxide to adjust the pH at 7 and 0.136g of imidazole.

3.1.3. Elution Buffer preparation

This buffer was used to wash away the unbound protein at first and it releases the desired protein at higher concentration and this buffer is prepared by using the same quantity of chemicals which

was used to prepare wash buffer in the above but the only difference was in this case amount of imidazole was 1.7g. Also this buffer works quickly without changing the function and activity of desired protein.

3.2. Methods

3.2.1. Sample Collection and Preparation

Two types of bananas (ripen and unripen) were purchased from a local store and 1 kg of each sample was pilled and soaked by using 5ml acetic acid and kept it at different range of soaking time (12, 24 & 36 hr) in a plastic beaker and homogenized with same solution by using mixer.

A) Unripen *B) ripen* *c) soaked banana*

Figure 1: Unripe, ripen and soaked banana

3.2.2 Extraction of lectin

The homogenate was kept in the refrigerator at 2^{o}C for 24hours in order to allow the foam to separate from the liquid. After 24 hr the sample was filtered by using cheese cloth. Then the filtrate was kept in an oven at three different temperatures (20, 25 and 30 ^{o}C) for 12 hr and then the pH was adjusted to different range (5.5, 7 and 8.5) by using NaOH. After this it was centrifuged at 6000rpm for 15 minute and kept the supernatant by discarding the pellet.

Figure 2: mixed sample of banana with acetic acid

3.2.3 Purification of the filtrate solution

Open column chromatography was used to purify the filtrate solution which contains lectin. 25 ml of lysis buffer was added to the column as a buffering agent. Then 1ml of the supernatant was applied which contains the banlec into the column. When the whole supernatant was absorbed by the column, 25ml of the wash buffer was added to it. Most of the wash buffers were allowed to leave the column. The last 1ml of wash was collected before proceeding on to the next step and then 25ml of elution buffer was added to pass through the column and finally 1ml fraction of it was collected.

Figure 3: open column chromatography

3.2.4 Quantitative Banana Lectin Analysis

Samples were hydrolyzed at different pH buffers (acidic, neutral and basic) and different temperatures (20, 25 and 30) within different times (12, 24 and 36 hours) in glass-distilled and sealed evacuated tubes. Amount of lectin protein was determined with spectrophotometric smethod using pectin as a standard.

Figure 4: spectrophotometry

3.2.5. Agglutination Assay of Banana Lectin

l00 µl of the lectin sample from ripen banana that had socked for 24 hours, at pH 7 and at 25^{o}C was dropped by using pippete into KHB kit in order to see its activity.

Figure 5: KHB kit

3.2.6. Optimization of lectin extraction and experimental design

Extraction was carried out to produce lectin from banana and optimize various process parameters influencing lectin extraction. The effect of pH (5.5, 7, and 8.5), temperature (20, 25 and 30^{o}C), and soaking time (12, 24 and 36hr) were examined for maximum lectin production. The laboratory experiment was done based on full factorial design with one replication. All parameters were analyzed for their single effects and their interaction effects on optimum lectin production.

Table 1: Experimental factors and levels for ripen sample of banana

Factors	**Levels**		
	Minimum	**Medium**	**Maximum**
Temperature (^{o}C)	**20**	**25**	**30**
pH	**5.5**	**7**	**8.5**
Soaking time (hr)	**12**	**24**	**36**

$$\textbf{Number of run} = r^{*} L^{k}$$

Where

r = replication

L = number of level **number of run = $r^{*} L^{k} = 1^{*}3^{3} = 27$**

K = number of factor

Table 2: Experimental factors and levels for unripe sample of banana

Factors	Levels		
	Minimum	Medium	Maximum
Temperature (^oC)	20	25	30
pH	5.5	7	8.5
Soaking time (hr)	12	24	36

$$\text{Number of run} = r^* L^k$$

Where

r = replication

L = number of level $\quad$ **number of run** $= r^* L^k = 1*3^3 = 27$

K = number of factor

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. Extraction and purification of lectin

Based on the extraction and purification method higher concentration of lectin were found from ripen sample of banana. This indicates that few lectin content were found in the unripe sample of banana and were followed similar procedures to extract and purify lectin from two types of banana the unripe and ripe. All purified samples were observed by using spectrophotometer in order to measure the concentration of lectin protein. Amount of lectin from each sample that were soaked in different pH, temperature and soaking time were recorded in the following table.

