International Journal of
Technology and Emerging Research
ISSN: 3068-1995 (Print) | 3068-109X (Online) | https://www.ioro.org/ijter

To Extract Lectin from Selected Fungal and Plant Sources and To Evaluate of Their Hemagglutination and antibacterial activities.

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Abstract

Lectins are carbohydrate- binding proteins that are widely distributed in a variety of biological systems including bacteria, fungi, plants, animals, and humans. These proteins possess the ability to recognize and bind specific carbohydrate molecules through reversible and non-covalent interactions. Unlike enzymes, lectins do not catalyze chemical reactions but instead interact specifically with sugar residues present on glycoproteins, glycolipids, and polysaccharides. Due to their high carbohydrate specificity and their ability to agglutinate erythrocytes, lectins exhibit significant hemagglutinating activity. In general, lectins are considered proteins of non-immune origin that can recognize and bind distinct carbohydrate structures. Because of these properties, lectins have been widely applied in several biological and biomedical fields. Lectins typically contain at least one non-catalytic structural domain known as the Carbohydrate Recognition Domain (CRD). The Carbohydrate Recognition Domain determines the carbohydrate specificity of the lectin molecule and enables interaction with glycoproteins and glycolipids present on the surface of erythrocytes, resulting in visible agglutination. In addition to their hemagglutinating properties, several lectins also exhibit antimicrobial activity. This biological activity is mainly attributed to their ability to interact with carbohydrate components present on microbial cell surfaces. Such interactions may lead to disruption of cell membrane integrity, inhibition of microbial adhesion, or interference with biofilm formation. Due to the increasing demand for economical diagnostic tools and alternative antimicrobial agents, lectins have attracted considerable research interest. The present study was therefore designed to extract and characterize lectins from selected plant and fungal sources and to evaluate their hemagglutination and antibacterial activity. The findings of this study are expected to contribute some in the field of development of natural lectin-based application in medical diagnostics & antimicrobial research.
Keywords: *Lectins*, *Hemagglutination*, *ABO Blood Group Typing*, *Antibacterial Activity*, *Fungal Lectins*, *FTIR Spectroscopy*.

1. Introduction

Proteins are macromolecules composed of one or more chains of amino acids linked together through peptide bonds (21). Among all the diverse classes of proteins “lectin” possess unique characteristic to bind with specific carbohydrate moiety (10).

Lectins occur widely across different forms of life including humans, plants, animals, algae, cyanobacteria, yeasts, mushrooms, and microfungi. (16). In plants, lectins occusrs in various tissues such as such as seeds, leaves, barks, roots, tubers, flower & fruits (8,6). Among fungal organisms, lectins have been reported from several genera including *Penicillium sp.*, *Fusarium sp.*, and *Aspergillus sp.* (31).

The term “lectin” was first introduced in 1954 by William c. Boyd and was derived from the latin word “legere” meaning “to select” (5). Proteins that possess the ability to agglutinate red blood cells (RBCs) based on definrd sugar specificity are referred to as lectins. with its known specificity of sugar referred to as lectins In cases where the sugar specificity is not yet known, these proteins are often described as hemagglutinins (16). Lectins are generally defined as carbohydrate-binding proteins or glycoproteins that interact specifically with carbohydrate structures through reversible and non-covalent interactions (10,8). Plant lectins that demonstrate hemagglutinating activity are commonly referred to as phytohemagglutinins (9).

Structurally, lectins typically contain at least one non-catalytic domain known as the Carbohydrate Recognition Domain (CRDs) (26). This domain determines the type of carbohydrate molecule that the lectin can recognize and bind. The interaction between the CRD and its target carbohydrate resembles a reversible “lock-and-key” mechanism in which the lectin selectively binds to specific sugar residues. Many lectins are multivalent molecules and may possess multiple CRDs (16). As a result, a single lectin molecule can bind simultaneously to several carbohydrate structures present on different cells or molecules. This multivalent binding results in cross-linking of cells and ultimately leads to visible agglutination or precipitation (25, 24).

Lectins exhibit a wide range of biological properties that make them an important subject of scientific investigation. Various studies have reported that lectin possess antitumor, immunomodulatory and antiinsects, antifungal, antibacterial, anti HIV and antigenic activities, analgesic activity, antioxidant, antiproliferative and many more (24, 12, 27). The biological activity of lectins is largely associated with their ability to recognize and bind specific carbohydrate residues such as mannose, galactose, lactose, N-acetylglucosamine, N-acetylgalactosamine, fucose, and rhamnose (12).

By interacting with carbohydrate structures present on glycoproteins, glycolipids, and polysaccharides located on cell surfaces, lectins participate in numerous biological processes including cell–cell communication, immune responses, and host defense mechanisms (4, 25).

Goldstein et al. stated that lectins are carbohydrate- binding proteins or glycoproteins of non immune origin that agglutinate cells. Among their various properties, hemagglutination is one of the most widely studied property of lectins (5, 26). Hemagglutination occurs when lectins bind to carbohydrate residues located on the surface of erythrocytes. Because lectins are multivalent proteins possessing multiple carbohydrate recognition domains, they can simultaneously bind several red blood cells. This results in cross-linking of erythrocytes and formation of visible aggregates. Due to this property, lectins have been explored as potential alternatives to commercial antisera used in ABO blood group typing. Lectin-based systems offer advantages such as natural origin, lower cost, and ease of production. Consequently, lectins have found important applications in laboratory diagnostics.

In addition to their diagnostics value, lectins have attracted considerable researches attention because of their antimicrobial properties (6). Several lectins have been reported to exhibit antibacterial, antifungal, and antiviral activities (15). These effects are primarily attributed to the interaction of lectins with carbohydrate components present on microbial cell surfaces, including those located on the cell wall and cell membrane. Binding of lectins to these carbohydrate structures may interfere with microbial growth, disrupt membrane integrity, inhibit microbial adhesion, or prevent biofilm formation.

Because of these properties, lectins are increasingly being considered as potential natural antimicrobial agents, particularly in the context of rising antibiotic resistance among pathogenic microorganisms. Despite the considerable progress made in lectin research, many aspects of their biological functions and mechanisms of action remain to be explored.

Therefore, the present study was designed to extract and characterize lectins from selected plant and fungal sources and to evaluate their hemagglutination activity as well as their antibacterial properties. The finding of this study may contribute to the development of lectin- based cost effective diagnostic tools and novel natural antimicrobial compounds.

