Indian Journal of Natural Products and Resources
Vol. 15(1), March 2024, pp. 73-86
DOI: 10.56042/ijnpr.v15i1.6562

In silico Approaches of *Azadirachta indica* polypeptides exhibiting antimicrobial and anticancer potential

Mohammed Al Saiqali[1]*, Krishnamurthy Nakuluri[2], A. Venkateshwar Reddy[3], Syed Safiullah Ghori[3] and Hemanth P. K. Sudhani[4]

[1]Department of Research and Innovation, Anwarul Uloom College, New Mallepally, Hyderabad 500001, Telangana state, India
[2]Department of Internal Medicine, Carver College of Medicine, University of Iowa, Iowa City IA, 52242 USA, 1-319-335-3918
[3]Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Telangana state, India
[4]Department of Biotechnology, SRM Institute of Science and Technology (SRMIST), Tiruchirappalli Campus, Tiruchirappalli 621105, Tamil Nadu, India

Received 03 November 2023; revised received 23 February 2024; accepted 29 February 2024

Medicinal plants are a vital source of natural bioactive peptides with broad therapeutic and pharmaceutical applications. These peptides are emerging as a viable alternative to traditional anticancer drugs and conventional antibiotics and potentially have the ability to reduce adverse side effects. This study concentrated on the anticancer and antibacterial efficacy of three bioactive low molecular weight polypeptides (11, 13, and 14 kDa) extracted from neem leaves. The analysis employed SDS-PAGE peptide profiling followed by sequence elucidation via MALDI TOF spectrometry. The three-dimensional (3D) structural models and the target proteins BCL-2 and Sortase A were constructed using Modeller9v7 software. The structural integrity of these models was assessed through molecular dynamics simulations and validated with the SAVES program. Molecular interactions between the peptides and cancer-associated protein BCL-2, alongside the bacterial enzyme Sortase A, were explored using molecular docking techniques facilitated by GOLD 3.0.1 software. The docking analysis revealed that the polypeptides, designated as polypeptide 1 (11kDa), polypeptide 2 (13kDa), polypeptide 3 (14 kDa), exhibited substantial binding affinity towards both BCL-2 and *Staphylococcus aureus* Sortase A, with binding energies of 26.96, 21.59, and 26.11 Kcal/mol, respectively. They also inhibited BCL-2 with binding energies of 27.00, 23.13, and 20.31 Kcal/mol, respectively. Remarkably, polypeptide 1(11 kDa) showed superior efficacy in both anticancer and antimicrobial activities, underscoring its potential as a candidate for developing novel therapeutic agents against anticancer and antimicrobial infections. The findings of this study highlight the promising role of plant-derived bioactive polypeptides in the treatment of cancer and microbial infections.

Keywords: Anticancer, Antimicrobial activity, BCL-2, Bioactive polypeptides, Docking, Sortase A

IPC code; Int. cl. (2021.01) – A61K 36/00, A61P 31/00, A61P 35/00

Introduction

Antimicrobial peptides (AMPs) occur naturally in various organisms, including bacteria, fungi, insects, amphibians, plants, and mammals. Plants produce several secondary metabolites as part of their defence mechanisms, including AMPs. These AMPs, derived from medicinal plants, usually consist of 10 to 100 amino acids and have a molecular weight of 2 to 10 kDa. They have a helical structure that contains a combination of hydrophobic and hydrophilic amino acids encoded by genes[1]. AMPs act rapidly to neutralise bacterial pathogens, including fungi, protozoa, parasites, and cancer cells[2]. AMPs play a critical role in the innate immune response of plants by serving as a component of the barrier defence system and providing the first line of defence against microbial diseases. These peptides can be extracted from various plant parts, such as flowers, stems, leaves, roots, and seeds, and they have a wide range of applications. Recent studies confirmed that peptides effectively regulate various diseases and promote human health, such as enhancing immunity, lowering blood pressure, lipid and cholesterol, and anti-inflammatory. AMPs were classified based on their mode of action as antihypertensive, immunomodulatory, antimicrobial, anticancer, antithrombotic, anti-oxidative, and opioid[3]. AMPs have been shown to inactivate bacterial cells via various metabolic mechanisms targeting extracellular,

*Correspondent author
Email: mohdalsaid11@gmail.com
Mob.: +91-9908907773

plasma membrane, and intracellular components[4]. Plant AMPs exhibit structural and functional diversity and can classified into cationic (CAMPs) and anionic AMPs[5,6]. The biological activity of plant AMPs is primarily based on their interactions with anionic membrane lipids through various mechanisms to eliminate pathogens specifically. Plant AMPs exhibit antimicrobial activities against human pathogens and possess anticancer and antiviral activities. Recently, plant antimicrobial peptides have gained attention as valuable tools for developing novel antimicrobial and anticancer drugs. It is vital to tackle the swift increase in multidrug resistance. Consequently, plant-derived AMPs are considered promising novel antibiotic compounds with significant potential applications in the pharmaceutical, agricultural, and biotechnology sectors[7,8]. Moreover, infectious diseases account for more than 20% of global deaths annually, making them the leading cause of death in children under five years of age[9]. These alarming statistics have led many researchers in the medical and pharmaceutical fields to conclude that we have entered a "post-antibiotic era" This era is characterised by the declining efficacy of commercially available antibiotics[10,11]. The plant antimicrobial peptides can serve as natural antimicrobial agents and alternatives to commercially available antibiotics, protecting against various infections in humans, animals, and plants[12]. One notable study focuses on the viscotoxin protein extracted from the leaves and stems of *Viscum album*. This protein exhibits anticancer and antitumor properties. It causes perforation of the cell membrane, leading to membrane destabilisation and disruption of the bilayer structure[13]. In addition, Ib-AMP4 and Ib-AMP1 proteins have shown the ability to inhibit the growth of various Gram-positive bacteria at low concentrations[14]. Neem, a plant used extensively in Ayurvedic, Unani and homeopathic medicine, offers a wide range of chemically and structurally more than 140 different bioactive compounds isolated from its various parts. Traditionally, these compounds have been used to treat numerous diseases and disorders[15,16].

The bioinformatics approach combines biological mass data using computer science, biology, mathematics and statistical analysis methods, which are considered simple and faster search tools for identifying new potential targets. These *in silico* approaches include diversified tools as databases of protein and peptide sequences, web-based applications for predicting the physicochemical and bioactivities of peptides, and identifying their structure-functional relationships. The downstream purification process of bioactive peptides can be accelerated by integrating *in-silico* methods with omics. The bioinformatics tools, including universal peptide and protein databases like UniProtKB, Swiss rot, BIOPEP and PepBank, are utilized to get high throughput related to *in silico* protein and peptide prediction information on potent bioactive peptide sequences. These computational approaches can easily recommend the use of appropriate ligands to produce potent peptides from native proteins and suggest the best target molecule. Therefore, bioinformatics is recognized as an effective mining tool to accelerate the discovery of bioactive peptides encrypted in different types of protein precursors. The collective results consistently indicate that various constituents of neem, such as seeds, bark, leaves, and extracts, have significant antibacterial, anticancer, and antiviral activities[17-20]. *In silico* studies have also revealed certain similarities in the tertiary structures of plant AMPs despite differences in their amino acid sequences[21-23]. This study aims to isolate small peptides from *Azadirachta indica* leaves, obtain their sequences, *in-silico* structure development and molecular docking for possible usage of these small peptides as anticancer and antimicrobial agents.

Materials and Methods

Plant collection and protein extraction

Fresh leaves of *A. indica* A. Juss (neem) were collected from the Botanical garden of Osmania University (O. U), Hyderabad, India, in August 2022. The fresh leaves were plucked into clean, dry, labelled plastic bags and frozen at -80°C until use. A taxonomist (Professor A. Vijay Bhaskar Reddy) from the Department of Botany, O. U, authenticated and classified the plant. A voucher specimen No. Aug/2022/2 was deposited at the herbarium of O. U. The collected leaves were washed and rinsed with distilled water to remove dust and impurities and then air-dried at room temperature. The dried neem leaves (1g) were pulverised in liquid nitrogen using pre-chilled mortar and pestle. Leaves fine powder was homogenised in a ratio of 1:10 W/V with Tris-HCl buffer pH 7.4. The mixture (Tris-HCl buffer pH 7.4 and sample) was rotated for 1 hour at 4°C, centrifuged, and the supernatants were collected. The pellets were extracted twice with fresh buffer, centrifuged, and supernatants were collected[24-26].

