

Research Article



Insilico analysis of Neem secondary metabolites against clumping factor A of *Staphylococcus aureus*

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ABSTRACT

Antimicrobial and antibiotic resistance is very common nowadays in hospital community due to bacteria, fungal, viral strains. All kinds of bacteria initially forms colonies in the part of the body and produces exo and endotoxins commonly to cause the severe infection, on the same path gram-positive bacteria *Staphylococcus aureus*, colonies on skin, soft tissues and nasal passage of healthy individuals in the anterior nares. MRSA is very common and major public health problem in india, hence the natural based drugs are necessary to treat this kind of disease. In this current study, one of the clumping factor was chosen from *Staphylococcus aureus* and docked with a multiple secondary metabolites from neem. The docking results it was concluded that neem ligands salanin, astragalgin and epoxyzadirone show high dock score among these three the ligand salanin shows highest antibacterial activity in Insilco screening.

Keywords: Antimicrobial, Clumping factor, Insilco screening, Secondary metabolites, *Staphylococcus aureus*.

INTRODUCTION

Antimicrobial and antibiotic resistance is very common nowadays in hospital community, The frequency of traveling and movement of food materials are the major risk for the spreading of antimicrobial resistance genes, Resistance genes were found reservoir commonly in the fecal flora and upper respiratory tract.¹ Multi drug resistance infections caused by Gram-positive bacteria, such as *Staphylococcus aureus* and *Enterococcus spp* these two organisms represents a major health burden to public.² All kinds of bacteria initially forms colonies in the part of the body and produces exo and endotoxins commonly to cause the severe infection, on the same path gram-positive bacteria *Staphylococcus aureus*, colonies on skin, soft tissues and nasal passage of healthy individuals in the anterior nares.³⁻⁴ This bacteria *Staphylococcus aureus* are capable of producing virulence factor like alpha toxin⁵, beta toxin⁵⁻⁶, leukocidin⁷, coagulase and clumping factor⁸⁻⁹, staphylokinase and exotoxins.¹⁰ Natural habitat of this bacteria is moist squamous epithelium, about 80% population were directly or indirectly affected by this bacteria in this 20% people carry permanently in the nasal way and another intermittent carriers are about 60%, By day-to-day activities this bacteria become very strong and get resistance to various treating drugs.¹¹⁻¹²

Drug resistance to be seen widely in *Staphylococcus aureus*, the frequency of methicillin resistant *S aureus* (MRSA)¹³ has been reported in different hospitals in various places in world. Apart, vancomycin resistant *Staphylococcus aureus* (VRSA), vancomycin intermediate resistant *Staphylococcus aureus* (VISA) and vancomycin resistant enterococci were also emerging in recent years,

this kind of resistance led to experimental failures of glycopeptides.¹⁴

A surface protein plays a vital role in causing the disease, the surface proteins of *Staphylococcus aureus* like Clumping factor A (ClfA), Clumping factor B (ClfB), iron-regulated surface protein (IsdA).¹⁵⁻¹⁶ Apart, serine-aspartic acid repeat proteins SdrC, SdrD and SdrE were studied recently in many research papers and reported that these proteins in *Staphylococcus aureus*, promotes cell adhesion to host desquamated epithelial cells in the nasal vestibule anterior part.¹⁷

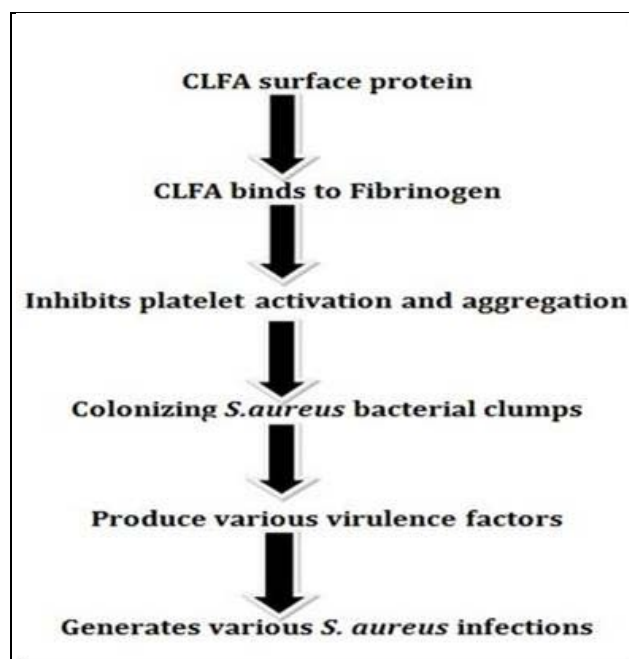


Figure 1: Disease mechanism pathway of clumping factor A in *S.aureus*

In this current study, Clumping factor A (ClfA) was taken as drug target protein the disease causing mechanism of the protein is shown in the figure 1. On other hand the secondary metabolites from neem was taken and docked using computational biology techniques.

