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Determination of Insecticidal Potential of Lentil (*Lens culinaris* L.) Lectin through Bio-computational Tools

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plant and animals are the chief source of lectins playing important role in defense system against various pathogens. Lentil (*Lens culinaris*) lectin (LL) binds to polysaccharides and other glycoconjugates in a reversibly manner which contain glucose or mannose type sugars. In present investigation, the insecticidal potential of LL is determined through *In-silico* study by using various bio-computational tools against mustard aphid. The physico-chemical property of LL also exhibited theoretical pI is 5.22, GRAVY value is -0.086, aliphatic index is 78.33 and instability index is 27.51. The functional domain exposure of LL showed metal interacting and N-linked glycosylated amino acid residues. The structural behavior and biological activity of LL protein is determined by secondary structure prediction and its other attributes. Present investigation explores the potential of insecticidal activity of lentil lectin against mustard aphid by using numerous *In silico* approaches. This probably helps in understanding of LL three dimensional protein structures.

Keywords: Legume lectins; functional domain; molecular docking; molecular modeling and Insecticidal potential.

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1. INTRODUCTION

The role of different economic insect and pest towards increasing the yield and production of crops cannot be neglected. These insects and pests not only causes severe physiological damage to the crops plants but also acting as vectors for transmitting viruses. Extensive research has been done to control these economic losses occurred through insect and pest. But, in the present scenario, the insecticidal toxins produced by transgenic plants through ectopic expression are derived from *Bacillus thuringiensis* (Bt). These Bt toxins are much effective against insects of lepidopteran and coleopteran class [1] and less or no effective against insects feed upon phloem sap such as bugs, hoppers or aphids [2]. Therefore, development of resistance against such insect need alternative toxin then Bt. Plant lectins are the most promising candidate proteins containing insecticidal activity against these insect's pests. The insecticidal potential of plant lectins exhibit enormous potential towards different group of insect species falls in Coleoptera, Homoptera, Diptera, Hemiptera and Lepidoptera order is well known [3].

In the present study, *In-silico* analysis revealed LL gene found in genome of lentil (*Lens culinaris* L.) exhibited homology with earlier reported lectins genes having insecticidal activity. The results of this findings also revealed that the protein contain a conserved mannose binding region which allow this lectins to be categorised into a superfamily of mannose-binding monocot lectins [4].

2. MATERIALS AND METHODS

2.1 Retrieval of Nucleotide Sequence and Primary Structure Prediction

In order to sequence analysis of LL protein, the sequence of amino acid from lentil lectin (LL) protein (accession #KU382474.1) was withdrawn from NCBI database. The InterPro server tool (www.ebi.ac.uk/interpro/) was run for functional domains characterization. Furthermore, various physico-chemical parameters such as amino acid composition, molecular weight (Mw), (Isoelectric point) pI, half-life of protein and instability index estimated using Protparam.

2.2 Secondary Structure Prediction and Homology-based Modelling

The *In silico* tool like, RaptorX (http://raptorx.uchicago.edu/Structure Prediction /predict/) were employed to predict the secondary structure and solvent accessibility of LL. Furthermore, the SWISS Model (https://swissmodel.expasy.org/) was employ to predict the three dimensional structure of LL protein based on homology modelling. Further, the authenticity of the predicted models was verified by ramachandran plot generated through by employing RAMPAGE tool (http://mordred.bioc.cam.ac.uk/~rapper/rampage. php).

2.3 Molecular Docking of LL Protein with APN Receptor

LIGPLOT online server automatically exhibits protein-ligand interactions from the built model for active site mapping [5]. The amino acid residues involve in ligand and receptor interaction were identified through PDBSum tool (http://www.ebi.ac.uk/pdbs um). Identified amino acids also interact with different sugar moieties for glycosylation. The Aminopeptidase N of *Acyrthosiphon pisum* (Accession # DQ440823), serve as template as receptor for LL protein. The molecular docking studies were performed by using ClusPro Docking server (http://cluspro.bu.edu/). The results were analysed under Discovery Studio 4.1 visualizer. The different amino acids from protein of LL which forming the cleft area were also identified by using same server.

