

Antimicrobial And Anticancer Activity Of A Novel- Mannose Specific Lectin From The Flower, *Ipomoea Pes-Caprae*

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Abstract

In the present study, a 35 kDa D-mannose specific lectin was purified from the flower extract of *Ipomoea pes-caprae* (IPL) by lactoferrin-Sepharose 4B in the econo column (Bio-Rad). Physico-chemical analysis revealed that lectin was stable the pH ranges from 6.5-9.5 and temperature ranges from 0°C-60°. The lectin was highly specific for rabbit erythrocytes. UV spectral analysis of lectin documented a characteristic peak at 230 nm and FTIR spectrum showed the IR absorption peaks correspond to amide I and II bands, carboxylic and O-H groups in the lectin. Antimicrobial activity of lectin was tested against four Gram positive bacteria, four Gram negative bacteria and four fungal pathogens. It was observed that the lectin was more effective with a maximum zone of inhibition of 22 mm and 19 mm against *B. subtilis* and *A. flavus* respectively. Moderate activities were observed against all the bacteria and fungus tested ranging from 12 mm to 18 mm. The IPL lectin was checked for its antiproliferative and cytotoxicity effect against three cancer cell lines and also with normal fibroblast cell line by MTT assay. The result shows MCF-7 was more sensitive to IPL with lowest IC₅₀ value of 161.01 µg/ml and exhibit low toxicity against normal cells with IC₅₀ value of 374.36 µg/ml. As a result, this research opens up new possibilities for lectin pharmacology.

Keywords: Lectin, affinity chromatography, mannose, antimicrobial, anticancer

1. Introduction

Lectins are a heterogeneous group of sugar binding proteins [1] with the ability to agglutinate mammalian red blood cells. They are abundant in plant species but the specific activities and structure varies from plant to plant [2,3]. Most plant lectins show specific binding [4] towards mannose [5,6] and structural diversity with high mannose specificity, highlights the importance of lectin in higher plants [7]. Affinity chromatography is the best purification method for the purification of plant lectins [8,9].

The interaction between carbohydrates and lectins aid in many biological processes, including bacterial and fungal growth inhibition [10], anti-HIV [11], immunomodulatory [12],

anti-insect [13] and antitumor [14]. Mannose-binding lectin (MBL) is an important host defense molecule that plays a key role in first-line of host defense against certain infectious agents [15] by recognizing and initiating the opsonization of microorganisms [16]. Mannose-binding lectin (MBL) can opsonize many bacterial and fungal pathogens and activate complement pathway [17]. MBL appears to be a prototypical pattern recognition molecule that is able to recognize a wide range of microorganisms [18] especially high-mannose type glycans.

Lectins can be toxic to cancer cells via different mechanisms, which are generally initiated by interaction with specific receptors, glycosylated or not, on the membrane of cancer cells. The lectins can be internalized through endocytosis and addressed to different compartments leading to activation of signaling pathways related to cell death [19]. Several lectins have been found to possess anticancer properties preferentially by binding to cancer cell membranes or their receptors, causing cytotoxicity, apoptosis and inhibition of tumor growth [20]. The ability of mannose specific plant lectins to discriminate between normal and cancer cells, and specifically the changes that have occurred in the high-mannose component covering tumor cells allows these proteins to be used as cytotoxic agents for various malignant cells [21]. Plant lectins were reported to induce death of cancer cells by binding to cancer cell membranes or their receptors [22,23]. Since they have shown remarkable anticancer properties in vitro /in vivo and clinical studies against human cancer cell lines [24,25], they are considered to be useful in cancer research and therapy [26].

Ipomoea pes-caprae is a valuable medicinal plant which belongs to Convolvulaceae family [27]. This plant was found to be very important as a source for bioactive principle and natural antioxidants [28]. The medicinal value of *Ipomoea* plant is reported for their antimicrobial, anticancer, anti-inflammatory and other ailments [29]. The lyophilized leaf powder of sweet potato (*Ipomoea batatas*) strongly suppressed the growth of Gram positive and Gram negative bacteria [30]. Medicinal herbs are proved to be a source of agglutinin and lectins have been isolated from *Convolvulus arvensis*, *Ipomoea batatas* and *Calystegiasepium* [13]. The *Ipomoea pes-caprae* plant was previously studied by different investigators on its phytochemical composition but the purification of agglutinins/lectin has not been documented. Thus, the present investigation reports the isolation, purification and characterization of lectin of *Ipomoea pes-caprae* flower and its biomedical applications such as antimicrobial activity against bacterial and fungal strains and cytotoxicity against cancer cell and normal fibroblast cells.

2. Materials and methods

Lectin purification

Lectin purified by biospecific adsorption

Rabbit erythrocytes were collected in Alsever's solution. Immediately upon receipt, rabbit erythrocytes were washed three times in 20 volumes of PBS, pH 7.5 (75 mM NaCl, 75 mM Na₂HPO₄) per packed cell volume by centrifugation at 4000 rpm for 5 minutes. The cells were suspended at a concentration by volume of 8% PBS (pH 7.5) and an equal volume of formalin (3% solution in PBS and adjusted to pH 7.5 with 0.1 M NaOH) was added. The mixture was incubated at 37°C for 16 hours with moderate shaking. The cells were then washed four times with five volumes of PBS (pH 7.5) per packed cell volume and stored at 4°C as 10% suspension in the same buffer [31]. The stored formalinized cells were prepared for use as an affinity reagent by washing six times in 10 volumes of TBS (50 mM Tris-HCl, 100

mM NaCl). The packed cells were then incubated with 20 volumes of clarified flower extract in TARSON centrifuge tube for 2 hours with moderate shaking at 4°C and then washed 3 times with 20 volumes of TBS, pH 7.5, containing 10 mM CaCl₂.

Elution of adsorbed hemagglutinin was accomplished by incubation of the cells with 10 volumes of elution buffer containing 10 mM disodium EDTA. The elution was continued for 2 hours with moderate shaking at 4°C and the elution mixture was then centrifuged for 10 minutes at 2800 rpm, to remove any residual particulate material and the resultant supernatant was dialyzed against 10 mM CaCl₂ at 4°C for 1 minute and then lyophilized and stored at -4°C.