2. Materials and Methods

2.1. Study Design

This experimental study was designed to extract, partially purify, and characterize lectins obtained from selected plant and fungal sources and to evaluate their biological activities like hemagglutination and antibacterial activity. Hemagglutination assays were used to observe lectin potential in ABO blood group typing, and antibacterial activity against selected bacterial culture.

2.2. Biological Materials

Plants including *Lablab purpureus*, *Pisum sativum*, and *Vigna unguiculata* were used. The seeds of these plants were collected from the local market.

Fungal cultures of *Aspergillus niger* and *Penicillium sp*. were obtained from the Microbiology Laboratory of Dolat Usha Institute of Applied Sciences.

Fresh fruiting bodies of Mushroom *Pleurotus ostreatus* were collected from Super Mushroom Farm, Dharampur, and transported to the laboratory for immediate processing.

Human blood samples were collected in EDTA tubes from Riddhi Advance Laboratory, Valsad. Standard clinical procedures were followed for the collection and with the consent. These blood samples (Blood group A, B and O) were used for hemagglutination and blood grouping experiments.

2.3. Culture Media and Reagents

Culture media including Potato Dextrose Agar (PDA), Czapek's Dox broth, Mueller–Hinton agar, and Nutrient broth were used for fungal and bacterial culture. Phosphate buffer saline (PBS, pH 7.2), ammonium sulphate, folin–ciocalteu reagent, alkaline copper reagent, and bovine serum albumin (BSA), normal saline (0.9%) and distilled water were used throughout the experimental procedures.

2.4. Equipment

The experiments were performed using standard laboratory equipment present in microbiological lab including a centrifuge, microcentrifuge, incubator, colorimeter (Equiptronics EQ-650A), micropipettes, magnetic stirrer, analytical weighing balance, grinder, and standard laboratory glassware.

2.5. Extraction of Lectins

2.5.1. Extraction of lectins from Fungal Cultures

Fungal cultures of *Aspergillus niger* and *Penicillium sp.* were initially grown on Potato Dextrose Agar (culture medium) slants at room temperature for 5–7 days to obtain sufficient biomass. Then the developed fungal biomass were transferred into Czapek's Dox broth, a production medium and incubated for 7–10 days under static condition. After incubation, the production broth was filtered using whatsman filter paper, filtrate was collected, centrifuged at 2000 rpm for 15 minutes, supernatant was used as the crude fungal lectin extract (28, 20).

2.5.2. Extraction of lectin from Pleurotus ostreatus

Fresh fruiting bodies of mushroom *Pleurotus ostreatus* were washed thoroughly with distilled water. Approximately 20 g of mushroom tissue was taken and cut into small pieces and then homogenized in phosphate buffer saline (PBS). The homogenate was filtered through muslin cloth filtrate was collected, centrifuged at 2000 rpm for 15 minutes, supernatant was used as the crude fungal lectin extract (29,16).

2.5.3. Extraction lectins from Plant Seeds

Plant sources for lectin *Lablab purpureus*, *Pisum sativum*, and *Vigna unguiculata* were collected from market. Peel were removed and seeds are collected. Seed were then washed and ground into a fine paste using a grinder. This paste were mixed in some amount of phosphate buffer. The mixture was filtered through muslin cloth and filtrate was collected, centrifuged it at 2000 rpm for 15 minutes, The supernatant obtained was collected as crude plant lectin extract (8).

2.6. Partial Purification of Lectin

Partial purification of lectins was performed by ammonium sulphate precipitation. Solid ammonium sulphate was measured according to the how much lectin had and how much saturation is needed. Then ammonium sulphate was gradually added to the crude extract with continuous stirring. The mixture was incubated in refrigerator at 4℃ overnight. Next day the sample was centrifuged , mixture was collected and resuspended it with phosphate buffer. Filled it in dialysis bag and placed it on magnetic stirrer in PBS for 24 hours. (23).

2.7. Protein Estimation

The protein content of lectin extracts was determined by using the Folin–Lowry method. Bovine serum albumin (BSA) was used as the standard protein. Different dilution of lectin was prepared. Then were treated with alkaline copper reagent after incubated for 15 minutes followed by Folin–Ciocalteu reagent, incubate it at room temperature for 30 minutes to develop color. The blank was set and absorbance of all the tubes were measured at 750 nm using a colorimeter, and protein concentration were calculated from the standard curve prepared using BSA (18).

2.8. Hemagglutination Assay

Hemagglutination activity of lectin extracts were done by using washed human red blood cells (RBCs). The RBCs were washed approx. three times with normal saline by centrifugation at 1500 rpm for 5 minutes. A 2% RBC suspension was prepared. Serial two-fold dilutions of lectin samples were prepared in microtiter plates by using 0.9% normal saline and equal volumes of RBC suspension were added to each well. The plates were incubated at room temperature for 60 minutes. Hemagglutination assay result were observed visually, and recorded as the hemagglutination titer (28, 5).

2.9. ABO Blood Group Typing

For blood group identification, blood sample from individuals with known ABO blood groups were collected. Drop of blood and a drop of lectin were mixed on a clear slide and observed the agglutination reactions were observed. The results were recorded to determine the specificity of lectin extracts for different ABO blood group antigens (5,14).

2.10. Antibacterial Activity

The antibacterial activity of lectin extracts were evaluated using disc diffusion method i.e Kirby–Bauer disc diffusion method (3). The different bacterial cultures were inoculated into nutrient broth and incubated at 37℃ until visible turbity is observed. The bacterial suspension was swabbed uniformly on Mueller–Hinton

agar plates using a sterile cotton swab. Sterile filter paper discs (6 mm diameter) which was impregnated with lectin extracts were placed on the agar surface. PBS was used as a negative control. The plates were incubated in the incubator at 37°C for 24 hours. After incubation, the plates were examined and measured for zones of inhibition.

2.11. Fourier Transform Infrared (FTIR) spectroscopy

FTIR of the crude lectin sample was performed at the Center of Excellence to obtain the infrared spectrum and confirm the presence of characteristic functional groups.

2.12. Data Analysis

Protein concentration, hemagglutination titers, antibacterial inhibition zones and result of FTIR were recorded and comparison is done to evaluate the biological activity of the extracted lectin.

3. Results and Discussion

3.1. Extraction of Lectins from Different Biological Sources

Lectin was extracted from six different biological sources including fungal species (*Aspergillus niger*, *Penicillium* sp., *Pleurotus ostreatus*) and plant sources (*Lablab purpureus*, *Pisum sativum*, and *Vigna unguiculata*). To confirm lectin activity, blood grouping experiments were performed based on lectins as a hemagglutinin and agglutinate erythrocyte by binding with its specific carbohydrate present on the surface of RBCs.

