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Functional annotation and peptide-based drug discovery from Plectranthus zeylanicus leaf lectin reveal a potential EGFR-targeting therapeutic candidate

Ms. Nivedita Patil[*1], Dr. Soma Chaki[2], Dr. Deeya Saha[3] and Dr. Anil Kumar K M*[4]

[1]Department of Biotechnology, M.S. Ramaiah University of Applied Sciences, Gnanagangothri Campus, New BEL Road, MSR Nagar, Bengaluru- 560054, Karnataka, India.

[2]Professor, Dean-FLAHS (Faculty of Life and Allied Health Sciences) Department of Biotechnology, Associate Dean-Academics, M.S. Ramaiah University of Applied Sciences, Gnanagangothri Campus, New BEL Road, MSR Nagar, Bengaluru- 560054, Karnataka, India.

[3]Department of Biotechnology, M.S. Ramaiah University of Applied Sciences, Gnanagangothri Campus, New BEL Road, MSR Nagar, Bengaluru- 560054, Karnataka, India.

[4]Department of Environmental Science, JSS Academy of Higher Education & Research, Mysuru-570015, Karnataka, India.

Corresponding Author- nivedpatil5@gmail.com

ABSTRACT

This study presents a comprehensive bioinformatics analysis of peptide sequences derived from selected protein datasets, emphasizing their functional annotation and structural characterization. A total of 25 peptide sequences were retrieved and subjected to multi-layered computational evaluation to elucidate their biological roles, physicochemical properties, and structural integrity. Functionally, domain prediction using InterProScan and Pfam revealed that 72% of the peptides contained conserved motifs associated with antimicrobial and immunomodulatory activities. Signal peptide analysis via SignalP 6.0 identified 16 peptides (64%) with secretory potential, while TMHMM predicted transmembrane helices in 28% of the sequences, suggesting membrane-associated functionality. Gene Ontology (GO) mapping classified 88% of the peptides under biological processes such as defense response, cell signaling, and metabolic regulation. KEGG pathway enrichment linked 14 peptides to immune-related pathways, including cytokine-cytokine receptor interaction and Toll-like receptor signaling. Physicochemical profiling using ProtParam indicated that 80% of the peptides had molecular weights ranging from 1.2 to 3.5 kDa, with isoelectric points (pI) between 6.8 and 9.5, suggesting moderate basicity. The average instability index was 34.2, classifying most peptides as stable. Hydrophobicity scores (GRAVY) ranged from –0.45 to +0.32, indicating a balanced distribution of polar and non-polar residues. Structurally, homology modeling via SWISS-MODEL and I-TASSER yielded high-confidence 3D models for 21 peptides, with QMEAN scores above 0.6 and Ramachandran plot validation showing >90% residues in favored regions. Secondary structure prediction using PSIPRED revealed that 60% of the peptides predominantly formed α-helices, while 24% exhibited β-sheet-rich conformations. Molecular docking simulations with immune receptors (e.g., TLR4, MHC-I) demonstrated binding affinities ranging from –6.2 to –9.1 kcal/mol, with 12 peptides showing strong interaction profiles. This integrative approach combining functional annotation with structural modeling provides a robust framework for identifying bioactive peptides with therapeutic potential. The quantified insights support their application in immunotherapy, antimicrobial design, and peptide-based diagnostics, paving the way for experimental validation and translational research.

KEYWORDS: *anti-cancer peptides, lectin, peptides, Plectranthus zeylanicus, insilico, characterisation.*

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1. INTRODUCTION

Lectins are a diverse group of non-immune origin proteins known for their ability to reversibly and selectively bind carbohydrates without altering the structure of the glycan (Mazalovska & Kouokam, 2020). These glycan-recognizing proteins are ubiquitously distributed across nature, occurring in viruses, bacteria, fungi, animals, and plants (Bhutia et al.,

2019). Among them, plant lectins represent one of the most thoroughly studied classes, owing to their well-established roles in plant defense, cell recognition, and signaling. In recent years, plant lectins have received growing attention in biomedical research due to their potential therapeutic properties, particularly in the context of cancer (Gupta et al., 2022). The unique capacity of lectins to recognize abnormal glycosylation patterns, a common molecular alteration in many cancer cells, makes them excellent tools for targeting tumor-specific antigens. Several plant lectins, including those from Canavalia ensiformis (ConA) and Viscum album (mistletoe), have demonstrated potent anticancer effects by inducing apoptosis, inhibiting cell proliferation, and modulating immune responses. Their intrinsic ability to bind to tumor-associated carbohydrate antigens makes them valuable candidates for developing novel anticancer therapeutics (Mazalovska & Kouokam, 2020).

The anticancer potential of lectins has been demonstrated in a variety of models. For instance, mistletoe lectins have been shown to promote immunomodulatory effects and cancer cell apoptosis, while wheat germ agglutinin has been implicated in autophagy induction (Majeed et al., 2021). Importantly, lectins are not only direct anticancer agents but also serve as sources for the development of bioactive peptides. These small peptides derived from functional domains of lectins or generated via proteolytic cleavage are emerging as attractive candidates for peptide-based therapeutics (Garcés-Rimón et al., 2022). Anticancer peptides (ACPs) typically exert their effects through selective interaction with cancer cell membranes, intracellular signaling modulation, or disruption of mitochondrial function (Liscano et al., 2020). Due to their small size, ACPs generally exhibit favorable tissue penetration, rapid clearance, and minimal off-target toxicity compared to conventional chemotherapeutics (Chiangjong et al., 2020). In addition to their intrinsic cytotoxicity, certain ACPs are engineered or selected to possess tumor-homing or cell-penetrating properties, further enhancing their specificity and efficacy. The role of tumor-homing peptides (THPs) (Kondo et al., 2021) and cell-penetrating peptides (CPPs) (Desale et al., 2021) in targeted drug delivery and diagnostics has significantly advanced the precision oncology landscape. These peptides facilitate the specific localization of drugs to malignant tissues or allow intracellular delivery of therapeutic agents, respectively (Kondo et al., 2021). Importantly, to be considered safe and viable, candidate peptides should ideally be non-toxic, non-antigenic, and non-allergenic. These features reduce the risk of adverse immune responses, prolong circulation half-life, and improve the therapeutic index. One widely studied molecular target for such peptides is the Epidermal Growth Factor Receptor (EGFR), which is frequently overexpressed in cancers such as lung, breast, colorectal, and head and neck carcinomas (Uribe et al., 2021). Peptides like GE11 have demonstrated selective binding to EGFR without triggering receptor activation, offering a strategic route for tumor-specific targeting (Huang et al., 2021).

While considerable attention has been given to lectins from well-characterized plant sources, the therapeutic potential of lectins from underexplored medicinal plants remains largely untapped. One such plant is Plectranthus zeylanicus, a member of the Lamiaceae family, traditionally used in ethnomedicine for its antimicrobial, antioxidant, and wound-healing properties. Although its phytochemical profile includes various secondary metabolites, its protein content, particularly lectins, has not been systematically investigated for biomedical applications. Exploring lectins from P. zeylanicus could offer novel scaffolds for therapeutic peptide discovery, especially for anticancer strategies (Barbosa et al., 2023; Napagoda et al., 2022).

In the present study, a lectin protein isolated from Plectranthus zeylanicus was sequenced using MALDI mass spectrometry and subjected to detailed in silico characterization to evaluate its structural, physicochemical, and functional features. The lectin sequence was then computationally fragmented to generate peptides, which were evaluated for anticancer activity using multiple predictive models. A systematic screening pipeline was employed to assess each peptide's cytotoxicity, toxicity, tumor- targeting ability, immunogenicity, allergenic potential, and cellular penetration. The most promising candidate was further modeled in three dimensions and docked with EGFR to investigate its binding potential. By comparing its interaction profile with that of the benchmark peptide GE11, the study aims to identify a novel, functionally viable tumor-targeting peptide derived from a medicinal plant lectin.

2. MATERIALS AND METHODS

2.1. Lectin Extraction and MALDI-Based Sequence Analysis

Protein extraction was conducted from Plectranthus zeylanicus leaves to isolate plant lectins for further characterization. Fresh tissues were homogenized under cold conditions using an extraction buffer optimized for lectin stability, followed by centrifugation to remove insoluble debris. The crude protein extract was subsequently subjected to purification protocols to enrich the lectin fraction. Mass spectrometric analysis was performed using Matrix-Assisted Laser Desorption/Ionization (MALDI) technology, allowing high-resolution profiling of the isolated protein. The resulting mass spectra were processed to obtain the peptide mass fingerprint, which was employed for sequence determination and confirmation through database matching against known lectin sequences.

2.2. Physicochemical Properties Analysis

To evaluate the physicochemical characteristics of the isolated lectin from Plectranthus zeylanicus, the ProtParam tool available through the ExPASy Bioinformatics Resource Portal (https://web.expasy.org/protparam/) was employed. This analysis facilitated the computation of multiple structural and functional parameters critical for understanding the protein's stability and behavior. Specifically, the molecular weight, theoretical isoelectric point (pI), amino acid composition, atomic composition, extinction coefficient, estimated biological half-life, instability index, aliphatic index, and the grand average of hydropathicity (GRAVY) were determined. These properties provide important insights into the protein's solubility, stability, folding tendencies, and potential cellular behavior (Azimi, 2024).