Purification of lectin by affinity chromatography

Since the hemagglutinating activity of the flower extract with rabbit erythrocytes was highly inhibited by lactoferrin, the flower agglutinin was purified using lactoferrin-Sepharose 4B affinity column. The strategies for adsorption and elution steps were developed from the identified optimal pH, temperature and calcium ion requirement [32].

Extract of the flower (20 ml) of the plant *Ipomoea pes-caprae* was applied to 3.5 ml of lactoferrin-agarose in an econo-column (Bio-Rad) previously equilibrated with TBS at 4°C. The eluant was collected at a rate of 0.6 ml/min. The column was washed with HSB until the A280 of the effluent was <0.002. The column was further washed with cold LSB (4°C) until the A280 of the effluent was <0.002 and again it was washed with warm LSB (32°C) until the A280 of the effluent was <0.002. This step eluted additional inert proteins and was necessary for obtaining homogenous lectin. The elution of lectin was done with elution buffer (EB) that contained the potent inhibitor 50 mM D-Mannose and collected as 1 ml fractions on ice in polypropylene tubes at a rate of 0.3 ml/min. The fractions were vortexed immediately after collection and stored at 4°C. The effluents (fractions) collected during adsorption, washing, re-equilibration and elution were tested for HA activity with 1.5% suspension of rabbit erythrocytes to determine the presence of lectin. The fractions that contained significant amount of lectin when eluted with the elution buffer were pooled on the same day and dialyzed against distilled water at 4°C for 3 minutes and the purified lectin obtained was lyophilized and stored at -20°C [33].

Hemagglutination and Hemagglutination inhibition assay

Hemagglutination assays were performed at room temperature (30±2°C). The purified lectin (25 µl) was serially diluted with TBS-BSA (25 µl, pH 7.5) and mixed with 25 µl of 1.5% erythrocyte suspension and incubated for 1 hour. HA titre was reported as the reciprocal of the highest dilution of lectin giving complete agglutination after 1 hour.

With the view to ascertain the nature of the binding specificity of the purified lectin, hemagglutination inhibition assays were performed with sialoglycoproteins. Inhibitors were serially diluted with TBS-BSA (25 µl) in a microtitre plate. To each well 25 µl of purified lectin in TBS-BSA at sub agglutination concentration (giving a HA titre of 2 with 1.5% suspension of rabbit erythrocytes) was added and mixed. After mixing, the lectin-inhibitor solution was incubated for 1 hour at room temperature (30±2°C) and was mixed with 25 µl of 1.5% suspension of rabbit erythrocytes. The HAI titre was reported as the reciprocal of the highest dilution of inhibitors giving complete inhibition of agglutination after 60 minutes.

Effect of pH and temperature on IPL

pH dependence of IPL was tested at different pH (5 to 11) and the effect of temperature on agglutinin was studied by incubating the IPL at different temperature (0°C to 100°C) for an hour before adding erythrocytes.

Protein concentration and evaluation of molecular mass

The protein concentration of the lectin was estimated by Folin-Ciocalteu method [34]. Sodium dodecyl sulphate polyacrylamide 12% slab gel electrophoresis was performed according to Laemmli [35]. The molecular mass marker used is (11-245kDa) pre stained marker purchased from HIMEDIA, Chennai, Tamil Nadu, India.

UV and FTIR analysis of IPL

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (UV-2450). In Fourier transform infrared (FTIR) spectroscopy measurements, the lectin was air dried and mixed with potassium bromide in the ratio of 1: 100. FTIR spectrum of the samples was recorded on Shimadzu IR Prestige-21 FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} . This range was used to study the fundamental vibrations and associated rotational vibrational structure [36].

Antimicrobial assay

In vitro antimicrobial assay was carried out by disc diffusion technique [37]. Lectin purified by formalinized erythrocyte adsorption and affinity column chromatography was used for the assay. Sterile discs of 6 mm were impregnated with lectin of 50 μl (50 μg and 100 μg concentration) and allowed to dry at room temperature inside laminar air flow. The lectin loaded discs were placed on agar plates seeded with microorganisms and incubated at 37°C for 24 hours. The susceptibility of the test organism was measured and determined by zone of inhibition in mm (using Himedia zone measuring scale). The streptomycin discs were used as a positive control for bacteria, fluconazole for fungi and sterile discs were used as a negative control. Lectins were tested in triplicate.

MTT Cytotoxicity Assay

To prepare the stock concentration of 1 mg/ml, one mg of sample was weighed and dissolved in 1000 μl of medium. Different concentrations ranging from 25, 50, 100, 200 and 400 $\mu\text{g/ml}$ were treated with the cells by incubating for 24 hours at 37°C in a 5% CO_2 atmosphere. Camptothecin at a concentration of 5.2 $\mu\text{g/ml}$ was used as a positive control for the study. After the incubation period, direct microscopic observations of sample treated cell lines by using Inverted biological microscope with the magnification of 10X was recorded. Further the spent media was removed and 100 μl of MTT reagent at a concentration of 0.5 mg/ml was added (Cat No: 4060, HIMEDIA, Chennai, Tamil Nadu, India) and incubated for 3 hours at 37°C for the reaction. After the incubation period, the formed formazan crystals were dissolved with 100 μl of DMSO (Cat No.1309, Sigma, Salem, Tamil Nadi, India) to solubilize the formed formazan crystals and the absorbance were recorded by 96 well plate ELISA Reader (ELX 800, Bio Tek) at 570 nm. High intensity of purple color indicated the number of viable cells indirectly and low intensity of purple color indicated less number of viable cells and more dead cells.

Statistical analysis

Data are expressed as mean \pm SD from three separate observations. The IC₅₀ value (the effective concentration of the compound having the ability to kill 50% of viable cells) was calculated using ED50V10 software [38].

