Original Research Article

EXPLORING NEOPHYTADIENE FROM *Ampelocissus araneosa*: A MOLECULAR DOCKING APPROACH TO INHIBIT BIOFILM FORMATION IN *Staphylococcus*

Abstract

Background: The urgent need to combat antimicrobial resistance and biofilm production has prompted the exploration of alternative remedies, such as plant extracts, as viable substitutes for antibiotics. **Aim:** This study aims to assess the phytocompound from *A. araneosa* and elucidate its inhibitory mechanisms against biofilm formation by *S. aureus* through molecular docking techniques. **Methodology**: Plant material collection and extract preparation were conducted, followed by GCMS analysis of *A. araneosa* and protein modeling of Biofilm_IcaR from *S. aureus*. Drug 3D prediction and drug docking studies were performed using Neophytadiene and Metronidazole, with the PatchDock server for the exploration of molecular interactions between the compounds and protein targets.

Results and Implications: The study identified bioactive compounds from *A. araneosa*, including neophytadiene, 3-eicosyne, phytol, and stigmasterol, known for their robust antimicrobial properties. Molecular docking studies demonstrated that neophytadiene exhibited a higher binding affinity to the Biofilm_IcaR protein compared to the control drug, Metronidazole, suggesting its efficacy as an inhibitor. These findings provide molecular insights into the potential of neophytadiene as a therapeutic agent against biofilm-producing *S. aureus*, with implications for the development of novel antimicrobial treatments.

Keywords: Biofilm production, *A. araneosa*, neophytadiene, Molecular docking, stigmasterol.

Introduction

Staphylococcus, a genus of bacteria that includes the well-known pathogen *Staphylococcus aureus*, has gained notoriety for its ability to produce biofilms. Biofilms are structured communities of microorganisms embedded within a self-produced extracellular matrix, adhering to surfaces and interfaces. Staphylococcus species, including *S. aureus*, have the remarkable capacity to form biofilms, and this unique attribute carries significant implications for various fields, ranging from healthcare to industry and beyond [1].

Biofilm production by Staphylococcus species represents a complex biological phenomenon that has far-reaching consequences. These biofilms are known for their resilience, making them challenging to eradicate and a major concern in clinical settings. Understanding the mechanisms behind biofilm formation by Staphylococcus is crucial for addressing healthcare-associated infections, antibiotic resistance, and diagnostic challenges. Moreover, biofilm-related issues extend beyond healthcare, impacting industries such as food processing and environmental microbiology.

The urgent action required for the observed scenario calls for our sincere attention to the use of alternative remedies to combat antimicrobial resistance and biofilm production. Studies have shown that plant extracts may be a viable alternative to antibiotics [2]. Relatively few documented records, including research publications, have reported on the efficacy of plant extracts in treating infections caused by multi-drug- resistant pathogens [3and 4].

Ampelocissus, belonging to the Vitaceae family, holds significant importance in traditional medicinal practices. Within the realm of Indian traditional medicine, Ampelocissus plant components are commonly employed in the form of aqueous infusions or decoctions. The tuberous root and aerial parts of this plant are particularly utilized in the preparation of aqueous decoctions, which find widespread application in addressing various health concerns. These

include the treatment of ulcers, dental issues, dysentery, fractured bones, gout, indigestion, dyspepsia, and tuberculosis. Furthermore, the plant is employed topically for wound care and serves as an antidote for snake bites. Its therapeutic applications extend to managing conditions such as whooping cough, abscesses, and facilitating easy labor and delivery [5].

Ampelocissus plants, encompassing numerous species, have been harnessed globally for a myriad of medicinal treatments. However, there is a notable lack of comprehensive research regarding the phytochemistry and biological properties of Ampelocissus, particularly concerning *Ampelocissus araneosa (A.araneosa*). Notably, within the existing body of literature, there is no record of the isolation and characterization of phytochemicals from *A. araneosa* or investigations into its potential inhibitory mechanisms against biofilm-producing *S. aureus*. In light of this knowledge gap, our present study is dedicated to assessing the inhibition of the *S. aureus* strain and elucidating the inhibitory mechanisms of phytocompound via molecular docking techniques.