Protein precipitation

The protein content of the fresh leaves of *A. indica* extracted by a Tris-HCl buffer was precipitated

by 15% TCA/acetone containing traces of 2-mercaptoethanol in a ratio of 4:1. Briefly, the tubes containing extraction and precipitation mixtures were placed for overnight in -20°C. The tubes were centrifuged under cold conditions, and the supernatant was removed. The precipitated protein pellets were rinsed with cold 90% acetone with βME thrice, centrifuged, and protein pellets were collected and kept to dry for 10 minutes till the acetone was evaporated. Finally, the protein pellets were placed in a −80°C deep freezer for further use. The concentration of the soluble protein was calculated by the Bradford method[16,25-27].

SDS-PAGE analysis

The 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis in vertical mini-slab gel (Bio-Rad, USA) was used to resolve the protein bands. The protein samples were solubilised using a small amount of mild alkali solution. In brief, after heating, protein samples were loaded into the wells of the gel. Where protein molecular marker was added alongside in a separate well. 50 and 70 V currents are used for stacking and resolving gel, respectively. After electrophoresis, the gel was removed, rinsed with water, and stained with Coomassie Brilliant Blue R-250[16,28]. The molecular weight of the protein bands was determined according to the standard protein marker. The gel images were taken and analysed by Gel Doc™ EZ Gel Documentation System. The experiment was performed in triplicates.

Protein sequence and template identification

The sequences of the low molecular weight polypeptides are identified using the mascot search engine following MALDI-TOF/TOF mass spectrometry analysis[16]. The proteins from neem leaves were subjected to *in-silico* computational analysis against the BCL-2 protein and the bacterial membrane protein Sortase A (SrtA). The three polypeptide sequences derived from neem leaves are investigated to identify related protein structures that could serve as templates. It was accomplished by utilising the Basic Local Alignment Search Tool (BLASTp) to search the Protein Data Bank (PDB) for sequences with high scores, significant e-values, maximum identity, and fewer negative or zero values. The identified sequences that met these criteria are selected as references for creating 3D structural models of the three polypeptides. The coordinates for structurally conserved regions (SCRs) of polypeptides were determined based on the selected template using the BLASTp tool and the Needleman-Wunsch algorithm[29]. In this process, the polypeptide sequences themselves served as the target sequences for alignment and modelling.

Homology modelling

For modelling, three low molecular weight polypeptides, namely Polypeptide 1 (11 kDa), Polypeptide 2 (13 kDa), and Polypeptide 3 (14 kDa), were identified and selected. Three templates, specifically 5XFD, 5F05_D, and 5F05-A, were downloaded from the PDB database to serve as references for generating their three-dimensional structures (3D). The modelling process was carried out using MODELLER9V7, and the resulting structures were analyzed using a Ramachandran plot to assess their reliability and quality. To evaluate the antimicrobial and anticancer activities, *Staphylococcus aureus* Sortase A (SrtA) and Homo sapiens breast cancer BCL-2 proteins were selected. The target protein sequences for both BCL-2 and SrtA were obtained from the PDB database[30,31].

Detecting the three-dimensional structure of the polypeptides

The initial models of the three peptides are generated using homology modelling techniques and the MODELLER9V7 software[32]. This software utilizes a comparative protein structure approach, where spatial restraints are obtained from the alignment expressed as probability density functions (PDFs) for the desired features. These PDFs impose restraints on Cα-Cα distances, N-O spaces in the core chain, and dihedral angles of side and main chains. By optimizing the molecular PDF 3D dimensional structures of the polypeptides were obtained, ensuring that the structure satisfies the input restraints to a significant extent. The molecular PDF was constructed by combining PDF files that restrict specific spatial features of the entire molecule. The optimization process employed a variable target function method, utilizing the conjugate gradients algorithm to adjust the positions of all non-hydrogen atoms[33].

Molecular docking studies of peptides

Molecular docking studies were crucial in evaluating the interactions between peptides derived from *A. indica* and two proteins, Sortase A (SrtA) and BCL-2, relevant in microbial pathogenicity and breast cancer progression, respectively. Utilizing the genetic optimization of ligand docking (GOLD 3.0.1) software, a genetic algorithm (GA) approach is employed to enhance accuracy. Configuration settings allowed limited flexibility for protein targets and flexibility for polypeptides, mirroring realistic interaction conditions. Fine-tuning the genetic algorithm involved specifying

parameters such as a selection pressure of 1.1, a population size of 100, and niche size and number of operations set at 10,000. These settings facilitated comprehensive exploration of the docking space. Each polypeptide's interaction with target proteins was analyzed individually, applying default cut-off values for hydrogen bonding (3.0 Å) and van der Waals interactions (6.0 Å) to assess interaction quality and binding affinity. Simulation defined binding sites within a 10 Å radius of protein active sites, generating 100 poses for each ligand and terminating when the top three bound conformations were within 1.5 Å RMSD of each other, ensuring identification of stable and probable binding poses[34,35].

Molecular dynamics simulations and active site identification with structure validation

The study employed NAMD 2.9 software for molecular dynamics simulation and improving equilibrium using the CHARMM-27 force field for proteins, lipids, and the TIP3P model for water molecules[36,37]. Simulations are conducted at 310 K temperature and 1 ATM pressure, with specific settings for Van der Waals interactions and solvation. The stepwise simulation process included energy minimization, gradual heating, and a 30 ns simulation for equilibration, ensuring the accuracy of the protein models. Active sites of Sortase A and BCL-2 proteins were identified using the CASTp server[38], employing computational geometry to map protein cavities and pockets precisely. Post-simulation structure validation involved selecting the lowest energy and RMSD structures, followed by Ramachandran plot analysis using PROCHECK software[36]. The validation process included a detailed assessment of stereo-chemical quality, residue distribution, and additional analyses for conformational integrity. This combined approach of molecular dynamics simulations, active site identification, and rigorous structure validation forms the cornerstone of the study, providing a detailed foundation for subsequent docking studies and functional analyses.

Gold score and fitness function

The GOLD docking software's calculation of the Gold Score is essential for evaluating ligand binding efficacy, incorporating four critical energy components to assess interactions between the ligand and protein. These components are external and internal hydrogen bond energies and external and internal Van der Waals energies, with the external VDW energy amplified by 1.375 to highlight the importance of hydrophobic interactions for binding stability and specificity. This amplification underscores the model's enhanced capability to predict hydrophobic contacts. The fitness function within the GOLD software integrates these energies, offering a nuanced assessment of ligand binding poses, thereby serving as a crucial tool in identifying potential therapeutic agents. This approach ensures a detailed metric for evaluating ligand-binding poses' favourability based on the aggregation of scores from four interactions: external hydrogen bond energy (S_hb_ext), external Van der Waals energy (S_vdw_ext), internal hydrogen bond energy (S_hb_int), and internal Van der Waals energy (S_vdw_int), maintaining the integrity and analytical depth of the docking studies[32,39].

Results

Plant collection and protein extraction

Plant leaves contain a complex matrix of various metabolites, e.g., phenolics, lipids, polysaccharides, pigments, and others that contaminate protein extracts and interfere with protein analysis and downstream applications. The 15% TCA/acetone precipitation protocols achieved good quality, quantity and clean protein. The extraction procedure resulted in minimal contamination, no smearing, high resolution, reproducible polypeptide bands (Fig. 1), and efficient elimination of interfering substances. The total protein yield of neem leaves was 118.54±5.37 mg/g of total dry weight.

SDS-PAGE analysis

The electrophoretic analysis of the protein extract exhibited protein bands of approximately 250 to 11 kDa compared to molecular marker analysis. Sharp, clear, distinct bands were well observed through the gel with a low background according to SDS-PAGE results. On the other hand, the lower gel region that contains the bands of interest from 11 to 14 kDa was excellently resolved (Fig. 1). These lowest three molecular weight polypeptides were selected for anticancer and antimicrobial studies using bioinformatic approaches.

Protein sequence template Identification

This study identified three specific polypeptides and assigned molecular weights based on their mass spectra. The peptide mass fingerprinting was matched against Swiss-Prot and NCBI protein databases with a Viridiplantae filter using the Mascot (URL http://www.matrixscience.com) database search engine (version 2.5.1, London, UK). Protein scores >58 were considered significant (P<0.05). These

polypeptides were labelled as polypeptide 1 (molecular weight of 11 kDa), polypeptide 2 (molecular weight of 13 kDa), and polypeptide 3 (molecular weight of 14 kDa) (Fig. 2). The search results showed that the template structure 5XDA has a high sequence identity with peptide 1 (11 kDa), template structure 5F05 has a high sequence identity with peptide 2 (13 kDa), and template structure 5F05 chain A has a high sequence identity with peptide 3 (14 kDa). The structurally conserved regions (SCRs) between the model and the template were determined by superimposing the two structures and performing BLAST analysis. The sequence identities were observed to be 42, 34, and 34 for peptides 1, 2, and 3, respectively, indicating a significant similarity between the model and the template structures.