3.2. Lectin Activity of Crude Extracts

Hemagglutination activity were performed to evaluate the lectin activity present in the extracts obtained from the different fungal and plant sources. Lectin activity was checked by adding drop of crude lectin of different soucres in different blood group on clean grease free slide.

The lectin activity of crude extracts was confirm of the fungal and plants extract by ABO blood group typing by the visible agglutination of blood samples as shown in figure 3.1.

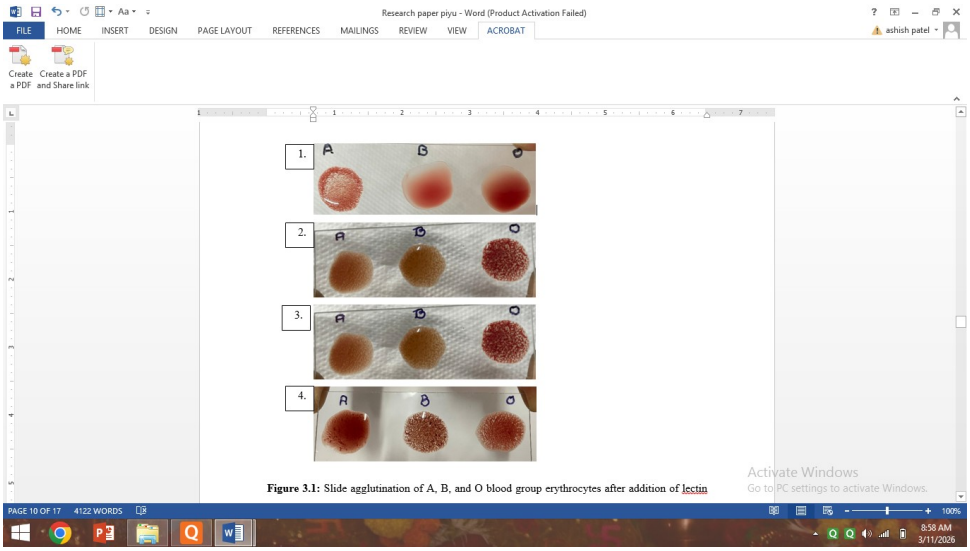


Figure 3.1: Slide showing ABO blood group typing of the extracted lectin. Panels (1-4) shows agglutination patterns of different lectins ANL, POL, LPL, and PSL respectively with different blood groups.

The results interpreted from the above experiment, that the lectin activity was successfully detected from the extracts collected from *Aspergillus niger*, *Pleurotus ostreatus*, *Lablab purpureus*, and *Pisum sativum* (8,20,21). But, extracts collected from *Penicillium* sp. and *Vigna unguiculata* did not show any detectable hemagglutination activity. The absence of detectable lectin activity may be attributed to the low lectin concentration in the crude extract,or due to growth conditions which is optimum for the growth for lectin was not obtained, or may be lectin activity is loss during the extraction and purification steps. Sharon & Lis have reported that lectin production can vary significantly depending on which species used, environmental conditions or optimum condition, and extraction procedures used for the downstream process (25).

Based on these observations, four lectin samples showing lectin activity were selected for further study are as follows:

- ANL – Lectin extracted from *Aspergillus niger* (Fungal source)
- POL – Lectin extracted from *Pleurotus ostreatus* (Fungi- mushroom source)
- LPL – Lectin extracted from *Lablab purpureus* (Plant seed source)
- PSL – Lectin extracted from *Pisum sativum* (Plant seed source)

These lectin extracts were subsequently used for further analysis for testing to evaluate its hemagglutination specificity, blood group recognition, antibacterial activity, and structural characterization.

3.3. Hemagglutination Activity of Extracted Lectins

The hemagglutination assay was performed using red blood cells of different ABO blood groups in order to determine the specificity of the lectin samples toward blood group antigens (5, 25). The assay revealed distinct agglutination patterns among the different lectins, indicating differences in carbohydrate recognition properties.

The lectin extracted from *Aspergillus niger* (ANL) shows selective hemagglutination toward blood group A and no visible agglutination was observed with the blood group B and O. These results suggest that lectin ANL exhibits binding affinity to carbohydrate structures associated with antigen A present on erythrocyte A membrane and not toward erythrocyte B and O (33).

Similarly, the lectin extracted from *Pleurotus ostreatus* (POL) shows selective agglutination toward blood group B and no visible agglutination was observed with the blood group A and O. So, we can interpret that lectin POL may have binding affinity to carbohydrate structures associated with antigen B present on erythrocyte B cell membrane and not toward erythrocyte A and O. This result suggests that the fungal lectins may have the ability for selective carbohydrate recognition domain (CRDs) for recoginition of specific carbohydrate residue on the surface of particular erythrocyte.

In the contradict, the lectins extracted from plant sources *Lablab purpureus* (LPL) and *Pisum sativum* (PSL), shows broad range of hemagglutination activity. Both LPL and PSL shows agglutination toward all type of blood groups A, B, and O (8, 10). This indicates that these lectins are non specific toward particular blood group antigen' carbohydrate and more likely recognize carbohydrate structures that are commonly present on the surface of human red blood cells.

The hemagglutination reactions were visually confirmed using U shaped microtiter plates by Hemagglutination assay (25). If the diffuse lattice across the bottom of the well is observed then its means a positive reaction, lectin have recognize the carbohydrate residue present on the surface of erythrocyte, formed a cross- linking of cells by binding with multiple RBCs at a time. If the compact button at the cell of the

well observed, its indicate that the lectin doesn't bind with the carbohydrate present on the RBCs and RBCs are settled down in well. These observation clearly suggests that the presence of lectin activity and provided clarification of the extracted lectin's specificity.

