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Original Article

Potential of *Nephrolepis exaltata* as a Promising Antibacterial Agent against Methicillin-Resistant *Staphylococcus aureus*: A Comprehensive *in silico* and *in vitro* Study

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Abstract

Recently, the emergence of bacteria impenetrable to antibiotics has led to a devastating situation concerning international health. This study identified one of the most threatening bacteria, methicillin-resistant *Staphylococcus aureus*. The potential antibacterial properties of *Neprolepis exaltata* include its ability to inhibit the growth of *Staphylococcus aureus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) is not effectively susceptible to methicillin antibiotics. Methanolic crude extracts of *Neprolepis exaltata* were tested against Methicillin-resistant *Staphylococcus aureus* at 30 µg/mL and 40 µg/mL concentrations using the agar disc diffusion technique; 18 mm and 20 mm inhibition zones were reported. Furthermore, the dilution method, the crude extract's minimum inhibitory concentration (MIC) was tested against Methicillin-resistant *Staphylococcus aureus* at concentrations of 20, 40, 60, 80, and 100 µg/mL. The MIC value of the crude extract was found to be 40 µg/mL. Furthermore, crude methanolic extracts of *Neprolepis exaltata* were subjected to GC-MS analysis, and 22 phytochemicals were identified. Compound 2, 3-Diazabicyclo [2.2.1] hept-2-ene, has shown the highest binding affinity of -6.6 kcal/mol based on molecular docking. Molecular dynamics (MD) simulations determine the structural dynamics of the sphingosine kinase 1 complex with ligands (2ME, BEN, ARC). Factors such as RMSD, RMSF, Rg, SASA, and H bonding were examined to investigate the dynamic changes in ligand binding. The RMSD revealed stable equilibration within 10 ns, illustrating stability throughout the 100 ns simulation. The RMSF results indicate that ligand binding influences the flexibility of the protein, as indicated by the lower fluctuations for 2ME and BEN. Ligand binding increased solvent accessibility, and the greater compactness of the complex systems was indicated by Rg and SASA values. The stable interactions of H bonds were examined, and principal component analysis confirmed the lower flexibility of the ligand-bound complexes. The MM-PBSA method revealed the binding affinity of van der Waal energy, electrostatic energy, polar solvation energy, and binding energy of 1MWS-2ME, 1MWS-BEN, and 1MWS-ARC.

***Keywords**: Nephrolepis exaltata, Methicillin-Resistant Staphylococcus aureus, Penicillin-Binding Proteins, in silico Docking*

Introduction

The global issue of antibiotic resistance necessitates strategies for successfully combating bacterial disease. To fight bacterial illness, it must adopt alternative methods in response to the difficulties of antibiotic resistance. The bacterium known as "staph," *Staphylococcus aureus*, is one of the main causes of this issue. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common bacterium

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found in sugar factories and other food production industries, the environment, and normal human flora on the skin and mucous membranes (Stapleton & Taylor, 2002; Peacock & Paterson, 2015). The most common human pathogen, gram-positive methicillin-resistant *Staphylococcus aureus*, is responsible for various clinical infections and illnesses. The annual incidence rate of Methicillin-resistant *Staphylococcus aureus* infection is 20–50 cases per 100,000 people, with a death rate of 10–30% worldwide (Baddour *et al*., 2015). *Staphylococcus aureus* is the most prevalent agent that causes infections of the skin and soft tissues, as well as skin abscesses, furuncles, and carbuncles. Severe muscle or bone infections can result from skin and soft tissue infections, which often begin as abscesses or small boils. They may also spread to the lungs or the heart valves, which can cause endocarditis. Since the main components of the bacterial cell wall are peptidoglycans, the integrity of the cell wall controls bacterial survival during growth and cell division. Penicillin-binding proteins (PBPs) use their transpeptidase domain to crosslink peptidoglycans. Because PBPs are essential for bacterial survival, they are attractive targets for antibiotics, particularly β-lactams. When β-lactam antibiotics attach to the transpeptidase domain of Penicillin-binding proteins, peptidoglycan crosslinking is prevented. Therefore, β-lactams can effectively block Penicillin-binding proteins and eradicate bacterial infection. In addition to native Penicillin-binding proteins, Methicillin-resistant *Staphylococcus aureus* has a resistance determinant called Penicillin-binding protein 2a, a low-affinity penicillin-binding protein. The methicillin resistance (mecA) gene encodes Penicillin-binding protein 2a (Tabassum *et al*., 2023).