3. RESULTS AND DISCUSSION

3.1 *In silico* Characterization of LL Protein

LL protein sequence were analysed through InterPro server for functional domain study which revealed the N-linked glycosylation site and metal interacting sites (Fig. 1). The residue annotation ASP^{151}, PHE^{153} and ASP^{159} were identified metal binding sites of LL along with their position in the polypeptide chain. The existence of different metal ions i.e., Mn^{2+} and Ca^{2+} in legume lectins determines their biological activity [6]. Similarly, the residue ASN^{135} of LL was identified as important for N-linked glycosylation. The computed pI value 5.22 (pI<7) of LL exhibited its amino acidic nature. The stability of this protein were explained by

instability index which is 27.51 results protein fall under stable group. The thermo stability under varying environmental conditions regulated through aliphatic index which is 78.33 for LL protein. Similarly, Gupta et al. [7] also reported and explain the optimum thermo stability of other lectin protein. GRAVY value for LL outcomes as -0.086, showed the probability of hydrophilic nature of LL protein that endorsed to charged amino acid residues (21 positively charged and 28 negatively charged residues), signifying about LL protein which may be associated as extrinsically in plasma-membrane. The same results were reported by Prajapat et al. [8] for *Cajanus cajan* lectin protein against APN receptor of *Acyrthosiphon pisum*.

3.2 Structural Analysis and Homology-based Modelling

Nearly 6% α helices, 41% of β pleated sheets and 51% of Coil were found in the secondary structure of protein chain through RaptorX prediction tool (Fig. 2A). It also exhibited protein solvent accessibility as 26% amino acids are underlying into structure, exposed residues were approximately 33% and 40% were medium as shown in Fig. 2B. Legume lectins intrinsically varied in 0–10% α-helix, 40–50% β-sheet and 35–45% β-turn and therefore form a structurally diverse class. The 3D structure of lentil lectin was predicted by SWISSMODEL online server by means of pea lectin (ID-2bqp.1) as template with identity of 89.70% and coverage of 85% (Fig. 3). The protein model for APN with identity of 30.59% and coverage of 89% was predicted for APN1 of *Anopheles gambiae* with same server as shown in Fig. 4. The RAMPAGE server finally verified the predicted 3D models. To access the reliability of predicted protein model, torsion angles ψ and ϕ were examined. The results exhibited during validation confirm that 94.7% of amino acids were present in favoured region and 6.1% of amino acid are present in allowed region (Fig 5). The rationale of APN shown 93.5% of amino acid are identified in most favoured region, while 4.7% and 0.2% of amino acid are present in allowed and outlier region as represented in Fig 5. The same results were reported by Prajapat et al. [9] for *Cajanus cajan* lectin protein toward APN receptor from *Acyrthosiphon pisum*.