3. Results and Discussion

Flowering plants express a whole battery of carbohydrate-binding proteins commonly known as lectins [38]. Lectins of *Ipomoea pes-caprae* of convolvulaceae family remain understudied and poorly characterized. This study reports the purification of a novel mannose specific lectin (IPL) from the flower of *Ipomoea pes-caprae*. The lectin was purified through

affinity column chromatography and by biospecific adsorption using formalinized rabbit erythrocytes. The flower agglutinin purified by affinity chromatography resulted in 667 purification fold and showed 1.0×10^7 increase in specific activity (Fig.1). The lectin was also purified by biospecific adsorption using formalinized rabbit erythrocytes and eluted using 10 mM disodium EDTA and it showed 225 purification fold and 3.6×10^6 increase in specific activity (Table 1).

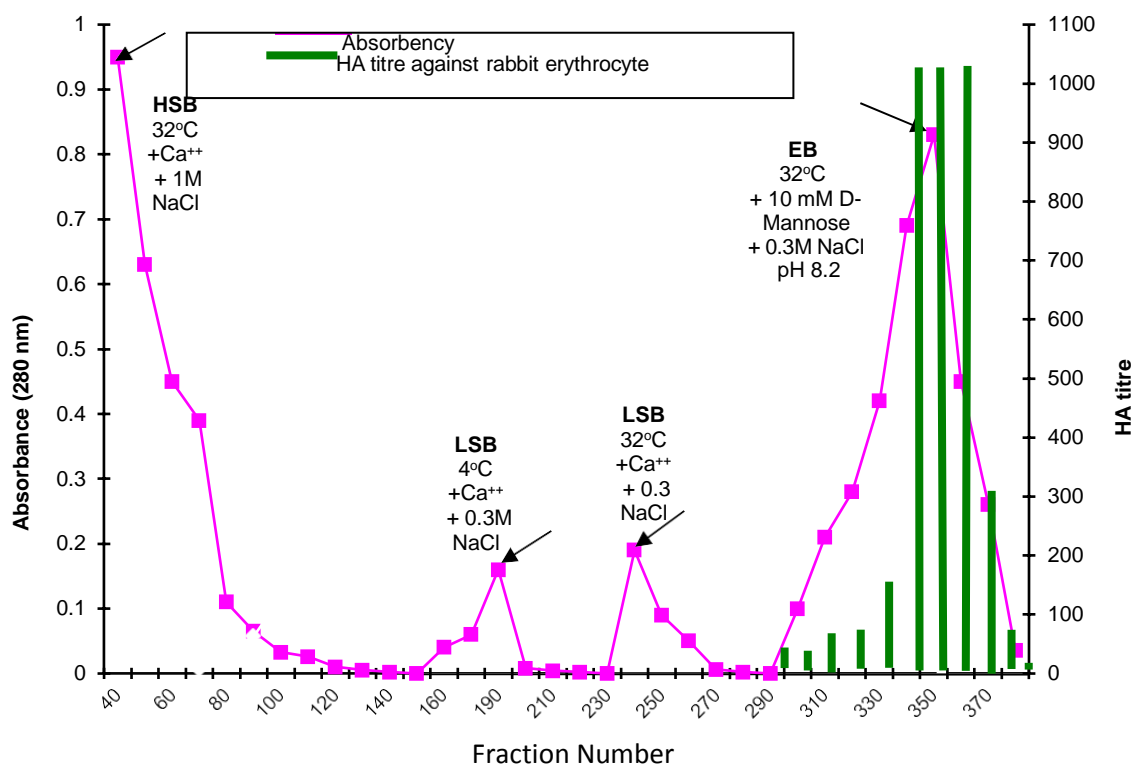


Figure 1. Lactoferrin – affinity elution profile of lectin from the flower of *Ipomoea pes-caprae*

Table 1. Purification of lectin from the flower of *Ipomoea pes-caprae*

Sample	Volume (ml)	Protein (mg)	Total activity (HA units)	Specific activity (HA units/mg)	Purification fold
Crude flower extract	50	251.25	4.09×10^6	1.6×10^4	1
Clarified extract	30	12.81	1.2×10^6	9.5×10^4	5.89
Lectin purified by biospecific adsorption method using formalinized rabbit erythrocytes	10	0.335	1.2×10^6	3.6×10^6	225

Lectin purified using lactoferrin affinity column	9	0.113	1.2×10^6	1.0×10^7	667
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The purified lectin was designated as IPL. The IPL purified by both biospecific adsorption and affinity column chromatography showed a single band of 35 kDa on SDS-PAGE and revealed it as a single homogenous lectin (Fig. 2). Most plant lectins have a molecular weight of 30 to 50 kDa [39,40,41,42]. The HA profile of the purified lectin showed maximum HA activity of 1024 with rabbit erythrocytes similar to crude agglutinin. It also agglutinated rat > Dog > Human O = mice erythrocytes (Table 2). The specificity of carbohydrate binding for the *Ipomoea pes-caprae* lectin was investigated using the hemagglutination inhibition assay. Though this method is semi-quantitative, it provides information regarding various saccharides that inhibit the lectin activity [43]. The lectin IPL was inhibited by glycoproteins: Lactoferrin > Apotransferrin > Fetuin > Transferrin > Thyroglobulin = BSM and sugars: D-mannose > ManNAc > Sucrose = D-Glucosamine = L-Fucose > α -lactose = D-galactosamine = D-glucose – 6 phosphate (Table 3). Hapten inhibition assay of the purified lectin IPL revealed lactoferrin and D-mannose as potent inhibitors as reported in *Canavalia grandiflora* which showed hemagglutination with rabbit erythrocytes and was specifically inhibited by the simple sugar D-mannose [44] and glycoprotein bovine lactoferrin [45]. Specificity of lectin to mannose can be related to its affinity to lactoferrin that contains mannose, galactose, fucose, N-acetyl neuraminic acid and N-acetyl galactosamine [46]. Mannose binding lectin (MBL) is a group of III type lectin that can recognize carbohydrates like mannose, glucose, fucose and N-acetyl mannosamine [47, 48]. Mannose-binding lectins are reported to occur in various vegetative tissues such as leaves [49], flowers [50], roots [51] and even in nectar [52].