Homology modelling

The selected templates, 5XFD, 5F05, and 5F05 chain A, were used as reference structures for modelling the three-dimensional structures of the three polypeptides (polypeptide 1, 2, and 3). Modeller9V7 software was employed for this purpose. The stability of the obtained polypeptide structures was evaluated, and the final stable structures are shown in (Fig. 3). During modelling, the coordinates of the reference proteins were assigned to the structurally conserved regions (SCRs), structurally variable regions (SVRs), C termini, and N termini of the target peptide sequences. This ensured that the alignment between the polypeptides and the templates was maintained. To determine the most suitable structures for further study, 20 PDB files were generated using Modeler software. The files with the lowest energy were selected for further analysis. SPDBV software was utilized to analyze the resulting structures. It was found that polypeptide 1 had two alpha helices and one beta-sheet, polypeptide 2 had four helices and one beta-sheet and polypeptide 3 had five helices and one beta-sheet. The Modeller9v7 software is widely used for modelling peptides and proteins and has proven to generate accurate structures and provide satisfactory results reliably.

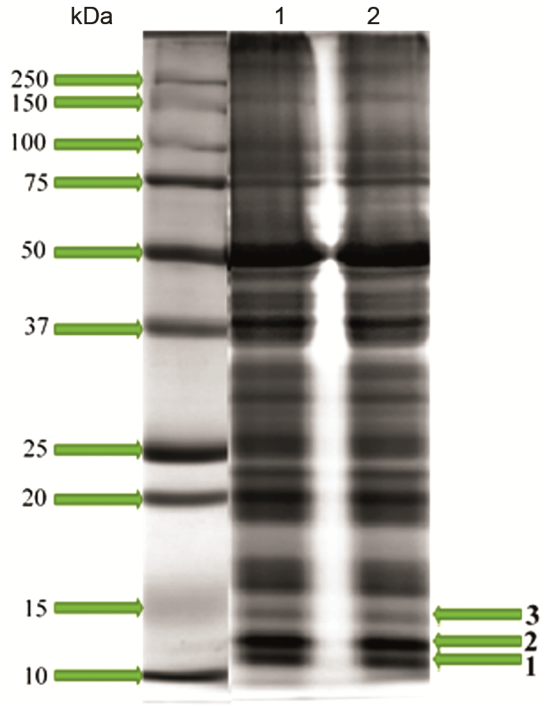

Fig. 1 — SDS-PAGE analysis of protein extracted from fresh neem leaves. Lines (1 and 2) show protein bands analyzed under denatured conditions. The electrophoresis showed a similar band pattern in all the lanes. The unmarked lane shows the molecular weight marker.

Structure validation

The structure with the lowest energy and root mean square deviation (RMSD), obtained using NAMD 2.8 software and the refinement procedure, was selected

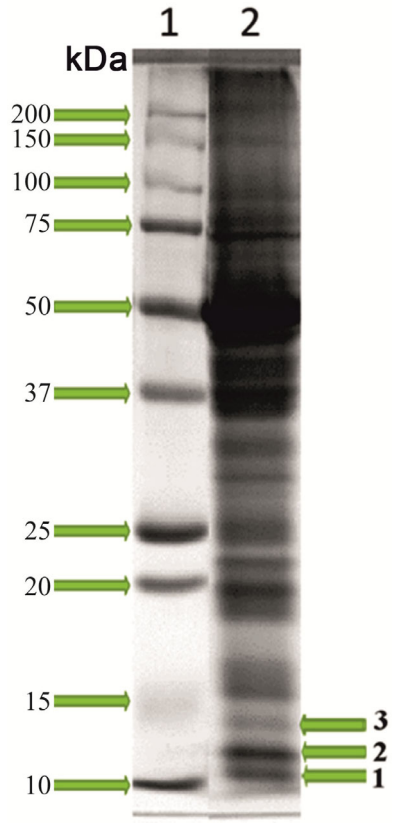

Fig. 2 — Shows the three low molecular weight polypeptides analysed by 10% SDS-PAGE. Lane No. 2 exhibited clearly the three low molecular weight bands named polypeptide1 (11 kDa), polypeptide 2 (13 kDa), and polypeptide 3 (14 kDa). Lane No.1 is a molecular weight marker.

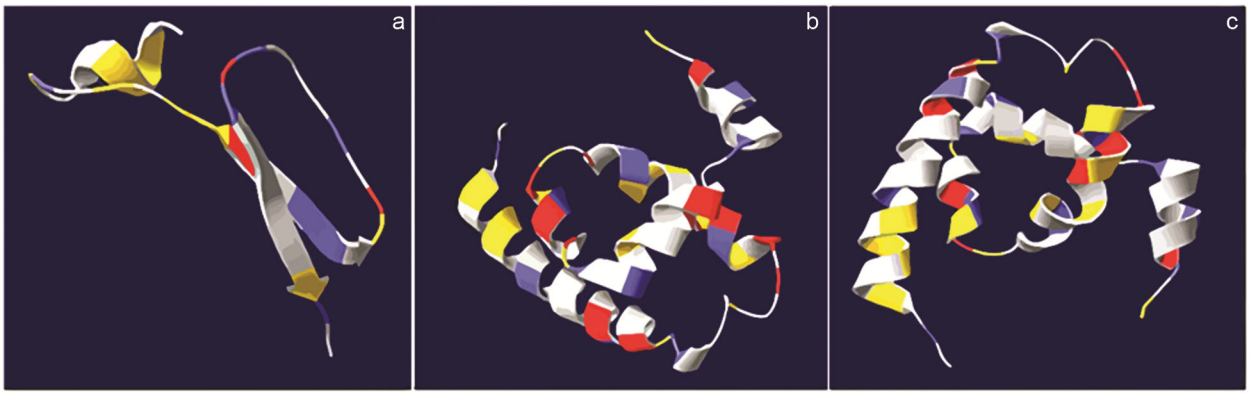

Fig. 3 — The 3D structures of three low molecular weight polypeptides extracted from neem leaves. a) Represent the 3D structure of 11 kDa polypeptide; b) Represent the 3D structure of 13 kDa polypeptide; and c) Represent the 3D structure of 14 kDa polypeptide.

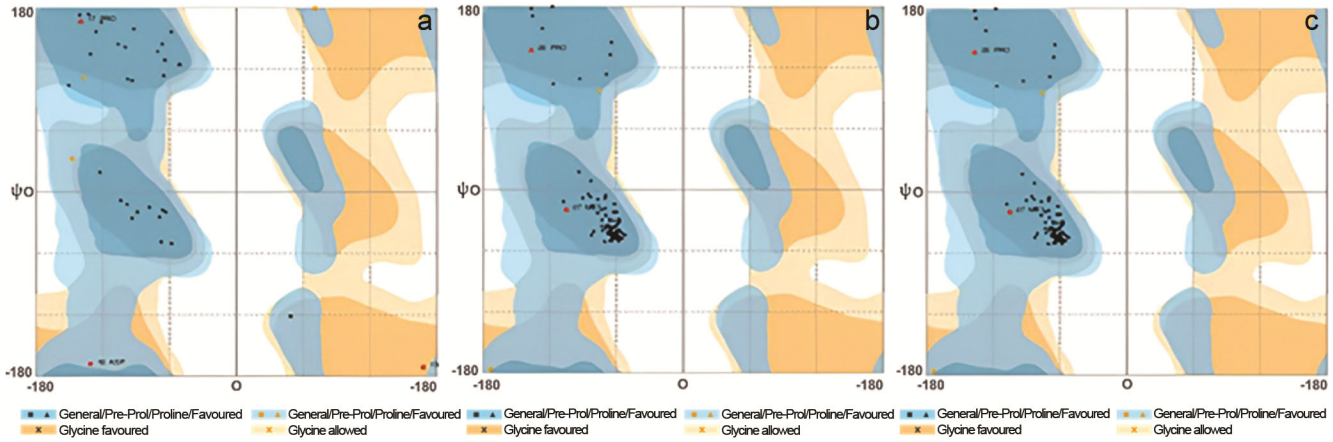

Fig. 4 — Ramachandran plot for amino acids analysis of the three polypeptides extracted from fresh neem leaves. The figure shows the distribution of the amino acids of the three polypeptides in favoured, allowed and outlier regions. a) Amino acids distribution of polypeptide 1(11 kDa);b) amino acids distribution of polypeptide 2 (13 kDa); and c) amino acids distribution of polypeptide 3 (14 kDa).

