

**THE EVALUATION OF ANTICARCINOGENIC  
POTENTIAL OF LECTIN ISOLATED FROM  
LEAVES OF *Aloe barbadensis* (Miller) AND  
*Bryophyllum pinnatum* USING HUMAN CANCER  
CELL LINE**

A Thesis

Submitted in partial fulfillment of the requirements for the award of  
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**DOCTOR OF PHILOSOPHY**

in

**(BIOCHEMISTRY)**

By

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### DECLARATION

I hereby declare that the thesis entitled, “**The Evaluation of Anticarcinogenic Potential of Lectin Isolated from leaves of *Aloe barbadensis* (Miller) and *Bryophyllum pinnatum* using Human Cancer Cell line**” submitted for Ph.D. Biochemistry Degree to Department of Biochemistry, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

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### CERTIFICATE

This is to certify that **Ms. Sruchi Devi** has completed the Ph.D. Biochemistry titled, **“The Evaluation of Anticarcinogenic Potential of Lectin Isolated from leaves of *Aloe barbadensis* (Miller) and *Bryophyllum pinnatum* using Human Cancer Cell line”** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma. The thesis is fit for the partial fulfillment of the condition for the award of the degree of Ph.D. in Biochemistry.

Signature of Supervisor

(Dr. Arvind Kumar)

Assistant Professor

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## ABSTRACT

Lectins are the structurally vary diverse and versatile proteins or glycoprotein bind with carbohydrates. Lectins are proteins that are present in plants, animals, and microorganisms. We can define lectins as ‘glycoprotein binds to particular monosaccharaides and oligosaccharides reversibly’. Lectins do not alter the structure of the ligand. Plant lectins have a reorganization role in many biological processes. Its use is spontaneously promoted in various biological, chemistry, and medical science research area. Plant lectins have a crucial role in biomedical sciences such as lectin has anticarcinogenic properties. Plant lectins like galactose and N-acetyl-D-galactosamine have been trials in biomedical sciences as a potential drug for cancer treatment. Binding of lectin to receptors present on the membrane of cancer cell, inhibit the growth of the tumor, cause cytotoxicity, apoptosis. Anticarcinogenic activity of lectins is due to different biochemical mechanisms like reduction of certain tumors, cytotoxic anti-tumor effect, by improving the antineoplastic effects of radiation and chemotherapy, restoration of control growth of cancerous cells, by promoting the immunogenicity of tumor cells. Lectins have different cytotoxicity for malignant or tumor cells w.r.t. normal cell.

Lectin isolated from *Aloe barbadensis* and *Bryophyllum pinnatum* has shown anticancer activity on HeLa (Cervical cancer cell line) and SW480 (Colon cancer cell lines). Aloctin-I and Aloctin-II lectins were isolated from the leaf pulp of *Aloe barbadensis*. Aloctin-I from when tested for specificity is not inhibited by different sugars and N-acetyl-D-galactosamine inhibited the activity of Aloctin-II. It is highly specific for malignant cells. Glycoprotein and polysaccharide are the two compounds of aloe vera have anticarcinogenic property. Different studies indicated that aloe vera gel has an anti-cancer effect. The gel extract suppresses tumor weight, shrinkage of tumor, necrosis, and decreases the survival rate of the tumor.

In clinical research, many herbs have been investigated phytochemically to evaluate and their anti-carcinogenic potential for various types of cancer. Thus, cancer patients who are burdened by chemotherapy and toxic side effects of drugs, have now turned to seek help from traditional medicine for a better cure.

*Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves juice utilized in these researches changed into weighed, minced, homogenized and purified through the

dialysis membrane. The 50% ammonium sulfate is used in the precipitation of lectin from the dialyzed extract. *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin could agglutinate these human erythrocytes. The pH stability of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin was determined by using different buffer ranging from 1pH -13pH. The antioxidant residences of *Aloe barbadensis* Lectin from the gel and *Bryophyllum pinnatum* Lectin from leaves extract had been additionally studied spectrophotometrically by evaluating the free radical-scavenging interest of the DPPH radical (1,1-Diphenyl-2-picrylhydrazyl) and ABTS [2, 2'-azinobis-(three-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging method.

HeLa (cervical cancer cell line) and SW-480 (colon cancer cell line) were selected to study the cytotoxic effects of isolated lectin. HeLa and SW-480 using SRB assay as indicated. The anticancer activity of the isolated lectin has been changed to indicate DNA fragmentation in the human cervical cancer cell line (HeLa) by using PI staining, which may also illustrate possible cell cycles, apoptosis, and necrosis.

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## PREFACE

The present research work was conducted to evaluate the outcome of plant lectin as an anti-cancer agent isolated from leaves of *Aloe barbadensis* and *Bryophyllum pinnatum* on human cancer cell lines i.e. HeLa (Cervical cancer cell line) and SW480 (Colon cancer cell line). The plants were collected from a local nursery of Jalandhar. The authentication of plants has done by Dr. Sanjay Kumar Uniyal (Sr. Principal Scientist & Curator) CSIR-IHBT, Palampur, Himachal Pradesh.

The present research has been done in Toxicological and Pharmacological Lab, CSIR-IHBT, Palampur (H.P.) and can be proved that the lectin extracted from leaves of *Aloe barbadensis* and *Bryophyllum pinnatum* has anticarcinogenic activities.

In this study, the investigation has been carried out on:

1. To isolate *Aloe barbadensis* (Miller) & *Bryophyllum pinnatum* lectin from leaves.
2. To characterize the purified plants Lectin.
3. To study the antioxidant activity of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* Lectin
4. To evaluate the anti-carcinogenic activity of plants lectin by *in vitro* methods using human cancer cell lines.

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## LIST OF ABBREVIATIONS

- #: Percentage
- °C: Degree Celsius
- ABL: *Agaricusbisporus* lectin
- ABTS: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
- APA: Abrin agglutinin
- APS: Ammonium persulfate
- ATCC: American type culture collection
- BSA: Bovine serum albumin
- ConA: Concanavalin A
- COX: Cyclooxygenase 2
- DMEM: Dulbecco's Modified Eagle Medium
- DMSO: Dimethyl Sulfoxide
- DNA: Deoxyribonucleic acid
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- EDTA: Ethylene Diamine Tetra Acetate
- FBS: Fetal Bovine Serum
- FITC: Fluorescein isothiocyanate
- gm.: Gram
- H: Hour
- HA: Haemagglutination Assay
- KDa: Kilo Dalton
- Kg: Kilogram
- L: Liter
- M: Molar
- mg: Milligram
- Min: Minute
- ML: Mistletoe lectin
- mL: Milli Liter
- mM: Milli molar
- mm: Millimeter
- MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

OD: Optical Density  
PBS: Phosphate Buffered Saline  
PCD: Programmed Cell Death  
PHA: *Phaseolus vulgaris* Agglutinin  
PI: Propidium iodide  
PNA: Peanut agglutinin lectin  
PS: Phosphatidylserine  
RAB: Rice Bran Agglutinin  
RBC: Red Blood Cells  
RCA: Ricin Agglutinin  
RIP: Ribosome inactivating protein  
RNase: Ribonuclease  
rpm: Revolutions per minute  
SA: Specific activity  
SBL: SoyBean Lectin  
SDS PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis  
SRB: Sulforhodamine B  
TEMED: Tetramethylethylenediamine  
TCA: *Trichloroacetic acid*  
*TNF- $\alpha$* : *alfa-tumor necrosis factor*  
UEA: *Ulex europaeus* Agglutinin  
VAA: *Viscum album* Agglutinin  
VCA: *Viscum album* colorectum  
WGA: Wheat Gram agglutinin  
 $\mu\text{g}$ : Microgram  
 $\mu\text{l}$ : Microliter  
 $\mu\text{m}$ : Micrometer  
nm: Nanometer

*CHAPTER-1*  
*INTRODUCTION*

## INTRODUCTION

Lectins are a gaggle of proteins that specifically and reversibly share the property of binding to carbohydrates, either free or forming part of many advanced structures. These proteins usually have a minimum of two binding sites per molecule: a selected sugar and a glycosylated molecule. As a specific feature tend to bind to cells (Goldstein *et al.*, 1980). This kind of molecule is distributed in nature, in numerous organisms like microorganisms, fungi, animals, and plants.

A medical student Hermann stillmark presented an initial step of his doctoral thesis in 1888 at the University of Dorpat (Estonia, the Baltic provinces of Russia) that the proteins have a property for agglutinating erythrocytes. As proteins were agglutinated in the blood so initially, these proteins were referred to as haemagglutinins. Stillmark has isolated the first haemagglutinin by the crude extracts from castor seeds botanical name *Ricinus communis* with four other plants from Euphorbiaceae family able to agglutinate blood (Sharon and Lis, 2004). It was a toxic substance and named ricin (regarded as enzyme "ferment") it has an enzymic subunit RNA N-glycosidase which is highly specific for 28S rRNA. As the protein was isolated from a plant so it was named phytoagglutinin also (Franz, 1988). The discovery of ricin played a significant role as a model antigen for immunological studies of Paul Ehrlich (Ehrlich, 1891).

The first pure haemagglutinin to be isolated by the James B. Sumner (American biochemist who got Nobel prize 1946) from jack beans was concanavalin A in short form ConA and its botanical name is *Canavalia ensiformis* by the crystallization of purified seeds extracts (Sumner, 1919). ConA can interact with RBCs as well as carbohydrates like starch, glycogen, and mucins. Carbohydrates like sucrose with low molecular weight prevented this interaction (Sumner *et al.*, 1936). This experiment showed that a carbohydrate bind agglutinin has a significant role in the study of cell membrane constituents. In 1940, Willan Boyd and his co-worker Karl Renkonen made a revolutionary invention of specificity of blood groups for haemagglutinins. By the study of two crude extracts of lima beans (*Phaseolus lunatus limensis*) and Legumiosae, they found that blood group A agglutinated and named as 'lectin'. Lectin is a Latin word '*legree*'. The meaning of the '*legree*' is to pick, choose, or to select. The extracts of *Lotus tetragonolobus* agglutinated the blood

group O (Boyd and Shapleigh, 1954). Landsteiner and van der Scheer described that competitive inhibition between antigen and antibody reaction by haptenic group which is structurally related (Landsteiner and van der Scheer, 1931), haemagglutination of blood group O is inhibited by *Anguilla Anguilla* (eel) blood serum and extract of *lotus tetragonolobus* by  $\alpha$ -linked L-Fucose and agglutination of type A blood group is inhibited by other plant lectins,  $\alpha$ -linked N-acetyl-D-galactosamine (Morgan and Watkins, 1953). These research works were further expanded for biochemical studies of human blood groups and specificity of antigen (Morgan and Watkins, 2000). Lectins are the structurally vary diverse and versatile proteins or glycoprotein bind with carbohydrates. Lectins are proteins that are present in plants, animals, and microorganisms. We can define lectins as 'glycoprotein binds to a particular monosaccharide and oligosaccharides reversibly'. Lectins do not alter the structure of the ligand. Lectin has a basic nature to bind with antibody without any stimulation in the immune system (Peumans and Van Damme, 1995; Brindha, 2016). The lymphocyte mitogenesis effects have been shown by lectins *in vitro* with stimulation and inhibition of lymphocytes in the GI tract. Lectins proteins are resistant to both temperature and digestion because proteolytic enzymes and HCl cannot lyse the lectin proteins. Lectins have been purified from plants and animal tissues by Rocca in 2004.

Subramanyam *et al.*, 2008 were classified lectins according to lectins carbohydrates binding specificity like; galactose binding (Tsvileva *et al.*, 2007), binding to mannose, binding to glucose (Naeem *et al.*, 2007). Based on structural differentiation, lectins can be classified as merolectins, hololectins, chimerolectin, and superlectins. Some lectins like lectin C-erythrine, C-concanavalin, *Ulex europaeuse* lectins, and C- type. Based on affinity, lectins are organized into four groups, galactose glucose/ mannose, N-acetyl-D-galactosamine, L-fucose & sialic acid, (Varki and Cummings, 2009). Due to highly specific interaction for carbohydrates plant lectins are very useful and most studied. Phytolectins play important role in biological research because plant lectins are readily accessible proteins that bind to carbohydrates. The glycoprotein lectins isolated or extracted from plants are specifically suitable to examine, purified, and separate glycoconjugates of animals and humans. Plant lectins have a recognition role in many biological processes

(Gabijs *et al.*, 2002). According to the structural and evolutionary relationship of plant, lectins has been classified into many different groups given as; Lectins of Legume family, Amaranthins, Lectin of Jacalins family (Van Damme, 1998). Some lectins such as proteins include pentavalent domain pentraxins, annexins, G domains glycans on  $\alpha$ -dextrglycan and CD18/CD11b (beta integrins, CR3), fungal glycans, and residues of Glc NAC exposed to glycoproteins (Varki *et al.*, 2009).

Most of the lectins on plants and animals are immune to high-temperature absorption and digestion. Several of these lectins are extremely stable, thermally (for about 30 minutes at 70 ° C), but complete degradation of lectins is not possible with cooking. While several lectins are degraded, the gut is reabsorbed into the blood flow in animals in terms of 1–5 percent, which is taken into consideration by a large quantity, decent to cause a reaction. The lectin's biological roles are hypothetical. Lectins may also be concerned with transporting sugar or storing sugar. Several lectins could also be related to making root nodules by binding-dependent rhizobia. Because of their proper role in adherence and agglutination, in each dependent and morbid interaction between certain microorganisms and hosts, lectins are considered vital. The lectins of the microorganism could play a crucial role in adhering to the microorganism-settled surfaces. Tomato lectins, for example, bind to tissue layer cells and resist acid and chemical process enzymes denaturation (Kilpatrick *et al.*, 1984). Many studies have accepted lectins as specific agglutination for various groups of erythrocytes in human blood. Because of their varied biological applications, lectins were later developed as an analysis of cell surface structures and functions by cell biology scientists. Mannose-binding lectin (MBL) is a predominant constituent of the innate immune system which initiates one of the complement activation, the lectin pathway. The activation of the complement system is also associated with many human diseases (Sunil *et al.*, 2016). Various lectins, together with B and T-lymphocytes, are familiar with fractionate creature cells and show changes in viral or parasite cell surface structure (Nicolson *et al.*, 1974). For the execution of chemotherapy specialists, lectins are utilized as bearers. Lectins are basic materials in microorganisms, protozoa, and more advantageous creatures for analyzing extracellular receptors. For the composing of microorganisms, parasites, and protozoa, the interactivities of plant lectins with microorganisms are relevant. Lectins

are helpful to portray cell parts of microorganisms and to examine receptors of bacteriophage. A few microorganisms contain related surface lectins that change these microorganisms to stick to surfaces. One of the primary endowments of natural science is that cell or surface receptor destinations are mostly portrayed by hapten hindrance (Damjanov, 1987). In humans, lectins, as well as a mass gastrointestinal disorder from the kidney, are reported to cause harm, and it causes hemolytic anemia and jaundice from Mexican fava beans. Lectins, as well as nausea and expulsion, could cause acute gastrointestinal signs. They bind inside the small digestion tracts to the luminal surface of springy erythrocytes. This will cause the intestinal microvilli to endure severe demolition, subsequently disarranging both assimilation and retention. Lectins may likewise advance the extension of the intestinal unsafe microorganism. Lectins conjointly aggravate proteins and supermolecule digestion. In macromolecule osmosis, gut lectins tie to erythrocytes causes irritation that hinders the gathering of catalyst, a macromolecule protein. Just in the event of supermolecule malabsorption, it lessens viscous aldohexose take-up by fiftieth. Nourishment counter-acting agents and various lectins will even tie to hypoglycaemic operator receptors on cells, disturbing aldohexose digestion. Grains can have a high quantity of lectins, which can cause fiery digestive system and celiac infections in humans (Mirelman *et al.*, 1975) and autoimmunity (Vojdani, 2015).

Cancer is the subsequent driving reason for death around the world, constituting the second most frequently diagnosed and deadliest pathology after cardiovascular diseases (Madia *et al.*, 2019). Commonly applied therapeutic options for cancer comprise operation, radiotherapy, and chemotherapy (Ward *et al.*, 2019). Chemotherapy is frequently applied on cancer patients can be divided into four different categories such as a permanent cure by removing malignant cell known as curative, elimination of cancer cells with surgery (adjuvant), shrinkage of the preoperative lesion (neoadjuvant), and symptom alleviation called palliative type (Vaidya *et al.*, 2018; Reoland and LeBlanc, 2016). Generally, the chemotherapy drugs modulate the different molecules using various pathways and suppress target cells. Unfortunately, these anticancer drugs have bad effects on normal cells (Schirrmacher, 2019). Cancer patients often experience poor quality of life during chemotherapy treatments due to side effects including fatigue, insomnia, pain, and nausea/vomiting.



In clinical research, there are many herbs have been evaluated and are investigated phytochemically to provide a better understanding of their anti-carcinogenic actions on various cancers. Thus, cancer patients who are burdened by chemotherapy and toxic side effects of drugs, have now turned to seek help from traditional medicine for a better cure. However, a myriad of many plant products exists that have shown very promising anti-cancer properties *in vitro* but have yet to be evaluated in humans. Further investigation is required to decide the viability of these plant items in treating tumors in people (Desai *et al.*, 2008).

Plant lectins show different binding patterns to cancer cells because of the glycosylation processes and are therefore not only used as an anticancer agent but also as a diagnostic tool (Bhutia *et al.*, 2019). Lectins are found in animals, plants, fungi, and some other microorganisms (Mbae *et al.*, 2018). Plant lectins have many important roles in biomedical sciences, and for example, lectin has anticarcinogenic properties. Plant lectins like galactose and N-acetyl-D-galactoseamine have been preliminaries in biomedical sciences as a potential medication for malignant growth treatment (Ernst *et al.*, 2003). Authoritative lectin to receptors present on the layer of malignant growth cell, hinder the development of the tumor, cause cytotoxicity, apoptosis (de Mejia, 2005). Different biochemical mechanisms such as reducing certain tumors, cytotoxic anti-tumor effects, improving the antineoplastic effects of radiation and chemotherapy, restoring the controlled growth of cancer cells, promoting the immunogenicity of tumor cells are caused by the anticarcinogenic activity of lectins. For malignant or normal tumor cells w.r.t., lectins have different cytotoxicity (Avarez *et al.*, 2002). The lectins obtained from various plant and animal sources can inhibit the growth of cancer cells depending on their concentration in different ways (Pryme, 2001). Lectins stimulate T-lymphocyte proliferation and activate the immune system and can induce apoptosis (Lyu *et al.*, 2001; Choi and Park, 2001). Previous studies focused on lectin cytotoxic such as ricin and abrin as potential cancer therapy (Lin *et al.*, 1970; Dickers *et al.*, 2003). Subsequently, some research using ConA (*Conavali aensiformis*) inhibits hamster tumor growth (Shoham, 1970). Some plant lectins either in their pure or semi pure structure are utilizing against various disease cell lines or tumor-like, Abrin-a, cyto-agglutination against refined human cell lines which are gotten from an all-around created T-cell leukemia

and intense lymphoblastic leukemia (Moriwaki *et al.*, 2000; Ohba and Nakamura, 2000), AHL (*Arisaema helleborifolium*), represses the expansion malignant growth cell lines of human 66% HEP-2, 92% HCT-15 and 95% HOP-62 (Kaur, 2006), DSA (*Datura stramonium*), irreversible separation enlistment of glioma C6 cells, portion subordinate multiplication hindrance and DNA combination concealment (Sasaki and Yamazaki, 2002). Lectin obtained from ML (Chinese Mistletoe Lectin) has some significant effect on T-cell cytotoxicity, cell death (apoptosis), and the production of cytokines. Mistletoe lectin (ML) (*Viscum album*) shows antitumor activity by cytotoxicity, increase tumor necrosis factor (TNF)-alfa inhibit the anti-inflammatory interleukin (IL)-10 (Gong *et al.*, 2004). Recently, plant lectins have shown their attention for selectivity and sensitivity for glycans present on the cancer cell surface (Yau *et al.*, 2015; Coulibaly and Youan, 2017). Glycans are diverse and complex consisting of different monosaccharides, binding sites for glycans, and bond with branching types (Varki *et al.*, 2017). Glycans are mostly linked to N (nitrogen) of asparagine called N-glycans or linked to the oxygen of serine or threonine called O-glycans on secreted or glycoproteins present on the cell membrane. The main applications of glycans are protein folding, cell-to-cell interactions, identification of pathogens, immunity reaction, presentation of antigens, and cell adhesion and migration (Pinho and Reis, 2015). Matured glycoproteins vary N-linked oligosaccharides according to types of cell, tissue, and different species (Goh and Ng, 2017).

The great importance of lectins is principally thanks to their biological properties like interaction with specific blood groups, agglutination lymphocytes, erythrocytes, spermatozoa, platelets, microorganism and tumor cells, induction of cellular division in lymphocytes, and cytotoxic effects on lymphocytes (Sharon and Lis, 2004; Lajolo and Genoves, 2002; Sharon and Lis, 1995). Some applications are; analysis of lymphoproliferative functions and cytotoxic mononuclear cells caused by bound medication, detection of chromosomal abnormalities like fluorescent markers to review structural changes in glycoconjugates on cell surfaces, and sleuthing changes malignant cells, among others (Hernandez *et al.*, 1999). However, it had been not till 1963 (Aub and Tieslau, 1963). It discloses that plant lectins will distinguish between traditional cells and cancer cells which the distinction is on the surface, in

different words, that alteration of cell surface property of cancer cells (Aub and Tieslau, 1963). Due to its specific properties, lectins are used as tools in organic chemistry, cell biology, immunology, genetic science biomedicine, and analytical functions and preparations further as for designation and medical care in cancer (Hernanz *et al.*, 1999; Sharon and Lis, 2004; Guillot *et al.*, 2004). Glycoconjugates on cell surfaces are legendary for their performance in the necessary cell, like recognition, communication, and adhesion (Gabius, 2001; Kobata, 1998). Such interactions also are necessary for tumorigenesis, growth progression, and metastasis (Kannagi *et al.*, 2001; Gabius *et al.*, 1997). Throughout cell differentiation and malignant transformation, the biogenesis of the carbohydrate chains of glycoproteins is commonly altered and these alterations are often detected by lectins (Fan, 2004; Dabelsteen, 1996). Within the studies rumored membrane exploitation lectins to research changes structural on cell surfaces (Sharon and Lis, 2004; Kitamura *et al.*, 2003). In the field of cancer therapy, the study of lectins has vied a crucial role. Different studies *in vivo* and *in vitro* with various plant lectins are shown to possess antineoplastic activity (inhibitory impact on tumor growth) and anticarcinogenic activity (inhibitory effect within the induction of cancer by carcinogens) (Abdullaev and de Meija, 1997). The work according to the victimization of different lectins plants in cancer cases enables the U.S. to know the mechanisms of action of those proteins are varied counting on various factors like the cellular origin, variety of tumor, and glycoprotein concentration.

Since the seventies, anticancer activities of plant lectins are reportable. Intraperitoneal administration of ricin toxin (RCA) and abrin (APA) in evoked mice inhibits the expansion of tumors derived from pathology malignant neoplastic disease Paul Ehrlich (Lin *et al.*, 1970) and up to date reports the mechanism of toxicity each ricin and abrin delineates (Doan, 2004; Dickers *et al.*, 2003). In another study, it was shown that injection of Con-A (*Canavalia ensiformis*) causes inhibition of tumor growth in hamsters (cells papovavirus reworked 3T3) (Shoham and Inbar, 1970). Then henceforth are reportable several studies with totally different plant lectins and their result in tumor cells. In succeeding studies it reportable that intraperitoneal administration of GS1 glycoprotein (*Griffonia simplicifolia*) in mice with pathology cells Ehrlich, conjointly inhibits the expansion of tumors by its cytotoxic impact

(Chen *et al.*, 1994; Knibbus *et al.*, 1994). Alternative authors examined the growth activity of various lectins: common bean plant (PHA), soya bean (SBA), and monocot genus *Triticum vulgare* (WGA) in murine malignant neoplastic disease cells in pathology *in vivo* and located that each lectin strangled growth and raised the chance of life (Ganguly and Das, 1994). Diet PHA in animals stirred up cell pathology growths. Krenzelok II caused tumors to develop additional slowly than the controls and determined that the whole range of tumor cells, proteins, DNA, polymer, and contents of polyamines were reduced compared with controls (Pryme *et al.*, 1994; Mukhopadhyay *et al.*, 1994). Examined the result of the independent agency in dietary supplementation with this glycoprotein in cell growth pathology murine cancer and immune operate within the host. They counsel that the doable antineoplastic mechanism independent agency is also thanks to the strengthening of the system of the host (Mukhopadhyay *et al.*, 1994). Different studies have shown that the result of TMA I and TMA II lectins (*Tricholo mamongolicum*) is to inhibit the expansion of cancer cells one hundred eighty and prolong the lifetime of mice with tumors (Wang *et al.*, 1997).

On the opposite hand, additionally reported studies demonstrating the cytotoxic impact of lectins on growth cells *in vitro*. The study with 5 different lectins: PHA, GSA, Con A, WGA, PNA (*Arachis hypogaea*), on cell growth of 3 cell lines of human body part cancer (LoVo, HCT15, and SW837) is affected otherwise betting on the concentration and sort glycoprotein, closing that these lectins have the potential to affect the expansion of cancer colonies *in vitro* (Kiss *et al.*, 1997). These are reported studies on the specificity of lectins and their binding to carbohydrates in 3 cell lines of human body part cancer (Caco2, HT29, and HCT8) victimization different lectins fluorescently labeled: DBA (*Dolichos biflorus*), PNA, LCA (*Culinary lens*), STL (*Solanum tuberosum*), UEAI (*Ulex europaeus* I), and WGA. The speed of binding to different cell lines has represented, reflective the glycosylation pattern of the cells (Garbor *et al.*, 1998). The particular binding of lectins on sugar residues has enabled additional studies with lectins as ABL (*Agaricus bisporus*) that bind to oligosaccharide I galactosylated expressed in keratinocytes. These studies determined that this glycoprotein reversibly inhibits the proliferation of neoplastic cell lines while

not toxicity and therapeutic potential in things like skin disorder (Parslew *et al.*, 1999).

Comparing binding patterns of lectins in several cell lines of human skin cancer has been analyzed. Glycosylation is typically altered in neoplasm cells compared to its traditional counterpart. This study relatively analyzed patterns of glycoproteins of human skin cancer cells victimization differently labeled glycoprotein (SNA: bourtree, MAA: *Maacki aamurensis* and PHA: rosid dicot genus vulgaris) and suggests that in human skin cancer, the expression of branching and sialilados advanced oligosaccharides N-type cells are augmented pathological process sites. It's additionally advised that carbohydrates are related to the acquisition of pathological process potential of neoplasm cells (Litynska *et al.*, 2001). Extracts from mistletoe are widely used as complementary treatments for cancer in Europe. In these extracts, the presence of lectins has been known because of the main activity. Cell proliferation of sixteen cell lines with liquid mistletoe extracts was investigated, the coverage that growth activity is shown within the cell line of carcinoma in extracts containing high amounts of lectins (Maier and Fiebig, 2002). On the opposite hand, it's already been reportable inhibition of cell human carcinoma with the employment of different lectins (Valantier *et al.*, 2003). It has additionally been created a comparative study of European mistletoe (*Viscum album*) and mistletoe Korean (*Viscum album* var. *Coloratum*), wherever the purification of glycoprotein latter (VCA) shows that the relative molecular mass of the A and B chains are different of the pure lectin European (VAA); but, they found that each showed similar activity toxicity against Molt4 cells (Lyu *et al.*, 2000). The toxicity of lectins is characterized by the power to inactivate ribosomes and is classified as macromolecule RIP II (Olsnes and Kozlov, 2001). Among the pathological lesions delineate with intake or administration of lectins in animals or humans, the presence of parenquimatosi is discovered, fatty degeneration, and a lump in numerous tissues. It's additionally disclosed that lectins bind to glycosylated teams' membranes of animal tissue cells of the canal, preventing the absorption of nutrients, and also the presence of clots within the capillaries of all organs, and native hemorrhage at the positioning of application (Vasconcelos and Oliveira, 2004). Studies aspect effects are rumored on the utilization of bound lectins as anticancer agents. The toxin has been tested by many

native intratumoral and intraarterial patients with tumor application routes; it's rumored a range of results. In many clinical tests with low doses of toxin administered intravenously to patients with cancer, they were tolerated. The symptoms bestowed were like those of a chilly, fatigue, and muscle pain, and typically with nausea and reflex, symptoms beginning four to 6 hours when the administration and lasting one to 2 days. However, for the case of abrin 2 deaths, they were rumored in phase I clinical trials; These patients had general attacks and signs of toxicity within the central nervous system (Franz and Jaax, 1997).

In another study the immunoreactivity was characterized in patients with aspect effects throughout treatment with binary compound extract of European mistletoe, being varied production of TH1 and TH2 that indicates that completely different mechanisms are concerned within the induction of aspect effects. During this study over that the aspect effects of mistletoe are rare and are dominated by a reaction at the positioning of application suggesting the involvement of delayed sort hypersensitivity reactions (Stein and Berg, 1999).

A patient with carcinoma was inoperable duct gland treated and peritumoral injections intraperitumoral extract mistletoe L, containing 5,700 metric weight unit/cubic centimeter glycoprotein. The third injection showed a marked symptom. The general condition of the patient was stable throughout treatment and over that the glycoprotein could also be related to hypereosinophilia additionally because of the production of TH1 and TH2 (Huber *et al.*, 2000). The intensity and therefore the time course of native reactions appear to rely upon the concentration of mistletoe lectins (Huber *et al.*, 2002).

In another study 3 clinical cases wherever there are severe hypersensitivity reactions when injection of mistletoe are according, among two cancer patients and a 3rd as a preventive proposal by the fact that a brother had cancer (Hutt *et al.*, 2001). There presymptomatic studies showing a cytotoxic result and immunoestimulado mistletoe, preponderantly within the immune cell system. The clinical info, however, isn't pretty much as good because of the experimental results. So far, there has been an instantaneous action opposed to cancer or associate improvement in time to neoplasm progression or overall survival of cancer patients. Medical aid with mistletoe has not noninheritable a stable in medicine place, however, within the

future, strategic clinical trials are needed to verify the primary positive conclusions concerning associate improvement within the quality of life of cancer patients (Stauder and Kreuser, 2002). Numerous studies *in vitro* and presymptomatic mistletoe have reported effects of immune stimulation, cytotoxic and proapoptotic. Translation of those effects in clinical responses continues to create a controversy. Whereas varieties of clinical studies have found improved quality of life, effectiveness information mistletoe prolonging survival are contradictory and variable qualities. The information from clinical trials about toxicity and pharmacological medicine of compounds of mistletoe with data *in vitro* or presymptomatic activity is deficient (Mansky, 2002). Given the properties of RIP lectin, few reports have centered on the employment of those for manufacturing immunotoxins for cancer, wherever the glycoprotein or its active half is hooked up to an antibody having a particular receptor website for tumor cells. Ricin, extremely hepatotoxic, has been studied as a part of those antitumor agents. Native albumin or solely the sequence therefrom is conjugated to organism antibodies specific to tumor cells. There are clinical trials with these compounds as anti-cancer agents. Though the results are promising, two factors seem to limit the effectiveness of albumin immunotoxin:

1. Deficiency of specificity to protein.
2. Important Immunotoxigenicidad the explanation for the poison, which ends in a speedy attack of immunity to the therapeutic agent (Franz and Jaax, 1997).

Furthermore, it conjointly reports that the most doses limiting adverse medical care in patients with the immunotoxin fashioned by the chain of albumin impact are vascular infiltration syndrome (Baluna *et al.*, 1996). Kreitman in 1999 could be a review regarding immunotoxin cancer medical care, suggesting that this medical care has potential clinical effectiveness in patients with malignant diseases that are untreatable with surgery, radiation, and therapy. Immunotoxins begin to develop as new antigens for the treatment of cancer (Kreitman, 1999).

*In vitro* and *in vivo* studies of lectins and their contribution to cancer are varied and show that they will evaluate the different biological role, like cell growth, coupling, adhesion, malignant, metastasis transformation, and cell death processes (Abdullaev and de Mejia, 1997; Jordison *et al.*, 1999; Yu *et al.*, 1999; Bantel *et al.*, 1999; Park *et al.*, 2001; Sasaki *et al.*, 2002; Yoon *et al.*, 2003). In recent years the

work that has been through with totally different plant lectins in cancer cells shows that the action of those ranges from sugar-binding specificity to the mechanism of action on the molecular level. The use of glycoproteins as marker molecules in reworked cells has shown that binding lectin is restricted in cell lines and this could mirror different routes of tumor cell progression individual lines (Litynska *et al.*, 2001; Lazaris *et al.*, 2000; Sherwan *et al.*, 2003). In the hunt for agents derived from plants as choices antineoplastic antitumor activity of plant lectins is confirmed, these being a useful gizmo in cancer analysis, yet as for designation and medical care in trendy drugs. The action of lectins ranges from sugar-binding specificity to the mechanism of action at the molecular level. Specificity within the different tumor cell lines might replicate different routes of progression of the latter. At the molecular level, they delineate some apoptotic mechanisms that trigger different lectins. However, it's necessary to stress that the response of the utilization of lectins clinically continues to cause a controversy, since conflicting results are reported, the continuity of analysis associated with plant lectins as an alternate to treatment is important in cancer. It is necessary to gift the adverse impact of lectins in some cases, even the anticarcinogenic properties of lectins are delineated within the studies (Castillo-Villaneva, 2005). Lectins are unhealthful, most of the lectins are isolated from each plant and animal cause different degrees of toxicity (Rhodes, 1999; Tareq *et al.*, 2001). Hypersensitivity reaction and administration route are factors to blame for the unhealthful impact of glycoprotein (de Mejia, 2005). Some studies are centered on the properties of Ribosome-inactivating supermolecule glycoprotein for immunotoxins production against neoplasm cells. RIPs are connected to neoplasm cells with specific receptor binding sites on the antibody. Once several clinical trials, it's been rumored that one of the most important adverse effects that limit of therapeutic dose in patients beneath the treatment for vascular infiltration syndrome (immunotoxins) by ricin toxin a series. Vascular infiltration syndrome (immunotoxins) is additional frequent and more severe within the patient's chemotherapy or radiotherapy (Castillo-Villaneva, 2005).

*Aloe barbadensis* (Botanical name; *Aloe barbadensis*, family; Asphodelaceae, common name; *Aloe vera*, Ghirita, Gawarpaltra, Lu hui, etc.) grow water storage tissue in the leaves. It is perennial succulent xerophytes. Among all 400 species of



*Aloe barbadensis*, 85 are under cultivation throughout the world. The most biologically active species is *Aloe barbadensis*, with 95% medicinal properties. (West and Zhu, 2003; Yagi, 2003). According to a report of WHO, traditional plants are the better source of a variety of allopathic drugs (Sahu *et al.*, 2013). *Aloe barbadensis* belongs to East and South Africa. *Aloe barbadensis* was subsequently produced in North Africa then naturalized in the Mediterranean part and also in other countries. *Aloe barbadensis* is the best commercial plant and cultivated in the United States of America, South Africa, Venezuela, Japan, India, China, Bonaire (Yeh *et al.*, 2003). The best quality *Aloe barbadensis* is found in the desert region of southern California.

In India major producers states of this crop are Maharashtra, Rajasthan, Gujarat, Andhra Pradesh, Tamil Nadu, and Kerala. The local or common names of *Aloe barbadensis* are Ghikanwar and Musambar in Hindi, Kumari in Sanskrit, Ghrithakumari in Bengali. *Aloe perfoliata*, *A. barbadensis*, *Aloe chinensis*, *Aloe littoralis*, *Aloe indica*, *Aloe abyssinica* Varieties of *Aloe barbadensis* cultivated in India (<http://www.agrifarming.in>). *Aloe barbadensis*, *Aloe vulgaris*, AL-1, IC111280, IC111269, IC111271 are some improved Commercial is basically a warm tropical region crop but can also grow in other climatic conditions like low rainfall area and dry regions with warm humidity but sensitive to extreme cold conditions. *Aloe barbadensis* is cultivated on a vast variety of soils from loamy soils to sandy soils. However, it thrives best in light soils. *Aloe barbadensis* plant is sensitive to water circulation, for its cultivation select well-drained soils. Alkaline pH 8.5 of soil can tolerate by the plant. Black cotton soil is good for faster growth. (<http://www.agrifarming.in>), (<http://aloeveracultivation.blogspot.co.in>).

The *Aloe barbadensis* has been used as a medicinal plant from ancient times for its therapeutic and curative properties. Two lectins were isolated, by Suzuki in 1979, from *Aloe arborescens*, a species of *Aloe vera* grown in India, China, the USA, and Japan. In 1993, Saito performed further purification to achieve its biological, chemical, and pharmacological applications and activities. Out of two lectins, one lectin named Aloctin A isolated from 'Kidachi Aloe' botanical name *Aloe arborescens* bind to mannose has haemagglutinating and mitogenic activities (Koike *et al.*, 1995). In 1981, Winters presented a report that fractions prepared from *Aloe barbadensis* leaf gel at different Centrifugation contain a lectin that inhibits cell

growth. This *in vitro*, isolated and characterized fraction of glycoprotein promoting proliferation in hamster & human cells (Yagi *et al.*, 1997). Winters (1993) made a comparative study between haem-agglutination and mitogenicity of lectin-like substances obtained from *Aloe arborescens* gel. 75 active ingredients with medicinal properties from the pulpy gel have been identified (Wiliamson, 2000; Hamman, 2008). It has various biological properties to improve bio-availability of co-administration and absorption of vitamins and drugs with wound healing of wounds, anti-microbial, stimulation of immune response, anti-inflammatory, anti-cancer, and anti-diabetic properties (Nejatzadeh-Barandozi, 2013; Azam *et al.*, 2003). Aloesin, Barbaloin, Lectin, and Aloin A and B are some biological active and chemopreventive constituents of *Aloe barbadensis* that possess anti-metastatic potential immunostimulating, anti-mutagenic, antiproliferative, apoptosis-inducing, and antioxidant (Choi and Chung, 2003). According to some scientific evidence, *Aloe barbadensis* possesses therapeutic (Niciforovic *et al.*, 2007) and preventive applications against various types of cancer (Joseph and Raj, 2010). In 1999, Akev isolated two types of lectin, from the pulp of leaves of *Aloe barbadensis* i.e. aloctin-I and II (Akev, 1999). Aloctin I when tested for specificity has not inhibited by different sugars and N-acetyl-D-galactosamine inhibited the activity of aloctin II. It is highly specific for malignant cells. Glycoprotein and polysaccharide are the two compounds of *Aloe barbadensis* have anticarcinogenic property. In 2007, Akev studied tumor preventive effect of *Aloe barbadensis* leaf pulp lectin (alocetin I) on *Ehrlich ascites* tumors in mice Different studies indicated that *Aloe barbadensis* gel has an anticancer effect. The gel extract suppresses tumor weight, shrinkage of tumor, necrosis, and decreases the survival rate of the tumor (Hamman, 2008). *Aloe barbadensis* is the most known commercialized succulent species. Within the food business, it is supplied as practical foods associate with Nursing as an ingredient in an alternative food product, for the assembly of gel as health juice and beverages. In the cosmetic and toiletries business, it is used as the base material for the assembly of creams, in lotions as a moisturizer agent, soaps, hair cleansers, and conditioners face cleansers and alternative products. In the pharmaceutical trade, it is used for the production of topical merchandise like ointments and preparations of gel, further as within the manufacturing of medicines like tablets and capsules (Eshun and He, 2004;

He *et al.*, 2005). Significant pharmaceutical properties as of late found for every gel of *Aloe vera* and entire leaf remove encapsulate the bioavailability of nutrients co-directed in people (Vinson *et al.*, 2005). Because of its retention improving impacts, *Aloe vera* gel could likewise be utilized to viably convey ineffectively absorbable prescriptions through the oral course of medication organization. Besides, the powder acquired from the gel was with progress acclimated fabricate straightforwardly compressible grid sort tablets (Jani *et al.*, 2007).

*Bryophyllum pinnatum* belongs to the family Crassulaceae. Its common names as Ghayamari, air plant, love plant, life plant, Zakhm-e-hyat, panfutti (Jain *et al.*, 2010). It grows worldwide and is used as folk medicine in many countries like Australia, tropical Africa, India, tropical America, and China. It has a sour taste, astringent but during the post-digestive effect, it has a sweet taste. The plants have many medicinal properties. It has hemostatic and wound healing properties (Kamboj & Saluja, 2009). The chemical compounds isolated from *Bryophyllum pinnatum* are used as folk medicines on large scale for the treatment of many diseases hypertension and kidney stones, (Lans, 2006) pulmonary infections, rheumatoid, arthritis, etc. (Majaz *et al.*, 2011). *Bryophyllum pinnatum* leaves are used as an antifungal (Sofowora, 1993) and antiallergic agent (Pal and Chaudhuri, 1991). *Bryophyllum pinnatum* is an emollient, refrigerant, hemostatic, mucilaginous, depurative, anodyne, disinfectant, constipating and antitonic. The plant proved to be beneficial in vitiated conditions (pitta and vata), epilepsy, piles (Hossan *et al.*, 2009), haematemesis, menorrhagia, healing of wounds, hemorrhoids, skin discolorations, boils, and ophthalmia (Rola *et al.*, 2011).

In the present study, the antioxidant activity with DPPH (1,1-Diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay and anticancer activity against HeLa and SW480 cancer cell line using SRB (Sulforhodamine B) assay with its cell cycle arrest and study of apoptosis and necrosis after 48hours with two lectins AL and BL isolated from two different plants *Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves of different family Asphodelaceae and Crassulaceae was investigated.

*CHAPTER-2*  
*REVIEW OF LITERATURE*

## 2. REVIEW OF LITERATURE

The present study “The evaluation of the anticarcinogenic potential of lectin isolated from leaves of *Aloe barbadensis* (Miller) and *Bryophyllum pinnatum* using human cancer cell lines” has been reviewed with the following headings:

1. Cancer, its types and causes
2. Biological role
3. Plant lectins and their types
4. Role of plant lectins in cancer therapy
5. Pharmaceutical activities of plant lectins
6. Biological applications of lectin
7. A review on *Aloe barbadensis*
8. Biochemical Constituents of *Aloe barbadensis*
9. Medicinal uses of *Aloe barbadensis*
10. Clinical significances of *Aloe barbadensis*
11. Anti-carcinogenic properties of *Aloe barbadensis*
12. A review on *Bryophyllum pinnatum*
13. Phytochemicals and commercial products of *Bryophyllum pinnatum*
14. Pharmacological and Clinical significances of *Bryophyllum pinnatum*

### 2.1. Cancer, its types and causes

(<http://www.midday.com/articles/over17lakhnewcancercasesinindiaby2020icmr/17248152>)

In the world, Cancer may be a frightened or dreadful illness. To combat this illness is a crucial step to public health (Madia, 2019). Therefore, its price evaluating the standard medicines like a plant-primarily based medical care, besides the rationalization of allopathic medication. The utilization of Ayurvedic medication is an alternate answer to embodied allopathic medicine with severe facet effects. In clinical analysis, there are several herbs are evaluated and are investigated phytochemically to give an improved understanding of their anti-carcinogenic actions on numerous cancers. Thus, cancer patients, UN agencies are burdened by therapy and nephrotoxic facet effects of medicine, have currently turned to hunt facilitate from the standard

medication for an improved cure. In the body, random or uncontrolled growth of abnormal cells is known as cancer. Once the body has started the development of cancer, the traditional management mechanism of the body has stopped operating. In this mismanagement, the abnormal continue the growth and division of cells without death (Saeland and Vam, 2011). These additional cells might type a mass of tissue, referred to as growth. Some cancers, like leukemia, type tumors. Cancer isn't one sickness. It's a bunch of quite a hundred completely different and distinctive diseases. Cancer will involve any tissue of the body and have many alternative forms in every body space. Most cancers are named for the sort of cell or organ within which they begin. If a cancer spreads (metastasizes), the new growth bears an identical name because of the original (primary) tumor.

The frequency of specific cancer could depend upon gender. Whereas carcinoma is that the commonest variety of malignancy for each male and female, the second commonest sort in males is adenocarcinoma, and in girls, breast carcinoma. Cancer frequency doesn't equate to cancer mortality. Skin cancers are typically curable. Carcinoma is that the leading reason for death from cancer for each male and female nowadays. Benign tumors aren't cancer; malignant tumors are cancer. Cancer isn't contagious. Cancer is that the Latin word for crab. The people used the word to mean a malignancy, likely due to the crab-like pertinacity a tumor typically appears to point out in grasping the tissues it invades. Cancer may additionally be referred to as malignancy, cancer, or a tumor (literally, a replacement growth).

The two things are found due to malignancy:

- Cancer cells try to manage the blood or fluid system of the body throughout the body's victimization, damaging healthy cells using the invasion method.
- A cancer cell has the capability to grow, divide, and creating new blood vessels to feed ontogenesis.

When a successful growth spreads to different body elements invasively and damages different healthy tissues, metastasizing is found to possess and called metastasis. Because of this significant condition i.e. terribly troublesome to treat is the result. Cancer is the 2<sup>nd</sup> most typical death cause in the U.S. approx one by four deaths. According to the most recent data of World Health Organization in 2019, 18 million

new cases of cancer were reported worldwide and death cases were 10 million deaths from cancer (Ferlay *et al.*, 2019; Bray *et al.*, 2018)

## **Types**

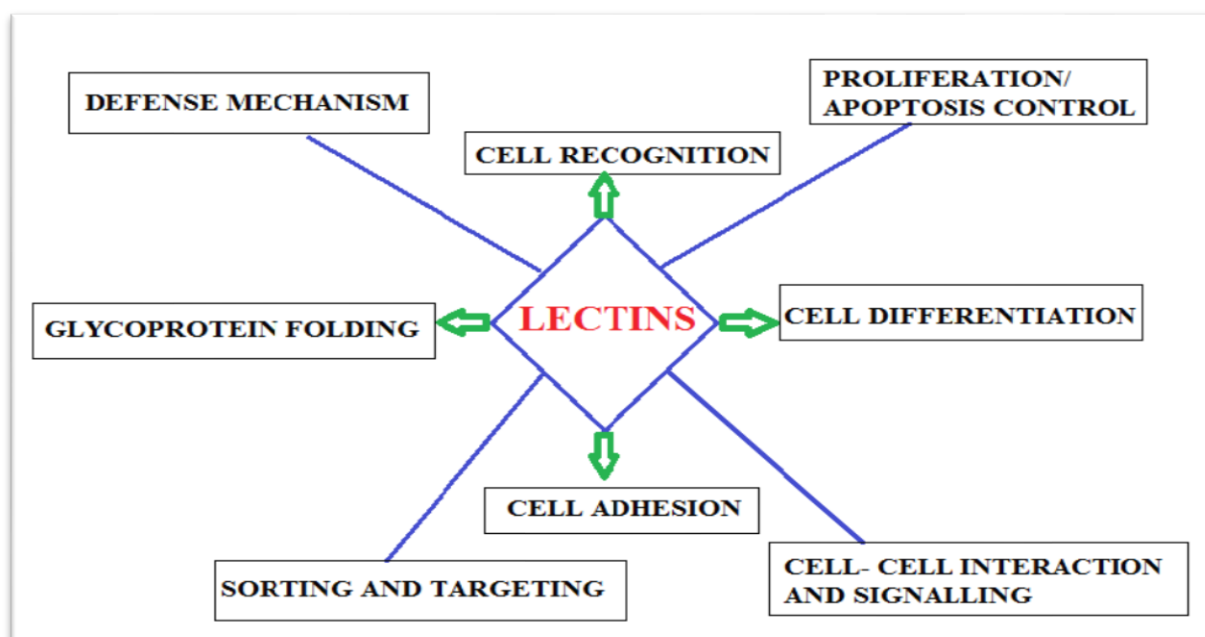
Two hundred differing cancer types have been found. Subsequent general cancer varieties listed in a particular data Center articles: Anal cancer, cancer of Bladder, cancer of bones, carcinoma, Cervical cancer, endometrial carcinoma, urinary organ cancer, Leukemia, liver disease, Lymphoma, sex gland cancer or prostate cancer, glandular cancer, abdomen cancer, Thyroid cancer, duct cancer.

- Cancer is considered one of the world's leading causes of morbidity and death.
- The risk of cancer is believed to be reduced by avoiding tobacco, reducing alcohol intake, limiting sunlight ultraviolet light exposure and tanning beds, and maintaining a healthy diet, fitness level, and seeking regular treatment.
- Screening will find early, treatable stage of cervical cancer, other body part cancer, and carcinoma.
- Immunizing vaccines such as human papillomavirus (HPV) help prevent certain cervical, vaginal, vulvar, and oral cancers. A hepatitis B immunizing agent will reduce the risk of liver disease.
- In line with the World Health Organization (WHO), it is predicted that the number of recent cancer cases will increase by 70 over the next twenty years.
- Respiratory organs, prostate, colon, rectum, abdomen, and liver are the most common cancer sites among men.
- In females, Breast cancer, colon cancer, rectum, lung, cervix, and abdomen are the most common cancer.

## **Causes**

Definitely, cancer is an outcome of randomly grown cells that are dividing continuously without die. In the body, traditional cells obey the associated degree of an ordered path i.e. growth of cells, division of cells, and finally death of cells. A programmed necrobiosis which is known as programmed cell death and cancer begins to occur once this method breaks down. In the case of cancer cells, programmed cell death does not take place like a regular or normal cell. The ending of anomalous cells growing occurs because of mismanagement.

## 2.2. Biological role



**Fig.1. Representation of biological function of Lectins in living organisms (Martinez, 2010)**

Lectins have essential roles in several biological and metabolic mechanisms (Nowell, 1960). Biological applications have been established very early. In immunology, major steps are given to define the role of lectin glycoproteins in lymphocytes (growth and division). Lectins from kidney beans known as phytohemagglutinin can undergo mitosis and stimulate mitosis. There are many types of research performed to evolve the significance of lectin in an immune response. Lectins have multi-face biological activities that are why lectins developed as a tool to study structure and functions of cell surface e.g. lectins can fractionate animal cell (B-lymphocytes and T-lymphocytes) to study parasite infection (Martinez *et al.*, 2010).

