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Molecular Modelling and Structure Analysis of Lectins from *Vigna linearis*

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ABSTRACT

A study was conducted during the year 2022–2023 for *in silico* analysis of two *Vigna linearis* lectins in the computer lab, CDFT, Jodhpur, Rajasthan, India. Amino acid sequences of both lectins were obtained from NCBI database and analyzed using different computational tools. Primary structure analysis using ProtParam server revealed acidic, stable and hydrophobic nature of both VLL proteins. Both proteins were predicted heavily glycosylated with a single signal peptide cleavage site (A_{26}-A_{27}). High sequence similarity of both VLL proteins was observed with adzuki, rice bean and moth bean lectins. Both VLL proteins had high proportions of β-sheets in their secondary structure. Good quality 3D structures for both VLL proteins were modelled using Modeller software. A Jelly roll fold, also known as β-sandwich structure was identified in the 3D structure of both VLL proteins. Profunc server annotated both lectins as a carbohydrate binding regulatory protein with significant role in plant defense (GO terms: 0065007, 0006952, 0050896, 0006950, 0009607, 0030246). Galaxy Site web server predicted galactose, A2G (2-acetamido-2-deoxy-α-D-galactopyranose), MFU (Methyl α-L-fucopyranoside) and adenine as common binding ligands for both VLL proteins. Lig Plot analysis revealed hydrogen bonding and hydrophobic interactions were the major bonding interactions between VLL proteins and putative ligands. The results of the study would provide a base for conducting future research on *Vigna linearis* lectins for different applications.

KEYWORDS: Computation, ligand prediction, protein annotation, VLL proteins, modelling

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1. INTRODUCTION

Lectins, a class of glycoproteins, have with the property of selectively binding and precipitating the carbohydrate moieties. They also known as hemagglutinins, for precipitating the glycoconjugates moieties of the cell surface receptor on the red blood cells (Tsaneva and Van Damme, 2020). Various Plant proteins have been explored for various biological activities and use them as a drug against various diseases (Ghosh et al., 2021). Due to specificity in carbohydrate binding, various biological activities such as antimicrobial, anticancer, antitumour, antiviral activities have been reported for lectins (Dias Rde et al., 2015, Gautam et al., 2018, Costa et al., 2022, Konozy et al., 2022, Fonseca et al., 2022). Based on their structure, lectins have been subdivided into merolectins (single lectin domain), hololectins, chimerolectins and superlectins (multiple lectin domains) (Van Damme, 2022). Multilectins is another group of lectins having two or more similar sugar binding sites (Jimenez et al., 2019). Moreover, depending on the structure and the sequence of carbohydrate binding motif, lectins have been classified into 12 different families (Mishra et al., 2019).

Lectins are ubiquitously distributed in nature. Among different families of lectins, plant lectins, particularly legume lectins are comprehensively researched and investigated (Cavada et al., 2019). Prior to storage, they are synthesized as pre-precursor molecules and thereafter go under some processing events and store in vacuole. During seed germination, they break down and provide essential amino acids to miniature plant (Dang and Van Damme, 2015). Inmature legume seeds, up to 10% of the total storage proteins are constituted by lectins. In vegetative plant parts, they are present in lower amount. Legume lectins are known for having high structural relatedness and sequential similarities. However, differences in binding specificity with carbohydrates and their quaternary structure have also been reported. The presence of β-sandwich structure (Dome like structure) with two hydrophobic cores (Lagarda-Diaz et al., 2017) is one of the main structural characteristic of legume lectins. High number of β-sheets and β-turns with low proportion of α-helix, is the important structural characteristic of secondary structure of legume lectins (Shevkani et al., 2019) whereas quaternary structure is an oligomeric structure having the assemblage of monomers in the form of homodimer or homotetrameric structure. The characteristic carbohydrate binding site of legume lectins is constituted by four loops *viz.*, A, B, C and D. Of which D loop and C loop determines the variation in carbohydrate binding specificity. The presence of metal ion binding site particularly with Ca^{+2} and Mn^{+2} ions is crucial to maintain the stability of carbohydrate binding site (Cummings et al., 2022).