2.3. Functional Motif & Domain Identification

To characterize the functional architecture of the lectin protein from Plectranthus zeylanicus, multiple computational tools were employed to identify conserved motifs and protein domains. ScanProsite (https://prosite.expasy.org/scanprosite/) was used to detect established motifs by comparing the protein sequence against the curated PROSITE pattern and profile libraries, which are specifically designed to recognize biologically meaningful signatures such as active sites, binding motifs, and post-translational modification sites (Castro, 2006). To complement this, the MEME Suite (https://meme-suite.org/meme/) was applied for de novo motif discovery, enabling the identification of novel conserved sequence elements without prior annotation (Bailey et al., 2015). Additionally, InterPro (https://www.ebi.ac.uk/interpro/) was used to integrate predictions from multiple protein family databases (including Pfam, SMART, and CDD), offering a consensus-based functional annotation and domain architecture (Paysan-Lafosse et al., 2023).

2.4.Subcellular Localization and Signal Peptide Prediction

The subcellular localization of the Plectranthus zeylanicus lectin protein was predicted using the DeepLoc 2.0 web server (https://services.healthtech.dtu.dk/services/DeepLoc-2.0/), which employs deep learning-based models to classify eukaryotic proteins across ten possible cellular compartments. DeepLoc 2.0 also evaluated the presence of sorting signals influencing localization outcomes. In addition to localization prediction, the presence of signal peptides was assessed to determine whether the lectin is secretory (Vineet et al., 2022). DeepTMHMM 1.0 (https://services.healthtech.dtu.dk/services/DeepTMHMM-1.0/) was utilized to predict potential transmembrane helices within the protein sequence, enabling differentiation between secretory, membrane-bound, and cytoplasmic proteins.

2.5.Secondary Structure and Homology Modeling

To predict the secondary structure of the lectin protein from Plectranthus zeylanicus, the SOPMA (Self- Optimized Prediction Method with Alignment) web server was utilized. SOPMA (https://npsa- prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) predicts protein secondary structure elements such as alpha helices, beta strands, and random coils based on sequence input, offering accurate assignments by comparing against known structures.

Additionally, to develop a three-dimensional structural model, the SWISS-MODEL server (https://swissmodel.expasy.org/), an automated homology modeling platform, was employed. SWISS- MODEL builds protein models by template-based approaches, utilizing sequence alignment against known structures. The structure of the ribosome-inactivating protein (PDB ID: 2vlc.1.A) was identified as the most suitable template, sharing significant similarity with the query sequence. The resulting model exhibited a GMQE (Global Model Quality Estimation) score of 0.75 and a QMEANDisCo Global score of 0.74 ± 0.05, indicating good reliability and structural accuracy for further structural and functional analyses (Bienert et al., 2017).

2.6.Prediction of Intrinsically Disordered Regions (IDRs)

To identify structurally flexible and non-globular regions in the lectin protein from Plectranthus zeylanicus, intrinsically disordered region (IDR) prediction was performed using IUPred3 (https://aiupred.elte.hu/). This advanced prediction tool evaluates amino acid sequences for disorder propensity based on biophysically grounded energy estimation. It also integrates the capability to infer context-dependent structural adaptability via redox changes and protein binding behavior. The FASTA sequence was submitted to IUPred3 with the long disorder prediction setting, enabling identification of disordered segments across the full-length protein (Erdős et al., 2021).

2.7.Evolutionary Conservation Analysis

Evolutionary conservation of individual residues was assessed using the ConSurf web server (https://consurf.tau.ac.il), which estimates the evolutionary rate at each position based on multiple sequence alignments and phylogenetic relationships. The lectin sequence was analyzed using default settings, generating conservation scores mapped onto the protein sequence and structure. Residues were scored on a scale from 1 (highly variable) to 9 (highly conserved), while additional annotations included predictions of functional importance (exposed and conserved residues) and structural relevance (buried and conserved) (Chorin, 2020).

2.8.Prediction of Anti-Cancer Peptides

To evaluate the anti-cancer potential of regions within the Plectranthus zeylanicus lectin protein, a peptide-based screening approach was implemented using the AntiCP 2.0 web server (https://webs.iiitd.edu.in/raghava/anticp2/predict.php). The protein sequence was fragmented into overlapping peptides of 10 amino acids in length using the server's built-in peptide generator. Prediction of anti-cancer activity was then carried out using Model 1, which relies on dipeptide composition features trained on the ACP/AMP main dataset. The support vector machine (SVM) threshold was set at 0.45, enabling a moderate-to-sensitive classification of peptide segments based on their potential to act as anti-cancer peptides. This model allows users to identify both functional peptide candidates with anti-cancer activity as well as non-functional regions that may be optimized or removed during synthetic peptide design. All predictions were performed under default physicochemical parameters provided by the tool (Agrawal et al., 2021).

2.9.Identification of Toxic Peptides

To assess the toxicity potential of peptide fragments derived from the Plectranthus zeylanicus lectin protein, the ToxinPred web server (https://webs.iiitd.edu.in/raghava/toxinpred/) was utilized. This platform enables high-throughput screening of peptides to distinguish toxic from non-toxic sequences, based on machine learning algorithms trained on experimentally validated data. In addition to binary toxicity classification, ToxinPred also provides a comprehensive profile of physicochemical parameters such as hydrophobicity, net charge, and isoelectric point (pI), which offer insights into peptide behavior in biological systems. Peptide fragments generated in the previous step were submitted for evaluation using the default prediction model, allowing for the identification of potentially harmful segments that may affect therapeutic applications or peptide safety (Rathore et al., 2024).

2.10.Identification of Tumour Homing Peptides

To evaluate the potential of Plectranthus zeylanicus lectin-derived peptides to function as tumor homing peptides (THPs), the TumorHPD web server was employed. This tool specializes in identifying short peptides (typically 7–12 residues) with an inherent ability to selectively bind tumor cells or tissues. Such peptides are of significant interest due to their ability to facilitate targeted drug delivery and act as diagnostic imaging agents. Peptides derived from the lectin sequence were screened against the TumorHPD database to identify sequence motifs with tumor-targeting capability, based on curated experimental data and sequence similarity features. The screening results provide insights into which peptide segments may possess tumor-specific affinity, which can then be considered for theranostic applications in oncology, particularly in enhancing the specificity and efficacy of cancer-targeted therapies (Sharma et al., 2013).

2.11. Antigenicity Prediction

The antigenicity prediction was performed using VaxiJen v2.0 (https://ddg- pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). VaxiJen is an alignment-independent computational tool that assesses a protein or peptide's probability of being antigenic based on its auto-cross covariance (ACC)-transformed physicochemical properties. The method is designed to distinguish likely antigens from non-antigens without the need for sequence homology. In the present study, the ACP sequences were submitted under the "Tumor" target class using the default threshold of 0.4, which optimally balances specificity and sensitivity (Zaharieva et al., 2019).

2.12. Allergenicity Prediction

To evaluate the allergenic potential of peptides, AllerTOP v.2.1 (https://www.ddg- pharmfac.net/allertop_test/) was employed. AllerTOP is a robust web-based platform designed to predict the allergenicity of proteins and peptides based on physicochemical properties and machine learning approaches. It classifies peptides as either allergenic or non-allergenic using an alignment- independent approach and auto cross covariance (ACC) transformation of amino acid sequences. The tool is trained on a dataset of known allergens and non-allergens and uses k-nearest neighbor (k-NN) algorithm for prediction (Dimitrov et al., 2014).

2.13. Identification of Cell Penetrating Peptides

To determine whether the peptides identified in this study possess cell-penetrating capabilities, the CellPPD tool (http://crdd.osdd.net/raghava/cellppd/) was employed. CellPPD is a specialized computational platform developed for the prediction and design of cell-penetrating peptides (CPPs) using machine learning algorithms. It is trained on a curated dataset of 708 experimentally validated CPPs and utilizes Support Vector Machine (SVM) models to evaluate input sequences based on physicochemical and sequence-derived features. This tool is particularly effective in identifying peptides that can translocate across biological membranes, making them ideal candidates for intracellular delivery of therapeutic agents (Gautam et al., 2015).

2.14. Structural Analysis and EGFR Docking of Predicted Peptide

The structural modeling of the lead tumor-homing peptide was performed using PEP-FOLD3.5, a de novo peptide structure

prediction tool available at PEP-FOLD3.5 Server. This method employs a structural alphabet (SA) approach to encode local peptide conformations and uses a greedy algorithm combined with a coarse-grained sOPEP force field to predict energetically favorable peptide structures. The simulation was configured to generate 100 structural models, which were ranked based on the sOPEP energy score (Lamiable et al., 2016). The model with the best energy profile was selected for downstream analyses. To assess the physicochemical properties of the modeled peptide, ProtParam was utilized (https://web.expasy.org/protparam/). The tool estimates key molecular descriptors including molecular weight, isoelectric point (pI), amino acid composition, instability index, and GRAVY score, offering insight into the peptide's stability and hydropathic nature (Azimi, 2024). For docking and interaction evaluation, the Epidermal Growth Factor Receptor (EGFR) was chosen due to its central role in multiple cancers. The GE11 peptide (YHWYGYTPQNVI), a well-characterized tumor-homing peptide targeting EGFR, served as the control/reference ligand for comparison. The EGFR receptor structure was retrieved from the Protein Data Bank (PDB ID: 8SC7), resolved by X-ray crystallography at 1.98 Å resolution. Protein–peptide docking was performed using the GalaxyPepDock server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=PEPDOCK). GalaxyPepDock is a structure-based peptide docking tool that predicts binding poses by aligning user-submitted peptide and protein sequences to a template complex from a curated library of known structures. The server uses a hybrid scoring function combining structural similarity, interface energy, and statistical potentials to generate accurate docking models. The tool outputs metrics such as TM-score, interaction similarity score, and estimated model accuracy, facilitating objective comparison of docking outcomes. The interaction between the predicted peptide and the EGFR receptor was analyzed using PDBsum Generate, a tool that provides detailed visualizations of protein–peptide or protein–protein interfaces including hydrogen bonding, contact residues, and interaction networks (Laskowski et al., 2018).