Table 3: **Detected Lectin**

Samples	Temperature (0C)	pH	Amount of lectin detected (mg/kg)		
			Fermentation time		
			12 Hours	24 Hours	36 Hours
Unripen banana	20	5.5	0.5	0.6	0.4
		7.0	0.9	1.0	0.6
		8.5	0.8	0.7	0.8
	25	5.5	0.7	0.6	0.5
		7.0	1.3	1.0	1.2
		8.5	1.0	0.9	1.0
	30	5.5	0.4	0.6	0.3
		7.0	1.0	0.9	0.8

		8.5	0.5	0.6	0.7
Ripped banana	20	5.5	1.2	1.5	1.3
		7.0	2.3	2.6	2.4
		8.5	1.8	1.7	2.0
	25	5.5	2.2	2.4	2.3
		7.0	3.4	3.6	3.3
		8.5	3.0	2.7	3.0
	30	5.5	1.0	1.1	1.0
		7.0	2.2	2.4	2.4
		8.5	1.3	1.7	1.2

4.2. Carbohydrate Agglutination Assay of Banana Lectin

100 µl of the lectin were dropped on the KHB kit. Color line were observed after 15 min(agglutination was observed).Throughout purification of the lectin, KHB kit were used as indicator. Agglutination was observed from ripen banana sample that was socked in pH 7, in 24 hours and at a temperature of 25^{o}C when dropped into KHB kit. This indicates that the purified samples had a lectin and this protein binds to the carbohydrate fragment of glycoprotein (gp120) of the KHB kit.

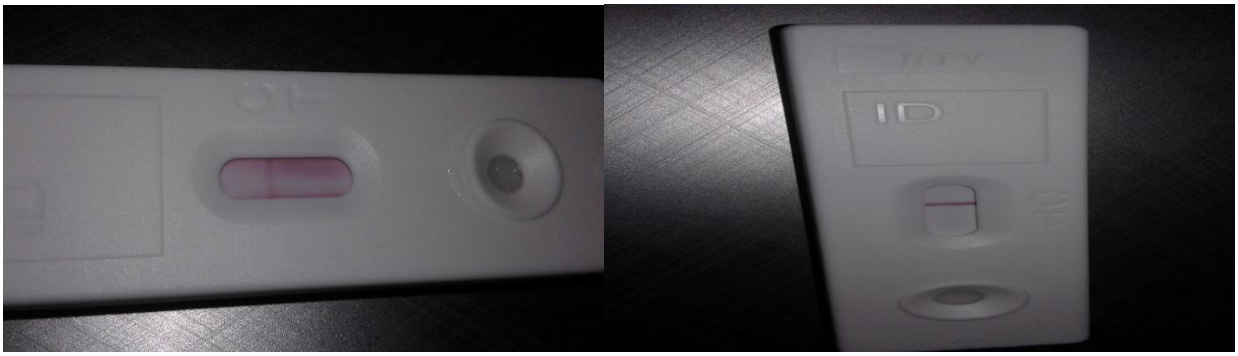

Figure 6: Lectin activity on KHB kit

4.3. Model adequacy check

In this experiment there were three kinds of factors (temperature, pH, and time) for two kinds of samples with one replicate and one response which was amount of lectin. Because of this, the overall experiments were become around 54. The result of each experiment was recorded in the table form. Finally each result was fed to design experiment in order to analyze every factor.

The adequacy of the model was further checked with analysis of variance (ANOVA) as shown at the end of the appendix and the graph of the predicted values obtained using the developed correlation versus actual values is also shown in the next figure.