Proteins sequence and structure determines their physicochemical properties and functions. Therefore it is essential to extract structural and sequential information for their proper characterization. Various reports on utilization of computational tool to analyse structural and functional characteristics of various legume lectins are available (Filho et al., 2017, Osman and Konozy, 2017, Leal et al., 2022), but there is little information is available on lectins from *Vigna linearis* legume. In this study, various bioinformatics tools for were used to obtain the preliminary information on structural and functional characteristics of *Vigna linearis* legume lectins (VLL), that would provide much needed base to conduct future studies on lectins from *Vigna linearis*.

2. MATERIALS AND METHODS

The study was conducted for *in silico* analysis of two *Vigna linearis* lectins in the computer lab, CDFT, Jodhpur, Rajasthan, India during the year 2022–2023. The amino acid sequence of *Vigna linearis* lectins (VLL-1, VLL-2) were retrieved from NCBI database [(i) VLL-1: Accession number: CAD43279.1, *Vigna linearis* var. linearis (ii) VLL-2: CAD43280.1, *Vigna linearis* var. latifolia]. Multiple sequence alignment (MSA) of VLL lectins was done using Clustal W. The MSA was curated with ESPript 2.2 server to identify conservedness in the sequences. Mega X software was used to construct phylogenetic tree (Kumar et al., 2018). ProtParam analysis was carried out (http://web.expasy.org/protparam) (Gasteiger et al., 2005) for the physiochemical characterization of VLL proteins. Analysis of secondary structure of VLL proteins was carried out using PSIPRED web server (http://bioinf.cs.ucl.ac.uk/psipred/) (Buchan and Jones, 2019). Signal P5.0 server (Almagro Armenteros et al., 2019) and Phobius server (http://phobius.sbc.su.se/) (Kall et al., 2007) were used for the prediction of signal peptide and transmembrane helixes in VLL proteins, respectively. Prediction of *N*-glycosylation sites in VLL proteins was doneusing NetNGly1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/) (Gupta et al., 2004). Sub-cellular localization of RbL protein was predicted with CELLO v2.5 (Yu et al., 2006). ProFunc server (http://www.ebi.ac.uk/thornton-srv/databases/ProFunc) (Laskowski et al., 2005) was used for functional annotation of VLL proteins. 3D structural modeling was carried out using MODELLER tool (Webb and Sali, 2016) available at MPI Bioinformatics Tool Kit (https://toolkit.tuebingen.mpg.de/tools/hhpred/). The modelled structure was visualized using UCSF Chimera 1.13.1 software (Pettersen et al., 2004). Evaluation and validation of the modelled structure was carried using PROCHECK server (http://servicesn.mbi.ucla.edu/PROCHECK/) (Laskowski et al., 1993). GalaxySite server (http://galaxy.seoklab.org) was used for prediction and analysis of binding of ligands with VLL proteins (Heo et al., 2014).

>CAD43279.1 lectin [*Helicotropis linearis* var. linearis]

MASSNFSTVLSLSLALFLVLLTHANSTNLVSFN-
FQTFNSPNLILQGDASISSSGQLRLTNVKANDIP-
TAKSLGRAFYSAPIQIWDSTTGNVANFATSFTF-
NISAPNESKTADGLAFALVPVGSKPKTNGGYR-
GLFENAAYDSSAQTVAVEFDTLSNHHWDPET-
GHIGINVNSIRSIKTVPWDLANGQNAEVLITY-
DSSTKLLVASLVYPSKRTSYIISETVDLKSVLPEWV-
SIGFSATTGLTADFIETHDVLSWSFASKLSDGT-
TSEGLNLANFVLNQIL

>CAD43280.1 lectin [*Vigna linearis* var. latifolia]

MASSNFSTVLSLSLALFLVLLTHANSTNVFSFN-
FQTFDSPNLILQGDASISSSGQLRLTKVNGNGK-
PAVGSLGRAFYSAPIQIWDSTTGNVANFATAFT-
FNIFAPNKSNSADGLAFALVPVGSQPKSNDG-
FLGLFENATYDNSVQTLAVEFDTYSNPKWD-
PENRHIGIDVNSIQSIRTTPWGLANGQNAEILI-
TYDSSTKLLVASLVHPSRRTSYIVSERVDVKSV-
LPEWVSIGFSATTGLLEGSIETHDVLSWSFASKLS-
DGTTSEGLNLANFVLNKIL