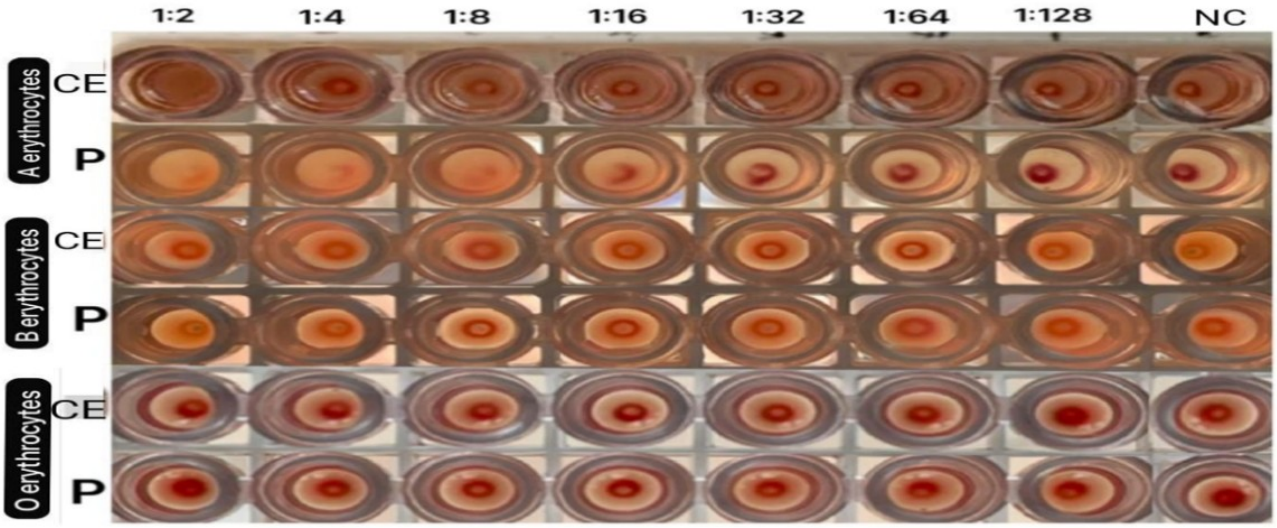
The patterns observed from the above experiment, These results highlight that lectin in context to carbohydrate- binding property are very diverse in nature. Lectins that can recognize and bind to specific carbohydrate present on particular antigen of RBCs, it may be used as blood group typing and have potential application in diagnostic assays. Lectins that agglutinate multiple blood groups may interact with the more common carbohydrate residue present on cell surfaces.

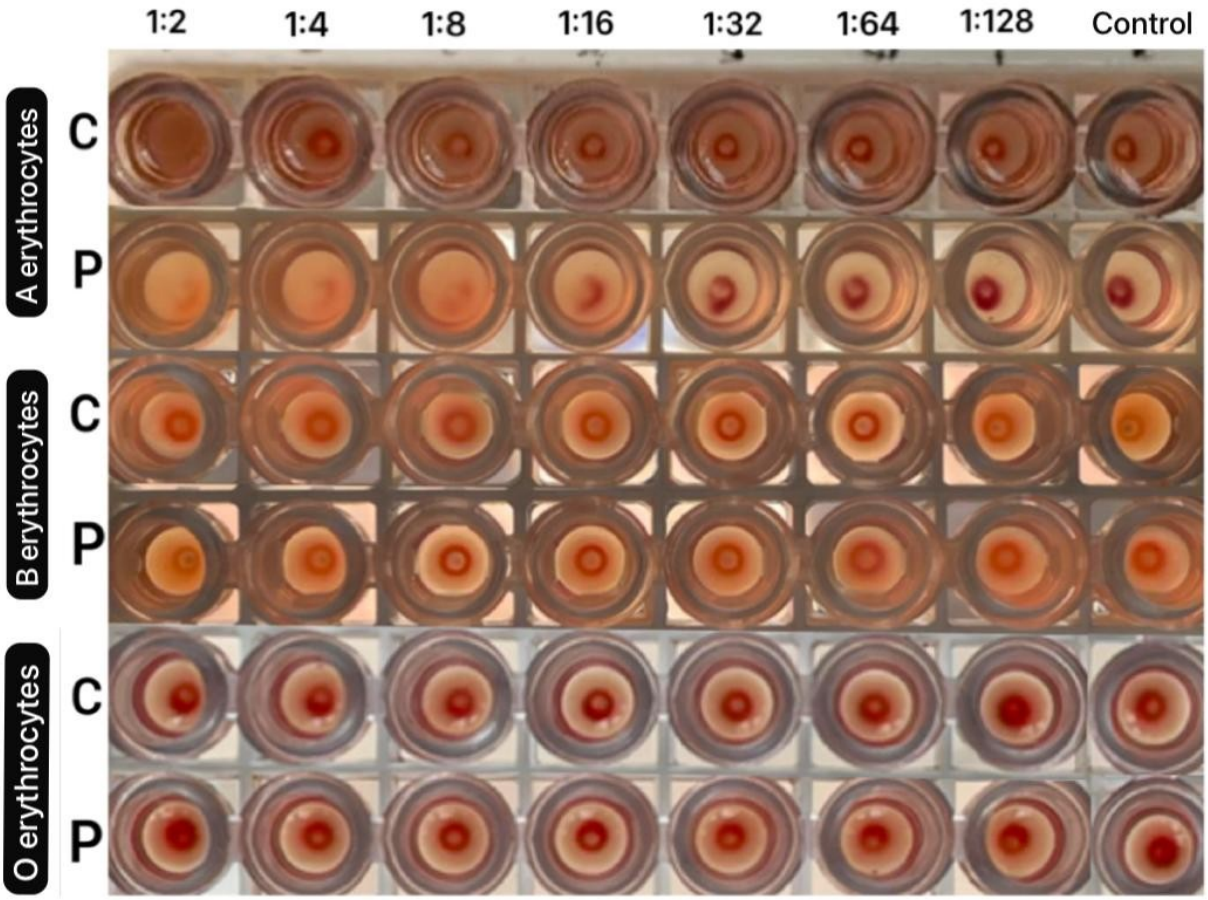
The ABO blood group typing of the extrated lectins were confirmed by the visible agglutination of bloods as shown in figure 3.1. and the result of Hemagglutination assay observed in microtitre plate well are shown in figure 3.2.

The hemagglutination patterns observed for each lectin sample are summarized below:

Table 3.1: Hemagglutination specificity of lectin samples toward different ABO blood groups

Lectin Sample	Source Organism	Hemagglutination Specificity
ANL	*Aspergillus niger*	Agglutinates Blood Group A
POL	*Pleurotus ostreatus*	Agglutinates Blood Group B
LPL	*Lablab purpureus*	Agglutinates A, B and O
PSL	*Pisum sativum*	Agglutinates A, B and O