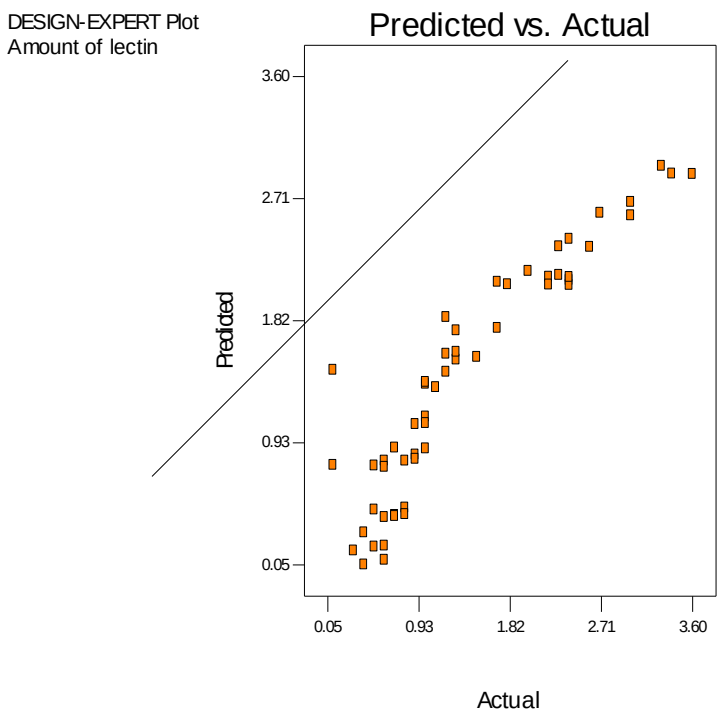

Figure 7: predicted versus actual lectin activity

This plot represents the line of perfect fit with points corresponding to zero error between predicted values and actual values and demonstrated that the regression model equation provided accurate description of the experimental data, in which all the points are close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the three variables to the lectin activity.

4.3.1. Effect of temperature

Temperature is one of the important factors that affect the amount of lectin extracted. Because proteins are highly sensitive to high temperature protein denaturation may result. Temperature shows significant effect on the amount of lectin protein as shown in figure 8. Temperature has both positive effect and negative effect on lectin amount and as temperature increased the amount of lectin was also increased until 25^{o}C but after this temperature lectin amount was decreased as temperature increased. This is due to temperature has significant effect on lectin amount. The present study indicates amount of lectin increases with increasing temperature until

optimum temperature conditions but further increasing of temperature would decrease the amount.

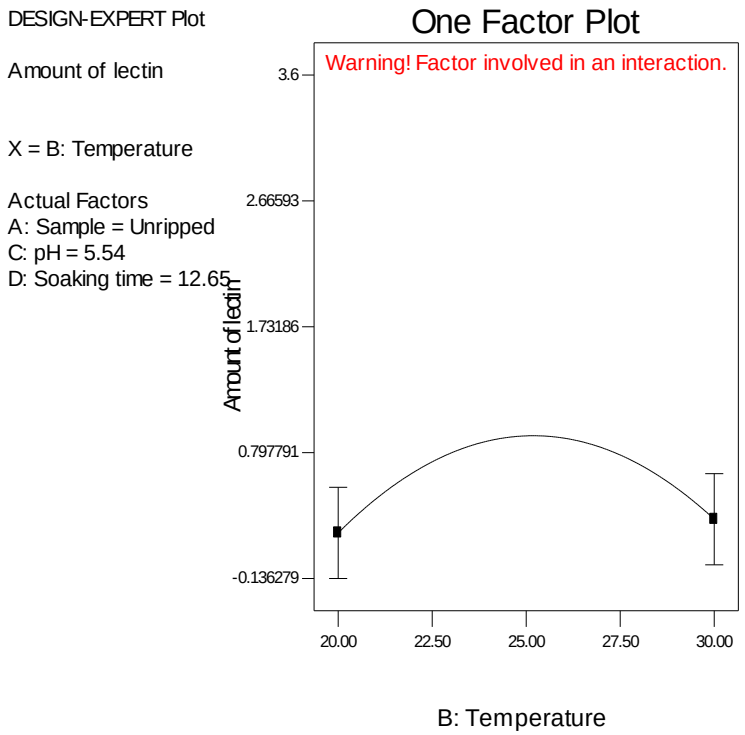

Figure 8: Effect of temperature on lectin amount

4.3.2. Effect of pH

Figure 9 shows the effect of pH on the amount of lectin protein in the present study. Higher amount of lectin was observed at pH of 7. Further increasing of pH decreases the lectin amount the same is true for acidic range.