Neprolepis exaltata is useful for removing harmful air pollutants from the environment by naturally adding moisture to the air. Boston Ferns not only restore interior air quality but also increase humidity. *Neprolepis exaltata* has been shown to have antioxidant and anti-inflammatory properties (Swain *et al*., 2025; Sharma, Dave & Shah, 2021). The antibacterial efficacy of *Neprolepis exaltata* against *Staphylococcus aureus* was investigated using the disc diffusion technique. The primary objective provides a visual representation of the antibacterial properties of *Neprolepis exaltata* and their impact on inhibiting bacterial growth. In addition to the disc diffusion method, laboratory-based MIC tests were also conducted to evaluate the antibacterial activity further. These MIC tests offered insights into the metabolic activity and potential growth inhibition induced by *Neprolepis exaltata* samples in the broth. This approach enables analysis of the minimum inhibitory concentration (MIC) of the substance, indicating that the resultant solution is effective (Arshad, Mohiuddin & Azmi, 2012; Manandhar, Luitel & Dahal, 2019). The discovery of chemical compounds that can exhibit antibacterial properties against *Staphylococcus aureus* in *Neprolepis exaltata via* molecular docking was attempted. Molecular docking involves analysing the various chemical compounds of plants and identifying those that effectively interact with specific bacterial targets. Our endeavour is centred on deploying *in silico* screening to determine the bioactive phytochemicals that increase the potential of *Neprolepis exaltata* as an antibacterial agent. The primary goal is to cautiously examine *Neprolepis exaltata* and determine the potential of antibacterial drugs *via* a computational approach. The use of molecular docking software energetically enhanced the molecular structure of the compounds, which were subsequently docked into the crystal framework of a key protein target in *Staphylococcus aureus*. Understanding the binding affinity of these bioactive compounds with the target protein and their activity in other locations. These analyses of docking scores and a detailed examination of intermolecular interactions, such as hydrogen bonding, were performed. These determinations set the level for further molecular dynamics simulations to elucidate the dynamic behavior and stability of the ligand-receptor structure, thus offering additional confirmation that the compound from *Neprolepis exaltata* could function as a positive antibacterial agent against *Staphylococcus aureus*. Molecular dynamics simulations significantly affected the dynamic behaviour of the ligand-receptor complex. The probable ligand-receptor configuration was the focus of a strong simulation software analysis. The simulation provided a dynamic view of interactions within the complex as well as stability and fluctuation over time. MD simulations were performed to investigate the dynamic changes that occur upon binding of the target protein. Several parameters, such as RMSD, RMSF, Rg, SASA, and inter- and intramolecular hydrogen bonding, were calculated for both the protein and protein-ligand complex. The Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) were used to examine the overall stability and flexibility of the complex during the simulation period. The Radius of Gyration (Rg) represents the distribution of atoms from their common centre of mass, and the Solvent Accessible Surface Area (SASA) measures the surface area of a biomolecular structure accessible to solvent molecules. Molecular simulation assists in analysing a molecule's interaction with its environment. It provides insight into how active compounds in *Neprolepis exaltata* combined with *Staphylococcus aureus* function as antibiotics at the molecular level (Gurung *et al*., 2021; Samad *et al*., 2022).

Materials and Methods

Preparation of Plant Material

The plant samples were collected from the Ooty Hills and subjected to a standard tap water rinse to eliminate any residual dust. Subsequently, the plants were thoroughly washed with distilled water, and the plant material was air-dried at 37°C for two to three weeks. Once dried, the plant material was pulverised using a mortar and pestle. A cold extraction method utilising methanol as the solvent was used to prepare the plant extract (Ouandaogo *et al*., 2023).

GC-MS Analysis

Plants are an abundant source of secondary metabolites with structural configurations and biological activities. Gas chromatography, particularly gas-liquid chromatography, is often employed to analyse these metabolites. A sample is vaporised and then injected into the head of the chromatographic column. An inert gaseous mobile phase transports the sample through the column, with an inert solid surface covered with a liquid phase. This separation is based on adsorption and partitioning, essential concepts in gas chromatography (Al-Rubaye, Kaizal & Hameed, 2017).

Kirby Bauer Disc Diffusion Method

In this method, a standardised suspension of microorganisms is spread onto the surface of an agar plate. Filter paper discs or sterile commercial blank discs are used for impregnation with the plant solution and allowed to dry before being placed on agar plates. A filter paper disc containing 30 µg/mL and 40 µg/mL substance was placed on the agar surface. Standard ciprofloxacin discs (30 µg/disc) were used as the positive control, and methanol as the negative control. After incubation, the plate was examined for zones of inhibition where bacterial growth was inhibited around the disc. The size of the zone of inhibition was measured and correlated with the susceptibility of the microorganism to the test substance (Bubonja-Šonje, Knežević & Abram, 2020; Septama & Panichayupakaranant, 2015).

Broth Dilution Method

The broth dilution test is the earliest method for assessing antibacterial susceptibility. This method includes preparing twofold dilutions of plant extract and broth in a liquid growth medium delivered in test tubes. A standardised bacterial suspension was introduced into concentrated plant extract-containing tubes. Methanol was used as the negative control, and standard ciprofloxacin discs (30 µg/disc) were used as the positive control. After an overnight incubation at 35°C, the tubes were checked for visible bacterial growth, as indicated by turbidity. The lowest inhibitory concentration (MIC) is the lowest concentration of antibiotic that prevents growth (Ács *et al*., 2018).

Molecular Docking

Molecular docking is an increasingly indispensable tool in drug discovery, enabling the simulation of small-molecule-protein interactions at the atomic level. This technique provides insights into the behaviour of small molecules within target protein binding sites and helps elucidate key biochemical processes. Docking involves two key steps: sampling ligand conformations within the protein's active site and ranking these conformations using a scoring function. Effective sampling methods should replicate the experimental binding mode, with the scoring function accurately ranking it highest among all conformations generated (Meng *et al*., 2011).