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1:4
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1:32
1:64
1:128
Control
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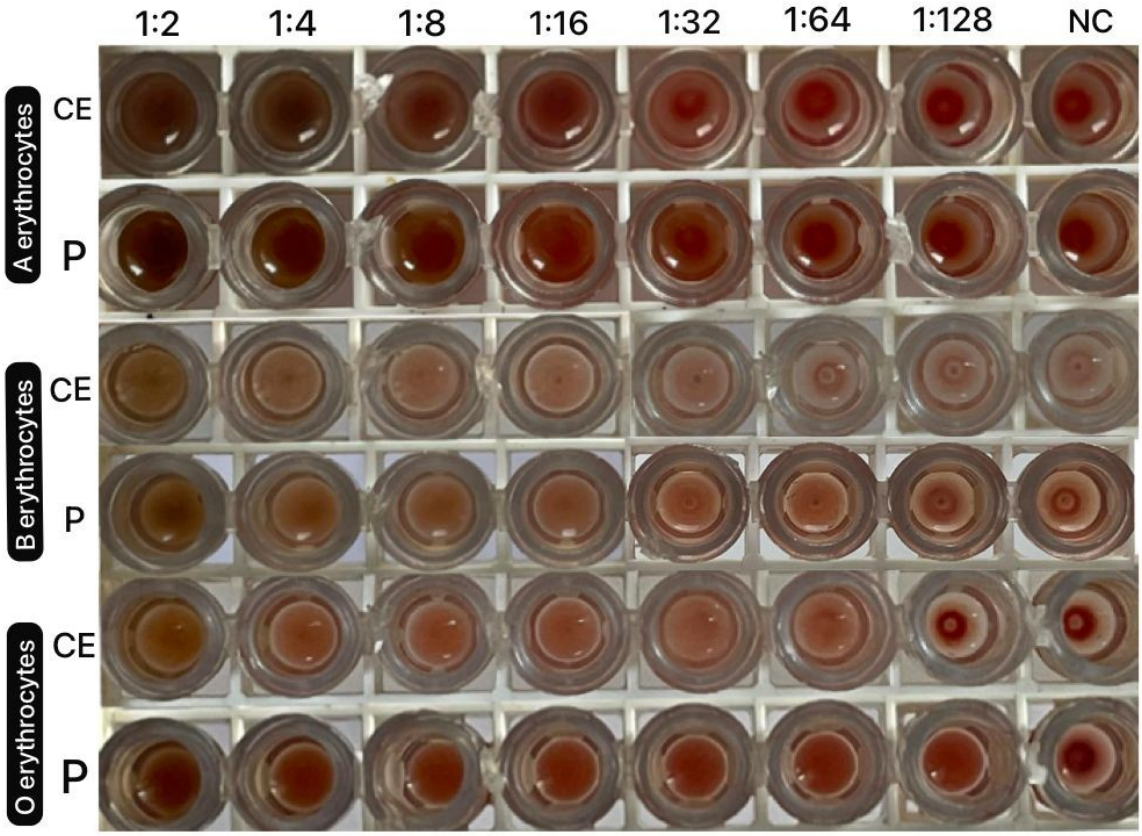
C
P
B erythrocytes
C
P
O erythrocytes
C
P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CE
P
B erythrocytes
CE
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O erythrocytes
CE
P

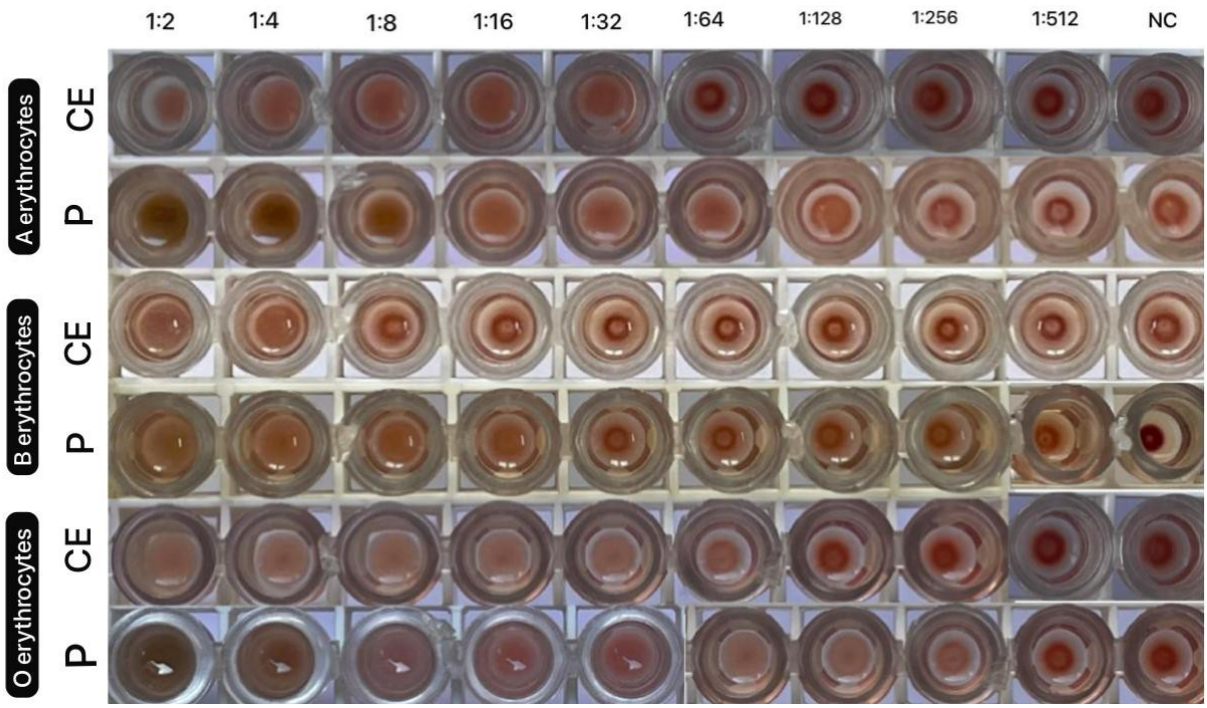


Figure 3.2: Hemagglutination activity of lectins extracted from different microbial sources. (A-B) ANL, POL, LPL, & PSL respectively. Positive result indicates by diffuse mat and negative was indicated by compact button.

Table 3.2. Purification and hemagglutination activity profile of lectins obtained from ANL, POL, LPL & PSL.