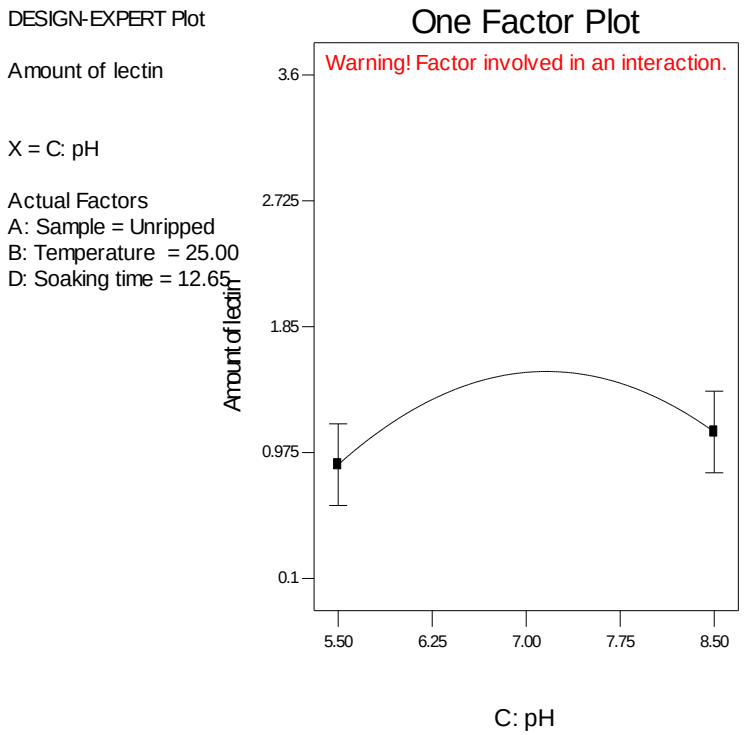

Figure 9: Effect of pH on lectin amount

4.3.3. Effect of socking time

In the present study the amount of lectin was increased from 12 – 24 hour of socking time. But further increment of socking time resulted decreasing of the lectin amount. The time of fermentation had a profound effect on lectin production. The amount of lectin varies with the time duration of the socking process. The reduction in lectin production might be the result of denaturation of the protein due to high temperature, pH and socking time.

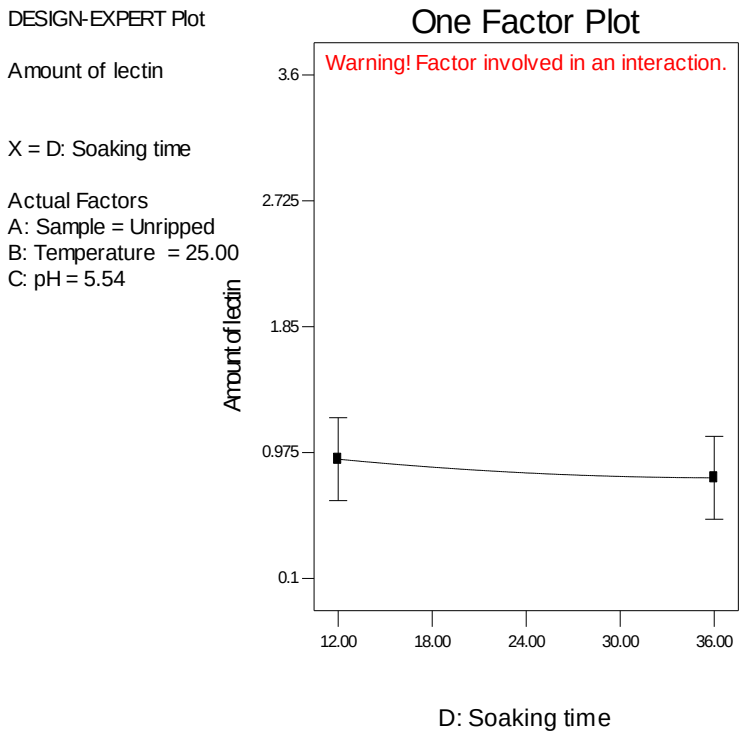

Figure 10: Effect of soaking time on lectin amount

4.3.4. Effect of interaction between the factors

The factors were found to have significant interaction effects. The following Figures shows the interaction effects between pH and temperature; and the other figure shows the interaction effects of temperature and soaking time on the activity of lectin. This indicates that the interaction effect between temperature and pH has significant effect on lectin amount.