### 2.2.1. Biosynthesis of Lectin

The synthesis of lectins from various and numerous plants has been studied. (Vitale & Chrispeels, 1992). Shortly, the synthesis of glycoprotein took place in ribosomes connected to ER (Endoplasmic reticulum), enters the endoplasmic lumen, and its transportation occurs through the Golgi bodies. In distinction to humor proteins in an exceedingly slender sense that moves towards the semipermeable layer by sac transportation and then escapes by exocytosis from the cell, lectins find within

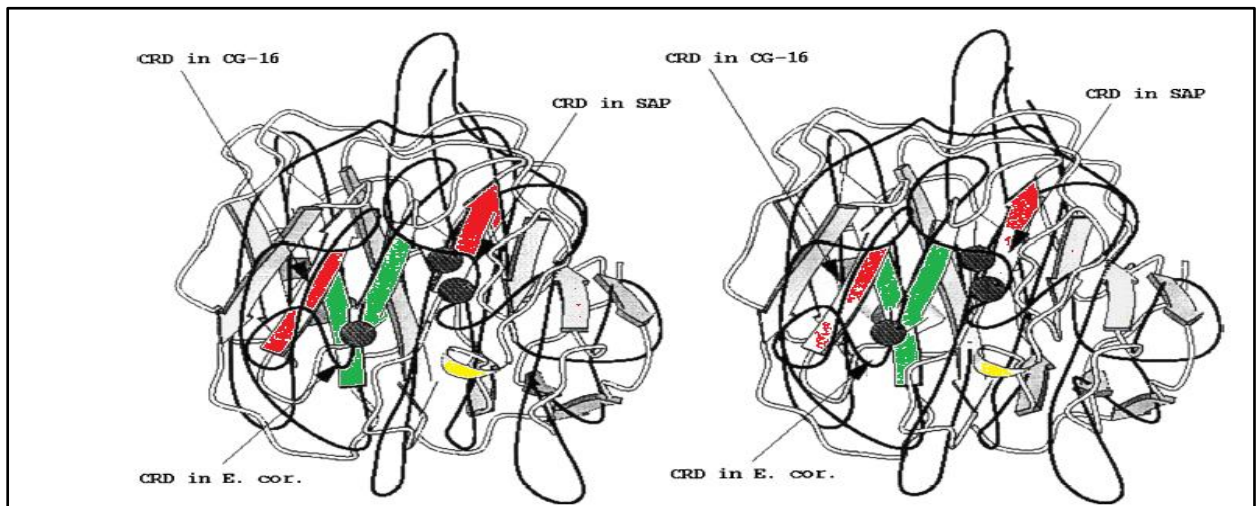


the cavity. In the time of growth of the napping seeds this organ exactly separates into smaller elements known as macromolecule bodies (storage vacuole). This conquering is troubled by the availability of monensin. It introduces into layers allow movement of cations as well as  $H^+$  ions, so that intrusive with the intracellular transportation.

Monensin (a polyether antibiotic isolated from *Streptomyces cinnamonensis*) completely reduces the partition of transportation paths to the external cytomembrane and vacuolar membrane, and successively glycoprotein can assemble extracellularly covered by such conditions (Bowles and Pappin, 1988).

### 2.3. Plant Lectins and their types

Carbohydrate binding proteins isolated from plants are considered as a heterogeneous group of glycoproteins, having different structures binding specificity of carbohydrate and other biological activities (Peumans, 1998).



**Fig.2.** Demonstration of the jelly-roll motif with its opposite beta-strand settlement in a pentraxin bound to  $Ca^{2+}$  ions (a legume lectin (1AXY)).

In the time of distribution of common bending arrangement the connecting sites in pentraxin (SAP) for ligands, *Erythrina corallodendron* lectin and galectin (Rudiger and Gabius, 2001).

All recent known plant lectins are classified into small and structurally related protein families. There are seven lectin families and named as; 1) Legume family of lectins, 2) Monocot-mannose binding lectins, 3) Chitin-binding domains proteins, 4) RIPs-II (Ribosome-inactivating protein-II), 5) The cucurbitaceae phloem lectins, 6) Jacalin family, 7) Amarnathaceae lectins (Peumans and Van Damme, 1998).

### 2.3.1. Legume superfamily of lectins

Among all plant lectin family, the legume superfamily of lectins is the best known (Sharon and Lis, 1990). Gene sequencing and protein helped to demonstrate that all legume lectins have two or four protomers (30K Da). The two chains of legume lectins promote further division in two small polypeptides. Legume lectins show variation with the relative molecular level in the specificity of carbohydrate-binding. Legume lectins are the most important lectins because it is abundant in many crops and in the symbiosis between legume and *Rhizobium* (Nitrogen-fixing bacteria) (Peumans and Van Damme, 1998). In this sense, the synthesis of lectins from the legume family has been studied totally. Legume lectins distributing enlarged sequence homology demonstration associate degree uncommon interspecies inconsistency of carbohydrate target choice. Commonly, selectivity for Glucose, GlcNAc, and Mannose, however additionally for Galactose, GalNAc, Fructose, and complex type oligosaccharides are described. Binding capability to sialic acids, a monosaccharides family does not synthesize by plants (Reuter and Gabius, 1996), to bound lectins ought to be examined with care. Supposed plant lectin bind sialic acid really binds Galactose or disaccharide, however, don't act with free Neu5Ac (Knibbs *et al.*, 1991). Their attraction for Galactose or other disaccharides, although perceptibly increased if an acidic cluster is in a closed locality. Binding of sialic acid to Galactose unit that consists the first site to provide examples for the significant role of carbohydrate cluster which consist negative charge (Imberty *et al.*, 2000; Bai *et al.*, 2001). A notable exception is an antibody and its binding to each GlcNAc and Neu5Ac. It has been proven that the stereochemical demands are satisfied by sialic acid the decisive property for GlcNAc (Wright, 1977). A mannose/glucose binding lectin with anticancer properties *Dioclea Violacea* lectin (DVL) has isolated from *Dioclea Violacea* seeds of legume protein. DVL was shown to inhibit U87 cells (Nascimento *et al.*, 2018; Nascimento *et al.*, 2019). *Dioclea lasiocarpa* lectin (DLL) has strong anticancer effects on A549, MCF-7, PC3. DLL suppresses glioma cell lines by inducing caspase-3 activation, autophagy, and cell death (Nascimento *et al.*, 2017). On the basis of quaternary structure, superfamilies of legume lectins are historically divided into 2 classes.

### **2.3.1.1. PHA (Phaseolus lectins).**

The primary group is the well-reported example given by the glycoprotein extraction from the French bean plant, its extraction, and resolution of isolectins of purified extract cited on top of Fleischmann *et al.*, 1985. This glycoprotein fraction will stimulate malabsorption and discomfort within the alimentary canal (Liener, 1986). Another issue is providing in its quality to analyze the abundance and isolation is so easy providing it standing of task model. Thus phaseolus lectins have been named “phytohaemagglutinin” (PHA), properly apply to plant lectins normally (Goldstein and Portez, 1986). Phaseolus agglutinin consists of 2 sub types. These are merchandise of tandem gene with eighty-two sequences specifically on the organic compound level. E- and L-subunits were frequently selected reflects their discriminatory affinity to red blood cells and leukocytes, severally. Their synthesis took place concomitantly within the endoplasmic reticulum which assembled in the formation of tetramers randomly, therefore providing the familiar series of five isolectins as mentioned on top of. The sequence of a signal of N-terminal release is average to phaseolus agglutinin as for alternative glycoproteins of the humor path.

rL (Recombinant lectins), having ragged ends however these will disagree from biological conditions. Some organic methods for such kind of adjustment, expect thought to spill out of degradative advances once homogenization, is misty. Phaseolus agglutinin subunits include a trademark N-glycosylation (Reuter and Gabius, 1999), at Asn12, Asn60, and Asn80 fiscal unit E, L is a worth related unit exclusively at Asn12 and Asn60. Glycosylation occurs solely on the primary two sites in mature proteins. At Asn12, glycan belongs to the high-Mannose kind, which at Asn60 to the complicated kind was containing wood sugar and Fuc within the stem region. High-Man sort glycans close to the N-terminus are uncommon among animal glycoproteins (Kinbbs *et al.*, 1991).

The presence of N-glycans affects additional transport; synthesis of lectin was performed within tunicamycin, associate degree substance of primary step in N-glycosylation. During the synthesis of lectin, a non-glycosylated in the presence of the matter however was processed and deposited usually (Bollini *et al.*, 1985).

### **2.3.1.2. SBA (Soybean agglutinin).**

For instance, glycans were developed nearness on the far side collapsing and directing, the outline of a particular work of soybean lectin is informative for glycans. Gal/GalNAc-restricting glycoprotein from soybean neutralizer might be a high-Mannose sort glycan protein (Dorland *et al.*, 1981). Obviously, the glycan moiety is fundamental for keeping the subunits together: Small Business Administration changed by guanidinium chloride recombines when weakening. It bombs, be that as it may, to attempt and do in this manner when evacuation of the glycan or within the sight of the aggressive high-mannose glycopeptide Man9GlcNAc2Asn (Nagai and Yamaguchi, 1993; Nagai *et al.*, 1993). Using various extra methods, the creators are incontestable for the last time that non-diminishing finishes of the glycans bound to SBA are covered within the subunits, although the center districts are uncovered (Masaok *et al.*, 1999). In this manner, Small Business Administration consolidates two starch restricting locales amid a solitary particle: One explicit for oligosaccharides and coordinated towards its own saccharide center, the other explicit for Gal and GalNAc, superficially uncovered and coordinated towards outer sugars.

### **2.3.1.3. Con A (Concanavalin A).**

In 1919, Sumner and his colleagues purified Concanavalin's glycoprotein, the goliath stock bean by crystallization, which is one of the premier aplenty lectins celebrated. ConA is routinely applied to isolate glycoproteins by affinity chromatography (Wu *et al.*, 2019). Investigation of its amalgamation and procedure was rendered generally uncomplicated by this powerful articulation. A high degree of resemblance was found when its amino alkanoic corrosive arrangement was contrasted with that of various vegetable lectins. Shockingly, arrangements were obtained ideal likeness sorted out the peptide chains in some manners that the N-and C-ends of ConA face bound buildups with in and around the center of the elective lectins chains and a different way (Cunningham, 1983). In 1985, it had been discovered that the ConA antecedent shows the "ordinary" grouping, which the round change is on account of partner degree up there to time unexampled posttranslational occasion in plants, inside which the amide chain is part at one site and solidified at another (Carrington *et al.*, 1985). Despite the fact that the important murein microorganism combination, transpeptidations had not resolved in life higher forms

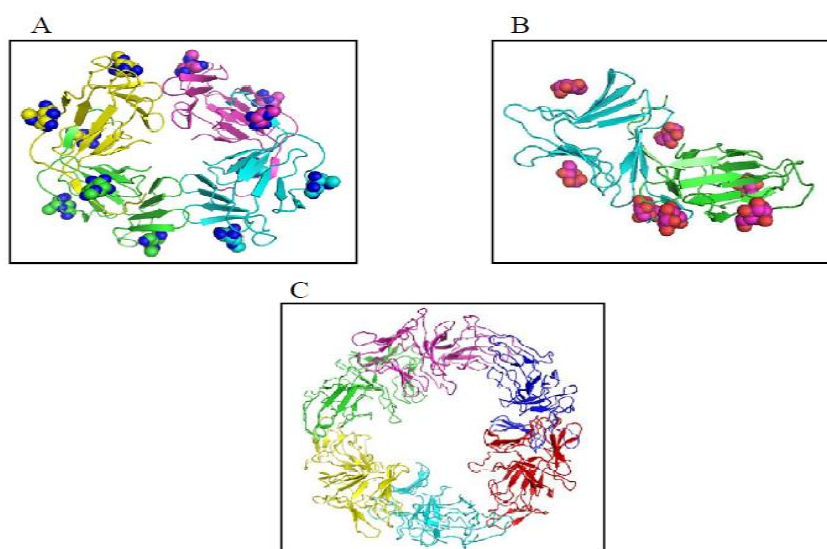
until that point. Indeed, even up to now the identification of equivalent occasions has stayed extraordinary in eukaryotes (Kane *et al.*, 1990), however, they're very normal among microscopic organisms (Heinemann *et al.*, 1995).

#### **2.4. Monocot –mannose binding lectin**

Monocot-mannose binding lectin is a new group of lectins. Lectins extracted from snowdrop bulbs show specificity for mannose (Van Demme *et al.*, 1987). Monocot family (Amaryllidaceae, Bromeliaceae, Alliaceae, Orchidaceae, Araceae, and Liliaceae) also containing some binding lectin (Van Damme *et al.*, 1995). All monocot mannose binding lectin is made up of 1-4 subunits of about 12kDa. A comparative study between the sequences demonstrates that monocot mannose-binding proteins belong to related proteins of the same superfamily. The basis of molecular modeling studies further indicated that all mannose monocot binding protein subunits are very similar in structure and variation between one and three per promoters of active binding sites. Mannose limiting plant lectins are especially restricted to monocots and common in six not in the slightest degree like monocot families named, *Araceae*, *Amaryllidaceae*, *Alliaceae*, *Bromeliaceae*, and *Liliaceae* (Van Damme *et al.*, 1998). To establish in leaves, blooms, bulbs, rhizomes, establishes and even in nectar however infrequently in seeds (Peumans *et al.*, 1997). The apparent qualities of a real piece of the monocot-mannose restricting lectins or *Galanthus nivalis* agglutinin (GNA) homologs are their blends as a couple of area forerunners. At the point when two domains are sorted out pair isolated by short part course of action and the domains are associated with N-terminal sign peptide (Van Damme *et al.*, 2007). Co-translational uprooting of sign peptide and post-translational handling of the peptide produces a couple of complete spaces in GNA lectins.

GNA sublimate from *Anemone quinquefolia* bulbs is likely the most effective studied mannose specific plant lectins and primary crystallized lectin (Wright *et al.*, 1990; Hester *et al.*, 1995). The 3-D appearance of this lectin has appeared beta-crystal II overlay, an element of this family. The monomer lies of three tandem showed I, II, and III sub-domains for every which displays a four-stranded beta-sheet. Each and every space is comprised of CRD (Carbohydrate Recognition Domain). The 3-sub-domains I, II, and III have nearby 3-overlay symmetries and framed three crystal fronts. These are joined by circles and make a 12-stranded beta-barrel consisting of 3-

mannose restricting destinations available in clefts shaped by three beta-sheet groups. These monomers can frame tense dimers through an H-bond interface adjusted by C-terminal strand trade. These dimers are additionally related to tetramers mainly through hydrophobic interfaces. The tetrameric structure of GNA molecule consists of twelve mannose-binding sites (Fig. 1.3 a). Daffodil (*Narcissus pseudonarcissus*) and bluebell (*Scilla campanulata*; SCA man) (Wood *et al.*, 1999; Sauerborn *et al.*, 1999) consisting of tetrameric mannose-binding lectins display an identical 3-D structure. Indeed, even so, these lectins are appearing for mannose, can likewise recognize glycoprotein comprising mannose because of that they can indicate inhibitory action of retroviruses showing on replication (Balzarini *et al.*, 1991, 1992). The complexed glycans are selected in advance from the oligomerization process of molecules. While on HIV, the tetrameric windflower, narcissus, and bluebell lectins tie to surface conjugation of proteins occurs, general expert one hundred twenty with high affinity, the dimeric garlic glycoprotein (Fig.1.3 b) doesn't recognize general professional one hundred twenty (Vijayan and Chandra 1999).



**Fig.3(a).** Three-dimensional structure of GNA combined with 1-methyl mannose. **(b).** The garlic bulb lectin (dimeric) complexed with  $\alpha$ -D-Mannose. **(c).** The crystal structure of SCAfet.

Aside from straight forward mannose particularity, bound lectins with cutting edge sugar explicitness have also been by happening in certain monocots, for instance, LacNAc (N-acetyl-D-lactosamine) explicit lectins of liliopsid family flavum

(Singh *et al.*, 2004); *aroid cucullata* (Kaur *et al.*, 2005a); liliopsid sort tortuosum (Dhuna *et al.*, 2005); reed (Kaur *et al.*, 2005b) and so on. *Dioclea lasiophylla* lectin displays cytotoxic effects against C6 glioma cells (Leal *et al.*, 2018).

The structure of such advanced sugar-specific glycoprotein from *squill campanulata* bulbs (SCAfet) was crystal shaped. Different monocot-mannose binding lectins (e.g. SCAMAN and GNA) folding into one domain (each domain consisting of 3 sub domains), SCAfet contains 2 domains (each domain containing 3 sub domains) with a series identification of about 55 percent linked by a peptide linkage. In the formation of each domain, 12 stranded beta prism II fold with three binding sites for carbohydrates, one on each domain occurs. SCAfet's lack of interaction with straight sugars may be due to the replacement of residues of aminoalkanoic acid i.e. Asn, Asp, Gln, and Tyr between bindings of hydrophobic residues with monosaccharide. *Remusatia vivipara* lectin (RVL) is another mannose-binding protein. It shows a high affinity towards N-glycan. It exerts cytotoxic effects on breast cancer MDA-MB-468 and MCF-7 cancer cells via apoptosis (Sindhura *et al.*, 2017).

## **2.5. Chitin binding lectins**

Lectins of a chitin-binding family composed of hevein domains. Due to the presence of hevein domains, chitin-binding lectins represent large glycoproteins (lectins). The word 'hevein domain' refers to a merolectin hevein, containing 43 amino acid residues found in latex of rubber tree with botanical name *Hevea brasiliensis* (Lee *et al.*, 1991). Chitin-binding lectins have been found in many plant families, Solanaceae, Gramineae, Papavaracaceae, Phytolaccaceae, Urticaceae, and Viscaceae (Raikhel *et al.*, 1993). These lectins are classified into different types, named as merolectins, hololectins, chimerolectins and superlectins (Van Damme *et al.*, 1998). The hevein's 3-D structure consists of 2 short  $\alpha$ -helices and a stretch of amino alkanoic acid residues located at the polypeptide chain's N-terminal finish, forming 2 parallel  $\beta$ -sheet strands followed by one more  $\alpha$ -helix (Fig. 1.4 a).

Four disulfide bonds help to stabilize the general configuration of hevein. Hololectins consist of polypeptides engineered with several tandemly arranged in repeating of hevein which found in numerous species of plants (Peumans *et al.*, 1984) e.g. monomeric glycoprotein of 2-hevein *Urtica dioica* domains, dimeric Sustenance Antibody and Graminae alternative lectin with four hevein domains (Reikhel *et al.*,

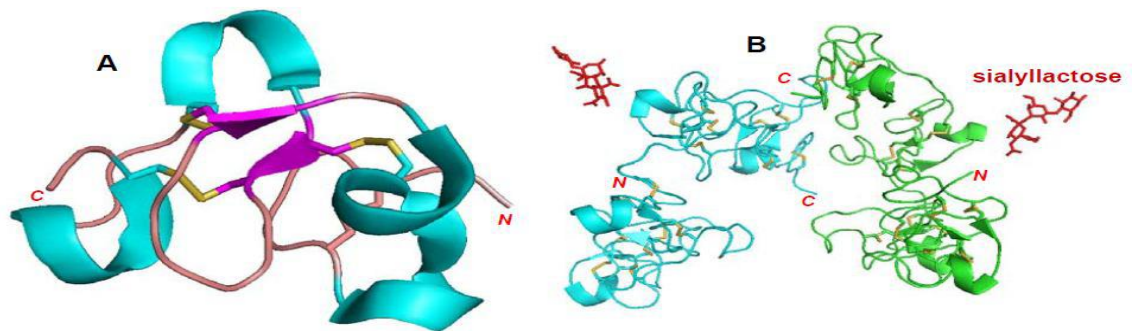
1993). There are four hevein domains are present in a monomer. These domains are folded and form a seven compressed globule which is bind with four disulfide linkages having 5-6 beta-turns (Fig. 1.4 b). These 4-domains are arranged in such a way to form a helical assembly. Monomers are arranged into head-to-tail structures and formed dimers to organize pair domains (Wright *et al.*, 2000). In a monomer, every 4-domains consists of a carbohydrate-binding site.

**Table 1. Classification of Lectins on structural bases (Martinez *et al.*, 2010)**

<b>Types of Lectin</b>	<b>Definition</b>
<b>Merolectins</b>	Merolectins are monovalent in nature, having a single carbohydrate-binding domain, that's why they cannot agglutinate cells and not take part in glycoconjugation.
<b>Hololectins</b>	Hololectins having double identical or homologous carbohydrate-binding domains. These domains can bind the same carbohydrate or structurally identical sugars. Due to their Di or multivalent properties, these can agglutinate cells.
<b>Chimerolectins</b>	Chimerolectins are fusion glycoproteins with single and numerous carbohydrate-binding domains and having an enzymatic domain. These domains participate in many biological activities like act independently. Because of their numerous carbohydrate-binding sites, they can also act like merolectins and hololectins.
<b>Superlectins</b>	Superlectins are a special type of chimerolectins containing two carbohydrate-binding domains that can bind even structurally non-identical domains.

The chitin-binding domain like hevein is present in many plant proteins. The chitin-binding anti-microbial proteins obtained from *Amaranthus caudatus* are built from a single hevein domain (Broekaert *et al.*, 1992). Lectins isolated from *Stinging nettle*, *Phytolacca americana*, and wheat are proteins bind with chitin and composed of two, three, or four hevein domains respectively (Raikhel *et al.*, 1993, Konami *et al.*, 1995).





**Fig.4(a).** Hevein 3-D configuration (1Q9B code). Cyan-colored alpha-helices and magenta-colored beta-sheets. Disulfide bonds are yellow in color. **(b).** Sialyl lactose present in WGA (PDB code 1WGC) (Wright *et al.*, 2000). Some chitin proteins are made up of unrelated or different hevein domains e.g. single hevein domain that bind to catalytic active chitinase domain is constituent of Class I chitinases, chimerolectins (Collinge *et al.*, 1993).

A deeply studied WGA (Wheat germ agglutinin) with hevein domains having anticarcinogenic property is a chitin-binding lectin. Inference showed that wheat germ agglutinin has a strong reducing effect on the pancreatic cancer cell. Wheat germ agglutinin binds to sialic acid residues on the cell membrane and produces toxic to human pancreatic cancer. WGA has an effect on T-lymphoma cell survival and on the basis of preceding research it has been found that it controls tumor growth (van Buul & Brouns, 2014). *In vitro* breast cancer cell growth was effectively controlled by WGA induced the condensation of chromatin, nuclear fragmentation, and discharge of DNA related to apoptosis (Wang *et al.*, 2000).

## **2.6. RIPs-II (Type II ribosome-inactivating proteins)**

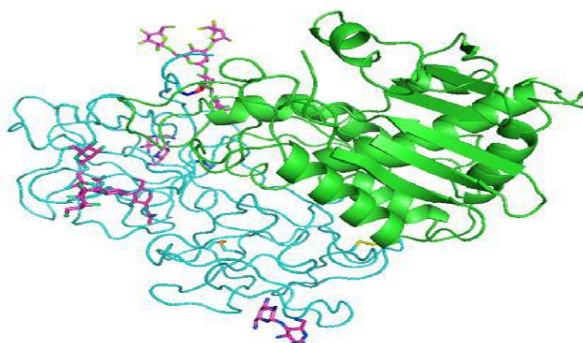
### **2.6.1. *Viscum album* (Mistletoe lectin)**

RIP are eukaryotic ribosomes that are catalytically inactive (Barbieri *et al.*, 1993). Type-I RIPs consisting of a PAG (polynucleotide adenosine glycosidase) enzyme of 30 kDa single polypeptide chain. On the opposite side, a catalytically active A-chain connecting with B-chain to Gal-binding domain in Type II RIPs (Endo *et al.*, 1987; Lord *et al.*, 1994).

RIPs-II (Type II ribosome-inactivating protein) is classified as *Viscum album* is the botanical name of Mistletoe is extensively studied because of their anticarcinogenic activities and other biological applications (Seifert, 2008). Mistletoe

composed of alpha-chain consists of three distinct individuals domains with a beta-chain comprised of two domains of the same configuration. It has been assumed that the specificity difference of sugar for their beta-chain has a significant status in the determination of cytotoxicity of tumor cells by interacting with receptors present on the cell surface (Siegle *et al.*, 2001). Lectins have cytotoxicity which appears to require both alpha and beta chains. Therefore it is concluded that internalization and recognition of beta-chain of RIPs II via appeared receptors prior for alpha chain and apply cytotoxicity to reduce the growth of tumor cells (Thies *et al.*, 2005). Supplementary, RIPs II are recognized to be applying anticarcinogenic activity on the growth of tumor cells in different manners by the involvement of distinct stages of apoptosis mechanism or programmed cell death (Martinez *et al.*, 2010).

There are a number of families, named Cucurbitaceae, Euphorbiaceae, Iridaceae, Fabaceae, Lauraceae, Passifloraceae, Ranunculaceae, Sambucaceae, and Viscaceae in Type-II RIPs. Ricin was the first isolated lectin from *Ricinus communis* seeds. The 3-D structure of ricin consisting of A and B subunits with a molecular weight of 32 kDa which hold by a single disulfide bond (Rutenber *et al.*, 1991) (Fig.1.4) showing specificity for GalNAc/Gal (Lord *et al.*, 1994). 3-D structure of A-chain consisting of a regular 2<sup>o</sup> structure which is catalytically active, on the other hand, B-chain lacks a secondary structure like alfa helices or beta-sheets consisting of coiled structure bound by loops and turns which involved in carbohydrate binding. The 2-domains of the B-chain showing a 3<sup>o</sup> structure of the beta-trefoil family (Murzin *et al.*, 1992)



**Fig.5.** 3-D structure of Ricin. Chain -A, in green color, consisting of regular secondary structure, but B-chain in magenta color lacks secondary structure.

### **2.6.2. Rice bran agglutinin**

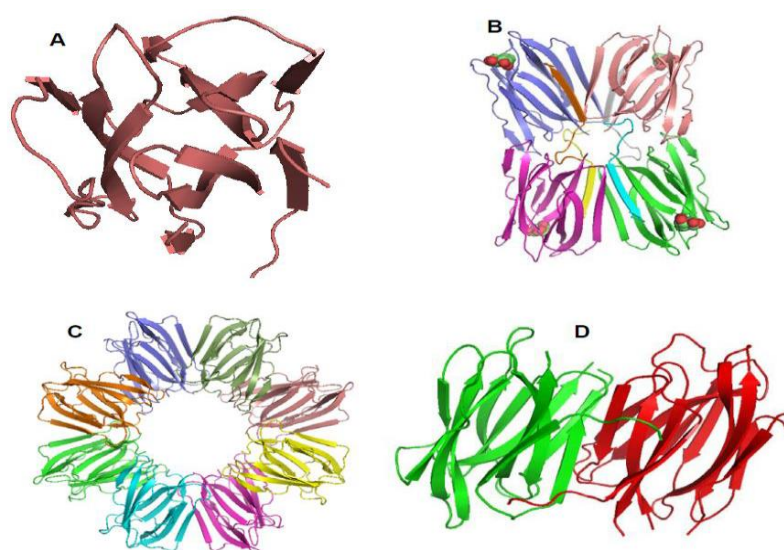
Rice bran agglutinin has a significant role in chromatin condensation, DNA releases nuclear fragmentation, and externalization of phosphatidyl serine present on the cell membrane in monoblastic leukemia U937 cell line (Miyoshi, 2001). A recent study has reported that Ricin, Type II ribosome-inactivating proteins induced apoptosis in cell line Hodgkin's lymphoma L540 by increasing the number of receptors of Caspase 3/7 and caspase-8 downstream (Letizia *et al.*, 2009). Ricins are known for synergic and antitumor effects with some anticarcinogenic agents to tumor cells. These researches are developed an important pharmaceutical application on a molecular basis.

### **2.6.3. Jacalin family: Galactose binding lectin**

The galactose-specific lectin, named Jacalin isolated from seeds of jack fruit, botanical name *Artocarpus integrifolia* it is a non-legume plant lectin. Jacalin family is a superfamily of lectin and also heterogeneous w.r.t. specificity binding for carbohydrate and found in taxonomic groups (Peumans and Van Damme, 1998; Esch, & Schaffrath, 2017). Jacalin is one of two lectins in mature seeds of *Artocarpus integrifolia* (Jackfruit). For all lectures that are structurally and biologically associated with jacalin, the term "jacalin connected lectins" is used. On the basis of their specific nature, these lectins are classified as galactose-specific sub-cluster contains jacalin and some alternative dicot family lectins, which show an affinity for sucrose and are engineered from subunits containing a brief beta-chain of regarding twenty residues and an extended alpha-chain of 133 residues.

In this family, the first isolated lectin was Jacalin which was sequenced, crystallized, and determined with methyl- $\alpha$ -D-galactose in its 3-D structure (Sankaranarayanan *et al.*, 1996). Jacalin lectins with a 3-D structure present a beta-prism I-fold property explained by a 3-fold symmetric beta-prism consisting of 3-4 stranded beta-sheets arranged in three prism fronts. These strands are just parallel to the 3-fold axis (Fig. 1.5 A). Each monomer has a single binding site for saccharides. The maximum number of lectins of these family-type tetramers as their useful biological units in 222-symmetry (Fig. 1.5 B) and are therefore power. In distinction, an eight-monomer glycoprotein from flower tuberosus (Heltuba) formed an octameric structure related to donut-shaped octamer (Fig. 1.5 C) with eight binding sites of

solvent carbohydrate (Bourne *et al.*, 1999). A lectin isolated from the Calsepa family (*Calystegia sepium*) revealing a dimeric assembly imitating a canonical 12-stranded beta-sandwich dimer (Fig.1.5D) commonly found in legume lectins (Bourne *et al.*, 2004). Even though jacalin-associated lectins are found in many tissues, lectins from Convolvulaceae family rich in rhizomes, on the other hand, lectins from Moraceae family are plentiful in seeds. Previous studies found that jacalin connected lectins also revealed certain insecticide properties (Van Damme *et al.*, 1998).



**Fig.6.(a).** In the monomer of jacalin lectin, a Three-fold symmetric beta-prism-I fold has been observed (Protein Data Bank code: 1UGX).

**(b).** 3-D structure of an active tetramer of jacalin lectin (PDB code: 1UGW).

**(c).** Heltuba lectin in octameric form (PDB code: 1C3K).

**(d).** Calsepa lectin with dimeric formation exhibiting similarity to a canonical dimer of legume lectins (PDB code: 1OUW) (Bourn *et al.*, 1999; Bourn *et al.*, 2004).

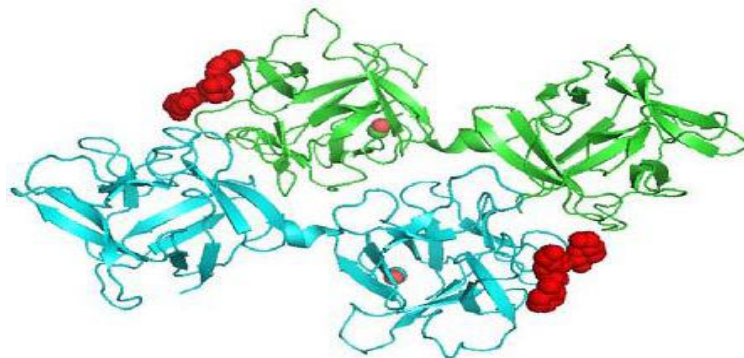
For instance, lectin of *Maclura pomifera* (Murdock *et al.*, 1990) retard the cowpea beetle growth (*Callosobruchus maculatus*) larvae, both *Maclura pomifera* agglutinin & jacalin strangled the growth of larvae of *Diabrotica undecimpunctata* (Southern corn rootworm) (Czapla and Lang, 1990), and dicot genus tomentose glycoprotein was anti-insecticidal for *Tribolium castaneum* (red darkling beetle) larvae (Gurjar *et al.*, 2000).

#### **2.6.4. Cucurbitaceae phloem lectin**

*Cucurbitaceae* species contain a lectin that shows specificity for oligomers of GlcNAc (Wang *et al.*, 1994). Lectin isolated from *Cucurbitaceae* phloem is dimeric proteins and comprised of two similar subunits 24 kDa (Peumans, 1998). Cucurbitaceae phloem lectin is found in the phloem of some plants i.e. a small part of the chitin-binding lectin of the Cucurbitaceae family. They're not associated with seed lectins of Cucurbitaceae plants (which are principally sorting two RIPs) and additionally, the hevein domain doesn't present. Cucurbitaceae families of lectins (also known as PP2) are full of proteins known within the exudates of the phloem of *Cucurbita*, *Coccinia*, *Citrullus*, *Cucumis*, *Sechium*, and *Luffa* species. Non-glycosylated subunits of 25 kDa which are dimeric in solution are present in these lectins (Bostwick *et al.*, 1994; Wang *et al.*, 1994). Two disulfide bonds were covalently joined to 2-subunit of *Cucurbita maxima* glycoprotein (Read and Northcote, 1983) and specific towards oligomers i.e. GlcNAc. In the defensive mechanism of the plant, the Cucurbitaceae phloem lectins may be concerned.

#### **2.6.5. Amaranthin lectin**

A seed lectin isolated from *Amaranthaceae caudatus* named Amaranthin, revealed by molecular cloning and X-ray diffraction analysis. The three-dimensional structures and amino acid sequence of this lectin do not resemble any other plant lectin. In the amaranthin lectin family, Amaranthin is considered a prototype (Peumans, 1998). Amaranthin lectins are homodimeric glycoproteins formed with two similar subunits with molecular weight 30kDa and show specificity for GalNAc (Rinderle *et al.*, 1989, 1990). 3-D structure of amaranthin lectin showed resolution at 2.2 Å (Fig.1.6). C-domains and N-domains monomers were joined with a short helix. In the formation of a dimer by substantial non-covalent, two monomers couple in head-to-tail form. In this arrangement, the one monomer of the N-terminal domain front on to another monomer of the C-terminal domain.



**Fig.7.** The 3-D structure of amaranthin lectin (*Amaranthus caudatus*)(PDB code 1JLX) formed a complex structure with T-antigen carbohydrates.

The dimer shows two undefended carbohydrate-binding sites that come across as schematic at the edge between the two fronts on monomers (N- domains and C- domains) (Transue *et al.*, 1997).

**Table 2. Some important Plant Lectin families with their specificity**

S.No.	Family	Related species	Specificity	Characteristics
1.	Amaranthins	<i>Amaranthus species</i>	<b>GalNAc(T-antigen)</b>	Seed supermolecule, homodimeric unglycosylated protomers of thirty-three kDa, $\beta$ -trefoil structure
2.	GNA- related lectins (Monocot-mannose binding lectins)	<i>Alliaceae</i> <i>Amaryllidaceae</i> <i>Dicots</i> <i>Gymnosperms</i> <i>Monocots</i> <i>Orchidaceae</i> <i>Taxaceae</i>	<b>Mannose</b>	N-glycosylation of different oligomerization cytoplasmic GNA $\beta$ - barrel only on precursor not on mature glycoprotein vacuolar location.
3.	Heveins (chitin-binding lectins)	<i>Viridiplantae</i>	<b>(GlcNAc)<sub>n</sub>, N-glycans</b>	All tissues, hevein domain, 43 AA different oligomerization, typically N-glycosylated folding of hevein

<b>4.</b>	Jackalins a. Galactose-specific b. Mannose-specific	<i>Moraceae</i> <i>Viridiplantae</i>	<b>Galactose</b> <b>T-antigen</b>  <b>Mannose</b>	N-glycosylated vacuolar location of seed and vegetative tissues totally different from oligomerization. Structure of $\beta$ -prism
<b>5.</b>	Legume lectins	<i>Fabaceae</i> <i>Lamiaceae</i>	<b>Man/Glc</b> <b>Fuc</b> <b>Sia<math>\alpha</math>2,3</b>	Seed and vegetative tissues of 30kDa metalloproteins (Mn <sup>2+</sup> and Ca <sup>2+</sup> ) heterogeneous cluster di-or tetrameric subunits
<b>6.</b>	Nictaba related proteins	<i>Cucurbitaceae</i> <i>Solanaceae</i>	<b>(GlcNAc)<sub>n</sub></b>	Phloem glycoprotein, unglycosylated homodimeric, 17-25 kDa subunits
<b>7.</b>	Ricin-B domain Plant lectins	<i>Cucurbitaceae</i> <i>Euphorbiaceae</i> <i>Iridaceae</i> <i>Leguminoceae</i> <i>Viscaceae</i>	<b>Gal</b> <b>GalNAc</b> <b>Sia<math>\alpha</math>2,3Gal/</b> <b>GalNAc</b> <b>GalNAc</b>	Mostly N-glycosylation, different glycosidase domain of oligomerization (type II RIPs)

## 2.7. Role of Lectins in cancer therapy

Although lectins have been proverbial for a generation became the central focus in intense interest only if the invention took place in 1963 (Aub *et al.* 1963) Proof is currently showing that the dynamic contribution of plant lectins to surface markers for tumor cell recognition, cell adhesion, localization, signal transduction across the membrane, mitogenic toxicity and necrobiosis. Because of their specific properties, lectins are used as a tool each for the analytical and preparatory function of organic chemistry, medicine, and cellular biology. However, for clinical and curative processes in cancer analysis (Sharon and Lis, 1989; Mody *et al.*, 1995) the purpose of this review is not to mirror the conflicting views on the effectiveness of various lectins in the treatment of cancer. Here, solely anticancer effects of lectins *in vivo* and *in vitro* which could be of chemopreventive interest are taken into consideration. The initial two studies of the antitumor activity of lectins of some plants were printed in

1970 (Lin *et al.*, 1970; Shoham *et al.*, 1970). One study (Lin *et al.*, 1970), has absolutely shown intraperitoneal injection of toxin (*Ricinus communis* antibody (RCA)) or abrin (*Abrus precatorius* agglutinin (APA)) in mice tumor reduction of expansion of tumor-derived from EAC (Ehrlich pathology cancer). Tumor-bearing mice that received seven to ten  $\mu\text{g}/\text{kg}$  weight of toxins and abrin, severally, had considerably longer (2-3 fold) life spans than untreated tumor-bearing animals. In another study (Shoham *et al.*, 1970), it absolutely was incontestable that injection (1 mg/kg body weight) of Con-A (*Canavalia ensiformis* antibody, jack bean) made inhibition of tumor development (polyoma remodeled 3T3 cells) in gnawing animal. Experiments with dimethylnitrosamine (DMNA) and simian virus forty (SV-40)-treated hamsters *in vivo* indicated that inhibition of tumor cell growth needed an area injection of 40mg Con-A. At this dose, eightieth of tested animals had no tumors. Shot of Con-A was distance concerning from the tumor failed to inhibit tumor growth.

In ensuant studies, many investigators (Robinson and Mekori, 1971; Refsnes *et al.*, 1974; Hsu *et al.*, 1974; Nachbar *et al.*, 1976; Fodstad and Pihl, 1978, 1980) found that plant lectins considerably inhibited the expansion of various tumor cells *in vitro* however no vital impact had on the growth of normal cells.

The studies enclosed that the abrin and toxin considered alone and in combination with Adriamycin on leukemia (murine L1210 leukemia cell line) (Fodstad and Pihl, 1978) PHA, abrin and ricin on murine Ehrlich Pathology tumor cells (Robinson and Mekori, 1971; Refsnes *et al.*, 1974) abrin and toxin on human cancer cells (Hsu *et al.*, 1974) and sustenance antibody (WGA, *Triticum vulgare*) on murine pathology (MM46) tumor cells (Esumi-Kurisi *et al.*, 1983). They suggested that the excellence among the structure of the surface membrane between ancient and reworked cells is chargeable for the different harmful impacts of plant lectins on traditional and malignant cells. In fascinating studies from Goldstein's laboratory (Eckhardt *et al.*, 1982; Maddox *et al.*, 1982a,b; Knibbs *et al.*, 1994), the researchers examined the impact of GS-1 plant conjugated protein on Ehrlich pathology growth cell growth in mice *in vivo*. These researchers terminated that intraperitoneal administration (100  $\mu\text{g}/\text{day}$  or 300  $\mu\text{g}$  on alternate days) of GS-1 (*Griffonia simplicifolia* isolectin) inhibits the enlargement of those tumor cells that lectin-



dependent, macrophage-mediated growth cell lysis may even be Associate in Nursing proof for the cytotoxic impact of conjugated protein. Ganguly and Das (1994) examined the antineoplastic activity of PHA, nonwoody plant agent conjugated protein (PWM), tiny business.

Administration of soya bean and WGA plant lectins on murine pathology malignant growth of cells *in vivo* and set that every 1/4 tested lectins restrained the growth and improved. In Pusztai's laboratory (Bardocz *et al.*, 1994; Pryme *et al.*, 1994a,b), it absolutely was shown that the animals injected intraperitoneally with chemist II pathology tumor cells so Greek deity a diet containing plant protein PHA developed tumors very slowly than the animals on a control diet. They found that the whole form of tumor cells and their molecule, DNA, matter, and polyamine contents were all reduced compared with the management once glycoprotein was enclosed within the diet of tumor-bearing animals. Mukhopadhyay and coworkers (1994) examined the impact of restrictions on diet and protein supplementation for enlargement of the pathology of murine malignant growth of cells and the host immune system. They found that there was a reduction within the amount of vitamins A and E in the liquid substance of tumor-bearing animals, however, it absolutely was raised to ancient levels following the addition of very little Business Administration inside the diet. Another study (Wang *et al.*, 1997) has incontestable the impact of 2 new lectins, TMA I and II (*Tricholoma mongolicum* agglutinin), isolated from mushroom, on tumor-bearing mice. They found that every lectin suppressed the enlargement of malignant growth unwellness one hundred eighty cells and prolonged the generation of growth-bearing animals. Friess and colleagues (1996) investigated the impact of treatment with mistletoe extract on patients with cancer. They found that mistletoe extract did not significantly influence growth in gland carcinomas, but did improve the quality of life for patients in their few remaining months. There are varieties of studies that have contestable *in vitro* cytotoxic results on cancer cells of plant lectins. The *in vitro* primary report of the anticarcinogenic activity of lectins was reported in 1970 (Shoham *et al.*, 1970). During this study, it had been shown that plant glycoprotein Con-A had a restrictive result on the expansion of various lines of cultured *Mesocricetus auratus* embryo cells reworked *in vitro* by the polyomavirus, or when treated with the chemical matter DMNA (Dimethyl nitrosamine), or derived

from SV-40 (Simian Virus-40) an oncogenic DNA virus-induced primary brain and bone cancer, malignant mesothelioma and lymphoma in laboratory animals. Con-A had no result on the growth of traditional 3T3 animal cells (mouse embryonic fibroblast cells) (Shoham *et al.*, 1970). Many connected types of research (Vilarem *et al.*, 1978; Warner and Krueger, 1979; Yawata *et al.*, 1983; Bazeed *et al.*, 1988; Kaplowitz and Haar, 1988; Yu *et al.*, 1993; Ryder *et al.*, 1994) throughout the last decades are administrated exploitation totally different approaches, numerous plant lectins, and growth cell lines. Once (3H)-thymidine, (3H)-uridine, and (3H) essential amino acid uptake were utilized as a criterion for growth of cells and proliferation, associate degree repressing result of plant lectins on macromolecule and macromolecule synthesis was found. It's fascinating to notice that totally different plant lectins had different effects on these processes in various growth and traditional cell lines.

Plant lectins like Con A and genus *Robinia pseudoaccacia* antibody (RPA) repressed DNA biosynthesis in murine respiratory organ malignant neoplastic disease cells additional impactive than supermolecule synthesis and had no restrictive effect on normal murine lung cells (Vilarem *et al.*, 1978). PHA, Con A, PWM, and RPA repressed deoxyribonucleic acid synthesis in murine spleen malignant neoplasm cells and had no impact on normal murine spleen cells (Warner and Krueger, 1979). RCA II repressed DNA and RNA synthesis in human bladder malignant neoplastic disease cells, however, Con A and WGA showed stimulatory impact on supermolecule synthesis (Bazeed *et al.*, 1988). Glycoprotein from common beans (*Phaseolus vulgaris*) repressed deoxyribonucleic acid and RNA synthesis in L929 (murine fibroblast cell line), muscle and liver cells from chick embryo, and human cancer Hela S3 cells (Yawata *et al.*, 1983). Con A and WGA had a repressive impact on deoxyribonucleic acid and supermolecule biogenesis in cultured human formative cells (Kaplowitz and Haar, 1988). RCA, APA, and ML-I (mistletoe glycoprotein, parasitic plant L.) repressed supermolecule biogenesis in human lymphocytic leukemia cells (Dietrich *et al.*, 1992). The antiproliferative effect of ML-I varies according to glycosylation patterns of cancer cell culture (Beztsinna *et al.*, 2018). Fungus genus bisporus glycoprotein (ABL) had a repressive impact on deoxyribonucleic acid biogenesis in human colon (HT29 and CaCo2) malignant

neoplastic disease cells, human breast (MCF7) malignant neoplastic disease cells, and rat exocrine gland fibroblast cell (Rama27), however, PNA (*Arachis hypogaea* antibody, peanut) had no impact on deoxyribonucleic acid biogenesis in CaCo2 cells and exciting DNA biosynthesis in HT29 cells (Yu *et al.*, 1993; Ryder *et al.*, 1992, 1994). Cytotoxic effects of plant lectins against completely different malignant cells were conjointly incontestible by mistreatment different approaches like the MTT (3-(4,5)-dimethylthiazolyl)-2,5 diphenyltetrazolium bromide) quantitative analysis, the Mossman assay, optical research, and flow cytometry. It absolutely was shown that *Griffonia simplicifolia* antibody (GSA) and WGA iatrogenic necrobiosis with the fragmentation of DNA of varied murine tumor cells (Kim *et al.*, 1993). Con A, PHA, and PA-1 (*Pseudomonas aeruginosa* lectin) had a restrictive impact on the growth of murine spleen malignant neoplastic disease (AKR) cells (Leibovici *et al.*, 1986), and PHA and ML-I, -II, and -III had cyanogenic effects on human neoplastic cell (MOLT four, U 937) lines (Gabius *et al.*, 1992; Janssen *et al.*, 1993; Schumacher *et al.*, 1994, 1995a,b).

Two *in vitro* studies reported that lectins reserved human immuno deficiency virus (HIV) sort I infection and had an impact on the receptor specificity of respiratory disease viruses were published recently (Hammar *et al.*, 1995; Ito *et al.*, 1997). It absolutely was ended that GNA (*Gananthus nivalis* lectin) neutralized HIV infection by binding to the virus at the surface of the cell with mannosyl residuals for modulation of infection with cytopathic HIV-INDK (Hammar *et al.*, 1995). Also, agglutinating assays with lectins like MAA and SNA (*Maacki amurensis* agglutinin and *Sambucus nigra* agglutinin) would be helpful in characterizing the receptor specificity of respiratory disease viruses (Ito *et al.*, 1997).

More recently, it absolutely was incontestible that five plant lectins, PNA, GSA, Con A, WGA, and PHA-L, had shown different effects on the growth of cells in three cancers of human body part (LoVo, HCT-15, and SW-837) cell lines. Apart from PNA, all lectins restrained the expansion of tested cells. The restrictive impact was time-dependent and dose-dependent and varied among completely different lectins and cell lines (Kiss *et al.*, 1997). From information like those reviewed here, it looks possible that plant lectins possess cytotoxic activities against completely

different malignant cells *in vivo* and *in vitro*, and principally had no restrictive impact on the growth of normal cells.

### **2.7.1. The Mechanism of Lectin Action**

It is typically accepted that cancer will be prevented by a range of artificial and present compounds. In spite of the increasing body of scientific proof, the mechanism of action of most of those chemo preventive agents remains poorly understood. Additionally, the precise molecular mechanism(s) of lectin antitumor impact is also not relevant in this case, although there are many hypotheses that advise that this impact is related to lectin flexibility to modulate the expansion, differentiation, proliferation, and necrobiosis of *in vivo* and *in vitro* premalignant and malignant cells. Most of those effects of plant lectins are sometimes mediated by receptors present on the cell surface, however alternative mechanisms might also be concerned.

A mechanism that has been planned for the repressive result of plant lectins on experimental tumorigenesis and chemical carcinogenesis is that the distinction in structure of surface membrane between normal and cancer cells (Sharon and Lis, 1995). It had been shown that the lectins have many combining sites and therefore the determination of specificities of lectins by the nature of residues of an organic compound to that the macromolecule is coupled and precise form of binding sites. Little changes within the structure of the sites might lead to marked changes in the specificity of lectins (Sharon and Lis, 1995). On the alternative side, an alteration in the structure of the macromolecule has been discovered in cancerous cells. The expression of branched and sialylated complex-type amendment in the differentiated state of cancerous cells seems to be related to pathological process potential (Dennis, 1991). The glycoprotein binding patterns are specific for cell lines and this might mirror distinct pathways of progression of individual neoplasm cell lines (Tsubura *et al.*, 1991; Rak *et al.*, 1992; Remani *et al.*, 1994; Werner and Kissel, 1996).

These observations will probably justify the differential result of various plant lectins on various forms of malignant cells. Though the surface glycoconjugates of traditional and reworked cells are unremarkably characterized by plant lectins, recently it absolutely was according to that class lectins are desirable as analysis tools in cancer research (Mann *et al.*, 1994). One in all the foremost recent and

systematically discovered organic chemistry effects of plant lectins are their repressing effects on cellular deoxyribonucleic acid, RNA, and supermolecule biogenesis. Of specific interest is that the observation that plant lectins reserved deoxyribonucleic acid, RNA, and supermolecule synthesis in malignant cells (irrespective of whether or not they originated from a tumor or from the transformation of traditional cells *in vitro*) however had no detectable inhibiting result on molecule synthesis in normal cells (Vilarem *et al.*, 1978; Warner and Krueger, 1979; Bazeed *et al.*, 1988; Kim *et al.*, 1993; Schumacher *et al.*, 1995a,b; Kiss *et al.*, 1997). Taken as an entire, these findings recommend that the repressing impact of lectins on molecule synthesis might represent a molecular basis for their inhibiting effect on neoplasm cell differentiation and proliferation. The question has therefore arisen on whether or not the inhibition of deoxyribonucleic acid, RNA, and macromolecule synthesis is of course an instantaneous impact of lectins, or is associate degree indirect results of another effect on the cells. Future experiments should be designed to look at the impact of plant lectins on deoxyribonucleic acid, RNA, and macromolecule synthesis during an acellular system. Many alternative mechanisms for the antineoplastic impact of plant lectins are projected. The observations that plant lectins evoked caspase-mediated cell death with DNA fragmentations which neoplasm cell lysis are often blocked within the presence of the precise sugar inhibitors, permit one to conclude that caspase-mediated cell death could management the response of neoplasm cells to treatment with antineoplastic medicine (Smets, 1994).

In the literature we have a tendency to found robust proof that administration of antitumor medication triggers apoptotic death of tumor cells (Nakamura and Ueda, 1993; Kim *et al.*, 1993; Mayer *et al.*, 1994; Darzynkiewicz, 1995; Lenartz *et al.*, 1996; Werner and Kissel, 1996); thus, we have a tendency to project that the cytotoxic result of lectins is mediate via programmed cell death. A second projected mechanism for the antitumor action of plant glycoproteins relies on the quantitative distinction in lectin labeling and sialyltransferase activity in numerous cancer cells.