Protein Preparation

The structure of penicillin-binding protein 2a (PBP2a) from methicillin-resistant *Staphylococcus aureus* strain 27r was retrieved from the Protein Data Bank (PDB) under the accession code 1MWS. The PDB files were imported into Discovery Studio for initial processing. The protein structure underwent preparation steps to optimise it for molecular docking studies. First, water molecules (B chains) were removed from the structure to streamline the model. Additionally, chloride ions present in the crystal structure were eliminated to maintain focus on the protein's primary structure. The modified protein structure was saved in PDB format for subsequent docking analyses (Mohamed *et al*., 2019).

Ligand Preparation

The chemical structures of the phytochemical compounds in *Neprolepis exaltata* were obtained from the PubChem website. Both two-dimensional (2D) and three-dimensional (3D) representations of these structures can be downloaded in PDB format (Gunasekharan *et al*., 2021).

Process of Docking

Docking was performed using the software applications "AMDOCK" and "AUTODOCK." A docking methodology was used to determine the binding affinity of the phytochemical compounds for the target protein. Initially, a project was defined by its name and location. Sample 1 was then loaded with 1MWS, the target protein that had previously been modified to eliminate its chloride and water chains. The 3D or 2D structures of the phytochemical substances found in the plant sample were then loaded into Sample-2 (Ligand). After the method was completed, the binding affinity between the ligand and target protein of the phytochemical compounds was evaluated (Morris & Lim-Wilby, 2008).

Molecular Dynamics Simulation

Molecular Dynamics (MD) simulations were used to analyse protein structure dynamics and ligand interactions. The MD simulations examine the molecular systems at the atomic level of protein-ligand interactions. Many metrics were computed, including RMSD, RMSF, Rg, SASA, and H bonding for the protein and the protein-ligand complex. Principal component analysis (PCA) and free energy landscape analysis were also performed on the simulation's 100 ns trajectory (Adejoro *et al*., 2020).

System Preparation for Molecular Dynamics Simulation

The protein-ligand complex active sites were submerged in a sphere of water molecules with a transferable intermolecular potential of 3 points. The systems included between 8,000 and 12,000 atoms, and a half-harmonic potential was provided at the solvent border to inhibit water molecule evaporation. The ligand, water molecules, and protein residues within 12 Å of the active centre were allowed to move. In contrast, other protein residues were restrained to the X-ray structure using a harmonic energy term in all molecular mechanics (MM), including energy minimisation and MD simulations (Adejoro, Babatunde & Tolufashe, 2020).

Results

Crystal Structure of Penicillin Binding Protein (PBP)

The structure of nitrocefin acyl-penicillin binding protein 2a (PBP2A) of methicillin-resistant *Staphylococcus aureus* strain 27r, which was resolved at 2.00 Å, provided important information on how PBP2A and Nitrocefin, an antibiotic analogue of cephalosporin, interact. Antibacterial medications and complex processes underlying antibiotic resistance are used to identify the targets *via* high-resolution guidance. This unique PBP2A variation proved the antibacterial activity of *Neprolepis exaltata* against *Staphylococcus aureus*.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The presence of 22 phytochemicals (Table 1) was identified using the crude methanolic extracts of *Neprolepis exaltata* by GC-MS. Each detected component of *Neprolepis exaltata*contributed to the overall chemical makeup of the plant extract, shedding light on the probable bioactive elements responsible for its synergistic impact on its antibacterial activity (Figure 2)