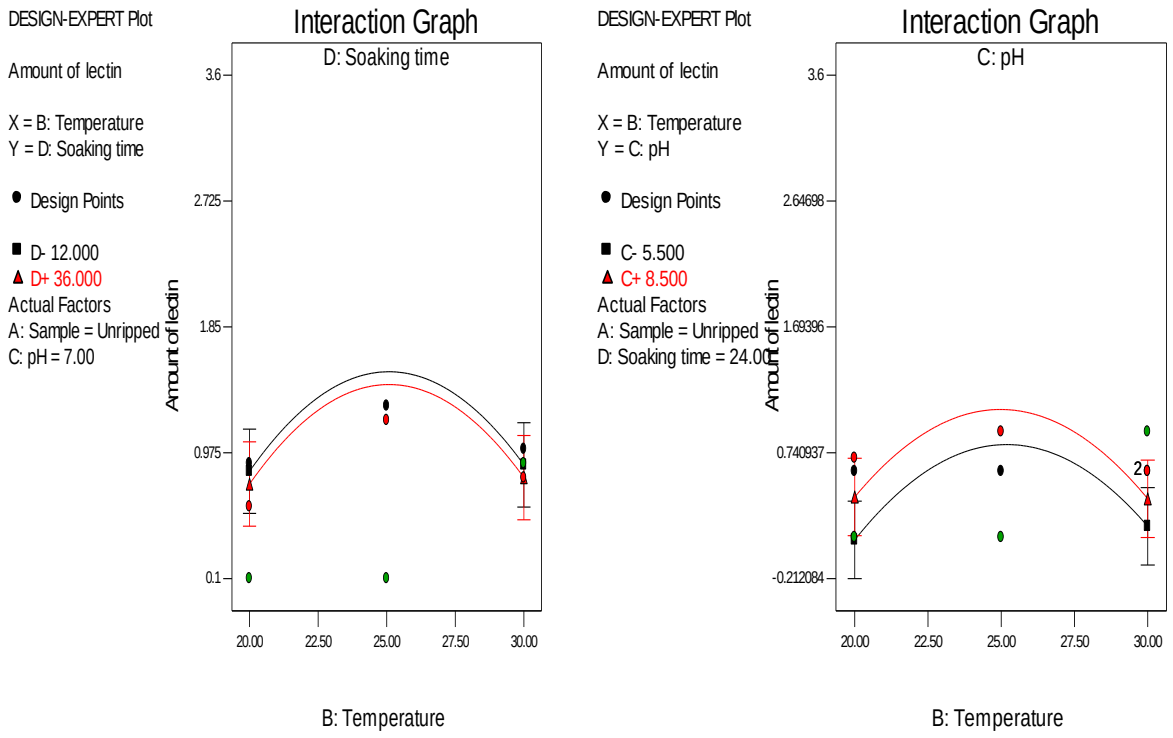

Figure 11: Interaction effects

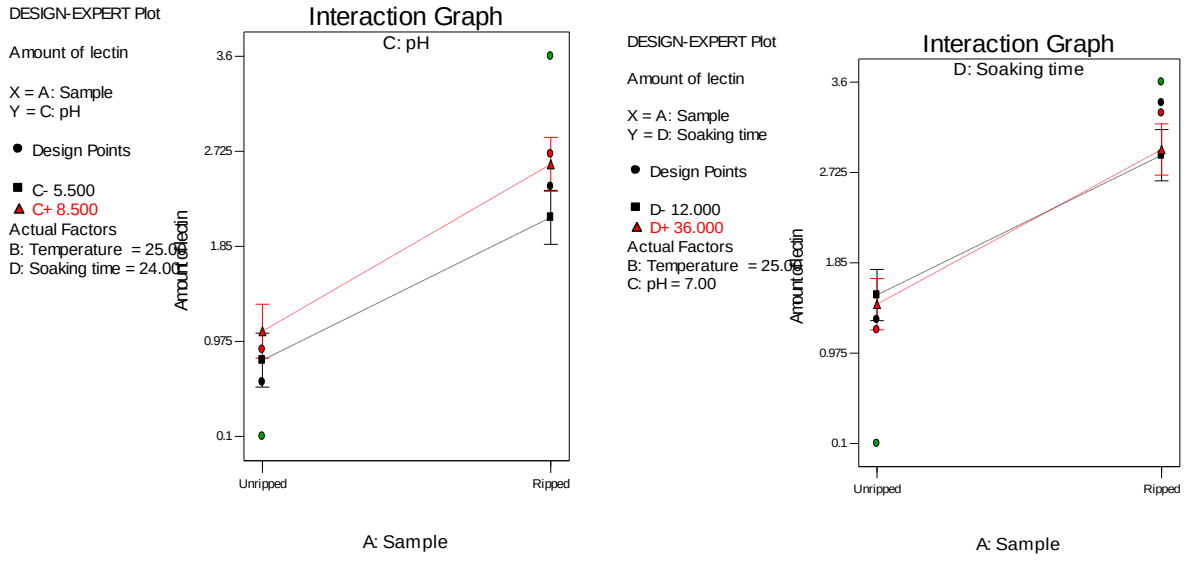

Figure 12: interaction effects between two samples

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, the extraction of lectin from banana using acetic acid for precipitate of the protein has been investigated. The effect of temperature, PH and socking time with their interaction were studied by using full factorial methodology by design expert software, 7.0 for maximum lectin production. After optimum condition was found, the lectin protein activity was observed by using KHB kit .The result shows that efficient lectin producing banana is from the ripen one.

Based on experimental results obtained, it is found that all the process parameters showed significant interaction effect on the yield of lectin protein. These effects were analyzed successfully using design experiment.

Lectin was extracted from ripen and unripen banana. The maximum amount of lectin (3.6mg) was found from ripen banana that was socked in neutral pH, at 25^{o}C and 24 hours. And it was checked its glycoconjugates activity by using KHB kit and agglutination was observed on the wall of the plate.

As socking time increased from 12-36 hours the amount of lectin also decreased. Large amount of lectin was observed in neutral pH than in acidic and basic solutions. Besides as temperature increased from 25-30^{o}C, still amount of lectin decreased. This was due to the break-down of protein molecules in long socking time, acidic and basic solutions and high temperature.