Table 1 — Individual amino acid residue distributions in each region of Ramachandran plot for the three polypeptides

Region	Number of residues	% of amino acids in each region	Expected % of the amino in each region
Peptide 1 (11 kDa)			
favoured region	29	80.6%	~98.0%
allowed region	4	11.1%	~2.0%
outlier region	3	8.3%	-
Peptide 2 (13 kDa)			
favoured region	80	95.2%	~98.0%
allowed region	2	2.4%	~2.0%
outlier region	2	2.4%	-
Peptide 3 (14 kDa)			
favoured region	54	84.4%	~98.0%
allowed region	8	12.5%	~2.0%
outlier region	2	3.1%	-

for further analysis. Validation of this structure was performed using Ramachandran plot analysis with PROCHECK software. RMSD assessment of the covalent bonds and angles in the peptides compared to the standard peptide dictionary yielded values of -4.27 and -0.85 Å, respectively. This indicates that the peptide structure agrees with the expected values for these parameters. In addition, 100% of the residues in the peptides were in the preferred location and allowed regions of the Ramachandran plot. The overall PROCHECK G factor, which measures the overall quality of the protein structure, was determined to be -2.32 for the polypeptides. This suggests a good quality 3D environment for the peptides. The results of this analysis can be found in (Table 1). They provide information about the conformational preferences of these amino acids in the peptide structure. The analysis obtained from the Ramachandran plot was crucial for checking the amino acid residues distribution and their position in the structure (Fig. 4).

Anticancer studies

Identification of activities of the BCL-2 domain

The initial BCL-2 protein structure was obtained from the PDB database with the corresponding PDB ID: 1GJH (Fig. 5). To prepare the structure for further analysis, unwanted heteroatoms and chains were removed using SPDBV software. Hydrogen atoms were then added to the protein to ensure accurate modelling of the active sites. Once the final model was created, the potential binding sites of BCL-2 were identified using structural comparison techniques. The CASTp server was used to analyze the structural features of the model and compare them with the template structure. The results of this analysis are shown in (Fig. 5), highlighting the predicted binding sites of BCL-2. Multiple predicted structures were submitted to the CASTp server to ensure comprehensive identification of BCL-2 active sites. This process helped determine the regions of the protein most likely to be involved in binding interactions with other molecules or ligands.

Molecular Docking studies

The docking of BCL-2 with the polypeptides was performed using GOLD 3.0.1, a flexible docking software. The program generates multiple rigid body alignments (poses) for each polypeptide within the binding pocket of BCL-2. Each pose is then compared against a negative image of the binding site, and any poses that clash with the bump map are removed. The remaining poses that pass the bump test are scored and ranked using a Gaussian shape function. The binding pocket for BCL-2 was defined using the ligand-free protein structure, and a box enclosing the binding site was created (Fig. 6). The size of the box was extended by 4 Å, based on the dimensions of a co-crystalized ligand (specified with the "add box" parameter of

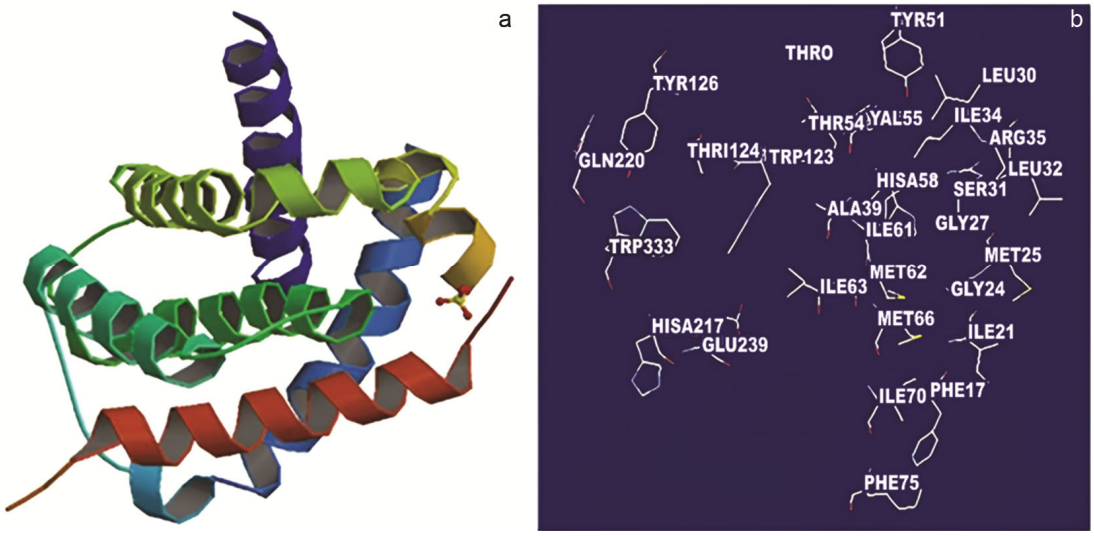

Fig. 5 —The structure of the cancer protein BCL-2. a) 3D structure; and b) The binding sites of the Homo sapiens cancer protein BCL-2.

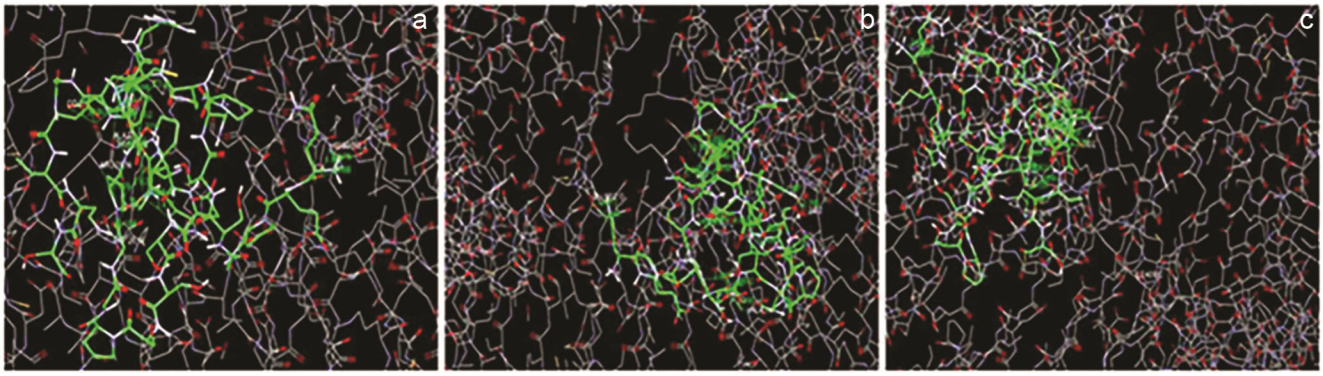

Fig. 6 — Docking studies between the active sites of three polypeptides extracted from neem leaves with the cancer protein BCL-2. a) Binding site between polypeptide 1 (11 kDa); b) binding site between polypeptide 2 (13 kDa); and c) binding site between polypeptide 3 (14 kDa).

FRED). After docking, a single special pose was selected for each of the best-scored peptides, which was then used for subsequent procedures. The peptide with the best docking score was converted into a 3D structure using OMEGA (a program for generating multiple conformations). The substrate-matching modelled protein was added to the structure. The docking scores of BCL-2 with the polypeptides are presented in (Table 2), providing an assessment of the binding affinity and potential interaction strength between BCL-2 and each polypeptide. Our results explained the details of hydrogen bond interactions between docked polypeptides and target proteins in hydrophobic binding pockets involving key amino acids that were critical for binding specificity and affinity. For example, in the case of BCL-2, the polypeptides can form hydrogen bonds with residues like Ser31, Gly27, and Asp108, which are known to be crucial in the binding site for various inhibitors. These interactions stabilize the peptide-BCL-2 complex, potentially blocking the protein's anti-apoptotic activity.