3. RESULTS

3.1. Lectin Extraction and MALDI-Based Sequence Analysis

The purified lectin protein extracted from Plectranthus zeylanicus was successfully analyzed by MALDI-TOF mass spectrometry. The mass fingerprinting data enabled accurate sequence determination of the lectin, which was further verified through database matching and peptide alignment. The resulting sequence was compiled and is presented below in the standard FASTA format. This verified amino acid sequence forms the basis for all subsequent bioinformatics analyses, including structural modeling, domain prediction, and functional characterization.

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>Lectin_Plectranthus_zeylanicus

MNGHLASRRAWVWYFLMLGQVFGATVKAETKFSYERLRLRVTHQTTGEEYFRFITLLRDYVSSGSFSNEIPLLRQSTIPVSD
AQRFVLVELTNEGGDSITAAIDVTNLYVVAYQAGDQSYFLRDAPRGAETHLFTGTTRSSLPFNGSYPDLERYAGHRDQIPLG
IDQLIQSVTALRFPGGSTRTQARSILILIQMISEAARFNPILWRARQYINSGASFLPDVYMLELETSWGQQSTQVQQSTDGV
FNNPIRLAIPPGNFVTLTNVRDVIASLAIMLFVCGERPSSSDVRYWPLVIRPVIADDVTCSASEPTVRIVGRNGMCVDVRDD
DFHDGNQIQLWPSKSNNDPNQLWTIKRDGTIRSNGSCLTTYGYTAGVYVMIFDCNTAVREATLWEIWGNGTIINPRSNLVLA
ASSGIKGTTLTVQTLDYTLGQGWLAGNDTAPREVTIYGFRDLCMESNGGSVWVETCVISQQNQRWALYGDGSIRPKQNQDQC
LTCGRDSVSTVINIVSCSAGSSGQRWVFTNEGAILNLKNGLAMDVAQANPKLRRIIIYPATGKPNQMWLPVP
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3.2. Physicochemical Properties Analysis

The lectin protein extracted from Plectranthus zeylanicus consists of 564 amino acids with a calculated molecular weight of 62.63 kDa and a theoretical isoelectric point (pI) of 6.55. Amino acid composition analysis revealed a high abundance of leucine (8.5%), glycine (8.0%), serine (8.0%), and threonine (7.8%), indicating a flexible and potentially interactive structure. The protein contains 49 positively charged residues (Arg + Lys) and 50 negatively charged residues (Asp + Glu), suggesting a nearly neutral charge balance at physiological pH. The atomic composition showed hydrogen as the most abundant element, followed by carbon, oxygen, nitrogen, and trace amounts of sulfur. The estimated extinction coefficient was 105,935 $M^{-1}cm^{-1}$, and the instability index value (32.80) classifies the protein as stable. Additional values include a high aliphatic index (87.62), indicating probable thermal stability, and a GRAVY score of –0.160, supporting overall hydrophilicity (Figure 1).

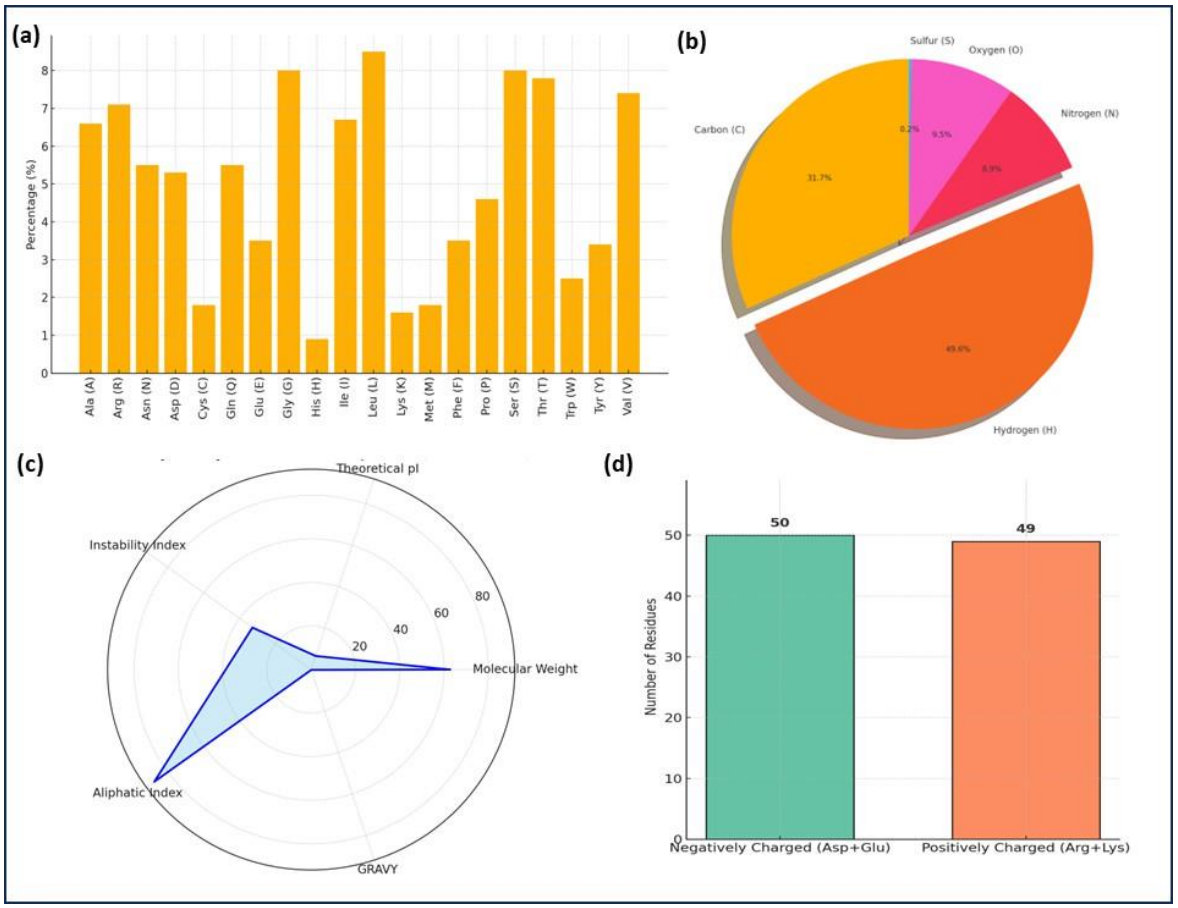

Figure 1. Physicochemical characteristics of Plectranthus zeylanicus lectin.

(a) Amino acid composition showing the percentage of each residue. (b) Atomic composition highlighting the elemental breakdown, with hydrogen being most abundant. (c) Radar plot displaying core physicochemical properties such as molecular weight, theoretical pI, aliphatic index, instability index, and GRAVY. (d) Charge distribution based on positively (Arg + Lys) and negatively (Asp + Glu) charged residues, reflecting an overall balanced ionic profile.

3.3. Functional Motif & Domain Identification

Analysis of the Plectranthus zeylanicus lectin protein revealed a well-defined arrangement of conserved domains and motifs consistent with its classification as a type-2 ribosome-inactivating lectin. InterPro domain analysis identified two Ricin B lectin-like domains toward the C-terminal region (amino acids~350–520), typically responsible for carbohydrate-binding specificity, particularly for β-galactoside residues. Additionally, upstream domains such as the Ricin A-subunit (Domains 1 and 2) and the RIP domain span the N-terminal half, implicating ribosome-inactivating enzymatic function. This architecture demonstrates the dual-functional nature of this lectin in binding and toxicity. The MEME Suite identified 10 conserved motifs, such as KQNQLW, RGAETK, and CMEGNGQRWVETC, distributed across the protein sequence. These motifs show strong statistical significance (e.g., $p = 1.7\times 10^{-67}$) and correspond well with predicted carbohydrate recognition sites and structural domains. ScanProsite further supported this, confirming characteristic β-galactoside-binding motifs, reinforcing its role in glycan recognition and defense response (Figure 2).

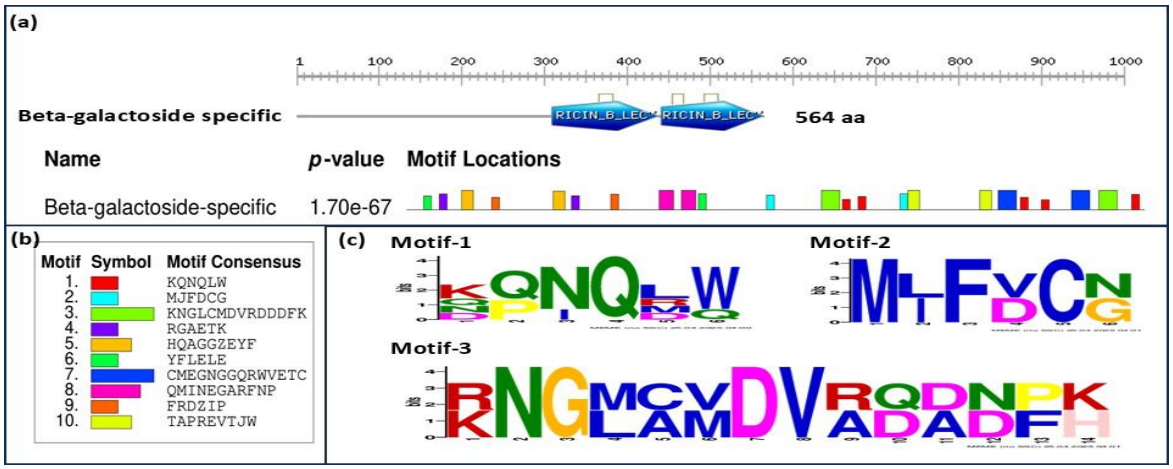

Figure 2. Conserved Motifs and Domain Architecture of Plectranthus zeylanicus Lectin

(a) Domain schematic showing the presence of two Ricin_B_lectin domains along with upstream A- subunit and RIP domains; (b) Distribution and positioning of 10 conserved sequence motifs, including their p-values and sequence consensus; (c) MEME sequence logos for three top motifs (Motif 1–3) indicating key residue enrichment.