It was found that the amounts of sialic acids in cancer cells are related to growth progression (Murayama *et al.*, 1997; Varki, 1997). A 3rd mechanism involves the impact of plant lectins on the activity of two enzymes (DNA enzyme, ribonucleic

acid polymerase) and on the regulation of the adenylation system in cell membranes (Umekawa *et al.*, 1992; San Jose *et al.*, 1993; Leist *et al.*, 1994; Leist and Wendel, 1996). The fourth mechanism that has been prompt the lectins on the surface of the malignant cells are changed and their expression is additionally altered when transformation (Gabijs *et al.*, 1986; Lotan and Raz, 1988). Additionally, it absolutely was reportable that the distinction in the quaternary structure of plant lectins may also play a crucial role within completely different biological properties of those proteins (Sanz-Aparicio *et al.*, 1997). Many studies resulted that, the current proof of anticancer activity of plant lectins. Such scientific findings, along with proof for his or her therapeutic activity against cancer (Ohsawa *et al.*, 1995; Lenartz *et al.*, 1996), offer a robust indication that plant lectins could convince a helpful tool in the analysis of cancer, also as for diagnostic and glycoprotein medical care in drugs. Future investigations specialized in an examination of those prospects in acceptable models of human diseases. Extra studies also will be needed to know the mechanism(s) of the anticancer result of plant lectins. Once a far better insight on glycoprotein action is achieved at the cellular and organic chemistry levels, it ought to be attainable to create a far better assessment of different agents (e.g., artificial compounds) that could act with plant lectins in a very synergistic manner. It ought to even be attainable to create higher predictions on that sorts of protocol (e.g., chemopreventive or chemotherapeutic) are presumably to achieve success, each in animal models and ultimately in human diseases. A lot of analysis is required clearly to clarify and extend a scientific understanding of the health effects of lectins. Basic analysis ought to continue, and extra large-scale randomized trials and clinical studies to be undertaken. Carbohydrates have the ability to encode biological information. Many biological interactions have been shown by glycolipids and glycoproteins between intracellular and extracellular environments (Taniguch *et al.*, 2011). Glycosylation of glycolipids and glycoproteins was changed for neoplastic cells (Pinho and Reis, 2015). There is an overproduction of Glycoproteins and glycolipids in cancer (Zheng *et al.*, 2019). These changes generate the cell membrane signaling molecules. These molecules can induce many biological processes which are related to cell adhesion (tumor progression) angiogenesis, cellular mitosis, and metastasis (Edson *et al.*, 2012). Some neoplastic cells show certain frequent changes in glycans and are known

as tumor-specific (Nangia, 2002). Carbohydrates have an intrinsic role in carcinogenesis, the glycosylation and identification of glycosylated antigens can vary and depend upon the stage of the diseases. Cancerous cells have abnormal patterns of carbohydrates in the glycosylation process. These carbohydrates are linked to ceramides and protein and present on the cell membrane (Hakomori, 1996). Alteration of membrane glycosylation process occurs in progression markers and cancer cells (Magalhaes *et al.*, 2017). All types of cancer have different altered patterns at different stages of diseases (Martinez *et al.*, 2010). Blockage of carbohydrates synthesis and neo-synthesis are two major changes that occur during glycosylation. Lectin-based microarrays help to study the structural alteration of glycans and enable screening and assessment of glycosylation profiles of a therapeutic protein (Pazitna *et al.*, 2020). Alteration of the glycosylation occurs in the cancerous cell may involve structural and functional changes, the appearance of modified structures involvement of truncated structure with their precursors. Carbohydrates present in cancer cells modulate the functions of adhesion receptors or adhesion molecules (Martinez *et al.*, 2010). An increase of N-glycans and sialic acid contents abnormally produced mucin, the expressed structure of Lewis X/A in glycol-sphingolipids and galactin as in their increased expression. In few types of cancer, glycoproteins that are present on the membrane are also modified and act as oncogenic antigens. Few studies in recent years implicated that lectin in cancer cells has cellular interactions, cell growth, adhesion metastasis, and cancer cell differentiation (Martinez *et al.*, 2010). The presence of lectin in cell and host-pathogen interaction, glycoproteins present in blood serum, and immune responses are relevant for tumor growth and metastasis. Alteration in response to cancer cells involves the change in glycosylation like interaction with endogenous and exogenous. The interaction of tumor cells with lectin and their effect on the biology of cancer explains by the role of carbohydrates (malignant status and its reduction). Lectin studies as biological tools have concluded main significance of lectins lied in their ability to cells recognition as tumor cells, RBCs, lymphocytes platelets, sperms, bacteria, viruses (Castillo-Villaneva *et al.*, 2005).

According to previous researches, lectins shows preferential agglutination on tumor cells that is why lectins are used to detect transformed cells and cancer cell by

malignant changes. It is observed that lectin has a higher affinity for human cancer cells (Kuwahara, 2002). The membrane glycoprotein has a low affinity for lectins, but it becomes stronger when formed by multiple binding sites of a weak joint. Lectin can induce cytotoxicity, apoptosis and inhibit the growth of the tumor by using this mechanism (de Mejia, 2005). Types of Mistletoe lectins are MLs, ML-1, ML-2, and ML-3, in which  $\beta$ -chain of carbohydrate inhibits their toxic effects (Frantz, 2000). DBA (*Dolichos biflorus*), PNA, *Lens culinaris* (LCA), *Ulex europaeus-I* (UEA-I) are the fluorescence-labeled lectins that show specific binding on three cancerous cell lines CaCO-2, HCT-8 HT-29 (human colon cancer) (Garbor *et al.*, 1998). Comparative analyses of glycoprotein patterns are using different lectins for human melanoma cells show an increase in N- oligosaccharides expression from metastatic sites (Litynska *et al.*, 2001). A relevant potential using 5637-bladder tumor cells with different plant lectins WGA (wheat gram agglutinin) and UEA (*Ulex europaeus* agglutinin) showed a strong interaction towards single-cell representing a presence of sialic acid, N-acetyl-D-glucosamine, and  $\alpha$ -1-fucose residue on the cell surface (Verena, 2008). ABL (*Agaricus bisporus*) lectins particularly bind to a galactosylated disaccharide present in keratin. The proliferation of cancer cells was inhibited by these lectins reversibly without producing cytotoxins (Parslew *et al.*, 1999). PNA (Peanut agglutinin lectin) binds with an oncofetal antigen named as Thomsen-Friedenrich i.e. increased in the colorectal cancer cell, adenomas, and inflammatory bowel disease. PNA increases the rate of mitosis for colorectal epithelial cancer cells are mediated by C-Met (Singh *et al.*, 2006). As per *in vivo* and *in vitro* studies of anticarcinogenic properties of lectins, binds to the membrane of a tumor cell or to receptors present on their surface, causing reduction of tumor growth and apoptosis (de Mejia, 2005). Different mechanisms are responsible for the anti-carcinogenic effect of lectins, such as certain tumors promoting the immunogenicity and induced the remission of tumor cells and because of cytotoxicity on tumor cells (Alvarez *et al.*, 2002). With the help of previous observations, it is concluded that lectins obtained from various sources like plants, animals, and micro-organisms reduce tumor cell growth in a different manner depending on their concentration (Pryme, 2001). Lectin induces apoptosis and stimulating the proliferation of T-lymphocyte activates the immune response (Lyu *et al.*, 2002). Lectins present in food material can stimulate the



differentiation of colorectal cancer cells (Jordison *et al.*, 1999). The surface receptors mediated the ability of lectins to apoptosis, proliferation, and differentiation (Abdullaev *et al.*, 1997). Previous studies have focused on cytotoxicity of lectins like Abrin (APA) and Ricin agglutinin (RCA) for the treatment of cancer (Lin *et al.*, 1970; Dickers, 2003).

After that, the studies and research performed using *Canavalia ensiformis* (Con A) showed a reduction of tumor growth in hamsters (Soham, 1970). There are many comparative types of research and studies have shown differential effects using lectins as anticancer and cytotoxic agents e.g. lectins isolated from wheat, common beans, and soybeans were studied on the lymphoma cell. These cells are inoculated into normal cells after *in vitro* treatment. All types of lectin have their own therapeutic effects, like inhibition of cancer growth and delaying cancer progression. Among the lectins obtained from food sources wheat germ lectin is most effective to uncontrolled growth and in activation of immune response (Watzl *et al.*, 2001). The multiplication capabilities of cells are probably disturbed; even the cell viability was retained. Macrophages-mediated cytolysis increases the susceptibility of tumor cells, which increases the non-specific cell lysis by increasing the binding capacity of cells (Watzl *et al.*, 2001). There are five different lectins with different concentration and type of lectin affect the growth of the tumor by a different way, e.g. *Phaseolus vulgaris* agglutinin (PHA), *Griffonia simplicifolia* agglutinin (GSA), Con-A (*Concavalia-A*), wheat germ agglutinin and *Arachis hypogeal* on three colorectal cancer cell lines (HCT-15, Lovo and SW 837). On basis of studies, it has concluded that lectins that were *in vitro* treated to have a good potential to inhibit tumor cell growth (Kiss, 1997). Lectins isolated from the common beans, botanical name *Phaseolus vulgaris* have mitogenic cells of the immune system and agglutinate malignant cells. This ability of lectin play important role in the inhibition of cancer growth (Riano *et al.*, 1997). European Mistletoe botanical name *Viscum album* used in cancer treatment or therapies as an anticancer agent has strong modulation of the immune system, more than 80 years (Ahmad *et al.*, 2018; Patel and Singh, 2018). With the Eurixor exposure in phase I and phase II, some important improvements have been found in patients suffering from pancreatic cancer stage III and stage IV (Friess *et al.*, 1996). On the other side, the patient suffering from head and neck cancer (squamous cell carcinoma)

did not find any improvement (Steuer *et al.*, 2001). *Viscum album* (VAA) a European variety and a Korean variety *Viscum album colorectum* (VCA) are the two Mistletoe lectins that have been studied for their effect on cancer, like cytotoxic activity against leukemia, T-cell lymphoblast (Molt-4 cells) (Lyu *et al.*, 2000). Lectins isolated from Chinese Mistletoe present some important effects on T-cell lymphoblast like cytotoxic effect, apoptosis, cytokine production, TNF- $\alpha$  (alfa-tumor necrosis factor) release anti-inflammatory IL-10 (interleukin-10) (Gong, 2007). Three major families that extensively studied due to their biological application are; RIPs II (Type-II ribosome-inactivating protein, Legume superfamily of lectins and GNA-related lectins are (Van Damme, 1998). Plant lectins act as a recognizing tool to differentiate the metastasis with a degree of glycosylation from malignant tumors. Plant lectins also reduce the side effect related to the treatment act as adjuvant agents during chemotherapy and radiotherapy (de Mejia, 2005; Choi *et al.*, 2004). Ricin, *Triticum vulgare* (WGA) and Mistletoe lectin (*Viscum album*) is possessing anticarcinogenic activity by inducing apoptosis in tumor cells (Chang, 2007; Lui *et al.*, 2009). LCA and *Aleuria aurantia* lectin (AAL) bind to an early diagnostic marker fucosylated prostate-specific antigen (PSA) of prostate cancer (Zhou *et al.*, 2017; Wang *et al.*, 2019). Due to the specificity of lectins, there is the development of numerous applications in Medical science. It is important to present the adverse effect of lectins in some cases, even the anticarcinogenic properties of lectins have been described in the studies (Castillo-Villaneva, 2005). Lectins are toxic in nature, most of the lectins are isolated from both plants and animals cause a different degree of toxicity (Rhodes, 1999; Tareq *et al.*, 2001). Allergy and administration routes are factors responsible for the toxic effect of lectin (de Mejia, 2005). Some studies have been focused on the properties of Ribosome-inactivating protein lectin for immunotoxins production against tumor cells. RIPs are attached to tumor cells with a specific receptor-binding site on the monoclonal antibody. After many clinical trials, one major adverse effect has been reported the therapeutic dose limit in patients under the treatment for immunotoxins (vascular infiltration syndrome) produced by the Ricin chain (Castillo-Villaneva, 2005). Immunotoxins were designed for selective delivery of a toxin to malignant cells by linking a toxic domain to a specific target (Mei *et al.*, 2019). Ricin has been assessed for such a purpose (Polito *et al.*, 2019).

### **2.7.2. The Molecular Biology: Apoptosis induced by a plant lectin**

Programmed cell death or apoptosis was pointed by condensation process of cytoplasm and cell nucleus, cell contraction, merging of chromatin, DNA-fragmentation, dynamic membrane blubbing, in nuclear periphery and phagocytosis. Programmed cell death is recognized as the most essential molecular mechanism and maintained by many cell signaling pathways for the suicide of cancer cells. Apoptosis is deeply related to cancer. The programmed cell death is required to suppress the carcinogenesis by the recognition of molecules. The carcinogenesis causes the adoption of modulation of apoptosis for new targeted chemotherapy (Abdullaev and de Mejia, 1997). Modification in vital components of cell lysis machinery is an important approach in the extraction of plant lectins (Pusztai *et al.*, 2008).

## **2.8. Pharmaceutical activities of Plant Lectins.**

### **2.8.1. Antiviral drug**

The D-mannose-specific lectin was isolated from *Gerardia savaglia* and first reported to prevent HIV-1 infection of H9 cells. The lectin also inhibited the formation of syncytium in the HTLV-IIIB / H9-Jurkat cell system and the HIV-1/human lymphocyte system by reacting with the HIV-1 gp120 side chains (Müller *et al.*, 1988). After a period, it is found that the lectins of concanavalin A, wheat germ agglutinin, *Lens culinaris* agglutinin, *Vicia faba* agglutinin, and *Pisum sativum* agglutinin had the capacity to tie up gp120 and repress combination of HIV-tainted cells with CD4 cells. In extreme intense respiratory disorder coronavirus, plant lectins indicated hostile to coronaviral action, especially mannose-restricting lectins. In the beginning time of the replication cycle, they meddle with viral connection and stifled development by interfacing toward the finish of the irresistible infection cycle. *Musa acuminata* lectin inhibited HIV replication (Swanson *et al.*, 2010). There was a further investigation into lectins the treatment of AIDS. Various lectins have different mechanisms for anti-HIV. More recently, lectin of *Chaetopterus variopedatus* (marine polychaete worm) (Wang *et al.*, 2005) inhibited HIV-1-induced cytopathic effect and p24 antigen production. Lectin of *Serpula vermicularis* (sea worm) suppressed HIV-1-induced viral p24 antigen production (Molchanova *et al.*, 2007). Lectin of *P. cyrtoneura* Hua reduced cytopathicity caused by HIV-I and HIV-II in MT-4 and CEM cells (An *et al.*, 2006). Lectin of *Gerardia savaglia* (Banana) was

straight forwardly bound to gp120 an HIV-1 envelope protein and obstructed the infection section into the cell, bringing down the dimensions of an early turn around translation solid stop item (Swanson *et al.*, 2010). HIV-1 invert transcriptase was repressed by lectin of purple bean (Fang *et al.*, 2010) and mushroom *Russula delica* lectin (Zhao *et al.*, 2009). Consequently, lectins are potential medicines for AIDS treatment. In addition to the above-mentioned practical lectin applications, antibacterial and anti-nematode activities were also reported from some isolated lectin (Ngai and Ng 2007; Wang *et al.*, 2005).

### **2.8.2. Immunomodulatory activity of Plant lectins**

An immune system is a system of biological structures and processes that protects an organism against diseases (Ferrero, 2007). The human immune system comprises numerous cells and biomolecules which act in a harmonious and agreeable way with a definitive target of destroying the harmful factors (Margetic, 2012). The innate immune response and adaptive immune response are two phases of activity in the immune system which were clearly identified. There is a non-specific activity of cells and cytokines in the innate immune response with the main purpose of rapidly annihilating the local harmful agents. Feature the neutrophils, eosinophils, basophils, and macrophages at this stage, cells with entrenched exercises yet with the basic capacity of cytokine creation and discharge. These cytokines are atoms with various fiery procedure capacities, for example, chemotaxis, initiation of certain cell gatherings, and expanded tissue perfusion (Hurwitz *et al.*, 2012). Then again, another arrangement of cells, the lymphocytes, makes the versatile insusceptible reaction. These cells are in charge of the creation of explicit antibodies for intrusive microorganisms and the apoptosis components in abnormal cells (Fiocchi, 1997). Traditionally used Korean mistletoe has analgesic, anti-spasmolytic, cardiotonic, sedative, and anticancer properties. This plant confined a significant lectin and investigated its immunomodulatory action (Lee *et al.*, 2007). KML has tweaked insusceptible reactions interceded by macrophages differentially. It additionally expanded the statement of IL-3, IL-23, and TNF-a cytokines, the age of reactive oxygen species (ROS), phagocytosis, and the surface dimensions of certain glycoproteins. In any case, the utilitarian actuation of bond atoms assessed by attachment occasions of cell-cell or fibronectin was up-controlled by treatment with

KML. An immunomodulating movement was reported by a lectin of *Canavalia brasiliensis* seeds (ConBr). ConBr could prompt splenocyte expansion *in vitro* with negligible harm to the structure of the cell. ConBr likewise expanded cytokine generation, for example, IL-2, IL-6, IFN- $\pi$  and diminished IL-10. These discoveries show this present lectin's potential immunomodulatory impact related to the characteristic job of sugars in the incendiary procedure related intercellular correspondence. An ongoing report (de Melo et al., 2010) assessed the impact of lectin removed from *Cratylia mollis* Mart seeds on lymphocyte lectin action. (Cramoll 1,4) on mice lymphocyte exploratory societies. In this investigation, viewpoints straight forwardly identified with amplification as generation of cytokine, cytotoxicity, and cell creation of nitric oxide were assessed. In addition, the tested concentrations for cytotoxicity were not demonstrated by Cramoll 1,4 but able to induce IFN- $\alpha$  and demonstrated anti-inflammatory activity by suppressing NO production.

### **2.8.3. Pro-healing activity of Plant lectins**

As of late, research has focused on the possible healing properties of some lectins (Brustein *et al.*, 2012). This objective is supported by the fact that the inflammatory process can be interfered with by such molecules. However peculiar and interesting results can be observed, this effect is not yet fully elucidated. An experiment conducted on a murine model, using a lectin isolated from *Bauhinia variegata* seeds (BVL) on wounds induced surgically, revealed the pro-healing potential of such molecules. In spite of that still, it is not clarified, this lectin is recommended to invigorate the mitogenic action of occupant cells, making them powerful chemotactic operators for the enrollment of neutrophils by discharging cytokines (Neto *et al.*, 2011). The BVL stimulated the differentiation of fibroblasts into myofibroblasts (Li and Wang, 2011).

### **2.8.4. Anti-oxidant activity of Lectins**

Lectins act as an inhibitor that protects cells from harm caused by free radicals. They conjointly slow down the digestion and therefore the absorption of carbohydrates, which can forestall sharp rises in glucose and high internal secretion levels. Early analysis is additionally observing the employment of non-toxic low amounts of lectins to assist stimulate gut cell growth in patients who are unable to eat

for long periods, and in antitumor treatments because of the power of lectins to cause neoplastic cell death (World Health Organization) (Vasconcelos & Oliveira, 2004; Liu *et al.*, 2013). In several massive population studies, lectin-containing foods like legumes, whole grains, and some other crops are related to lower rates of disorder, weight loss, and sort a pair of polygenic disease (Reban *et al.*, 1994; Liu *et al.*, 1999; Aune *et al.*, 2013; de Munter *et al.*, 2007). These foods are wealthy sources of B vitamins, protein, fiber, and minerals, and healthy fats. Thus, the health advantages of overwhelming these foods way outweigh the potential hurt of lectins in these foods.

## 2.9. Biological applications of Lectin

### 2.9.1. Anti-insect activity

Lectins show the activity of anti-insects. They either increase mortality or delay insect development. Lectins were suggested as the most promising anti-insect pest agents. Lectins were successfully engineered into wheat, rice, and potatoes, and in some other crops. It is an integrated strategy that could be used for pest management and attack of pest caveats. Overall, enormous scale usage of herbicide-tolerant plants and transgenic insecticidal does not seem to have huge unfriendly natural impacts. In addition, there are some transgenic plants which help to improve the environmental conditions and health of human being, as the significantly reduced production load of herbicides and chemical insecticides (Velkov *et al.*, 2005). When lectin of *Arisaema jacquemontii* is incorporated into an artificial diet has adverse effects on the growth of larvae of *Bactrocera cucurbitae* (Kaur *et al.*, 2006). Anti-insect effects were shown by lectin of *Arisaema helleborifolium* in the direction of larvae Cucurbitae (Kaur *et al.*, 2006).

**Table 3. The anti-insect activity of Plant Lectins**

Source of lectin	Insect affected	Anti-insect activity	Sugar specificity	References
Garlic bulbs ( <i>Allium sativum</i> )	<i>Acyrtosiphon Pisum</i>	Mortality rate increased	Mannose	Fitches <i>et al.</i> , 2008

<i>Arisaema intermedium</i> and <i>Arisaema wallichianum</i> (Araceae)	<i>Bactrocera Cucurbitae</i>	(1) Long-term development (2) Pupation and emergence inhibited	Non-specific	Kaur <i>et al.</i> , 2009
Red alga ( <i>Gracilaria cornea</i> )	<i>Boophilus Microplus</i>	(1)Reduced post-oviposition female body weight, (2) egg mass weight, and (3) hatching period	Fetuin, porcine stomach mucin	Lima <i>et al.</i> , 2005
<i>Gracilaria ornate</i>	<i>Callosobruchus Maculates</i>	Development delayed	N-acetyl-D-glucosamine	Leite <i>et al.</i> , 2005
<i>Myracrodruon urundeuva</i>	<i>Aedes aegypti</i>	Increased mortality	Fetuin, porcine Mucin	Sa <i>et al.</i> , 2009
<i>Xerocomus chrysenteron</i>	<i>Myzus persicae</i>	(1) Increased mortality (2) Reduced body weight, developmental duration, and fecundity	Fetuin, porcine stomach mucin	Jaber <i>et al.</i> , 2007

The enzymatic activity inside larvae has shown the insecticidal property of lectins. The enzyme esterases when treated with different lectins have shown increased activity in larvae, while the activity was reduced in the case of acid phosphatase and alkaline phosphatase. Microvilli disruption is caused by Galectin-1 treated larvae of *Plutella xylostella* and anomalies in these epithelial cells (Chen *et al.*, 2009). The growth of *Helicoverpa armigera* larvae was inhibited by lectin of *Dioscorea batatas* by binding to the border of the larval brush and the peritrophic membrane (Majumder *et al.*, 2005). Lectin of *Olneya tesota* bound to midgut

glycoconjugates and microvillae of larvae of *Zabrotes subfasciatus* that cause decreased oviposition of adult beetles (Lagarda-Diaz *et al.*, 2009). Lectin of *Annona coriacea* demonstrated poisonous quality in *Anagasta kuehniella*, which obviously came about because of changes in the gut film condition and ensuing interruption of the components of stomach-related catalyst reusing by an official to midgut proteins (Coelho *et al.*, 2009). When lectin of *Bauhinia monandra* leaf was incorporated into an artificial diet, caused an increased rate of mortality in *Zabrotes subfasciatus* and *Callosobruchus maculatus*. *B. Monandra* leaf lectin resulted in the weight decrease of Larvae of *A.kuehniella* by 40 percent.

The leaves were separated from transgenic plant of tobacco communicating *Allium sativum* lectins have diminished the added weight to improve the *Spodoptera littoralis* hatchlings and their transformation. The hatchlings were additionally hindering during the pupal stage, weight reduction, and deadly variations from the norm (Sadeghi *et al.*, 2008). *Rhopalosiphum maidis* nymph production on *Galanthus nivalis* agglutinin-expressing plants has been significantly reduced (Wang *et al.*, 2005).

### **2.9.2. Antifungal activity**

Regardless of the huge number of lectins and hemagglutinins purged, antifungal action was shown by just a couple of them. The parasite *Trichoderma viride* emphatically initiated the declaration of *Gastrodia elata* lectins in the vascular cells of roots and stems, demonstrating that lectin are a significant safeguard protein in plants (Sá *et al.*, 2009). The germination of *Botrytis cinerea*, *Colletotrichum lindemuthianum*, and *T.Viride* has been significantly reduced after insertion of precursor gene of stinging nettle isolectin-I into tobacco (Does *et al.*, 1999). In order to protect them from fungal attacks, lectins can be introduced into plants. The plant lectins do not penetrate the cytoplasm and do not bind with glycoconjugates because of the cell wall barrier. Even by modification of the structure and permeability of the fungal membrane, lectins do not inhibit fungal growth directly. However, the binding capacity of lectins to carbohydrates on the surface of the fungal cell wall can affect indirectly. Chitinase-free stinging nettle (*Urtica dioica* lectin) impeded the growth of the fungus. Due to attenuated synthesis and deposition of chitin, cell wall synthesis was interrupted (Van *et al.*, 1991).



**Table 4. Antifungal activity of Plant Lectins**

Source of lectins	Effect on fungal species	Carbohydrate specificity	References
<i>Amaranthus viridis</i>	<i>Botrytis cinerea</i> ,	T-antigen, N-acetyl-D-galactosamine	Kaur <i>et al.</i> , 2006
<i>Astragalus mongholicus</i>	<i>Borrytis cinerea</i>	D-galactose, lactose	Yan <i>et al.</i> , 2005
<i>Capparis spinosa</i>	<i>Valsa mali</i>	D(+)-galactose, $\alpha$ -lactose,	Lam <i>et al.</i> , 2009
<i>Capsicum frutescens</i>	<i>Aspergillus flavus</i> , <i>Moniliforme</i>	D-mannose, glucose	Nagai and Ng, 2007
<i>Curcuma amarissima</i> <i>Roscoe</i>	<i>Colectrotrichum cassiicola</i> ,	Non-specific	Kheeree <i>et al.</i> , 2010
<i>Dendrobium findlayanum</i>	<i>Alternaria alternata</i> , <i>Colletrichum sp.</i>	Non-specific	Sattayasai <i>et al.</i> , 2009
<i>Phaseolus vulgaris</i>	<i>Mycosphaerella arachidicola</i>	Non-specific	Xia and Ng, 2005
<i>Phaseolus vulgaris</i>	<i>Valsa mali</i>	Non-specific	Lam and Ng, 2010
<i>Phaseolus coccineus</i> Seeds	<i>Gibberalla sanbinetti</i> , <i>Helminthosporium maydis</i> ,	Sialic acid	Chen <i>et al.</i> , 2009
<i>Phaseolus vulgaris cv</i>	<i>Coprinus comatus</i> , <i>Fusarium oxysporum</i> ,	Lactoferrin, Thyroglobulin	Ye <i>et al.</i> , 2001

<i>Pouteria torta</i>	<i>Saccharomyces carevisiae, C. musae</i>	Fetuin, asialofetuin, heparin	Boleti <i>et al.</i> , 2007
<i>Talisia esculenta</i>	<i>Microsporum canis</i>	D-mannose	Pinheiro <i>et al.</i> , 2009
<i>Withania somnifera</i>	<i>Fusarium moniliforme,</i>	Non-specific	Ghosh, 2009
<i>Zea mays</i>	<i>Aspergillus flavus</i>	D(+)galactose	Baker <i>et al.</i> , 2009

A few other plant lectins repress the development of the organism. The major family comprises of little chitin-restricting merolectins with one chitin-restricting space, for example, hevein (elastic tree latex) (Van *et al.*, 1991) and *Amaranthus caudatus* seed chitin-restricting polypeptides (Broekaert *et al.*, 1992). Chimerolectins are only planted lectin belonging to the chitinases-I that can be considered as a protein with anti-fungal properties. However, these proteins' antifungal activity is attributed to their catalytic domain.

### 2.9.3. Anti-microbial effects of some Plants Lectin

A lot of, human micro-organisms, bacteria, pathogens use cell surface glycans as each of two receptors to introduce adhesion and infection (Sharon and Lis, 1989; Sharon and Lis, 2003; Zem *et al.*, 2006; Hyun *et al.*, 2007; Oppenheimer *et al.*, 2008; Magalhaes *et al.*, 2009; Mukhopadhyay *et al.*, 2009). *E.Coli* (*Escherichia coli*), e.g. when influenza viruses fix with host sialic acids cleft to host mannosidase (Mukhopadhyay *et al.*, 2009). The specificities of various host cell surface carbohydrate moieties have been found in different strains of *E.coli* (Khan *et al.*, 2000; Buts *et al.*, 2003). *Streptococcus pneumonia* binds the pentasaccharide and *Neisseria gonorrhoea* binds the N-acetylgalactosamine selectively in addition to the internal tetra and trisaccharides respectively, the reproductive organ pathogens (micro-organism, bacteria, and virus). In particular, *Pseudomonas aeruginosa* binds fucose (L-Fuc) (Barthelson *et al.*, 1998). Microbes will isolate in precisely one hydroxyl bunch between two comparable glycans (Sharon, 2006). Such host-pathogen interfaces are multivalent, making the coupling occasions profoundly partial and

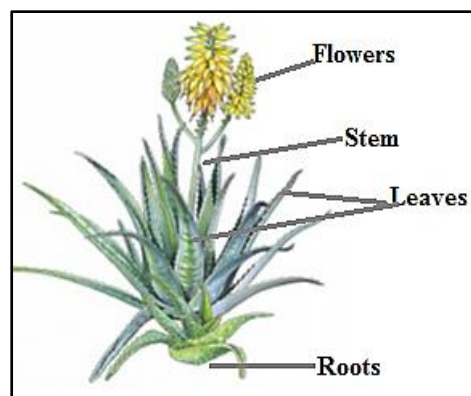
reasonable for intrusion (Nimrichter *et al.*, 2004; Mukhopadhyay *et al.*, 2009). Cytotoxic response of lectins might be uncovered through the enemy of viral and hostile to tumor movement just as unfavorable consequences for small scale living beings. Diverse starch explicit lectins can expand pathogens (microscopic organisms and parasites) restraint of development. Hostile to the bacterial movement of lectins on microscopic organisms (Gram-positive and Gram-negative microorganisms) happens through the perceptive of lectin with parts of the bacterial cell surface which incorporates lipopolysaccharides, teichuronic acids, and peptidoglycans; examination announced that the isolectin-I separated from seeds of *Lathyrus ochrus* tie to mixes muramic corrosive and muramyl dipeptide by H-bonding among ribose and starch restricting site of lectin and hydrophobic active alongside chains of Tyr100 and Trp128 buildups of isolectin-I (Bourne *et al.*, 1994).

Despite a large number of refined lectins and hemagglutinins, only a few of them showed antifungal activity. Fungal growth inhibition will occur through the binding of glycoprotein to fungal hyphae resulting in poor nutrient absorption as well as interference with the germination method of reproductive structure (Lis and Sharon, 1981). Polysaccharide polyposis is a segment of semipermeable membrane growths and chitin-binding lectins have antifungal movement; impedance of combination, as well as imprecation of polyosis in the semipermeable layer, may likewise be the reason for antifungal activity (Selitrennikoff, 2001). No doubt, inside the antifungal mechanisms, the carbohydrate restricting property of glycoprotein is concerned and lectins of various specificities will advance unmistakable impacts. It is trusted that plant agglutinins assume a job in plant insurance against phytopathogens (Sa *et al.*, 2009a). The execution of *Gastrodia elata* lectins in stem and roots tube cells was emphatically brought about by the parasitic *Trichoderma viride*, demonstrating that glycoprotein is a vital protein in plants (Sa *et al.*, 2009b). The addition of the *Urtica dioica* isolectin-I indicator factor to tobacco has significantly reduced the spread of *Botrytis cinerea*, *Colletotrichum lindemuthianum*, and *Trichoderma virides* (Does *et al.*, 1999). In order to protect them from flora attack, lectins could also be introduced into plants. Plant lectins will not bind or penetrate the cytoplasm attributable to the cytomembrane barrier to glycoconjugates on the plant membranes. Even so, on the fungal membrane, the binding capacity of lectins to

carbohydrates could also create indirect effects. Chitinase-free *Urtica dioica* lectin blocked the growth of the fungus (Van Parijs *et al.*, 1991). Synthesis of plasma membranes was unfinished due to extended synthesis or demotion of chitin. The impact of *Urtica dioica* glycoprotein on the membrane of fungal cells and the advice of hyphal morphology that endomycorrhizal is managed by nettle lectins in rhizomes. Many different plant lectins inhibit the growth of the fungus. The primary cluster comprises small chitin-binding merolectins with one chitin-binding domain, e.g. rubber tree latex hevein (Van Parijs *et al.*, 1991) and chitin-binding peptide from amaranth seeds (Broekaert *et al.*, 1992).

Chimerolectins in the chitinases category-I is the only plant lectins to be considered as antifungal proteins. However, the antifungal activity of these proteins is attributed to their chemical processes.

#### 2.10. A Review on *Aloe barbadensis*:

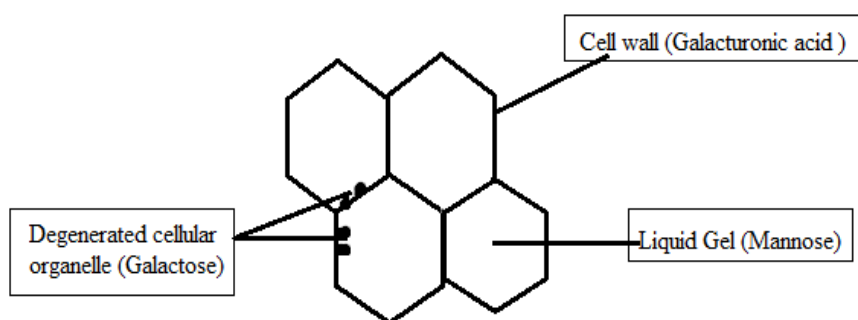


**Fig.8. *Aloe barbadensis* plant (IARC Monograph 108).**

India is truly called the "Botanical garden of the World" because of the largest producer of medicinal plants in India. Plants have natural therapeutic and medicinal values against various diseases (Shakya, 2016). The *Aloe barbadensis* plant has many medicinal, homeopathic, and allopathic uses. In the 20<sup>th</sup> century, herbs have been used as home remedies (Williamsons *et al.*, 1999). In Asia, the products and drugs are derived from medicinal plants have been used therapeutically (Parthen, 1998). On the basis of recent research, the population of the world has become more aware and interested in the use of herbs in the 21<sup>st</sup> century decline the use of herbal remedies began in the USA (Saeed *et al.*, 2003). Due to the chemical synthesis, structural

analogs, and isolation, herbal remedies are disappeared. After 50 years medicinal plants became the best source of a variety of drugs. According to a survey, it has been shown that about 60 million people of the total population of the world are using medicinal plants as home remedies (Williamson *et al.*, 1999). *Aloe barbadensis* is a vital important plant that has been used in ancient times for different diseases e.g. in the treatment of burns, wound healing (Parthen, 1998; Mahor *et al.*, 2016).

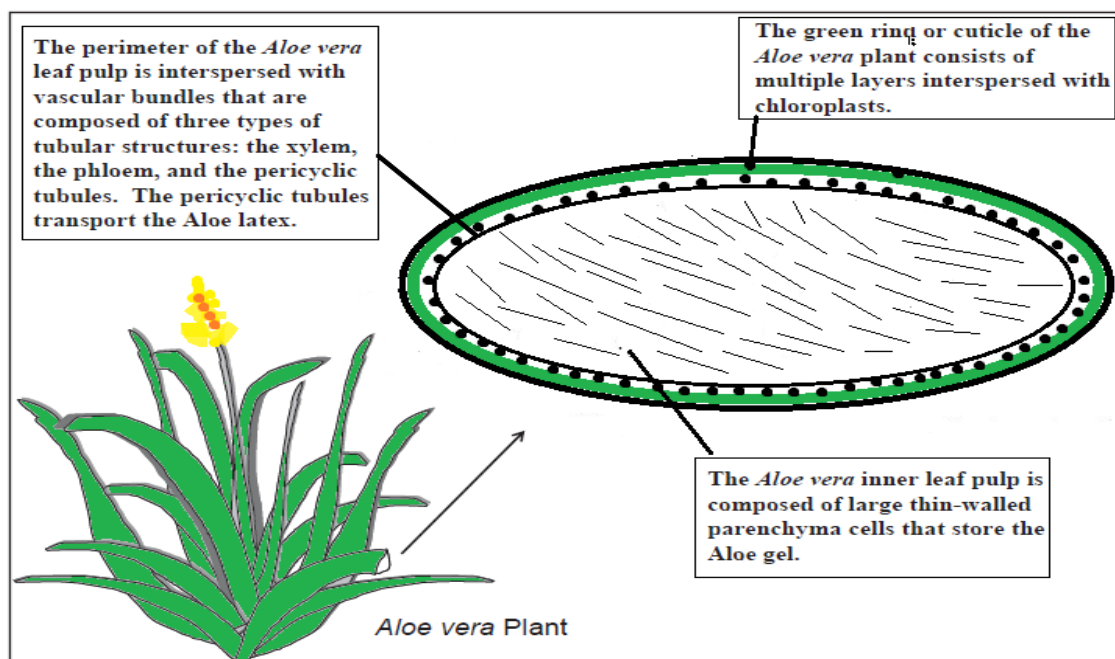
The name *Aloe barbadensis* has been picked from an Arabic word ‘Alloeh’ that means ‘substance which is bitter in taste’ (Mendal, 1980). Aloe is a common and traditional medicine both in China and in India. The gel of *Aloe barbadensis* is cool and moist, that is used to cure constipation and burns (Sahu *et al.*, 2013). The gel of *Aloe barbadensis* has various uses including, anticancer, cell reinforcement, antimicrobial, antiallergic, immunomodulatory, hepatoprotective, antiulcer, and antidiabetic (Sanchez *et al.*, 2017). The common names of Indian *Aloe barbadensis* are Ghritika kumari, True aloe Barbados, Aloe Lu hui, Aloe indica, Kwareyan, Kwar gandhal. Belong to the family Liliaceae and genus Aloe (Kumar *et al.*, 2012). *Aloe barbadensis* is a perennial succulent or xerophytes grow in dry chalky soil or sandy loam is the best favorable condition (Grindlay and Reynold, 1986). The variation in the variety and quality of products and extract isolated from the *Aloe barbadensis* plant is due to different environmental conditions, processing, and storage (Boudreall *et al.*, 2013). USA China and Thailand were the countries that have the highest production of *Aloe barbadensis* (Rodriguez *et al.*, 2010).



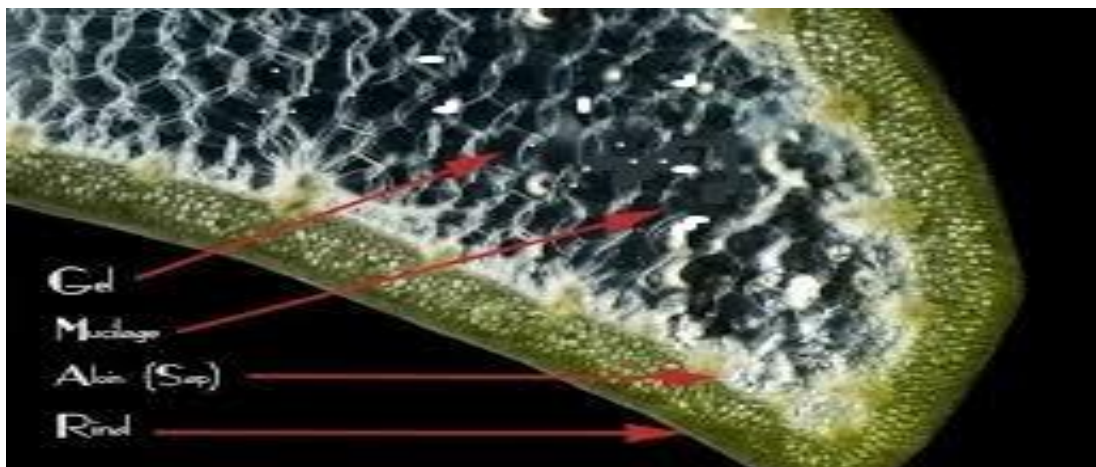
**Fig.8.1. *Aloe barbadensis* leaf pulp with its components (Hamman, 2008).**

*Aloe barbadensis* is a very short stem plant with a maximum height of 80-100cm spreading by root sprouts and offsets. *Aloe barbadensis* plants can store a large

volume of water in their tissues. *Aloe barbadensis* plant has green thick fleshy leaves are enveloped with thick cuticle and transparent pulp present in the inner side of leaves. The leaves are thick and fleshy, green in color. The tissues of *Aloe barbadensis* leaves contain gel and pulp which are transparent in color. The flowers grow up to 90 cm. *Aloe barbadensis* plants contain a major part of liquid material (Kumar *et al.*, 2010). Latex is located under a vascular layer of leaves having bitter in taste and yellow color. This bitter yellow latex has a high concentration of a compound named anthraquinone. The 2<sup>nd</sup> part is a clear layer gel present in the inner zone and produced by thin-walled tubular cells. This gel has been used in the earlier time to treat wounds and burns (Sahu *et al.*, 2013). This gel also prevents infection (Joseph and Raj, 2010). 3<sup>rd</sup> liquid is obtained by macerating the leaves. A number of the process such as mashing, pressing or grinding, filtration, decolorization, stabilization, heat processing with the addition of stabilizers and preservatives have involved in the production of products of *Aloe barbadensis* (Ahlawat and Khatkar, 2011). Harvesting of *Aloe barbadensis* leaves is manually performed like leaves of *Aloe barbadensis* plant cut from leaf base by hand (Grindlay and Raynold, 1986). These leaves are individually wrapped, crated, and transplanted to the process area.



**Fig. 8.2. Cross sectional view of *Aloe barbadensis* leaf (IARC Monograph 108).**



**Fig.8.3. *Aloe barbadensis* leaf with three cell layers, the protective layer, the middle thick layer, and the inner layer (Rudiger and Gabius 2001).**

The components of *Aloe barbadensis* can be affected by environmental factors such as light, temperature, air, humidity, etc. i.e. why within few hours after harvesting the *Aloe barbadensis* leaves processed (Palz *et al.*, 2000). Before processing the *Aloe barbadensis* leaves are treated with water and diluted chlorine solution (Grindlay and Raynold, 1986).

## **2.11. Biochemical Constituents**

The *Aloe barbadensis* gel, composed of some solid constituents, is divided into two parts; 1) Small molecules form 0.4% part. These small molecules have low molecular weight. These constituents are anthraquinones, vitamins, enzymes, saponins, sterol, etc. 2) Large molecules form 0.1% are polysaccharides such as glucomannan and acemannan, glycoproteins and lectins (Mendal, 1980).

### **2.11.1. Low molecular weight constituents (Misir *et al.*, 2014)**

#### **2.11.1.1. Amino acids**

*Aloe barbadensis* contains seven amino acids (essential) which cannot be synthesized by the body. These amino acids are components of proteins (functional enzymes and hormones (Joseph and Raj, 2010).

#### **2.11.1.2. Anthraquinones**

The main pharmacologically active anthraquinones are aloin, aloe-emodin, barbaloin, and emodin. Anthraquinones are used as an anti-inflammatory, anti-protozoal, antioxidant agent. Anthraquinones are present in latex, resin, or sap. Anthraquinones are glycosides and soluble in water, split by bacteria present in the

intestine into a form of glycones which affect the laxative activity (Brown, 1980). For medicinal purposes, the residues of dried leaves are used as an herbal laxative. The product of *Aloe barbadensis* (anthraquinone) is usually taken at night. The oral administration took a long time in the absorption of these products after bacterial hydrolysis gets well absorbed in the body. After absorption, they are excreted in the urine, bile, breast milk, and feces. Due to the risk of stimulating uterus contraction most herbalists are not recommended in pregnancy and lactation, because of its excretion in breast milk. The *Aloe barbadensis* has a stronger laxative action such as senna, cascara, cramping, nausea, and diarrhea (Brown, 1980).

#### **2.11.1.3. Enzymes**

Enzymes amylase, alkaline phosphatase, carboxypeptidase, catalase, peroxidase, and lipase are the enzymes isolated from the *Aloe barbadensis*. These enzymes help in the breakdown of fats, sugar and cause anti-inflammatory action (Joseph and Raj, 2010).

#### **2.11.1.4. Minerals**

There are nine minerals present in *Aloe barbadensis* are calcium, chromium, copper, iron, magnesium, manganese, potassium, sodium, and zinc. All the minerals are essential for good health. The combination of certain minerals and vitamins are known to work with each other such as histidine decarboxylase activity inhibited by magnesium lactate. This mechanism helps to prevent the formation of histamine from histidine. Histamine formed during an allergic response and cause redness, itching, and pain (Joseph and Raj, 2010).

#### **2.11.1.5. Hormones**

Auxins and gibberellins are the two hormones, present in *Aloe barbadensis*. Both the hormones play a significant role in healing wounds and inflammation (Saeed *et al.*, 2010).

#### **2.11.1.6. Salicylic acid**

It is an aspirin-like compound and acts as an analgesic. Help to dilute the blood circulation (Joseph and Raj, 2010).



#### **2.11.1.7. Saponins**

Saponins are present in the form of glycosides. Saponins are a soapy substance form three percent of the total gel part and have antiseptic properties (Saeed *et al.*, 2004).

#### **2.11.1.8. Sterols**

*Aloe barbadensis* has 4 plant sterols that act as anti-inflammatory agents Cholesterol, campesterol, lupeol,  $\beta$ -sitosterol. Lupeol possesses analgesic and antiseptic properties (Choi and Chung, 2003).

#### **2.11.1.9 Vitamin**

Vitamins found in *Aloe barbadensis* are A, E, C, B<sub>6</sub>, B<sub>12</sub>, choline, and Folic acid. Vitamin B<sub>12</sub> and folic acid improve the production of RBCs (Choi and Chung, 2003).

### **2.11.2. High molecular weight constituents**

#### **2.11.2.1. Polysaccharides**

The polysaccharides formed about 20% of the total solid mass. The polysaccharides are composed of linear highly acetylated  $\beta$ -1,4 mannan, branched dextrans, peptic acid, and negatively charged polysaccharides. Hexose polysaccharide formed about 10% of total solid contents. *Aloe barbadensis* gel contains 99% water with a 3.5 pH value and is used as a conditioner (Mendol and Das, 1980). The gel contains glucomannan an emollient polysaccharide used as a moisturizer in most cosmetics. Acemannan is the main water-soluble long-chain mannose polymer carbohydrate present in the gel, which helps to modulate immune response such as macrophages activation, cytokinesis, wound healing, and inhibition of antiviral activities (Hamman, 2008).

#### **2.11.2.2. Proteins**

Proteins formed 1% of the total solid contents of the gel extract with a low molecular weight of 11000 and 14000 KDa for two peptide chains. The proteins are specific for carbohydrates and probably associated with lectins (Winters, 1991).

#### **2.11.2.3. Glycoproteins**

Homogenous glycoprotein 34% of carbohydrates has also been isolated from *Aloe barbadensis* with a molecular weight of 40KDa. Glycoproteins stimulate the DNA synthesis in kidney cells of body hamsters. Glycoproteins isolated from *Aloe*

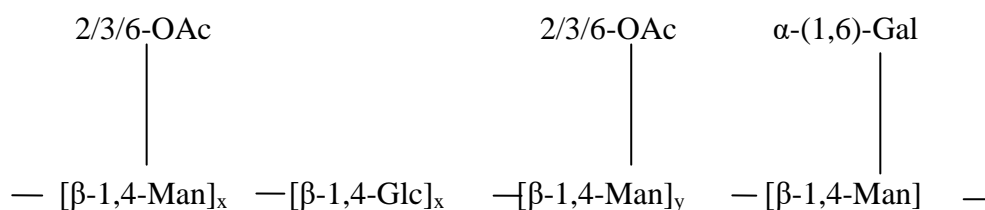
*barbadensis* gel that promotes human and hamster cell proliferation and improve wound healing (Choi and Chung, 2003).

#### 2.11.2.4. Lectins

There are two types of lectins isolated from the leaf pulp, Aloctin I and Aloctin II (Akev, 1999). The activity of aloctin II was inhibited by N-acetyl-D-galactosamine. The lectins have both mitogenic and haemagglutinating properties. The lectin protein sequence is homologous to snowdrop-binding lectin (Koike, 1995).

#### 2.11.2.5 Mannon

Mannon act as a hemicellular that binds celluloses that bind cellulose and play a structural role. Linear mannons have a linear chain of (1→4)-D-mannopyranosyl residues with galactose (less than 5%) (Moreira *et al.*, 2008) and are homopolysaccharides. Acetylated mannon or acemannan is a primary polysaccharide of the *Aloe barbadensis* leaf gel and known as carriesin, with β-(1→4)-D-mannopyranosyl residues as its backbone, acetylated on C-2 and C-3 positions with ratio 1:1 (approximately) of mannosyl and acetyl and also consist galactose side chains of C-6 positioned (Stenkemp and Stewart, 2007).



**Fig. 8.4. Chemical structure of Acemannan (Hamman, 2008).**

In this, the acetylated mannon (acemannan) is treated with endo-β-D-mannose, and a fraction of C-4 and C-6 resonances were scrutinized by NMR (C<sup>13</sup>) method. On the basis of analysis, it has been reported that acemannan has a single chain of β-(1→4)-mannose with a backbone of β-(1→4)-glucose and α-β-(1→6) galactose branching (Femenia *et al.*, 1999)

#### 2.11.2.6. Maloyl glucans

*Aloe barbadensis* gel contains three malic acids acetylated carbohydrates are; Veracylglucan A, Veracylglucan B, and Veracylglucan C (Esua and Rauwald, 2006).  
 i) Veracylglucan A; chemical formula (C<sub>10</sub>H<sub>16</sub>O<sub>10</sub>) with a molecular weight of 296 Da. During ester group hydrolysis it became very unstable. It is present in a very small quantity in *Aloe barbadensis* gel. ii) Chemical formula of Veracylglucan B is

C<sub>16</sub>H<sub>26</sub>O<sub>15</sub> with a molecular weight of 458 Da with 3.8 pH iii) Chemical formula of Veracylglucan C is C<sub>56</sub>H<sub>82</sub>O<sub>51</sub> with a molecular weight of 1570 Da and 4.7 its pH value (Esua and Rauwald, 2006).

#### **2.11.2.7. Pectic substance**

Pectin is closely related with polysaccharides consist  $\alpha$ -(1→4) linkage. This linkage occurred between polygalacturonic acid and intrachain rhamnose addition, unbiased sugar side chains, and methyl esterification. Pectin substances include pectic acid and arabinogalactan; it composed of arabinose, galactose, and galacturonic acid. It forms the neutral side chain of pectins (Hamman, 2008).

#### **2.12. Commercial and Technical products of *Aloe barbadensis* extracts**

There are three types of *Aloe barbadensis* extracts used as commercial products; *Aloe barbadensis* gel, *Aloe barbadensis* gel of the whole leaf, and decolorized extract of the whole leaf (Boudreau *et al.*, 2013). Dried latex is the fourth commercial material and used traditionally as a laxative (Eur Ph., 2008).

##### **2.12.1. *Aloe barbadensis* leaf gel**

The gel is produced by the leaf pulp of *Aloe barbadensis* from large thin-walled cells. It is clear, mucilaginous, and aqueous extract about 70% gel yield from the inner central part of leaf pulp. Aloe gel has 99-99.5% of water. Water is an energy storage component of a plant (Femenia *et al.*, 1999). A linear chain of glucose and mannose carbohydrate molecules form polysaccharides consist a repeating unit of glucose: mannose (1:3) (Femenia *et al.*, 1999 and Boudreau *et al.*, 2013). Fresh *Aloe vera* gel preserved or stored at 40<sup>o</sup>C temperature for 48 hours incubation. This period has exhibited degradation of the rheological properties of the gel. As the polysaccharide's contents decreased, the ratio of glucose: mannose increased from fresh gel 2:9 to incubated gel 13:4 (Yaron, 1993).

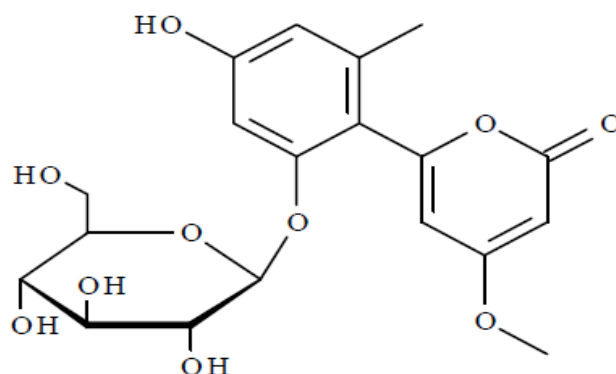
##### **2.12.2. Extract of *Aloe barbadensis* whole leaf**

The extract of *Aloe barbadensis* whole leaf is also called *Aloe barbadensis* juice or aloe juice. This latex has phenolic nature, mostly are anthraquinones (Park *et al.*, 1998). Four C-glycosyl constituents are found in Aloe latex: aloeresin A, aloesin, aloin A and aloin B (Saccu *et al.*, 2001). Aloin A is a C-glycosyl anthrone is a major component of latex also called Barbaloin. Aloin B is an epimer of aloin A and is also known as isobarbaloin, having a  $\beta$ -D- glucopyranosyl substituents and 9-anthrone

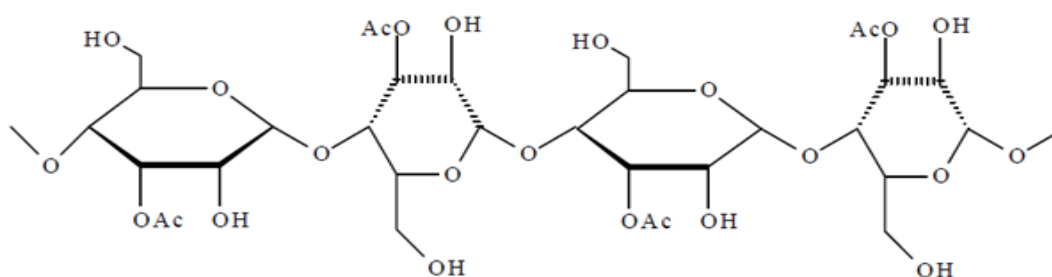
skeleton. Aloesin is a 5-methyl chromone with an 8- $\beta$ -D-glucopyranosyl substituent, also known as aloeresin B. Aloeresin A is a 5-methyl chromone with 8- $\beta$ -D-glucopyranosyl-2-O-trans-p-coumarol substituents.