Drug docking studies, a pivotal component of computational drug discovery, are essential for understanding molecular interactions between potential drug compounds and target proteins. In these studies, computer algorithms predict and analyze the binding affinity and binding modes of drug candidates within the active sites of biological macromolecules [6]. This approach offers numerous advantages, such as accelerating drug development, optimizing drug design, and gaining insights into the molecular basis of diseases. By simulating these interactions, drug docking studies play a crucial role in the early stages of drug discovery, aiding researchers in identifying promising compounds and advancing them toward the development of effective pharmaceuticals [7]. The present study also seeks to isolate and identify phytochemical compounds from *A. araneosa* and elucidate their inhibitory mechanisms against biofilm formation by *S. aureus* through molecular docking analysis.

Methodology

Plant material collection and extract preparation

In this study, we utilized a leaf of *A. araneosa* collected from Salem District, Tamil Nadu, and India. After cleaning and air-drying, the leaves were ground to create a powder. Extraction was carried out using a soxhlet extractor with 200 mL of ethanol and chloroform as solvents. The extraction process continued until colorless extracts from the top of the extractor. Each extract was then concentrated separately under reduced pressure. After complete evaporation, the dry extracts were weighed and used for subsequent investigations. The extracts were stored at temperatures ranging from 2°C to 8°C for further studies [8].

GCMS analysis of *A. araneosa*

Following the identification of the fraction with significant antimicrobial activity through the aforementioned procedure, the compound responsible for this activity was selected for further analysis. The chosen compound underwent GC-MS analysis, which was conducted using a modified version of the analytical method described by [9]. The relative amount of each component was calculated by comparing its average peak area to the total area, software adopted to handle mass spectra and chromatograms was a Turbo mass.

Protein Modeling:

In the initial phase of this study, the protein sequence of Biofilm_IcaR from *Staphylococcus aureus* was obtained. To gain insights into its structural characteristics and functional domains, the protein sequence was subjected to domain analysis using the ScanProsite server (https://prosite.expasy.org/scanprosite/). Following this, an automated homology

modeling approach was employed utilizing the Swiss Model server (https://swissmodel.expasy.org/). This process effectively transformed the primary amino acid sequence into a 3D structural representation. To ensure the reliability of the generated 3D model, rigorous validation was conducted using the ProCheck server (https://saves.mbi.ucla.edu/). The final step involved visualizing the validated 3D protein structure through the utilization of molecular visualization software, Discovery Studio.

Drug 3D Prediction:

For the drug docking studies, two compounds were selected: the control drug Metronidazole (CID: 4173), retrieved from the NCBI – PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the test compound Neophytadiene (CID: 10446), sourced from a GC-MS instrument. To facilitate the subsequent molecular docking investigations, the 2D structure of the control drug was converted into a 3D structure employing cheminformatics protocols.

Drug Docking:

The core of this study involved the molecular docking experiments, which were carried out using the PatchDock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/). PatchDock is a versatile automated molecular drug docking tool that allows for the exploration of molecular interactions between compounds and protein targets. In this study, both the control drug, Metronidazole, and the test compound, Neophytadiene, underwent separate docking simulations with the Biofilm_IcaR protein from *Staphylococcus aureus*. These docking experiments aimed to elucidate the molecular binding affinities between the chemical compounds and the target protein. Subsequently, the obtained results were meticulously compared to evaluate their respective binding characteristics.

Results and discussion

The isolation of phytochemicals from plant samples is essential for scientific investigation, allowing researchers to identify, analyze, and understand the properties and potential applications of individual compounds. This process is fundamental to various fields, including pharmacology, medicine, and plant science. The number of methods were utilized for the determination of phytochemicals; among them, the choice of GC-MS for phytochemical analysis in plants was driven by its ability to separate, identify, and quantify a wide range of volatile and semi-volatile compounds present in plant extracts [10]. It is a versatile and powerful tool for researchers studying the chemical composition of plants and their potential applications in various fields such as medicine, nutrition, and agriculture [11]. In the present study, GCMS was selected for the identification of phytocompounds. From this technique, various antimicrobial natures of bioactive compounds such as 3-eicosyne, Neophytadiene, Phytol, and Stigmasterol, as well as the fatty acid compounds of 9-Octadecenoicacid, Hexadecanoicacid and Pentadecanoicacid were observed.