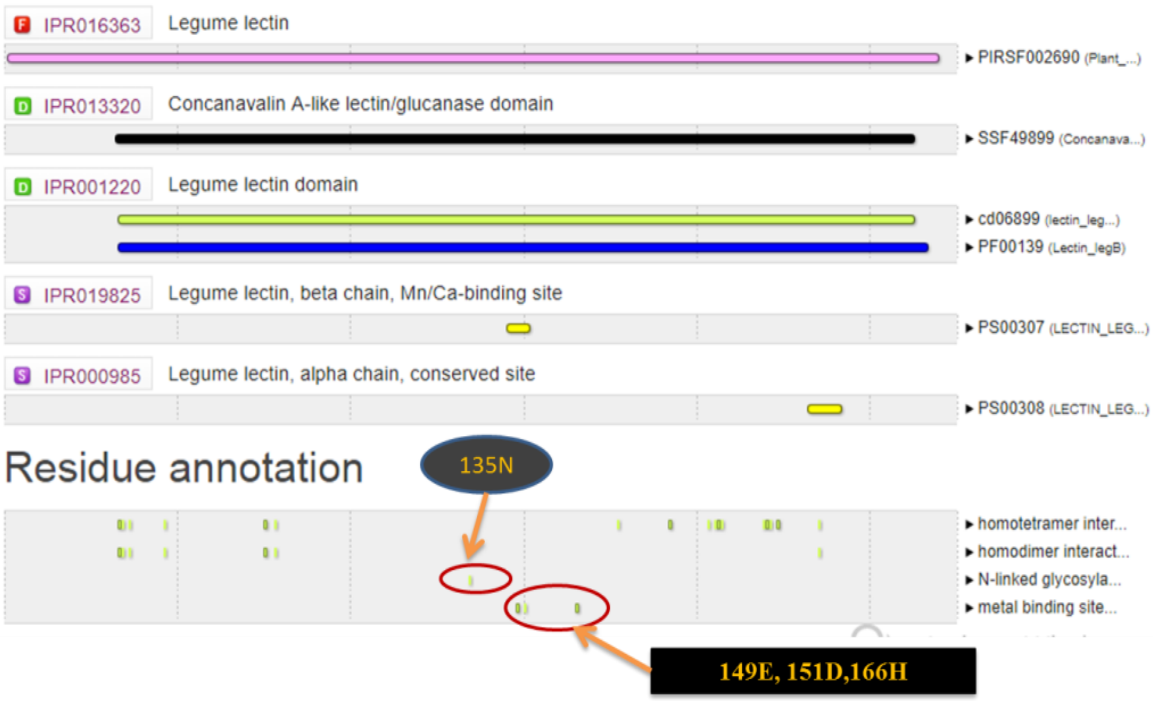

Fig. 1. Metal binding and N-linked glycosylation domains analysis of LL protein

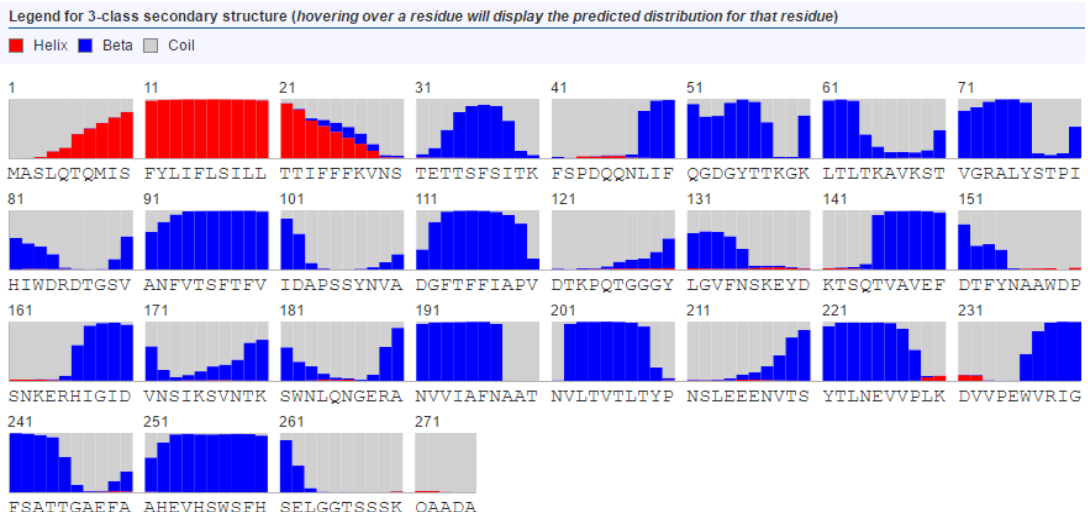

Fig. 2A. Secondary structure content from individual amino acid residue of LL Protein