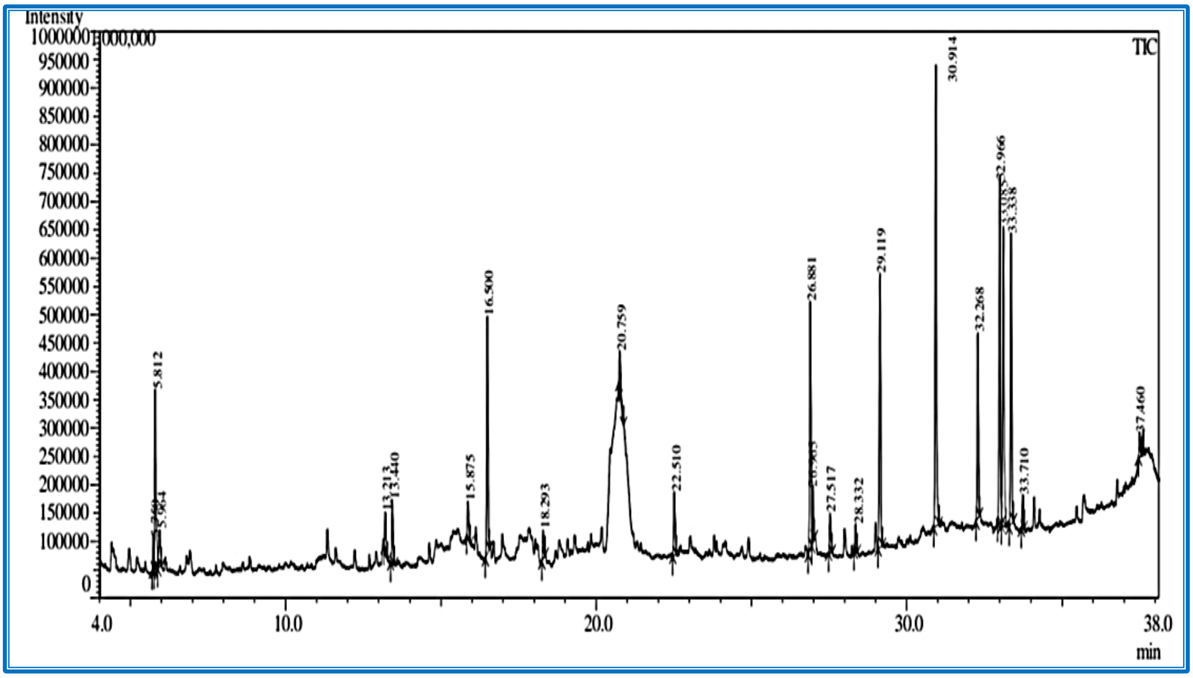

Figure 2: *GC-MS Data of* NeprolepisExaltata *- 22 Phytochemicals*

Table 1: *Phytochemical Profile of* NeprolepisExaltata

Peak #	R. Time	Area	Area%	Name
1	5.750	180293	1.12	1,2.3-Propanetriol
2	5.812	1007849	6.26	2-(Methoxymethyl) Pyrrolidine
3	5.964	249324	1.55	Butanedioicacid, methoxy-, dimethyl ester
4	13.213	201883	1.25	DL-Proline, 5-oxo-, methyl ester
5	13.440	329614	2.05	Alfa.- Copaene
6	15.875	198645	1.23	Benzoic acid,4-Hydroxy-Methyl
7	16.500	1223967	7.60	1H-Cyclopenta [1,3] cyclopropa[1,2] benzene
8	18.293	155687	0.97	Cyclohexene, 6-Ethenyl-6-Methy
9	20.759	241713	1.50	N,3-Diethyl-3-octanamine
10	22.510	343664	2.13	2H-Cycloprop[c]indene-2.3(3ah)-dione,Hexa
11	26.881	1348966	8.37	Neophytadiene
12	26.965	309035	1.92	3-(Methoxymethyl)-2,5,5,8A-Tetra
13	27.517	223017	1.38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	28.332	138195	0.86	7,10,13-Hexadecatrienoicacid, methylester
15	29.119	1394255	8.66	Hexadecanoicacid, Methyleste
16	30.914	2497138	15.50	(S, Z)-Heptadeca-1.9-dien-4,6-diyn-3-ol
17	32.268	890720	5.53	2,3-Diazabicyclo [2.2.1] hept-2-ene
18	32.966	1707232	10.60	9,12-Octadecadienoicacid (Z, Z)-, methyl est
19	33.085	1691612	10.50	9,12,15-Octadecatrienoicacid, methyl ester.
20	33.338	1469305	9.12	2-Hexadecen-1-OL,3,7,11,15-Tetram
21	33.710	149933	0.93	Methyl stearate
22	37.460	155673	0.97	3-Phenylpropanoicacid, dodec-9-ynylester

Kirby Bauer Antibacterial Efficacy of Neprolepis exaltata

An antibacterial experiment performed on crude extracts of *Neprolepis exaltata*against methicillin-resistant *Staphylococcus aureus* revealed good antibacterial activity. The agar disc diffusion method showed considerable zones of inhibition at 30 µg/mL and 40 µg/mL doses, with reported values of 18 mm and 20 mm, respectively (Table 2).

Table 2: *Antibacterial Assay of Methicillin-Resistant Staphylococcus Aureus Using Neprolepis exaltata*

Concentration of plant sample (µg/mL)	**Zone of Inhibition (mm)**	**Interpretation**
30 (µg/mL) of sample	18	Sensitive
40 (µg/mL) of sample	20	Sensitive
Ciprofloxacin-30µg/disc (positive control)	25	Sensitive
Methanol (negative control)	0	Resistant

Minimum inhibitory concentration (MIC) determination

The broth dilution technique evaluated various quantities of crude extracts from *Neprolepisexaltata* against methicillin-resistant *Staphylococcus aureus*. The observed minimum inhibitory concentration was significant, with a reported value of 40 µg/mL (Figure 3). These findings indicate that *Neprolepisexaltata* has potential antibacterial effects against Methicillin-resistant *Staphylococcus aureus*, suggesting a dose-dependent influence on bacterial growth inhibition.

Molecular docking

The significant interactions between the ligand and protein provide insights into the activity of penicillin-binding protein 2a. Among those phytochemical compounds, 2,3-diazabicyclo [2.2.1] hept-2-ene showed the highest binding affinity of -6.6 Kcal/mol (Table 3).