MATERIALS AND METHODS

Drug target Retrieval of form PDB database

The drug target protein Clumping Factor A from *Staphylococcus aureus* of ID 1N67 and its X-ray crystallographic structure with 1.90 Å resolution with UniProtKB unique identification number is Q53653 with amino acid sequence of 359 was retrieved from PDB database. (<http://www.rcsb.org/pdb/home/home.do>).

Preparation of protein and active site prediction

The water and ligand that bound to the protein Clumping Factor A was removed, initially force field CHARMM was applied to remove the bad clashes and non-bonded interactions followed by the respective drug target protein was saved in current mode of protein data bank. Now active site of the protein was automatically predicted using flood filling algorithm available through acclerys discovery studio 2.1v

Retrieval of natural compounds

The neem secondary metabolites such as Meldenin (CID:4114), Myricetin (CID:497359), Isomorgololone (CID:189727), Morgolonone (CID:189726), Morgolone (CID:189728) Epicatechin (CID:72276), Mahmoodin (CID:126566), Nimbin (CID:108058), Azadirachtin (CID:5281303), Azadirone (CID:185587), Catechin (CID:9064), Salannin (CID:6437066), Sugioli (CID:94162), Gallic acid (CID:370), Astragalin (CID:5282102) Kaempferol (CID:5280863), Nimbolide (CID:100017), Nimolicinol (CID:184937), Gedunin (CID:12004512), Azadiradione (CID:363978) were retrieved from pubchem database (<https://www.ncbi.nlm.nih.gov/pccompound>)

Screening of compounds based on molecular docking

The concept of structure based drug designing was applied to screen the more than one compounds based on receptor ligand interaction studies. The appropriate active site was defined initially it starts with the site 1, if the compounds doesn't show the no more interaction again. Next site was preceded consequently until the compounds docks, if the compounds shows non docking then it was concluded that there is no shape complementarity for these compounds in the active site of the drug target.

RESULTS AND DISCUSSION

Theoretical prediction of cavities in drug target

The protein cavities considered as binding site for the compounds, xenobiotics are capable of binding to cavities. These cavities are predicted theoretically using different kind of algorithm here in discovery studio 2.01v eraser and flood filling algorithm¹⁸ automatically predicts

the active site of the proteins from increasing to decreasing order, That is site 1 shows more volume and atoms when compared with site 2, site 3 and site 4 respectively. The protein Clumping Factor A shows eight different sites is shown in the figure 2.

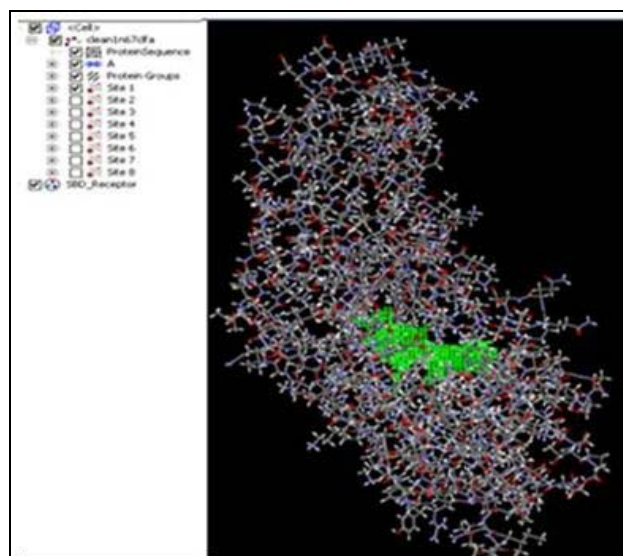


Figure 2: Different binding sites of the protein

Molecular docking of neem metabolites

A series of neem metabolites compounds such as Isomargololone, mahmoodin, margololone, margolone, nimbin, azadiractin, azadirone, catechin, myricetin, salanin, sugiol, gallic acid, Linoleic acid, astragalin, kaempferol, meldinin, nimbolide, nimolicinol, gedunin and azadiradione. were docked to drug target protein Clumping Factor A in the active site 1 of 767.75 volume with 6142 point counts with equal grid Spacing of 0.5 X 0.5 X 0.5 and grid angles of 90° in three dimensions of 30.703(X), 4.31(Y), and 36.442(Z) directions respectively. Interaction between the active site amino acid and neem metabolites were measured in angstrom and dock score. the docking of with neem compounds gives dock scores (Kcal/mol) according to biological activity, from the list of compounds salanin shows high biological activity against staphylococcus protein with dock score of 65.094 interacting at residue ARG395, the results of biological activity of neem ligands are represented.