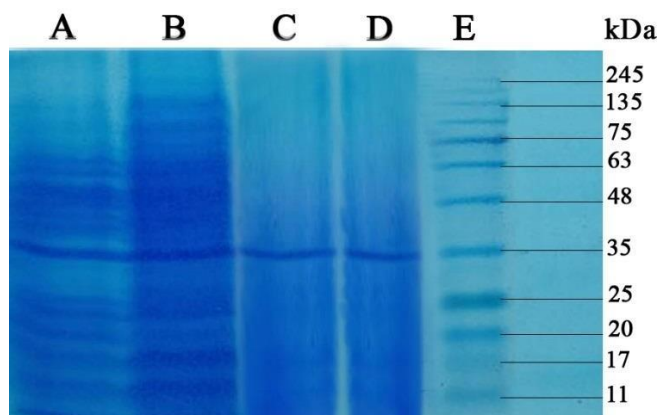


Figure 2. SDS-PAGE of purified lectin from the flower of *Ipomoea pes-caprae*. Lane A - Crude extract, Lane B - Clarified extract, Lane C - Lectin purified by biospecific adsorption, Lane D - Lectin purified by affinity chromatography, Lane E - Molecular weight marker

Table 2. Hemagglutination titre of the purified lectin from the flower of *Ipomoea pes-caprae*

Erythrocytes (n=3)	HA Titre		Common species of sialic acid	Reference
	Crude	Purified		
Rabbit	1024	1024	NeuAc / 9-O-Ac NeuAc/ NeuGc/9-O-Ac NeuGc	Murayama 1981
Rat	512	64	NeuGc/NeuAc/O-Ac Sia	Nowak 1986
Dog	128	64	NeuGc α , 2-3 Gal β 1-4 GluNAc β 1-	Yamakawa 2005
Human O	64	16	NeuAc	Berman 1984
Mice	16	16	NeuGc/ NeuAc	Gowda 1984 and Varki 1980
Human A	2	64	NeuAc	Berman 1984
Human B	2	64	NeuAc	Berman 1984

n = Number of times tested

Table 3. Hemagglutination inhibition of lectin from the flower of *Ipomoea pes-caprae* by glycoproteins

Inhibitors Glycoprotein/ Sugars (n=3)		HAI Titre	Minimum concentration required for inhibition (μ g/ml)	Relative inhibitory Potency (%)
glycoprotein	Lactoferrin	2048	2.441	100
	Apotransferrin	512	9.765	25
	Fetuin	512	9.765	25
	Transferrin	8	625	0.39
	Thyroglobulin	4	1250	0.195
	BSM	4	1250	0.195
Sugars	D-Mannose	1024	0.097	100

	ManNAc	512	0.195	50
	Sucrose	128	0.78	12.5
	D-Glucosamine	128	0.78	12.5
	L-Fucose	128	0.78	12.5
	α -Lactose	32	3.125	3.25
	D-Galactosamine	32	3.125	3.25
	D-Glucose-6-phosphate	32	3.125	3.25

n = Number of times tested

Melibiose, Raffinose, GalNAc and GluNAc failed to inhibit the hemagglutination nature of lectin

HA titre was reported as the reciprocal of the highest dilution of lectin giving complete agglutination after 1 hour. The HA titer was stable between pH 6.5 and 9.5 (Fig. 3 A). The pH stability of plant lectins varies with different plant species. It is reported that *Morus rubra* [53] and *Ipomoea asarifolia* [53] leaf lectin are stable at pH 7.5 but in *Kalanchoe crenata* leaf lectin pH ranges from 2 to 12 [54]. Lectins are thermosensitive and temperature may change their activity [55]. The flower agglutinin of *Ipomoea pes-caprae* was stable from 0°C to 50°C and declined above 60°C suggesting the proteinaceous nature of the agglutinin (Fig. 3 B). The UV spectral analysis of lectin measured at a wavelength range from 200-800 nm documented a characteristic peak at 230 nm for IPL (Fig. 4 A). *Aspidistra elatior* lectin (AEL) of 13.5 kDa and 14.5 kDa at far-UV resulted in two broad minima at 206nm and 218 nm [56]. The FTIR spectrum of pure lectin represented the characteristic IR absorption regions of proteins showing distinct bands at 1580 cm⁻¹, 1061 cm⁻¹ and 1407 cm⁻¹ which corresponds to amide I and II bands with C-O and C-NH₂ stretching of side chain primary amines. The sharp peak at 3424 cm⁻¹ corresponds to the absorption of carboxylic and O-H groups with stretching mode of amide N-H. A weaker band of 1329 cm⁻¹ related to C-N, C-O and C-C-O vibrations of the protein backbone and amino acid residue was observed. The FTIR spectrum of *Ipomoea pes-caprae* lectin represented by 1407 cm⁻¹, 1580 cm⁻¹ and 3424 cm⁻¹ bands (Fig. 4B) coincides with the amide I and II region and is in agreement with data reported earlier [57]. These FTIR band assignments may be helpful to detect the lectin crystal structure and its conformations.