*Aloe barbadensis* latex contains endogenous free anthraquinone and anthrones formed by oxidation processed by glycosides (Boudreau *et al.*, 2013). The latex of *Aloe barbadensis* contains aldehyde and ketones (Saccu *et al.*, 2001).

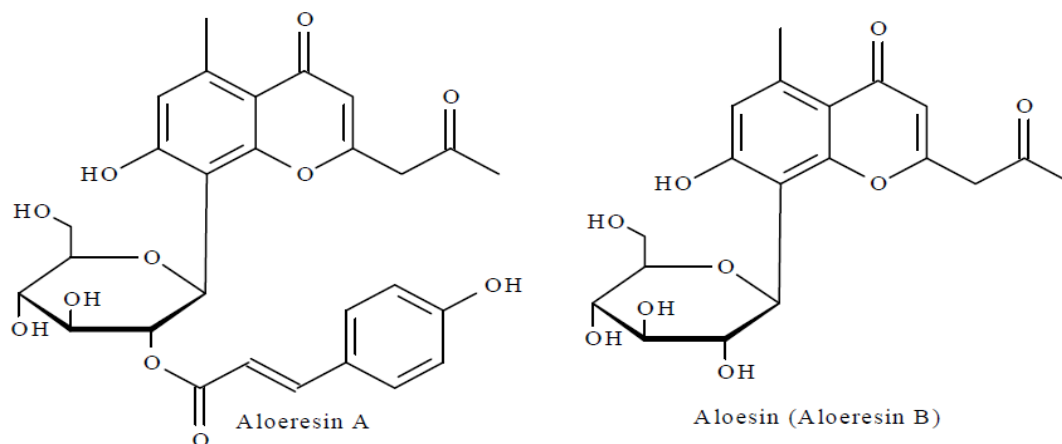
The C-C bonding is resistant to an acidic and alkaline condition. Intestinal bacterias of humans and animals cleaved the  $\beta$ -C- glycosyl bond. Due to the cleavage of the  $\beta$ -C- glycosyl bond, aloe-emodin is formed. This is a cathartic principle for free anthraquinones, anthrones, and latex during storage at a higher temperature; whole-leaf extracts (Boudreau *et al.*, 2013).



Aloenin



Acetylated mannan (Acemannan)



**Fig.8.5. Whole leaf *Aloe barbadensis* extracts (IARC Monograph 108).**

### 2.12.3. Decolorized extract of *Aloe barbadensis* whole leaf

Removal of color and bitterness caused by the anthraquinones compound of the latex of *Aloe barbadensis* whole leaf extract by activated carbon treatment, the product formed by this process called decolorized whole leaf extract (Boudreau *et al.*, 2013). Aloin content is <10ppm of decolorized extract (an industry standard). The toxicological assessment reported a commercial decolorized extract contained 0.9 ppm of Aloin A 1.3ppm of Aloin B, and 0.2ppm of aloe-emodin approximately (Sehgal *et al.*, 2013). A combination of aloin A and aloin B at < 0.1ppm has been assessed by a decolorized extract of aloe leaf for safety (Shao *et al.*, 2013). Although the decolorized whole leaf and *Aloe barbadensis* gel extract has similarities in physical and chemical properties i.e. each contain little amount of latex or latex anthraquinones are totally absent. But both differ during incubation exhibit degradation in rheological properties approximately 19-23% losses of polysaccharide contents (IARC Monograph, 108).

### 2.12.4. Dried latex of *Aloe barbadensis* as a pharmaceutical product

Dried aloe latex is used for pharmaceutical or medicinal purposes. Dried aloe latex is a solid form of thick liquid; originates from the cell of pericycle and adjacent leaf parenchyma spontaneously grows from leaf cut. This material allows drying with the aid of external heat. (WHO, 1990).

### 2.13. Medicinal uses and Clinical significance of *Aloe barbadensis*

*Aloe barbadensis* is an important component of traditional medicines in India, China, and Japan for more than 2000 years in folk medicines (Grindlay and Reynolds,

1986). Extract of aloe gel, aloe whole leaf extract, decolorized Aloe whole leaf extract, and dried Aloe latex is commercial extracts of *Aloe barbadensis*, possess many pharmaceutical activities. In the 1930s, X-ray burns have been treated successfully with the use of *Aloe barbadensis*, and these results gave popularity and increase the use of *Aloe barbadensis* as home remedies in the USA (Ulbricht *et al.*, 2007). Anthraquinone isolated from *Aloe barbadensis* has cathartic properties and laxative effects; mucus secretion is stimulated by anthraquinones and, increases water content in intestine. *Aloe barbadensis* contain glycosides 1,8-dihydroxyanthracene, aloin A and aloin B. After administration of aloin A and aloin B (remain unabsorbed by upper intestine) from the oral route are hydrolyzed intestinal bacterias in the colon and form active metabolites aloe-emodin 9-anthrone a reduced form (Sahu *et al.*, 2013).

## **2.14. Clinical significance of *Aloe barbadensis* leaf gel (Gupta & Rawat, 2017)**

### **2.14.1. Antidiabetic effects**

*Aloe barbadensis* gel lowers the blood glucose level. On the basis of a report on induced Streptozotocin diabetic rats, the oral route administration of aloe gel consistently lowering the glucose level in the blood (fasting), hepatic transaminases enzyme activity, blood cholesterol, phospholipids, and free fatty acids significantly increase the insulin hormone (Hamman, 2008). The level of LDL (low-density lipoprotein) decreased and level of HDL (high-density lipoprotein) increased and the level of cholesterol in Streptozotocin-induced rats comes in their better condition when treated with extract of *Aloe barbadensis* leaf gel (Rajasekaran, 2006).

### **2.14.2. Immunomodulatory effects**

Immunomodulating properties of gel occurring when nitric oxide is generating by activated macrophage cells, cell surface markers (biomarkers), and cytokines (Hamman, 2008). Doses of gel extract Acemannon stimulate the production of tumor necrosis factor (TNF- $\alpha$ ), IL-1 (interleukin-1), interleukin-6 (IL-6), and interferon-gamma (INF- $\gamma$ ) (Zhang *et al.*, 1996). *Aloe barbadensis* gel extracts reduced inflammation and blocks the production of thromboxane and prostaglandin from arachidonic acid. Acemannan isolated from *Aloe barbadensis* gel increases the stimulation of WBC, splenic cellularity, absolute number of neutrophils, lymphocytes,

and monocytes in mice. Gel decreases the production of interleukin-10 during exposure to UV radiation, block mast cell inflammation to antigen-antibody complexes by reduction of delayed type of hypersensitivity (Ro *et al.*, 1998 and Yamamoto, 1993).

#### **2.14.3. Anti-inflammatory properties of *Aloe barbadensis* gel**

A study on enzyme Bradykinase (present in Aloe gel) activity through *in vitro* or *in vivo* methods has revealed the anti-inflammatory action (Zhang *et al.*, 1996). The bradykinase helps to reduce excessive inflammation and break the peptide bond in bradykinin. Bradykinin is a protein and inflammatory substance that induces pain (Chow, 2005).

#### **2.14.4. Antiseptic effects**

There are six antiseptic agents are found in *Aloe barbadensis* gel named; salicylic acid, lupeol, nitrogen, phenols, cinnamonic acid, and sulfur. These antiseptic agents inhibit the activity of fungi, bacteria, and viruses.

#### **2.14.5. Anti-oxidant effects**

The enzymes Glutathione peroxidases and superoxide dismutase are phenolic act as an anti-oxidant has found in the gel of *Aloe barbadensis* (Singh *et al.*, 2016). The dose-dependent anti-oxidant effect of *Aloe barbadensis* gel has shown during incubation with inflamed colorectal mucosal biopsies. To study the scavenging effect of both superoxide and peroxy radicals, cell-free techniques are used (Langmead *et al.*, 2004).

#### **2.14.6. Healing wound effect**

Healing is a three-phase dynamic process; 1st phase consists, inflammation, leukocyte infiltration, and hyperemia. 2nd phase consists removal of dead cells and tissues. 3rd phase consists of regeneration of epithelial and the formation of fibrous tissue (Reddy, 2011). According to recent reviews (Maenthaisong *et al.*, 2007) aloe gel heals first to second-degree burns. The gel contains mannose-6-phosphate has wound healing properties. Plant growth hormone gibberellin and glucomannan stimulate mannose-6-phosphate and proliferation and increase the synthesis of collagen by interacting with growth factors and receptors of fibroblasts (Sahu *et al.*, 2013). The oral administration of *Aloe barbadensis* influences collagen composition and improving breaking strength by increasing cross-linkage with collagen for wound

contraction (Reynolds and Dweck, 1999). The synthesis of dermatan sulfate and hyaluronic acid increases the synthesis of collagen in granulation tissue and healing the wound (Chithra *et al.*, 1998). Acemannan present in *Aloe barbadensis* increase wound healing and reduces the radiation on the skin (Witte *et al.*, 1993).

#### **2.14.7. Gastrointestinal activity**

*Aloe barbadensis* gel has anti-ulcer properties and is attributed to several mechanisms including inflammatory action; wound healing effect stimulates and regulates mucus and gastric secretion (Suvitayavat *et al.*, 2004). Gastric mucosa damage has been found in rats as the effect of ethanol-water *Aloe barbadensis* extract on the secretion of HCL and gastric acid. The inhibition of gastric acid depends on the concentration of aloe gel extract, explained by interaction with H<sub>2</sub> receptors present on parietal cell direct interaction with cells producing acid. Effect on gastric ulcer was observed at the lowest dose administration. Because at low concentration *Aloe barbadensis* extract possesses cytoprotective activity (Yusuf, 2004).

#### **2.15. Use in cosmetics**

##### **2.15.1. Moisturizing and skin protection application**

Mucopolysaccharide helps to moisturize the skin. It has been studied that gel improved skin integrity, decreases the appearance of acne and wrinkles. *Aloe barbadensis* gel act as a moisturizing agent and also provides a cooling effect to the skin (Sahu *et al.*, 2013). The gel is used in hydrating ingredients in liquids, creams, sunscreen lotions healing ointments, and face packs because of these entire features, in cosmetic industries *Aloe barbadensis* products are used as skin tonic (Newton, 2004). *Aloe barbadensis* gel has antifungal and antibacterial properties, which helps in the treatment of boils, benign skin cysts, and other skin infections. It also inhibited the fungal infection tinea (Sumbul *et al.*, 2004)

##### **2.15.1.1. Skin hydration application**

Cosmetics with skin hydration application consist of 0.25% of commercial freeze and dried *Aloe barbadensis* gel in a ratio of 200:1. Water contents of stratum corneum improved by this concentration (Dal *et al.*, 2006). Anthraquinone present in *Aloe barbadensis* gel extracts absorbs the UV radiation of the sun. *Aloe barbadensis* should contain at least 5g/100g of cosmetic products produced by regular authorities in Germany (IARC Monograph-108).



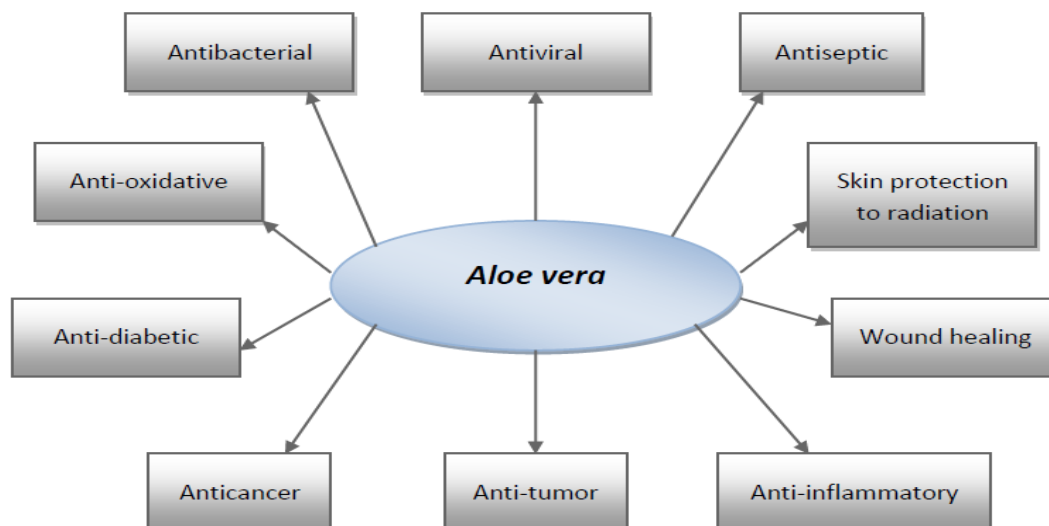
## **2.15.2. Antimicrobial applications**

### **2.15.2.1. Antibacterial activities**

The *Aloe barbadensis* gel contains 99.3% of water content and 0.7% part contains carbohydrate constituents (El-Shemy *et al.*, 2010). The *Aloe barbadensis* gel extract has bacterial activity against *Pseudomonas aeruginosa* and acetylated mannan prevented it in a monolayer culture from adhering to epithelial cells of lungs (Azghani *et al.*, 1995 and Cera *et al.*, 1980). Gel extract of *Aloe barbadensis* leaves is used for the treatment of hemorrhoid because of its laxative properties. Glucomannan and acemannan have activated macrophages; accelerate healing of wound stimulating the immune system and antimicrobial activities (Lordache *et al.*, 2015). There are two micro-organisms *Streptococcus pyogenes* and *Streptococcus faecalis* have been inhibited by concentration gel extract of *Aloe barbadensis* leaves. The extracts obtained from *Aloe barbadensis* are very effective against three microbial strains of Mycobacterium such as *M. kansasii*, *M. fortuitum*, and *M. smegmatis*, potent antibacterial activity against *Mycobacterium tuberculosis* and *E. coli*, *Staphylococcus aureus*, *P. aeruginosa*, and *Staphylococcus typhi* (Sahu *et al.*, 2013).

### **2.15.2.2. Anti-viral activities**

*Aloe barbadensis* gel contains antiviral agents. Acemannan reduced infections in two target cultured cell lines have been reduced by acemannan. Inhibited the activity cytomegalovirus in inhibited by lectins isolated from aloe gel proliferation in cell culture (Sahu, 1990). Aloe-emodin has an effective result on infectivity of type-I and types II Herpes simplex virus. It also inactivates Varicellazoster virus, influenza viruses, and Pseudorabies virus. A study by electron monograph represented that anthraquinone partially damage the outer covering of Herpes simplex virus. This observation result that anthraquinone extract from different plants is directly kills enveloped virus due to stimulation of immune response and the indirect effect of anthraquinone. Anthraquinone aloin also inhibits the action of Herpes simplex, Varicella zoster and influenza, and some other enveloped viruses.



**Fig.8.6. Therapeutic and Medicinal uses of *Aloe barbadensis* (Kar and Bera, 2018)**

### **2.15.2.3. Anti-fungal activities**

Extensive studies were carried out on *Colletotrichum coccodes*, *Fusarium oxysporum* and *Rhizoctonia solani* for mycelium development which yielded a result that indicated the inhibited the effect of *Aloe barbadensis* pulp at  $10^4 \mu\text{l L}^{-1}$  on *Fusarium oxysporum*. Same time growth of colony has been observed in *Colletotrichum coccodes*, *Fusarium oxysporum*, and *Rhizoctonia solani* at  $10^5 \mu\text{l}$  concentration with a reduced liquid fraction (Rodriguez, 2005). The *Aloe barbadensis* juice has reported anti-inflammatory, anti-arthritic activity, hypoglycemic effects, and anti-bacterial action. *Aloe barbadensis* gel reduced the growth of *Streptococcus* and *Shigella* (Ferro, 2003). *Trichophyton mentagrophytes* growth inhibited by gel and leaves inhibited growth of *Candida albians* and *Pseudomonas aeruginosa* (Agarry *et al.*, 2005). But there is a species named *Xanthomonas*, *Aloe barbadensis* extract failed to report antibiotic effects. Low molecular weight protein saponins, a soapy substance found in the gel have antiseptic antibacterial, antiviral, antifungal properties (Sahu *et al.*, 2013).

### **2.16. Anti-carcinogenic properties of *Aloe barbadensis***

Anticarcinogenicity of *Aloe barbadensis* has not yet evolved very well. According to a hypothesis, the chronic abuse of anthranoid that has laxative properties plays an important role in colon cancer (Chouhan *et al.*, 2016). But there is no causal relationship between anthranoid laxative effects on colon cancer (Siegers *et al.*, 1992).

*Aloe barbadensis* juice helps to recover the cells or tissues which have been damaged by radiotherapy and chemotherapy. Anthraquinone has antineoplastic activities so it can reduce the growth of malignant cells (Sahu, 2013). Glycoprotein and polysaccharide are the two compounds of *Aloe barbadensis* have anticarcinogenic property. Different studies indicated that *Aloe barbadensis* gel has an anticancer effect. The gel extract suppresses tumor weight, shrinkage of tumor, necrosis, and decrease the survival rate of the tumor (Hamman, 2008).

#### **2.16.1. Experimental studies of anticarcinogenic activities of *Aloe barbadensis* (animal-based F344/N Rats and B6C3F1 Mice)**

The study of *Aloe barbadensis* whole leaf extract for carcinogenicity after oral administration in 1-2 years experimental study in mice (B6C3F1 animal model) and both male, female rats (F344/N). Mixing of *Aloe barbadensis* leaf extract in drinking water increased the process of adenoma and cancer of the large intestine (colon and caecum), development of the tumor is very rare in rats. In the case of mice, in 2 years experimental study, no increased process of any type of tumor in males or females even with leaf extract in drinking water has given (Boudreau *et al.*, 2013).

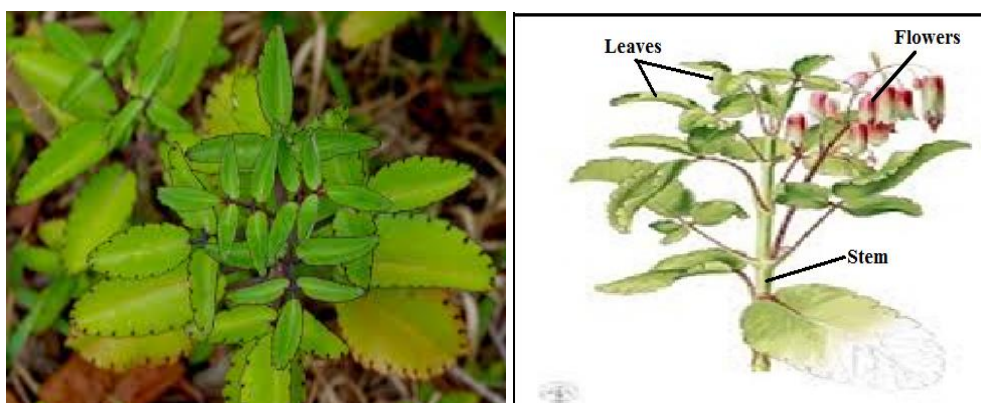
#### **2.16.2. Lectins extract from *Aloe barbadensis***

There are two lectins that have been separated, partially purified from *Aloe barbadensis* (Akev, 1999 and Suzuki, 1979). These lectins have different haemagglutinating properties. These isolated *Aloe barbadensis* lectins are named Aloctin-I and Aloctin II (Koike, 1995). N-acetyl-D-galactosamine inhibited the action of aloctin II but when aloctin-I is tested for specificity, aloctin I have not been suppressed by any of the sugars. The glycoprotein (P-2) isolated from *Aloe arborescence*, has a molecular weight of 18000 kDa and sugar (S-1) contains 50% neutral carbohydrates by weight. Both P-2 and S-1 have haemagglutinating properties (Akev, 1999). P-2 also has mitogenic property. These lectins are composed of Asp and Glu (acidic amino acids) in higher concentration but Met and His in low concentration. A lectin has isolated from the skin part of *Aloe barbadensis* also has both mitogenic and haemagglutinating properties. These lectins showed specificity for mannose sugar (mannose-binding lectin) from a snowdrop bulb (Saeed *et al.*, 2004). Recently, in 2015, Arif Husain, present research on the evaluation of the suitable combination of cisplatin with *Aloe* crude extract (ACE), with growth inhibition and apoptosis by

drugs of chemotherapy at lower concentration of doses with its side effects get reduced on normal cells.

### 2.17. A Review on *Bryophyllum pinnatum*

*Bryophyllum pinnatum* belongs to the family Crassulaceae. Its common names are Ghayamari, air plant, love plant, life plant, Zakhm-e-hyat, panfutti (Jain *et al.*, 2010).



**Fig.9. *Bryophyllum pinnatum*.**

It grows worldwide and is used as folk medicine in many countries like Australia, tropical Africa, India, tropical America, and China. It has a sour taste, astringent but during the post-digestive effect it has a sweet taste. The plants have many medicinal properties. It has hemostatic and wound healing properties. (Kamboj and Saluja, 2009).

The chemical compounds isolated from *Bryophyllum pinnatum* were used as folk medicines on large scale in the treatment of many diseases like hypertension and kidney stones, (Lans, 2006) pulmonary infections, rheumatoid arthritis, etc. (Majaz *et al.*, 2011). *Bryophyllum pinnatum* leaves are used as an antifungal (Sofowora, 1993) and antiallergic agent (Pal and Chaudhuri, 1991). *Bryophyllum pinnatum* is an emollient, refrigerant, hemostatic, mucilaginous, depurative, anodyne, disinfectant, constipating and antitonic. The plant proved to be beneficial in vitiated conditions (pitta and vata), epilepsy, piles (Hossan *et al.*, 2009), haematemesis, menorrhagia, healing of wounds, hemorrhoids, skin discolorations, boils, and ophthalmia (Rola *et al.*, 2011).

## **2.18. Phytochemistry and Commercial products of *Bryophyllum pinnatum***

The phytochemicals are present in *Bryophyllum pinnatum* are phenols, alkaloids, flavonoids, tannins, carotenoids, saponins, glycosides, anthocyanins, sitosterol, malic acid, tocopherol, quinines, lectins, bufadienolides, and Coumarins. Different substance constituents found in leaves are 1-octane-3-O- $\alpha$ -L-arabinopyranosyl-(1-6)-glucopyranoside (Mishra and Dixit, 1979), isorhamnetin-3-O- $\alpha$ -L-1C4-rhamnopyranoside, 40-methoxy-myricetin-3-O- $\alpha$ -L-1C4-rhamnopyranoside and protocatechuic-40-O- $\beta$ -D-4C1-glucopyranoside (Recknagel, 1967), 24-epiclerosterol [24(R)-stigmasta-5, 25-dien-3 $\beta$ -ol], 24(R)-5 $\alpha$ -stigmasta-7, 25-dien-3 $\beta$ -ol, 5 $\alpha$ -stigmast-24-en-3 $\beta$ -ol and 25-methyl-5 $\alpha$ -ergost-24 (28)-en-3 $\beta$ -ol (Quazi *et al.*, 2011). Another steroidal subordinate, Stigmast-4, 20 (21), 23-trien-3-one was likewise detached from the plant leaves separate alongside stigmata-5-en-3 $\beta$ -ol,  $\alpha$ -amyrin- $\beta$ -D-glucopyranoside, nundecanyl n-octadec-9-en-1-oate and n-dodecanyl noctadec-9-en-1-oate (Afzal *et al.*, 2012).

### **2.18.1. Commercial products of *Bryophyllum pinnatum***

Amantol Cream: Its indications are respiratory disorders, Sinusitis, bronchitis, allergic reactions, blocked nose. Ingredients are Mint extract (*Mentha Viridist*), escanel extract (*Iresine difusa*), yantria extract (*Lippia alba*), ginger extract (*Zingiber officinalis*), Pakipanga extract (*Bryophyllum pinnata*), ajodemonte extract (*Mansia alliacea*), mentol, alcanfor, water cream base, external use only. *Parnabija savarasa*: anti-obesity (Majaz, 2011).

## **2.19. Pharmacological and Clinical significance of *Bryophyllum pinnatum***

### **2.19.1. Antimicrobial activity**

4,3,5,7 tetrahydroxy 5 methyl 5 propenamine anthocyanidins and 5 methyl 4,5,7 trihydroxyl flavones are two flavonoids that control microbial activities e.g. *E.coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, and *Aspergillus niger* (Okwu and Nnamdi, 2007). At 25 mg/ml concentration of 60% methanolic concentrate of *Bryophyllum pinnatum* leaf represses the bacterial development (Akinpelu, 2000). Based on some examination it has been accounted for that the dynamic constituents secluded from *Bryophyllum pinnatum* like Bufadienolides: bryophyllin A and bryophyllin C have solid insecticidal movement (Veitch and Grayer, 2007).

### **2.19.2 Anti-ulcer activity**

The leaf extract of *Bryophyllum pinnatum* with methanol inhibits the development of a variety of acute ulcers (Ghasil *et al.*, 2011).

### **2.19.3. Anthelmintic activity**

The *in-vitro* assessment of anthelmintic action against *Pheretima posthuma* (Annelida) and *Ascardia galli* (nematode) *Bryophyllum pinnatum* roots were exposed to natural solvents like chloroform, oil ether, methanol, and fluid dissolvable individually. The inferences reveal that except petroleum ether, chloroform, methanolic and aqueous extract of *Bryophyllum pinnatum* root has significant activity against helminth. But the methanolic extract of roots of *Bryophyllum pinnatum* was reported to be most effective against helmentic activity of all the extract. 100 mg/ml concentration of roots extract is very high to caused paralysis and deaths of worms, in the short time period than to referred drug e.g. Piperazine citrate. Phytochemical analysis of the crude extracts of *Bryophyllum pinnatum* reported that the presence of tannins with anthelmintic activity has been revealed (Majaz *et al.*, 2011).

### **2.19.4. Antihypertensive activity**

According to a study, the leaf extract of *Bryophyllum pinnatum* in aqueous form showed an effect on the liver, kidney, blood pressure, the status of anesthetized rabbits and cats. On the basis of this study, it has been reported that the concentrate caused a little fall in the circulatory strain and furthermore decreased the raised adrenaline which caused hypertension of the anesthetized cat. It was presumed that the pharmacological reason for the utilization of *Bryophyllum pinnatum* bring down the pulse was set up. However, the *Bryophyllum pinnatum* leaf extract is organotoxic in nature and it has a slight effect on the reduction in blood pressure which neglects it as an antihypertensive agent (Ghasil *et al.*, 2011).

### **2.19.5. Wound healing activity**

The wound healing activity of *B. pinnatam* has been evaluated by using a wound model of rats. On the basis of the histological analysis, it has shown that the extract of plant leaf exhibited significant potential for wound healing. The presence of steroid glycosides in leaf extract may be attributed to wound healing (Nayak *et al.*, 2010).

#### **2.19.6. Anti-inflammatory activity**

Using fresh egg albumin-induced paw edema; rats were investigated for the study of anti-inflammatory activity of *Bryophyllum pinnatum* plant extract. The inflammation in fresh egg albumin is significantly inhibited by plant extract (John, 2005). In addition, the antinociceptive effect of the plant's aqueous leaf extract on thermally and chemically induced nociceptive pain stimuli in mice was studied and the plant showed significant antinociceptive effects. Further, a new Stigmast-4, 20 (21), 23-trien-3-one, a steroidal derivative with analgesic and anti-inflammatory activity has been obtained from the leaves extract of the plant has also been evaluated (Afzal *et al.*, 2012).

#### **2.19.7. Hepatoprotective activity**

The hepatoprotective activity of *ryophyllum pinnatum* was reported by various scientists. It was found very effective hepatoprotective as it lowers the activity of liver enzymes SGOT, SGPT, SALP, and SBLN levels. The increased level of these enzymes is a sensitive indicator of liver disorders (Molander *et al.*, 1955). The juice of its leaves and the ethanolic extract were studied against CCl<sub>4</sub>- induced hepatotoxicity in rats and found to be potential hepatoprotective (Shihadi *et al.*, 1999).

#### **2.19.8. Antidiabetic activity**

The anti-diabetic effect of *Bryophyllum pinnatum* plant extract had been investigated in rats using streptozotocin (STZ)-induced diabetes mellitus. The aqueous extract of *Bryophyllum pinnatum* has significantly reduced the level of blood sugar (an obscure mechanism) of the normal and STZ-treated diabetic rats in fasting (John, 2005).

#### **2.19.9. Nephroprotective activity**

The investigators reported nephroprotective activity of aqueous extract *Bryophyllum pinnatum* leaves possess potent for gentamycin-induced nephrotoxicity in rats (Harlalka and Patil, 2007). It has also been stated that the plant extract has shown significant diuretic and antiurolithitic activity when hydroalcoholic extract of *Bryophyllum pinnatum* leaves was given by oral administration to male rats and i.p. route (Patil *et al.*, 2008).

#### **2.19.10 Antioxidant activity**

The imbalanced homeostatic phenomenon between oxidants and antioxidants is caused by physiological excess of free radicals in the body. This randomness causes oxidative stress that is being suggested as the principal cause of aging, arteriosclerosis, neurodegenerative diseases such as Alzheimer's and Parkinson's disease, diabetes and cancer in human (Jain, 2010). The oxidative activity is detected by DPPH and Nitric oxide free radical scavenging method. The leaf extract of *Bryophyllum pinnatum* has significant potential free radical scavenging activity for the treatment of many diseases (Halliwell and Gutteridge, 1997). Morales and colleagues suggested that cadmium-induced nephrotoxicity is reduced by quercetin. It increases, a small cysteine-rich protein Metallothionein, and the activity of enzyme endothelial nitric oxide synthase (eNOS) that inhibit the enzyme activity of COX-2 (cyclooxygenase-2) and iNOS (inducible nitric oxide synthase) (Michelle *et al.*, 2006).

#### **2.19.11. Anticancer Properties of *Bryophyllum pinnatum***

The ethanolic extract of *Bryophyllum pinnatum* has anticancerous activity (Jaki *et al.*, 1999). Five bufadienolides are isolated from *Bryophyllum pinnatum* leaves. Bufadienolides inhibit the activation of Epstein-Barr virus early antigen (EBV-EA) in 12-Otetradecanoylphorbol-13-acetate induced Raji cells which is a tumor promoter. All bufadienolides have inhibitory activity, and among the tested compounds a marked inhibition has been exhibited by bryophyllin A. Bryophyllin C is a reducing agent for Bryophyllin A and bersaldegennin-3-acetate. These compounds are less active and lack the orthoacetate moiety. These investigations suggested that the bufadienolides isolated from *Bryophyllum pinnatum* are potentially cancer chemopreventive agents (Veitch and Grayer, 2007). MTT testing of a highly metastatic human fibrosarcoma cell line HT-1080 has shown a mild antiproliferative activity of methanolic aqueous extract (Jun *et al.*, 2002).

A few previous types of research are even introductory; even so, there is vital advancement of both *in vitro* and *in vivo* anti-tumor mechanisms of lectins with the help of biochemical and molecular involvement autophagic route are effectively understood, the new curative methodology would, without doubt, be evolved and generate other medicinal strategies in cancer researches. The involvement of other



studies clinical experiments with the implementation of molecular level reactions help the oncologist and physician to more studies the therapeutic outcomes, dietetic advantages, and toxicity of lectins. However, a strong anticarcinogenic effect of many plant lectins harmful and cause many side effects on clinical patients. Thus the inflexibility of pre-clinical studies keeps in mind so that patient protection has to be assured. Additionally, due to genesis in plants, *in vivo* encouragement of immunity in plant lectins which have side-effects on both their functions and health of the understudy clinical patients. Consequently, a few identical plant lectins reagents or broad-spectrum infections must be planned for future applications. In a word, a few comprehensively examined plant lectins are supporting a succession of procedures a key stage and in this manner can be utilized as gainful remedial medications for malignancy explore. Notwithstanding more examinations into plant lectins are likely to be required as unmistakable techniques that could moderate malignancy reversion in the pre-clinical and clinical examination are basically required. Based on the above confirmations plant lectins can be utilized as a future enemy of neoplastic medications from seat to clinical in further malignant growth cures (Liu *et al.*, 2010).

According to the review of literature, the previous studies presented the effects of *Aloe barbadensis* and *Bryophyllum pinnatum* whole leaves extracts on cancer cells. Both plants are with lots of medicinal properties but have very little work is present for the antioxidant and anticancer activities with lectin. Antioxidants may enhance the anticancer activity of cancer chemotherapy. A primary mechanism of many chemotherapy drugs against cancer cells is the formation of reactive oxygen species or free radicals which can cause cancer. So the antioxidant activity has been performed to study the more effectiveness of plant lectins on cancer cells. There is no work is present on cell cycle arrest and apoptotic and necrotic study of lectin isolated from *Aloe barbadensis* gel and *Bryophyllum pinnatum*. Here the presented researches also make comparative studies on its anticancer potential, cell cycle arrest, and apoptotic and necrotic studies of both plants lectin.

**Table.5. Anti-cancer and cytotoxic effects of some Plant Lectin.**

<b>Plant Lectin extracts</b>	<b>Anti-cancer effects of plant lectins</b>	<b>References</b>
<b><i>Agrocybe aegerita</i>(AAL)</b>	Growth of HL-60, MGC80-3, SW480, SCG-7901, HeLa cancer cell lines, and Murine S-180 sarcoma were inhibited. The cytotoxic effects occurred with DNAase activity and apoptosis.	Zhao <i>et al.</i> , 2003
<b><i>Algaricus bisporus</i> (ABL)</b>	Inhibition of HT29 cancer cells proliferation. NLS-dependent nuclear channels were blocked.	Kent, <i>et al.</i> , 2003 Yu <i>et al.</i> , 1999
<b>Abrin-a</b>	Lymphoblastic leukemia (Blood cancer) cell lines were agglutinated but mature T- cell Leukemia showed weak agglutination for normal lymphocytes.	Moriwaki <i>et al.</i> , 2000
<b>Abrin</b>	Cytotoxic and anti-tumoral effects on mice (transplanted).	Ramnath <i>et al.</i> , 2002
<b><i>Arisaema helleborifolium</i> (AHL)</b>	In different human cancer cell lines such as PC-3(39.4%), HOP-62(95%), HT-29 (68%), HCT-15 (92%), A-549(20.7%) and HEP-2 (66%) proliferation were inhibited.	Kaur <i>et al.</i> , 2006
<b><i>Alocasia cucullata</i></b>	Growth inhibition of cervix cancer cell line SiHa.	Kaur <i>et al.</i> , 2005
<b><i>Arisaema tortuosum</i> (ATL)</b>	<i>in vitro</i> inhibition of proliferation of SiHa, HT-29, and OVCAR-5	Dhuna <i>et al.</i> , 2000
<b><i>Datura stramonium</i> (DSA)</b>	Identification of glycoreceptors between astrocytic and neuronal. Irreversible differentiations were induced on glioma-C6. Synthesis of DNA was suppressed.	Sasaki <i>et al.</i> , 2002
<b><i>Griffonia simplicifolia</i> (GS-1)</b>	Reduction of tumor growth in mice.	Chen <i>et al.</i> , 1994 Knibbs <i>et al.</i> , 1994
<b><i>Viscum album</i> (Iscador M)</b>	Life span of Mice and rats were increased which were suffering from lung cancer and lymphoma by inhibiting tumor growth.	Kuttan <i>et al.</i> , 1997
<b><i>Viscum album</i> (Iscador P.</b>	In the preparation, the concentration of lectin is high	

<p><b>Aqueous Mistletoe extracts Iscador M and Q special)</b></p>	<p>which has shown antitumoral activity in MAXF-401NL (mammary cancer cell line). Both apoptotic and cytotoxic processes were positively linked at medium and low concentrations.</p> <p>The effects of lectins were observed after a long interval of time. Proliferation was inhibited on sixteen tumor cell lines.</p>	<p>Maier and Fiebig, 2002</p>
<p><b><i>Viscum album</i> (Isorel Mistletoe extract)</b></p>	<p>Inhibition of protein synthesis in different tumor or malignant cells. Lectin extracted from mistletoe inhibited tumor growth by increasing apoptotic and necrotic processes and reduced mitosis.</p>	<p>Zarkovic <i>et al.</i>, 2001</p>
	<p>Sustained life span and reduced tumor growth. Histology disclosed a decrease in mitosis and increase apoptotic and necrotic processes. Immune system variations collaborated with the reduction of tumor growth.</p>	<p>Schaffrath <i>et al.</i>, 2001</p>
<p><b>KM-110 (a Korean mistletoe)</b></p>	<p>Natural killer cells were activated and stimulate the host defense system. Melanoma and colon cancer cell metastasis were inhibited. Liver and spleen metastasis was dose-dependent.</p>	<p>Yoon <i>et al.</i>, 1998</p>
<p><b>KML-C <i>Viscum album var. coloratum</i> (Korean mistletoe lectin extract)</b></p>	<p>Natural killer cells and macrophages got activated to stimulate the immune system.</p>	<p>Yoon <i>et al.</i>, 2003</p>
<p><b>KuroKawa mushroom (<i>Boletopsis leucomelas</i>)</b></p>	<p>Due to apoptotic induction, the reduction of proliferation of U937 (human monoblastic leukemia) was dose-dependent.</p>	<p>Koyama <i>et al.</i>, 2002</p>
<p><b>Mesquite seed lectin (<i>Prosopis</i>)</b></p>	<p>Inhibition of proliferation effect on HeLa (cervical cancer cell line). There was no side-effect on normal cell lines.</p>	<p>Gonzalez <i>et al.</i>, 2002</p>

<p><b>Mistletoe lectin (ML)</b> (<i>Viscum album</i>)</p>	<p>Cytotoxic effect and anti-tumoral activity. Bladder cancer was reduced. The life span of mice was increased.</p> <p>Reduction of growth on Molt-4, KPL-1, HeLa-S3, VM-CUB 1, and MFM-223.</p>	<p>Menge <i>et al.</i>, 2000 Knopfl <i>et al.</i>, 2005 Urech <i>et al.</i>, 2006</p>
<p><b>Mistletoe lectin (ML-I)</b> (<i>Viscum album</i>)</p>	<p>Inactivate the ribosomal functions. Induce apoptosis on T and B leukemia cell line.</p>	<p>Bantel <i>et al.</i>, 1999</p>
	<p>Murine non- Hodgkin Lymphoma tumor growth has reduced.</p> <p>Reduction of mitosis.</p>	<p>Pryme <i>et al.</i>, 2002 Pryme <i>et al.</i>, 2004</p>
<p><b>Mistletoe lectin (ML-II)</b> (<i>Viscum album</i>)</p>	<p>The growth of U-937 (myeloleukemic cell line) was reduced by apoptotic cell death.</p>	<p>Kim <i>et al.</i>, 2003</p>
<p><b>ML-I and ML-III Mistletoe lectins</b></p>	<p>There was an induction of apoptosis on T-cell leukemia (Molt-4) and B-cells from B-lymphocytic leukemia.</p>	<p>Bussing <i>et al.</i>, 1999</p>
<p><b><i>Polygonatum cyrtonema</i> Lectin (PCL)</b></p>	<p>Induction of apoptosis in HeLa cell line.</p>	<p>Liu <i>et al.</i>, 2008</p>
<p><b><i>Phaseolus vulgaris</i> (PHA) (Common bean agglutinin)</b></p>	<p>Kreb-II tumor cells were reduced in number in the ascetic fluid of mice. Polyamine oxidase gets activated.</p> <p>A diet containing lectins, reduced subcutaneous non-Hodgkin lymphomas, and intraperitoneal tumor in mice.</p>	<p>Bardcoz <i>et al.</i>, 1997 Rabellotti <i>et al.</i>, 1998 Pryme <i>et al.</i>, 1999 a Pryme <i>et al.</i>, 1999 b Pryme <i>et al.</i>, 1994</p>
<p><b><i>Phaseolus acutifolius</i> (Tepary bean lectin extracts)</b></p>	<p>Lectin extract has shown different cytotoxicity on colon, breast, and cervical cancer cell lines.</p>	<p>Castaneda <i>et al.</i>, 2007</p>

<b><i>Pleurotus ostreatus</i> Lectin</b>	Lectin has different effects on H3B (Human hepatoma), human choriocarcinoma, osteosarcoma cell lines of rat and mouse melanoma. The lectin was more effective and inhibits tumor growth on sarcoma S-180 than on H-22 hepatoma.	Wang <i>et al.</i> , 2000
<b><i>Ricin</i></b>	Lectins bind ribosomes and inhibit protein synthesis.	Fang, 1998
<b>Recombinant Mistletoe Lectin (rML)</b>	Growth of tumor in breast cancer influenced by immunomodulation There was no relation between inhibitory effects and IFN- $\gamma$ (interferon-gamma) dependent mechanism in rat	Hostanska <i>et al.</i> , 2003 Elsasser <i>et al.</i> , 2001
<b>Soybean agglutinin (SBA)</b>	Stimulation of immune system and reduction of the ascitic lymphoma cell line. Proliferation was reduced in MCF-7 cell lines (breast cancer) and Hep G2 cell lines (Hepatoma).	Mukhopadhyay <i>et al.</i> , 1994 Lin <i>et al.</i> , 2008
<b><i>Sauromatum venosum</i> (SVL)</b>	<i>In vitro</i> reduction of proliferation on the colon (SW-620), liver (HEP-2), cervix (SiHa), breast (T-47D), colon (HT-29), prostate (PC-3), and ovary (OVCAR-5) cell lines.	Single <i>et al.</i> , 2005
<b><i>Viscum album</i> agglutinin(VAA)</b>	Stimulation of lung metastasis.	Timoshenko <i>et al.</i> , 2001
<b>VAA-1 <i>Viscum album</i> agglutinin</b>	VAA has shown anti-neoplastic activity alone but when combined with chemotherapeutic drugs on human lung carcinoma cell lines (A-549).	Siegle <i>et al.</i> , 2001
<b>VCA (<i>Viscum album</i> var. <i>corolatum</i>)</b>	The effect on HL-60 was dose-dependent and induction of apoptosis via caspase-3.  VCA's dose-dependent effect on cell growth of B16-BL6 melanoma, apoptosis induction. The survival time of inoculated mice has been increased due to the anti-metastasis effect. Dose-dependent angiogenesis inhibition.	Lyu <i>et al.</i> , 2001  Park <i>et al.</i> , 2001

<b><i>Vicia faba</i> (VFA)</b>	SW1222, LS174T, and HT-29 are colorectal cell lines that have shown cell aggregation, different morphological changes, and dose-dependent inhibition of proliferation.	Jordison <i>et al.</i> , 1999
<b>Wheat germ agglutinin (WGA)</b>	Inhibition of growth of AR42J (Pancreatic tumor cell line of rat) with a little reduction of alpha-amylase secretion.	Mikkat <i>et al.</i> , 2001
	It was very toxic <i>in vitro</i> for human pancreatic tumor cells, with high membrane binding with lectin to sialic acid residues, apoptosis was induced.	Schwarz <i>et al.</i> , 1999
	Reduction of growth of tumor cells of lymphoma.	Ganguly and Das, 1994
	Isolectins have shown different connections with blood cancer cells and different cytotoxicities.	Ohba <i>et al.</i> , 2003
	Effects of lectin vary on the growth of different human breast cancer cell lines (BT-20, MCF-7, and HBL-100) <i>in vitro</i>	Valentiner <i>et al.</i> , 2003

*CHAPTER-3*  
*HYPOTHESIS OF*  
*RESEARCH*

### **3. Hypothesis for Research.**

#### **3.1. Plant constituents provide a better action on various cancers**

Plant lectins have a crucial role in biomedical sciences such as lectin has anticarcinogenic properties. A dose of plant lectins has been trialed in biomedical sciences as a potential drug for chemotherapy. Binding of lectin to receptors present on the membrane of cancer cell, inhibit the growth of the tumor, cause cytotoxicity and apoptosis.

#### **3.2. Use of plant lectin in chemotherapy has not established well**

*Aloe barbadensis* and *Bryophyllum pinnatum* plants are with lots of medicinal properties but have very little work is present for cancer with lectin. There is no work is present on lectin isolated from *Bryophyllum pinnatum* against cancer. Here we can make a comparative study on its anticancer potential. Based on the traditional uses and scientific reports, lectins have a significant role in cancer treatment. There is a broad scope to isolate a purified anticancer agent from medicinal plants, and which needs thorough study and research.

Hence, a systematic study and understanding of *Aloe barbadensis* & *Bryophyllum pinnatum* lectin are considered to develop as allopathic drugs in cancer treatment by pharmaceutical industries. Relative frequency to use medicinal plants and their commercial products hold a prominent position so the cultivation of medicinal plants can be a good source of income for poor farmers.

#### **3.3. *In vitro* evaluation of the anticarcinogenic potential of Lectin**

To develop lectin-based anticancer treatment it is important to understand the underlying mechanism involved during human tumor cell apoptosis induced by lectins.



*CHAPTER-4*  
*OBJECTIVES OF THESIS*

#### 4. OBJECTIVES OF THE STUDY

**1. To isolate *Aloe barbadensis* and *Bryophyllum pinnatum* lectin from leaves.**

- (i) Preparation and purification of leaves extract
- (ii) Determination of protein concentration by Lowry's method
- (iii) Preparation of 2% erythrocytes
- (iv) Agglutination test for the conformation of lectin
- (v) Sugar specificity test/ agglutination inhibition test
- (vi) Effect of different pH on lectin

**2. To characterize the purified plant lectin**

- (i) To observe the protein mixture pattern.
- (ii) The homogeneity of the purified protein mixture shall be determined.
- (iii) To determine the purified enzyme's molecular weight.

**3. To study the antioxidant activity of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin.**

- (i) DPPH (1,1-Diphenyl-2-picrylhydrazyl) Free Radical-Scavenging Activity
- (ii) ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method

**4. To evaluate the anti-carcinogenic activity of plants lectin by *in vitro* methods using human cancer cell lines.**

- (i) Sulforhodamine B assay
- (ii) PI (Propidium iodide) staining for the study of cell cycle analysis
- (iii) Annexin V fluorescein isothiocyanate (FITC) for the study of apoptosis by morphological changes in nuclei of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin treated HeLa cells with flow cytometry.

*CHAPTER-5*  
*MATERIALS & METHODS*

## 5. MATERIAL AND METHODS

### 5.1 (a). Materials

*Aloe barbadensis* and *Bryophyllum pinnatum* plants are grown commercially were purchased from a local nursery at Jalandhar, Punjab, India. The authentication of plants has been done by Dr. Sanjay Kumar Uniyal (Sr. Principal Scientist & Curator) CSIR-IHBT, Palampur, and Himachal Pradesh. Specimen no. PLP15366 and PLP15367.

These plants were already grown nursery in favorable conditions. These plants can grow in pots as well in the field. These plants can grow in both shallow and sunny conditions. The main characteristics of both the plants do need not much quantity of water. Plants have grown in pots in the lab. The leaves of these plants were harvested within 5 to 7 weeks. The fully grown leaves were collected from both plants. For haemagglutination assay i.e. for conformation of lectins we need human blood groups of healthy donors. A<sup>+</sup>, B<sup>+</sup> and O<sup>+</sup> blood groups were obtained from healthy donors under the guidance of a Medical Lab Technician.

Malignancy cell lines HeLa (Cervical cancer cell line) and SW480 (Colon cancer cell line) and were gotten from ATCC (American Type Culture Collection) in CSIR-IHBT, Palampur. The cell lines were kept up in DMEM (Dulbecco's Modified Eagle Medium) enhanced with 10% Fetal Bovine Serum. 95% moistness is required to keep up Cancer cell lines in a CO<sub>2</sub> (5%) incubator and enabled the way of life to grow up to 80-90% confluency.

### 5.1. (b). Chemicals and consumables

#### Chemicals

ABTS, Acetic acid, Acrylamide, Agarose, Ammonium persulphate (APS), Bisacrylamide, Bovine serum albumin (BSA), Bromophenol blue, Copper sulfate (CuSO<sub>4</sub>), D-Fructose, D-Galactose, D-Glucose, D-Lactose, D-Mannose, (Nice Chemicals Ltd.) DMEM (Sigma Aldrich), DMSO, DPPH, Ethanol, Folin-ciocalteu phenol reagent, Fetal Bovine Serum (FBS), FITC, Glycerol, Glycine, HCl, N-acetylgalactosamine, N, N, N, N-tetramethylethylenediamine (TEMED), Milli-Q water, Molecular weight marker (Bio-Rad, India), Penicillin, Propidium iodide, RNase-A, Sodium acetate buffer, Sodium bicarbonate, Sodium chloride (NaCl), Sodium hydroxide (NaOH), Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), Sodium phosphate buffer, Sodium

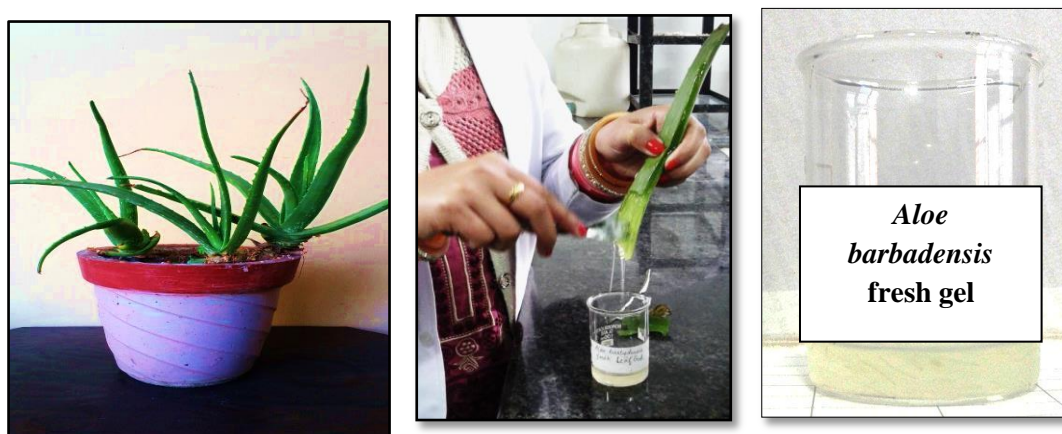
dodecyl sulfate (SDS) (Himedia, India) Streptomycin, Sulforhodamine B, Triton-X 100, TCA, Trypsin solution (Sigma Aldrich).