Antibacterial studies

The crystal structure of Sortase A (PDB ID: 1IJA) was downloaded from the Protein Data Bank (PDB) to prepare the protein. After obtaining the crystal structure, the binding sites of the Sortase A protein were explored using the CASTP server. The CASTP server identifies and calculates the properties of pockets, mouth openings, and cavities within the protein structure. (Fig. 7) showed the residues present within the active site of the Sortase A protein. In the case of Sortase A, hydrogen bonds will be formed with Thr180, Arg197, and His120, which are part of the active site. These interactions could inhibit the enzyme's function by stabilizing the polypeptide in a manner that blocks substrate access. These specific interactions contribute significantly to the docking energy, influencing the potential therapeutic efficacy of the peptides. These residues likely play a crucial role in the binding and interaction of Sortase A with other molecules or ligands. The analysis of the binding sites of Sortase A revealed that the binding pockets are similar in all chains of the protein. Therefore, the main binding pocket was selected for subsequent docking analysis. As the crystal structure of Sortase A was the same across all chains, a representative structure was chosen for the docking analysis. The docking of ligands to the active sites of Sortase A was carried out using the GOLD software, which utilizes a genetic algorithm approach. The docking results were evaluated based on the Gold

Table 2 — Total docking score of the three polypeptides extracted from neem leaves with the cancer protein BCL-2

Name of polypeptide	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_int)	S (int)
Polypeptide 1 (11kDa)	27.00	12.72	19.01	0.00	-11.87
Polypeptide 2 (13kDa)	23.13	13.05	17.34	0.00	-13.76
Polypeptide 3 (14kDa)	20.30	11.19	18.66	0.00	-16.54

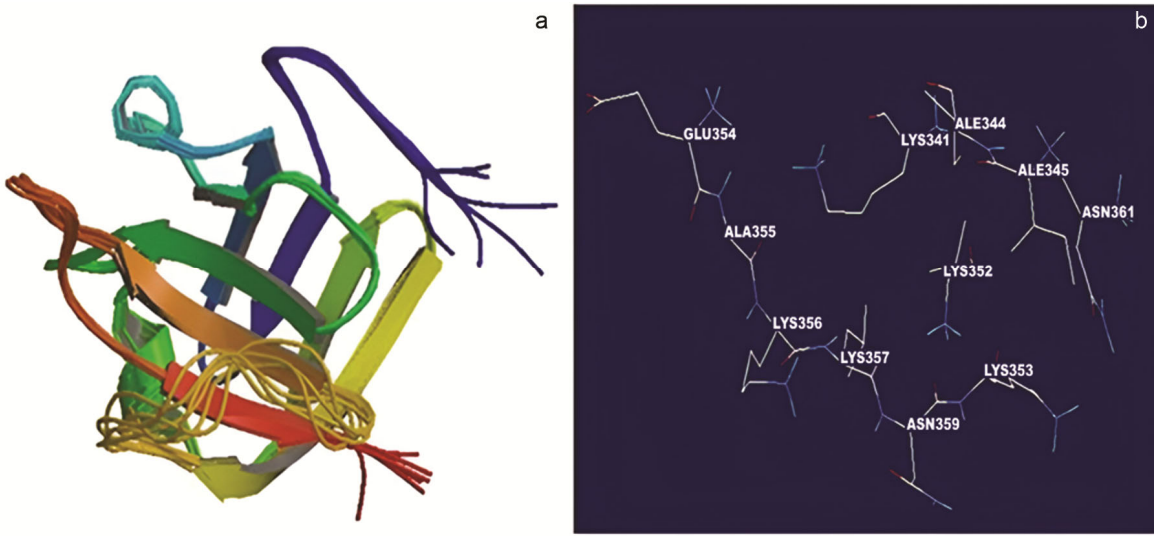

Fig. 7 — The structure of the microbial protein Sortase A. a) 3D configuration of sortase A; and b) binding sites of the *S. aureus* Sortase A protein.

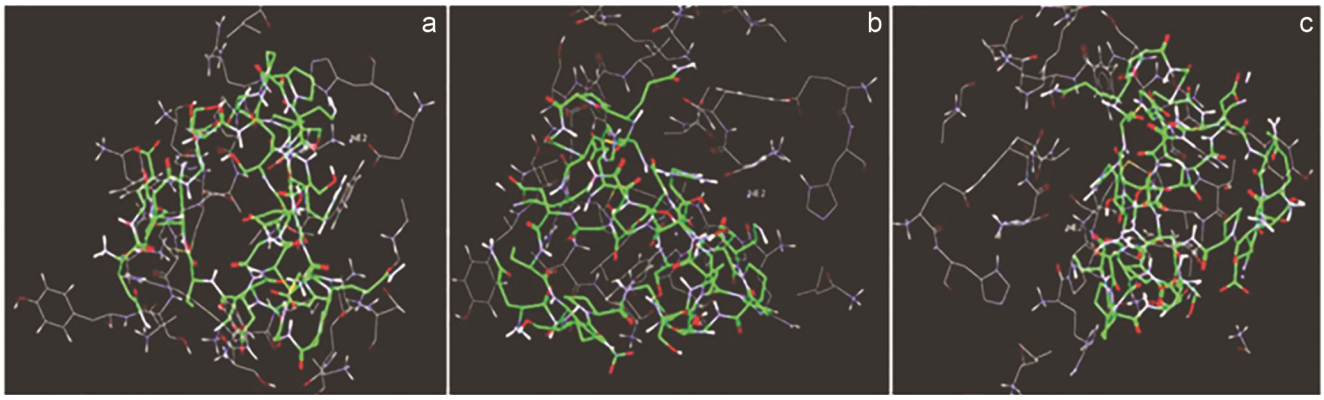

Fig. 8 — Docking studies between the active sites of three extracted from neem leaves with the ligand microbial protein Sortase A. a) The binding sites between polypeptide 1 (11 kDa) and Sortase A protein; b) The binding sites between polypeptide 2 (13 kDa) and Sortase A protein; and c) The binding sites between polypeptide 3 (14 kDa) and Sortase A protein.

Table 3 — Total docking score of the three polypeptides extracted from neem leaves with the microbial protein Sortase A

Name of polypeptide	Fitness	S (hb_ext)	S (vdw_ext)	S (hb_int)	S (int)
Polypeptide 1 (11kDa)	26.96	12.04	19.05	0.00	-11.27
Polypeptide 2 (13kDa)	21.59	12.60	18.91	0.00	-17.02
Polypeptide 3 (14kDa)	26.11	12.44	18.55	0.00	-11.83

Score fitness function, which takes into account various energy terms and interactions between the ligand and the protein (Fig. 8). (Table 3) presents the docking scores obtained for the ligands docked to the active sites of Sortase A. The docking scores provide information about the predicted binding affinity of each ligand to the protein. The ligands with higher docking scores are expected to have stronger interactions with Sortase A and are considered more favourable in terms of binding.

Discussion

Medicinal plants are highly valued as a rich source of bioactive compounds that have potential applications in pharmaceutical and chemotherapeutic fields. Researchers are increasingly focusing on studying pharmacologically active compounds from ethnobotanical plants[36]. There is a need for novel antimicrobial drugs to overcome current multidrug resistance to commercially available antibiotics and even anticancer drugs to reduce or replace chemotherapy and its side effects. These compounds offer several advantages, including high efficacy, cost-effectiveness, non-narcotic origin, and reduced side effects, making them attractive candidates for drug development[40]. To exploit the therapeutic potential of medicinal plants. It is crucial to obtain high-quality protein extracts. To achieve that, a well-established extraction protocol was developed. This protocol has proven highly efficient in extracting significant amounts of proteins from neem leaves. Therefore, the current research results are consistent with those obtained from banana, neem leaves, and tomato pollen[25].

In many cases, extracting high-quality protein from plant tissues is challenging because they contain interfering substances that can hinder downstream analyses and investigations. Considering this, the precipitation protocol using 15% TCA-acetone resulted in clean, white-coloured, non-viscous protein pellets[16,26]. The dilute alkaline dissolution solution reduced elevated pH, preventing protein variation, modification, and alteration during SDS-PAGE analysis (Fig. 1). The 10% SDS-PAGE was exploited successfully for analyzing low molecular weight polypeptides. Electrophoresis results of the protein bands showed better resolution with no smears, streaks, and almost clear background (Fig. 1). The low molecular weight polypeptides of interest identified as polypeptide 1 (11 kDa), polypeptide 2 (13 kDa), and polypeptide 3 (14kDa) (Fig. 2). These findings are in agreement with our previous results[16]. In this context, MALDI-TOF/TOF mass spectrometry has been used successfully to detect low molecular weight polypeptides by generating peptide profiling mass fingerprinting (PMF). This technique enables precise characterization of protein content and provides valuable insight into the composition of

extracted proteins. By using this technique, scientists can achieve an understanding of the bioactive compounds present in medicinal plants and their potential applications in the emergence of new and novel therapeutics. Mascot compares the experimental peptide masses with the protein sequence database, enabling the identification of specific polypeptides based on their mass-to-charge ratio.