The interaction scores of this neem compounds are as follows, the dock score of with isomargololone is 46.157 at residues SER447, mahmoodin is 47.613 at residues SER290, astragalin 57.842 at residues ARG395 azadiradione is 51.927 at residues TYR399. Similarly, Catechin is 53.814 at residues ASP385, epicatechin 48.135 at residues VAL288, epoxyazadiradione 53.909 at residues TYR399, gallic acid 29.896 at residues ASP385, VAL447, kaempferol dock score is 21.358 at residues ARG395, linoleic acid is 40.547 at residues ARG395, myricetin is 43.524 at residues TYR399, nimbin 44.187 at residues VAL450, sugiol the dock score is 47.673 at residues TYR399.

Table 1: Interaction Analysis of Protein with Neem Compounds

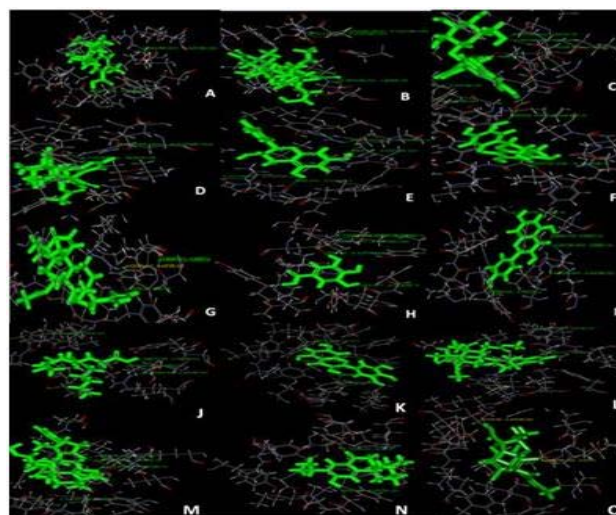
Compound name	Interacting atoms of compounds with active site amino acid atoms	H-bond length in Å
Salanin	A:ARG395:HH21-O2	2.36774
Astragalin	A: ARG395:HH12-O6	1.81177
Epoxyazadiradione	TYR399:HH-O4	2.3107
Catechin	H33-A:ASP385:OD1	1.1001
Azadiradione	A:TYR399:HH-O4	2.21379
Epicatechin	H28-A: VAL288: O, H34-A: VAL450: O	2.0496, 2.19949
Sugiol	A:TYR399:HH-O1	1.85417
Mahmoodin	A: SER290:HG-O5	1.69872
Isomargolonone	H45-A: SER447: O	1.90206
Nimbin	A:VAL450:HN-O8	1.99188
Myricitin	A:TYR399:HH-O2	1.82972
Linoleic acid	A:ARG395:HH12-O1, H50-A:ASP385:OD1	2.26372, 1.5304
Gallic acid	H16-A:ASP385:OD1, H17-A:SER447:OD1	1.03762, 1.84235
Kaempferol	A:ARG395:HH22-O4	1.8238
Standard-Floxacillin	A:ARG395:HH22-F3, H41A:ASP340:OD1	1.66734,1.34225

Table 2: Docking Results of Neem Compounds with Protein

Compound name	Dock score in kcal/mol
Salanin	65.094
Astrageline	57.842
Epoxyazadiradione	53.909
Catechin	53.814
Azadiradione	51.927
Epicatechin	48.135
Sugiol	47.673
Mahmoodin	47.613
Isomargolonone	46.157
Nimbin	44.187
Myricitin	43.524
Linoleic acid	40.547
Gallic acid	29.896
Kaempferol	21.358
Standard-Floxacillin	67.159

Among all compounds, salanin shows high anti-bacterial activity. The protein is docked with standard drug floxacillin its dock results gives dock score of 67.159 interacting at residues ARG395, ASP340 whereas Salanin also shows similar results with the standard drug. Table 1 and table2 describes about interaction analysis and dock

score respectively. Figure 3 shows the docking of compounds to the active site of protein.



A: Isomargolonone, B: Mahmoodin, C: Astragalin, D: Azadiradione, E: Catechin, F: Epicatechin, G: Epoxyazadiradione, H: Gallic acid, I: Kaempferol, J: Linoleic acid, K: Myricitin, L: Nimbin, M: Salanin, N: Sugiol, O: Standard-Floxacillin

Figure 3: Docking of compounds to the active site of protein

CONCLUSION

Staphylococcus aureus contains clfA cell surface proteins, these proteins are involved in binding to the human fibrinogen. After binding to the human fibrinogen, it inhibits the platelet activation and plays a major role in staphylococcus infections. The protein CLFA is retrieved from uniprot, its structure is retrieved from pdb databases. The neem compounds which already shown anti-microbial activity are retrieved from chemical databases, docking was performed between the CLFA protein and neem ligands using discovery studio, from the docking results it was concluded that neem ligands salanin, astragalin and epoxyazadiradione show high dock score among these three the ligand salanin shows highest antibacterial activity in insilico screening. Thus, further in-vitro and in-vivo studies need to be conducted to prove the biological activity of this compound.

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