3. RESULTS AND DISCUSSION

3.1. *Physicochemical characterization of Vigna linearis legume lectins*

Computation analysis of physicochemical parameters of proteins provides information on their amino acids composition, molecular weight, pI, extinction coefficient, estimated half-life, instability index, aliphatic index and hydrophobicity (Grand average of hydropathicity) (Nivetha et al., 2021). The physicochemical analysis of VLL proteins (VLL-1, VLL-2) using Protparam server revealed 280 amino acids in both lectins with 30.28 kDa and 30.85kDa molecular weight, respectively. Both lectins can be formulated as $C_{1350}H_{2088}N_{348}O_{413}S_1$ and $C_{1353}H_{2092}N_{354}O_{421}$S1, respectively. Both proteins had high percentage of serine amino acids (13.60%) followed by leucine (11.10%) and threonine amino acids (9.30% and 7.90%, respectively).Total 22 negatively charged amino acids were computed in VLL-1 and VLL-2 proteins. The number of positively charged amino acids in both lectins was 15 and 16, respectively. The amino acids composition of both proteins has been presented in Figure 1. In the study, VLL-1 and VLL-2 lectins have shown considerable sequential similarity to *Erythrina* lectin (Osman and Konozy, 2017) with high percentage of hydroxylic, aliphatic and acidic amino acid residues with low proportion of methionine amino acid. For VLL-1 and VLL-2 proteins, the computed theoretical pI was 5.12 and 5.14, which indicated the presence of high number of negatively charged amino acids, thus their acidic nature. Both *Vigna linearis* lectins (VLL-1 and VLL-2) were predicted stable

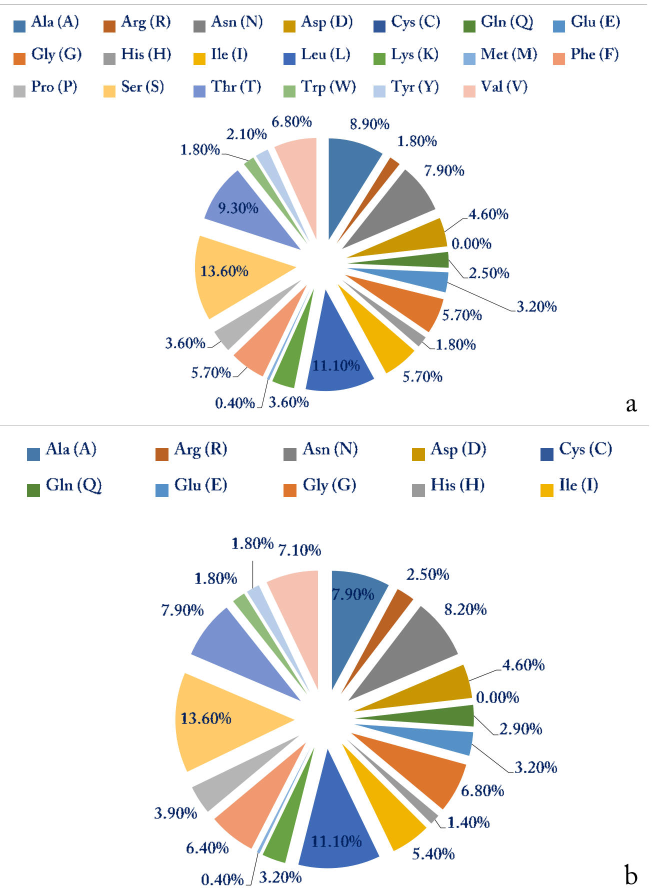

Figure 1: Amino acid composition of (a) VLL-1 and (b) VLL-2 proteins

(Instability index value =27.94 and 34.40, respectively) with estimated half-life of 30 hrs. ProtParam analysis revealed that VLL-1 and VLL-2 proteins had high aliphatic index (94.07 and 92.64, respectively) and positive GRAVY value (0.079 and 0.051, respectively) that indicated thermal stability and hydrophobic nature of both lectins. Using ProtScale server, hydrophobicity was confirmed for VLL-1 and VLL-2 proteins.