5.2. Recommendations

Depend on the experiment obtained from this study, the following recommendations should be considered for the future work;

- Extraction & purification of lectin have to be performed on highly developed laboratories
- Further studies have to be performed on the activity of lectin for industrial applications
- Further studies on maximizing of lectin amount have to be performed.
- Whether the sample is ripe or not should be studied.

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APPENDIX

Appendix A buffer and reagent preparation

1) Lyses buffer preparation

50mM NaH_2PO_4 0.69g $NaH_2PO_4.H_2O$ (MW 137.99g/mol)

300mM NaCl 1.754g NaCl (MW 58.44g/mol)

10mM imidazole 0.068g imidazole (MW 68.08g/mol)

Adjust pH to 7.0 using NaOH

2) Wash Buffer

50mM phosphate buffer pH 7

300mM NaCl

20mM imidazole (0.136)

Adjust pH to 7.0 usingNaOH

3) Elution Buffer

300mM NaCl

250mM imidazole (1.7)

Adjust pH to 7.0 using NaOH

50mM phosphate buffer pH 7

Appendix B ANOVA for lectin activity

Response: Amount of lectin

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	38.01	13	2.92	17.06	< 0.0001	significant
A	*27.45*	*1*	*27.45*	*160.12*	*< 0.0001*	
B	*0.12*	*1*	*0.12*	*0.71*	*0.4030*	
C	*1.28*	*1*	*1.28*	*7.49*	*0.0092*	
D	*2.500E-003*	*1*	*2.500E-003*	*0.015*	*0.9045*	
B^2	*5.38*	*1*	*5.38*	*31.37*	*< 0.0001*	