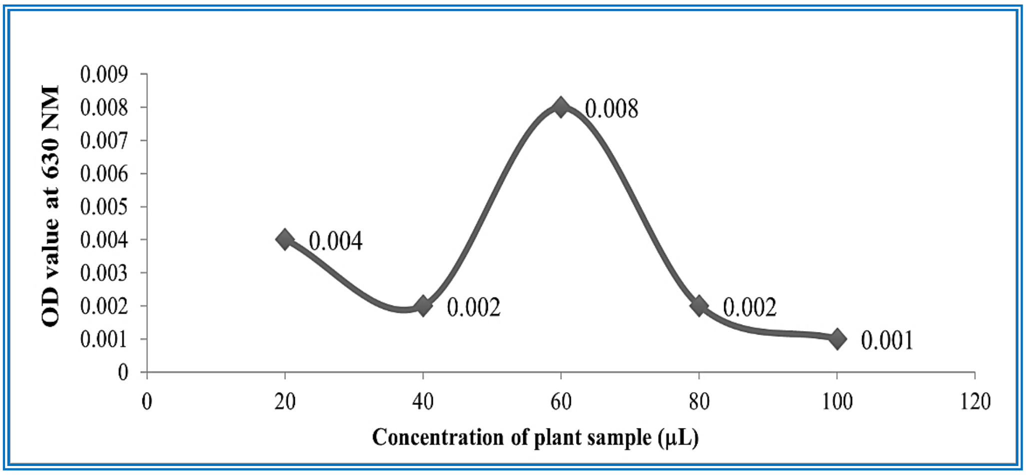

Figure 3: *Graphical Representation of The Minimum Inhibitory Concentration of The Broth Dilution Method*

Table 3: *Molecular Docking Analysis of Phytochemical Compound*

Poses	**2, 3-Diazabicyclo [2.2.1] hept-2-ene**			
	Affinity (KCAL/MOL)	**Estimated Ki**	**Ki Units**	**Ligand**
1	-6.6	14.53	uM	-0.44

Molecular Dynamics Simulation

In the molecular simulation, all atoms were analysed for the structural dynamics of the sphingosine kinase 1 complex and interactions with ligands (2ME, BEN, ARC).

Structural Dynamics and Stability

According to the RMSD data, both the APO form and ligand-bound complexes achieved equilibrium within 10 ns, and the remaining stability was attained throughout the 100 ns simulation. The RMSD of 1MWS-APO decreased after binding with the ligands (APO, BEN, and ARC), implying that these complexes are stable and exhibit minimal fluctuations (Figure 4)

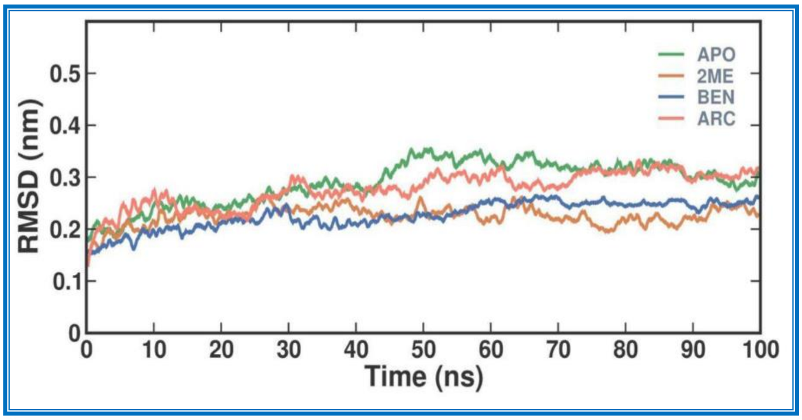

Figure 4: *Conformational dynamics of 1MWS-APO, 1MWS-2ME, 1MWS-BEN, and 1MWS-ARC complex*

Residue fluctuation

RMSF analysis was carried out to assess the effect of ligand binding on the fluctuation of each residue in the protein. The results showed that the binding of ligands (2ME, BEN, ARC) did not considerably alter the overall RMSF distribution. Compared with those of the APO form, the fluctuations of the 2ME and BEN complexes decreased, suggesting a stable effect on ligand binding (Figure 5).

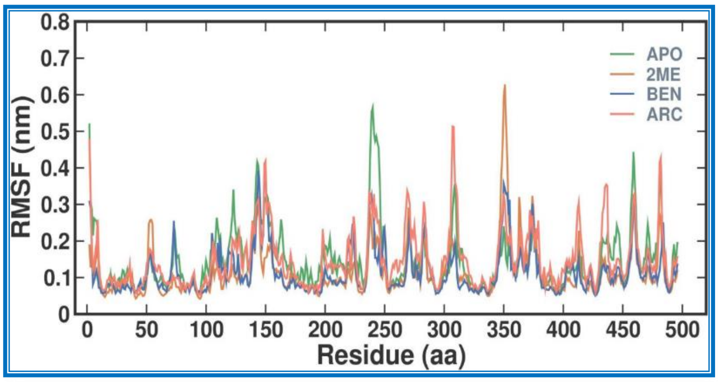

Figure 5: *Conformational Dynamics of 1MWS-APO, 1MWS-2ME, 1MWS-BEN and 1MWS-ARC Complex*

Compactness and Stability

The Radius of Gyration (Rg) was then calculated to determine the dynamic stability and compactness of the protein and its complexes in terms of space-occupied dimensions. The Rg values for the complexes (1MWS-2ME, 1MWS-BEN, and 1MWS-ARC) are slightly smaller than the Rg values of the apo complex, indicating that the complex system is more compact and more stable than the apo complex. The Rg Values are well supported and confirmed by the RMSD and RMSF analysis results, indicating that oriented and docked protein complexes are stable (Figure 6).