In proteins, signal peptide serve the function of a localization signal for their entry into secretary pathway. Signal peptide sequence is mainly present at N-Terminal end of the proteins (Dang and Van Damme, 2015) and depending upon the presence of signal peptide, legume lectins have been classified into constitutive and inducible legume lectins. Constitutive legume lectins synthesize with signal peptide and stored either in plant vacuole or extracellular space whereas inducible legume lectins don't have signal peptide and accumulate in nucleus or in cytoplasm (Dang and Van Damme, 2015). A single signal peptide cleavage site (A_{26}-A_{27}) was predicted for both legume lectins (VLL-1 and VLL-2) with probability of 0.5536 and 0.5398, respectively. Filho et al. (2017) also predicted a single signal peptide cleavage site (A_{26}-A_{27}) in *Vigna unguiculata*

lectin sequence. The α-helical region in signal peptide mediates its interaction with hydrophobic interior of plasma membrane. Phobius server predicted 10 to 21 amino acids in transmembrane region of signal peptide in both legume lectins. Both VLL-1 and VLL-2 lectins were predicted to have ~34% β-sheets, ~55% random coil and ~11% α-helix in their secondary structure (Figure 2). A large number of β-sheets and negligible proportion of α-helix, is the important structural characteristic of legume lectins (Shevkani et al., 2019).

3.2. Multiple sequence and phylogenetic analysis Vigna linearis legume lectins

Legume lectins have high sequence similarity and have been considered an efficient tool to analyse evolutionary and phylogenetic relationships (Lagarda-Dias et al., 2017). Multiple sequence analysis revealed highest similarity in sequences of VLL proteins with *Vigna umbellate*, *Vigna angularis*, *Vigna aconitifolia* and *Vigna unguiculata* lectins. Multiple sequence alignment of VLL proteins and other legume lectins revealed high sequence similarity and conservedness, essential for metal ion binding (Glutamic acid[150], Aspartic acid[160], Histidine[165]) and monosaccharide binding (Aspartic acid[112], Glycine[130], Tyrosine [154] and Asparagine[156]). A conserved leucine[260]amino acid, the cleavage signal to remove C-terminal peptide was also identified in both VLL proteins. Moreover, the presence of α-helix at C-terminal end was also confirmed (Figure 3). DBL (*Dolichos biflorus* lectin), SBA (Soybean agglutinin) and BVL-II (*Bauhinia variegate* lectin-II) legume lecins also have α-helix in C-terminal region their structure (Moreira et al., 2013) which provide stability to their oligomaric