C^2	*3.34*	*1*	*3.34*	*19.50*	*< 0.0001*
D^2	*0.011*	*1*	*0.011*	*0.065*	*0.7995*
AB	*0.23*	*1*	*0.23*	*1.36*	*0.2500*
AC	*0.11*	*1*	*0.11*	*0.65*	*0.4255*
AD	*0.047*	*1*	*0.047*	*0.27*	*0.6037*
BC	*0.020*	*1*	*0.020*	*0.12*	*0.7318*
BD	*0.000*	*1*	*0.000*	*0.000*	*1.0000*
CD	*0.010*	*1*	*0.010*	*0.061*	*0.8066*
Residual	6.86	40	0.17		
Cor Total	44.87	53			

The Model F-value of 17.06 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case A, C, B^2, C^2 are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.
If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Std. Dev.	0.41	R-Squared	0.8472
Mean	1.40	Adj R-Squared	0.7975
C.V.	29.61	Pred R-Squared	0.7322
PRESS	12.01	Adeq Precision	13.760

The "Pred R-Squared" of 0.7322 is in reasonable agreement with the "Adj R-Squared" of 0.7975.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.760 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.18	1	0.15	1.87	2.48	
A-Sample	0.71	1	0.056	0.60	0.83	1.00
B-Temperature	-0.058	1	0.069	-0.20	0.081	1.00
C-pH	0.19	1	0.069	0.049	0.33	1.00
D-Soaking time	-8.333E-003	1	0.069	-0.15	0.13	1.00
B^2	-0.67	1	0.12	-0.91	-0.43	1.00
C^2	-0.53	1	0.12	-0.77	-0.29	1.00
D^2	0.031	1	0.12	-0.21	0.27	1.00
AB	-0.081	1	0.069	-0.22	0.059	1.00
AC	0.056	1	0.069	-0.084	0.20	1.00
AD	0.036	1	0.069	-0.10	0.18	1.00
BC	-0.029	1	0.085	-0.20	0.14	1.00

BD	0.000	1	0.085	-0.17	0.17	1.00
CD	0.021	1	0.085	-0.15	0.19	1.00

Final Equation in Terms of Coded Factors:

Amount of lectin	=
+2.18	
+0.71	* A
-0.058	* B
+0.19	* C
-8.333E-003	* D
-0.67	* B^2
-0.53	* C^2
+0.031	* D^2
-0.081	* A * B
+0.056	* A * C
+0.036	* A * D
-0.029	* B * C
+0.000	* B * D
+0.021	* C * D

Final Equation in Terms of Actual Factors:

Sample	Unripped
Amount of lectin	=
-27.77531	
+1.37056	* Temperature
+3.44228	* pH
-0.021991	* Soaking time
-0.026778	* Temperature ^{2}
-0.23457	* pH^2
+2.12191E-004	* Soaking $time^2$
-3.88889E-003	* Temperature * pH
+1.15741E-003	* pH * Soaking time

Sample	Ripped
Amount of lectin	=
-26.20679	
+1.33833	* Temperature
+3.51636	* pH
-0.015972	* Soaking time
-0.026778	* Temperature ^{2}
-0.23457	* pH^2
+2.12191E-004	* Soaking $time^2$