3.4. Subcellular Localisation and Signal Peptide Prediction

The Plectranthus zeylanicus lectin protein was predicted to be extracellular with a very high localization probability of 0.9367, as determined by the localization analysis tools. Minor probabilities were also detected for compartments like the lysosome/vacuole (0.3349) and endoplasmic reticulum (0.2928), but the extracellular assignment remained dominant. Signal peptide prediction by SignalP 6.0 indicated the presence of a Sec/SPI-type signal peptide, with a predicted cleavage site between positions 28 and 29 and a high confidence probability of 0.812. DeepTMHMM analysis revealed that the lectin has zero predicted transmembrane helices (TMHs), suggesting that it is not membrane-embedded but rather secreted or soluble outside the cell (Figure 3).

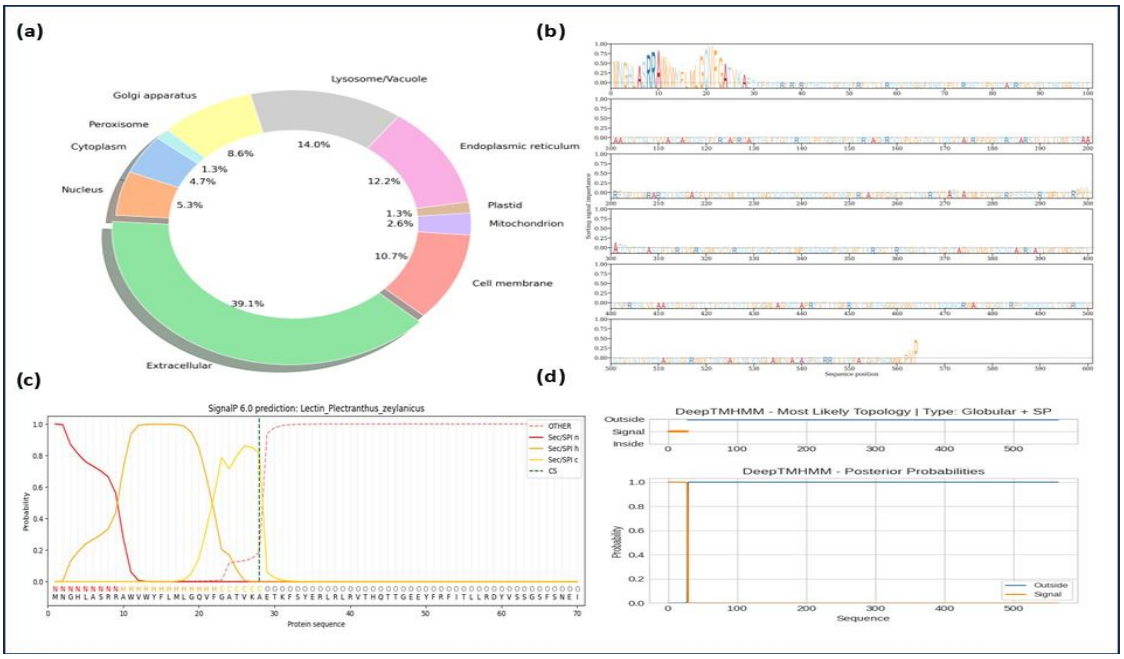

Figure 3. Computational prediction of extracellular localization and signal sequence architecture of Plectranthus zeylanicus lectin.

(a) Probability distribution of subcellular localization, with a predominant extracellular prediction; (b) Absence of transmembrane helices confirmed by DeepTMHMM topology analysis; (c) SignalP 6.0 prediction showing a Sec/SPI-type signal peptide with a predicted cleavage between amino acids 28 and 29; (d) Posterior probability analysis confirming the protein's secretory nature and lack of membrane-spanning domains.

3.5. Secondary Structure and Homology Modeling

The secondary structure analysis (Figure 4a) of the Plectranthus zeylanicus lectin protein revealed a significant proportion of random coils (56.91%), followed by extended β-strands (24.65%), and alpha helices (18.44%). The absence of other structural motifs such as 310 helices, beta bridges, or bends suggests a simplified, yet functionally optimized folding pattern (Figure 4b). The dominance of coil regions indicates a flexible backbone, likely facilitating conformational adaptability during ligand binding, while the β-strands reflect conserved carbohydrate-binding domains characteristic of lectins. Homology modeling using SWISS-MODEL, with the ribosome-inactivating protein (PDB ID: 2vlc.1.A) as a template, produced a structurally robust model (Figure 4c). Validation metrics revealed a MolProbity score of 1.50, a very low clash score (0.61), and 90.36% residues in favored Ramachandran regions, confirming a stereochemically sound model. Only 3.02% residues were Ramachandran outliers, within acceptable thresholds for homology-based predictions (Figure 4d).

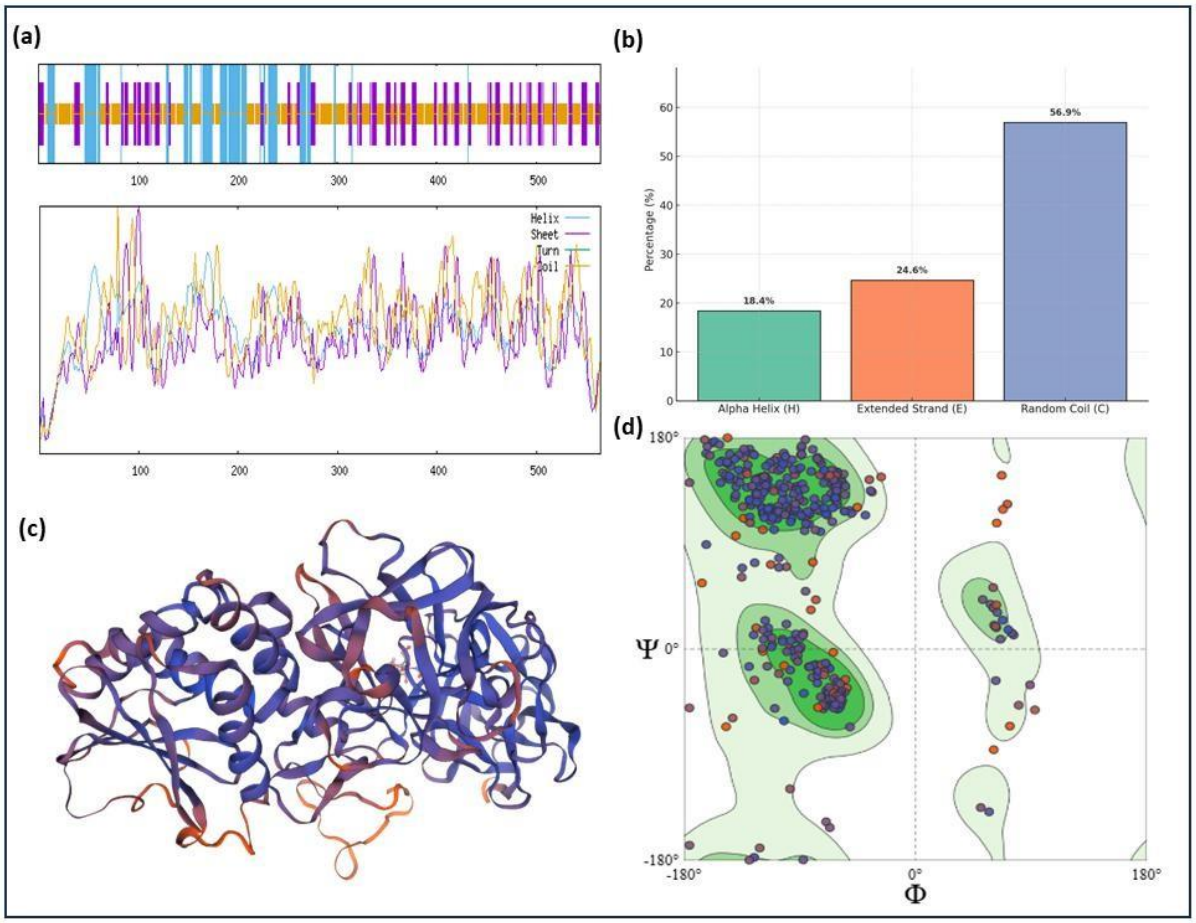

Figure 4. Secondary and Tertiary Structural Representation of the Lectin Protein

(a) SOPMA-based prediction of secondary structure showing the distribution of helices, strands, and coils across the sequence and confidence plots. (b) Bar plot indicating quantitative proportions of secondary structure elements, highlighting the dominance of random coils (56.9%) over β-strands (24.6%) and α-helices (18.4%). (c) Homology-modeled 3D structure of the protein built using SWISS- MODEL with template 2vlc. 1.a, colored by structural domains. (d) Ramachandran plot validating backbone geometry, showing 90.36% residues in favored regions, and confirming the structural quality of the predicted model.