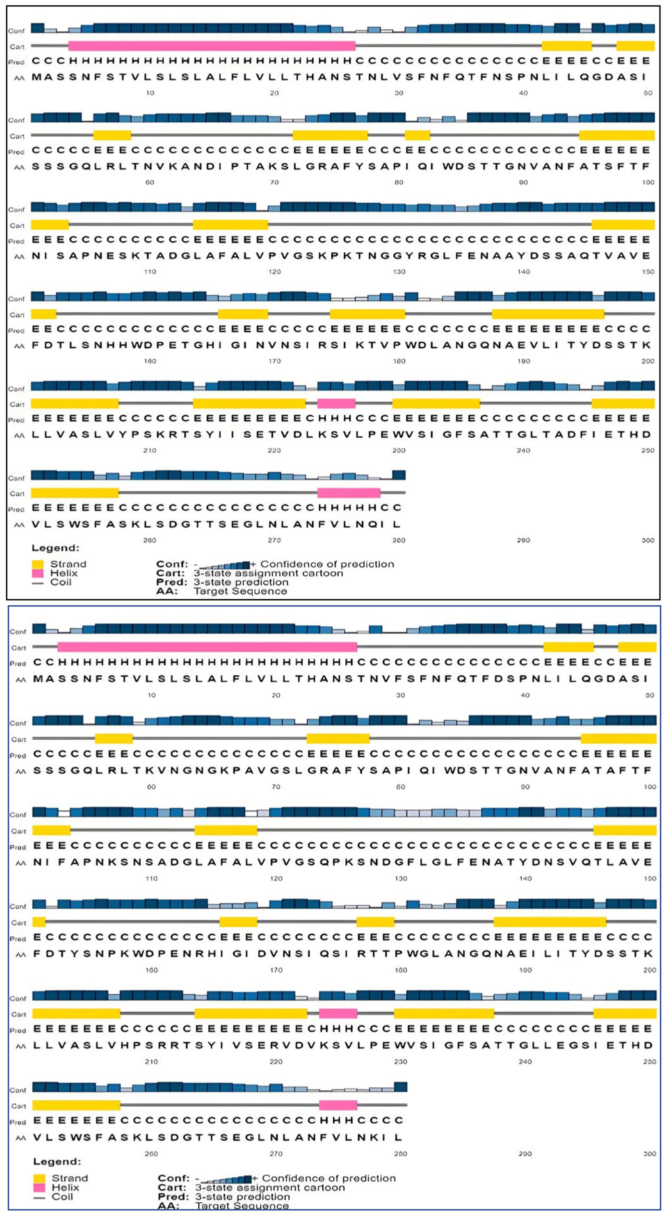

Figure 2: Secondary structure analysis of (a) VLL-1 and (b) VLL-2 Proteins

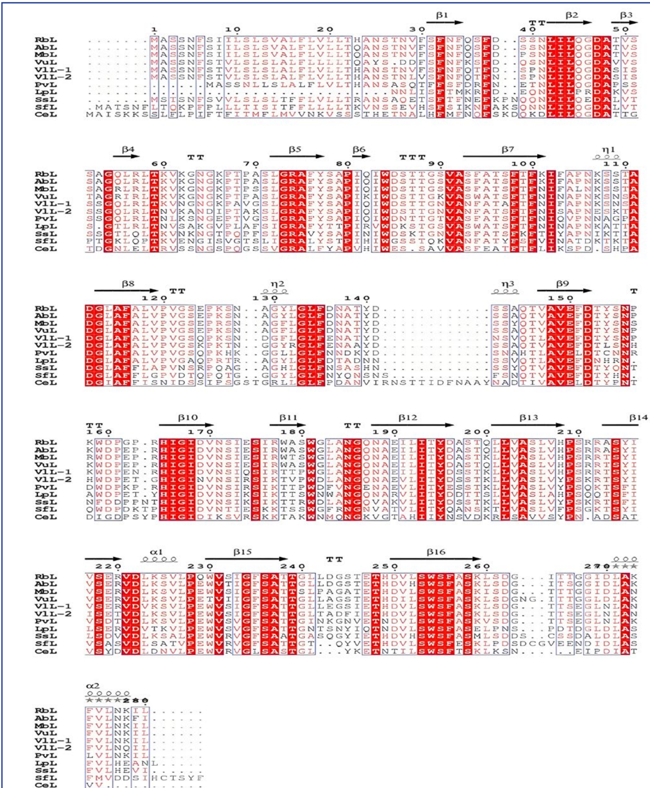

Figure 3: Multiple sequence alignment of VLL proteins with other legume lectins; RbL, Rice bean lectin (QUB37040.1), AbL, Adzuki bean lectin (XP_017413483.1), MbL, Moth bean lectin; AEE88306.1), VuL, *Vigna unguiculata* lectin precursor (CAF18557.1), VLL-1&VlL-2, Vigna linearis lectins; (CAD43279.1,CAD43280.1), PvL, *Phaseolus vulgaris* lectin; (CAD28675.1), LpL, *Lablab purpureus* lectin (ABM92662.2), SsL, *Spatholobus suberectus* lectin (TKY53520.1), SfL, *Sophora flavescens* lectin (AUD40029.1,) CeL, *Canavalia ensiformis* lectin (AAL09432.1)

structures. In addition to the conserved amino acids, cis-peptide bond between Ala[111] and Asp[112] was also identified in VLL protein sequences. N-linked glycosylation of soluble and membrane bound proteins helps in maintaining their correct folding state (Jayaprakash, and Surolia, 2017). Four glycosylation sites were predicted in the both VLL lectins sequences (VLL-1: N[5]FS, N[25]ST, N[101]IS, N[106]ES, VLL-2: N[5]FS, N[25]ST, N[106]KS, N[137]AT) (Figure 3). Phylogenetic analysis is essential to decipher the evolutionary relationships between the proteins. Phylogenetic analysis established high sequence similarity of VLL proteins with *Vigna umbellate*, *Vigna aconitifolia, Vigna angularis*, and *Vigna unguiculata* lectins (Figure 4). Moreover, the grouping of legume lectins in the phylogenetic tree also revealed common evolutionary origin based on functional, structural and sequential similarity.