Lectins	Blood type	Purification step	Volume (ml)	HA activity (Titre/ml)	Total HA activity (Titre)	Protein conc. (mg/ml)	Total protein (mg)	Specific activity (Titre /mg)	Fold purification	Yield (%)
ANL	A	Crude	30	2	60	0.58	17.4	3.44	1	100
		Purified	5	8	40	0.93	4.65	8.60	2.50	66.67
POL	B	Crude	30	4	120	1.93	59.4	2.02	1	100
		Purified	5	16	80	2.66	13.3	6.01	2.97	66.67
LPL	A	Crude	30	16	480	0.92	27.6	17.39	1	100
		Purified	5	32	160	0.39	1.95	82.05	4.72	33.33
	B	Crude	30	4	120	0.92	27.6	4.35	1	100
		Purified	5	16	80	0.39	1.95	41.02	9.43	66.67
	O	Crude	30	64	1920	0.92	27.6	2.32	1	100
		Purified	5	128	640	0.39	1.95	65.64	28.28	33.33
PSL	A	Crude	30	32	960	1.17	35.1	27.35	1	100
		Purified	5	128	640	1.92	9.6	66.67	2.44	66.67
	B	Crude	30	4	120	1.17	35.1	3.44	1	100
		Purified	5	16	80	1.92	9.6	8.33	2.42	66.67
	O	Crude	30	64	1920	1.17	35.1	54.70	1	100
		Purified	5	256	1280	1.92	9.6	133.33	2.44	66.67

3.4. Antibacterial Activity of Extracted Lectin

In addition to their hemagglutination properties, lectins also have others properties from which antibacterial activity attracted many. The antibacterial property of lectins is due to their ability to bind to the carbohydrate

residue present on microbial cell surfaces and block them to bind with targeted cells. Here, antibacterial activity of the extracted lectins was evaluated by using disc diffusion method Kirby- Bauer technique (3).

The zone is observed and measured, among all the lectins extract tested ANL (*Aspergillus niger* lectin) shows a strongest antibacterial activity. This means ANL may have ability to bind with carbohydrate residue present on bacterial CWs or CMs, which eventually leads to interference in growth of bacteria and thereby inhibiting bacterial survival.

The lectin extract POL (*Pleurotus ostreatus* lectin) a zone of inhibition was observed against *Pseudomonas aeroginosa (P.a.)* shows selective antibacterial activity toward it (29). POL doesn't show and zone of inhibition against other bacterial cultures. This selective inhibition observed may be due to different carbohydrate residue or composition present on bacterial cell surfaces. It can be said that POL have a CRDs only specific to a carbohydrate present on *P.a.* surface.

The lectin extract PSL (*Pisum sativum* lectin) shows an antibacterial activity toward some bacterial strains, zone of inhibition is observed in some bacterial species. As compare to ANL, in the PSL zone of inhibition is less observed, however, the antibacterial effect was weaker compared with ANL but its still have antibacterial property (10).

In the contrast, the lectin extract LPL (*Lablab purpureus*) did not show any detectable antibacterial activity against selected bacterial cultures at the tested condition. There was no clear zones of inhibition were observed. Interestingly, despite having its strong hemagglutination ability toward all blood groups (A, B or O blood group), but LPL did not show any antimicrobial effects toward selected bacterial culture. So, clearly stated that the ability of lectins to agglutinate RBCs does not interfere with the antibacterial property of lectins.

From the above finding, we have interpreted that all the lectin varies from each other in the antibacterial property. The variation in antibacterial activity among the lectin samples increase the need to study more about lectin structure and carbohydrate- binding specificity in determining in biological activity.

These observations indicate that lectins extracted from different biological sources vary in their antimicrobial properties. The antibacterial activity of the extracted lectins is illustrated in table 3.2, where clear zones of inhibition indicate suppression of bacterial growth.

Table 3.2 Antibacterial Activity of ANL, POL, LPL and PSL

Test Organism	**Gram Reaction**	**Zone of Inhibition (mm)**			
		ANL	**POL**	**LPL**	**PSL**
Escherichia coli	Gram-negative	4	-	-	-
Proteus vulgaris	Gram-negative	5	-	-	6
Pseudomonas aeruginosa	Gram-negative	6	5	-	-
Klebsiella pneumoniae	Gram-negative	-	-	-	-
Salmonella sp.	Gram-	4	-	-	-

(Green colour colonies)	negative				
Salmonella sp. (Brown colour colonies)	Gram-negative	4	-	-	-
Bacillus subtilis	Gram-positive	-	-	-	4
Bacillus cereus	Gram-positive	-	-	-	>4

3.5. FTIR Analysis of Lectin Samples

Fourier Transform Infrared (FTIR) Spectroscopy is an analytical technique which is used to identify functional groups and chemical bonds present in a molecule by measuring the absorption of infrared radiation (IR). Further characterization of lectin was performed by using FTIR. FTIR analysis of the crude lectin samples was carried out at the Center of Excellence. The spectra was obtained which shows the characteristic absorption peaks corresponding to functional groups typically present in protein molecules.

The functional group region (4000-1500 cm^{-1}) is useful for identification of major functional groups present in sample.

Table 3.3: Interpretation of the FTIR Graph

Range (cm^{-1})	Functional Group	Interpretation	References
3700-3200	O-H stretching	Indicates presence of alcohols, phenols and hydrogen-bounded molecules	Barth, 2007
3500-3300	N-H stretching	Indicates presence of amine or amide (amide II group)	Kong & Yu, 2007
3000-2850	C-H stretching	Indicates aliphatic compounds	Movasaghi et al., 2008
1700-1600	C=O stretching	Indicates presence of amide I band	Barth, 2007

In this FTIR spectrum, 3435 cm^{-1} corresponds to O-H stretching , the presence of hydroxyl groups indicating that the compound is alcohols or phenolic (7). And for protein- related functional groups where detected by 1637 cm^{-1} corresponds to C=C stretching vibration or amide I band (2). The presence of absorption bands related to amide confirmed the proteinaceous nature of the lectin samples.

Although the FTIR spectrum of the lectin sample didn't prove that the given sample is lectin but it did revealed characteristic absorption peaks of the sample ANL, POL, LPL, and PSL corresponding to functional groups typically associated with protein structures (Figure 3.3.1-3.3.4).