3.6. Identification of Intrinsically Disordered Region

The IUPred3-based disorder prediction for the lectin protein of Plectranthus zeylanicus revealed six discrete segments of intrinsic disorder, comprising approximately 7.8% of the total amino acid sequence. The disordered residues are spread across specific regions such as 1–5, 35–37, 87–98, 347– 349, 405–416, and 545–553, with the longest disordered segments spanning 12 residues. These regions showed moderate to high disorder strength, with values ranging between 0.52 and 0.80 (Table 1, Figure 4a). The low average prediction score of 0.1768 indicates that the protein predominantly assumes a structured conformation under native physiological conditions. However, the presence of scattered flexible segments suggests possible roles in dynamic molecular functions such as conformational switching, molecular recognition, or

induced fit during ligand binding.

Table 1. Predicted Disordered Regions in Plectranthus zeylanicus Lectin

Segment No.	Residue Range	No. of Residues	Average Disorder Score	Comment
1	1–5	5	0.8044	Highly disordered region
2	35–37	3	0.5241	Moderately disordered
3	87–98	12	0.534	Longest disordered stretch
4	347–349	3	0.5472	Short disordered loop
5	405–416	12	0.703	Flexible region near C-terminus
6	545–553	9	0.645	Disordered tail

3.7. Evolutionary Conservation Analysis

The evolutionary conservation analysis of the Plectranthus zeylanicus lectin protein using ConSurf revealed that a substantial number of residues are highly conserved, particularly those with conservation scores of 8 and 9. These conserved residues are predominantly clustered within the core β-strand regions and predicted carbohydrate-binding domains. Many of these were annotated as either functionally important (f) being highly conserved and exposed on the surface or structurally important (s) highly conserved and buried. The presence of such residues indicates strong evolutionary pressure to maintain both the protein's structural framework and its glycan-binding functionality (Figure 5b and 5c). The disordered segments predicted by AIUPred3 are generally located in low-conservation regions, such as N- and C-terminal loops or flexible linkers, suggesting these regions tolerate greater sequence variability. This non-overlapping distribution implies functional specialization, where conserved residues maintain core lectin structure and carbohydrate-binding activity, while disordered, variable segments may facilitate dynamic interactions or structural flexibility.

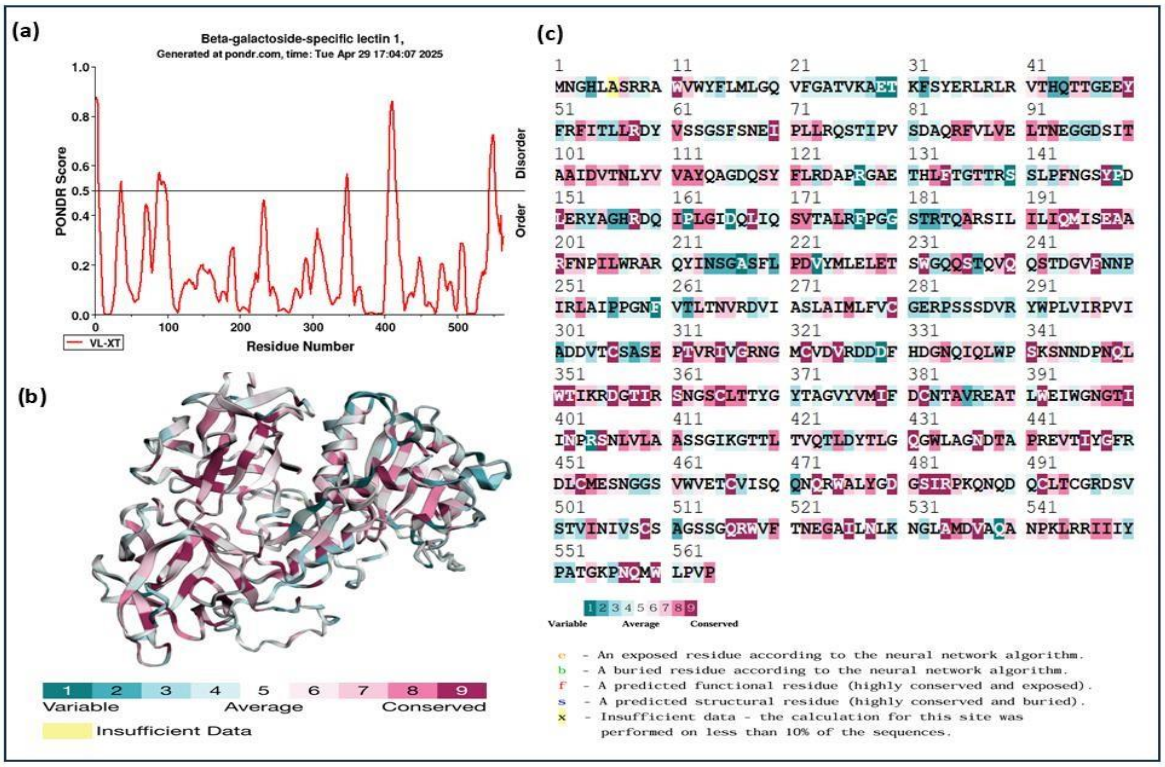

Figure 5. Structural Flexibility and Evolutionary Conservation of Plectranthus zeylanicus Lectin Protein

(a) Disorder prediction plot from PONDR showing disorder probability (red curve) across the sequence; regions above 0.5 threshold are considered disordered; (b) 3D model colored by ConSurf conservation scale, where dark pink indicates highly conserved residues and blue denotes variable sites; (c) Annotated amino acid sequence showing conservation scores (1–9) with labels for predicted functional (f) and structural (s) residues, and depth of burial (e = exposed, b = buried).

3.8. Prediction of Anti-Cancer Peptides

Anticancer peptides (ACPs) have emerged as promising therapeutic agents due to their selective toxicity against cancer cells and their ability to disrupt malignant cellular pathways. In the present study, the Plectranthus zeylanicus lectin sequence was systematically fragmented and screened using AntiCP 2.0 to evaluate its anticancer potential. A total of 553 peptide fragments were generated from the protein sequence, out of which 309 peptides (55.87%) were predicted to possess anti-cancer activity, while 244 peptides (44.13%) were classified as non-anticancer (Figure 6a).

3.9. Identification of Toxic Peptides

The presence of toxic peptides within biologically active sequences is a critical concern during the design and development of therapeutic peptides. Toxic peptides are defined as sequences that may cause cytotoxicity, immunogenic responses, or off-target cellular damage. These effects can emerge from high binding affinities to unintended targets, membrane disruption, or metabolic instability. The identification of toxic segments is thus essential in peptide drug discovery, especially for compounds with anticancer potential where therapeutic selectivity and safety are paramount. In this study, only 3 out of 309 predicted ACPs (0.97%) were identified as potentially toxic by ToxinPred, highlighting the high biocompatibility profile of the lectin-derived peptides (Table 2). These toxic peptides share a conserved core motif (GRNGMCVDVR) with slight variations in N- and C-terminal residues. Their negative hydrophobicity and elevated hydrophilicity suggest an ability to interact with aqueous environments or biological membranes, which might underlie their cytotoxic potential. Additionally, their variable net charges could influence their interaction with negatively charged cellular membranes or proteins. This low toxicity ratio indicates a strong therapeutic index for most peptides in the ACP pool, making them promising candidates for further development.

Table 2. Toxic Peptide Fragments Identified from Anti-Cancer Peptide Pool

Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
IVGRNGMCVDVR	0.15	Toxin	-0.16	0.39	0.05	1	1318.75
VGRNGMCVDVRD	0.03	Toxin	-0.28	-0.27	0.45	0	1320.67
GRNGMCVDVRDD	0.13	Toxin	-0.38	-0.92	0.83	-1	1336.62

3.10. Identification of Tumour Homing Peptides

The THPs are a distinct class of short peptides (typically 7–12 amino acids) that demonstrate the ability to selectively bind tumor tissues or cells via specific interactions with overexpressed receptors or aberrant extracellular matrix components found in the tumor microenvironment. This property makes them ideal for targeted drug delivery, tumor imaging, and minimizing systemic toxicity in cancer therapies. Unlike traditional chemotherapy agents that distribute nonspecifically, THPs offer a means to enhance therapeutic index by focusing treatment directly at the pathological site. In the current study, 90 of the 306 non-toxic ACP were predicted to exhibit tumor-homing characteristics (Figure 6b). This proportion (~29.4%) underscores the inherent targeting potential of peptide fragments derived from Plectranthus zeylanicus lectin, which already displays selective bioactivity through its known carbohydrate-binding and anti-proliferative properties. The enrichment of THPs within this pool may be attributed to the presence of motifs or structural features that mimic naturally occurring tumor- targeting ligands. Moreover, since these peptides have also passed toxicity filters, they form an ideal subpopulation for translational studies, where efficacy, safety, and specificity are all critical.

3.11. Antigenicity Prediction

In the context of peptide-based tumor therapy, non-antigenic peptides are of paramount importance. While antigenic peptides are designed to elicit an immune response (e.g., in cancer vaccines), they may lead to immune clearance, hypersensitivity, or adverse immunological reactions when used as drug delivery agents or targeting ligands. On the other hand, non-antigenic peptides evade immune detection, ensuring longer circulation time in the body, minimizing inflammation, and enhancing delivery to tumor tissues. Since the aim of this study is to develop tumor homing anti-cancer peptides with minimal off- target effects and high specificity, non-antigenic peptides offer superior therapeutic profiles. These peptides are more suitable for roles such as tumor targeting, molecular imaging, or site-specific drug delivery, where immune neutrality is essential for bioavailability and safety. Therefore, the 49 non- antigenic peptides (54.4%) predicted here were prioritized for downstream screening and development (Figure 6c).