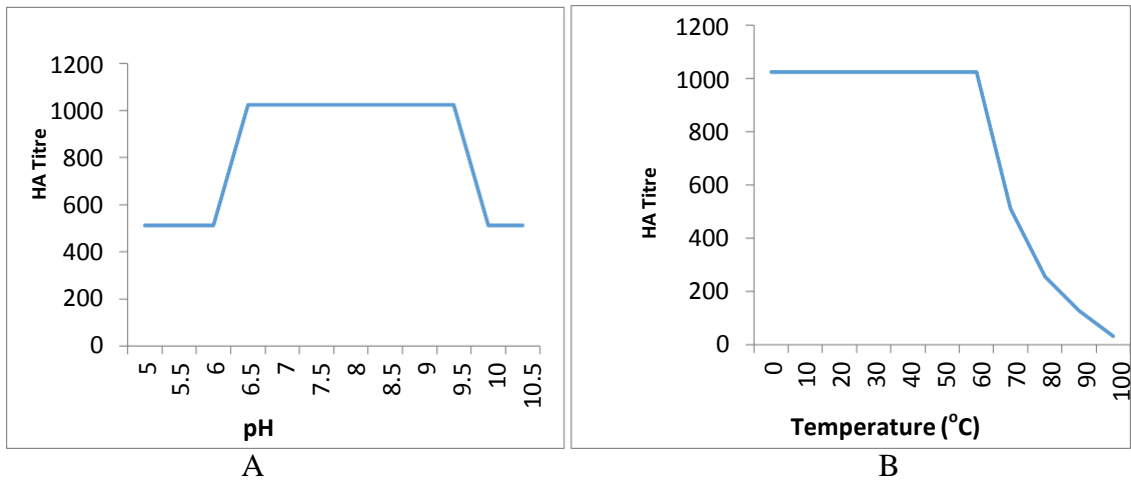


Figure 3. Impact of pH (A) and temperature (B) on HA titre of the IPL

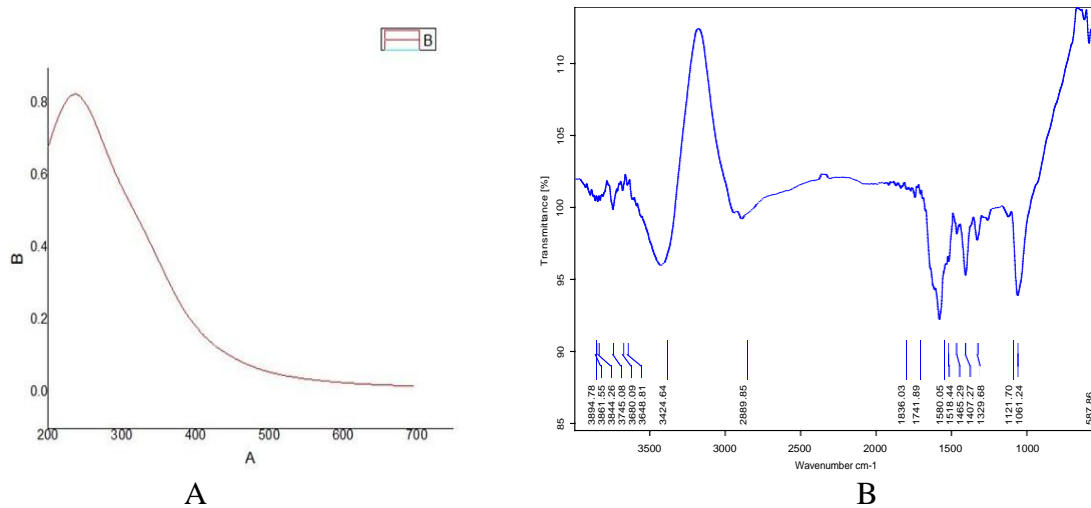


Figure 4. Characterization of lectin by UV- Spectra (A) and FTIR – Spectra (B)

MBL appears to be a prototypical pattern recognition molecule that is able to recognize a wide range of microorganisms. Infectious agents recognized by MBL include Gram positive and Gram negative bacteria, yeasts, parasites, mycobacteria and viruses [58]. Antimicrobial property of D-mannose specific lectin (IPL) purified by both the formalinized RBC (FRBC) adsorption and affinity column chromatography was tested against bacteria and fungi. The purified IPL was highly effective against gram positive *Bacillus subtilis* with a maximum zone of inhibition of 22 mm and 18 mm zone of inhibition was observed with Gram negative bacteria *P. aeruginosa*. Lectin purified through both the methods at 100 µg concentration recorded a similar inhibition zone of 16mm against *E. faecalis* and 13 mm against *K. pneumoniae*. 100 µg concentration of affinity column purified lectin showed 14 mm against both *S. aureus* and *P. vulgaris* and 12 mm against *E. coli* while FRBC purified lectin showed 11 mm against both *S. aureus* and *P. vulgaris* and was completely resistant to *E. coli* (Table 4 and Fig. 5A). The concentration needed for bacterial growth inhibition by lectin is similar to those presented by *Indigofera heterantha* lectin [59] and by *Canavalia ensiformis* [60]. Naturally occurring molecules such as proteins and peptides found in microbes, plants and animals can interfere with the microbial growth by

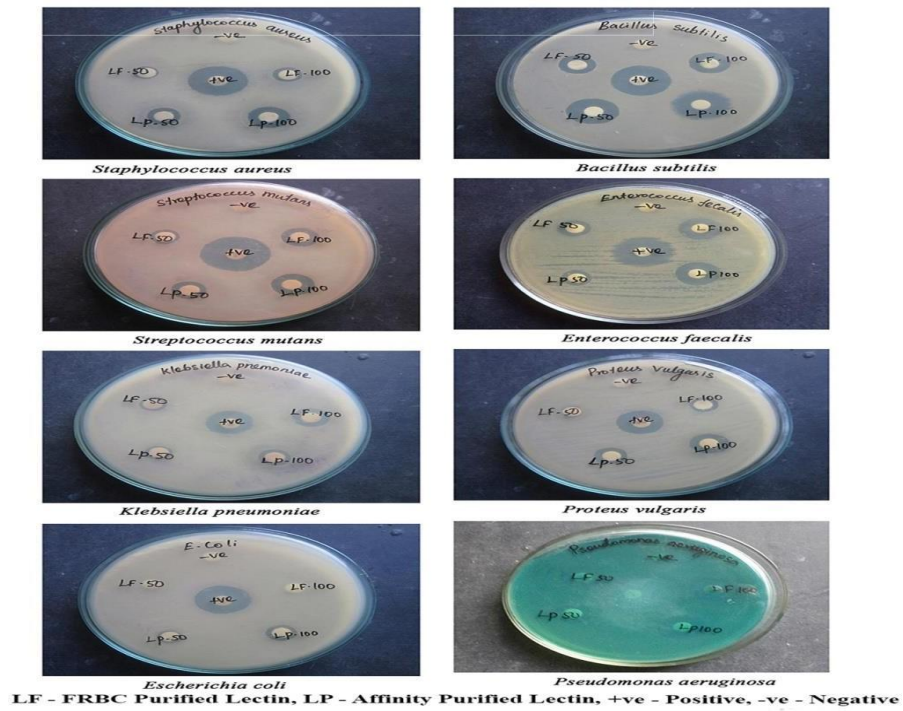
different mechanisms [61]. Lectins can mediate the identification of microbes by interaction with the carbohydrate moieties expressed on the cell surface thus promoting defense activation and cell-cell signaling [62]. The components of the bacterial cell wall, such as lipopolysaccharides (LPS), peptidoglycans, teichuronic and teichoic acids, are responsible for the antibacterial effects of lectin [63].