Mass spectrometry analysis allowed us to accurately identify and characterize the low molecular weight antimicrobial polypeptides extracted from neem leaves. The current research utilized the application of various approaches, such as bioinformatics applications and anticancer and antimicrobial studies, to explore the properties and potential applications of these polypeptides[40-41]. To model the three identified polypeptides from neem leaves, it was crucial to find template structures with high sequence identity. In this regard, a BLAST search of the Protein Data Bank (PDB) database was performed to identify template structures with significant sequence similarity to the target peptides. It is generally accepted that sequence similarity of at least 30% is required for reliable modelling of peptides or proteins. Mass spectrometry analysis allowed us to accurately identify and characterize the low molecular weight antimicrobial polypeptides extracted from neem leaves. The current research utilized various approaches, such as bioinformatics applications and anticancer and antimicrobial studies, to explore the properties and potential applications of these polypeptides[39-41]. Modelling the three identified polypeptides from neem leaves necessitated template structures with high sequence identity. In this regard, the BLAST search tool of the Protein Data database (PDB) was performed to identify template structures with significant sequence similarity to the target peptides. It is generally accepted that sequence similarity of at least 30% is required for reliable modelling of peptides or proteins.

In selecting a template for homology modelling, especially when the similarity percentage is low, the choice is based on several criteria beyond just sequence similarity. Key factors include the structural conservation within the protein family, the presence of a similar active site or binding domain, and the overall fold of the protein. Structural features tend to be more conserved than sequences so that a template can provide valuable insights into the likely 3D conformation of the target protein, even with low sequence similarity. Besides, the quality and resolution of the template structure and its experimental validation are crucial considerations. This approach allows for the modelling of proteins when direct analogues are not available, supporting the prediction of function and interaction mechanisms. The identified sequence identities between the target peptides and the template structures were above this threshold, indicating sufficient similarity for accurate alignment and modelling. This provides confidence in the modeling process and shows that resulting three-dimensional structures of neem polypeptides can predicted based on the selected templates[29]. GOLD is a widely used ligand docking program that uses a genetic algorithm to explore the conformational flexibility of the ligand and account for the partial flexibility of the protein during the docking process. The docking process aims to predict the optimal binding conformation and orientation of the three polypeptides within the active sites of Sortase A and BCL-2. The genetic algorithm of GOLD generates an ensemble of different rigid body alignments (poses) for each polypeptide within the binding pocket. These poses are then scored based on their fitness, which is determined using a scoring function that considers factors such as hydrogen bonding, van der Waals interactions, and electrostatic forces. During the docking process, GOLD considers the flexibility of the ligand, allowing it to displace loosely bound water molecules and heteroatoms that may be present in the active site. This consideration is important because it reflects the dynamic nature of ligand binding and the need for effective interaction of the ligand with the protein in a physiological context. GOLD software and the genetic algorithm predicted ligand binding conformations and orientations of neem-derived polypeptides within Sortase A and BCL-2 active sites. This approach allowed for the exploration of conformational space, identifying energetically favourable positions for the polypeptides. The scoring function within GOLD assessed these poses based on hydrogen bonding, van der Waals, and electrostatic interactions, highlighting the therapeutic potential of the polypeptides. Specific interactions targeted within the active sites included hydrophobic interactions and hydrogen bonding. The polypeptides interacted with specific amino acid residues within the hydrophobic binding pockets of Sortase A and BCL-2, stabilizing the binding through these interactions. This detailed examination of binding dynamics provides details into the potential

mechanism of action of the bioactive compounds, highlighting their therapeutic potential against Sortase A and BCL-2 and setting a foundation for further drug development efforts. This information will used in designing and developing potential therapeutic agents targeting these proteins. The high fitness scores obtained indicate favourable binding interactions and highlight the potential of these polypeptides as therapeutic candidates for Sortase A and BCL-2. These excellent fitness score results are consistent with the docking results obtained by analyzing the gold score fitness functions of Iturin A in the active site of lectin[32].

Furthermore, the current study revealed that the Gold fitness score software provided better results than the Chemscore fitness software[42]. The GOLD fitness score has been optimized for developing ligand binding positions and includes factors such as H-bonding energy, van der Waals energy, metal interaction, and ligand torsion strain. Also, GOLD docking software is capable of selecting well-known substrates and ligands. The CASTp server is used to predict the binding sites of the target polypeptides. The identification and selection of binding pockets for docking studies were based on the results of the binding site analysis to ensure that these regions were studied. All docking results were evaluated by GOLD fitness function analysis[36,42]. To ensure that the BCL-2 protein structure is suitable for the subsequent binding studies. It was modified using the SPDBV software (Swiss-Pdb Viewer). The modification includes refining the structure, optimizing the hydrogen bonding network, adjusting the side chain conformation, and ensuring proper geometry. SPDBV is a molecular graphics program that allows the visualization and manipulation of protein structures to suit the required function.

Swiss-PdbViewer (SPDBV) is also used for protein structure modification; the typical modifications include adjusting side-chain conformations to reduce steric clashes, optimizing hydrogen bond networks, or adding missing atoms in incomplete structures. These adjustments are critical for ensuring the structural model accurately represents the protein in its native or functionally relevant state, essential for reliable binding studies. The modifications could influence how the protein interacts with ligands by affecting the shape and properties of the binding site, thus altering docking results and interaction analyses. On the other hand, hydrogen bonds enhance the stability of peptide-protein complexes by creating energetically favourable interactions. They also ensure specificity by matching unique geometric patterns of amino acids between the peptide and target proteins like BCL-2 and Sortase A. This specificity and stability are crucial for the therapeutic efficacy of peptides, as they can precisely inhibit protein functions involved in diseases.

The Ramachandran plot analysis is crucial in assessing the structural validation by analyzing the distribution of amino acid residues and their positions within the peptide structure. Further analysis of the Ramachandran plot focused on the distributions of θ and Ψ angles for the non-proline and non-glycine amino acids[31,37]. In the case of BCL-2, it helps ensure that the conformation of the peptide bonds falls within energetically favourable regions, indicating a correctly folded structure. It's a critical step for verifying that the computational model of BCL-2 is structurally viable and accurately represents the protein's native conformation, essential for reliable binding studies and functional predictions. The amino acids involved in these interactions include hydrophobic residues such as leucine, isoleucine, and valine, contributing to the binding affinity through hydrophobic interactions

Moreover, polar and charged residues like arginine, glutamine, and aspartic acid are crucial in forming hydrogen bonds and electrostatic interactions with the peptide ligands. These interactions are pivotal for the inhibitory activity of the peptides against the target proteins, highlighting their therapeutic potential. Hydrogen bonding interactions play a vital role in the structural stability of the compound. The consensus sequences derived from the alignment of BCL-2 and Sortase A were separately investigated and used for docking analysis. The results of the consensus sequences showed that more than 84.50% of the conserved regions were found in BCL-2 and Sortase A. These results were by those of[39,41], who reported that compounds from neem have excellent binding affinity with conserved regions of the target protein. The analysis of BCL-2 and Sortase A proteins using the CASTP server provided valuable information about their specific binding sites, such as identification of binding buckets. The docking studies revealed that the three polypeptides were located within the hydrophobic binding pockets surrounding the binuclear site of the proteins[37]. It indicates that the polypeptides interacted with the proteins through hydrophobic interactions that involved the interaction between oxygen atoms of the polypeptides and specific amino acid residues within the binding pockets.

Furthermore, hydrogen bonding interactions between the docked polypeptides and target proteins inside the binding pocket are analyzed. These hydrogen bonds play a crucial role in stabilizing the binding and facilitating specific interactions between the polypeptides and the proteins. The hydrogen bonding interactions with the adjacent amino acids within the hydrophobic binding pocket are studied extensively. This analysis provides insights into the specific molecular interactions that contribute to the binding of the polypeptides to the target proteins[39,43].

BCL-2 is a protein that plays an important role in regulating apoptosis, the process of programmed cell death. It blocks apoptosis and promotes cell survival. The BCL-2 gene is located on chromosome 18, and the abnormalities in its expression or function are associated with various types of cancer, particularly B-cell leukaemias and lymphomas. The over-expression led to increased production of the BCL-2 protein that helps cancer cells evade apoptosis and promotes their survival and proliferation. The significant involvement of BCL-2 in cancer development and progression makes it a potential target for cancer therapies[44,45]. By studying the interactions between BCL-2 and various polypeptides using docking studies, the authors aim to discover the potential anticancer effects of agents that can modulate BCL-2 activity and induce cell death in cancer cells[46]. The docking score results show that the lowest molecular weight polypeptide (11 KDa) possessed remarkable anticancer potential. These results are consistent with the observations obtained from our previous wet lab studies, where protein was extracted from leaves of A. Indica possessed the highest antiproliferative activity of IC_{50} of 64.82±1.64 and 59.61±0.75 against BT549 cell lines for 24 and 48 hours, respectively, using MTT assay. In addition, the other two polypeptides of 13 and 14KDa have good anticancer activity according to the docking score analysis (23.13 and 20.30 Kcal/mol, respectively). These results agree with our analyses from the previous study[16].