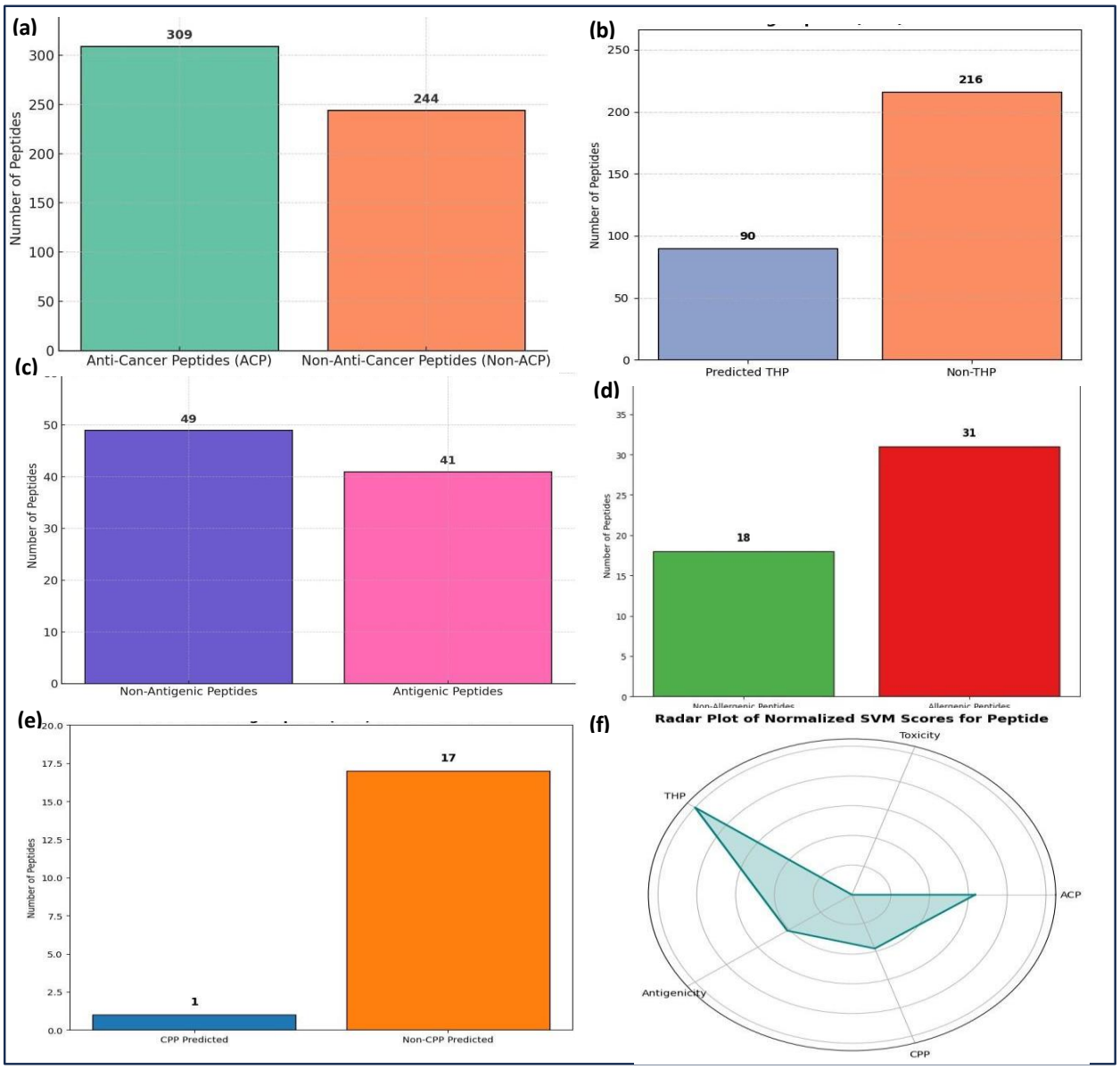

Figure 6. Functional Screening and Classification of Peptide Candidates

(a) Anti-cancer peptide (ACP) classification showing 309 peptides predicted as ACPs and 244 as non- ACPs; (b) Tumor Homing Peptide (THP) prediction results: 90 peptides identified as THPs and 216 as non-THPs; (c) Antigenicity prediction indicating 49 non-antigenic peptides (preferable for cancer therapy) and 41 antigenic; (d) Allergenicity results showing 18 non-allergenic and 31 allergenic peptides; (e) Cell Penetrating Peptide (CPP) prediction identified only 1 peptide as a CPP; (f) Radar plot showcasing the normalized SVM scores for the peptide HLASRRAWVWYF across key categories: ACP, Toxicity, THP, Antigenicity, and CPP.

3.12. Allergenicity Prediction

Allergenicity assessment plays a crucial role in therapeutic peptide development, especially when considering systemic administration. Allergenic peptides, if introduced into the body, can provoke immunoglobulin E (IgE)-mediated reactions, ranging from mild inflammation to severe anaphylaxis. Such immunological responses compromise safety and limit the clinical usability of peptides. In contrast, non-allergenic peptides are far safer and preferred for repeated administration, long-term delivery, or use in tumor-targeting strategies. The tool predicted 31 peptides (73.8%) to be allergenic, while 18 peptides (26.2%) were classified as non-allergenic (Figure 6d).

3.13. Identification of Cell Penetrating Peptides

CPPs are short peptides capable of translocating across cellular membranes, either alone or attached to therapeutic cargo. These peptides are crucial for intracellular drug delivery systems, especially in targeted cancer therapies, where selective

delivery to tumor cells is a major challenge. In the present study, CPP profiling was critical to identify peptides with enhanced functional versatility. Among the screened 18 peptides, only HLASRRAWVWYF met the criteria for a functional CPP, based on CellPPD's SVM-based prediction. Its SVM score of 0.11 exceeds the threshold for classification (Figure 6e), suggesting the presence of intrinsic features such as amphipathicity, cationic charge, and structural motifs known to promote membrane penetration. The model is available in ModelArchive at https://www.modelarchive.org/doi/10.5452/ma-l51b4.

3.14. Structural Analysis and EGFR Docking of Predicted Peptide

3.14.1. Physicochemical Properties of the Predicted Peptide

The predicted tumor homing peptide (THP) consists of 12 amino acids, with a molecular weight of approximately 1591.84 Da and a theoretical isoelectric point (pI) of 10.84, indicating it is basic in nature. The peptide is moderately rich in hydrophobic amino acids like Trp (16.7%) and Ala (16.7%), while it lacks any acidic residues such as Asp or Glu. Positively charged residues include Arg, and Lys (total: 2), with no negatively charged residues detected. The peptide exhibits strong hydrophilicity (−0.84), suggesting good solubility. Its amphipathicity score (0.53) indicates potential for membrane interaction, which is critical for tumor targeting and CPP functionality. The instability index (65.88) classifies it as unstable in vitro, though its estimated half-life (e.g., >10 h in E. coli) supports good cellular persistence. The GRAVY score (−0.142) further confirms a mild hydrophilic tendency, complementing its targeting and solubility characteristics (Figure 7).