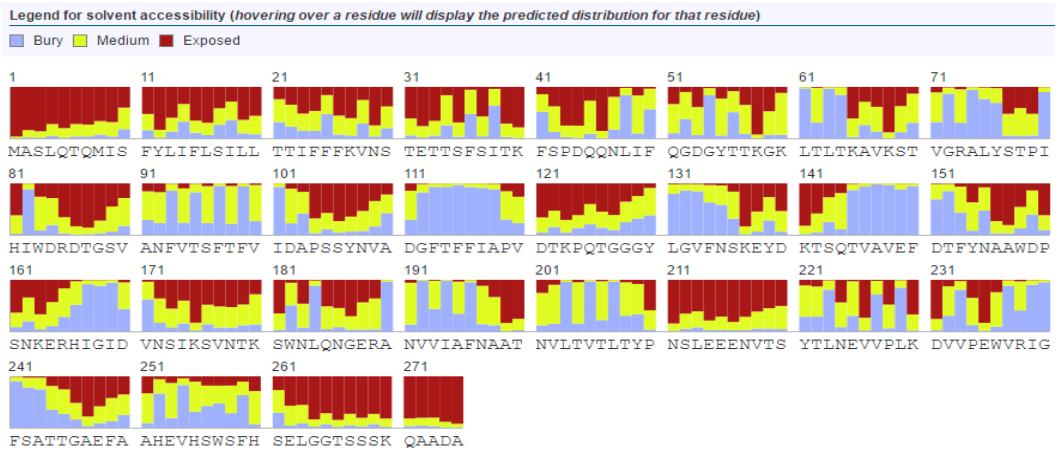

Fig. 2B. Solvent accessibility of individual amino acid from LL protein

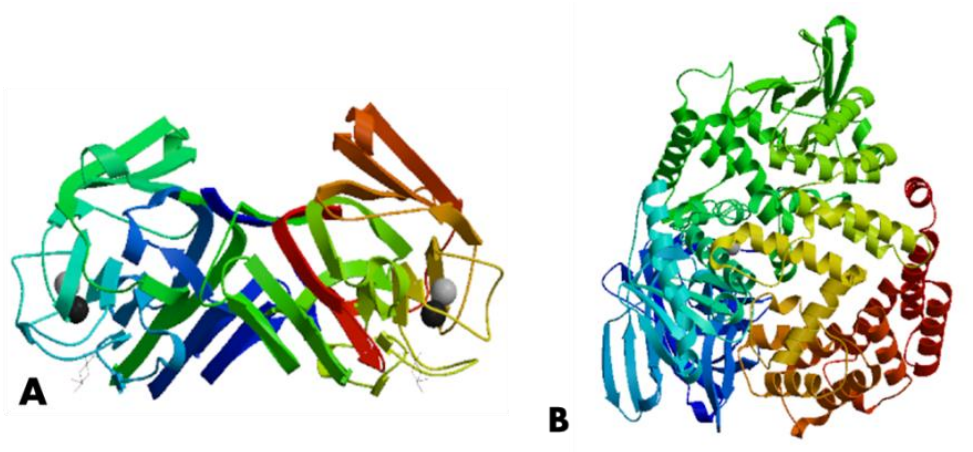

Fig. 3. Protein model structure of (A)LL protein (B) APN receptor

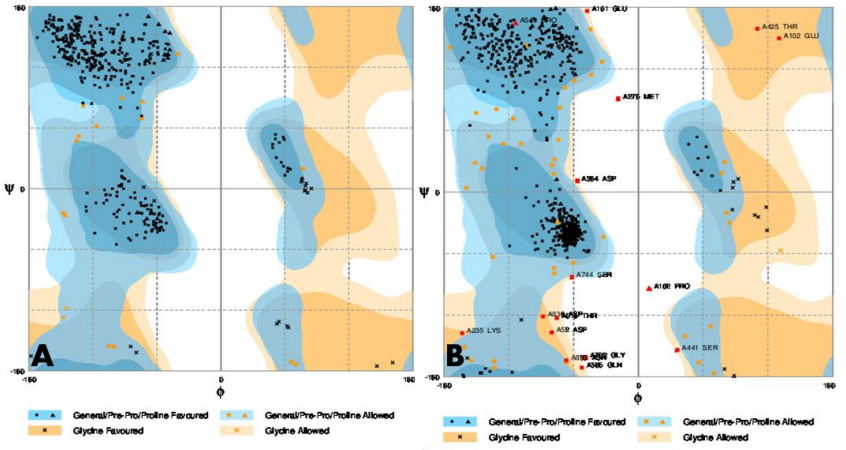

Fig. 4. RAMPAGE analysis of (A) LL protein (B) APN receptor

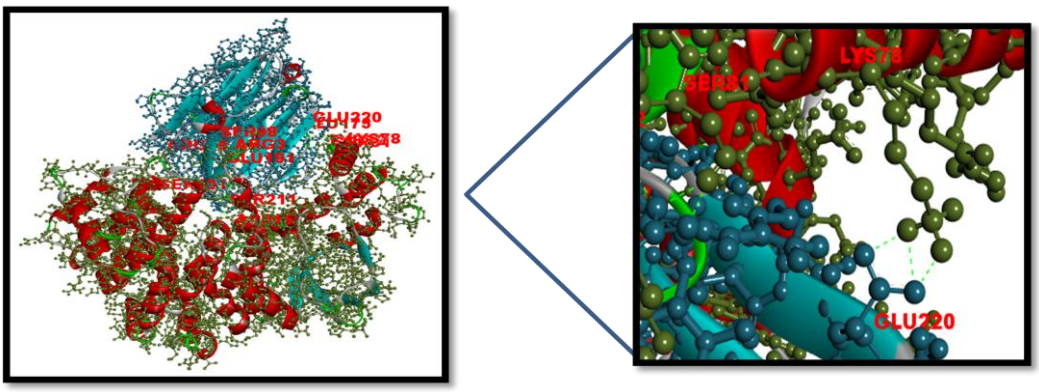

Fig. 5. Docking studies of LL protein and APN receptor, showing hydrogen bond (green dotted line) at interaction sites