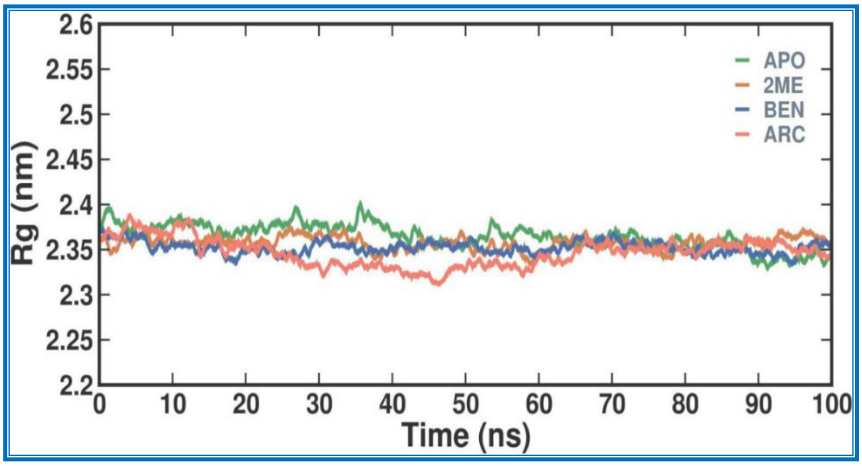

Figure 6: *Dynamic Stability and Compactness of APO and its 2ME and BEN Complexes*

Solvent Accessibility

The solvent-accessible surface area (SASA) was used to determine the protein's accessibility in the solvent, but a slight increase in SASA values of APO upon binding with 2ME, BEN, and ARC as ligand binding can expose the residues on the inside to the surface; hence, the fair equilibration of SASA values in a span of simulation represents balanced solvent accessibility (Figure 7).

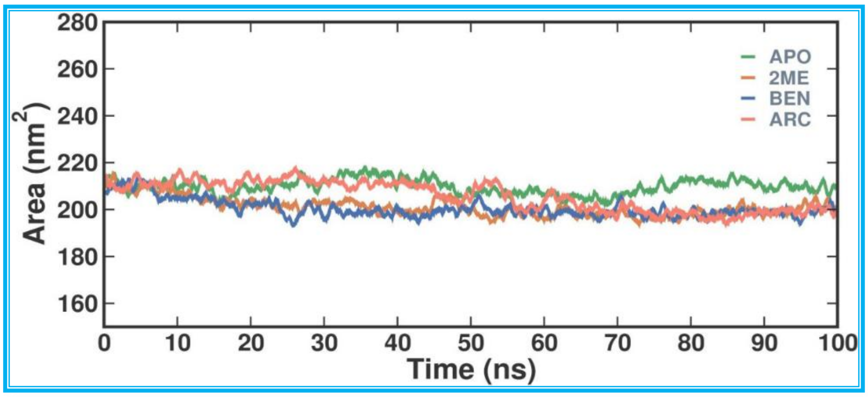

Figure 7: *SASA Values Determine the Impact of 2ME, BEN, And ARC Binding on The Solvent Accessibility of 1MWS*

Hydrogen Bonding

Investigating time-dependent intramolecular and intermolecular hydrogen bonds revealed the stability of protein-ligand interactions. Despite these fluctuations, many H bonds were maintained between 2ME, BEN, and ARC, supporting the stability of the docked complexes (Figure 8 and 9).

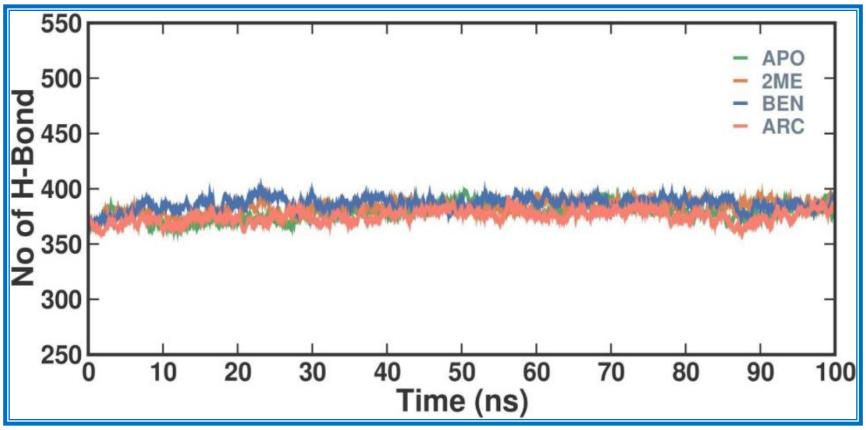

Figure 8: *Intramolecular H Bonds Between 2ME, BEN, and ARC During the Simulation Time*