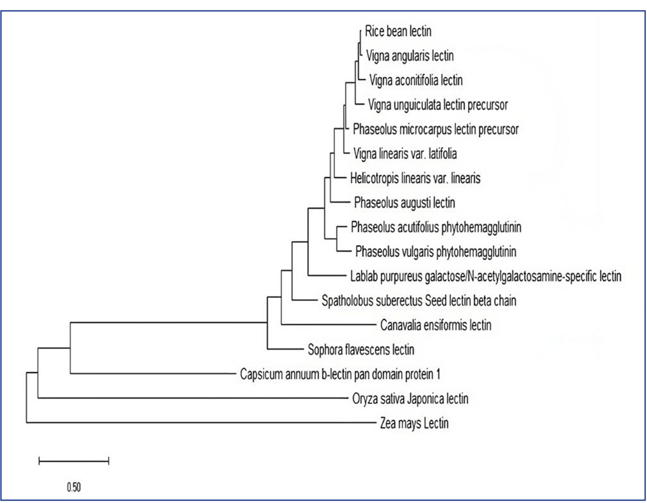

Figure 4: Phylogenetic analysis of VLL proteins with other legume lectins; Rice bean lectin (QUB37040.1), Adzuki bean lectin (XP_017413483.1), Moth bean lectin; AEE88306.1), *Vigna unguiculata* lectin precursor (CAF18557.1), Vigna linearis lectins(CAD43279.1, *Vigna linearis* var. linearis (ii) VLL-2: CAD43280.1, *Vigna linearis* var. latifolia], *Phaseolus vulgaris* lectin (CAD28675.1), *Lablab purpureus* lectin (ABM92662.2), *Spatholobus suberectus* lectin (TKY53520.1), *Sophora flavescens* lectin (AUD40029.1,) *Canavalia ensiformis* lectin (AAL09432.1), *Capsicum annuum* b-lectin pan domain protein 1, AJF21091.1, *Zea mays* Lectin (PWZ11033.1), *Oryza sativa* Japonica lectin (AAA20873.1)

3.3. Homology modelling, prediction of putative ligands and gene ontology analysis of Vigna linearis legume lectins

Homology modelling of VLL proteins was done in three steps: (i) Template selection and pairwise query-template alignment (ii) Model building and (iii) Visualization. *Vigna unguiculata* lectin (PDBID: 1G7Y_B, Probability: 99.49%, E-value: and 3.4e−9) was selected as a template to model 3D structure of both VLL proteins. The 3D structure analysis of both VLL proteins revealed the presence of dome like structure also known as *lectin fold*, *jelly roll fold* and *β-sandwich* structure and site for metal binding and a domain for carbohydrate recognition and binding (Figure 5). The characteristic lectin fold was consisted of seven- curved anti-parallel β-sheets (front face) and six flat stranded anti-parallel β-sheets (back face). Four binding loops namely A, B, C and D with conserved amino acids critical for determining the binding specificity of legume lectins with different carbohydrate moieties, have been also identified in both VLL proteins (Figure 5). For example,

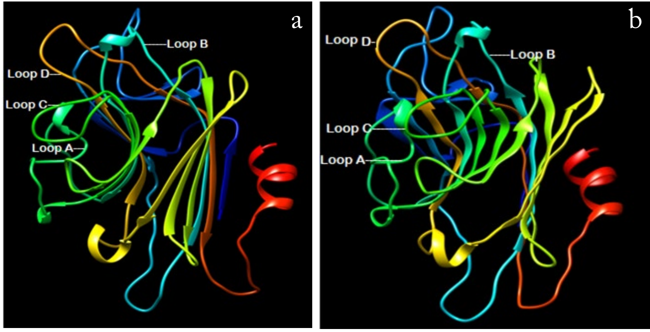

Figure 5: 3D- structure of (a) VLL-1 and (b) VLL-2 proteins

tyrosine amino acid, critical for stacking with non-polar face of carbohydrates was found conserved in Loop C of both lectins (Cummings et al., 2022). Ramachandran plot analysis for both VLL proteins revealed that>than 88% amino acids are distributed in most favourable region, >7% amino acids are in additional allowed regions, <0.5% of amino acid in disallowed regions of the plot (Figure 6).