On the other hand, SrtA is present in almost all Gram-positive bacteria, including *S. aureus*. Therefore, it was selected as a template for performing antimicrobial activities. *S. aureus* Sortase A (SrtA) is a small transpeptidase protein/peptide consisting of 144 amino acids from *S. aureus* genomic DNA[46]. It was reported to play a role in the cell wall of Gram-positive bacteria. It is responsible for the covalent binding of secreted proteins to peptidoglycan to cross-bridge peptides of the cell wall of Gram-positive bacteria. Sortase A displays surface proteins that mediate bacterial adhesion to host tissues. Therefore, it is an important virulence factor supporting host cell invasion, evasion, and suppression of the immune response[47,48]. Docking score analysis proves that the three low molecular weight polypeptides possess excellent antimicrobial activities, with polypeptide 1 showing the most significant results. These results are well correlating with our previous wet lab finding, where the lowest three polypeptides showed the most remarkable antimicrobial activities against methicillin-resistant *S. Aureus* (MRSA) and *S. aureus* with a zone of inhibition of 19.0±0.57 mm and 28.0±1.53 mm, respectively[16]. The docking studies provided information on the binding orientation of polypeptides in the binding pockets of BCL-2 and Sortase A, resulting in inhibition of their activities. It supports the previous *in vitro* data and confirms the antimicrobial and anticancer activities of the polypeptides. Exploiting *in silico*and *in vitro*, approaches in determining the remarkable activities of polypeptides against cancer and human pathogens are considered a significant achievement. Overall, this research not only confirms the antimicrobial and anticancer activities of the polypeptides but also lays the foundation for the development of potential universal drugs targeting cancer and human pathogens. The consistency of these results with previous in silico studies further strengthens the validity and reliability of the findings[49,50].

Conclusion

This study is the first investigation of the antimicrobial and anticancer potential of low molecular weight polypeptides of neem leaves using bioinformatics analysis. Remarkable results were achieved in terms of their activities by using computational approaches. The three identified polypeptides exhibited significant antimicrobial activity by inhibiting sortase A protein and anticancer activity by inhibiting BCL-2 protein. These findings highlight the potential use of these polypeptides as therapeutic agents to combat microbial infections and anticancer drug development. The *in silico* computational approach utilised in this study provides valuable insights into the molecular interactions between the polypeptides and their target proteins, paving the way for further experimental validation and potential future drug development efforts.

Conflict of interest

The authors declare no conflict of interest.

References

1 Wang W and De Mejia E G, A new frontier in soy bioactive peptides that may prevent age-related chronic diseases, *Compr Rev Food Sci Food Saf*, 2005, **4**(4), 63-78, doi: 10.1111/j.1541-4337.2005.tb00075.x.

2 Kim S Y, Je J Y and Kim S K, Purification and characterisation of antioxidant peptide from hoki (*Johniusbelengerii*) frame protein by gastrointestinal digestion, *J Nutr Biochem*, 2007, **18**(1), 31-38, doi: 10.1016/j.jnutbio.2006.02.006.

3 Sunkar S, Archana V S, Naveen Kumar N H and Hariharan V, Computational analysis of *Linum usitatissimum*-derived peptides: Toward therapeutic applications, *Indian J Nat Prod Resour*, 2023, **14**(4), 542-555, doi: 10.56042/ijnpr.v14i4.6821.

4 Singh B P, Vij S and Hati S, Functional significance of bioactive peptides derived from soybean, *Peptides*, 2014, **54**, 171-179, doi: 10.1016/j.peptides.2014.01.022.

5 Cavazos A and Gonzalez de Mejia E, Identification of bioactive peptides from cereal storage proteins and their potential role in prevention of chronic diseases, *Compr Rev Food Sci Food Saf*, 2013, **12**(4), 364–380, doi: 10.1111/1541-4337.12017.

6 Bhat Z F, Kumar S and Bhat H F, Bioactive peptides from egg: A review, *Nutr Food Sci*, 2015a, **45**(2), 190–212.

7 Danquah M K and Agyei D, Pharmaceutical applications of bioactive peptides, *OA Biotechnol*, 2012, **1**, 1-7, doi.org/10.13172/2052-0069-1-2-294.

8 Carrasco-Castilla J, Hernández-Álvarez A J, Jiménez-Martínez C, Gutiérrez-López G F and Dávila-Ortiz G, Use of proteomics and peptidomics methods in food bioactive peptide science and engineering, *Food Eng Rev*, 2012, **4**, 224–243, doi: 10.1007/s12393-012-9058-8.

9 Martens E and Demain A L, The antibiotic resistance crisis, with a focus on the United States, *J Antibiot*, 2017, **70**(5), 520–526, doi: 10.1038/ja.2017.30.

10 Wang C H, Hsieh Y H, Powers Z M and Kao C Y, Defeating antibiotic-resistant bacteria: Exploring alternative therapies for a post-antibiotic Era, *Int J Mol Sci*, 2020, **21**(3), 1061, doi: 10.3390/ijms21031061.

11 Streicher L M, Exploring the future of infectious disease treatment in a post-antibiotic era: A comparative review of alternative therapeutics, *J Glob Antimicrob Resist*, 2021, **24**, 285–295, doi: 10.1016/j.jgar.2020.12.025.

12 Kitts D D and Weiler K, Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery, *Curr Pharm Des*, 2013, **9**(16), 1309–1323, doi.org/10.2174/1381612033454883.

13 Hartmann R and Meisel H, Food-derived peptides with biological activity: from research to food applications, *Curr Opin Biotechnol*, 2007, **18**(2), 163–169, doi: 10.1016/j.copbio.2007.01.013.

14 Arrutia F, Rubio R and Riera F A, Production and membrane fractionation of bioactive peptides from a whey protein concentrate, *J Food Eng*, 2016, **184**, 1–9, doi.org/10.1016/j.jfoodeng.2016.03.010.

15 Marina R, Wylie D and Merrel S, The antimicrobial potential of the neem tree *Azadirachta indica*, *Front Pharmacol*, 2022, **13**, 891535, doi.org/10.3389/fphar.2022.891535.

16 Al Saiqali M, Tangutur A D, Banoth C and Bhukya B, Antimicrobial and anticancer potential of low molecular weight polypeptides extracted and characterised from leaves of *Azadirachta indica*, *Int J Biol Macromol*, 2018, **114**, 906–921, doi: 10.1016/j.ijbiomac.2018.03.169.

17 Senadheera T R L, Hossain A, Dave D and Shahidi F, *In silico* analysis of bioactive peptides produced from underutilised sea cucumber by-products-a bioinformatics approach, *Mar Drugs*, 2022, **20**(10), 610, doi.org/10.3390/md20100610.

18 Kandemir-Cavas C, Pérez-Sanchez H, Mert-Ozupek N and Cavas L, *In silico* analysis of bioactive peptides in invasive sea grass *Halophila stipulaceacagin*, *Cells*, 2019, **8**(6), 557.

19 Ibrahim N and Kebede A, *In vitro* antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria, *Saudi J Biol Sci*, 2020, **27**(9), 2261–2268, doi: 10.1016/j.sjbs.2020.06.047.

20 Al Saiqali M, Tangutur A D and Bhukya B, Peptides and low molecular weight polypeptides of *Azadirachta indica* seeds as new weapons against cancer cells and superbugs, *Phytomed Plus*, 2021, **1**(4), 100118, doi.org/10.1016/j.phyplu.2021.100118.

21 Capriotti A L, Caruso G, Cavaliere C, Samperi R, Ventura S, *et al.*, Identification of potential bioactive peptides generated by simulated gastrointestinal digestion of soybean seeds and soy milk proteins, *J Food Compos Anal*, 2015, **44**, 205–213, doi.org/10.1016/j.jfca.2015.08.007.

22 Dziuba M and Dziuba B, *In silico* analysis of bioactive peptides. In *Bioactive proteins and peptides as functional foods and nutraceuticals*, edited by Y Mine, E Li-Chan, B Jiang, (Wiley-Blackwell, Oxford, UK), 2010, 325–340, doi:10.1002/9780813811048.CH22.