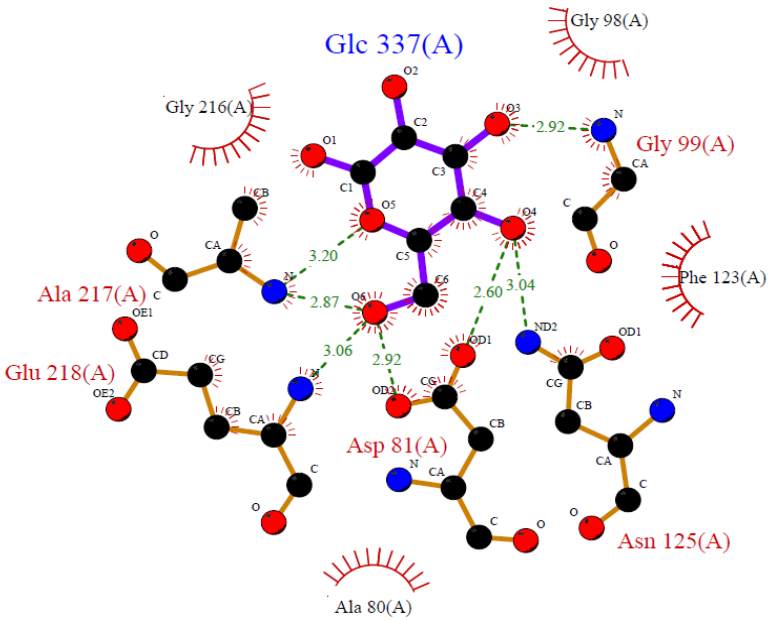

Fig. 6. LigPlot analysis of LL protein and its interaction with ligand XMM303 (A)

Table 1. Amino acid residues with their respective positions involved in interaction through H-bonding in molecular docking

LL Protein	APN Receptor (Aphid gut)
GLU220	LYS78
GLU175	TYR82
GLU175	SER81
SER98	ARG3
GLU191	ARG3
ARG56	SER401
SER211	ASP15

Table 2. Amino acid residue and their respective position involved in interaction with ligand XMM with H-bond length in LigPlot

Target protein (LL)		Ligand (XMM)	
Res. name	**Res .position**	**Res. position**	**Distance (A^0)**
ASP	81	303	2.92
GLY	99	303	2.92
ASN	125	303	3.04
ALA	217	303	3.20
ALA	217	303	2.87
GLU	218	303	3.06

3.3 Molecular Docking of LL Protein with APN Receptor

Active site mapping are best approach to determine residues involved in ligand binding i.e. XMM [(5-Bromo-4-Chloro-3-Indolyl)-A-D-Mannose] for lentil lectin protein (Fig. 6). The LIGPLOT results showed the interactions between ligand and amino acid residues of protein molecules with hydrogen bond (Table 2). Different lectins, exhibited mannose or glucose sugar binding sites, exposed reflective anti-metabolic effects on homopteran insects both under *in vitro* [9,10] and *in planta* [11,12] (Gatehouse et al.,1996). The sugar specificities articulated by lectins towards Mannose, Mannose/Glucose, Mannose/Maltose, Fucose, Galactose/N-acetylgalactosamine, N-acetylglucosamine, sialic acid and complex glycan groups (Peumans and Van Damme, 1998). Del Carmen Fernandez-Alonso et al. [13] reported the nature of lectins and carbohydrates interaction in detail. In order to find the amino acid residues involved interaction along with their position in polypeptide chain, docking studies were significant (Table 1). ClusPro docking server revealed the amino acid residues with positions of interaction between lentil lectin and APN receptor (Fig. 6). The results revealed affinity of lentil lectin protein to its receptor profoundly showed strong that may affect the lectin binding in insect mid gut during ingestion. After binding to gut lectin may interfere the normal metabolic and physiological activity by changing normal cellular metabolism. This ultimately affects insect normal growth and development and may lead to insect mortality.

4. CONCLUSION

This study proves insecticidal potential of lentil lectin protein through *in silico* and computational approaches. The various primary and secondary structural attributes were analysed through various bio computational tools. The 3D structure of the LL protein was predicted through SWISS MODEL via homology-based modelling. The 3D model structure from the study explains the structural and biological activity of this protein. The binding potential of LL protein against APN receptor from aphid could prove this as potent insecticidal after *Bt* genes against *hemipteran*s. This investigation open a pave for characterizing various lectins from plant species since no templates are available for the same in the protein model database.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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