Table 1. Isolation of phytocompounds on *A. araneosa* by GCMS

RT	Name of Compound	Molecular Formula	Molecular Weight
14.96	4a,8,8-trimethyl-3á-oxa-tricyclo[5.4.0(1,4)]undecane	$C_{13}H_{22}O$	194
14.96	Oxetano[2,3-c]indane, perhydro-6,6,8a-trimethyl-	$C_{13}H_{22}O$	194
20.24	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-	$C_{20}H_{40}O$	296

Commented [ny1]: Explain Druglikeness- ADME and Toxicity Prediction of compounds ?

	,[R-[R*,R*-(E)]]-(CAS)		
20.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
20.24	3-eicosyne	$C_{20}H_{38}$	278
20.24	Neophytadiene	$C_{20}H_{38}$	278
20.24	Oxirane,hexadecyl-	$C_{18}H_{36}O$	268
20.24	Phytol,acetate	$C_{22}H_{42}O_2$	338
22.66	9-Octadecenoicacid(Z)-(CAS)	$C_{18}H_{34}O_2$	282
22.66	Hexadecanoicacid(CAS)	$C_{16}H_{32}O_2$	256
38.66	Stigmasterol	$C_{29}H_{48}O$	412
22.01	Pentadecanoicacid,14-methyl-,methylester(CAS)	$C_{17}H_{34}O_2$	270

Numerous studies have consistently demonstrated the robust antimicrobial activity of the aforementioned compounds, with neophytadiene [12], 3-eicosyne [13], phytole [14], and stigmasterol [15 and 16] standing out for their efficacy from various plant sources. These compounds exhibit their antimicrobial effects through a range of diverse mechanisms, encompassing modulation of membrane permeability, interference with enzyme activity, induction of protein damage, cessation of metabolic activity, inhibition of electron chain transport, disruption of cell wall integrity, and degradation of cellular proteins [17].

The distinctive neophytadiene exhibits antibacterial properties by disrupting the cell wall. The therapeutic compounds, known as neophytadiene, establish robust bonds with porins spanning the cell membrane. This interaction impedes the passage of nutrients and other compounds into and out of the bacteria, ultimately leading to the demise of the bacterial cells

[18]. Bacteria that produce biofilm are significant virulence factors. The resistance of biofilm is mainly due to the presence of an extracellular polymeric matrix (EPS), which encourages strong microbial attachment to surfaces, leading to restricted antibiotic penetration. Moreover, the heightened activity of efflux pumps in cells expels antimicrobial agents [19]. A previously published study suggests that this neophytadiene inhibits biofilm formation by interfering with QS [20]. However, there is a lack of understanding regarding the specific mechanism through which phytocompounds suppress biofilm formation. Consequently, this study employs molecular docking to specifically investigate the mechanism of action of the bioactive compounds, focusing on neophytadiene.

In the primary step of our study, we retrieved the Biofilm_IcaR protein (CPM65595.1) in FASTA format from the NCBI database, with a length of 186 amino acids Fig.1. Utilizing the ScanProsite server, we conducted protein domain analysis to identify motifs and domains in the Biofilm_IcaR protein. Fig. 2 illustrates the diverse multi-domain amino acid ranges present in Biofilm_IcaR. In vivo, protein kinase C exhibits a preference for phosphorylating serine or threonine residues proximate to a C-terminal basic residue. The presence of additional basic residues at the N- or C-terminal of the target amino acid was observed to enhance both the Vmax and Km of the phosphorylation reaction. Moreover, the covalent addition of myristate, a C14-saturated fatty acid, to the N-terminal residue through an amide linkage was noted to acylate numerous eukaryotic proteins [21 and 22].