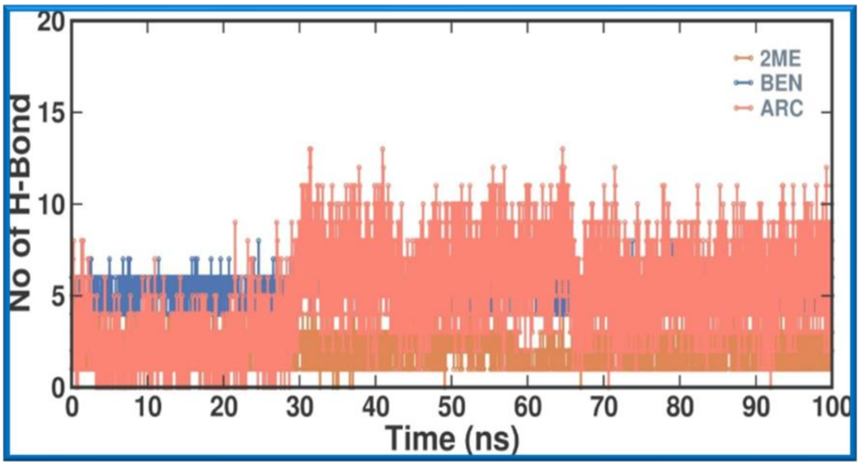

Figure 9: *Intermolecular H Bonds Between 2ME, BEN, and ARC During the Simulation Time*

Principal Component Analysis (PCA)

PCA explores the collective movement in the complexes. The reduced flexibility observed in the 2ME, BEN, and ARC complexes implies stability. The overlap in conformational motions between APO and the complexes further supports the stability of the 2ME complex, suggesting that 2ME did not significantly affect the conformation or dynamics of the 1MWS complex.

Table 4: *MM-PBSA Binding Affinity of 1MWS-2ME, 1MWS-BEN, 1MWS-ARC*

System	van der Waal energy	Electrostatic energy	Polar solvation energy	Binding energy
1MWS-2ME	-69.954 +/-9.586 kJ/mol	-58.492 +/-6.341 kJ/mol	79.504 +/- 14.396 kJ/mol	-58.678 +/- 14.532 kJ/mol
1MWS-BEN	-60.426 +/-7.354 kJ/mol	-199.199 +/-8.792 kJ/mol	179.786 +/- 15.228 kJ/mol	-93.762 +/- 12.717 kJ/mol
1MWS-ARC	-265.600 +/- 16.280 kJ/mol	-107.006 +/- 20.892 kJ/mol	325.010 +/- 43.566 kJ/mol	-77.507 +/- 13.443 kJ/mol

Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) Binding Affinity Analysis

The MM-PBSA method was employed to determine the binding affinity of the complexes (1MWS-2ME, 1MWS-BEN, 1MWS-ARC). The computed energy components provide interactions, including van der Waals, electrostatic, polar solvation, and binding energies. Further detailed analysis is required to draw comprehensive conclusions about each energy component, relative binding strengths, and contributions (Table 4). The diverse analyses support the stability and structural integrity of the Sphingosine Kinase 1 complexes with 2ME, BEN, and ARC. These findings contribute valuable insights for understanding the molecular behaviour of the complexes, laying the groundwork for future drug design and discovery.