### Consumables

Beakers, conical flasks, Dialysis tube, EDTA tubes, T-25, T-75, T-150 culture flasks, 96-well plates, 6-well plates, 3 MM Whatman Chromatography filter paper, 1L flasks, Ependroff tubes, Test tubes (Tarson, India)

## 5.2. Methodology

### 5.2.1. Preparation of leaves extract

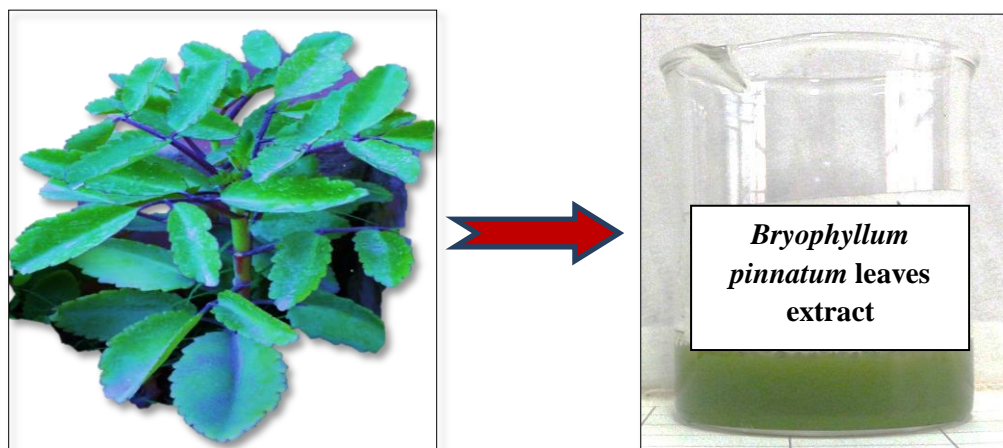


**Fig.10. Extraction of gel from washed and cleaned leaves of *Aloe barbadensis*.**

Leaves (100g) of *Aloe barbadensis* and *Bryophyllum pinnatum* were washed two-three times under tap water and then distilled water and clean with tissue paper. Harvesting of leaves is manually performed, such as leaves of plants cut from the base by hand. Remove the green skin part of the leaf and collected gel of *Aloe barbadensis* which contains 98% of water and leaf juice of *Bryophyllum pinnatum*. The gel is produced by the inner leaf pulp of *Aloe barbadensis* from large and thin-walled cells. It is a transparent, clear, mucilaginous, and aqueous extract, about 70% gel yield from the inner central area of leaf pulp. Approx 50ml of gel and 50ml of *Bryophyllum pinnatum* leaves juice were collected in a beaker.

50ml of gel and 50ml of leaves extracts of *Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves juice were homogenized in 500ml of ice-chilled 0.9% of normal saline solution. The homogenized extracts were allowed to settle at 4°C for 30 minutes after which the supernatants were extracted then filtered using 3MM Whatman chromatography paper. Both filtrates were centrifuged for 10 minutes at

10,000 rpm at 10°C. The supernatants obtained were stored at 4° C (Patil and Despande, 2015).



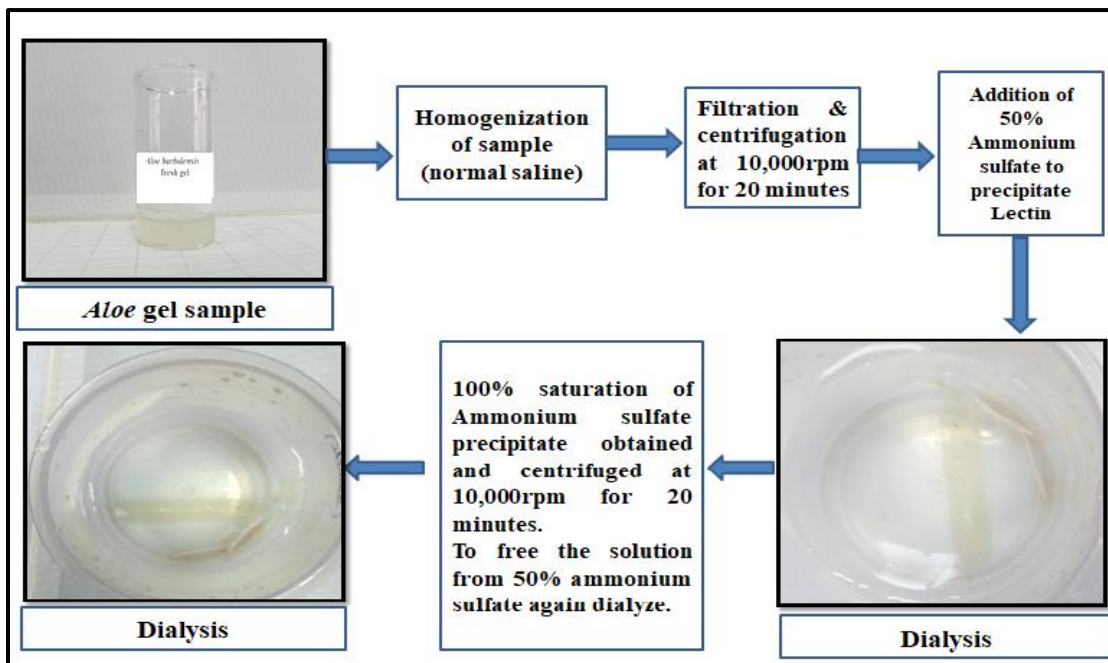
**Fig.10.1.** *Bryophyllum pinnatum* leaves juice.

#### **5.2.2 Purification of lectin of *Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves extract by dialysis method.**

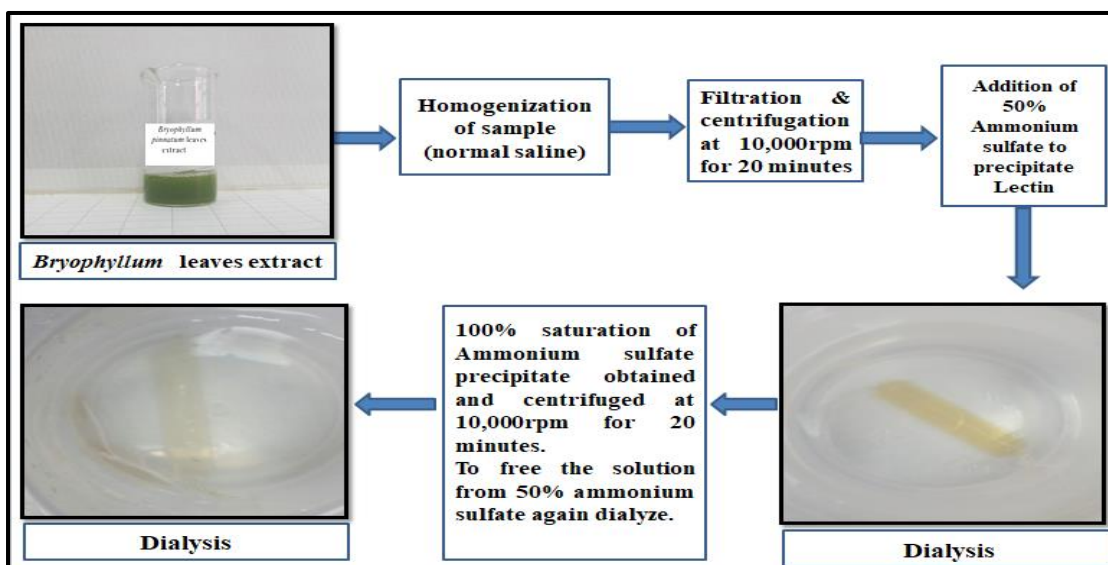
To separate the protein, small molecules and to remove the impurities of crude leaves extract of *Bryophyllum pinnatum* and *Aloe barbadensis* gel were dialyzed by dialysis tube (membrane) in normal saline at 4°C. This dialysis tube contains micropores through which the micromolecules escaped. Therefore the dimension of protein molecules is significantly large than the diameter of the pore retained inside the dialysis tube. The 50% of ammonium sulfate is used in the precipitation of lectin from the dialyzed extract. At 4°C with the gradual addition of 50% ammonium sulfate in dialyzed extracts, the lectin gets precipitated. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged for 20 minutes at 10,000 rpm. In normal saline, precipitates have been dissolved. To free the solution from ammonium sulfate fraction again dialyzed (Patil and Despande, 2015). The dialyzed sample was stored at 4°C for long use.

#### **Reagents requirement**

1. 0.9% of normal saline solution
2. 3MM Whatman chromatography paper
3. Ammonium sulfate
4. Dialysis tube



**Fig.10.2.Purification of lectin from *Aloe barbadensis* gel by dialysis method.**



**Fig.10.3.Purification of lectin from *Bryophyllum pinnatum* extracts by dialysis method.**

### 5.2.3 Determining the concentration of protein using Lowry's method

#### Principle of Method

The method of Lowry is based on the chemical reactions also called the Biuret test between  $\text{Cu}^+$  and peptide bonds in alkaline conditions. Sample containing Protein is mixed with an alkaline solution containing  $\text{CuSO}_4$  that reacts to  $\text{Cu}^+$  ions with peptide-protein bonds. It is a powerful agent for reduction. Thereafter the reagent of

Folin-Ciocalteu was added to the solution. In solution, a reaction occurs between  $\text{Cu}^+$  ions and Mo (VI) ions and the formation of Molybdenum blue (a complex of Mo (IV) and Mo (V) ion). 600 nm wavelength is used to measure the blue color of the dye. The produced color is the inference of protein concentration. Standards are used to determine protein concentration (Lowry *et al.*, 1951).

### **Procedure**

Lowry's method measured the protein concentrations of crude extracts by using a standard protein BSA (bovine serum albumin).

### **Requirement of reagents**

1. 1 mg/ml of BSA (Bovine serum albumin) stock solution

#### **2. Analytical Reagents:**

I.  $\text{Na}_2\text{CO}_3$  in 50ml (i.e. sodium carbonate is in 20%) mixed with 50ml of 0.1 N sodium hydroxide solution (i.e. 0.4gm of sodium hydroxide in 100ml distilled water).

II.  $\text{CuSO}_4$  (1.56%) solution in 10ml of 10ml of 2.37% sodium potassium tartrate solution. to prepare the analytical reagent, 2ml of solution II with 100 ml of solution I were mixed.

3. The dilution of 1N of solution Folin's -ciocalteu reagent to 2N commercial reagent on the day of use with the same volume of water (distilled water in 2ml and 2ml of reagent).

Dilutions of different stock solutions of BSA were taken. Protein sample of 0.2ml to which mixing of 2 ml of alkaline  $\text{CuSO}_4$  and then kept for incubation of 10 min. It was then added to 0.2 ml of Folin's reagent and incubated for 30 minutes in the dark spot. The optical density is then measured at 600 nm using the Perkin Elmer 2030 reader. The graph is diagrammed to determine the unknown protein concentration.

#### **5.2.4 Preparation of 2% erythrocytes**

The human blood of healthy donors A+ve, B+ve and O+ve were collected in heparinized tubes or EDTA tubes to prevent coagulation and stored at  $4^\circ\text{C}$  for 20 minutes. To prepare 2% erythrocytes whole blood was taken in Falcons tube and centrifuged for five minutes at 3000 rpm. Plasma and buffy layers were discarded. These RBC were resuspended in 0.9 % of NaCl (normal saline) with approximately two times the volume of RBCs and to mix it invert the tube. This tube was centrifuged



for five minutes at 3000 rpm thereafter washing of RBCs 2-3 times with normal saline until the supernatant was removed. The washed RBCs are called 2% erythrocytes (Olsen, 1944). These 2% erythrocytes were used for haemagglutination assay.

### **Reagents requirement**

1. Human blood group A, B, O
2. EDTA tubes
3. 0.9 % of normal saline

### **5.2.5 Haemagglutination Assay (Liener and Hill, 1953)**

#### **Principle**

Due to their characteristic property, lectins are called agglutinins to agglutinate cells such as lectin can bind on the surface of RBCs to specific receptors. Therefore, haemagglutination assay has vital importance in the characterization of isolated protein as lectin. Haemagglutination is macroscopically visible and detects lectin presence. This amount of lectins is possible to quantify by the use of haemagglutination assay present in suspensions. The method of serial dilutions is used to determine the suspension of lectin in a well plate of 96. Thus, the result obtained can be used in the suspension to quantify agglutinin expressed as a title of haemagglutination.

#### **Procedure**

For the detection of lectin in the samples, samples were added to series of wells by two-fold dilution so that each well with half of the concentration with normal saline than the previous well. Each well maintained a volume of 50 $\mu$ l of lectins with the addition of 50 $\mu$ l of 2% erythrocyte suspension. At 37<sup>0</sup>C 96 wells plate was kept for 5 hours. The agglutination was observed visually. The Haemagglutination unit is the strength of the titer was calculated with the last agglutination dilution reciprocally. Specific activity is the calculation of the unit of haemagglutination per mg protein.

### **5.2.6 Sugar specificity/ Agglutination inhibition assay**

Plant lectin can bind a particular carbohydrate or sugar. When lectins bind a specific carbohydrate it inhibited the agglutination. Agglutination inhibition testing was performed by testing different sugar capabilities (Kurokawa *et al.*, 1976). Here seven sugars were used for agglutination inhibition assay i.e. D-glucose, sucrose, lactose, fructose, mannose, galactose, and N-acetylgalactosamine.

To inhibit the agglutination equal volumes of sugars and lectin were used so 1ml of 500mM sugar solution were incubated with 500µl of lectin and 500µl of 2% of erythrocytes (1ml) for 30min at room temperature. The minimum agglutination of erythrocytes was taken.

### **5.2.7 Study of pH stability**

The determination of pH stability of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin was done by using a buffer of different ranging from 1pH -13pH. For pH 1 HCL is used (0.1N), for pH 2-3 glycine-HCl buffer (0.2M) is used. For pH 4-5, sodium acetate buffer is used (0.2 M). For 6-7, pH sodium phosphate buffer (0.2 M) is used. For pH 8, Tris-HCl (0.2 M) buffer is used. For 9 pH glycine-NaOH (0.2 M) buffer is used. Carbonate-bicarbonate (0.2 M) buffers were used for pH 10-13 (Suseelan *et al.*, 1997).

### **Procedure**

Incubation of 1ml of lectin with 1ml of solution of different buffer for 30min at room temperature and then 2% erythrocytes were used to test the agglutination.

### **5.2.8. Electrophoresis (SDS-PAGE) (Laemmli, 1970)**

#### **Principle**

Method SDS-PAGE electrophoresis is used in the separation of proteins. However, the migration of protein in denatured form, unlike the native PAGE. The velocity of migrated proteins is a function of their mass, size, shape, and the number of charges per unit of mass they carry. If two proteins carry the same mass and shape, the movement of protein with a large charge density will be faster through the gel. Similarly, if two proteins having the same charge density and shape, the one of smaller mass or size will migrate faster than the large size protein. The complete disruption of protein-protein interactions and denaturation of proteins in the unfolding of protein has been done by SDS [C<sub>12</sub>H<sub>25</sub>NaO<sub>4</sub>]. Rapidness, sensitivity, and a high degree of resolution capability are the main features of SDS-PAGE.

#### **5.2.8 (a) To accomplish the following SDS-PAGE was performed:**

- To observe the protein mixture pattern.
- The homogeneity of the purified protein mixture shall be determined.
- To determine the purified enzyme's molecular weight.

**Procedure:**

- First separating gel was prepared: 12%
- In the setting of the casting frames on the casting tray clamp plates of glass in the casting frames).
- Preparation of gel solution in a small beaker
- Filled the gap between two glass plates with an appropriate amount of separating gel solution.
- Filled the top of separating gel horizontal with water until overflow.
- Water was removed after the gel polymerized.
- **The composition of various components is given below:**

S.no	Component	Volume
1.	dH <sub>2</sub> O	7.9ml
2.	30% Acrylamide mix	6.7 ml
3.	Tris base (pH 8.8) 1.5M	5.0 ml
4.	SDS 10%	200 µl
5.	APS10%	200 µl
6.	TEMED	10 µl

Total volume = 20 ml

Then, stacking gel was prepared: 5%

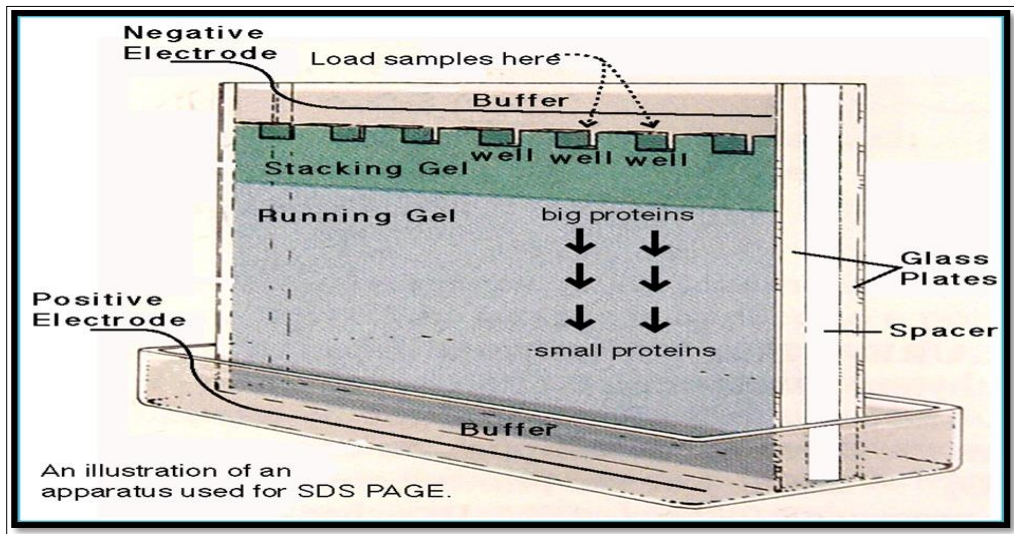
- **The composition of various components is given below:**

S.no	Components	Volume
1.	dH <sub>2</sub> O	3.4 ml
2.	30% Acrylamide mix	830 µl
3.	TrisHCl(pH 6.8) 1M	630 µl
4.	SDS10%	50 µl
5.	APS10%	50 µl
6.	TEMED	5 µl

Total volume = 5 ml

- The solution was poured carefully over the resolving gel.
- Inserted the comb and wait for 20-30 min to let it polymerized.

- After solidification of the gel comb. Set the glass plates in buffer dam after took the glass plates out of the casting frame



**Fig.11.An illustration of apparatus used for SDS-PAGE**

- **Preparation of running buffer:**

S.No	Component	Quantity
1.	Tris base (25mM)	3.0 g
2.	Glycine (192mM, pH 8.3)	14.4 g
3.	SDS (0.1%)	1.0 g

- Dissolved the above component in 750 ml water and placed on a stirrer for 15-20 min and made final volume to 1000 ml after dissolving it well.
- The running buffer or electrophoresis buffer was poured into the inner chamber and kept pouring until the surface of the buffer reached the required level after overflow in the outer chamber.

### **Preparation of Sample**

- Samples were prepared by adding 2X protein loading dye and lectin samples in MCTs.
- Loaded the prepared lectin samples into wells. Then covered the top and connected with power supply.
- Set an appropriate voltage and run the electrophoresis.

- When the front of the dye reached the gel bottom stop the run of electrophoresis when the dye front reached the bottom of the gel.
- Disassembled the gel sandwich and gel is taken out

### **5.2.8 (b) Protein band staining and characterization**

Prefixing of resolved proteins has done by holding gels in TCA (12.5 percent) for 1h. Provided overnight staining in 0.1% of Coomassie Brilliant Blue G-250 in methanol, acetic acid, water in the ratio of 50:10:40, and a staining mixture of methanol, acetic acid, water in the ratio of 10:7:83. The appearance of the protein band in the gel and the determination of the molecular weight of each band have been done by their mobility from the wells and extrapolating against their log molecular weights by the marker protein mobility from the plotted standard curve. Lastly, the photographs of the appeared bands of protein separation were taken for permanent record. Computer-assisted densitometry conducted a quantitative assessment of the relative protein content. Using the Quantity-one Software (BioRad), the SDS-PAGE gels were scanned and protein was reported in relative quantities per gel.

#### **Reagents requirement:**

1.15% Tris-HCl gel

2.Coomassie Brilliant Blue

### **5.3. Antioxidant Activity of Lectin**

#### **5.3.1. DPPH Free Radical-Scavenging Assay**

The study of antioxidant activity of *Aloe barbadensis* lectin from the gel and *Bryophyllum pinnatum* lectin from leaf extract have been done spectrophotometrically with the evaluation of DPPH free radical-scavenging assay (Ferreira *et al.*, 2009).

#### **Principle:**

**DPPH** (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical and is absorbed in 517nm. DPPH will generate its yellow color after scavenging free radicals.

#### **Procedure:**

The different concentrations of lectins of both plants were mixed with DPPH (2mg of DPPH in 100ml of methanol) containing methanolic solution. Ascorbic acid is the strongest antioxidant agent and used as a standard in different concentrations.

Shake the mixture vigorously and left it in the dark for 30 min till the decoloration from purple to yellow. The experiment was carried out in triplicates. The determination of the degree of DPPH radical reduction was done by measuring the absorbance at 517 nm of the mixture (Hatano *et al.*, 1988). The radical-scavenging activity (RSA) was determined as the level of DPPH %.

A mixture of methanol and DPPH is used as blank and a mixture of methanol, DPPH, and various concentrations of lectin extract of both plants were utilized as a sample, while DPPH solution in addition to methanol was utilized as a control. IC50 qualities signify the centralization of the example required to search half of DPPH radicals.

**DPPH Free Radical-Scavenging Activity % =  $[(\text{Abs}_{(\text{Cont})} - \text{Abs}_{(\text{Sample})} / \text{Abs}_{(\text{Cont})}] \times 100$**

DPPH has taken control.

### **5.3.2. ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method (Sithisarn *et al.*, 2005).**

#### **Principle**

The oxidation of ABTS generated the pre-formed radical monocation ABTS with a blue chromogen potassium persulfate and the reduction of ABTS occurred by hydrogen donating antioxidants.

#### **Procedure:**

Different substances can scavenge ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) in comparison with a standard i.e. ascorbic acid. To prepare a radical cation 36mg of ABTS is dissolved in 100ml of distilled water i.e. preparation of a stock solution of 7mM ABTS mixed with 57mg of sodium persulfate in 100ml of distilled water i.e. 2.45mM potassium persulfate in an equal ratio of 1:1. Placed this mixture in dark at room temperature for 16hrs incubation until the reaction has been completed. The optical density (OD) was set at 0.7 of ABTS solution. 50 % methanol used for dilution. Lectin extracts of both plants with different concentrations were added to every 1ml of ABTS solution. These mixtures were incubated for 30 minutes. After incubation, the absorbance was taken at 745nm of samples of different concentrations.

**ABTS Free Radical-Scavenging Activity % =  $[(\text{Abs}_{(\text{Cont})} - \text{Abs}_{(\text{Sample})} / \text{Abs}_{(\text{Cont})}] \times 100$**

ABTS has taken control.

#### **5.4. Cell Culture maintenance**

**HeLa (Cervical cancer)** and **SW480 (Colon cancer)** Cell lines were kept in a CO<sub>2</sub> incubator to maintain their confluency up to 80% to 90% at 37 °C temperatures and 5.0% of CO<sub>2</sub>. Cell lines took 24hrs to 48 hrs to be fully confluent.

##### **5.4.1. Media preparation for Cell Culture**

Media prepared for cell culture, thawing of cells, culturing, maintenance of cell lines (HeLa and SW480 cancer cell lines), trypsinization of cells, freezing.

##### **5.4.2. Culture medium (Stock)**

A stock solution of Dulbecco's Modified Eagle Medium (DMEM) media has been prepared by dissolving 13.5g of DMEM medium (Sigma, USA) + 3.76 g NaHCO<sub>3</sub> in 1000 ml Milli-Q water, taken in an autoclaved 2L flask. The filter assembly (Tarsons, India) with 0.22 µg/ml filter was used for sterilizing the media.

A peristaltic vacuum pump with negative pressure was used and the medium passed through the filter to the lower chamber of the filter assembly. The filter-sterilized medium was then stored in an autoclaved screw-capped reagent bottle at 4°C.

##### **5.4.3. Culture medium (Working)**

By using the prepared stock of Dulbecco's Modified Eagle Medium (DMEM) media, working media has prepared by adding 10% of Fetal Bovine Serum, FBS (HiMedia, India) supplemented with Penicillin (10,000 units/100 ml) and Streptomycin (10 mg/100 ml) (Sigma, USA).

##### **5.4.4. Trypsin solution**

For 1000ml

800ml of autoclaved PBS + 200mg of EDTA and adjust pH above 8.0 + 500mg of trypsin (Sigma, USA) and adjust the pH 7.3-7.4. The filtration of the solution has done through a 0.22 µg/ml filter and the solution was stored at 4°C for routine use and -20°C for long-term storage.

##### **5.4.5. Maintenance of cell line in media**

DMEM medium containing Fetal Bovine Serum of 10%, 2g/L Sodium Bicarbonate and supplemented with Streptomycin (10 mg/100 ml) & Penicillin (10,000 units/100 ml) was used for cell maintenance in routine. Each of the procedures was carried out in a laminar flow hood using aseptic techniques. T-25

flasks were used to grow the cell line and kept in 5% of humidity at 37°C in a CO<sub>2</sub> incubator. The culture medium has changed at regular intervals.

**Procedure:**

- Observe adherent cells microscopically (if too many cells are floating, it is induction of low viability, media of the cells needed to be changed).
- Discard media from the culture flask to the discard beaker.
- Wash the culture flasks, two times each by adding 3ml of PBS.
- Discard PBS into discard beaker and add fresh media to each culture flask which is required for the proper growth of the cells.
- Keep it inside a 37°C CO<sub>2</sub> incubator.

**5.4.6. Sub-culturing of cells:**

On reaching confluency (80-90%) the cells were subcultured.

- Observe the cells microscopically (75% confluent).
- Remove the media and discard it.
- PBS washes to each culture flask by adding 3ml of PBS.
- Discard the PBS in the discard beaker and a culture flask add 3ml of Trypsin-EDTA (0.25%) solution.
- Keep it inside the CO<sub>2</sub> incubator for 2-3 minutes at 37<sup>0</sup>C.
- Visualize Cells under an inverted microscope (detached from substratum) and immediately add complete media to each culture flask (to inhibit the activity of trypsin).

**5.4.7. Seeding of the cells**

Spreading of a defined volume or cell number of a cell suspension is called seeding. On reaching confluency (80-90%) the cells were subcultured into a new culture flask or in a plate etc.

- Observe the cells under a microscope.
- Discard the media; give PBS wash to the cells for the removal of extra media left over the surface of the flask.
- Discard the PBS and add trypsin. Keep the flasks in a CO<sub>2</sub> incubator for 2-3 minutes.
- After that add media to the flasks to inhibit the activity of the trypsin.



- Transfer this to the centrifuge tubes and centrifugation for 5 minutes at 800 rpm.
- Discard supernatants, add 1ml of media to the centrifuge tube, and gently mixed the pellet.
- As we were preparing cells for seeding so we add 10ml of media to the flask and mixed the pellet properly.
- Count cells with the help of a hemocytometer under the inverted microscope.
- Calculate the sets of experiments Eg:- (We were using two plant lectin extracts, one timeline, five concentrations (10µg/ml, 20µg/ml, 50µg/ml, 100µg/ml & 200µg/ml) and in triplicates, also 3 wells used as a blank, 3 as control media and 3 wells were used as positive control)
- Label 96 well plate and add 100 µl of media (containing an appropriate number of cells ) to each well ( we had used two cell lines so a total number of 96 well plates had been used was four and for each cell line there were one timeline 48 hours).
- Kept it inside a CO<sub>2</sub> incubator for 24 hours.
- Add cell suspension to the centrifuge tubes and centrifuge for 5 minutes at 800 rpm.  
(For NCCS – 800rpm 5 Min and for ATCC – 130g ~ 900rpm for 7 Min)
- Discard Supernatant; the pellet was mixed gently by adding 1ml media.
- Incubate again in CO<sub>2</sub> incubator in 5% CO<sub>2</sub> at 37°C.
- For seeding of cells, Count cells with the help of a hemocytometer under the inverted microscope, remaining again subculture in 25ml culture flasks.

## **5.5. Screening Methods (*in vitro*) of anticancer activity of *Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves extract**

### **5.5.1. Sulforhodamine B assay (Skehan, 1990)**

SRB (Sulforhodamine B) Assay is an aminoxanthene dye in bright pink color. In mild acidic conditions, a dye can bind to basic amino acids and dissociate in basic conditions. 5000-19000 cell/well are plated in a 96-well flat-bottom plate. The adjustment for differences in the number of cells plated different cell lines for

differences in growth rate. Adhering of cells to wells overnight, then samples were added in 3-fold serial dilutions to wells in triplicate. Water is added in the medium at a 1:10 dilution to control wells. Incubated the plates at 37°C temperature and 5% CO<sub>2</sub> for 36 hrs, then SRB assay has done for growth inhibition. Fixation of cells to a final concentration of 10% has been done with the addition of cold trichloroacetic acid (50%). Cells are washed with deionized water (5 times) after incubation for one hour at 4°C. Thereafter 0.4% of Sulforhodamine B dissolved in 1% acetic acid is used for staining of cells for 15-30 min. To remove the unbound stain again washed 5 times with 1% acetic acid. Plates are left to dry at room temperature. 10mm tris base is used to solubilize bound dye and the analysis of plates has done on a microplate reader (Molecular Devise) at 595nm.

$$\% \text{ growth inhibition is calculated as } = \frac{\text{Control sample}}{\text{Control}} \times 100$$

#### **Reagents requirements**

1. Sulforhodamine B
2. 96 well flat bottom plate
3. 50% trichloroacetic acid (cold)
4. Deionized water
5. 1% acetic acid
6. 10mm tris base

#### **5.5.2 Morphological Examination:**

Morphological Examination has been done with an Inverted Microscope with a camera (Nikon) to study the cell image without any treatment and after treatment.

#### **5.6. Cell cycle analysis with PI (Propidium Iodide) staining**

##### **PRINCIPLE:**

**PI (Propidium Iodide)** is a popular counter-stain atomic red-fluorescent and chromosome. Because propidium iodide is not permeable to live cells, it is also

commonly used in a population to detect dead cells. PI binds to DNA by intercalating with little or no sequence preference between bases. The dye has an excitation/emission maximum of 493/636 nm in an aqueous solution. Once the dye is bound, its fluorescence is increased 20-to30-fold, the maximum excitation of fluorescence is shifted to red ~30–40 nm and the maximum emission of fluorescence is shifted to blue ~15 nm, resulting in maximum excitation at 535 nm and maximum emission of fluorescence at 617 nm. PI is widely used in microscopy of fluorescence, microscopy of confocal laser scanning, cytometry of flow, and fluorometry.

## **5.7. FLOW CYTOMETRY:**

It is a technique in which microscopic objects suspended in a fluid are counted, examined, and sorted based on their optical properties. It helps in the measurement and analysis of multiple physical characteristics of a single object (generally a cell) as the flow of the objects through a beam of light in a fluid stream.

### **5.7.1. Cell cycle and apoptosis analysis with Flowcytometry**

Cell cycle evaluation by way of DNA content material dimension is a way that maximum often employs go to differentiate cells with the help of flow cytometric technique in distinctive phases of the cellular cycle. The cells are treated with a fluorescent dye to be permeabilized for quantitative staining of DNA, including propidium iodide (PI) before evaluation. The fluorescent intensity of stained or recolored cells corresponds with the measurement of fused DNA. Since the content material of DNA pairs at some phase in the S stage, the DNA content material (and along these lines profundity of fluorescence) of cells inside the G<sub>0</sub> area and G<sub>1</sub> stage (sooner than S), inside the S stage, and the G<sub>2</sub> segment and after S phase stage M recognizes cell cycle segment position inside the most significant stages (G<sub>0</sub>/G<sub>1</sub> rather than S instead of G<sub>2</sub>/M stage) of the cellular cycle. Character cells ' DNA content material has often drawn as a frequency histogram to provide records of relative frequency (percent) of cells in cell cycle's significant levels. Cell cycle peculiarities found in the DNA content recurrence histogram are routinely seen after particular cell lysis, for example, DNA lysis that interferes with the improvement of the versatile cycle at specific checkpoints. Such arrest of the progression of the cell cycle can lead to an effective repair of DNA that can also prevent carcinogenesis,

often through the apoptosis mode. Due to nutrients loss (boom factors), for example after serum deprivation, cell cycle arrest in G0 or G1 is often visible. In 1969, the assessment of cell cycle was first described by a team of researchers from the University of California at the Los Alamos Scientific Laboratory using the Feulgen staining technique (Van dilla *et al.*, 1969). In 1975, Awtar Krishan from Harvard scientific school provided a primary protocol for cellular cycle evaluation of the usage of staining with propidium iodide. This protocol is still extensively stated these days (Krishna, 1975).

Flow cytometry is one of the maximum famous and flexible programs for reading apoptosis and cell cycle. It offers the capacity to take a look at massive numbers of cells personally as opposed to a combined populace, examining simultaneously the expression of large numbers of proteins along with cell precise markers and signs of apoptosis. One component to don't forget before you start your experiments is that apoptosis is not a static process and sure exams are time unique. As an example, if most of your cells are getting into late apoptosis you can now not be capable of stumble on early ranges of apoptosis the usage of a few reagents. The effective technique of flow cytometry can be used to both locate and quantify the extent of apoptosis in a population of cells at static points or in a time route. Flowcytometry lets in the have a look at all components of apoptosis from induction via surface receptors, too late levels in which DNA fragmentation happens.

**Procedure:**

- Trypsinized cell culture washed with phosphate-buffered saline (PBS) and incubated with 5ml of a complete medium in 60 mm x 15 mm at a density of  $5 \times 10^5$  cells / well (Corning, USA, polystyrene-treated tissue culture dish) Petri plates.
- Cells present in Dulbecco Modified Eagle Medium (DMEM) were grown in addition to bovine fetal serum (10%) and antibiotic and antimycotic (1%).
- Dimethyl sulfoxide (DMSO) dissolved test sample and produced 10mg/ml stock solution.
- Finally, the test compounds diluted in 1ml of the complete medium were added to the working concentration.

- Cells are used as control alone or untreated.
- The plates were then incubated at 37°C in a 5% CO<sub>2</sub> incubator for 12 to 24 hours.
- Following incubation, harvested adherent and floating cells for centrifugation at 200g for 5min.
- Centrifuged the PBS washed pellets again at 200g for 5 minutes.
- Cell pellets were subsequently resuspended in 1ml ethanol (70%) and fixed for 2 hrs at 4°C. Washed the fixed cells with PBS (14ml) and centrifuged at 200g for 5min.
- 50µg/ml PI solution in 0.1% sodium citrate, 0.1% Triton-X 100 and RNAsA20 µg/ml) cell pellets were resuspended for 30 minutes in dark.
- The Amnis flow cytometer was used to process samples.
- Cell distribution was calculated using IDEA software in each stage of the cell cycle.

**5.8. For the apoptotic study by morphological changes in the nuclei of HeLa cells treated with *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin, Annexin V Fluorescein isothiocyanate (FITC):**

Annexin V is a protein that binds phospholipid to Ca<sub>2</sub><sup>+</sup>. This protein has the highest affinity for phosphatidylserine (PS) but it may bind to various phospholipids. Based on the affinity of Annexin V to PS can be used as a sensitive probe for PS cell surface exposure. The protein was labeled with fluorescein isothiocyanate (FITC) to use Annexin V as a sample for apoptosis. This protein can be used to quantify apoptotic cells in this form directly. The effective discrimination between apoptosis and necrosis has done with the measurement of binding of Annexin V when simultaneously performed with propidium iodide.

**Procedure:**

- Spin down cells, at 3,000 rpm for 1 minute, decant supernatant.
- PBS ice-cold wash cells, pH 7.4. Resuspend the pellet carefully.
- Spin down cells at 3,000 rpm for 1 minute, discarded the supernatants, and with pipettor remove excess PBS.

- 1X buffer (10 mM Hepes / NaOH, pH 7.4, NaCl (140 mM), CaCl<sub>2</sub> (2.5 mM), 0.2 µm pore filter is used for filtration) is used for resuspension of cells; adjust cell density to 2-5x 10<sup>5</sup> cells / ml.
- In 190 µl of the cell suspension, add 10 µl of V-FITC and mix it smoothly.
- Incubate the mixture for 10 minutes at room temperature.
- 1X cells were washed with buffer binding. In 190 µl binding buffer, spin cells down, 1 minute at 3,000 rpm, decant supernatant and resuspend.
- Add a stock solution of 10 µl of 20 µg / ml of PI.
- Use flow cytometry or fluorescent microscopy to analyze cells.

### **5.9. Statistical Analysis:**

The statistical analyses of triplicate readings were expressed as mean±SD. Analysis of data has been done by linear regression with 95% confidence band and IC<sub>50</sub> value of DPPH, ABTS, and ascorbic acid was calculated using software Graph Pad Prism 7.04. The statistical comparisons are performed by One-way analysis of variance (ANOVA) is used to study the statistical comparison. If the p-values are less than 0.05 then results are considered significant.

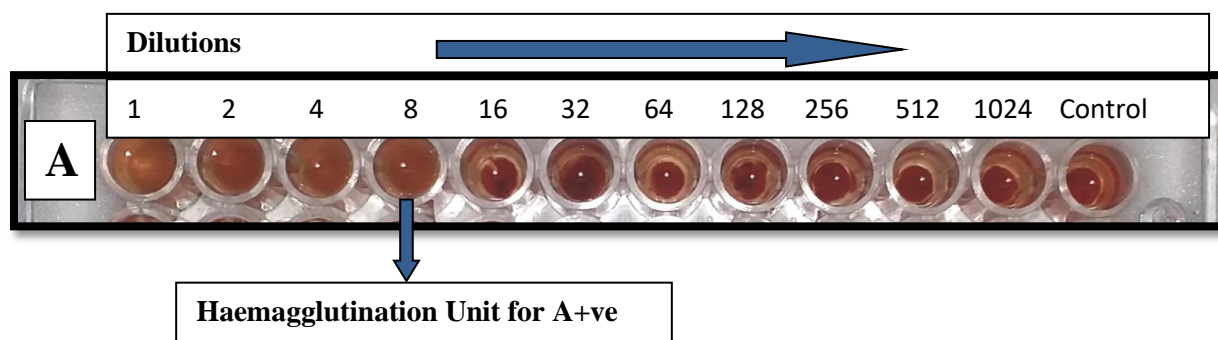
*CHAPTER-6*  
*RESULTS*

## 6. RESULTS

### 6.1. Haemagglutination Assay:

To characterize the lectin in *Aloe barbadensis* & *Bryophyllum pinnatum* plant extracts, a haemagglutination assay was performed by using human erythrocyte suspension (A+ve, B+ve, and O+ve).

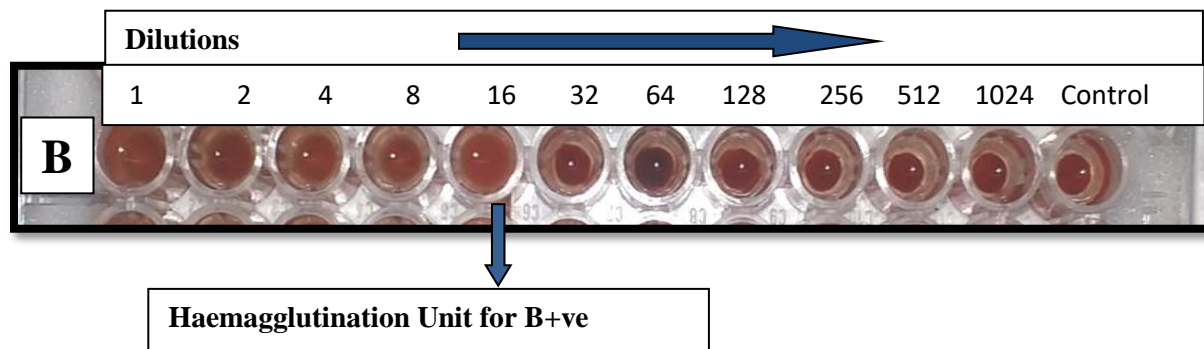
#### 6.1.1. Haemagglutination Assay with *Aloe barbadensis* gel extract



**Fig. 12.1. Haemagglutination Assay of A+ve human blood group with *Aloe barbadensis* gel extract**

#### Inference:

The haemagglutination assay has been performed by serial dilution with an A+ve human blood group to confirm the lectin in *Aloe barbadensis* gel extract. A+ve blood has been taken as control. From figure 15.1, it has been observed that the Haemagglutination unit is the strength of the titer was calculated with the last agglutination dilution reciprocally i.e. 8.

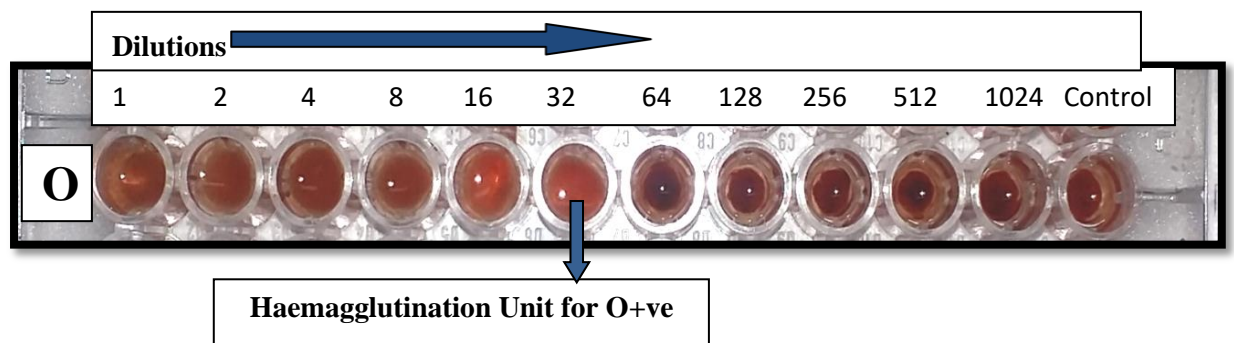


**Fig.12.2. Haemagglutination Assay of B+ve human blood group with *Aloe barbadensis* gel extract**



### Inferences:

The haemagglutination activity of *Aloe barbadensis* plant extract has been performed with a B+ve blood group to confirm the lectin in the plant extract. B+ve blood has been taken as control. From figure 15.2, it has been observed that the Haemagglutination unit is the strength of the titer was calculated with the last agglutination dilution reciprocally i.e. 16 which is more than the A+ve blood group.



**Fig.12.3. Haemagglutination Assay of O+ve human blood group with *Aloe barbadensis* gel extract**

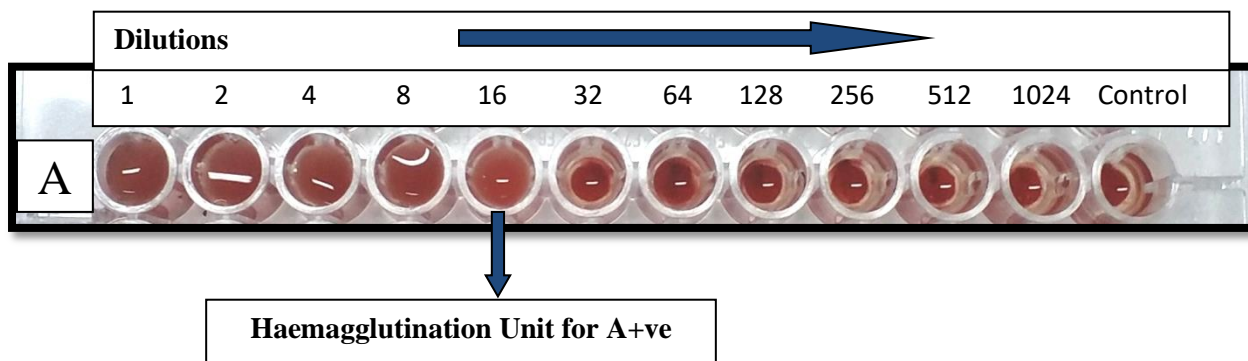
### Inferences:

The haemagglutination activity of *Aloe barbadensis* gel extract has been performed with an O+ve blood group to confirm the lectin in the gel extract. O+ve blood has been taken as control. From figure 15.3, it has been observed that the Haemagglutination unit with the last agglutination dilution reciprocally i.e. 32 which more than the B+ve blood group. O+ve blood group has shown its maximum haemagglutination as compared to B+ve and A+ve i.e. 32, 16, and 8 respectively.

The above results confirmed the presence of lectin in the *Aloe barbadensis* gel extract because the plant extract has agglutinated the A+, B+ve, and O+ve blood group.

### 6.1.2. Haemagglutination Assay with *Bryophyllum Pinnatum* leaves extract

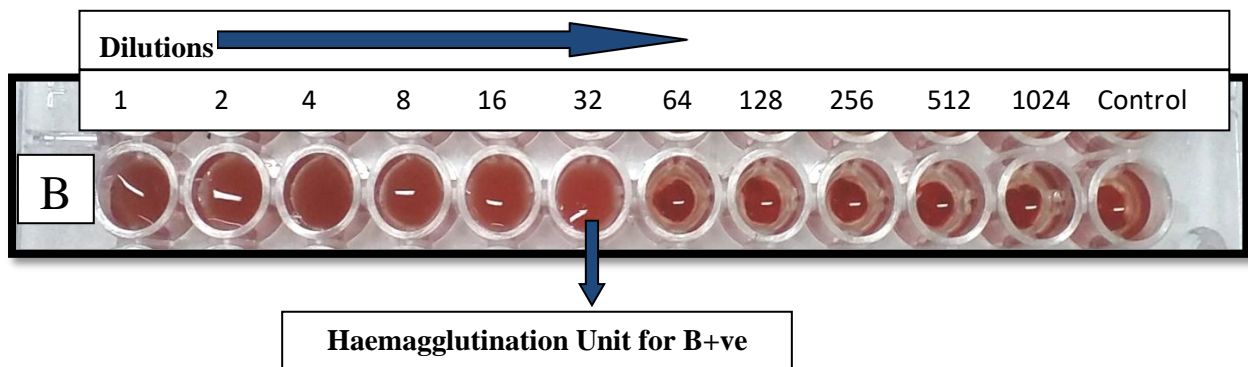
The haemagglutination assay has been performed with A+, B+ve, and an O+ve human blood group to confirm the presence of lectin in *Bryophyllum Pinnatum* leaves extract.



**Fig.13.1. Haemagglutination Assay of A+ve human blood group with *Bryophyllum Pinnatum* leaves extract**

**Inference:**

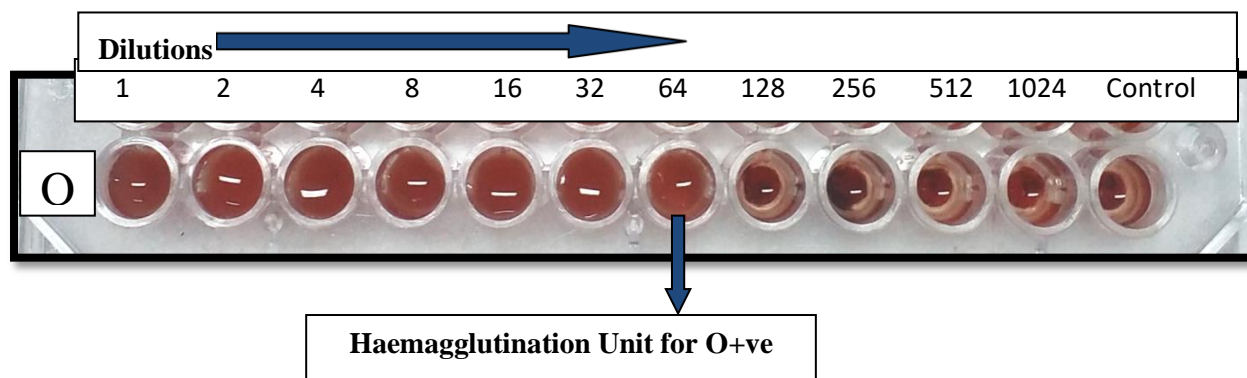
The haemagglutination assay of *Bryophyllum Pinnatum* leaves extract has been done with an A+ve blood group with serial dilution. A+ve blood has been taken as control. From figure 16.1, it has been observed that the Haemagglutination unit with the last agglutination dilution reciprocally i.e. 16.



**Fig.13.2. Haemagglutination Assay of B+ve human blood group with *Bryophyllum Pinnatum* Lectin**

**Inferences:**

The haemagglutination activity of *Bryophyllum pinnatum* leaves extract has been performed with a B+ve blood group to confirm the lectin in the leaves extract. B+ve blood has been taken as control. From figure 16.2, it has been observed that the Haemagglutination unit (HAU) is the strength of the titer was calculated with the last agglutination dilution reciprocally i.e. 32 which is more than the A+ve blood group.



**Fig.13.3. Haemagglutination Assay of O+ve human blood group with *Bryophyllum Pinnatum***

**Inferences:**

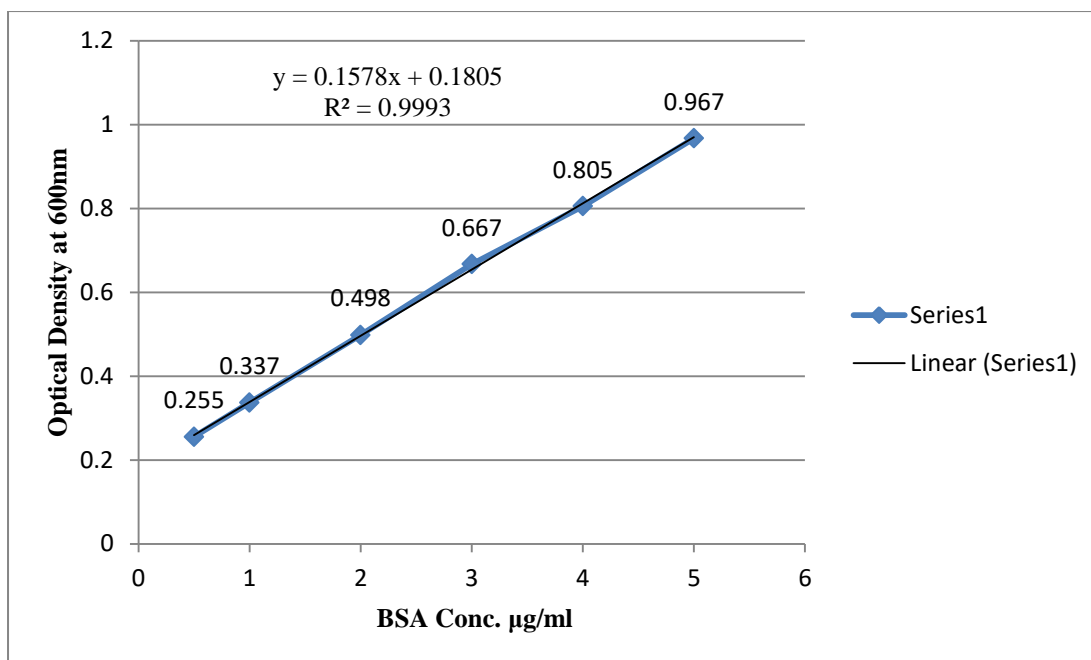
The haemagglutination activity of *Bryophyllum Pinnatum* extract has been performed with the O+ve blood group to confirm the lectin in the gel extract. O+ve blood has been taken as control. From figure 16.3, it has been observed that the Haemagglutination unit with the last agglutination dilution reciprocally i.e. 64 which is more than the B+ve blood group. O+ve blood group has shown its maximum haemagglutination as compared to B+ve and A+ve i.e. 64, 32, and 16 respectively.

The lectin present in both plant extracts react with human blood and agglutinate it. The above results confirmed the presence of lectin in the *Aloe barbadensis* gel and the *Bryophyllum Pinnatum* leaves extract.