Fig 1: Protein sequence of *Biofilm_IcaR*

>CPM65595.1 Biofilm operon icaABCD HTH-type negative transcriptional regulatorIcaR [*Staphylococcus aureus*]

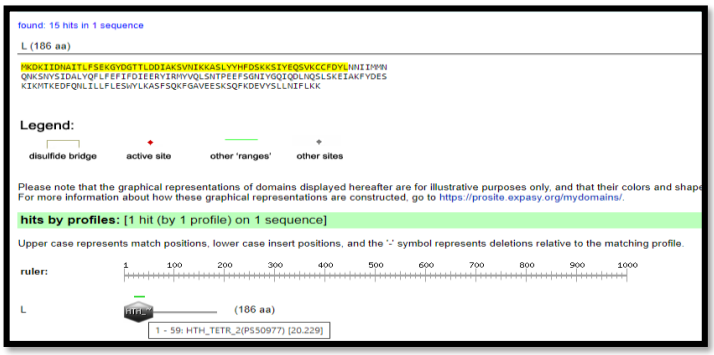
MKDKIIDNAITLFSEKGYDGTTLDDIAKSVNIKKASLYYHFDSKKSIYEQSVKCCFDYLNNIIMMNQNKS

NYSIDALYQFLFEFIFDIEERYIRMYVQLSNTPEEFSGNIYGQIQDLNQSLSKEIAKFYDESKIKMTKED

FQNLILLFLESWYLKASFSQKFGAVEESKSQFKDEVYSLLNIFLKK

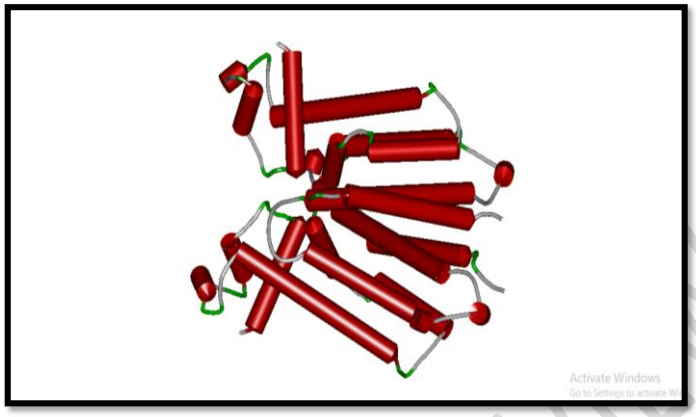
FASTA format: *Biofilm_IcaR* - *Staphylococcus aureus*

Fig 2: Protein Domain Analysis (*Biofilm_IcaR*)



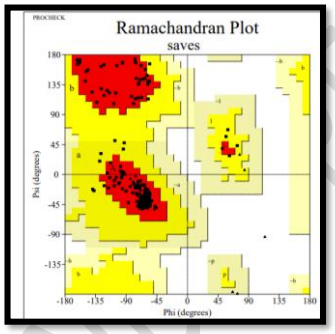
In the subsequent step, we predicted the 3D structure of the Biofilm_IcaR protein using the Swiss-Model server [23 and 24]. The automated homology modeling server converted the amino acid sequence into a 3D structure (Fig. 3), which was further visualized using Discovery Studio software. To validate the modeled structure, we employed the ProCheck server [25], which indicated high accuracy with a 96.1% error-free Ramachandran plot (Fig. 4). This rigorous evaluation ensured the reliability of the predicted 3D structure for subsequent molecular docking studies.

Fig 3: Protein Modelling: 3D structure of *Biofilm_IcaR*



The above picture shows the 3D view of the protein structure of*Biofilm_IcaR*in secondary structure colour with a solid ribbon model visualized using discovery studio software.