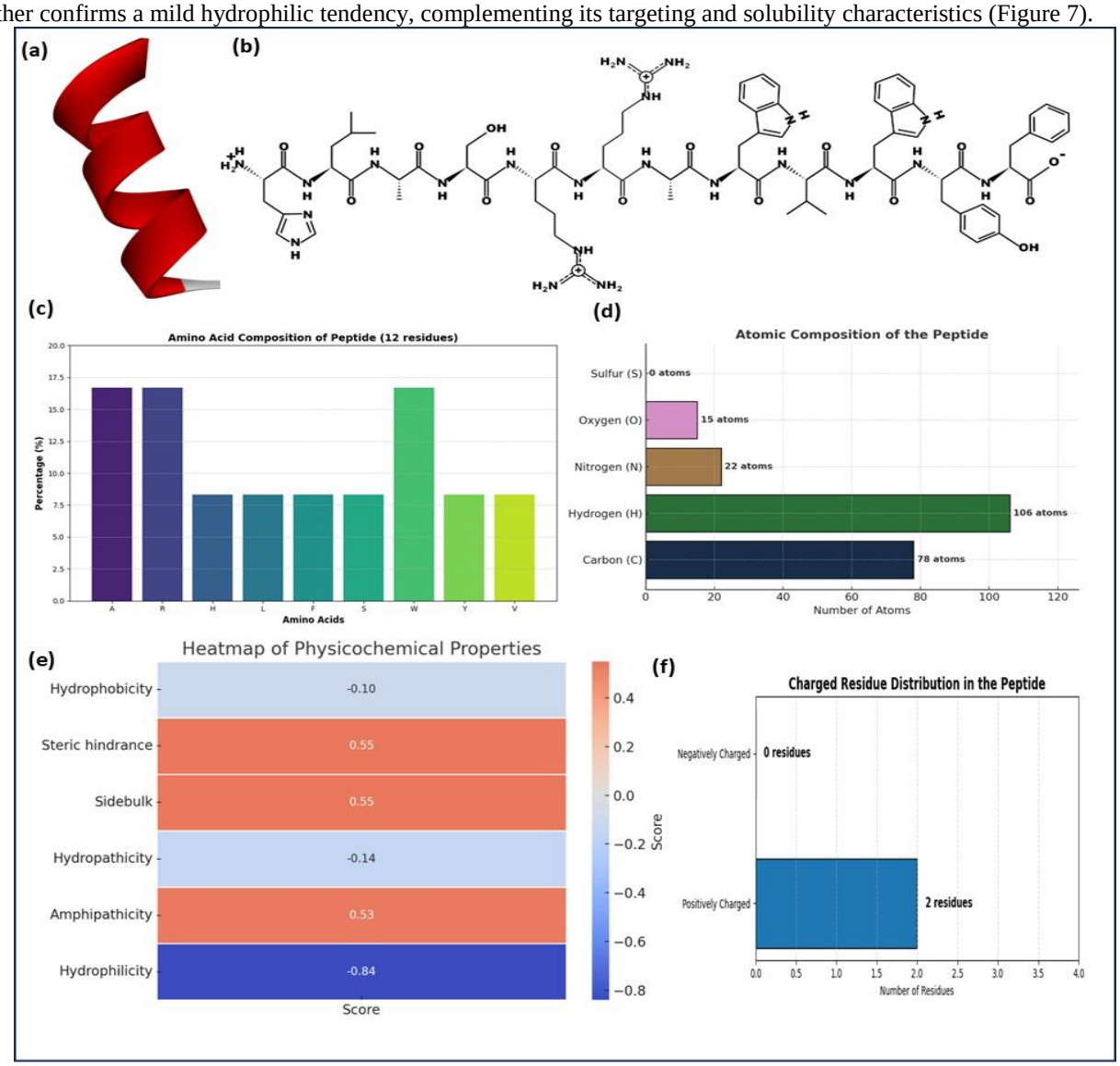

Figure 7. Physicochemical Properties and Structure of Modeled Tumor-Homing Peptide

(a) 3D ribbon structure from PEP-FOLD 3.5; (b) 2D chemical structure; (c) Amino acid composition bar plot; (d) Atomic composition distribution; (e) Heatmap of physicochemical features (e.g., hydrophobicity, amphipathicity); (f) Charged residue distribution plot

3.14.2. Peptide–EGFR Docking Analysis

The predicted THP demonstrated a higher interaction similarity score (74.0) compared to the standard GE11 (65.0), suggesting improved mimicry and binding interface alignment with EGFR. The TM-score (0.979) in both cases confirms near-native folding and excellent structural overlap with the EGFR binding domain (Table 3).

Table 3: Docking Score Summary for Modeled Peptide and Reference GE11 Peptide

Model Type	Protein Template	Peptide Template	TM-score	Interaction Similarity Score	Estimated Accuracy
Modeled Peptide	**2GS6_A**	**2GS6_B**	**0.979**	**74**	**0.772**
GE11 Reference Peptide	**2GS6_A**	**2GS6_B**	**0.979**	**65**	**0.752**

The modeled peptide formed six hydrogen bonds and two salt bridges with key residues on the EGFR receptor, indicating strong and specific interactions. Notably, residues such as ASP107, ASP162, and LYS220 of EGFR were involved in stabilizing interactions through side-chain participation with HIS327, ARG331, SER330, and ALA333 of the peptide. Most of these hydrogen bonds were well within the optimal range for stable interactions (2.83–3.30 Å), suggesting favorable geometry and energetic complementarity. In contrast, the standard GE11 peptide established only three hydrogen bonds, and its interaction surface was slightly less extensive. EGFR residues ASN149, VAL183, and ILE185 engaged with peptide residues TYR330, TYR332, and TRP329, but with fewer contact points and no salt bridges. Although the GE11 peptide has been validated experimentally as an EGFR-binding sequence, the interaction profile suggests that the modeled peptide may offer enhanced binding strength and possibly better receptor engagement due to its richer hydrogen bonding network and broader surface complementarity (Table 4; Figure 8).

Table 4: Hydrogen Bond Interactions Between EGFR and Peptides

Modelled Peptide				
EGFR Residue	**Peptide Residue**	**EGFR Atom**	**Peptide Atom**	**Distance (Å)**
ASP107	SER330	N	OG	3.3
ASP107	HIS327	OD2	N	2.83
ASP107	SER330	OD2	N	3.27
ASP162	ARG331	OD1	NH1	2.92
ASP162	ARG331	OD2	NH2	2.83
LYS220	ALA333	NZ	O	2.99
Control Peptide (GE11 Peptide)				
ASN149	TYR330	OD1	OH	2.73
VAL183	TYR332	N	O	2.81
ILE185	TRP329	N	O	3.31

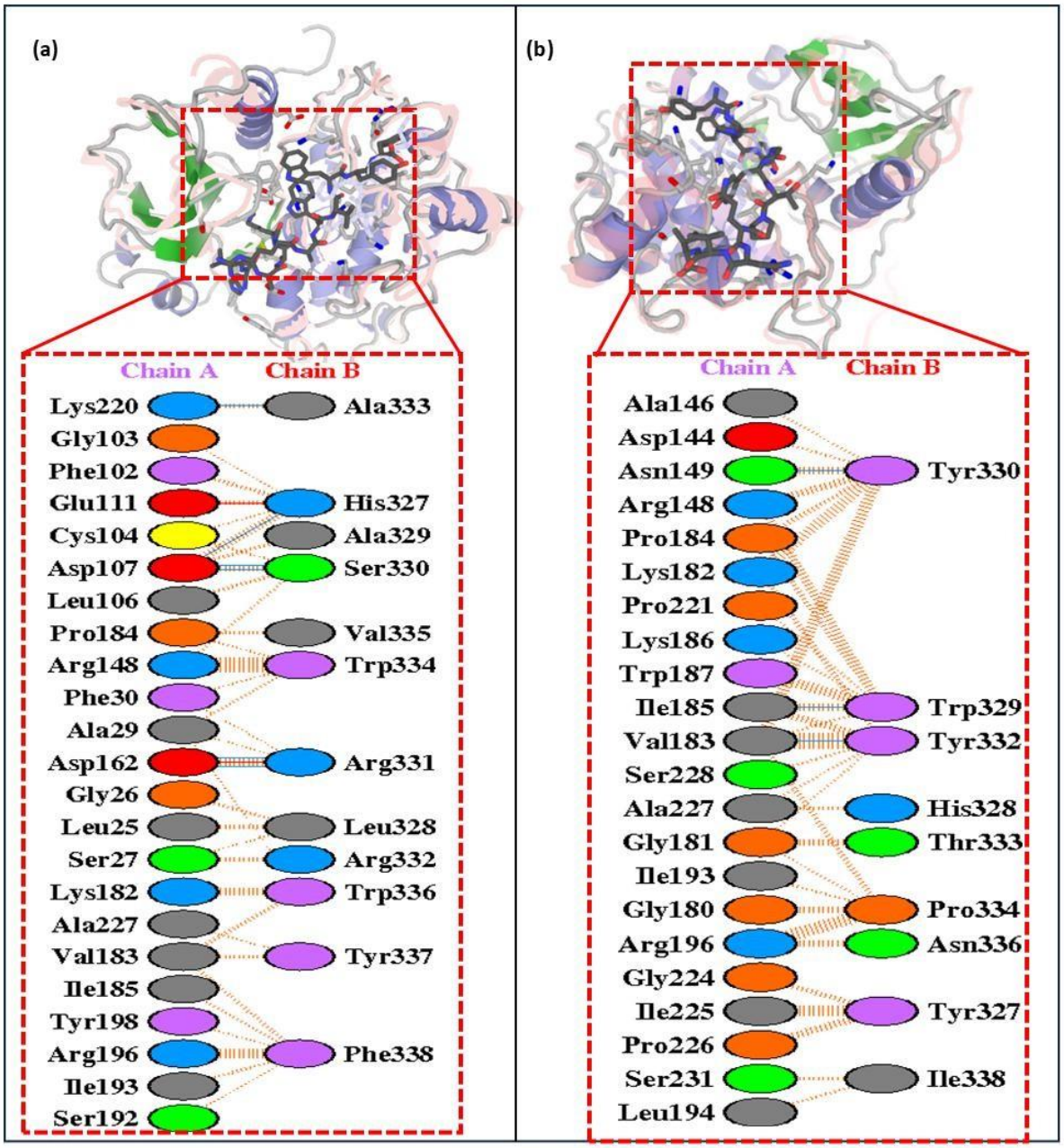

Figure 8: Interaction Mapping Between Peptides and EGFR

(a) The modeled peptide engages 23 EGFR residues via 6 hydrogen bonds and 2 salt bridges; (b) The GE11 control interacts with 22 EGFR residues via 3 hydrogen bonds. Residues involved in interactions are labeled on both receptor (Chain A) and peptide (Chain B).

4. DISCUSSION

Plectranthus zeylanicus, a member of the Lamiaceae family, is a perennial herb widely utilized in traditional Sri Lankan medicine for managing fevers, gastrointestinal ailments, infections, and inflammatory conditions (Napagoda et al., 2022). Commonly known as "Iruveriya," the plant has historically been incorporated into Ayurvedic and folk remedies, indicating its therapeutic significance. Although research on its direct anticancer properties remains limited, several studies have highlighted its pharmacological potential, particularly its antimicrobial and anti-inflammatory effects (Napagoda et al., 2022). Phytochemical analyses of P. zeylanicus have revealed a diverse spectrum of bioactive compounds including diterpenoids such as 7α-acetoxy-6β-hydroxyroyleanone and 7β,6β- dihydroxyroyleanone, which have demonstrated significant antibacterial activity against pathogens like methicillin-resistant Staphylococcus aureus (MRSA). These royleanones are also potent inhibitors of 5-lipoxygenase, implicating them in the downregulation of leukotriene-mediated

inflammatory pathways that are often associated with cancer progression (Napagoda et al., 2014). Additionally, the plant's essential oil composition, rich in terpenoids such as β-caryophyllene, p-cymene, and geraniol which provides indirect chemopreventive implications given the established roles of these compounds in reducing oxidative stress and modulating cellular redox states (Napagoda et al., 2022).