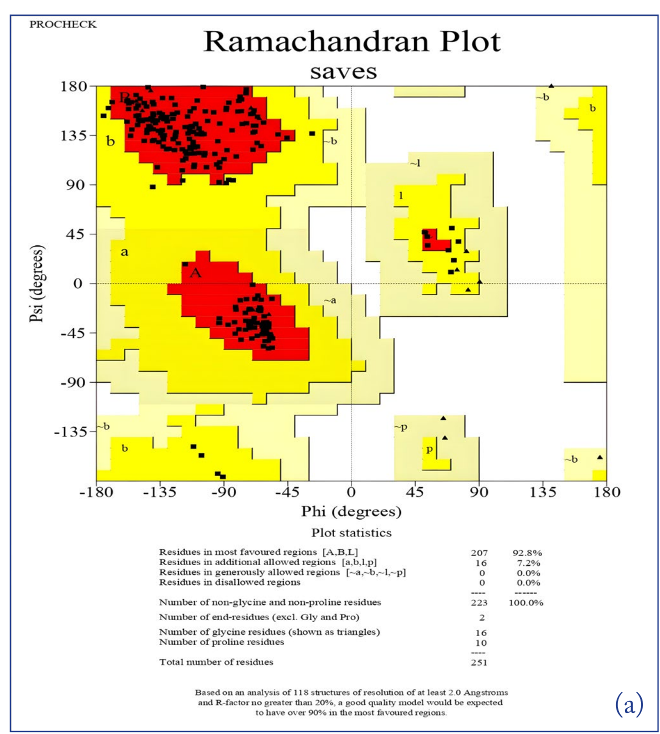

Figure 6: Continue...

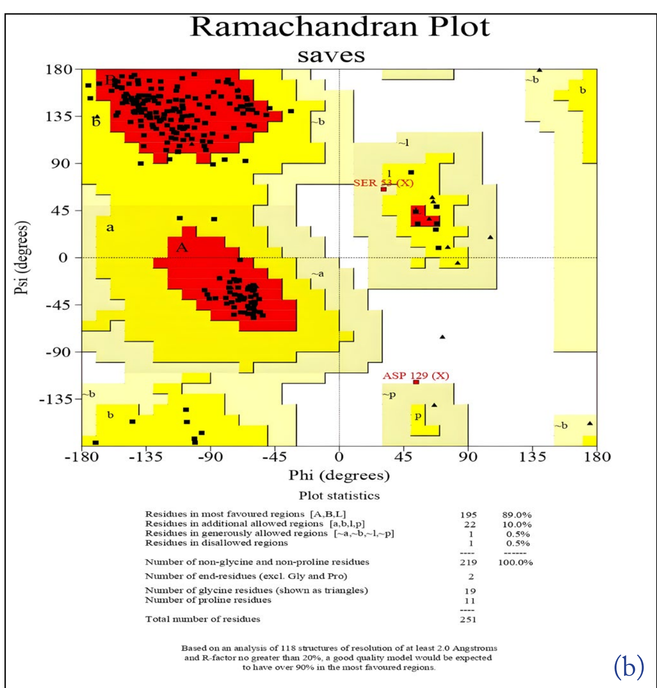

Figure 6: Ramchandran plot analysis of 3D structure of (a) VLL-1 and (b) VLL-2 proteins. The plot shows residues in most favored (red), additionally allowed (yellow), generously allowed (pale yellow) and disallowed regions (white color)

These results validated the good quality of structured 3D model of both VLL proteins. Binding of proteins with different ligands is essential for their biological activity. Therefore, prediction and identification of different ligand binding sites is helpful in functional characterization of proteins. Using GalaxySite web server predicted galactose, A2G (2-acetamido-2-deoxy-alpha-D-galactopyranose), MFU (Methyl α-L-fucopyranoside) and adenine as common binding ligands for both VLL proteins (Table 1 and Table 2). Though the binding ligands are same, but their interactions with different amino acid residues are different.