Fig 4: Protein Structure Validation: 3D structure of *Biofilm_IcaR*



Plot statistics

Residues in most favoured regions [A,B,L]	342	96.1%
Residues in additional allowed regions [a,b,l,p]	14	3.9%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	356	100.0%
Number of end-residues (excl. Gly and Pro)	4	
Number of glycine residues (shown as triangles)	10	
Number of proline residues	2	
Total number of residues	372	

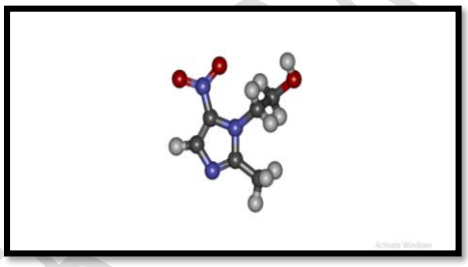
Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Assessment of the Ramachandran plot for the predicted mutated protein sequence of the modeled *Biofilm_IcaR*

Moving forward to molecular drug-protein interaction, we predicted the 3D structures of Metronidazole and Neophytadiene (Fig. 5 and 6). The discovery studio software facilitated the automated conversion of 2D to 3D structures for the docking procedure. The PatchDock server [26] was employed for molecular docking, involving a two-step process of ligand conformation

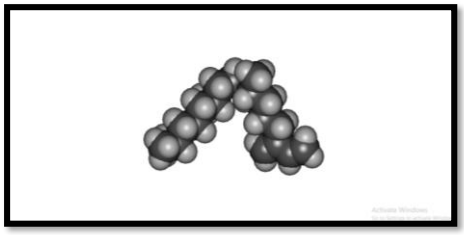
sampling and scoring (Table 2). The results of the docking experiments revealed that the existing drug molecule, Metronidazole, exhibited an atomic contact energy value of -164.93 Kcal/mol with Biofilm_IcaR (Fig. 7, 8, 9). Interestingly, Neophytadiene demonstrated a stronger interaction, with an atomic contact energy value of -202.96 Kcal/mol (Fig. 10 and 11), suggesting its potential as an inhibitor of the Biofilm_IcaR protein. Notably, clinical evidence pointed to specific domain regions of Biofilm_IcaR, located between amino acid positions 159-161 (PS00005) and 108-113 (PS00008), confirming the importance of these regions in the protein's function (Fig. 12).

Fig 5: Cheminformatics -3D Structure of Metronidazole



The above picture shows the 3D structure of *Metronidazole* with coloured atoms: Grey-Carbon, Blue-Nitrogen, Yellow-Sulphur, and White –Hydrogen using Discovery Studio Software.

Fig 6: Cheminformatics -3D Structure of Neophytadiene



The above picture shows the 3D structure of *Neophytadiene* with coloured atoms: Grey-Carbon, Red –Oxygen, and White –Hydrogen using Discovery Studio Software.

Table: 2 Results of Molecular drug docking (Patch dock server)

Target Protein	**Neophytadiene (CID: 10446) (GC/MS : Compound)**	**Metronidazole (CID: 4173) (Control drug)**
Biofilm_IcaR (NCBI Acc.no: CPM65595.1)	-160.20 kcal/mol	-118.39 kcal/mol

Fig 7: Molecular Drug Docking: *Biofilm_IcaR*with Metronidazole

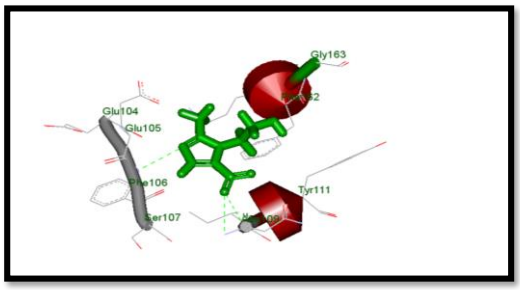
Receptor	Ligand	Complex Type	Clu
S.aubiofilm.pdb	Metronidazole.pdb	drug	4.0