Table 4. Antimicrobial activity of different concentration of lectin of *Ipomoea pes-caprae* flower

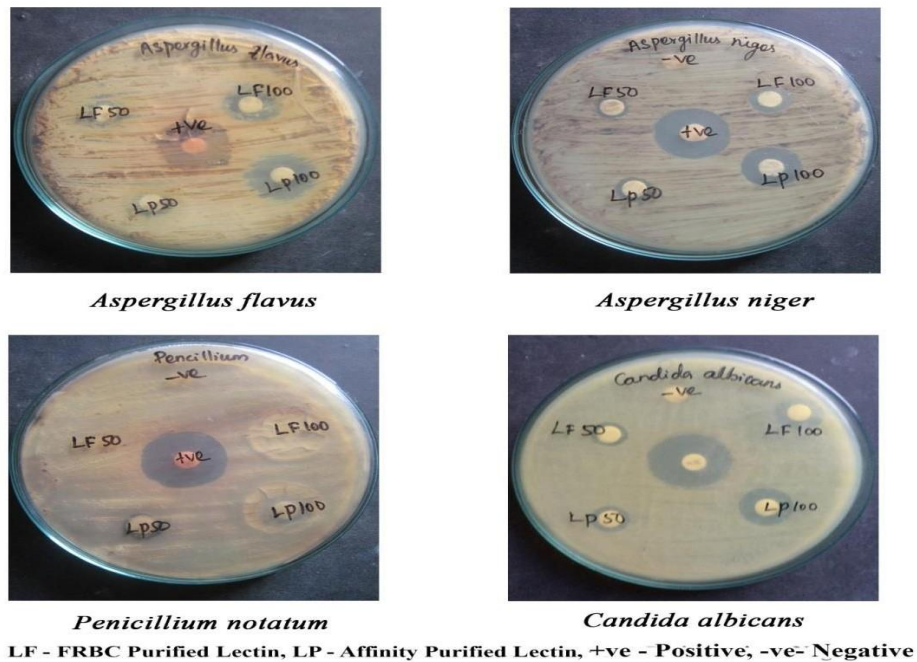
Microbial Strains	Zone of inhibition (mm) (Mean \pm SD)					
	LF (50 μ g)	LF (100 μ g)	LP (50 μ g)	LP (100 μ g)	PC	NC
<i>S.aureus</i> (G+)	9 \pm 0.5	11 \pm 0.5	12 \pm 1.3	14 \pm 0.5	19 \pm 0.5	-
<i>B. subtilis</i> (G+)	14 \pm 0.5	15 \pm 0.5	18 \pm 0.5	22 \pm 1.3	21 \pm 0.5	-
<i>S. mutans</i> (G+)	11 \pm 1	13 \pm 1	12 \pm 0.5	15 \pm 0.5	23 \pm 0.5	-
<i>E. faecalis</i> (G+)	11 \pm 0.5	16 \pm 0.5	11 \pm 1.5	16 \pm 1.2	21 \pm 0.5	-
<i>K. pneumoniae</i> (G-)	10 \pm 1.5	13 \pm 1.2	10 \pm 0.5	13 \pm 0.5	16 \pm 1.1	-
<i>P. vulgaris</i> (G-)	8 \pm 0.5	11 \pm 0.5	12 \pm 0.5	14 \pm 0.5	17 \pm 0.5	-
<i>E. coli</i> (G-)	-	-	9 \pm 1.2	12 \pm 0.5	19 \pm 1.1	-
<i>P. aeruginosa</i> (G-)	15 \pm 1	16 \pm 0.5	13 \pm 0.5	18 \pm 1.5	22 \pm 0.5	-
<i>A. flavus</i>	10 \pm 1.1	15 \pm 0.5	9 \pm 1.3	19 \pm 0.5	20 \pm 0.5	-
<i>A. niger</i>	10 \pm 1.5	11 \pm 1.2	10 \pm 0.5	16 \pm 1.3	20 \pm 1.1	-
<i>P. notatum</i>	-	-	9 \pm 1.5	13 \pm 1.1	22 \pm 0.5	-
<i>C. albicans</i>	10 \pm 0.5	12 \pm 1	11 \pm 1.5	15 \pm 1.2	22 \pm 0.5	-

Table values are expressed as mean \pm SD for three observation

PC- Positive control; NC – Negative control; (G+) - Gram Positive; (G-) - Gram Negative; LF – FRBC purified lectin; LP – Affinity purified lectin



A



B

Figure 5. Antimicrobial activity of IPL against different bacterial strains (A) and fungal strains (B)

Lectins which are multimeric proteins may undergo oligomerization after binding to the cell surface thus increasing the functional affinity and favoring binding of the lectin to the