-3.88889E-003 * Temperature * pH
+1.15741E-003 * pH * Soaking time

Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Student Leverage	Cook's Residual	Outlier Distance	Run t
1	0.50	0.18	0.32	0.356	0.973	0.037	0.972	49
2	1.20	1.58	-0.38	0.356	-1.146	0.052	-1.150	42
3	0.70	0.90	-0.20	0.245	-0.550	0.007	-0.545	6
4	2.20	2.14	0.060	0.245	0.166	0.001	0.164	48
5	0.40	0.28	0.12	0.356	0.362	0.005	0.358	23
6	1.00	1.36	-0.36	0.356	-1.087	0.047	-1.090	15
7	0.90	0.85	0.054	0.245	0.149	0.001	0.147	46
8	2.30	2.36	-0.061	0.245	-0.170	0.001	-0.168	5
9	1.30	1.54	-0.24	0.176	-0.633	0.006	-0.628	1
10	3.40	2.89	0.51	0.176	1.352	0.028	1.367	45
11	1.00	0.89	0.11	0.245	0.304	0.002	0.300	12
12	2.20	2.08	0.12	0.245	0.324	0.002	0.321	53
13	0.80	0.46	0.34	0.356	1.023	0.041	1.024	11
14	1.80	2.09	-0.29	0.356	-0.861	0.029	-0.859	51
15	1.00	1.12	-0.12	0.245	-0.341	0.003	-0.337	33
16	3.00	2.59	0.41	0.245	1.147	0.031	1.152	2
17	0.50	0.45	0.054	0.356	0.162	0.001	0.160	17
18	1.30	1.75	-0.45	0.356	-1.355	0.073	-1.370	24
19	0.60	0.081	0.52	0.245	1.443	0.048	1.463	29
20	1.50	1.56	-0.057	0.245	-0.158	0.001	-0.156	40
21	0.60	0.80	-0.20	0.176	-0.537	0.004	-0.532	22
22	2.40	2.12	0.28	0.176	0.754	0.009	0.750	35
23	0.60	0.18	0.42	0.245	1.157	0.031	1.162	44
24	1.10	1.34	-0.24	0.245	-0.660	0.010	-0.656	4
25	0.100	0.77	-0.67	0.176	-1.786	0.049	-1.838	14
26	2.60	2.36	0.24	0.176	0.643	0.006	0.638	52
27	0.100	1.46	-1.36	0.148	-3.567	0.158	-4.265 *	32
28	3.60	2.89	0.71	0.148	1.861	0.043	1.923	38
29	0.90	0.82	0.084	0.176	0.224	0.001	0.221	16
30	2.40	2.08	0.32	0.176	0.850	0.011	0.847	54
31	0.70	0.41	0.29	0.245	0.817	0.016	0.814	3
32	1.70	2.10	-0.40	0.245	-1.124	0.029	-1.128	21
33	0.90	1.07	-0.17	0.176	-0.448	0.003	-0.444	47
34	2.70	2.61	0.094	0.176	0.251	0.001	0.248	41
35	0.60	0.39	0.21	0.245	0.578	0.008	0.573	25
36	1.70	1.77	-0.068	0.245	-0.189	0.001	-0.187	26
37	0.40	0.046	0.35	0.356	1.065	0.045	1.067	34
38	1.30	1.59	-0.29	0.356	-0.887	0.031	-0.884	7

39	0.50	0.77	-0.27	0.245	-0.743	0.013	-0.738	37
40	2.30	2.15	0.15	0.245	0.405	0.004	0.401	8
41	0.30	0.15	0.15	0.356	0.454	0.008	0.450	13
42	1.00	1.38	-0.38	0.356	-1.129	0.050	-1.133	43
43	0.60	0.76	-0.16	0.245	-0.438	0.004	-0.433	19
44	2.40	2.42	-0.017	0.245	-0.046	0.000	-0.046	18
45	1.20	1.45	-0.25	0.176	-0.663	0.007	-0.658	39
46	3.30	2.95	0.35	0.176	0.939	0.013	0.937	36
47	0.80	0.80	-1.852E-003	0.245	-0.005	0.000	-0.005	27
48	2.40	2.14	0.26	0.245	0.726	0.012	0.722	30
49	0.80	0.41	0.39	0.356	1.165	0.054	1.171	9
50	2.00	2.18	-0.18	0.356	-0.552	0.012	-0.547	50
51	1.00	1.08	-0.075	0.245	-0.210	0.001	-0.207	10
52	3.00	2.68	0.32	0.245	0.877	0.018	0.874	31
53	0.70	0.40	0.30	0.356	0.906	0.032	0.904	20
54	1.20	1.85	-0.65	0.356	-1.949	0.150	-2.023	28

* Case(s) with |Outlier T| > 3.50