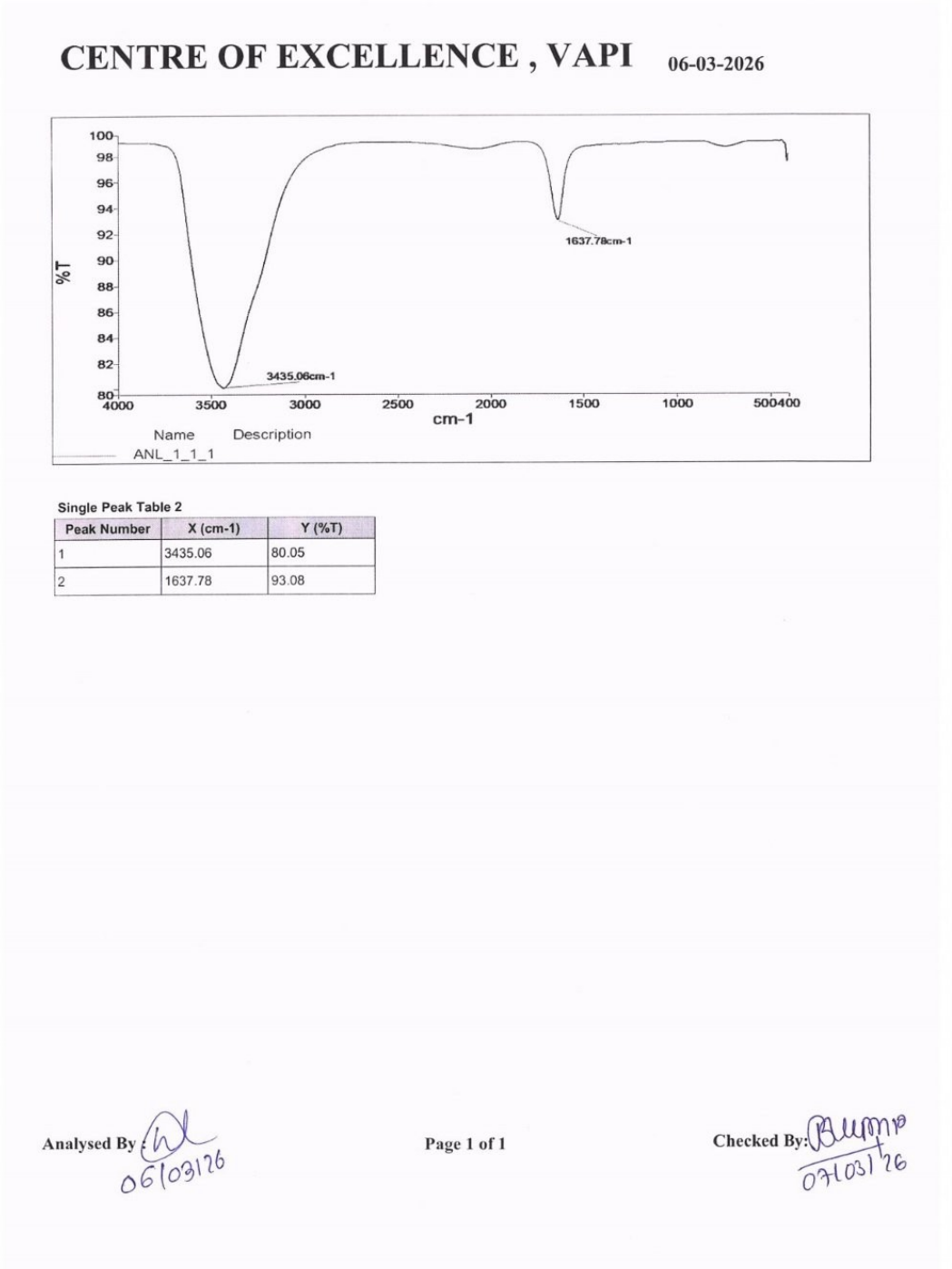


Figure 3.3.1: Result of the FTIR analysis of ANL

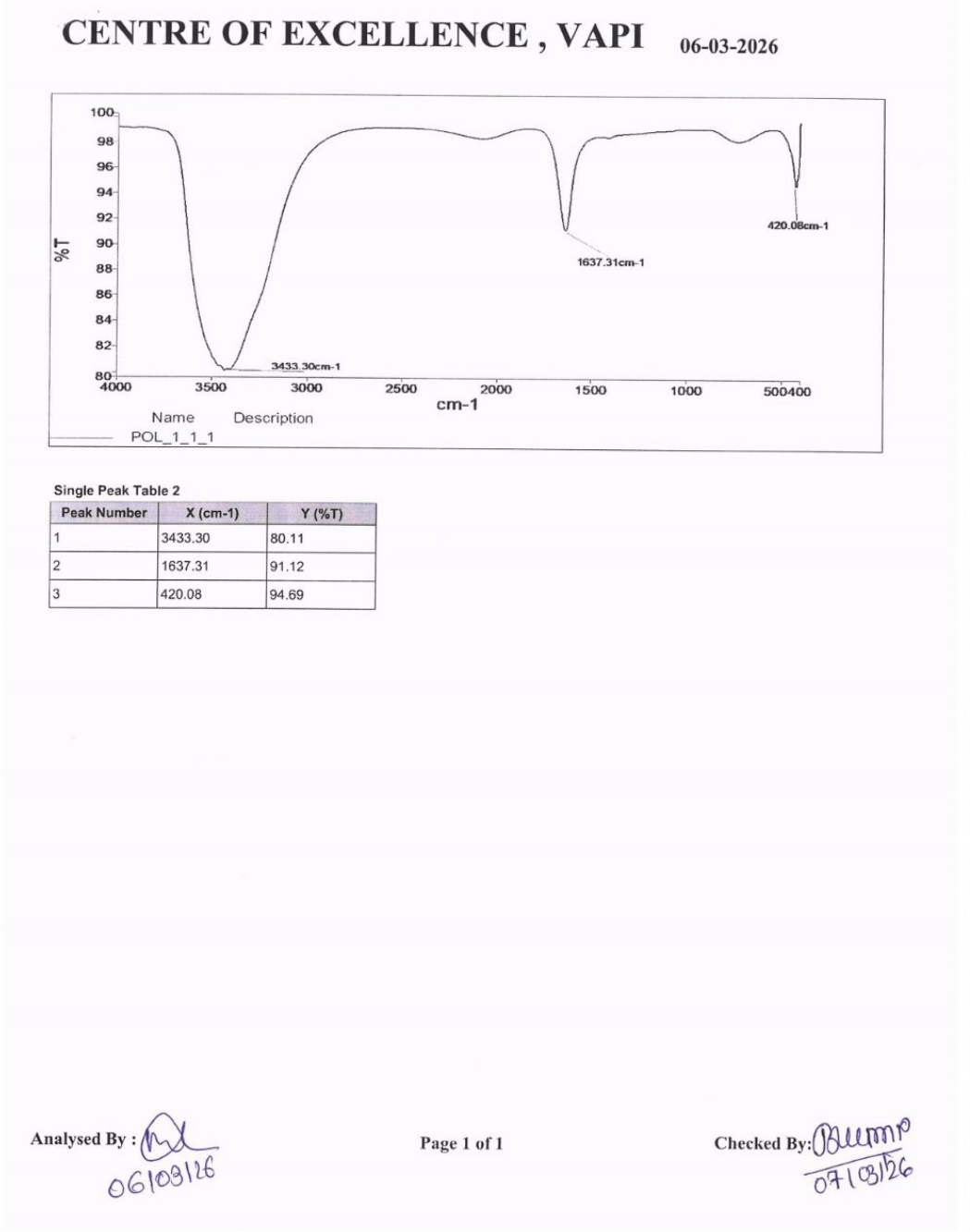
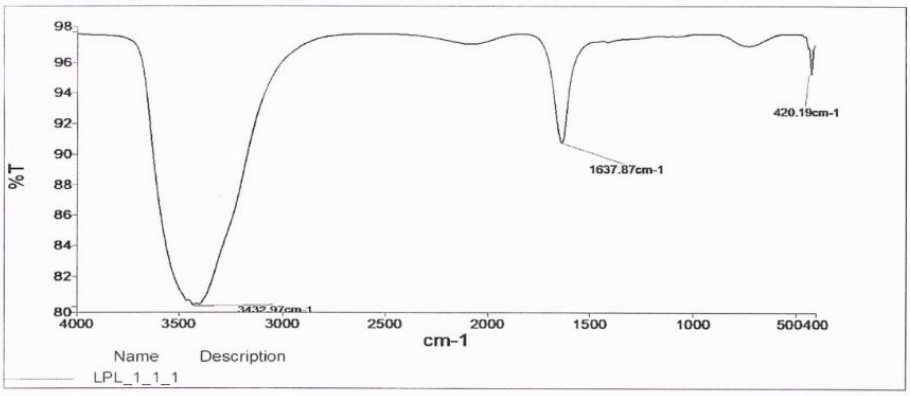