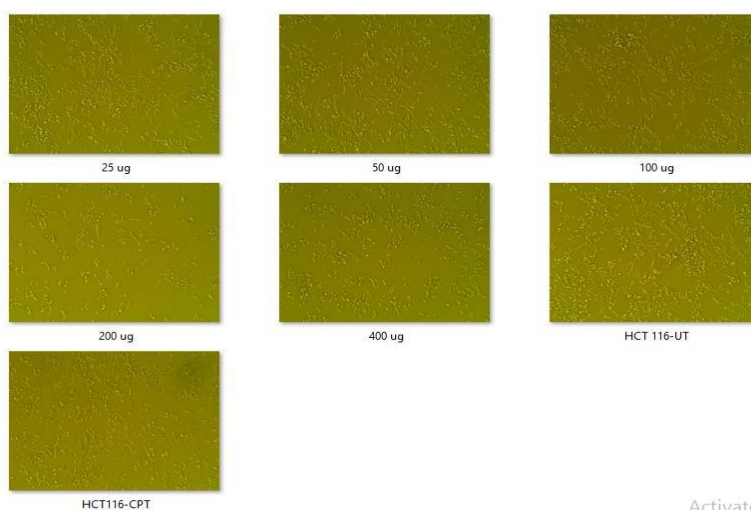
microbe [63]. From the above reports it is clear that lectins can function as antimicrobial proteins and is also evident from our study. The high antibacterial activity observed against *B. subtilis* may be due to the recognition of the sugars present in peptidoglycans like galactosamine and glucosamine [65]. The moderate activity in the other microbes *S. aureus*, *S. mutans*, *E. faecalis*, *K. pneumoniae*, *P. vulgaris* and *P. aeruginosa* may be due to the recognition of sugars sucrose, glucose, GlcNAc, glucosamine, galactosamine, fucose, mannose and muramic acid [66, 67]. Macrophage mannose receptor (MMR; 180-kDa), a prototype member of a family of multilectin receptors, recognize carbohydrates on cell walls of infectious organisms [68]. The receptor recognizes mannose, fucose or N-Acetyl glucosamine sugar residues on the surfaces of these microorganisms [69].

Antifungal assay of IPL demonstrated a maximum inhibition zone of 19 mm against *A. flavus* and 16 mm against *A. niger* followed by 15 mm zone of inhibition against *C. albicans*. Mannan is a major component of fungal cell walls, and MBL binds with high avidity to *Candida albicans* and *Aspergillus* sp [70]. Antimicrobial activity was also observed with FRBC adsorption purified lectin with a maximum zone of inhibition of 15 mm against *A. flavus* (Table 4 and Fig. 5B). The mechanism of action for lectins is based on their binding to the carbohydrates located on the surface of the fungal cell wall [71] which in turn interrupts the chitin synthesis thereby inhibiting the fungal growth. The fungal cell walls are thick and rigid with chitin as the main cellular component composed of monosaccharide units of N-Acetyl glucosamine [72]. Glucan is a polymer of repeating 1,3-linked glucose molecules, and fungal cell walls are comprised of mannan, glucan, and chitin components, all covalently cross-linked in a network [73]. Cell wall composition of *A. niger* showed the presence of six sugars, glucose, galactose, mannose, arabinose, glucosamine and galactosamine [74]. *C. albicans* cell wall is composed of three major polysaccharides: β -1, 3- glucan, β -1, 6- glucan and chitin [75]. The expression of N-acetyl-D-glucosamine, L-fucose, D-galactose and glucose/mannose on the cell wall surface of most of the *Aspergillus* species contribute to the inhibitory effect of the lectin.

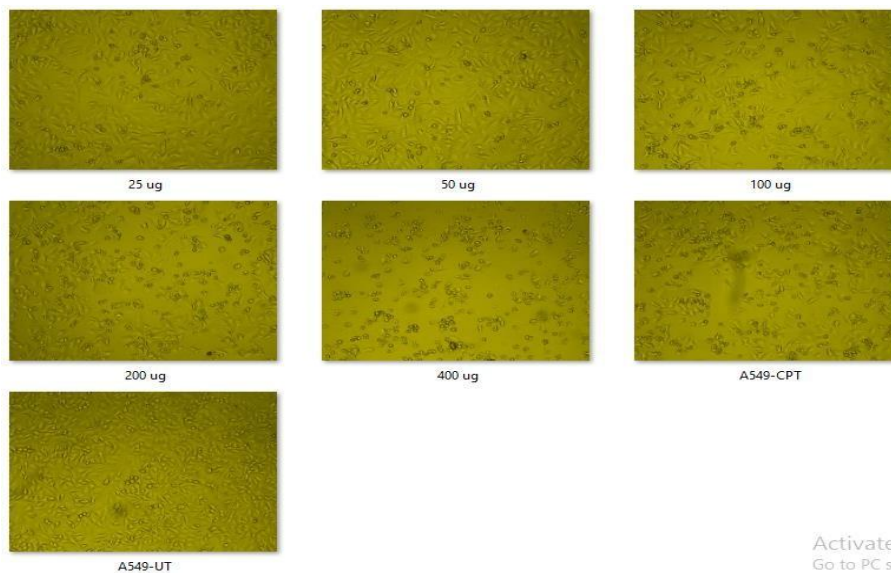
The anticancerous property of D-Mannose specific IPL was studied against cancer cell lines: breast cancer cell line (MCF-7), lung cancer cell line (A549) and colon cancer cell line (HCT116) and the effect was observed to be in a dose dependent manner. The IC_{50} value of the breast cancer cell line was 161.01 μ g/ml when compared to IC_{50} value of A549 and HCT116 which was 225.37 μ g/ml and 439.15 μ g/ml respectively. The cytotoxic effect of IPL was tested against normal fibroblast cell line (L929) and an IC_{50} value of 374.36 μ g/ml was recorded (Table 5). The sensitivity of MCF-7 cell line to the lectin may be due to the high selectivity and specificity for certain glycan structures on the cell surface and other biological mechanism like apoptosis, autophagy and inhibition of tumor growth [76]. The MCF-7 cells showed various changes in the microscopical observation with cell shrinkage and membrane blebbing (Fig. 6). Distorted cell membranearchitecture is characteristic of cells undergoing apoptosis and it may be due to the externalization/translocation of phosphatidylserine from the cytosol into the plasma membrane [77]. Lectins have the inherent capacity to inhibit the growth of cancer cells depending on their concentration and plant lectins have proved the ability to stop multiplication of cancer cells [78]. Lectins with potent anticancerous activity have been documented in different plant species. Concanavalin A (Con A) and *Sophora flavescens* lectin (SFL) lectins display antitumor activities against human breast cancer cells (MCF-7), both in vitro and in vivo [79]. D-Mannose specific *Aspidistra elatior* lectin (AEL) has been reported to be cytotoxic to Bre-04 (Breast), Lu-04 (Lung), HepG2 (Liver), and Pro-01 (Prostate) tumor cell lines [80].