**6.2. Concentration of Protein determined by Lowry’s Method:**

**Table. 6. The concentration of Protein by Lowry’s Method**

BSA (ml)	Water (ml)	Extract Conc. (ml)	Extract Vol. (ml)	Alk. CuSO <sub>4</sub> (ml)	Reagent (ml)	O.D. at 600nm
0.25	4.75	0.05	0.2	2.0	0.2	0.165
0.50	4.50	0.1	0.2	2.0	0.2	0.255
1.0	4.0	0.2	0.2	2.0	0.2	0.337
2.0	3.0	0.4	0.2	2.0	0.2	0.498
3.0	2.0	0.6	0.2	2.0	0.2	0.667
4.0	1.0	0.8	0.2	2.0	0.2	0.805
5.0	0.0	1.0	0.2	2.0	0.2	0.967



**Graph.1.** Determination of Protein Concentration.

Protein Concentration in gel of *Aloe barbadensis* = **4.28mg/ml**

Protein Concentration in leaves extract of *Bryophyllum pinnatum* = **5.09mg/ml**

### 6.3. Haemagglutination Unit with specific activity:

Haemagglutination unit (HAU) which represents the titer strength was calculated with the reciprocal of the last dilution of agglutination. Specific activity (SA) which is HAU per mg protein was also calculated. Haemagglutination units and protein concentration were calculated, lectin specific activity was calculated by using the formula (Liener & Hill, 1953).

$$\text{Lectin Specific activity} = \text{HA units/ml} \div (\text{protein concentration i.e. mg/ml})$$

From haemagglutination assay, the HAU (Haemagglutination unit) and SA (specific activity) of A, B, O blood groups for *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin were calculated.

### 6.3. (a) *Aloe barbadensis* Agglutination

**Table.7. Haemagglutination unit with a specific activity of *Aloe barbadensis***

Protein Conc. (mg/ml)	HAU/ml			Specific Activity (HAU/mg)		
	A	B	O	A	B	O
4.28	34.24	68.48	136.96	8	16	32

#### **Inferences:**

The Protein Concentration in gel of *Aloe barbadensis* was 4.28mg/ml. Haemagglutination unit/ml and specific activity (SA/mg) of *Aloe barbadensis* lectin for A+ was 34.24, for B+ was 68.48, and O+ was 136.96 HAU/ml, and the specific activity for A+, B+, and O+ was 8, 16, and 32 SA/mg respectively.

### 6.3. (b) *Bryophyllum pinnatum* Agglutination:

**Table.8. Haemagglutination unit with a specific activity of *Bryophyllum pinnatum***

Protein Conc. (mg/ml)	HAU/ml			Specific Activity (HAU/mg)		
	A	B	O	A	B	O
5.09	81.44	162.88	325.76	16	32	64

#### **Inferences:**

The Protein Concentration in leaves extract of *Bryophyllum pinnatum* was 5.09mg/ml. For *Bryophyllum pinnatum*, Haemagglutination unit/ml and specific activity (SA/mg) of A+ was 81.44, for B+ 162.88, and for O+ was 325.76 HAU/ml, and the specific activity for A+, B+, and O+ was 16, 32, and 64 SA/mg respectively.

### 6.4. Sugar specificity test/Agglutination Inhibition Assay

A sugar specificity test was performed by testing the ability of various seven sugars. 1.0mL sugar volumes of test solutions have been mixed with the lectin solution of the same volume. Addition of 2% erythrocyte suspension in an equal volume after incubating for 3 hours at room temperature and left the mixtures for another 30 min. The agglutination was then examined visually.

**Table.9. Sugar specificity of *Aloe barbidensis* with different sugars**

Blood Group	D-Glucose	Sucrose	Lactose	Fructose	Mannose	Galactose	N-acetyl-D-galactosamine
<b>A</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>
<b>B</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>	Absence of Inhibition
<b>O</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>	Absence of Inhibition	Absence of Inhibition

**Inferences:**

- Agglutination activity of *Aloe barbadensis* Lectin was inhibited due to specific sugar:
- **A+ve blood group:** N-acetyl-D-galactosamine.
- **B+ve:** D-galactose
- **O+ve:** D-Mannose

**Table.10. Sugar specificity of *Bryophyllum pinnatum* Lectin with different sugars.**

Blood Group	Glucose	Sucrose	Lactose	Fructose	Mannose	Galactose	N-acetyl-D-galactosamine
<b>A</b>	<b>Inhibition (500mM)</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>
<b>B</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>	Absence of Inhibition
<b>O</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition	<b>Inhibition (500mM)</b>	Absence of Inhibition	Absence of Inhibition	Absence of Inhibition

**Inferences:**

- Agglutination activity of *Bryophyllum pinnatum* Lectin was inhibited due to specific sugar:
- **A+ve blood group:** D-glucose, N-acetyl-D-galactosamine.
- **B+ve:** D-galactose
- **O+ve:** L-Fructose

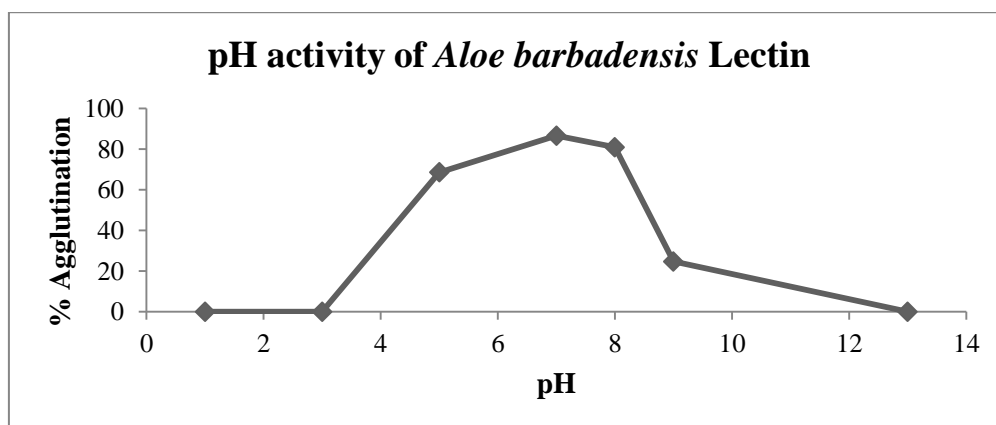
### 6.5. Study of pH activity of Lectin

The pH stability of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin was determined by using different buffer ranging from 1pH -13pH spectrophotometrically at 616 nm. (Suseelan *et al.*,1997). Lectin extract with 2% erythrocytes has been taken as control

$$\% \text{ pH activity} = \frac{(\text{Abs}_{(\text{Cont})} - \text{Abs}_{(\text{Sample})})}{\text{Abs}_{(\text{Cont})}} \times 100$$

**Table.11. pH activity of *Aloe barbadensis* Lectin**

Control	pH	%age
0.89	1	0
0.89	3	0
0.89	5	68.53
0.89	7	86.51
0.89	8	80.89
0.89	9	24.71
0.89	13	0



**Graph.2.** pH activity of *Aloe barbadensis* lectin with different buffers at room temperature.

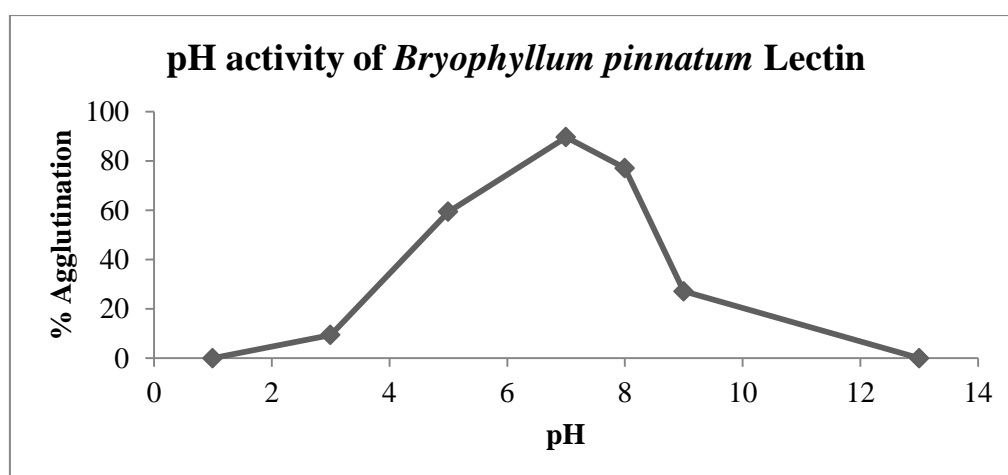
#### Inferences:

The effect of different pH on *Aloe barbadensis* lectin at room temperature has been shown in graph.2. From the graph.2 it has been observed that at 1-3pH the activity of lectin was 0%. At 5pH the activity of lectin in the graph has shown increased activity i.e. 68.53%. At 7pH the maximum activity of lectin has shown in the graph i.e. 86.51%. At 8pH the activity of lectin was 80.89%. As the pH increased

there was a downfall has observed in the graph. At 9pH the activity of lectin was 24.71 and at 13pH there was no activity has been observed i.e.0%. The maximum activity of *Aloe barbadensis* lectin has been observed at 5pH to 9pH in the graph.

**Table.12. pH activity of *Bryophyllum pinnatum* Lectin**

Control	pH	%age
0.96	1	0
0.96	3	9.37
0.96	5	59.37
0.96	7	89.58
0.96	8	77.08
0.96	9	27.08
0.96	13	0



**Graph.3.** pH activity of *Bryophyllum pinnatum* lectin with different buffers at room temperature.

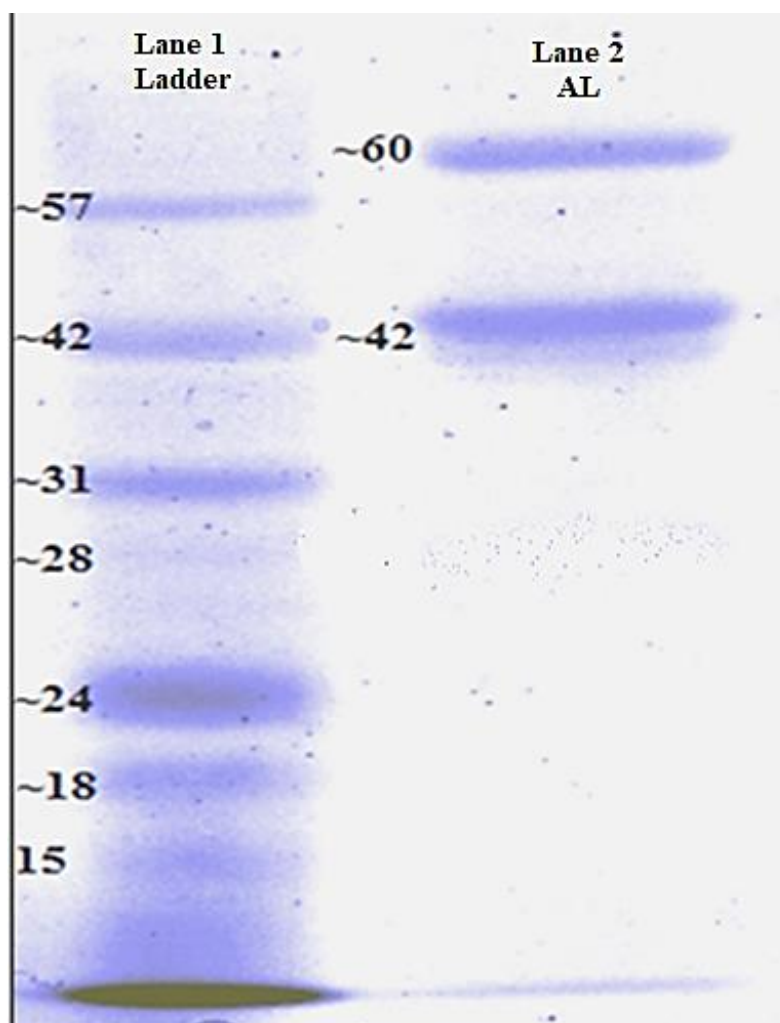
**Inferences:**

The effect of different pH on *Bryophyllum pinnatum* lectin at room temperature has been shown in graph.3. From the graph.3 it has been observed that at 1pH there was no activity of lectin has been observed i.e. 0%. At 3pH lectin was showed minimum activity i.e. 9.39 in the graph. At 5pH has shown increased activity i.e. 59.37%. At 7pH the maximum activity of lectin has been observed in the graph i.e. 89.58%. At 8pH the activity of lectin was 77.08%. At 9pH the activity of lectin was 27.08 and at 13pH there was no activity has been observed i.e.0%. As the pH increased there was a downfall has observed in the graph. The maximum activity of *Bryophyllum pinnatum* lectin has been observed at 5pH to 9pH in the graph.



## 6.6 Electrophoresis (SDS-PAGE) for the confirmation of purity of lectins:

### 6.6.1 *Aloe barbadensis* Lectin:

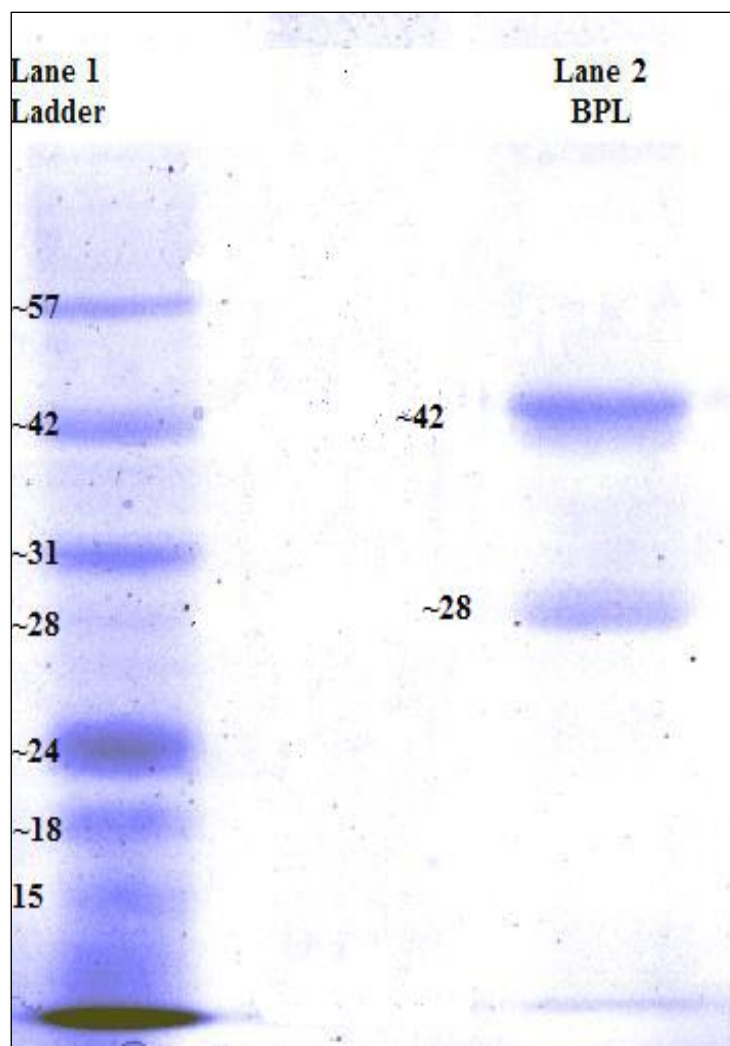


**Fig.14.1.** The appearance of the band resulted in SDS-PAGE with molecular weights of 42kDa and 60kDa in the purified lectin of *Aloe barbadensis* Lectin (50 $\mu$ l). Here Lane 1 is standard protein marker (BioRed) AL is *Aloe barbadensis* Lectin.

#### **Inferences:**

The 50 $\mu$ l lectin extracts were prepared by adding 2X protein loading dye in MCTs. As the prepared lectin extracts were loaded into wells then connected with a power supply 50V-70V voltage and run the electrophoresis. The protein present in the crude *Aloe barbadensis* lectin has started separating according to their molecular weight i.e. 42kDa and 60kDa respectively.

### 6.6.2. *Bryophyllum pinnatum* lectin:



**Fig.16.2.** Band pattern of *Bryophyllum pinnatum* lectin on SDS-PAGE has shown the presence of lectin with 28kDa and 42kDa molecular weight (50 $\mu$ l). Here Lane 1 is the standard protein marker (BioRed) and Lane 2 BPL is *Bryophyllum pinnatum* lectin.

#### **Inferences:**

The 50 $\mu$ l *Bryophyllum pinnatum* lectin extracts were prepared by adding 2X protein loading dye and loaded into wells then connected with a power supply 50V-70V voltage and run the electrophoresis. The protein present in the crude *Bryophyllum pinnatum* lectin has started separating according to their molecular weight i.e. 28kDa and 42kDa respectively.

### 6.7. Anti-oxidant Activity of Lectin:

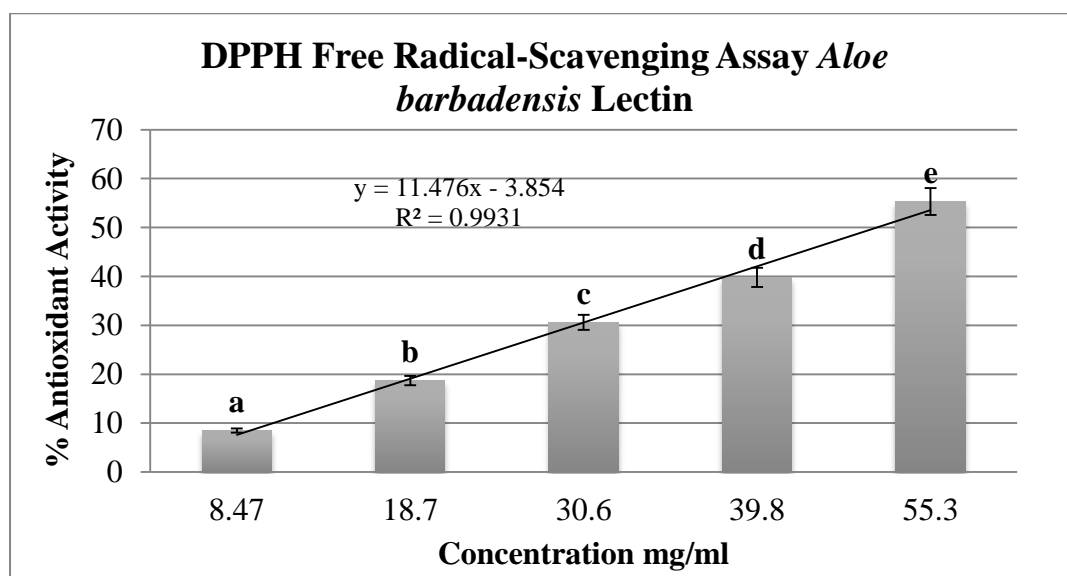
According to the DPPH Free Radical-Scavenging assay and ABTS Scavenging assay lectin has antioxidant activity at different concentrations.

#### 6.7.1(a). DPPH Free Radical-Scavenging Assay of *Aloe barbadensis* Lectin

**DPPH** (1,1-Diphenyl-2-picrylhydrazyl) can be reduced by antioxidants because it is a free radical. The different concentrations of *Aloe barbadensis* lectin 4, 8, 12, 16, and 20mg/ml were taken.

**Table.13.a. DPPH Free Radical-Scavenging Assay of *Aloe barbadensis* Lectin extract:**

Conc. of extract (mg/ml)	DPPH (Control)	Mean±SD
4	0.35	8.47±1.4
8	0.35	18.7±1.1
12	0.35	30.6±1.0
16	0.35	39.8±1.1
20	0.35	55.3±1.1



**Graph 4.** DPPH Free Radical-Scavenging Assay of *Aloe barbadensis* lectin (AL) extract. Antioxidant activity of *Aloe barbadensis* lectin (AL) at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by Tukey's test so it is statistically significant.

**Inferences:**

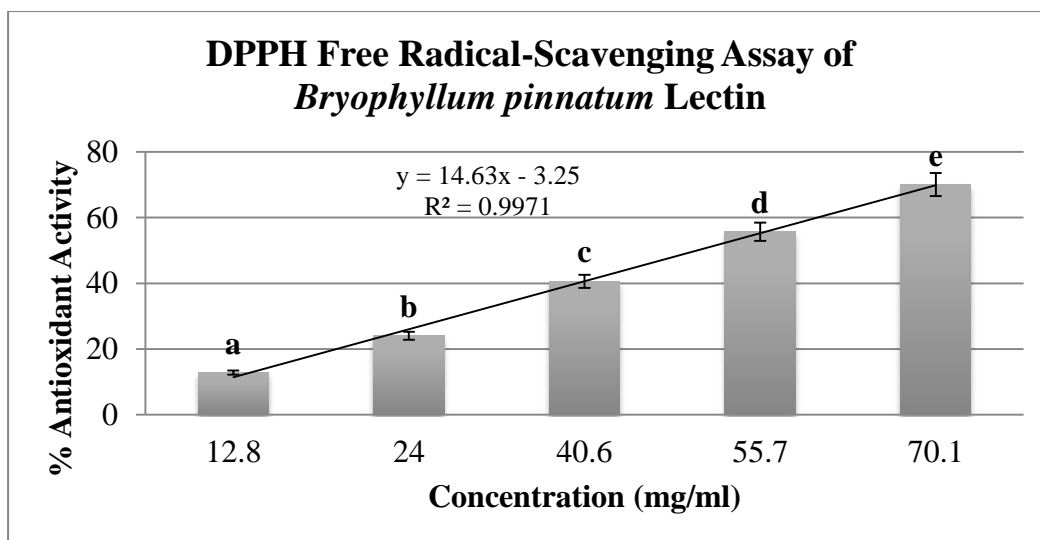
The scavenging effect of *Aloe barbadensis* lectin on DPPH was increased as the concentration of lectin was increased. From Graph.4 the antioxidant activity of *Aloe barbadensis* lectin at different concentrations has been observed. The readings have been taken in triplicates. The antioxidant activity of *Aloe barbadensis* lectin at 4mg/ml concentration was  $8.47 \pm 1.1$ , at 8mg/ml concentration the antioxidant activity was  $18.7 \pm 1.1$ , at 12mg/ml concentration the antioxidant activity was  $30.6 \pm 1.0$ , at 16mg/ml the antioxidant activity was  $39.8 \pm 1.1$  and at 20mg/ml concentration the activity was  $55.3 \pm 1.1$ . The maximum antioxidant activity of *Aloe barbadensis* lectin has been observed at 20mg/ml as shown in graph.4. The maximal inhibitory concentration i.e. IC50 value of *Aloe barbadensis* lectin was 18.5 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 1.15mg/ml.

**6.7.1 (b). DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* Lectin extract:**

DPPH (1,1-Diphenyl-2-picrylhydrazyl) can be reduced by antioxidants because it is a free radical. The different concentrations of *Bryophyllum pinnatum* lectin i.e. 4, 8, 12, 16, and 20 mg/ml were taken.

**Table.13.b. DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum*****Lectin extract:**

Conc. of extract (mg/ml)	O.D. of DPPH (Control)	Mean±SD
4	0.35	$12.8 \pm 1.1$
8	0.35	$24.0 \pm 1.1$
12	0.35	$40.6 \pm 1.1$
16	0.35	$55.7 \pm 1.1$
20	0.35	$70.1 \pm 1.0$

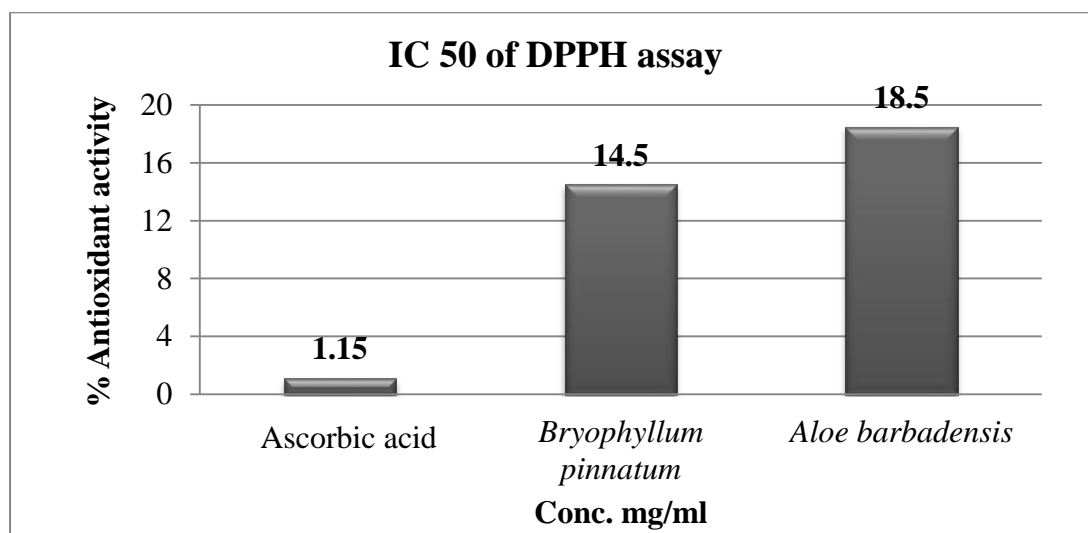


**Graph.5.** DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* lectin extract. Antioxidant activity of *Bryophyllum pinnatum* lectin at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by Tukey’s test so it is statistically significant.

**Inferences:**

From Graph.5 different antioxidant activity of *Bryophyllum pinnatum* lectin at different concentration i.e. 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20mg/ml has been observed. The readings have been taken in triplicates. The antioxidant activity of *Bryophyllum pinnatum* lectin at 4mg/ml concentration was 12.8±1.1, at 8mg/ml concentration the antioxidant activity was 24.0±1.1, at 12mg/ml concentration the antioxidant activity was 40.6±1.1, at 16mg/ml the antioxidant activity was 55.7±1.1 and at 20mg/ml concentration the activity was 70.1±1.0. The maximum antioxidant activity of *Bryophyllum pinnatum* lectin has been observed at 20mg/ml as shown in graph.4. The maximal inhibitory concentration i.e. IC50 value of *Bryophyllum pinnatum* lectin was 14.5 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 1.15mg/ml.

### 6.7.1 (c) IC50 of DPPH assay



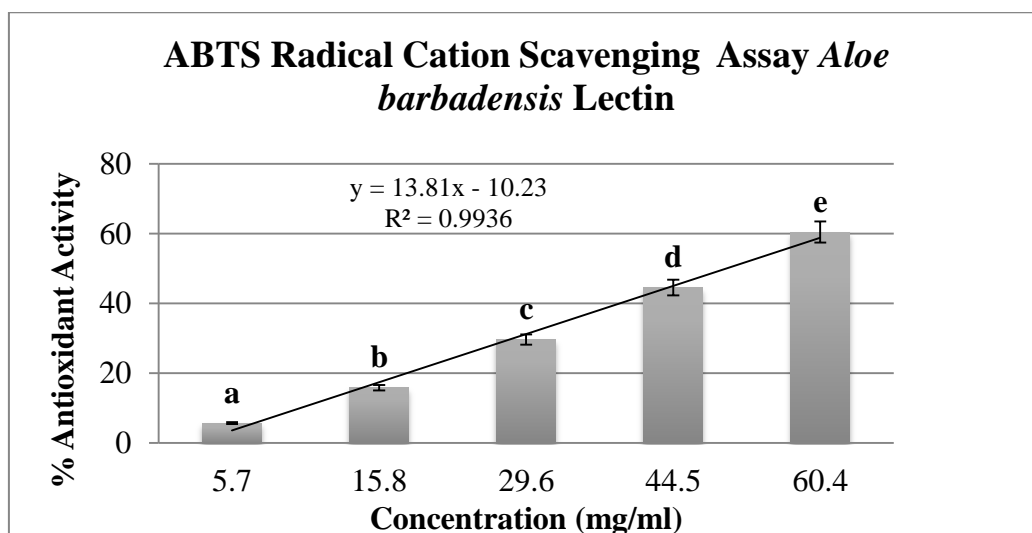
**Graph 5(a)** The above graph showed the IC50 value of DPPH assay of Ascorbic acid i.e. **1.15mg/ml**, *Bryophyllum pinnatum* i.e. **14.5mg/ml**, and *Aloe barbadensis* i.e. **18.5mg/ml**. As the ascorbic acid used as standard is the strongest antioxidant agent that is why it is showing the maximal inhibitory concentration than *Aloe barbadensis* and *Bryophyllum pinnatum*.

### 6.7.2 (a).ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging assay for *Aloe barbadensis* Lectin extract:

The production of pre-formed radical monocation of 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) with potassium persulfate (a blue chromogen) by oxidation of ABTS and in the presence of hydrogen (antioxidant donors) gets reduced. Different substances are capable of scavenging ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) compared to a standard i.e. ascorbic acid.

**Table.14.a. ABTS Radical Cation Scavenging Assay of *Aloe barbadensis* Lectin extract:**

Conc. of extract (mg/ml)	ABTS (Control)	Mean±SD
4	0.7	5.7±1.0
8	0.7	15.8±1.3
12	0.7	29.6±1.5
16	0.7	44.5±1.1
20	0.7	60.4±1.1



**Graph.6.** ABTS Radical Cation-Scavenging Assay of *Aloe barbadensis* lectin extract. Antioxidant activity of *Aloe barbadensis* lectin at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by Tukey's test so it is statistically significant.

**Inferences:**

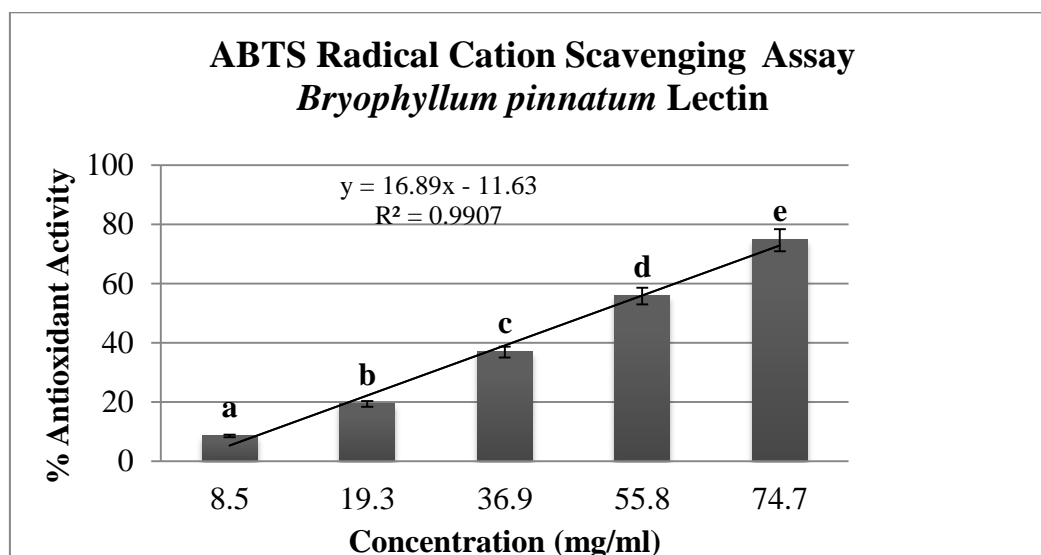
ABTS Radical Cation-Scavenging Assay has been performed to study the antioxidant activity of *Aloe barbadensis* lectin. The different concentrations of *Aloe barbadensis* lectin 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20 mg/ml were taken. The scavenging effect of lectin on ABTS was increased as the concentration of lectin was increased. The readings have been taken in triplicates. The antioxidant activity of *Aloe barbadensis* lectin at 4mg/ml concentration was 5.7±1.0, at 8mg/ml concentration the antioxidant activity was 15.8±1.3, at 12mg/ml concentration the antioxidant activity was 29.6±1.5, at 16mg/ml the antioxidant activity was 44.5±1.1 and at 20mg/ml concentration the activity was 60.4±1.1. The scavenging effect of *Aloe barbadensis* lectin on ABTS has been observed at 20mg/ml as shown in graph.6. The maximal inhibitory concentration i.e. IC50 value of *Aloe barbadensis* lectin was 17.2 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 0.96mg/ml.

**6.7.2 (b). ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin:**

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation scavenging assay has been performed to study the antioxidant activity of *Bryophyllum pinnatum* Lectin at different concentration of lectin.

**Table. 14. b. ABTS Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin extract:**

Conc. of extract (mg/ml)	ABTS (Control)	Mean±SD
4	0.7	8.5±1.0
8	0.7	19.3±1.2
12	0.7	36.9±1.1
16	0.7	55.8±1.1
20	0.7	74.7±1.3



**Graph.7.** ABTS Radical Cation Scavenging Assay of *Bryophyllum pinnatum* lectin extract. Antioxidant activity of *Bryophyllum pinnatum* lectin at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by Tukey's test so it is statistically significant.

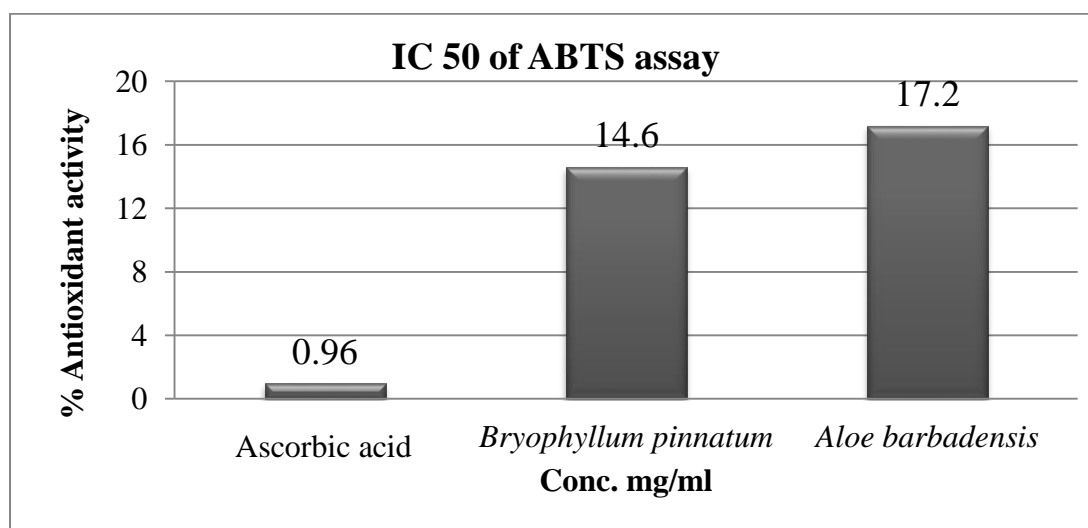
**Inferences:**

The antioxidant activity of *Bryophyllum pinnatum* lectin has been studied at different concentrations with ABTS Radical Cation-Scavenging Assay. The different



concentrations of *Bryophyllum pinnatum* lectin 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20 mg/ml were taken. The readings have been taken in triplicates. The antioxidant activity of *Bryophyllum pinnatum* lectin at 4mg/ml concentration was  $8.5\pm 1.0$ , at 8mg/ml concentration the antioxidant activity was  $19.5\pm 1.2$ , at 12mg/ml concentration the antioxidant activity was  $36.9\pm 1.1$ , at 16mg/ml the antioxidant activity was  $55.8\pm 1.1$  and at 20mg/ml concentration the activity was  $77.7\pm 1.3$ . The scavenging effect of lectin on ABTS was increased as the concentration of lectin was increased. The scavenging effect of *Bryophyllum pinnatum* lectin on ABTS has been observed at 20mg/ml as shown in graph.7. The maximal inhibitory concentration i.e. IC50 value of *Bryophyllum pinnatum* lectin was 14.6 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 0.96mg/ml.

#### 6.7.2(c) IC 50 of ABTS assay



**Graph 7(a).** The inhibitory concentration IC50 of ABTS assay of Ascorbic acid which is used as standard is **0.96mg/ml**, IC50 of *Bryophyllum pinnatum* is **14.6mg/ml** and *Aloe barbadensis* is **17.2mg/ml**.

According to the observation, both the *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin is showing antioxidant activity. But *Bryophyllum pinnatum* lectin extract has shown good anti-oxidant activity as compare to *Aloe barbadensis* lectin extract. On the basis of results, these can consider a good antioxidant agent.

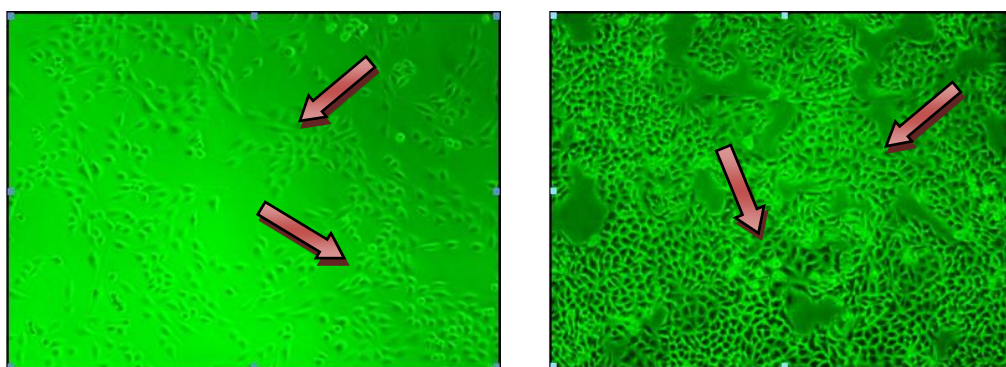
## 6.8. Anticarcinogenic activity of Plant Lectins:

Two cell lines have been cultured (HeLa and SW 480). There was a lead in two cell lines that were cervical cancer cell line HeLa and Colon cancer cell line SW480 in 48 hours timeline at 10 $\mu$ g/ml, 20 $\mu$ g/ml, 50 $\mu$ g/ml, 100 $\mu$ g/ml, and 200 $\mu$ g/ml concentrations. Vinblastine drug (an anti-cancer drug) was used as a positive control. DMEM medium is used to maintain HeLa and SW480 cancer cell lines supplemented with 10% fetal bovine serum, Penicillin (10,000 units/100 ml) and Streptomycin (10 mg/100 ml) were used to prevent the culture from contamination and grown in a humidified 5% CO<sub>2</sub> incubator at 37°C to achieve 80-90% confluency and harvested using trypsinization.

### 6.8.1. Morphological Examination:

HeLa cells in the control were seen as adherent, elongated cells with tapering ends. Round cells with clear inner cell mass and distinct boundaries were dividing, living cells. SW480 cells in the control were seen as adherent, grow as a monolayer, and polygonal in shape.

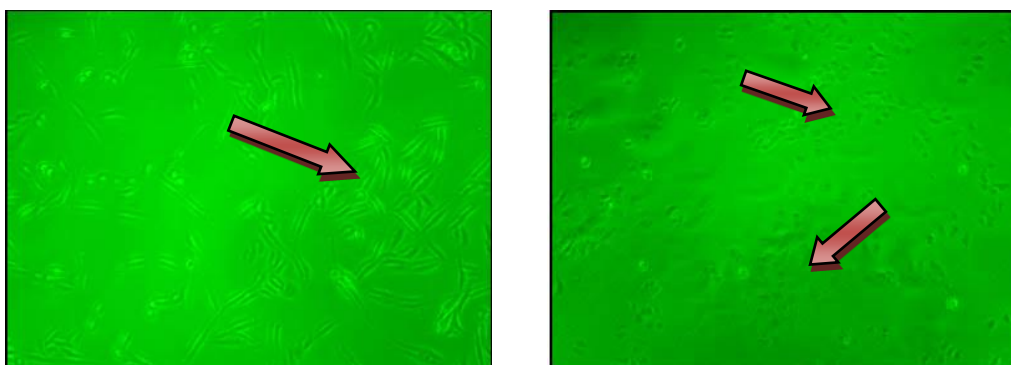
#### 6.8.1 (a). Images of cells without treatment



**Fig.15. (a) HeLa cancer cells without treatment (b) SW480 cancer cells without treatment**

The above figure showed the 80-90% growth of cancer cell line without any treatment under the inverted microscope.

**6.8.1 (b). Cells treated with the positive control (vinblastine 0.81µg/ml)**

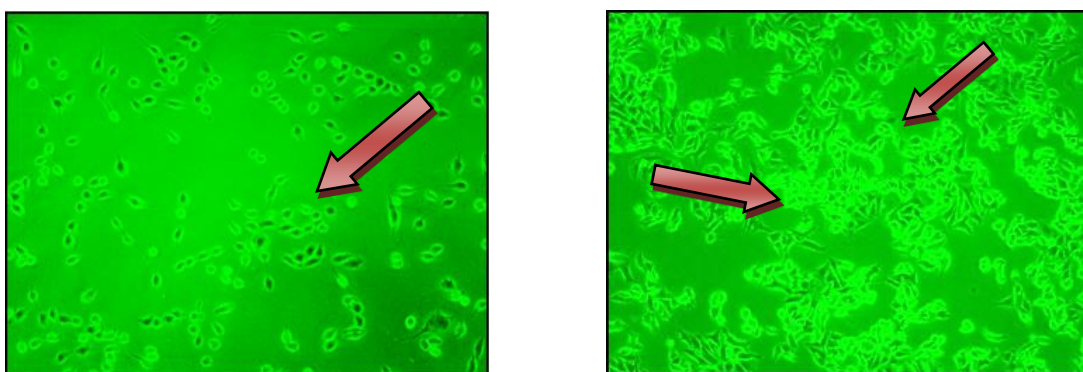


**Fig.16. (a) Treated HeLa cells with Vinblastine (b) Treated SW480 cells with Vinblastine**

**Inferences:**

The Vinblastine (Anticancer drug) is used as a positive control. Figure 19.a and b. has shown the treated cancer cell lines with Vinblastine. Vinblastine has affected the growth of both the cancer cell lines. The above figure has shown the morphologic changes and decreased growth of cancer cells.

**6.8.1 (c). *Aloe barbadensis* Lectin treated HeLa and SW 480 cells at 200µg/ml concentration**

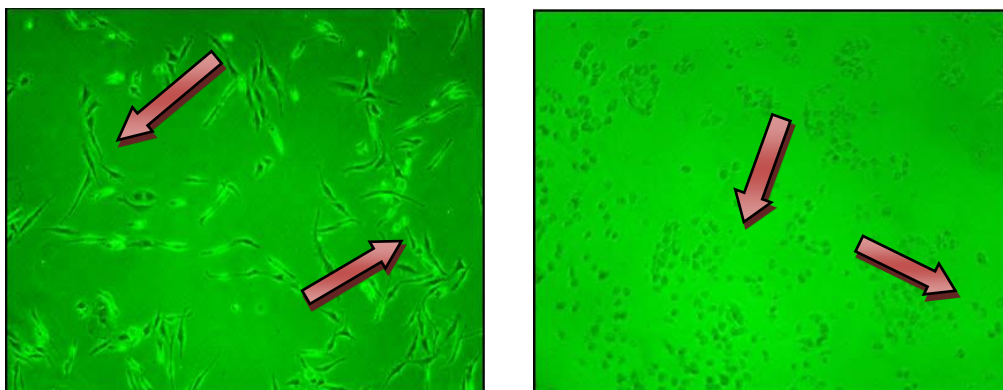


**Fig.17. (a) HeLa cancer cells (200µg/ml) (b) SW-480 cancer cells (200µg/ml)**

**Inferences:**

HeLa and SW480 have been treated with different concentrations of *Aloe barbadensis* lectin i.e. 10, 20, 50, 100, and 200µg/ml for 48hrs incubation. The above figure has shown the cell lines treated with 200µg/ml. 200µg/ml concentration killed maximum cancer cells and affected the growth of cell lines as compared to other concentrations. The *Aloe barbadensis* lectin has shown its maximum effectiveness on HeLa than the SW480 cancer cell line.

**6.8.1(d). *Bryophyllum pinnatum* Lectin treated HeLa and SW-480 cells at 200µg/ml concentration.**



**Fig.17(c) HeLa cancer cells (200µg/ml) (d) SW-480 cancer cells (200µg/ml)**

**Inferences:**

*Bryophyllum pinnatum* Lectin treated HeLa and SW-480 cells at different concentration i.e. 10, 20, 50, 100 and 200µg/ml. The above images have shown the effect of 200µg/ml on the growth of cell lines. *Bryophyllum pinnatum* Lectin has shown its maximum effectiveness on HeLa than SW480.

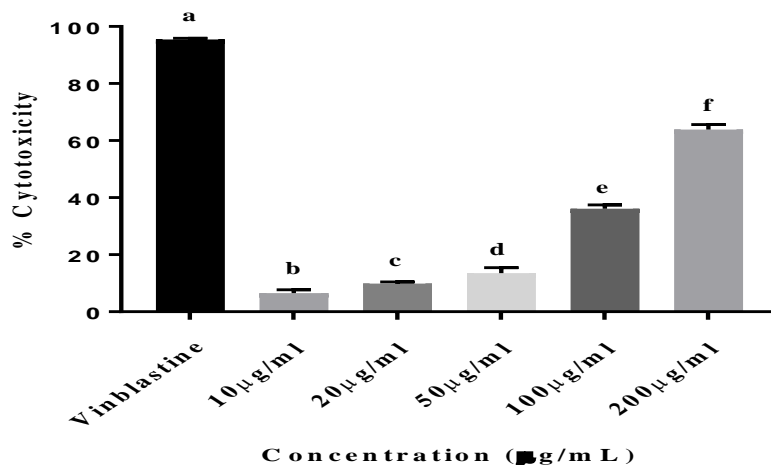
**6.9. Cytotoxicity Analysis**

SRB assay had been performed for cytotoxicity evaluation of *Aloe barbadensis* lectin extract and *Bryophyllum pinnatum* lectin extract compounds. The read had been taken at 540nm. We analyzed the data and represented it in graphical form as shown below:

**Table.15.a.Anticancer activity of *Aloe barbadensis* lectin on HeLa cell line by SRB assay:**

<b>Compounds</b>	<b>Mean ±SD</b>
Vinblastine 0.81µg/ml	95.4±0.3
AL 10 µg/ml	6.5±1.2
AL 20µg/ml	9.8±0.6
AL 50 µg/ml	13.5±1.9
AL 100 µg/ml	36.1±1.2
AL 200 µg/ml	63.8±1.7

**Anticancer activity of *Aloe barbadensis* lectin on HeLa cell line**



**Fig.18.1.**Anticancer activity of AL (*Aloe barbadensis* Lectin) on HeLa cell line at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value is less than 0.05 by Tukey’s test so it is statistically significant.

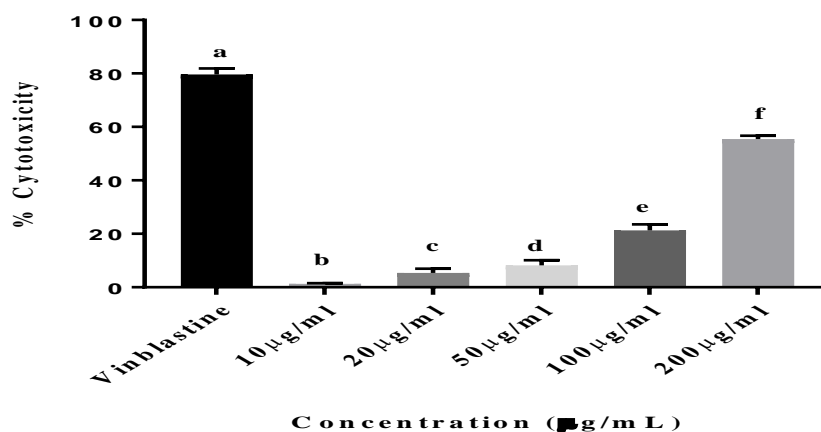
**Inferences:**

Vinblastine is an anticancer drug and used as a positive control in SRB assay at 0.81µg/ml concentration showed **95.4±0.3** mean activity. At 10µg/ml *Aloe barbadensis* lectin (AL) has showed **6.5±1.2** activity, at 20µg/ml **9.8±0.6** activity, at 50µg/ml **13.5±1.9** activity, at 100µg/ml **36.1±1.2** activity and at 200µg/ml **63.8±1.7** activity. In the SRB assay, 200µg/ml concentration of *Aloe barbadensis* lectin (AL) has shown its maximum anticancer activity after 48 hrs incubation.

**Table.15.b.Anticancer activity of *Aloe barbadensis* lectin on SW480 cell line by SRB assay:**

<b>Compounds</b>	<b>Mean±SD</b>
Vinblastine 0.81µg/ml	79.65±2.2
AL 10µg/ml	1.26±0.2
AL 20µg/ml	5.37±1.5
AL 50µg/ml	8.19±1.9
AL 100µg/ml	21.28±2.2
AL 200µg/ml	55.43±1.3

**Anticancer activity of *Aloe barbadensis* lectin on SW480 cell line**



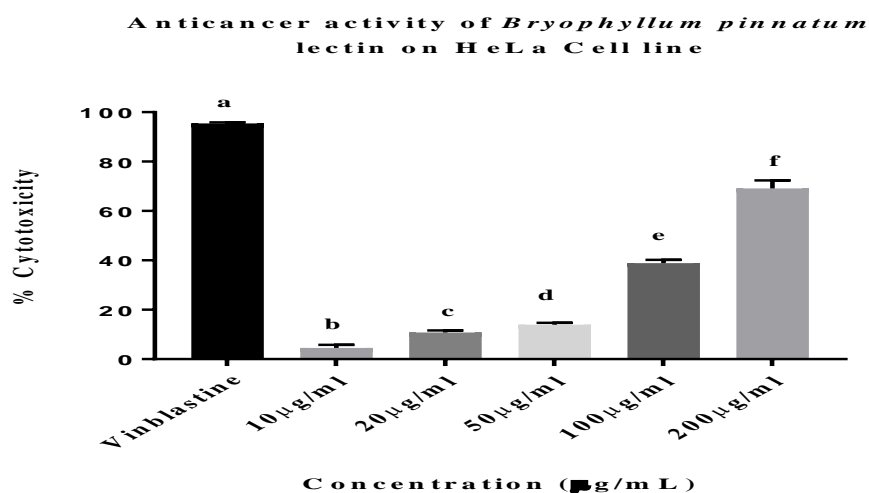
**Fig.18.2.**Anticancer activity of AL (*Aloe barbadensis* Lectin) on SW480 cell line at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by Tukey’s test so it is statistically significant.

**Inferences:**

In the SRB assay of the SW480 cancer cell line, Vinblastine (anticancer drug) is used as a positive control. At 0.81µg/ml concentration of vinblastine, the anticancer activity was **79.65±2.2**, the anticancer activity of *Aloe barbadensis* lectin (AL) at 10µg/ml was **1.26±0.2**, at 20µg/ml concentration the anticancer activity of *Aloe barbadensis* lectin (AL) was **5.37±1.5**, at 50µg/ml the activity was **8.19±1.9**, at 100µg/ml the activity was **21.28±2.2** and at 200µg/ml the activity was **55.43±1.3**. The maximum anticancer activity of *Aloe barbadensis* lectin was observed at 200µg/ml after 48 hrs incubation.

**Table.16.a.Anticancer activity of *Bryophyllum pinnatum* lectin on HeLa cell line by SRB assay :**

Compounds	Mean ±SD
Vinblastine 0.81 µg/ml	95.4±0.3
BL 10µg/ml	4.5±1.2
BL 20µg/ml	10.7±0.8
BL 50µg/ml	13.9±0.7
BL 100µg/ml	38.8±1.3
BL 200µg/ml	69.1±3.7



**Fig. 18.3.** Anticancer activity of *Bryophyllum pinnatum* lectin on HeLa cell line at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value <0.05 by Tukey's test so it is statistically significant.