Solution No	Score	Area	ACE
1	2702	293.10	-84.38
2	2676	291.80	-76.74
3	2438	283.30	-49.06
4	2412	261.40	-1.96
5	2364	252.80	14.82
6	2348	258.60	-85.51
7	2294	290.50	-56.54
8	2284	242.30	10.50
9	2276	253.30	-96.41
10	2266	239.10	-50.25
11	2256	259.90	-27.75
12	2252	268.70	-111.36
13	2246	271.10	-118.39
14	2222	249.60	14.87
15	2210	290.30	-20.74
16	2182	235.20	-72.51
17	2176	281.00	27.77
18	2166	241.80	17.33
19	2164	235.20	-35.53
20	2162	231.30	-28.49

The above picture represents the PatchDock result page showing the drug docking score of the control drug, Metronidazolewith the modelled protein target, Biofilm_IcaR. The negatively high ACE (*Atomic Contact Energy*) value is -118.39 kcal/mol.

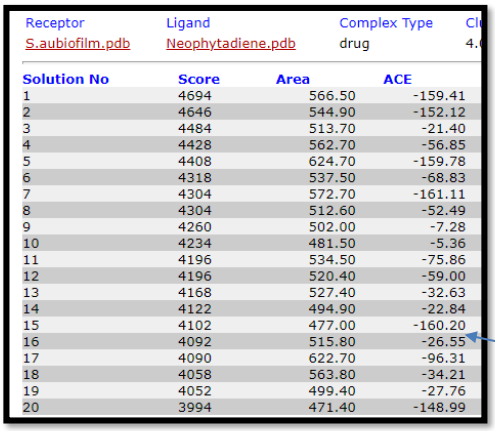
Fig 8: Molecular Drug Docking

3D *Biofilm_IcaR*with Metronidazole complex



The above picture represents the existing drug molecule, Metronidazole docked with the Biofilm_IcaR protein structure with drug binding amino acid labels. The Green colour indicates Metronidazole in the stick model using discovery studio software (H-Bond -Ligand Binding site prediction: Model).

Fig 9: Molecular Drug Docking: *Biofilm_IcaR* with Neophytadiene



Receptor	Ligand	Complex Type	Cl
S.aubiofilm.pdb	Neophytadiene.pdb	drug	4.

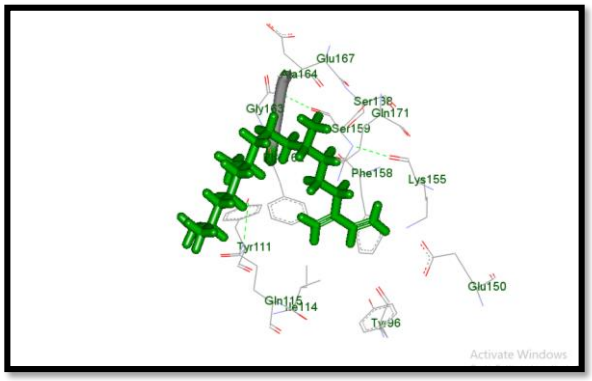
Solution No	Score	Area	ACE
1	4694	566.50	-159.41
2	4646	544.90	-152.12
3	4484	513.70	-21.40
4	4428	562.70	-56.85
5	4408	624.70	-159.78
6	4318	537.50	-68.83
7	4304	572.70	-161.11
8	4304	512.60	-52.49
9	4260	502.00	-7.28
10	4234	481.50	-5.36
11	4196	534.50	-75.86
12	4196	520.40	-59.00
13	4168	527.40	-32.63
14	4122	494.90	-22.84
15	4102	477.00	-160.20
16	4092	515.80	-26.55
17	4090	622.70	-96.31
18	4058	563.80	-34.21
19	4052	499.40	-27.76
20	3994	471.40	-148.99

The above picture represents the PatchDock result page showing the drug docking score of the testmolecule, *Neophytadiene*with the modeled protein target, *Biofilm_IcaR*. The negatively high ACE (Atomic Contact Energy) value is -160.20 kcal/mol.