Table 5. Anticancer and cytotoxic effect of different concentrations of IPL

Cell lines	Percentage (%) of viability					IC ₅₀ value µg/ml
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	
HCT116	98.22	84.64	74.01	63.85	51.38	439.15
A549	96.01	86.98	72.82	70.42	53.57	225.37
MCF-7	83.73	72.77	56.62	47.08	32.66	161.01
L929	96.12	89.72	81.77	73.89	46.12	374.36



A



B

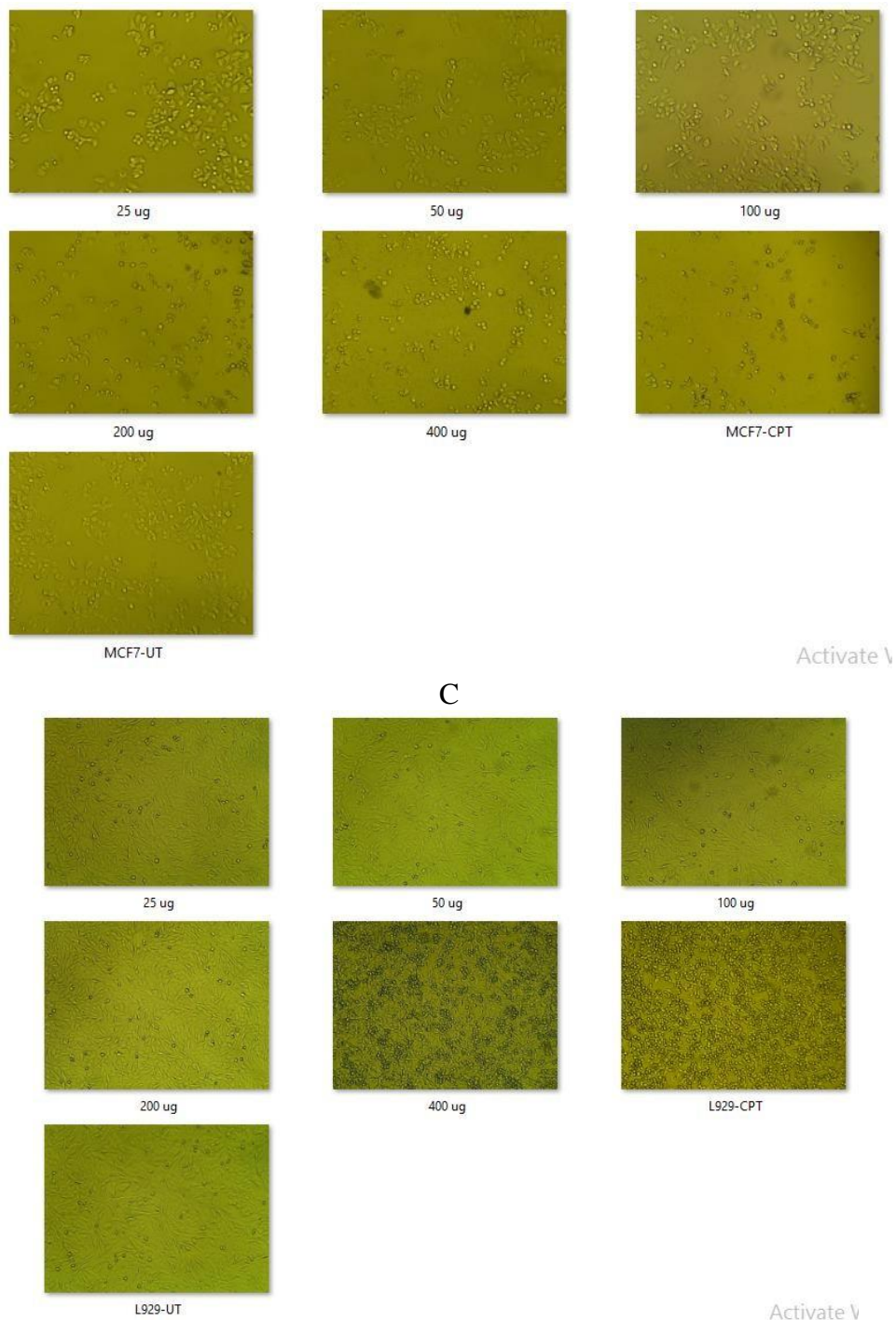


Figure 6. Inverted Phase contrast microscopical view of different cell lines treated with *Ipomoea pes-caprae* flower lectin (IPL) against HCT116 (A), A549(B) MCF-7 (C) and L929 cell line(D). Morphological difference can be observed in all different cell lines treated with IPL and standard drug treated . Cells appear normal in untreated

Conclusion

Theresearchers have paid close attention to the role of lectins in cancer detection, imaging, and treatment. Despite the fact that practical translation of these results remains a major challenge, lectinology is projected to grow even quicker in the next years. New research will almost certainly be required to investigate safe and effective lectin drug delivery system methodologies in order to maximize their use and raise the chance of clinical translation. In reality, lectin mediated inflammation, toxicity and digestive enzymes resistance are just a few of the key concerns against these powerful proteins. Lectins are being studied for their role and/or therapeutic potential in diseases such as HIV, rheumatic heart disease, obesity-induced adipose tissue fibrosis and diabetes, in addition to cancer. As the future of breast cancer treatment lies in a combination of patient-tailored and multi-targeting strategies, we believe that lectin because of their profile of expression and their progressive function will be considered potent molecular targets.

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