**Inferences:**

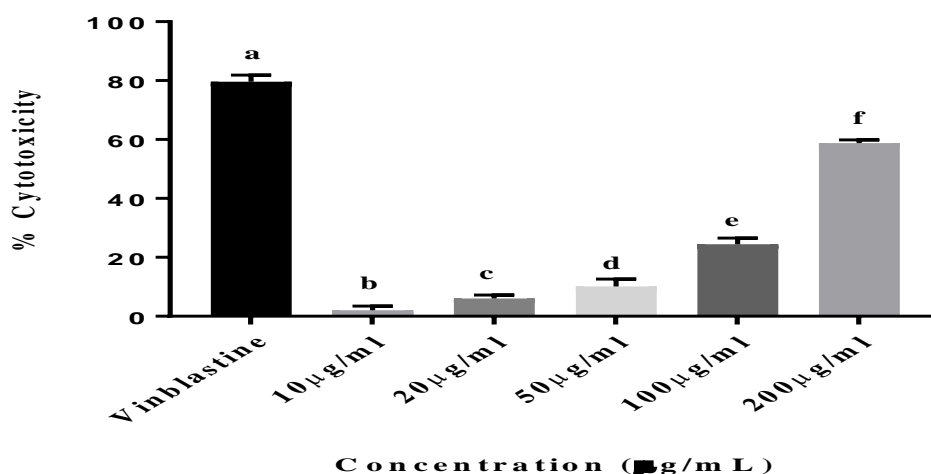
The anticancer activity of *Bryophyllum pinnatum* lectin (BL) was studied at different concentrations on the HeLa cell line by SRB assay. At 10µg/ml of BL, the anticancer activity was **4.5±1.2**, at 20µg/ml the activity was **10.7±1.2**, at 50µg/ml the activity was **13.9±0.7**, at 100µg/ml the activity was **38.8±1.3** and at 200µg/ml the activity was **69.1±3.7**. Hence the maximum anticancer activity of *Bryophyllum pinnatum* lectin (BL) on the HeLa cell line was observed at 200µg/ml concentration after 48hrs incubation.

**Table.16.b.Anticancer activity of *Bryophyllum pinnatum* lectin on SW480 cell**

**line by SRB**

Compounds	Mean± SD
Vinblastine 0.81µg/ml	79.65±2.2
BL 10µg/ml	2.02±1.4
BL20µg/ml	6.02±1.2
BL 50µg/ml	10.1±2.4
BL 100µg/ml	24.4±2.0
BL 200µg/ml	58.8±1.0

**Anticancer activity of *Bryophyllum pinnatum* lectin on SW 480 Cell line**



**Fig. 18.4.** Anticancer activity of *Bryophyllum pinnatum* lectin on SW480 cell line at different concentrations (mean±sd, n=3) with One-way analysis of variance (ANOVA) P-value < 0.05 by +Tukey's test so it is statistically significant.

**Inferences:**

In the study of anticancer activity of *Bryophyllum pinnatum* lectin (BL) on SW480 cell line by SRB assay, 0.81µg/ml of vinblastine was used as positive control and showed **79.65±2.2** activity. The anticancer activity of *Bryophyllum pinnatum* lectin on the SW480 cell line was different at different concentrations. At 10µg/ml the activity was **2.02±1.4**, at 20µg/ml the activity was **6.02±1.2**, at 50µg/ml the activity was **10.1±2.4**, at 100µg/ml the activity was **24.4±2.0** and at 200µg/ml the activity was **58.8±1.2**. Hence 200µg/ml of *Bryophyllum pinnatum* lectin (BL) showed its maximum anticancer activity on SW480 after 48hrs incubation by SRB assay.

Anticancer activity of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin by SRB assay resulted that the effect of both plants lectins at 200µg/ml concentration on HeLa (cervical cell line) is more effective than SW-480 (colon cancer cell line) after 48 hrs. The further studies are in continuity with the HeLa cell line followed by the analysis of cell cycle and apoptosis.

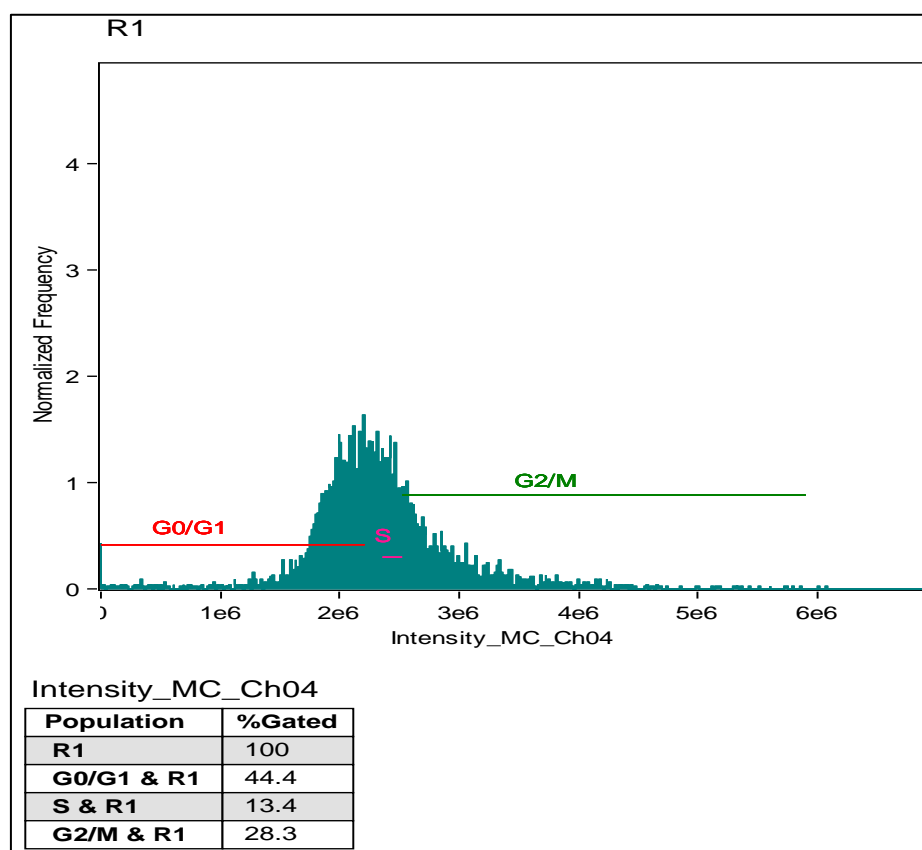


## 6.10. FLOW CYTOMETRY:

### Analysis of Cell Cycle against HeLa cell Line at 48h

To understand the mechanism involved in the cytotoxicity of AL (*Aloe barbadensis* Lectin) and BL (*Bryophyllum pinnatum* Lectin), we first investigated the cell cycle arrest and the lectin mediated morphological changes in HeLa cells. The distribution of cells in different stages of the cell cycle was evaluated by exposing the HeLa cells to both the plant lectins at 200 $\mu$ g/ml concentration for 48hrs followed by PI staining and cell cycle analysis. The untreated cells exhibit all three phases G0/G1, S, G2/M of the cell cycle. The effect of both plant lectins on HeLa cells appeared similar and time-independent, arresting the cells at G0/G1 phase, with a parallel decrease in cell population in the S and G2/M phase respectively.

#### 6.10 (a).Result with vinblastine at concentration 0.81 $\mu$ g/ml



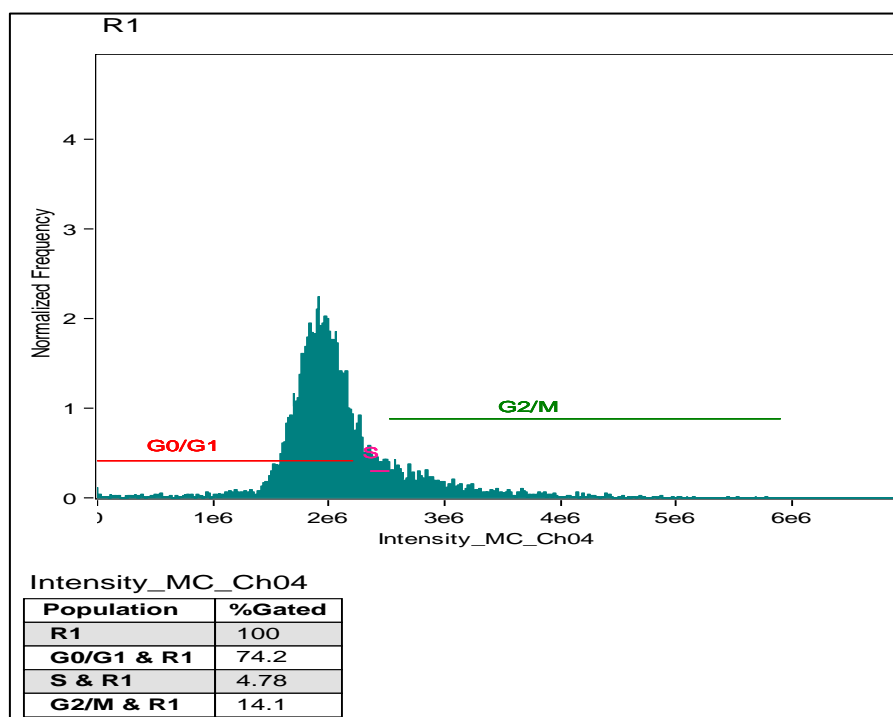
**Fig.19.1.** Analysis of Cell cycle of Vinblastine treated HeLa cell line at concentration 0.81 $\mu$ g/ml showing different phases.

### Inferences:

In the above figure 22.1, R1 is the population of single-cell without debris i.e. 100% has been studied in cell cycle analysis of vinblastine treated HeLa cells at 0.81 $\mu$ g/ml concentration after 48hrs, **G0/G1phase** where cell cycle has stopped dividing/ cells are ready for DNA synthesis i.e. **44%**. In the **S phase** DNA replication occurs in this phase i.e. **13.4%**. In the **G2/M phase** In the G2 phase, cells are ready for mitosis and start dividing / In the M phase cell growth has stopped and cells are ready to complete the cell division i.e. **28.3%**.

The cell cycle has been arrested in G0/G1phase with a total of 44% cell population till 48hrs in vinblastine treated HeLa cells at 0.81 $\mu$ g/ml concentration.

### 6.10. (b). Results with *Aloe barbadensis* lectin (AL) at 200 $\mu$ g/ml concentration:



**Fig. 19.2.** Cell cycle analysis of HeLa cell line treated with *Aloe barbadensis* (AL) lectin at 200 $\mu$ g/ml concentration showing different phases.

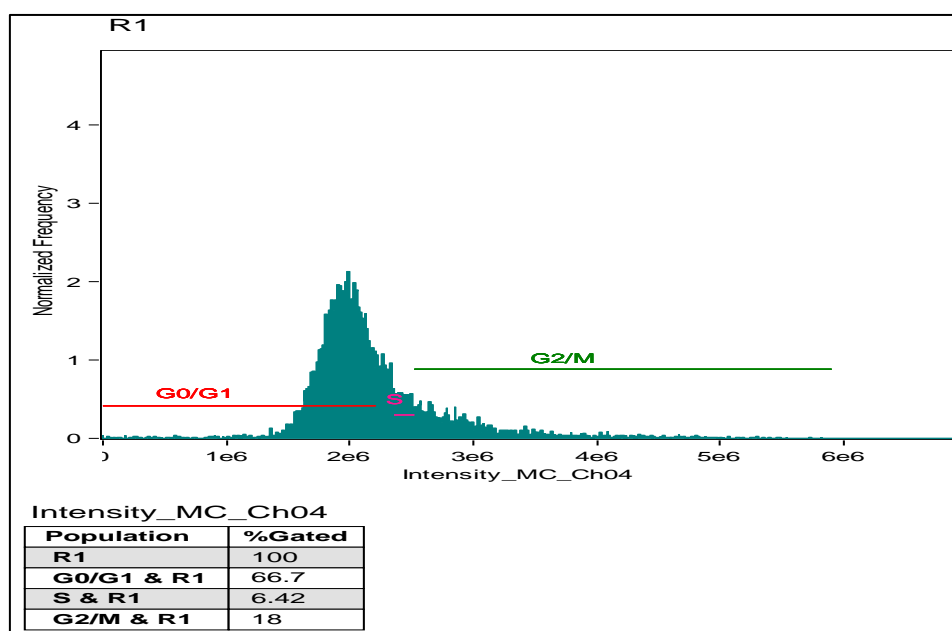
### Inferences:

In the above figure22.2, R1 is a single-cell population of HeLa cells treated with *Aloe barbadensis* (AL) lectin at 200 $\mu$ g/ml concentration without debris i.e. 100% has been studied, In the **G0/G1 phase**; the total cell population was **74.2%**. In

the **S phase**, DNA replication phase and the population of cells was **4.78%**. In the **G2/M phase**, cells are ready for mitosis and start dividing in the G2 phase. In the **M phase** cell growth has stopped and cells are ready to complete the cell division i.e. **14.1%**.

The cell cycle has been arrested in G0/G1phase with a total of 74.2% cell population till 48hrs in HeLa cells treated with *Aloe barbadensis* lectin (AL) at 200µg/ml concentration.

**6.10.(c). Results with *Bryophyllum pinnatum* lectin (BL) at 200µg/ml concentration:**



**Fig.19.3.** Analysis of Cell cycle of HeLa cell line treated with *Bryophyllum pinnatum* lectin (BL) at 200µg/ml concentration showing different phases.

**Inferences:**

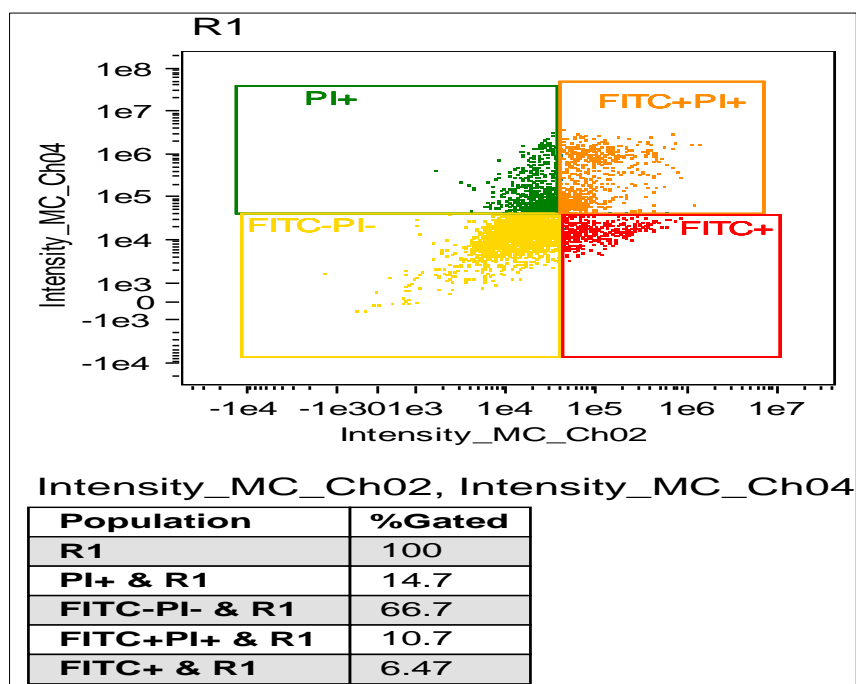
In figure 22.3, R1 is a single-cell population of HeLa cell line treated with *Bryophyllum pinnatum* lectin (BL) at 200µg/ml concentration without debris i.e. 100% has been studied, In the **G0/G1phase**; the total cell population was **66.7%**. In the **S phase**, DNA replication phase and the population of cells was **6.42%**. In the **G2/M phase**, cells are ready for mitosis and start dividing in the G2 phase. In the **M phase** cell growth has stopped and cells are ready to complete the cell division i.e. **18%**.

The cell cycle has been arrested in G0/G1phase with a total of 66.7% cell population till 48hrs in HeLa cells treated with *Bryophyllum pinnatum* lectin (BL) lectin at 200µg/ml concentration.

### 6.11. Apoptosis assay against HeLa cells at 48Hrs:

Annexin V is a Ca<sup>2+</sup>-dependent phospholipid-binding protein. The morphological analysis by Annexin V-FITC/ Propidium iodide staining demonstrated that lectin-treated cells undergo apoptosis. When cells undergo apoptosis, phosphatidylserine (PS) which usually present on the inner side of the membrane gets exposed to the surface, acting as a key marker for apoptotic bodies. The exposed PS is detected by Annexin V FITC (apoptosis) and PI stains the necrotic cells, which have leaky DNA content that help to differentiate the apoptotic and necrotic cells. Anti-tumor agents induce cell death by arresting the cell cycle (Lakshman *et al.*, 2012). In normal cells, the cell cycle checkpoint ensures damage repair, whereas in malignant cells the apoptosis eliminates them.

#### 6.11 (a). Result with Vinblastine at 0.81µg/ml concentration:



**Fig.20.1.** Apoptosis analysis of HeLa cell line with Vinblastine at concentration 0.81µg/ml at 48 hrs.

In the above figure 23,

**R1** is a single-cell population without debris where 100%

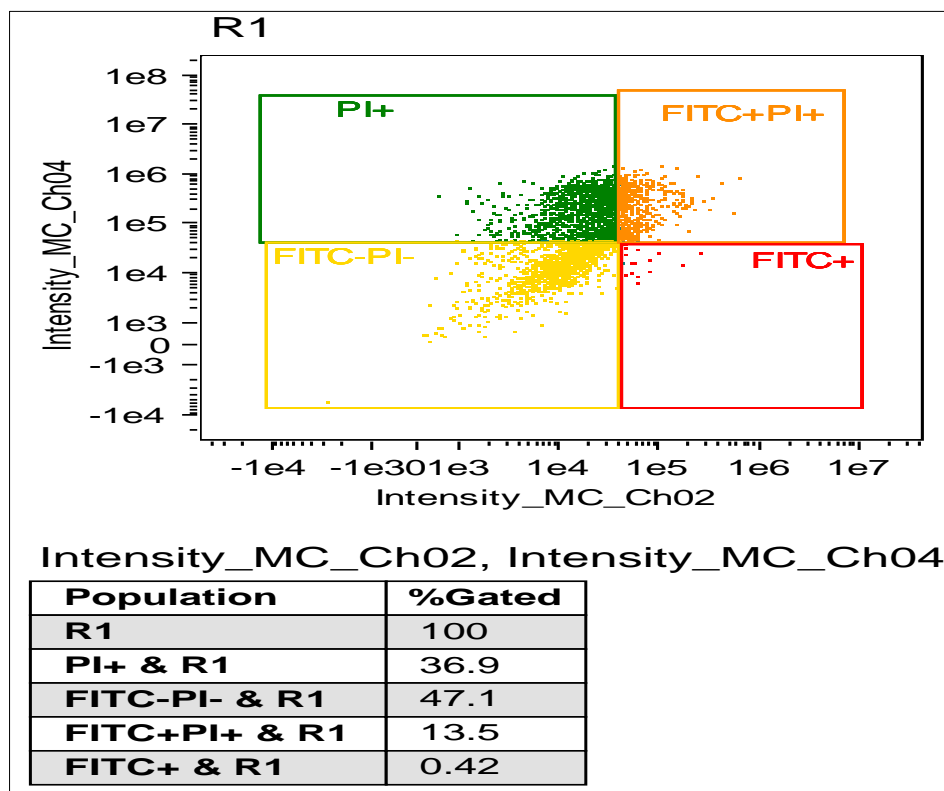
**PI+R1** (propidium iodide) is used to detect the necrotic cells population were **14.7%**

**FITC-PI** and **R1** is live cells population without apoptosis and necrosis were **66.7%**

**FITC+PI+R1** are population of both apoptosis and necrosis were **10.7%**

**FITC+R1** are population of apoptotic cells were **6.47%**

**6.11. (b). Result with *Aloe barbadensis* lectin at 200 µg/ml concentration:**



**Fig.20.2.** Apoptosis analysis of HeLa cell line with *Aloe barbadensis* lectin (AL) at concentration 200µg/ml at 48 hrs.

In the above graph,

**R1** is single cell population without debris were 100%

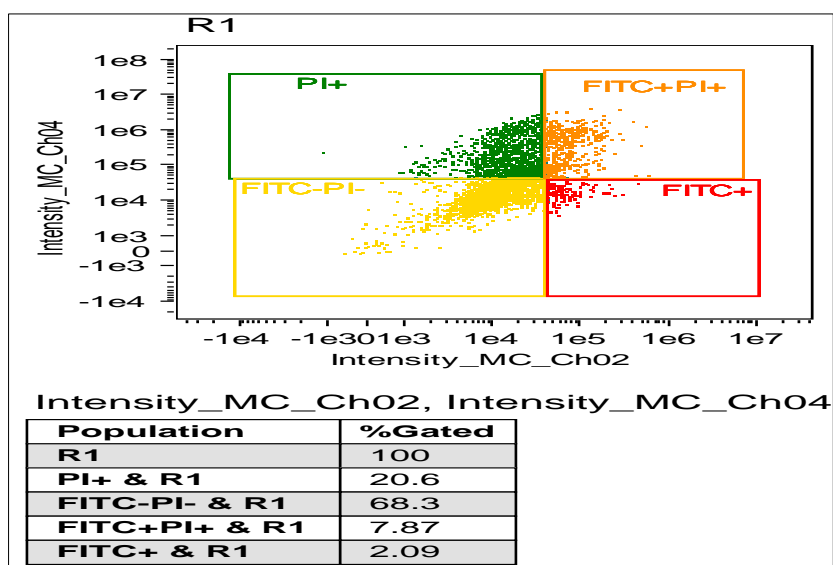
**PI+R1** (propidium iodide) is used to detect the necrotic cells population were **36.9%**

**FITC-PI** and **R1** is live cells population without apoptosis and necrosis were **47.1%**

**FITC+PI+R1** are population of both apoptosis and necrosis were **13.5%**

**FITC+R1** are population of apoptotic cells were **0.42%**

**6.11 (c). Result with *Bryophyllum pinnatum* at 200µg/ml concentration:**



**Fig.20.3.** Apoptosis analysis of HeLa cell line with *Bryophyllum pinnatum* lectin (BL) at concentration 200µg/ml at 48 hrs.

In figure 23.3,

**R1** is a single-cell population without debris where 100%

**PI+R1** (propidium iodide) is used to detect the necrotic cells population were **20.6%**

**FITC-PI-** and **R1** are live cells population without apoptosis and necrosis were **68.3%**

**FITC+PI+R1** are a population of both apoptosis and necrosis were **7.87%**

**FITC+R1** are a population of apoptotic cells were **2.09%**

**INFERENCE**

The apoptotic potential of both plant lectins (AL and BL) was quantified using Annexin V-FITC (Fluorescein isothiocyanate)/ Propidium iodide staining and also differentiates viable, apoptotic, and necrotic cells. There has been a time-dependent increase in Annexin V-FITC positive cells at 48hrs. for both plant lectins, indicating that these two plant lectins (AL and BL) induced apoptosis in HeLa cells. The diagrams depict the percentage of apoptotic cells after AL and BL exposure over a while.

The vinblastine-treated HeLa cancer cells at 0.81µg/ml concentration for 48hrs have shown the total population of HeLa cells with necrosis was 14.7% and the total population of HeLa cells with apoptosis was 6.47. The total population of HeLa cancer cells was treated with *Aloe barbadensis* lectin (AL) at 200µg/ml concentration with necrosis was 36.9% for 48hrs. *Bryophyllum pinnatum* lectin (BL) a treated HeLa cancer cell at 200µg/ml concentration for 48hrs with necrosis was 20.6% and the total population of HeLa cancer cells with apoptosis was 2.09%. From the above observation, it has been concluded that *Bryophyllum pinnatum* lectin (BL) has shown more apoptosis and necrosis than *Aloe barbadensis* lectin (AL) on the HeLa cancer cell line.

*CHAPTER-7*  
*DISCUSSION*



## 7. DISCUSSION

We will outline lectins as ‘glycoprotein binds to particular monosaccharides and oligosaccharides reversibly’. Lectins do not modify the structure of the ligand. Lectin has a fundamental nature to bind with antibodies without any stimulation in the immune system (Peumans and Van Damme, 1995). The lymphocyte mitogenesis results were shown through lectins *in vitro* with stimulation and inhibition of lymphocytes in the GI tract. Lectins proteins are immune to both temperature and digestion due to the fact proteolytic enzymes and HCl cannot lyse the lectin proteins. Lectins have biochemical and binding properties by which, carbohydrate affinity chromatography, is very handy for its purification. Carbohydrate binding lectins is a non-covalent and reversible process related to the interactions of hydrogen bonds, electrostatic, hydrophobic, van der Waals, and dipole enchantment.

*Aloe barbadensis* plant has many medicinal, homeopathic, and allopathic uses. The anticarcinogenic of *Aloe barbadensis* has not been evolved thoroughly yet. *Aloe barbadensis* juice allows recovering the cells or tissues which have broken with the aid of radiotherapy and chemotherapy. Distinct research indicated that *Aloe barbadensis* gel has an anti-cancer effect. The gel extract suppresses tumor weight, shrinkage of tumor, necrosis, and reduces the survival fee of tumor (Hamman, 2008).

*Aloe barbadensis* leaves had lectin-like properties and to decide if those substances markedly promoted restoration, more positive attachment, and inspired boom of human cells *in vitro*. Our goal is to separately isolate and examine the properties of *Aloe barbadensis* leaf gel lectins. The purification and a few *Aloe barbadensis* lectins residences are defined in this research.

*Bryophyllum pinnatum* has anticancerous properties (Jaki *et al.*, 1999). There are 5 bufadienolides remote from the leaves of *Bryophyllum pinnatum*. The bufadienolides inhibit the activation of EBV-EA (Epstein-Barr virus early antigen) in Raji cells prompted through 12-Otetradecanoylphorbol-13-acetate i.e. a tumor promoter. All bufadienolides have inhibitory activity. It has been confirmed that the MTT test on metastatic HT-1080 (human fibrosarcoma cell line) the methanolic concentrate have antiproliferative action (Jun *et al.*, 2002).

*Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves juice utilized in these researches changed into weighed, minced, homogenized and purified through the

dialysis membrane. The 50% ammonium sulfate is used in the precipitation of lectin from the dialyzed extract. At 4°C with the gradual addition of 50% ammonium sulfate in dialyzed extracts, the lectin receives triggered. After 100% saturation of ammonium sulfate precipitates acquired had been centrifuged for 20 minutes at 10,000 rpm. Saline used to dissolve the precipitates. To free the solution from ammonium sulfate fraction once more dialyzed (Patil and Despande, 2015). As defined for the smooth *Aloe* leaf gel extract and *Bryophyllum* leaves extract. The purified *Aloe* lectin (AL) and *Bryophyllum* lectin (BL) were then focused and refrigerated at 4°C. The concentrations of protein of crude extracts were measured by way of Lowry's method and use of BSA (bovine serum albumin) as the same sample protein. The protein concentration for *Aloe barbadensis* was 4.28mg/ml and for *Bryophyllum pinnatum* was 5.09mg/ml. Lectin can agglutinate the RBCs.

For haemagglutination assay blood group A+ve, B+ve, and O+ve of healthy human donors had been accrued in heparinized tubes and kept at 4°C for 20 mins. 2% erythrocytes were prepared. Washed RBCs had been used as a 2% erythrocytes solution (Olsen, 1944). *Aloe* lectin and *Bryophyllum* lectin could agglutinate these human erythrocytes. Haemagglutination unit/ml and specific activity (SA/mg) of *Aloe barbadensis* lectin for A+, B+, and O+ were 34.24, 68.48, and 136.96 HAU/ml and 8, 16, and 32 SA/mg respectively. For *Bryophyllum pinnatum*, Haemagglutination unit/ml and specific activity (SA/mg) of A+, B+ and O+ were 81.44, 162.88, and 325.76 HAU/ml and 16, 32, and 64 SA/mg respectively. Lectin present in both plant extracts react with human blood and agglutinate it. The above results confirmed the presence of lectin in the *Aloe barbadensis* gel and the *Bryophyllum Pinnatum* leaves extract.

Sugar specificity or the binding ability of *Aloe barbadensis* lectin at 500Mm concentration had shown by N-acetyl-D-galactosamine, D-galactose, and D-Mannose and for *Bryophyllum pinnatum* lectin D-glucose, N-acetyl-D-galactosamine, D-galactose, and L-Fructose. The determination of pH stability of *Aloe* lectin and *Bryophyllum* lectin has done by using different buffer solutions ranging from 1pH - 13pH (Suseelan *et al.*, 1997). pH activity of each *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin at 0-4 pH was nil. At 5-7, pH lectins were displaying their maximum interest. At pH 7 lectins were neutral. At 8-10, there's a downfall and

at 12-13 again the activity of lectin was very less. The purity of those proteins becomes confirmed through SDS-PAGE which has been shown by the characterization of AL (*Aloe barbadensis* lectin) protein of MW (molecular weight) 42kDa and 60kDa. BL (*Bryophyllum pinnatum* lectin) protein it's MW (molecular weight) 28kDa and 42kDa.

The anti-oxidant activity of AL (*Aloe barbadensis* Lectin) and BL (*Bryophyllum* lectin) were also studied spectrophotometrically by way of DPPH free radical scavenging assay at 517 nm wavelength and ABTS Radical Cation Scavenging Method at 745nm wavelength. Readings of both assays were analyzed with Graph pad prism software. AL had shown very little anti-oxidant activity than BL. Ascorbic acid is used as a standard because it is a strong antioxidant agent.

The evaluation of the free radical-scavenging activity of the DPPH radical (1,1-Diphenyl-2-picrylhydrazyl) (Ferreira *et al.*, 2009) and ABTS [2, 2'-azinobis-(three-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging methods have been additionally studied spectrophotometrically for antioxidant activity of *Aloe* Lectin from the gel and *Bryophyllum* Lectin from leaves extract (Sithisarn *et al.*, 2005). The Antioxidant activity of polysaccharides (Kardosova *et al.*, 2006 and Chun Hui *et al.*, 2007) turned into reported. Lectin is known to have a glycoprotein structure (Akev *et al.*, 1999). Efforts in the determination of whether the antioxidant activity of *Aloe barbadensis* suggested in the literature (Hu *et al.*, 2003), came from the glycoprotein, phenolic compounds, nutrients, polysaccharides. The antioxidant interest of the purified lectin turned into assessed by the DPPH radical scavenging approach. The scavenging effect of *Aloe barbadensis* lectin on DPPH increases as the concentration s of lectin i.e. 4mg/ml, 8mg/ml, 12mg/ml 16mg/ml and 20mg/ml increase as 8.47, 18.7, 30.6, 39.8 and 55.3 respectively. The antioxidant activity of *Aloe barbadensis* lectin is highest at 20mg/ml. The maximal inhibitory concentration i.e. IC<sub>50</sub> value of *Aloe barbadensis* lectin is 11.5 mg/ml. Antioxidant activity of *Bryophyllum pinnatum* lectin at different concentration i.e. 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20mg/ml as 12.8, 24.0, 40.6, 55.7 and 70.1 respectively. The readings have been taken in triplicates. The maximum antioxidant activity of *Bryophyllum pinnatum* lectin has been observed at 20mg/ml. The maximal inhibitory concentration i.e. IC<sub>50</sub> value of

*Bryophyllum pinnatum* lectin was 14.5 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent.

ABTS Radical Cation-Scavenging Assay has been performed to study the antioxidant activity of *Aloe barbadensis* lectin. The different concentrations of *Aloe barbadensis* lectin are 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20 mg/ml. The scavenging effect of lectin on ABTS increases as the concentrations of lectin increase as 5.7, 15.8, 29.6, 44.5 and 60.4 respectively. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 0.96mg/ml.

The antioxidant activity of *Bryophyllum pinnatum* lectin has been studied at different concentrations with ABTS Radical Cation-Scavenging Assay. The different concentrations of *Bryophyllum pinnatum* lectin 4mg/ml, 8mg/ml, 12mg/ml, 16mg/ml and 20 mg/ml were taken. The antioxidant activities of *Bryophyllum pinnatum* lectin at above concentrations are 8.5, 19.5, 36.9, 55.8 and 77.7. The scavenging effect of lectin on ABTS increases as the concentrations of lectin are increasing. The scavenging effect of *Bryophyllum pinnatum* lectin on ABTS is maximum at 20mg/ml. The maximal inhibitory concentration i.e. IC50 value of *Bryophyllum pinnatum* lectin was 14.6 mg/ml. Ascorbic acid is used as a standard because it is a strong antioxidant agent. The maximal inhibitory concentration i.e. IC50 value of ascorbic acid was 0.96mg/ml.

The capacity of the purified lectin i.e. on these observations *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin have been changed to indicate fragmentation of DNA in HeLa by using PI staining, which may also illustrate possible cell cycles, apoptosis, and necrosis. Anticancer properties of *Aloe barbadensis* lectin (AL) and *Bryophyllum pinnatum* lectin (BL) by SRB assay resulted that the impact of both plants lectins at 200µg/ml attention on HeLa (cervical cancer cell line) is extra effective than SW-480 (colon cancer cell line) after 48hrs. Further researches were carried out with the HeLa cancer cell line accompanied by using cell cycle and apoptosis analysis by flow cytometry. PI staining was used to analyze the cell cycle. PI (Propidium Iodide) is a well-known nuclear and chromosome counter-stain crimson-fluorescent. Given that propidium iodide is not always permeable to live cells, it is also commonly used in a population to find lifeless cells. PI binds to DNA

by intercalating with very little preference for collection between bases. In cell cycle analysis of HeLa cancer cell line dealt Vinblastine at concentration 0.81 $\mu$ g/ml displaying one of a kind phases. In different phases of the cell cycle out of 100% of the cell population 28.3% cell division had stopped. In the case of *Aloe barbadensis* lectin (AL) at 200 $\mu$ g/ml concentration, out of 100% cell population, 14.1% cell division had stopped. For *Bryophyllum pinnatum* lectin (BL) it was 18% out of 100% cell population.

Further research on cell death initiating pathway (apoptotic) of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin were essential to clarify some fundamental mechanisms of a lectin-expedited cell passing in disease cells as an approach to give more prominent facilities to pick up information on the possibility of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin in therapeutics for explicit most malignant growths types in the close fate.

*CHAPTER-8*  
*CONCLUSION*

## 8. CONCLUSION

The *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin are explicit for D-galactose, D-mannose, and D-galactosamine buildups at non-diminishing terminal places of glycoconjugates, was effectively confined from *Aloe barbadensis* gel and *Bryophyllum pinnatum* leaves squeeze through dialysis strategy with half of the ammonium sulfate.

In the current research, cervical cancer cell line; HeLa, and colon cancer cell line; SW-480 were selected to study the cytotoxic effects of purified crude lectin on anti-cancers because both cervical cancer (in female) and colon cancer in most cancer-associated deaths in India are the second most important cause. It has been shown that the purified plants lectins *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin show marked cytotoxicity towards the proliferation of most cellular strains viz human cancers. HeLa and SW-480 using SRB assay as indicated.

The homogenized sample of *Aloe barbadensis* lectin and *Bryophyllum* lectin were filtered with 3MM Whatman chromatography paper. The dialysis of crude leaves extract of *Bryophyllum pinnatum* and *Aloe barbadensis* gel had been used to separate proteins, eliminate small molecules and some impurities by way of a dialysis tube (membrane). The 50% ammonium sulfate is utilized in the precipitation of lectin from the dialyzed extract. The determination of protein concentration was performed by Lowry's method. The protein concentration for *Aloe barbadensis* was 4.28mg/ml and for *Bryophyllum pinnatum* was 5.09mg/ml. Lectin can agglutinate the RBCs.

Haemagglutination assay has vital importance to characterize protein as lectin. Even without a microscope, Haemagglutination is visible and the presence of lectin was detected. The amount of lectin present in suspensions has been quantified by haemagglutination assay. From haemagglutination assay, the HAU (Haemagglutination unit) and SA (specific activity) of A, B, O blood groups for *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin were calculated. Haemagglutination unit/ml and specific activity (SA/mg) of *Aloe barbadensis* lectin for A+, B+, and O+ were 34.24, 68.48, and 136.96 HAU/ml and 8, 16, and 32 SA/mg respectively. For *Bryophyllum pinnatum*, Haemagglutination unit/ml and specific activity (SA/mg) of A+, B+ and O+ were 81.44, 162.88, and 325.76 HAU/ml and 16, 32, and 64 SA/mg respectively. Sugar specificity or the binding ability of *Aloe*

*barbadensis* lectin at 500Mm concentration had shown by N-acetyl-D-galactosamine, D-galactose, and D-Mannose and for *Bryophyllum pinnatum* lectin D-glucose, N-acetyl-D-galactosamine, D-galactose, and L-Fructose. pH activity of each *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin at 0-4 pH was nil. At 5-7, pH lectins were displaying their maximum interest. At pH 7 lectins were neutral. At 8-10, there's a downfall and at 12-13 again the activity of lectin was very less. The purity of those proteins becomes confirmed through SDS-PAGE which has been shown by the characterization of AL (*Aloe barbadensis* lectin) protein of MW (molecular weight) 42kDa and 60kDa. BL (*Bryophyllum pinnatum* lectin) protein it's MW (molecular weight) 28kDa and 42kDa.

The anti-oxidant activity of AL (*Aloe barbadensis* Lectin) and BL (*Bryophyllum* lectin) were also studied spectrophotometrically by way of DPPH free radical scavenging assay at 517 nm wavelength and ABTS Radical Cation Scavenging Method at 745nm wavelength. Readings of both assays were analyzed with Graph pad prism software. AL had shown very little anti-oxidant activity than BL.

The anticancer activities of *Aloe barbadensis* lectin (AL) and *Bryophyllum pinnatum* lectin (BL) had elucidated by SRB assay, cell cycle analysis, and apoptosis assay. Both the Cancer cell lines HeLa and SW-480 were obtained from ATCC (American Type Culture Collection) and maintained in DMEM in a CO<sub>2</sub> incubator. Different lectin concentrations for HeLa and SW-480 cancer cell lines were used in the SRB assay to study the maximum effect. Vinblastine was used as a positive control. SRB assay has been performed for cytotoxicity assessment of *Aloe barbadensis* lectin (AL) extract and *Bryophyllum pinnatum* lectin (BL) extract compounds. The readings had been taken at 540nm. We analyzed the data with the help of Graph pad prism software. The *Aloe barbadensis* lectin has shown its maximum effectiveness on HeLa than the SW480 cancer cell line. *Bryophyllum pinnatum* Lectin has shown its maximum effectiveness on HeLa than SW480. In the SRB assay, 200µg/ml concentration of *Aloe barbadensis* lectin (AL) has shown its maximum anticancer activity after 48 hrs incubation. the maximum anticancer activity of *Bryophyllum pinnatum* lectin (BL) on the HeLa cell line was observed at 200µg/ml concentration after 48hrs incubation. Anticancer activity of *Aloe barbadensis* lectin and *Bryophyllum pinnatum* lectin by SRB assay resulted that the



effect of both plants lectins at 200 $\mu$ g/ml concentration on HeLa (cervical cell line) is more effective than SW-480 (colon cancer cell line) after 48 hrs. The further studies are in continuity with the HeLa cell line followed by the analysis of cell cycle and apoptosis.

FITC (Annexin V Fluorescein isothiocyanate) took a look at apoptosis through morphological modifications in nuclei of *Aloe barbadensis* lectin (AL) and *Bryophyllum pinnatum* lectin handled HeLa cells. Annexin V Fluorescein isothiocyanate is a Ca<sup>2+</sup> structured phospholipid-binding protein. The size of Annexin V binding when finished concurrently with a PI contained dye exclusion test may be used to effectively discriminate among apoptosis and necrosis. BL (*Bryophyllum pinnatum* lectin) had shown more apoptosis than AL (*Aloe barbadensis* lectin).

*CHAPTER-9*  
*BIBLIOGRAPHY*

## 9. BIBLIOGRAPHY

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## ANTIOXIDANT ACTIVITY OF LECTIN ISOLATED FROM LEAVES OF *BRYOPHYLLUM PINNATUM*

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### ABSTRACT

The *Bryophyllum pinnatum* refers to the family Crassulaceae. The glycoprotein from the 0.9% of normal saline extracts of *Bryophyllum pinnatum* leaves was refined by dialysis with 50% of ammonium sulfate. Protein concentration was invented by Lowry's method. The dialyzed sample allowed for SDS-PAGE to determine the molecular weight (M). The antioxidant activity of crude lectin extract has been assessed by the DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and ABTS methods. The present study aims to verify the antioxidant activity of lectins detached from the leaves of *Bryophyllum pinnatum*.

**Keywords:** Antioxidant activity; ABTS; *Bryophyllum pinnatum*; DPPH; Lectin; SDS-PAGE.

### INTRODUCTION

*Bryophyllum pinnatum* belongs to the family Crassulaceae. Its common names as Ghayamari, air plant, love plant, life plant, Zakhm-e-hyat, panfutti (Jain *et al.*, 2010). It grows worldwide and is used as folk medicine in many countries like Australia, tropical Africa, India, tropical America, and China. The plants have many therapeutic attributes (Kamboj *et al.*, 2009). The chemical compounds isolated from *Bryophyllum pinnatum* are used as folk medicines against many ailments such as hypertension and kidney stones, (Lans, 2006) pulmonary infections, rheumatoid arthritis, etc (Quaziet *al.*, 2011). *Bryophyllum pinnatum* leaves are used as an antifungal (Sofowora, 1993) and anti-allergic agent (Pal *et al.*, 1991). The oxidative activity was detected by DPPH and Nitric oxide free radical scavenging method. The leaf extract of *Bryophyllum pinnatum* has vital potential to scavenge the free radicals (Halliwell *et al.*, 1997).

The isolation of lectins from leaves of *Bryophyllum* and its antioxidant activity is an important step to study the nature, structural and biological properties of lectins. The study on isolation and partial characterization of a lectin with its antioxidant activity from the leaves of *Bryophyllum pinnatum* is presented here.

### MATERIAL AND METHODS

#### Plant material

Leaves of *Bryophyllum pinnatum* were collected from the nursery at Jalandhar, Punjab, India. The plant has been validated by Dr. Sanjay Kumar Uniyal, Principal

Scientist, CSIR-IHBT, Palampur, Himachal Pradesh. The specimen no. PLP15367.

#### Preparation of leaves extract

10ml extract of *Bryophyllum pinnatum* leaves was homogenized in 100ml of ice-chilled 0.9% of normal saline solution. The homogenized extracts were allowed to settle for 30 minutes at 4°C after which the supernatant was extracted and filtered using 3MM Whatman chromatography paper. The filtrates were centrifuged at 10,000 rpm, for 10 minutes at 4°C the obtained supernatants were stored at 4°C (Patil *et al.*, 2015).

#### Purification and isolation of lectin from *Bryophyllum pinnatum* leaves extract

The 50% ammonium sulfate is utilized in the precipitation of lectin from the dialyzed extract. The gradual addition of 50% ammonium sulfate in dialyzed extracts at 4°C causes the precipitation of lectins. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged at 10,000 rpm for 20 minutes. Precipitates were dissolved in normal saline to free the solution from ammonium sulfate fraction again dialyzed (Patil *et al.*, 2015).

#### Determination of Protein concentration by Lowry method

The protein concentrations of crude extract were measured by Lowry method using BSA (bovine serum albumin) as the standard protein (Lowry *et al.*, 1951).

#### Sodium dodecyl sulfate-Polyacrylamide gel Electrophoresis



**Fig. 1:** *Bryophyllum pinnatum*

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 12% separating gel (Tris-HCl 1.5M, pH 8.8, SDS 10%, APS 10%, TEMED 10 µl) for 1.5min. Stacking gel with pH 6.8. Electrophoresis buffer (Tris HCl 25mM, Glycine 192mM, SDS 0.1% at pH 8.3). The lectin sample was prepared by heating a protein solution in a sample buffer (2% SDS, 10% glycerol, 0.02% Bromophenol, 5% 2-mercaptoethanol) at 100° C for 5 min (Laemmli et al., 1973).

**Antioxidant Activity of Lectin**

**DPPH Free Radical-Scavenging Activity**

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) can be reduced by antioxidants because it is a free radical. The different concentrations of crude lectin 4, 8, 12, 16, and 20mg/ml were used for antioxidant activities (Hatano et al., 1988). The radical-scavenging activity (RSA) was calculated as the percentage of DPPH. Methanol and different concentrations of plant extract was used as a blank, while DPPH solution and methanol was used as a control. IC50 values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

$$\text{DPPH Free Radical-Scavenging Activity \%} = \left[ \frac{\text{Abs}_{(\text{Cont})} - \text{Abs}_{(\text{Sample})}}{\text{Abs}_{(\text{Cont})}} \right] \times 100$$

DPPH has taken as control.

**ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method Principle**

The oxidation of ABTS generated the pre-formed radical monocation ABTS with a blue chromogen potassium persulfate and the reduction of ABTS occurred by hydrogen donating antioxidants.

**Procedure**

Different substances have ability to scavenge ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) in comparison with a standard i.e. ascorbic acid. To prepare a radical cation 36mg of ABTS is dissolved in 100ml of distilled water i.e. preparation of a stock solution of 7mM ABTS mixed with 57mg of sodium persulfate in 100ml of distilled water i.e. 2.45mM potassium persulfate in an equal ratio of 1:1. Placed this mixture in dark at room temperature for 16hrs incubation until the reaction has been completed. The optical density (OD) was set at 0.7 of ABTS solution. 50 % methanol used for dilution. Lectin extracts of both plants with different concentrations were added

in every 1ml of ABTS solution. These mixtures were incubated for 30 minutes. After incubation the absorbance were taken at 745nm of samples of different concentrations(Sithisarn et al., 2005).

$$\text{ABTS Radical Cation Scavenging Activity \%} = \left[ \frac{\text{Abs}_{(\text{Cont})} - \text{Abs}_{(\text{Sample})}}{\text{Abs}_{(\text{Cont})}} \right] \times 100$$

ABTS has taken as control.

**Statistical Analysis**

The statistical analyses of triplicate readings or data were expressed as mean ±SD. The data analyzed by linear regression with 95% confidence band and IC50 value of DPPH and ascorbic acid was calculated using software Graph Pad Prism 7.04.

**Table 1:** DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* Lectin extract

Conc. of sample (mg/ml)	O.D. of DPPH (Control)	% of Antioxidant activity
4	0.35	12.8
8	0.35	24.0
12	0.35	40.6
16	0.35	55.7
20	0.35	70.1

**Table 2:** ABTS Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin extract

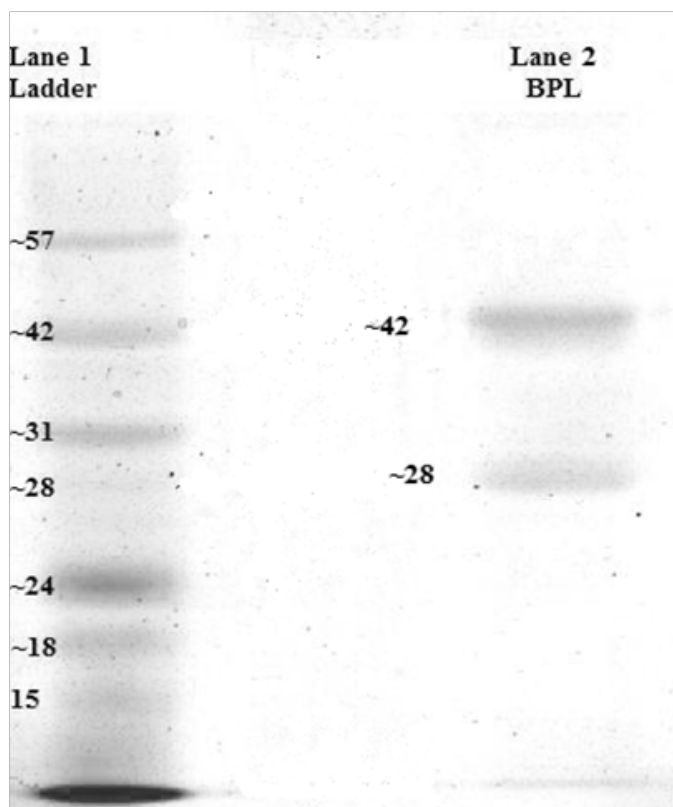
Conc. of sample (mg/ml)	O.D. of ABTS (Control)	% of Antioxidant activity
4	0.7	8.5
8	0.7	19.3
12	0.7	36.9
16	0.7	55.8
20	0.7	74.7

## RESULTS AND DISCUSSION

### Determination of Protein concentration by Lowry's Method

The total protein content in the dialyzed extract of (*Bryophyllum* Lectin) BL was found to be 5.09mg/ml.

### Electrophoresis (SDS-PAGE)



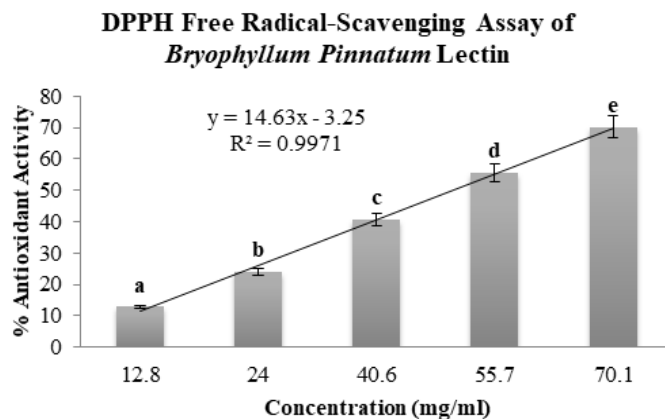
**Fig. 2:** Band pattern of *Bryophyllum* lectin on SDS-PAGE

SDS-PAGE resulted into the appearance of band with molecular weight of 28kDa and 42kDa in the purified lectin of *Bryophyllum pinnatum*.

In this study, the different concentrations of crude lectin 4, 8, 12, 16, and 20 mg/ml were taken. The scavenging effect of crude lectin on DPPH was significantly increased with the increase in the concentration of crude lectin. The antioxidant activity of *Bryophyllum* lectin with DPPH assay has shown in Table.1 and Graph. 1. The IC<sub>50</sub> value of crude lectin is **14.3 mg/ml**. Ascorbic acid is used as standard at a concentration of 4, 8, 12, 16, 20 mg/ml. IC<sub>50</sub> value of Ascorbic acid show its 50% activity at very low concentration i.e. **11.5mg/ml**.

### ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin extract

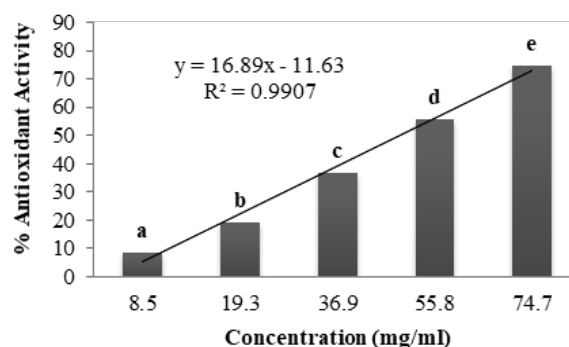
The different concentrations of crude lectin 4, 8, 12, 16 and 20 mg/ml were taken. The scavenging effect of crude lectin on ABTS was increased as the concentration of



**Graph 1:** DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* crude lectin extract. Antioxidant activity of *Bryophyllum* lectin (BL) at different concentration (mean  $\pm$  SD, n=3) with One way analysis of variance (ANOVA) P- value < 0.05 by Tukey's test so it is statistically significant.