Figure 3.3.2: Result of the FTIR analysis of POL

CENTRE OF EXCELLENCE , VAPI 06-03-2026



Single Peak Table 2

Peak Number	X (cm-1)	Y (%T)
1	3432.97	80.08
2	1637.87	90.80
3	420.19	95.31

Analysed By : 06/03/26

Page 1 of 1

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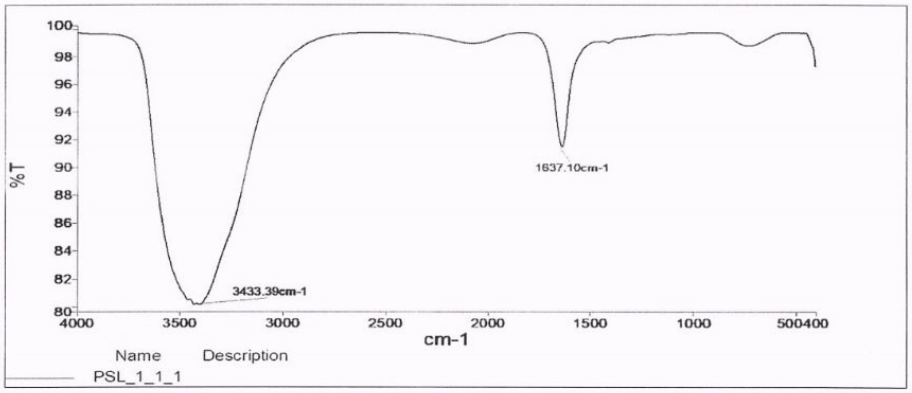
Figure 3.3.3: Result of the FTIR analysis of LPL

CENTRE OF EXCELLENCE , VAPI 06-03-2026



Single Peak Table 2

Peak Number	X (cm-1)	Y (%T)
1	3433.39	80.13
2	1637.10	91.51

Analysed By : 06/03/26

Page 1 of 1

Checked By: 07/03/26

Figure 3.3.4: Result of the FTIR analysis of PSL

Figure 3.3.1-3.3.4, Fourier Transform Infrared (FTIR) spectrum of the lectin sample showing characteristic functional group peaks.

Table 3.4: Result and interpretation of the tested sample ANL, POL, LPL, and PSL

Sr. No.	Graph	Peak Position (cm^{-1})	Funtional Group	Type of Vibration	Protein Band	Interpretation
1.	ANL	3435	N-H / O-H	Stretching	Amide A	This represent the peptide bond and confirms presence of protein functional group
		1637	C=O	Stretching	Amide I	This means the carbonyl group is presence. Amide I band is the most important indicator of protein secondary structure.
2.	POL	3433	N-H	Stretching	Amide A	Indicates peptide bond present in molecules.
		1637	C=O	Stretching	Amide I	Means carbonyl group is present
		420	Skeletal vibration	Bending	-	This low frequency vibration may indicate structural interactions between the protein and surrounding matrix or polymer structure.
3.	LPL	3432	N-H	Stretching	Amide A	Indicates peptide bond present in molecules.
		1637	C=O	Stretching	Amide I	Means carbonyl group is present
		420	Skeletal vibration	Bending	-	This low frequency vibration may indicate structural interactions between the protein and surrounding matrix or polymer structure.
4.	PSL	3433	N-H / O-H	Stretching	Amide A	Indicates peptide bond present in molecules.
		1637	C=O	Stretching	Amide I	Means carbonyl group is present

4. Conclusion

This study investigated the extraction and characterization of lectins from selected fungal and plant sources and evaluated their hemagglutination and antibacterial activities. Lectins were successfully extracted from

Aspergillus niger, Pleurotus ostreatus, Lablab purpureus, and Pisum sativum samples are as ANL, POL, LPL, and PSL respectively whereas no detectable lectin activity was observed in the lectin of Penicillium sp. and Vigna unguiculata under the experimental conditions used in this study.

Hemagglutination assays were performed on all successfully extracted lectins. The results clearly indicated that all the lectins have distinct hemagglutination patterns. ANL showed specificity toward blood type A, while POL shows selective agglutination with blood type B. But in contrast, plant lectins LPL and PSL shows agglutination to all blood type A, B and O.

The result of antibacterial activity and comparative study of this, revealed that the ANL have antibacterial property against many bacterial cultures, suggesting that it possess antimicrobial potential. Whereas POL shows antibacterial against one organism *Pseudomonas aeruginosa* among the selected bacterial culture. The plant lectin PSL exhibited moderate antibacterial activity against some tested bacteria but the LPL did not show any antibacterial activity against tested bacterial culture under the tested condition. FTIR analysis confirmed the presence of functional groups associated with protein molecules, which means its confirming the proteinaceous nature of the isolated lectins.

Overall, the findings of this study highlight the biological significance of microbial lectins and their potential applications in blood group recognition and antimicrobial research. Further studies focusing on purification, structural characterization, and molecular analysis may provide deeper insights into their mechanisms and potential biomedical applications.

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