LigPlot analysis for β-galactose and A$_2$G ligands binding with VLL-1 proteins revealed hydrogen bonding with aspartic acid^{112}, glycine^{130}, asparagine^{156}, leucine^{241} and threonine^{242} whereas with leucine^{154}, phenylalanine^{245} and glycine^{240} amino acids the interactions were hydrophobic. In case of VLL-2 proteins, galactose interacted through hydrogen bonding with aspartic acid$^{112, 129}$, glycine ^{130} and leucine^{241,242} and with Serine^{245} and Glycine^{240} and Tyrosine^{154} via hydrophobic interactions. A2G ligand linked to aspartic acid^{112}, glycine ^{130} by hydrogen bonding, however with Glycine^{240}, Serine^{245}, leucine^{242}, Tyrosine^{154}, Aspartic acid^{129}, and Tryptophan 159, the binding interactions were hydrophobic. The binding analysis of VLL-1 proteins with MFU ligand revealed hydrogen bond interactions with aspartic acid^{112}, glycine^{130} and asparagine^{156} and hydrophobic interactions with alanine^{111}, glycine^{129}, leucine^{154}, glycine ^{240} and leucine^{241} amino acids. H-bonding with aspartic acid$^{112, 129}$, glycine^{130} and asparagine^{156} and hydrophobic interactions with serine^{205}, tyrosine^{154}, alanine^{111}, glycine ^{240}and leucine ^{242} amino acid residues were the major binding interactions. A hydrophobic site constituted by leucine^{192}, threonine^{194} and serine^{205} amino acids was also identified in the modelled structures of VLL proteins. Binding with adenine suggests the probable biological functions of VLL

Table 1: Ligand details and binding interactions of VLL-1 lectin

Ligand name and structure	Templates for protein-ligand complex	Binding amino acid residues	LigPlot analysis
GAL	3UJQ_C, 1G9F_A, 1SBF_A, 1AX2_A	112D 130G 154L 156N 240G 241L 242T 245F	
A2G	1LU2_B, 4U2A_A	112D 129G 130G 131Y 154L 156N 240G 241L 242T 245F	

Table 1: Continue...

Ligand name and structure	Templates for protein-ligand complex	Binding amino acid residues	LigPlot analysis
ADE	1BJQ_H	192L 193I 194T 203V 205S	
MFU	1JXN_C	111A 112D 129G 130G 154L 156N 240G 241L	

Key

Ligand bond

Non-ligand bond

Hydrogen bond and its length

Non-ligand residues involved in hydrophobic contact(s)

Corresponding atoms involved in hydrophobic contact(s)

Table 2: Ligand details and binding interactions of VLL-2 lectin

Ligand name and structure	Templates for protein-ligand complex	Binding amino acid residues	LigPlot analysis
GAL	3UJQ_C, 1G9F_A, 1SBF_A, 1AX2_A, 1DBN_B	112D 129D 130G 154Y 156N 240G 241L 242L 245S	
Adenine	1BJQ_H	192L 193I 194T 203V 205S	

Table 2: Continue...

Ligand name and structure	Templates for protein-ligand complex	Binding amino acid residues	LigPlot analysis
MFU	1JXN_C	111A 112D 129D 130G 154Y 156N 240G 242L 245S	
A2G	1LU2_B, 4U2A_A	112D 129D 130G 154Y 156N 159W 240G 241L 242L 245S	

Key

Ligand bond

Non-ligand bond

Hydrogen bond and its length

Non-ligand residues involved in hydrophobic contact(s)

Corresponding atoms involved in hydrophobic contact(s)

proteins in regulation such as storage and utilization of adenine-derived hormones (Cummings et al., 2022) and defense response such as during stress *via* binding with extracellular ATP (Tripathi et al., 2018, Jewell et al., 2019). Extracellular compartment and plasma membrane as main localization sites for both VLL proteins in a cell which may enable the role of VLL proteins in extracellular communication particularly their role in defense via binding with PAMP's (Pathogen Associated Molecular Pattern) and their subsequent agglutination on cell surface. The presence of legume lectins in extracellular milieu or their association with plasma-membrane attributes their crucial role in in ion binding, kinase activity and enzyme regulator (Filho et al., 2017). Both VLL proteins were annotated as carbohydrate binding and metal ion binding with significant role in different biological processes associated with plant defense response, biological regulation and regulation of catalytic activity.

4. CONCLUSION

The computational analysis carried out in the present study provides insights into the various structural and functional properties of *Vigna linearis* lectins. Both VLL proteins were annotated as regulatory proteins with significant role in plant defense. However, future studies on functional characterization of both proteins could generate molecular mechanisms associated with their biological functions. In the study, information generated on putative binding ligands and their interactions with VLL proteins would also be useful in their implications for different glycobiological interventions.

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