The antioxidant activity of *Bryophyllum* lectin with ABTS assay has shown in Table .2 and Graph. 2. The IC<sub>50</sub> value of crude lectin is 14.6mg/ml. Ascorbic acid is used as standard at concentrations of 4, 8, 12, 16 and 20 mg/ml. IC<sub>50</sub> value of Ascorbic acid show its 50% activity at very low concentration i.e. 9.6mg/ml.

### ABTS Radical Cation Scavenging Assay *Bryophyllum Pinnatum* Lectin



**Graph 2:** ABTS Radical Cation Scavenging Assay of *Bryophyllum pinnatum* lectin extract. Antioxidant activity of *Bryophyllum* lectin at different concentration (mean  $\pm$  SD, n=3) with One way analysis of variance (ANOVA) P- value < 0.05 by Tukey's test so it is statistically significant.

## CONCLUSION

Antioxidant can neutralize and remove the free radicals from blood stream. The scavenging of free radicals occur by two mechanism i.e. reduction of ROS (reactive oxygen species) or by antioxidant defense mechanism (Faruq *et al.*, 2017). The lectin extract of *Bryophyllum pinnatum* has antioxidant activity (Saha *et al.*, 2014). The IC<sub>50</sub> values of DPPH assay of Ascorbic acid i.e. 11.5mg/ml, *Bryophyllum pinnatum* i.e. 14.5mg/ml. Lectins has the anti-oxidant property. However, further studies are

required to analyze the therapeutic effects of lectins in cancer related studies. There is a great deal of expectation for this neglected region of translational exploration to additional utilization in cancer-related ailments.

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# ***In vitro* anticancer activity of *Bryophyllum pinnatum* Lectin extract and its effects on Cell Cycle Progression and Apoptosis in HeLa cell line**

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## ABSTRACT

Lectins are the glycoprotein that has the ability to agglutinate all types of human blood. The glycoprotein from the 0.9% of normal saline extracts of *Bryophyllum pinnatum* leaves was purified by dialysis with 50% of ammonium sulfate. At 4°C with the gradual addition of 50% ammonium sulfate in dialyzed extracts, the lectin gets precipitated. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged at 10,000 rpm for 20 minutes. Precipitates were dissolved in normal saline to free the solution from ammonium sulfate fraction again dialyzed. The dialyzed sample allowed for SDS-PAGE to determine the molecular weight (M<sub>r</sub>). Protein concentration was determined by Lowry's method. The haemagglutination assay was done to confirm the presence of lectin in the crude extract. Sugar specificity assay was carried out to determine the effect of different carbohydrates on haemagglutination and thus find specific carbohydrates for *Bryophyllum* lectin (BL). The glycoprotein has shown its maximum activity over the 4-7 pH range. The anticancer activity was assessed by SRB assay after 48 hours. The Cell cycle analysis and Apoptosis has been done with Propidium iodide and Annexin V FITC (Fluorescence isothiocyanate).

**Keywords:** Anticancer activity, Apoptosis, *Bryophyllum pinnatum*, Cell cycle analysis, Haemagglutination assay, Lectin, SDS-PAGE, SRB Assay.

## INTRODUCTION

*Bryophyllum pinnatum* belongs to the family Crassulaceae. Its common names are Ghayamari, air plant, love plant, life plant, Zakhm-e-hyat, panfutti [1]. It grows worldwide and is used as folk medicine in many countries like Australia, tropical Africa, India, tropical America, and China. It has a sour taste, astringent but during the post-digestive effect, it has a sweet taste. The plants have many medicinal properties. It has haemostatic and wound healing properties [2].

The chemical compounds isolated from *Bryophyllum pinnatum* are used as folk medicines on a large scale for the treatment of many diseases hypertension and kidney stones [3], pulmonary infections, arthritis, etc. [4]. *Bryophyllum pinnatum* leaves are used as antifungal [5] and antiallergic agents [6]. *Bryophyllum pinnatum* is an emollient, refrigerant, haemostatic, mucilaginous, depurative, anodyne, disinfectant, constipating and antitonic. The plant proved to be beneficial in vitiated conditions (pitta and vata), epilepsy, piles [7], haematemesis, and menorrhagia, healing of wounds, haemorrhoids, skin discolorations, boils, and ophthalmia [8]. An imbalanced homeostatic phenomenon between oxidants and antioxidants is caused by the physiological excess of free radicals in the body. This randomness causes oxidative stress that is being suggested as the principal cause of aging and arteriosclerosis, diabetes, cancer, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease in humans [9]. The oxidative activity is detected by DPPH and Nitric oxide free radical scavenging method. The leaf extract of *Bryophyllum pinnatum* has significant potential free radical scavenging activity for the treatment of many diseases [10].

The isolation of lectin extract from leaves of *Bryophyllum pinnatum* and its anticancer activity is an important step to study the structural and biological properties and also to investigate the nature of the lectins. The study on isolation and partial characterization of lectin extract with its anticancer activity from the leaves of *Bryophyllum pinnatum* with cell cycle analysis and apoptosis in HeLa human cancer cell line is presented here.

## MATERIALS AND METHODS

### **Plant material, Cancer cell culture, and Preparation of leaves extract**

*Bryophyllum pinnatum* plants procured from a local nursery in Jalandhar and have been grown in the green field of the Lovely Professional University, Punjab, India. The authentication of plants has been done by Dr. Sanjay Kumar Uniyal (Sr. Principal Scientist & Curator) CSIR-IHBT, Palampur, Himachal Pradesh. A<sup>+</sup>, B<sup>+</sup>, and O<sup>+</sup> blood groups were obtained from healthy donors under the guidance of a Medical Lab Technician.

Malignancy cell lines HeLa (Cervical cancer cell line) was purchased from ATCC (American Type Culture Collection) in CSIR-IHBT, Palampur. The cell lines were kept up in DMEM (Dulbecco's Modified Eagle Medium) enhanced with 10% Fetal Bovine Serum. 95% moistness is required to keep up Cancer cell lines in a CO<sub>2</sub> (5%) incubator and enabled the way of life to grow up to 80-90% confluency.

Leaves (100g) of *Bryophyllum pinnatum* were washed two-three times under tap water then with distilled water and soaked with clean tissue paper. Harvesting of leaves is manually performed, such as leaves of plants cut from the base by hand. Approximately 50ml of *Bryophyllum pinnatum* was collected in a beaker. 50ml of leaves juice of *Bryophyllum pinnatum* were homogenized in 100ml of ice-chilled 0.9% of normal saline solution. The homogenized extracts were allowed to settle for 30 minutes at 4°C after which the supernatant was extracted and filtered using 3MM Whatman chromatography paper. Both the filtrates were centrifuged at 10,000 rpm, for 10 minutes at 4°C. The obtained supernatants were stored at 4°C [11].

### **Purification of lectin extracts from *Bryophyllum pinnatum* leaves**

The crude leaves extract of *Bryophyllum pinnatum* dialyzed for separation of proteins and removal of impurities and small molecules by dialysis tube (membrane) in normal saline at 4°C. This dialysis tube contains micropores through which the micromolecules escaped. Therefore the dimension of protein molecules significantly greater than pore diameter is retained inside the dialysis tube. The 50% ammonium sulfate is used in the precipitation of lectin from the dialyzed extract. At 4°C with the gradual addition of 50% ammonium sulfate in dialyzed extracts, the lectin gets precipitated. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged at 10,000 rpm for 20 minutes. Precipitates were dissolved in normal saline to free the solution from ammonium sulfate fraction again dialyzed [11].

### **Determination of Protein concentration by Lowry's method**

The protein concentration of crude extract was determined by Lowry's method using BSA (bovine serum albumin) as the standard protein [12].

### **Preparation of 2% erythrocytes**

The human blood A, B, and O of healthy donors were collected in heparinized tubes and stored at 4°C for 20 minutes. For the preparation of 2% erythrocytes blood was centrifuged at 3000 rpm for 10 minutes thereafter RBCs were washed 2-3 times with normal saline until the supernatant was removed. The washed RBCs were used as 2% erythrocyte solution. These 2% erythrocytes were used for haemagglutination assay [13].

### **Agglutination Assay**

For the detection of lectin in the crude extract of *Bryophyllum pinnatum*, the extract was added to a series of wells by two-fold dilution so that each well with half of the concentration with normal saline than the previous. Each well maintained a volume of 50µl of lectins with the addition of 50µl of 2% erythrocyte suspension. At 37°C 96 wells plate was kept for 5 hours. The agglutination was observed visually [14]. Haemagglutination unit (HAU) is the strength of the titer calculated with the last agglutination dilution reciprocally. Specific activity (SA) is the calculation of the unit of haemagglutination per mg protein [14].

### **Sugar specificity test**

Plant lectin can bind a particular carbohydrate or sugar. Binding of lectins with carbohydrate results in the inhibition of agglutination. Agglutination inhibition testing was performed by testing different sugar capabilities. To inhibit the agglutination equal volume of sugars and lectin were used. For this, the mixture of 1ml of 500mM sugar solution with 500µl of lectin was incubated at room temperature for 30 minutes along with 500 µl of 2% of erythrocytes (1ml). The minimum agglutination of erythrocytes was taken [15].

### **Study of pH stability**

The pH stability of *Bryophyllum pinnatum* lectin was determined by using different buffers ranging from 1pH -13pH. 0.1N



HCl is used for pH 1, 0.2M glycine-HCl buffer is used for pH 2-3. For pH 4-5, 0.2 M sodium acetate buffer is used. 0.2 M sodium phosphate buffer is used for pH 6-7. For pH 8, 0.2 M Tris HCl buffer is used. 0.2 M glycine-NaOH buffer is used for pH 9. 0.2 M Carbonate-bicarbonate buffers were used for pH 10-13 [16]. 1ml of lectin was incubated with 1ml of different buffer solutions for 30min at room temperature and then 2% erythrocytes were used to test the agglutination [16].

### **Sodium dodecyl sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 12% separating gel (Tris-HCl 1.5M, pH 8.8, SDS 10%, APS 10%, TEMED 10 $\mu$ l) for 1.5mm. Stacking gel with pH 6.8. Electrophoresis buffer (Tris HCl 25mM, glycine 192mM, SDS 0.1% at pH 8.3). The lectin sample was prepared by heating a protein solution in a sample buffer (2% SDS, 10% glycerol, 0.02% bromophenol, 5% 2-mercaptoethanol) at 100 $^{\circ}$ C for 5 min [17].

### **Anticancer Activity of Lectin Cell Culture maintenance**

**HeLa (Cervical cancer cell line)** was kept in T-25 flasks in a CO<sub>2</sub> incubator to maintain their confluency up to 80% to 90% at 37 $^{\circ}$ C temperatures and 5.0% of CO<sub>2</sub> in Dulbecco's Modified Eagle Medium (DMEM). Cell lines took 24hrs to 48 hrs. to be fully confluent.

### **Sulforhodamine B assay**

SRB (Sulforhodamine B) Assay is an amino xanthene dye in bright pink colour. 5000-19000 cells/wells are plated in a 96-well flat-bottom plate. The adjustment for differences in several cells plated of cells plated different cell lines for differences in growth rate. Adhering of cells to wells overnight, then samples was added in 3-fold serial dilutions to wells in triplicate. Water is added in the medium at a 1:10 dilution to control wells. Incubate the plates at 37 $^{\circ}$  C temperature and 5% CO<sub>2</sub> for 36 hrs, then SRB assay has done for growth inhibition. Fixation of cells to a final concentration of 10% has been done with the addition of cold trichloroacetic acid (50%). Cells are washed with deionized water (5 times) after incubation of one hour at 4 $^{\circ}$ C. Thereafter 0.4% of Sulforhodamine B dissolved in 1% acetic acid is used for staining of cells for 15-30 min. To remove unbound stains again, wash 5 times with 1% acetic acid. Plates are left to dry at room temperature. 10mm tris base is used to solubilize bound dye and the analysis of plates has been done on a microplate reader (Molecular Devices) at 595nm [18].

$$\frac{\% \text{ growth Inhibition}}{\text{Control sample}} = \frac{\text{Control}}{\text{Control}} \times 100$$

### **Propidium Iodide staining for the study of cell cycle analysis, Annexin V FITC for apoptosis morphological changes in nuclei of Lectin treated cancer cell lines with Flow cytometry.**

PI (Propidium Iodide) is a popular red-fluorescent nuclear and chromosome counterstain. Since propidium iodide is not permeating to live cells, it is also commonly used to detect dead cells in a population [19].

### **Principle of Flow cytometry**

Flow cytometry is a technique for counting, examining, and sorting microscopic objects suspended in a fluid based on their optical properties. It simultaneously measures and then analyzes multiple physical characteristics of a single object (usually cell) as they flow in a fluid stream through a beam of light [20].

Annexin V FITC (Fluorescein isothiocyanate) is a Ca<sup>2+</sup>-dependent phospholipid-binding protein. This protein can bind to a variety of phospholipids, but it has the highest affinity for phosphatidylserine (PS). To use the Annexin V protein as a probe for apoptotic cells, the protein has been labeled with fluorescein isothiocyanate (FITC). In this form, the protein can be used directly for the quantification of apoptotic cells. The measurement of Annexin V binding when performed simultaneously with a dye exclusion test (such as propidium iodide) can be used to effectively discriminate between apoptotic and necrotic cells.

## **RESULTS AND DISCUSSION**

*Bryophyllum pinnatum* plants were collected from a local nursery of Jalandhar, Punjab for identification of agglutination activity. The crude lectin extract of *Bryophyllum pinnatum* leaves juice showed haemagglutination activity with 2% erythrocytes of different blood groups of humans. The crude extract of *Bryophyllum pinnatum* showed good agglutination activity against blood group A+ve, B+ve, and O+ve. Lectin has the ability to agglutinate human blood cells. This property of lectin is recognized by the physiological effect that depends on their specificity for a particular carbohydrate moiety on

the cell surface. The haemagglutination inhibition assay has performed to determine the sugar specificity or specific glycoprotein of the lectin. BL (*Bryophyllum* Lectin) has shown good agglutination activity against blood group A,B and O.

**Determination of Protein concentration by Lowry’s Method**

The total protein content in the dialyzed extract of BL (*Bryophyllum* Lectin) was found to be 5.09mg/ml.

**Agglutination assay**

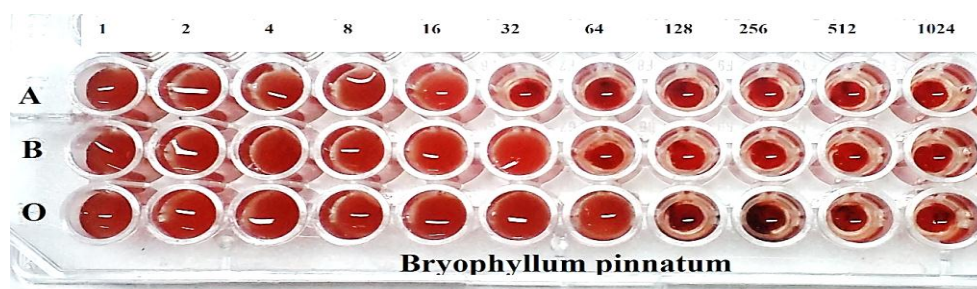
Haemagglutination results in Table 1. revealed that blood group A+ve and B+ve showed the least agglutination as compared to O+ve. Blood group O+ve showed strong agglutination with lectin extracted from *Bryophyllum pinnatum*. The carpet pattern is given by blood group A, B, and O is 4, 5 and 6-time dilution respectively as shown in figure 1. Further dilution showed no more precipitation of lectins.

**Table. 1. Agglutination assay of *Bryophyllum* lectins (BL) with human erythrocytes.**

S.No.	2% erythrocytes	Agglutination
1.	A +ve	+
2.	B +ve	++
3.	O +ve	+++

**Haemagglutination assay**

Haemagglutination assay results revealed that human blood groups O+ve showed stronger agglutination than B+ve and A+ve with *Bryophyllum* lectin (BL).



**Figure 1. Hemagglutination titer with blood group ABO.**

**Table 2. Protein concentration, HAU/ml, and the specific activity of *Bryophyllum* lectin.**  
 Here HAU/ml is haemagglutination unit

Blood group	HAU/ml	SA
A	81.44	16
B	162.88	32
O	325.76	64

**Agglutination inhibition assay/ Sugar specificity**

Agglutination inhibition assay was done with the help of different sugars or carbohydrates. Agglutination activity of *Bryophyllum* lectin was inhibited by D-Glucose, D-Fructose, D-Galactose, and N-acetyl-D-galactosamine as mentioned in table 3. The results indicated that inhibition of lectin was due to sugar specificity for D-Glucose, D-Fructose, D-Galactose and N-acetyl-D-galactosamine.

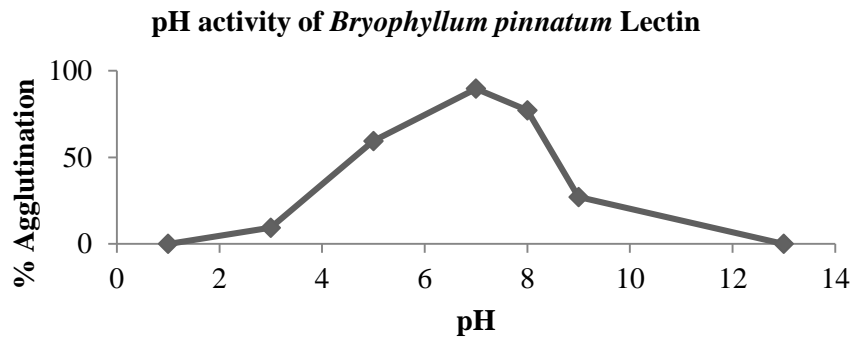


**Table 3. Agglutination inhibition assay was done with different sugars by *Bryophyllum* lectins.**

Sugars	Effect on agglutination
D-glucose	Inhibition at 500mM
Sucrose	Absence of inhibition
Lactose	Absence of inhibition
Fructose	Inhibition at 500mM
Mannose	Absence of inhibition
Galactose	Inhibition at 500mM
N-acetyl-D-galactosamine	Inhibition at 500mM

**Study of pH activity of Lectin**

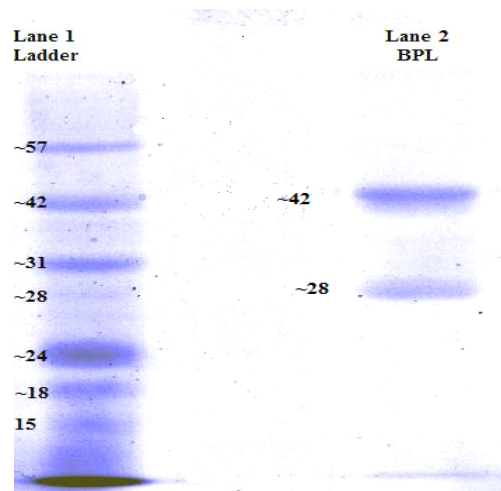
The pH stability of *Bryophyllum pinnatum* lectin was determined by using different buffers ranging from 1pH -13pH. According to the result, the activity of *Bryophyllum* lectin varies in pH ranging from 4 to 11. The optimum pH for maximum agglutination by lectin was found to be neutral (pH 7) as mentioned in figure2.



**Figure.2. pH activity of *Bryophyllum pinnatum* Lectin with erythrocytes**

**Electrophoresis (SDS-PAGE):**

SDS-PAGE resulted in the appearance of bands with a molecular weight of 28kDa and 42kDa in the purified lectin of *Bryophyllum pinnatum*.



**Figure.3. Band pattern of *Bryophyllum* lectin (BL) on SDS-PAGE showing the presence of lectin with 28kDa and 42kDa molecular weight. Here BPL is *Bryophyllum pinnatum* lectin.**

**Anticarcinogenic activity of Plant Lectins**

HeLa cell line has been cultured in a 48 hours timeline at 10µg/ml, 20µg/ml, 50µg/ml, 100 µg/ml and 200µg/ml concentrations. Vinblastine drug (an anti-cancer drug) was used as positive control. DMEM medium is used to maintain HeLa cancer cell line supplemented with 10% fetal bovine serum, Penicillin (10,000 units/100 ml) and Streptomycin (10 mg/100 ml) were used to prevent the culture from contamination and grown in a humidified 5% CO2 incubator at 37°C to achieve 80-90% confluency and harvested using trypsinization.

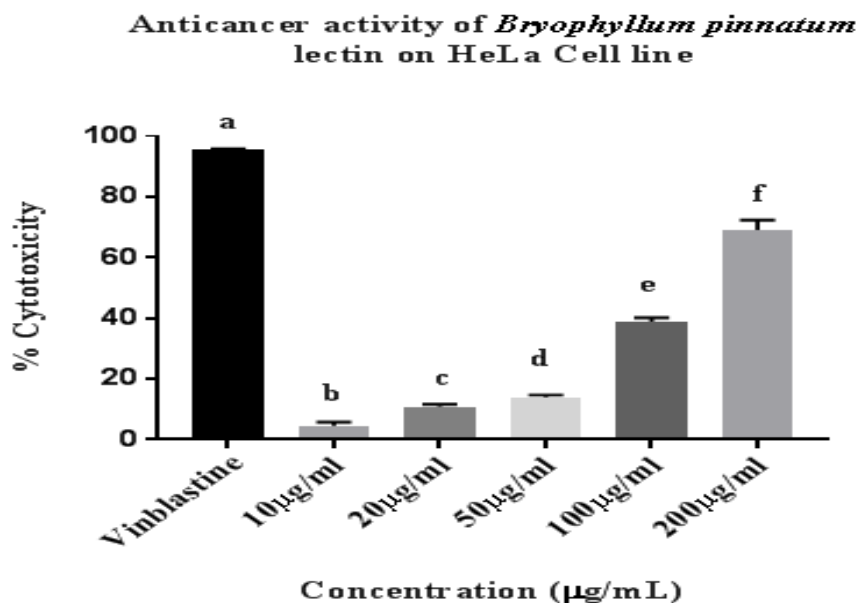
**CYTOTOXICITY ANALYSIS**

SRB assay has been performed for cytotoxicity evaluation of *Bryophyllum pinnatum* lectin extract. The reading has been taken at 540nm. We analyzed the data and represented it in graphical form as shown in figure 4.

In SRB assay of the HeLa cell line, Vinblastine is used as positive control. The anticancer activity of vinblastine at 1µg/ml concentration was **95.4%**. The anticancer activity of *Bryophyllum* lectin (BL) was studied at different concentrations on the HeLa cell line by SRB assay. At 10µg/ml of BL, the anticancer activity was **4.5%**, at 20µg/ml the activity was **10.7%**, at 50µg/ml the activity was **13.9%**, at 100µg/ml the activity was **38.8%**, and at 200µg/ml the activity was **69.1%**. Hence the maximum anticancer activity of *Bryophyllum* lectin (BL) on the HeLa cell line was observed at 200µg/ml concentration after 48hrs incubation. The IC50 value is found to be 13.18µg/ml.

**Table.4: Cytotoxic analysis of HeLa by Lectin extract of *Bryophyllum pinnatum* with SRB assay**

Test samples with Concentration	% of Cytotoxicity	IC50 Value (µg/ml)
Vinblastine 1 µM	95.4	13.18
BL 10µg/ml	4.52	
BL 20µg/ml	10.7	
BL 50µg/ml	13.9	
BL 100µg/ml	38.8	
BL 200µg/ml	69.1	



**Figure.4. Anticancer activity of *Bryophyllum* Lectin on HeLa cell line at different concentration**

**Flow cytometry**

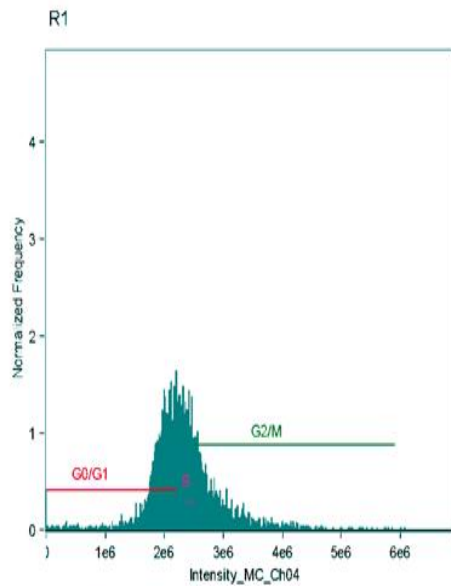
**Analysis of cell cycle against HeLa cell line at 48h**

**Result with vinblastine at concentration 1µm**

In figure.5, R1 is a population of a single cell without debris i.e. 100%, G0/G1 phase where cell cycle has stopped dividing and, cells are ready for DNA synthesis i.e. 44%. S phase: DNA replication occurs in this phase i.e. 13.4%. G2/M phase: In the G2 phase, cells are ready for mitosis and start dividing / In the M-phase cell growth has stopped and cells are ready to complete the cell division i.e. 28.3%.

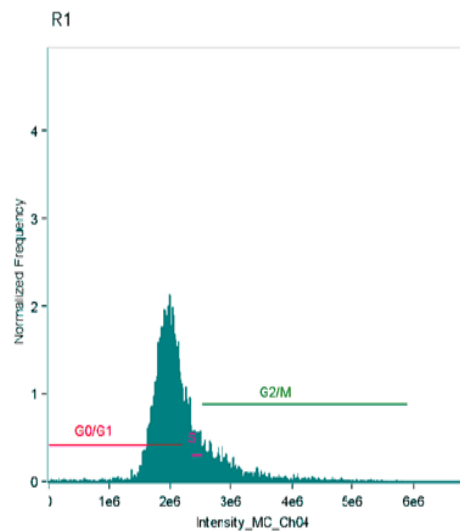
**Results with *Bryophyllum* lectin (BL) at 200µg/ml concentration**

The Cell population in the G0/G1 phase is 66.7%. In the S phase, the cell population is 6.42%. In the G2/M phase, the population of cells is 18%.



Population	%Gated
R1	100
G0/G1 & R1	44.4
S & R1	13.4
G2/M & R1	28.3

R1



Population	%Gated
R1	100
G0/G1 & R1	66.7
S & R1	6.42
G2/M & R1	18

**Figure.5. Analysis of Cell cycle of Vinblastine treated HeLa cell line at concentration 1µM**

**Figure.6. Analysis of Cell cycle of HeLa cell line treated with *Bryophyllum* lectin (BL) at 200µg/ml concentration.**

**Apoptosis assay against HeLa cells at 48Hrs**

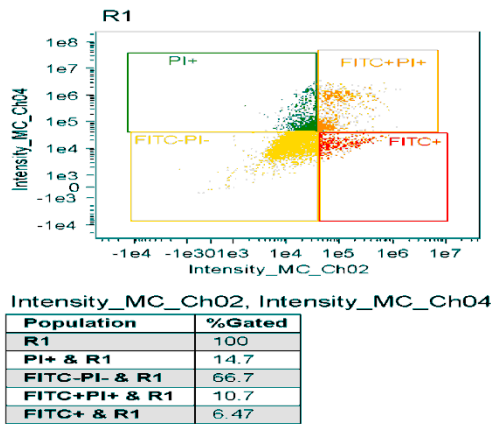
**Result with Vinblastine at 1µM concentration**

In Figure 7, R1 is a single cell population without debris, which is 100%. PI+R1 (propidium iodide) is used to detect the necrotic cell population was 14.7%. FITC-PI and R1 are live cells population without apoptosis and necrosis were 66.7%. FITC+PI+R1 are the population of both apoptosis and necrosis, which is 10.7%. FITC+R1 are a population of apoptotic cells that is 6.47%.

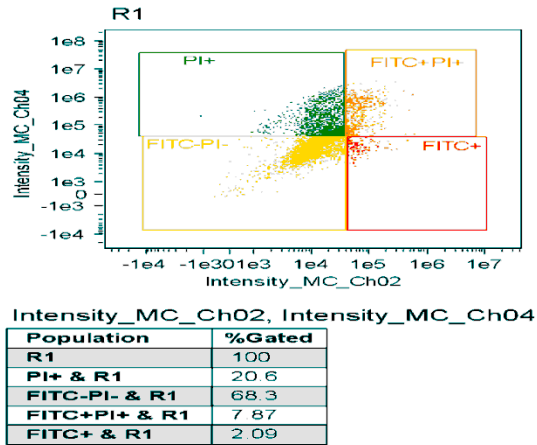
**Result with *Bryophyllum pinnatum* at 200µg/ml concentration:**

In Figure 8, R1 is a single cell population without debris, which is 100%. PI+R1 (propidium iodide) is used to detect the necrotic cell population was 20.6%. FITC-PI and R1 are live cells population without apoptosis and necrosis were 68.3%. FITC+PI+R1 are a population of both apoptosis and necrosis were 7.87%. FITC+R1 are the population of apoptotic cells

that was 2.09%.



**Figure.7. Apoptosis analysis of Vinblastine treated HeLa cell line at concentration 1µM**



**Figure.8. Apoptosis Analysis of HeLa cell line treated with Bryophyllum lectin (BL) at 200µg/ml concentration.**

### CONCLUSION

Lectin can agglutinate the RBCs. Haemagglutination assay has a vital importance to characterize protein as lectin. From haemagglutination assay, the HAU (Haemagglutination unit) and SA (specific activity) of A, B, O blood groups for *Bryophyllum* lectin were calculated.

Haemagglutination unit/ml and specific activity (SA/mg) of *Bryophyllum* lectin for A+, B+ and O+ were 81.44, 162.88 and 325.76 HAU/ml and 16, 32 and 64 SA/mg respectively. Sugar specificity or the binding ability of *Bryophyllum* lectin at 500Mm concentration has been shown by D-glucose, D-galactose, and Fructose. pH activity of *Bryophyllum* lectin at 0-4 pH was null. At 4-7 pH, lectins were showcasing their maximum interest. At pH 7 lectins were neutral. At pH 8-11, there was a downfall and at 12-14 again the activity of lectin was very less. The purity of those proteins becomes confirmed through SDS-PAGE which has been shown by the characterization of BL (*Bryophyllum* lectin) protein of MW (molecular weight) 28kDa and 42kDa. The anticancer activities of *Bryophyllum* lectin (BL) had elucidated by SRB assay using human cancer cell line HeLa. We analyzed the data with the help of Graph pad prism software. Anticancer properties of *Bryophyllum* lectin (BL) by SRB assay resulted in the impact of plant lectin at 200µg/ml concentration on HeLa (cervical cancer cell line) is effective after 48hrs. The cell-cycle analysis and apoptosis by flow cytometry with Propidium iodide and Annexin V FITC (Fluorescein isothiocyanate) have been performed with 200µg/ml of *Bryophyllum* lectin because at this concentration, the cytotoxicity of *Bryophyllum* lectin was high. From the above observation, it has been concluded that the *Bryophyllum* lectin (BL) has shown apoptosis on the HeLa cancer cell line.

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# IN VITRO ANTICANCER ACTIVITY OF LECTIN ISOLATED FROM GEL OF ALOE BARBADENSIS (MILLER) AGAINST HELA AND SW480 HUMAN CANCER CELL LINES

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**Abstract** - Lectin is a glycoprotein also called haemagglutinating protein which has ability to agglutinate all types of human blood. This paper presents the anticancer activity of lectin isolated from gel of *Aloe barbadensis* (Miller). The glycoprotein from the 0.9% of normal saline extracts of *Aloe barbadensis* leaves was purified by dialysis with 50% of ammonium sulfate. Protein concentration was determined by Lowry's method. The haemagglutination assay was done to confirm the presence of lectin in crude extract. Sugar specificity assay was carried out in order to determine the effect of different carbohydrate on haemagglutination and thus find specific carbohydrate for *Aloe* lectin. The dialyzed sample allowed for SDS-PAGE to determine the molecular weight ( $M_r$ ). The anticancer activity has assessed by SRB assay after 48hours.

**Keywords** - Anticancer Activity, *Aloe Barbadensis*, Haemagglutination Assay, Lectin, SRB Assay

## I. INTRODUCTION

*Aloe barbadensis* (Botanical name; *Aloe barbadensis*, family; Asphodelaceae, common name; *Aloe vera*, Ghirita, Gawarpaltra, Lu hui etc.) grow water storage tissue in the leaves. It is perennial succulent xerophytes. Among all 400 species of *Aloe barbadensis*, 85 are under cultivation throughout world. The most biologically active species is *Aloe barbadensis*, with 95% medicinal properties<sup>1,2</sup>. According to a report of WHO, traditional plants are the better source of variety of allopathic drugs<sup>3</sup>. *Aloe barbadensis* is belongs to East and South Africa. *Aloe barbadensis* was subsequently produced into North Africa then naturalized in the Mediterranean part and also in other countries. *Aloe barbadensis* is a best commercial plant and cultivated in the United State of America, South Africa, Venezuela, Japan, India, China, Bonaire<sup>4</sup>. The best quality *Aloe barbadensis* is found in desert region of South California.

In India major producers states of this crop are Maharashtra, Rajasthan, Gujarat, Andhra Pradesh, Tamil Nadu, and Kerala. The local or common names of *Aloe barbadensis* are Ghikanwar and Musambar in Hindi, Kumari in Sanskrit, Ghrithakumari in Bengali. *Aloe perfoliata*, *A. barbadensis*, *Aloe chinensis*, *Aloe littoralis*, *Aloe indica*, *Aloe abyssinica* Varieties of *Aloe barbadensis* cultivated in India. The *Aloe barbadensis* has been used as a medicinal plant form ancient time for its therapeutic and curative properties. In 1981, Winters presented a report that fractions prepared from *Aloe barbadensis* leaf gel at different Centrifugation contain a lectin that inhibits cell growth<sup>5</sup>. This *in vitro* study isolated and characterized fraction of glycoprotein promoting proliferation in hamster & human cells<sup>6</sup>.

## II. MATERIAL AND METHODS

*Aloe barbadensis* plants are grown commercially were purchased from local nursery at Jalandhar, Punjab, India. The authentication of plants has been done by Dr. Sanjay Kumar Uniyal (Sr. Principal Scientist & Curator) CSIR-IHBT, Palampur, Himachal Pradesh.

A<sup>+</sup>, B<sup>+</sup> and O<sup>+</sup> blood group were obtained from healthy donors under the guidance of Medical Lab Technician.

Malignancy cell lines Hela (Cervical cancer cell line) and SW480 (Colon cancer cell line) and were purchased from ATCC (American Type Culture Collection) in CSIR-IHBT, Palampur. The cell lines were kept up in DMEM (Dulbecco's Modified Eagle Medium) enhanced with 10% Fetal Bovine Serum. 95% moistness is required to keep up Cancer cell lines in a CO<sub>2</sub> (5%) incubator and enabled the way of life to grow up to 80-90% confluency.

## III. CHEMICALS AND CONSUMABLES

### Chemicals

Acetic acid, Acrylamide, Agarose, Ammonium persulphate (APS), Bisacrylamide, Bovine serum albumin (BSA), Bromophenol blue, Copper sulphate (CuSO<sub>4</sub>), D-Fructose, D-Galactose, D-Glucose, D-Lactose, D-Mannose, (Nice Chemicals Ltd.) DMEM (Sigma Aldrich), DMSO, Ethanol, Folin-ciocalteau phenol reagent, Fetal Bovine Serum (FBS), FITC, Glycerol, Glycine, HCl, N-acetylgalactosamine, N,N,N',N'' tetramethylenediamine (TEMED), Milli-Q water, Molecular weight marker (Bio-Rad, India), Penicilline, Propidium iodide, RNase-A, Sodium dodecyl sulphate (SDS) (Himedia, India), Streptomycin, Sulforhodamine B,

Triton-X 100, TCA, Trypsin solution (Sigma Aldrich).

#### **Consumables**

Beakers, conical flasks, Dialysis tube, EDTA tubes, T-25, T-75, T-150 culture flasks, 96-well plates, 6-well plates, 3 MM Whatman Chromatography filter paper, 1L flasks, Ependroff tubes, Test tubes (Tarson, India).

### **IV. METHODOLOGY**

#### **Preparation of crude extract**

Leaves (100g) of *Aloe barbadensis* were washed two-three times under tap water and then distilled water and clean with tissue paper. Remove the green skin part of leaf and collected gel of *Aloe barbadensis* which contain 98% of water. Approximately 50ml of gel was collected in a beaker.

50ml of gel was homogenized in 100ml of ice chilled 0.9% of normal saline solution. The homogenized extracts were allowed to settle at 4°C for 30 minutes after which the supernatants were extracted then filtered using 3MM Whatman chromatography paper. The filtrates were centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatants obtained were stored at 4°C<sup>7</sup>.

#### **Purification of crude extract by dialysis method.**

To separate the protein, small molecules and to remove the impurities of crude leaves extract of *Aloe barbadensis* gel were dialyzed by dialysis tube (membrane) in normal saline at 4°C. This dialysis tube contains micropores through which the micromolecules escaped. Therefore the dimension of protein molecules is significantly large than the diameter of pore retained inside the dialysis tube. The 50% of ammonium sulfate is used in precipitation of lectin from dialyzed extract. At 4°C with gradual addition of 50% ammonium sulfate in dialyzed extracts the lectin gets precipitated. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged for 20 minutes at 10,000 rpm. In normal saline, precipitates have been dissolved. To free the solution from ammonium sulfate fraction again dialyzed<sup>8</sup>. The dialyzed sample stored at 4°C for long use.

#### **Determining the concentration of protein using the Lowry's method<sup>9</sup>**

The Lowry method measured the protein concentrations of crude extracts by using a standard protein BSA (bovine serum albumin).

#### **Preparation of 2% erythrocytes**

The human blood of healthy donors A+ve, B+ve and O+ve collected in heparinized tubes and stored at 4°C. Erythrocytes were centrifuged for five minutes at 3000 rpm. Plasma and buffy layers were discarded. These RBC were resuspended in 0.9 % of NaCl

(normal saline) there after washing of RBCs for 2-3 times with normal saline until supernatant were removed<sup>10</sup>. The washed RBCs called as 2% erythrocyte. These 2% erythrocytes were used for haemagglutination assay.

#### **Agglutination Assay**

2% erythrocytes suspension is used for the agglutination test of purified extract<sup>11</sup>. 96 well plate is used to determine the haemagglutination activity by serial dilution method. The agglutination was observed visually after 5 hours. Haemagglutination unit is the strength of the titer was calculated with the last agglutination dilution reciprocally. Specific activity is the calculation of the unit of haemagglutination per mg protein.

#### **Sugar specificity/ Agglutination inhibition assay**

Plant lectin can bind a particular carbohydrate or sugar. Agglutination inhibition testing was performed by testing different sugar capabilities<sup>12</sup>. Here seven sugars were used for agglutination inhibition assay i.e. D-glucose, sucrose, lactose, fructose, mannose, galactose and N-acetylgalactosamine. The minimum agglutination of erythrocytes was taken.

#### **SDS-PAGE electrophoresis**

Method SDS-PAGE electrophoresis was done to determine the molecular weight of lectin of *Aloe barbadensis*. A 12% of separating gel and 5% of stacking gel was prepared and used in SDS-PAGE. Prefixing of resolved proteins has done by holding gels in TCA (12.5 percent) for 1h. An overnight staining was provided in 0.1% of Coomassie Brilliant Blue G-250. Lastly, the photographs of the appeared bands of protein separation were taken for permanent record<sup>13</sup>.

#### **Cell Culture maintenance**

**HeLa (cervical cancer cell line)** and **SW480 (colon cancer Cell line)** were kept in T-25 flasks in CO<sub>2</sub> incubator to maintain their confluency up to 80% to 90% at 37 °C temperatures and 5.0% of CO<sub>2</sub> in Dulbecco's Modified Eagle Medium (DMEM). Cell lines took 24hrs to 48 hrs. to be full confluent.

#### **Sulforhodamine B assay**

SRB (Sulforhodamine B) Assay<sup>14</sup> is aminoxanthene dye in bright pink colour. 5000-19000 cell/wall are plated in 96-well flat bottom plate. The adjustment for differences in number of cells plated different cell lines for differences in growth rate. Adhering of cells to wells overnight, then samples was added in 3-fold serial dilutions to wells in triplicate. Water is added in the medium at a 1:10 dilution to control wells. Incubated the plates at 37°C temperature and 5% CO<sub>2</sub> for 36 hrs, then SRB assay has done for growth inhibition. Fixation of cells to a final concentration of 10% has done with addition of cold trichloroacetic



acid (50%). Cells are washed with deionized water (5 times) after incubation of one hour at 4°C. Thereafter 0.4% of Sulforhodamine B dissolved in 1% acetic acid is used for staining of cells for 15-30 min. To remove unbound stain again washed 5 times with 1% acetic acid. Plates are left to dry at room temperature. 10mm tris base is used to solubilize bound dye and the analysis of plates has done on microplate reader (Molecular Devices) at 595nm.

$$\% \text{ growth inhibition} = \frac{\text{Control sample}}{\text{Control}} \times 100$$

## V. RESULTS

*Aloe barbadensis* plants were collected from a local nursery of Jalandhar, Punjab for identification of agglutination activity. The crude lectin extract of Aloe leaves gel showed haemagglutination activity with 2% erythrocytes of different blood group of human. The crude extract of *Aloe barbadensis* showed good agglutination activity against blood group A+ve, B+ve and O+ve.

### Determination of protein concentration

The concentration of protein from dialyzed extract of *Aloe barbadensis* was found to be 4.28mg/ml by using Lowry's method.

### Agglutination assay

Agglutination study of *Aloe barbadensis* lectin with 2% human erythrocytes showed in Table 1.

Blood Group	Agglutination
A	+
B	++
O	+++

Haemagglutination results in Table 1. revealed that blood group A+ve and B+ve showed least agglutination as compared to O+ve. Blood group O+ve showed strong agglutination with lectin extracted from *Aloe barbadensis* gel.

Carpet pattern given by blood group A, B and O is 4,5 and 6 time dilution respectively as shown in figure 1. Further dilution showed no more precipitation of lectins.

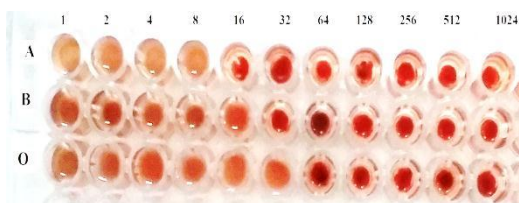


Figure 1. Haemagglutination assay

On the basis of results showed in Table 1. it was observed that minimum concentration of lectin was

required for agglutination of blood group O than B and A.

HAU (Haemagglutination Unit) and SA (specific activity) were also calculated as shown in Table 2.

Blood group	HAU/ml	SA
A	34.24	8
B	68.48	16
O	136.96	32

### Sugar specificity Test/Agglutination inhibition Assay

Table 3. showed Inhibition of agglutination activity of Aloe lectin was tested on seven sugars.

Sugars	Effect on agglutination
D-glucose	Absence of inhibition
Sucrose	Absence of inhibition
Lactose	Absence of inhibition
Fructose	Absence of inhibition
Mannose	Inhibition at 500mM
Galactose	Inhibition at 500mM
N-acetyl-D-galactosamine	Inhibition at 500mM

### Study of pH activity of Lectin

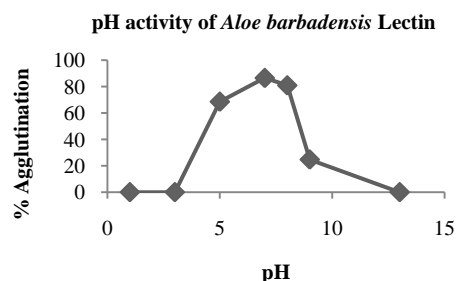


Figure 2. pH activity of *Aloe barbadensis* lectin with different buffers at room temperature.

### Electrophoresis (SDS-PAGE) for the confirmation of purity of lectins:

*Aloe* Lectin:

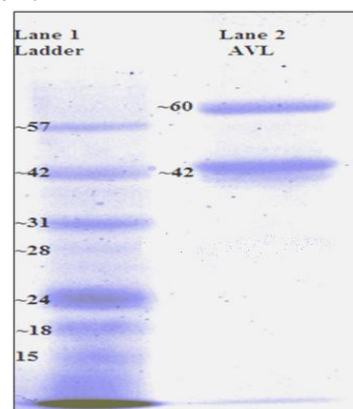


Figure 3. The appearance of band resulted into SDS-PAGE with molecular weight of 42kDa and 60kDa in the purified lectin of *Aloe barbadensis* Lectin (50µg). Here Lane 1 is standard protein marker (BioRed) AVL is *Aloe* Lectin

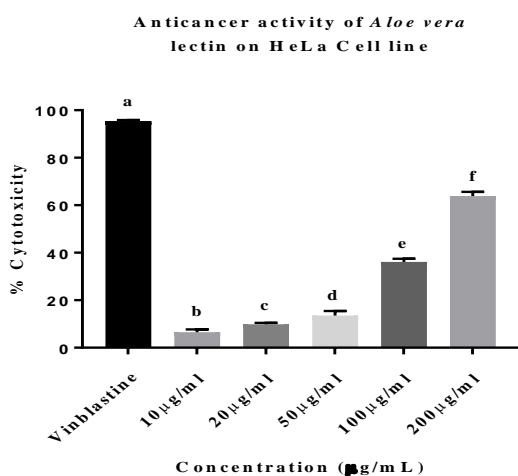


### Anticarcinogenic activity of Plant Lectins:

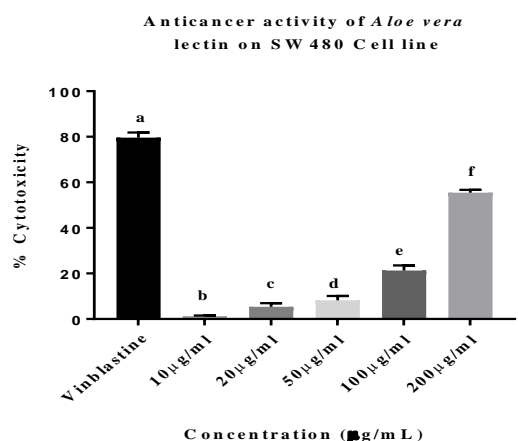
Two cell lines have been cultured (HeLa and SW 480). There was a lead in two cell lines that were Cervical cancer cell line HeLa and Colon cancer cell line SW480 in 48 hours timeline at 10µg/ml, 20µg/ml, 50µg/ml, 100 µg/ml and 200µg/ml concentrations. Vinblastine drug (an anti-cancer drug) was used as positive control. DMEM medium is used to maintain HeLa and SW480 cancer cell lines supplemented with 10% fetal bovine serum , Penicillin (10,000 units/100 ml) and Streptomycin (10 mg/100 ml) were used to prevent the culture from contamination and grown in a humidified 5% CO2 incubator at 37°C to achieve 80-90% confluency and harvested using trypsinisation.

### Cytotoxicity Analysis

SRB assay has been performed for cytotoxicity evaluation of *Aloe barbadensis* lectin extract. The reading has been taken at 540nm. We analyzed the data and represented it in graphical form as shown in figure 4 and 5.



**Figure.4. Anticancer activity of AL (*Aloe* Lectin) on HeLa cell line at different concentration (mean±sd, n=3) with One way analysis of variance (ANOVA) P- value is less than 0.05 by Tukey's test so it is statistically significant.**



**Figure.5. Anticancer activity of AL (*Aloe* Lectin) on SW480 cell line at different concentration (mean±sd, n=3) with One way analysis of variance (ANOVA) P- value is less than 0.05 by Tukey's test so it is statistically significant.**

### VI. CONCLUSION

Lectin can agglutinate the RBCs. Haemagglutination assay has a vital importance to characterize protein as lectin. From haemagglutination assay the HAU (Haemagglutination unit) and SA (specific activity) of A, B, O blood groups for *Aloe* lectin was calculated.

Haemagglutination unit/ml and specific activity (SA/mg) of *Aloe barbadensis* lectin for A+, B+ and O+ were 34.24, 68.48 and 136.96 HAU/ml and 8, 16 and 32 SA/mg respectively.

Sugar specificity or binding ability of *Aloe* lectin at 500Mm concentration has shown by N-acetyl-D-galactosamine, D-galactose and D-Mannose. pH activity of *Aloe* lectin at 0-4 pH was null. At 5-7 pH lectins were displaying its maximum interest. At pH 7 lectins were neutral. At 8-10 there's a downfall and at 12-13 again the activity of lectin was very less. The purity of those proteins becomes confirmed through SDS-PAGE which has been shown by the characterization of AL (*Aloe* lectin) protein of MW (molecular weight) 42kDa and 60kDa.

The anticancer activities of *Aloe* lectin (AL) had elucidated by SRB assay using human cancer cell line HeLa and SW480. We analyzed the data with the help of Graph pad prism software. Anticancer properties of *Aloe* lectin (AL) by SRB assay resulted that the impact of both plants lectins at 200µg/ml attention on HeLa (cervical cancer cell line) is extra effective than SW-480 (colon cancer cell line) after 48hrs.

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31<sup>st</sup> August, 2018

**TO WHOM IT MAY CONCERN**

This is to certify that **Ms. Sruchi Devi**, a student of Ph.D. Biochemistry, Lovely Professional University, Jalandhar, Punjab undertook her research training on the topic "***In vitro* Cytotoxic Evaluation of Lectin Extract of *Aloe barbadensis* (Aloe vera) and *Bryophyllum pinnatum* on Human Cancer Cell Lines**" under the supervision of **Dr. Yogendra S. Padwad**, Scientist, Pharmacology and Toxicology lab, Food and Nutraceuticals Division w.e.f. 01<sup>st</sup> February, 2018 to 31<sup>st</sup> August, 2018 at this Institute. During the training programme, she was found to be diligent, hardworking and capable of working in any team.

I wish her all success in her future endeavours.

गिरीश नड्डा  
31/8/18  
(Gireesh Nadda)



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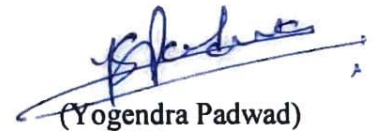
Date: August 31, 2018

To Whom It May Concern

It is a true pleasure for me to write this recommendation letter in support of Ms. Sruchi Devi. I know her from last six months, since she joined in my lab as a Research Trainee (Ph. D. scholar of Lovely Professional University, Jalandhar, Punjab) at CSIR-IHBT, Palampur (H.P.). She worked on '*In vitro* Cytotoxic Evaluation of Lectin extract of *Aloe barbadensis* (Aloe vera) and *Bryophyllum pinnatum* on Human Cancer Cell Lines'. She is well versed in various lab techniques like animal cell culture, biochemistry and molecular biology. She demonstrates excellent laboratory skills, a good bench worker and believes in team spirit.

In short, she has the motivation, intelligence and analytical aptitude for research. She has been always enthusiastic to ascertain new ideas and skill.

To the best of my knowledge, she bears a good moral character and conduct. I wish her all the best in her all endeavors.

  
(Yogendra Padwad)





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## ICEFABT-NEW DELHI

# *Certificate*

*This is to certify that Sruchi Devi has presented a paper entitled "In Vitro Anticancer Activity of Lectin Isolated from Gel of Aloe Barbadensis (Miller) against Hela and SW480 Human Cancer Cell Lines" at the International Conference on Environmental, Food, Agriculture and Bio-Technology (ICEFABT) held at New Delhi, India on 01<sup>st</sup> September, 2019.*

IF-FABT-IDRE-25089-2918

Paper ID



Chairman

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## Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. SRUCHI DEVI  
 has Presented E-poster/Oral presentation on Anti-oxidant activity of Lectin isolated from  
leaves of Bryophyllum pinnatum.  
 presentation in International Conference of Pharmacy (ICP-2019) on the theme of " Pharmacy: Realigning the focus  
 on health" held on 13 -14th September 2019 organized by School of Pharmaceutical Sciences, Lovely Professional  
 University, Punjab in a collaboration with Indian Pharmacy Graduates' Association (IPGA) Phagwara chapter.

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