Discussion

Fern species belonging to the oldest vascular plants have been widely used as traditional medicines by indigenous communities, especially in the humid regions of South East Asia (Salazar-Chacon *et al*., 2024). The fern possesses medicinal features such as antioxidant, radical scavenging, and cytotoxic activity against K562 leukaemic cells (Chai *et al*. 2015, Kaur, 2017). Pharmacological effects of fern plants are related to the production of diverse phytochemicals, including alkaloids (Dong *et al*. 2012), flavonoids (Xia *et al*. 2014), polyphenols (Socolsky *et al*. 2012), terpenoids (Socolsky, Asakawa & Bardon, 2007), and steroids (Ho *et al*. 2012). The ferns *O. vulgatum* and *O. pedunculosum* have been shown to contain several flavonoids, glycerides, and amino acids (Wan *et al*. 2012; Clericuzio *et al*. 2012; Hu, Meng & Wang, 2016) with antioxidant, anti-inflammatory, antibacterial, and antimutagenic properties (Pietta 2000; Choi *et al*. 2001; Yoshizumi *et al*. 2001; Schroeterh *et al*. 2001). *Dryopteris* spp. are rich in secondary metabolites, including glycosides, steroids, alkaloids, phenols, terpenes, flavonoids, and tannins, which are responsible for biological activities like antibacterial, antifungal, nematicidal, and antioxidant activities (Soare *et al*. 2012; Egorova *et al*. 2021; Valarmathi *et al*., 2023). *Diaportheueckerae* was another endophytic fungus isolated from the same fern in China (Gao *et al*., 2022) and shown to produce some known and undescribed cytochalasans such as ueckerchalasins A−E, 4′ hydroxycytochalasin J3, cytochalasin H, cytochalasin J, cytochalasin J1−J3, longichalasin B, RKS-1778, and phomopchalasin A which displayed antibacterial activity against *Staphylococcus aureus* (SA) and methicillin resistant *S. aureus*, selective activity against human carcinoma HeLa and HepG2 (Razaghi & Abdel-Azeem, 2024; Binjawhar *et al*., 2024). Methicillin-resistant *Staphylococcus aureus*'s resistance involves mutated penicillin-binding proteins, particularly PBP2a, which have a decreased affinity for β-lactam antibiotics, therefore, it produces resistance not only to methicillin but also to all members of the extended-spectrum β-lactam antibiotics (Kim *et al*., 2012; Idrees & Saeed, 2021; Mohammed *et al*., 2025), which permits cell wall construction to continue even in the presence of antibiotics, and this is the main mechanism underlying Methicillin-resistant *Staphylococcus aureus* uses the accessory gene regulator regulatory system, efflux pumps, and biofilm development to improve virulence and biofilm formation, contributing to its resistance (Ali *et al*., 2021; He *et al*., 2024). Traditional herbal medicine to treat infectious diseases has gained global prominence as a leading alternative medicine (Mustafa *et al*., 2022; Gandhiraj *et al*., 2021; Normile, 2003). In this study, the phytochemical extracts of *Nephrolepis exaltata* possessed antibacterial activity against Methicillin-resistant *Staphylococcus aureus* with 18 mm and 20 mm Zone of Inhibition (ZoI) at 30 µg/mL and 40 µg/mL dose concentration. Similarly, the essential oils from the *Nephrolepis exaltata*-2,4-Hexadien-1-ol (16.1%), nonanal (14.4%), β-Ionone (6.7%), and thymol (2.7%), and *Nephrolepis cordifolia* (β-Ionone (8.0%), eugenol (7.2%), and anethol (4.6%)) showed cytotoxicity in breast, colon, and lung carcinoma cells (El-Tantaw, Shams & Afifi, 2015). The 2.00 Å resolution structure of the nitrocefin acyl-PBP2A molecule from the methicillin-resistant *Staphylococcus aureus* strain 27r revealed intricate molecular interactions that laid the foundation for understanding antibiotic resistance (Fishovitz *et al*., 2014; Al-Mijalli *et al*., 2025). During an *in silico* study, the compound 2, 3-Diazabicyclo [2.2.1] hept-2-ene exhibited high stable binding affinity to Methicillin-resistant *Staphylococcus aureus* proteins, indicating their potential as effective antibacterial agents. MD simulation revealed that the compounds maintained stable interactions with the target proteins over 100 ns, with minimal deviations and fluctuations, whereas derivatives of Oxadiazoles and Napthyridine compounds have potential dual inhibition of PBP-2a and FemA proteins with the interactions of 250 ns (Bourhia *et al*., 2024). Various medicinal plants, such as *Rosmarinus officinalis*, *Ocimum basilicum*, *Eucalyptus globulus*, and *Thymus vulgaris*, were docked against β-lactamase inhibitors by molecular docking analysis (Etminani *et al*., 2023). No research has been conducted on the docking, simulation, or antibacterial activity of *Neprolepis exaltata*.

Limitations and Future Scope

Despite the promising antibacterial activity demonstrated by *Nephrolepis exaltata* against Methicillin-resistant *Staphylococcus aureus*, the persistence of the limitations should be addressed, the investigation may also proceed with various solvent extraction, which give more specificity of the results, secondly, while *in silico* methods such as molecular docking and MD simulations provided insights into the binding interactions and stability, these results require further validation through *in vitro* and *in vivo* models which is essential to determine the therapeutic safety of the extract and its components. Future studies can explore the isolation and purification of individual bioactive compounds from *Nephrolepis exaltata* to enhance the understanding of specific antibacterial agents responsible for the observed activity. Furthermore, a broader screening against various clinical Methicillin-resistant *Staphylococcus aureus* isolates and other multidrug-resistant bacteria would establish the spectrum of antibacterial potential. Expanding molecular docking and simulation to other bacterial targets could offer a more comprehensive understanding of the mode of action. Lastly, these bioactive compounds formulated into stable, deliverable pharmaceutical forms may pave the way for novel antibacterial therapies.

Conclusion

The study confirms the potent antibacterial potential of *Nephrolepis exaltata* methanolic extract against methicillin-resistant *Staphylococcus aureus*. The extract exhibited notable inhibition zones and an MIC of 40 µg/mL, supporting its effectiveness. GC-MS analysis identified 22 phytochemicals, with 2,3-Diazabicyclo [2.2.1] hept-2-ene showing the highest binding affinity in docking studies. Molecular dynamics simulations and MM-PBSA energy profiling confirmed the interaction strength and stability of the ligand and protein. These findings highlight the potential of *Nephrolepis exaltata* as a source of novel antibacterial agents and encourage further exploration for drug development against resistant pathogens.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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