Fig 10: Molecular Drug Docking

3D structure of *Biofilm_IcaR*with

Neophytadiene (H-Bond interaction)

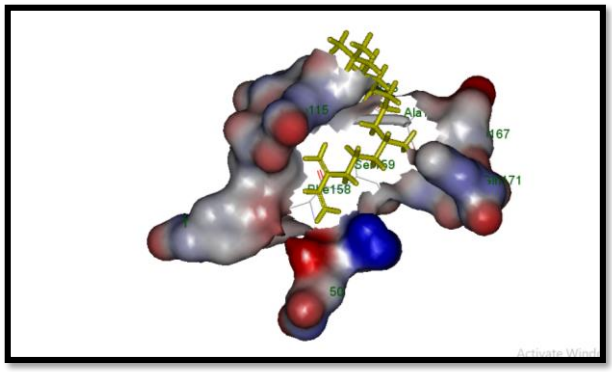


The above picture represents the test molecule Neophytadiene docked with the Biofilm_IcaR protein structure with drug binding amino acids labels. Green colour indicates Neophytadiene in the stick model using Discovery studio software (H-Bond - Ligand binding sites prediction: model).

Fig 11: Molecular Drug Docking

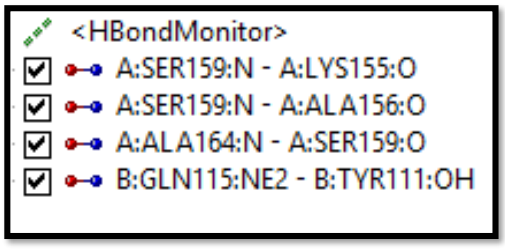
3D structure of *Biofilm_IcaR*with

Neophytadiene(H-Bond interaction -1)



The above picture represents the test molecule Neophytadiene docked with *Biofilm_IcaR* protein structure with drug binding amino acids labels. Green colour indicates Neophytadiene in the stick model using discovery studio software. (*Vander waals interaction*: Model-1)

Fig 12: Molecular Dynamics: H-Bond interactions



H-bond interaction:Neophytadiene – *Biofilm_IcaR*

(Molecular Acceptor and Donor Atoms Interaction (Discovery Studio Software)

The molecular docking study additionally gives information about molecules binding to active site. This study provides scientific evidence to structure-based drug design against biofilm producing *S.aureus*. Numerous researchers have investigated the potential of neophytadiene in combating various bacteria and inhibiting their virulence factors through molecular docking analyses [27, 28, 29 and 30]. However, the molecular docking-based mechanism of neophytadine against *S. aureus* has not been elucidated by any researcher.

Overall, the in silico results strongly indicate that neophytadiene binds directly to the functional domains of the Biofilm_IcaR protein. These findings hold substantial promise for advancing the development of novel antimicrobial treatments, and providing molecular evidence supporting the potential efficacy of neophytadiene as a therapeutic agent against biofilm-producing *S. aureus*. However, to translate these in silico results into practical therapeutic applications, further clinical research and experimental validation are imperative.

The interaction between the drug and the protein is characterized by atomic contact energy (ACE), indicating a significantly negative binding score. Notably, the binding of Neophytadiene with Biofilm_IcaR demonstrates a higher binding score compared to the control drug, Metronidazole. The docking results, effectively depicted through visual representations, clearly indicate that the protein Biofilm_IcaR is down-regulated as a result of the binding with Neophytadiene within its functional domain regions.

Conclusion

The phytochemical analysis of *A. araneosa* led to the identification of several bioactive compounds, including neophytadiene, 3-eicosyne, phytol, and stigmasterol, known for their robust antimicrobial properties. GC-MS analysis revealed the presence of these compounds, emphasizing their potential therapeutic significance. Molecular docking studies demonstrated

that neophytadiene exhibited a higher binding affinity to the Biofilm_IcaR protein compared to the control drug, Metronidazole, suggesting its efficacy as an inhibitor. The findings provide molecular insights into the potential of neophytadiene as a therapeutic agent against biofilm-producing *S. aureus*, with implications for the development of novel antimicrobial treatments. However, further experimental validation and clinical researches are essential for translating these promising in silico results into real-world therapeutic applications. This study contributes valuable information for structure-based drug design targeting biofilm formation in *S. aureus*.

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