While P. zeylanicus has not yet been extensively studied for direct anticancer activity, related species within the Plectranthus genus have demonstrated promising therapeutic potential. Plectranthus amboinicus (syn. Coleus aromaticus), for instance, has been shown to possess strong cytotoxic effects against various cancer cell lines (Lukhoba et al., 2006). Ethanolic and methanolic extracts of P. amboinicus exhibit significant antiproliferative activities, attributed to the presence of flavonoids, phenolic acids, and terpenoids (Rajesh & Gayathri, 2015). These bioactive constituents are known to induce apoptosis, disrupt cell cycle progression, and attenuate oxidative stress, the mechanisms that are critical in cancer control (Hasibuan & Sumaiyah, 2019). Additionally, P. amboinicus displays notable antioxidant (Nguyen et al., 2020) and anti-inflammatory activities (Chen et al., 2014), which further support its utility in chemoprevention and adjunctive therapy (Arumugam et al., 2016). While, Plectranthus barbatus (also known as Coleus forskohlii), is recognized for its active diterpene forskolin. Forskolin has been extensively documented for its anticancer activity (Sapio et al., 2017), particularly through activation of adenylate cyclase and elevation of intracellular cAMP levels, which ultimately leads to the inhibition of tumor cell proliferation and induction of apoptosis (Salzillo et al., 2023). Studies have shown its efficacy against breast (Illiano et al., 2018), colon (Cristóbal et al., 2014), and liver cancers (Yang et al., 2024), with further investigations highlighting its ability to sensitize tumors to conventional therapies (Cordeiro et al., 2021). Other less-studied species such as Plectranthus scutellarioides (Aziz, 2021) and P. neochilus (Caixeta et al., 2011) have exhibited antioxidant and antimicrobial properties, suggesting that the genus as a whole is a rich reservoir of bioactive molecules with chemotherapeutic potential. Plectranthus zeylanicus itself remains underrepresented in current oncological research, the documented bioactivity of its secondary metabolites, coupled with the anticancer evidence from its phylogenetic relatives, provides a compelling rationale for its exploration as a source of novel anticancer agents.

Lectins are a structurally diverse family of carbohydrate-binding proteins, predominantly found in plants, that play pivotal roles in intercellular signaling, pathogen recognition, and defense. Their unique ability to selectively bind specific glycan motifs, especially those overexpressed or aberrantly glycosylated on tumor cell surface, has made them valuable candidates for cancer diagnostics and therapeutics (Mayer et al., 2017). Numerous studies have validated their use as bioactive agents capable of recognizing and targeting malignant cells via glycan-encoded signals (Kaltner & Gabius, 2019). Mechanistically, plant lectins execute anticancer effects through several pathways: (1) Ribosome inactivation, as observed with type-2 ribosome-inactivating proteins (RIPs) like ricin, which depurinate rRNA and halt protein synthesis (Fatima & Bashir, 2025); (2) Mitochondrial disruption via lectin internalization and induction of cytochrome c release, leading to apoptosis and autophagy (Bhutia et al., 2019); and (3) Binding to cell surface glycoproteins, modulating pro-survival pathways such as PI3K/Akt and promoting tumor suppressive signals via p53 or caspase activation. These mechanisms collectively drive selective cancer cell death while sparing normal cells (Huldani et al., 2022).

In the present investigation, we explored the therapeutic viability of lectin derived from Plectranthus zeylanicus, a traditionally valued medicinal plant with established anti-inflammatory and antimicrobial activity. The characterized lectin displayed hallmark features of RIPs, including Ricin B-like domains for glycan binding and RIP-A domains for translational inhibition, indicating dual functionality. Subcellular localization studies predicted it to be secreted, consistent with extracellular defense functions. Structural analysis confirmed conserved β-sheet frameworks and intrinsically disordered regions associated with flexible binding dynamics. The evolutionary conservation of key disordered residues further emphasized their adaptive relevance, suggesting functional plasticity in glycan interactions. These features, along with favorable physicochemical attributes and modeled stability, support the hypothesis that P. zeylanicus lectin could mimic known anticancer lectins in selectively targeting cancer cell glycomes. Through peptide fragmentation and subsequent screening, this lectin was further exploited to identify novel anticancer peptides, one of which showed high potential in targeting EGFR, a key oncogenic receptor.

Peptides are emerging as vital agents in cancer therapy due to their specificity, low toxicity, and high biocompatibility. They are used as direct anticancer agents, tumor-targeting ligands, delivery vectors, and immunomodulators. Anti-cancer peptides (ACPs), typically 10–60 amino acids long, exert their effects by inducing apoptosis, disrupting tumor vasculature, and targeting mitochondrial integrity (Chinnadurai et al., 2023). Tumor-homing peptides like RGD target integrins overexpressed in cancers, enabling targeted drug delivery. Peptide-drug conjugates (PDCs) and functionalized nanocarriers, such as TAT-conjugated dendrimers, enhance tumor penetration and payload delivery. In immunotherapy, peptides derived from tumor-associated antigens stimulate cytotoxic T-cell responses, improving vaccine efficacy. Their utility lies in their ability to penetrate cell membranes, bind selectively to cancer biomarkers, and be chemically modified to improve half-life and bioavailability. Examples like tLyP-1 and HER-2 derived peptides have demonstrated real-world impact in inhibiting tumor growth and enhancing treatment specificity. Collectively, peptides offer modularity and

multifunctionality, allowing for customized cancer therapies with minimal side effects (Xie et al., 2020).

In the present study, the lectin protein identified from Plectranthus zeylanicus was enzymatically fragmented to generate a peptide library with potential anticancer functionality. This approach builds on the evidence that many plant-derived lectins possess domains similar to ribosome-inactivating proteins and exhibit high glycan-binding specificity traits associated with potent anticancer activity. Using advanced computational tools, the generated peptides were screened across several biological criteria to assess their therapeutic viability. The peptides were analyzed for anticancer potential using the AntiCP 2.0 server, identifying sequences with significant cytotoxic signatures. These were subsequently filtered for key therapeutic attributes such as tumor homing ability, antigenicity, allergenicity, and toxicity. Furthermore, cell-penetrating potential was evaluated through CellPPD. Importantly, from this pipeline, non-antigenic peptides were prioritized to avoid immune recognition. In therapeutic contexts such as targeted cancer therapy, peptides that are non-antigenic and non- allergenic are favored, as they evade immune clearance, reduce the risk of hypersensitivity reactions, and enhance circulation time, factors essential for repeated dosing and long-term effectiveness. This strategy aligns with current peptide-based delivery platforms, where the goal is to deliver cytotoxic or modulatory payloads specifically to tumors while minimizing systemic immune activation.

The EGFR is a critical cell surface tyrosine kinase involved in the regulation of cellular proliferation, differentiation, migration, and survival. In many cancers, including lung, breast, colorectal, and head and neck tumors, EGFR is frequently overexpressed or mutated, leading to constitutive activation of downstream signaling pathways such as PI3K/Akt and MAPK, which promote oncogenesis, metastasis, and resistance to apoptosis. Therapeutic strategies targeting EGFR, including monoclonal antibodies and tyrosine kinase inhibitors, have shown clinical success; however, peptides offer a promising alternative due to their high specificity and low immunogenicity (Sigismund et al., 2018). The GE11 peptide (YHWYGYTPQNVI) is a well-characterized tumor-homing peptide that binds EGFR with high affinity without activating its kinase function, making it ideal for targeted delivery systems. It selectively accumulates in EGFR-overexpressing tumors and has been employed to guide drugs, imaging agents, and nanoparticles directly to cancer cells. In the present study, GE11 served as a reference standard for comparing the binding efficacy and therapeutic potential of the modeled peptide targeting EGFR. Docking studies revealed that the modeled peptide exhibited superior interaction with EGFR, including higher hydrogen bond formation, increased surface contact, and stronger interaction similarity scores. These findings indicate enhanced receptor binding, which may translate to better internalization and therapeutic outcomes (Hailing et al., 2022). Thus, the identified P. zeylanicus- derived peptide represents a promising candidate for EGFR-targeted cancer therapy, with potential advantages over existing control peptides. The peptide characterized herein demonstrates potent interaction with EGFR, encouraging its future validation. Further experimental assays and clinical modeling are warranted to translate these findings into viable peptide-based therapeutics for precision oncology.

5. CONCLUSION

This research integrates a multi-layered computational strategy to explore anticancer peptide discovery from Plectranthus zeylanicus lectin, a plant species long valued in traditional medicine yet underexplored in oncology. The lectin was structurally and functionally profiled, revealing features characteristic of ribosome-inactivating proteins with glycan-binding potential. Systematic peptide mining followed by rigorous screening for anticancer traits led to the identification of a lead peptide fulfilling all essential therapeutic criteria. Docking studies with EGFR, a clinically validated cancer target demonstrated superior interaction characteristics over a benchmark peptide, reinforcing its therapeutic promise. This peptide stands out for its predicted tumor selectivity, low immunogenicity, and capacity to engage critical cancer receptors. While the findings provide a compelling basis for therapeutic advancement, they remain computational and require experimental validation. Future directions should include biochemical synthesis, cytotoxicity assays, and in vivo modeling to establish clinical relevance and develop peptide-based strategies for targeted cancer therapy.

Declarations
Authors Contribution:
All the authors have equally contributed to the manuscript.

Conflict of Interest
The authors declare no conflict of interest.

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All datasets generated and analyzed during the current study are included within the manuscript.

Ethical Approval:
Not applicable

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