23 Masroor H, Parvateesam M, Daddam J R and Naidu N, *In silico* docking of meliantrol, β-sitosterol, curcumin, vanillic and syringic acids to penicillin binding protein 2a on methicillin resistant *Staphylococcus aureus*, *Onl J Bioinform*, 2015, **16**(1), 88-97.

24 Altayb H N, Yassin N F, Hosawi S and Kazmi I, *In-vitro* and *in-silico* antibacterial activity of *Azadirachta indica* (Neem), methanolic extract, and identification of Beta.d-Mannofuranoside as a promising antibacterial agent, *BMC Plant Biol*, 2022, **22**(1), 1-14, doi: 10.1186/s12870-022-03650-5.

25 Sheoran I S, Ross R S A, Olson J H D and Sawhney V K, Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis, *J Plant Sci*, 2009, **176**(1), 99–104, doi.org/10.1016/j.plantsci.2008.09.015.

26 Hao R, Adoligbe C, Jiang B, Zhao X, Gui L, *et al.*, An optimized trichloroacetic acid/acetone precipitation method for two-dimensional gel electrophoresis analysis of Qinchuan cattle longissimusdorsi muscle containing high proportion of marbling, *PLoS One*, 2015, **10**(4), 1–12, doi.org/10.1371/journal.pone.0124723.

27 Bradford M M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein–dye binding, *Anal Biochem*, 1976, **72** (1-2), 248–254.

28 Sambrook J and Russell D W, SDS-polyacrylamide gel electrophoresis of proteins, *CSH Protoc*, 2006, 1-9, doi.org/10.1101/pdb.prot4540.

29 Papathoti N K, Saengchan C, Daddam J R, Thongprom N, Tonpho K, *et al.*, Plant systemic acquired resistance compound salicylic acid as a potent inhibitor against SCF (SKP1-CUL1-F-box protein) mediated complex in *Fusariumoxysporum* by homology modeling and molecular dynamics simulations, *J Biomol Struct Dyn*, 2022, **40**(4), 1472-1479.

30 Wannaporn T, Papathoti N K, Daddam J R, Hoang N H, Thanh T L, *et al.*, *In vitro* and *in silico* studies of salicylic acid on systemic induced resistance against bacterial leaf blight disease and enhancement of crop yield, *J Integr Agric*, 2022, **22**(1), 170-184, doi: 10.1016/j.jia.2022.08.112.

31 Kurjogi M, Satapute P, Jogaiah S, Abdelrahman M, Daddam J R, *et al.*, Computational modeling of the *Staphylococcal enterotoxins* and their interaction with natural antitoxin compounds, *Int J Mol Sci*, 2018, **19**(1), 133, doi: 10.3390/ijms19010133.

32 Kumar P N, Swapna T H, Khan M Y, Daddam J R and Hameeda B, Molecular dynamics and protein interaction studies of lipopeptide (Iturin A) on α-amylase of *Spodopteralitura*, *J Theor Biol*, 2017, **415**, 41-47, doi: 10.1016/j.jtbi.2016.12.003.

33 Suresh B B, Mangamoori L N and Jayasimharayalu D, *In Silico* docking studies of *Elytrariaacaulis* gas chromatography-mass spectroscopy derived compound against breast cancer target proteins, *World J Pharm Pharm Sci*, 2017, **6**, 1053-1062.

34 Daddam J R, Sreenivasulu B, Umamahesh K, Peddanna K and Rao D M, *In silico* studies on anti-stress compounds of ethanolic root extract of *Hemidesmus indicus* L, *Curr Pharm Biotechnol*, 2020, **21**(6), 502-515, doi: 10.2174/1389201021666191211152754.

35 Daddam J R, Sreenivasulu B, Peddanna K and Umamahesh K, Designing, docking and molecular dynamics simulation studies of novel cloperastine analogues as anti-allergic agents: Homology modeling and active site prediction for the human histamine H1 receptor, *RSC Adv*, 2020, **10**(8), 4745-4754, doi: 10.1039/c9ra09245e.

36 Reddy G S, Venkatappa B, Jayanna B and Daddam J R, Antimicrobial studies on silkworm (*Bombyxmori.* L) with special reference to hemolymph and hemocytes, *Int J Anal Pharm Biomed Sci*, 2012, **1**, 39-48.

37 Ali A, Kuo W W, Kuo C H, Lo J F, Chen M Y C, *et al.*, E3 ligase activity of carboxyl terminus of Hsc70 interacting protein (CHIP) in Wharton's jelly derived mesenchymal stem cells improves their persistence under hyperglycemic stress and promotes the prophylactic effects against diabetic cardiac damages, *Bioeng Transl Med*, 2021, **6**(3), e10234, doi: 10.1002/btm2.10234.

38 Suguna R, Jeya J and R Renuka, *in silico* screening of phytocompounds in *Simarouba glauca* towards identification of potential lead compounds to treat dengue viral infection, *Indian J Nat Prod Resour*, 2023, **14**(4), 556-563, doi: 10.56042/ijnpr.v14i4.6288.

39 Desai T D, Wen Y T, Daddam J R, Cheng F, Chen C C, *et al.*, Long term therapeutic effects of icariin-loaded PLGA atmicrospheres in an experimental model of optic nerve ischemia via modulation of CEBP-β/G-CSF/noncanonical NF-κB axis, *Bioeng Transl Med*, 2022, **7**(2), e10289, doi: 10.1002/btm2.10289.

40 Karmegam N, Mani J and Subbiah K, Synergistic antibacterial activity of four plants collected from Dharapuramtaluk of Tirupur district, South India, *J Plant Sci*, 2012, **24**, 328, doi: 10.3923/jps.2012.32.38.

41 Ahmad A, Javed M R, Rao A Q and Husnain T, Designing and screening of universal drug from neem (*Azadirachta indica*) and standard drug chemicals against influenza virus nucleoprotein, *BMC Complement Altern Med*, 2016, **16**(1), 1-8, doi: 10.1186/s12906-016-1469-2.

42 Parameshwar J, Khan M Y, Rayulu J D, Hameeda B, Narendrakumar P, *et al.*, *In silico* and *in vitro* studies of fungicidal nature of lipopeptide (Iturin A) from *Bacillus amyloliquefaciens* RHNK 22 and its plant growth promoting traits, *Indian Phytopathol*, 2016, **69**, 569–574.

43 Singh N K, Pakkkianathan B C, Kumar M, Daddam J R, Jayavel S, *et al.*, Computational studies on molecular interactions of 6-thioguanosine analogs with anthrax toxin receptor 1, *Interdisciplinary Sci*, *Comput Life Sci*, 2012, **4**, 183-189, doi: 10.1007/s12539-012-0126-9.

44 Youle R and Strasser A, The BCL-2 protein family: Opposing activities that mediate cell death, *Nat Rev Mol Cell Biol*, 2008, **9**(1), 47–59, doi.org/10.1038/nrm2308.

45 Campbell K J and Tait S W G, Targeting BCL-2 regulated apoptosis in cancer, *Open Biol*, 2018, **8**(5), 180002, doi: 10.1098/rsob.180002.

46 Ralte L, Khiangte L, Thangjam N M, Kumar A and Singh Y T, GC-MS and molecular docking analyses of phytochemicals from the underutilised plant, *Parkiatimoriana* revealed candidate anti-cancerous and anti-inflammatory agents, *Sci Rep*, 2022, **12**(1), 3395, doi: 10.1038/s41598-022-07320-2.

47 Mao H, Hart S A, Schink A and Pollok B A, Sortase-mediated protein ligation: a new method for protein engineering, *J Am Chem Soc*, 2004, **126**(9), 2670-2671, doi: 10.1021/ja039915e.

48 Spirig T, Weiner E M and Clubb R T, Sortase enzymes in Gram-positive bacteria, *Mol Microbiol*, 2011, **82**(5), 1044-1059, doi: 10.1111/j.1365-2958.2011.07887.x.

49 Harathi N, Reddy S, Sura M and Daddam J R, Structure prediction, molecular simulations of RmlD from *Mycobacterium tuberculosis*, and interaction studies of Rhodanine derivatives for anti-tuberculosis activity, *J Mol Model*, 2021, **27**(3), 75, doi.org/10.1007/s00894-021-04696-2.

50 Shaik A H, Shaik S R, Daddam J R, Ali D, Manoharadas S, *et al.*, Maslinic acid and gallic acid protective efficacy on lipids, lipoproteins and lipid metabolising enzymes against isoproterenol administered cardiotoxicity: An *in vivo* and *in silico* molecular docking evidences, *J King Saud Univ-Sci*, 2021, **33**(1), 101230, doi.org/10.1016/j.jksus.2020.101230.