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INSILICO BINDING STUDIES OF ANTI-MRSA COMPOUNDS ON PENCILLIN BINDING PROTEIN 2A

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ABSTRACT: The study evaluates a few selected plant derived products including Sesamin, Pellitorine, Guineesine, Brachystamide B and Pipataline from piper longum for their binding with and inhibitory capacity on Penicillin Binding Protein 2a of Methicillin Resistant *Staphylococcus aureus* (MRSA) using computational methods. The crystal structure of Penicillin Binding Protein 2a was taken from the Protein Data Bank (PDB_ID:1VQQ). Possible binding sites of Penicillin Binding Protein 2a were searched with CASTP server. Molecular docking was performed using the Gold (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA), to study the binding orientation of compounds into the Penicillin Binding Protein 2a structure. The efficiency and drug-likeness of various plant compounds were identified by using pre-ADMET software. In this study, all docked compounds were found to have some interaction between an oxygen atom of the Compounds and Penicillin Binding Protein 2a. In the binding pocket, common H-bonding interactions were formed between all docked compounds and ASP-65, CYS-66, CYS-88. The docking results agreed well with the observed in vitro data, which showed that the Penicillin Binding Protein 2a inhibitory activity of pepataline was higher than those of other compounds.

KEYWORDS: MRSA, Penicillin Binding Protein 2a, Docking, dithymoquinone and ADMET studies.

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1.INTRODUCTION

The multiple antibiotic resistances of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) has become a major clinical problem worldwide. Rates of MRSA infection are increasing [1]. In

some centers, MRSA is becoming less susceptible to vancomycin, and these strains have been associated with worse clinical outcomes [2]. Intermediate or fully resistant vancomycin strains of MRSA have emerged clinically, whereas community acquired MRSA has become epidemic. Since the major difference between methicillin sensitive *Staphylococcus aureus* (MSSA) and MRSA is gene mecA, studies to find novel inhibitors of PBP2a Protein are being carried out in order to counter the resistance of MRSA against β-lactam antibiotics [3, 4]. According to recent findings, Linezolid, daptomycin and tigecycline have been approved during the last decade to treat infections due to MRSA. Although these agents are extremely valuable in the fight against MRSA, each one has limitations because of their side effects. New lipoglycopeptides (telavancin, dalbavancin and oritavancin) are in advanced phase of clinical development. One of the new approaches for the discovery of compounds with anti MRSA activity is to search for molecules from a wide array of photochemicals from plants. Review of literature indicated that the active substances present in many plants could be used as therapeutic alternatives for MRSA infection. Screening of phytochemicals from plant extracts have shown that higher plants and their secondary metabolites provide diverse bioactive compounds with antibacterial activity [5-8]. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are discovered on a rational basis, using computational methods to simulate drug – receptor interactions insilico saving a lot of time, effort and expenditure in the search for lead compounds. The present study is an attempt to find such compounds using Bioinformatic tools on selected plant products for their binding and inhibiting capacity of the target Protein PBP2a, responsible for resistance of MRSA [9-11].

2. MATERIALS AND METHODS

Selection of ligands

Following plant derived compounds were selected based on their anti-*staphylococcus aureus* and anti- MRSA activities. Sesamin, Pellitorine, Guineesine, Brachystamide B and Pipataline from piper longum were selected for docking studies. The structures of these compounds were constructed and optimized using chemsketch software (Figure 1A to1E).

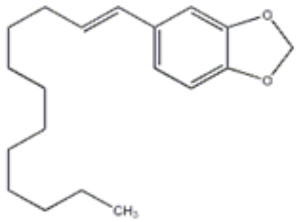

Fig 1A:Pipataline

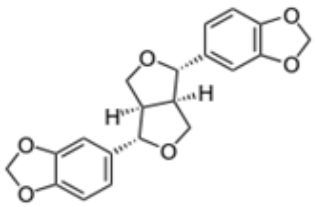

Fig 1B:Sesamin

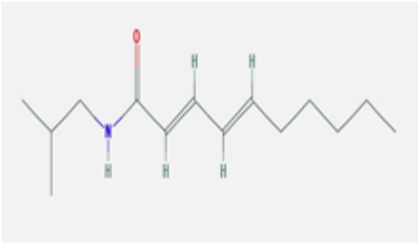

Fig 1C:Pellitorine

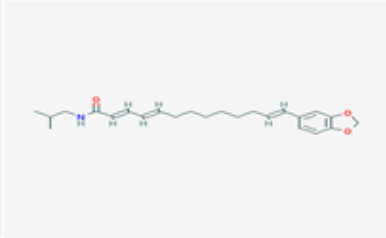

Fig 1D: Guineesine

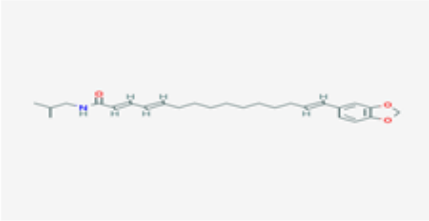

Fig 1E: Brachystamide B

Fig 1A-1E: Structure of Compounds

Selection and preparation of the Target

The crystal structure of Penicillin Binding Protein 2a was taken from the Protein Data Bank (PDB_ID:1VQQ) (Fig 2). Statins were removed from the binding site and the chain A was selected for docking studies. Hydrogen atoms were added to the Enzyme (Fig 2).

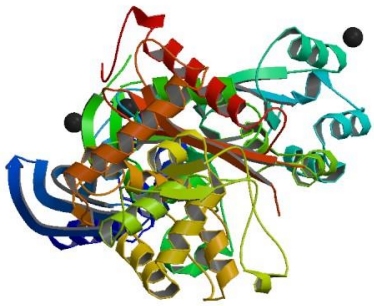

Fig 2: structure of Penicillin Binding Protein 2a

Docking Method

Molecular docking was performed using the Gold version 3.0.1 (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA), to study the binding orientation of compounds into the Penicillin Binding Protein 2a structure. This method allows partial flexibility of protein and full flexibility of compounds. The compounds are docked to the active site of the

Penicillin Binding Protein 2a . The binding site identification of Penicillin Binding Protein 2a structure was carried out using CastP server (Fig 3). CastP identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings [12-18].

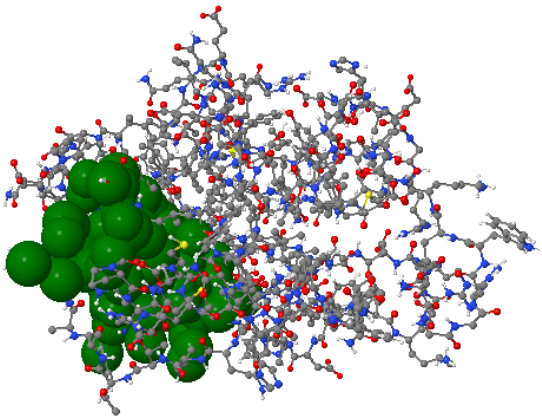

Fig 3: Active site of Penicillin Binding Protein 2a

The interaction of these compounds with the active site residues are studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the binding site in the Penicillin Binding Protein 2a was defined within a 10 A° radius with the centroid as CE atom of LEU55. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a compound were within 1.5A° RMSD. After docking, the individual binding poses of each compound were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each compound was selected [19-22].

Gold Score fitness function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3.Ligand internal vander Waals energy (internal vdw); 4.Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

ADMET Studies

The plant compounds with anti MRSA activity were used for absorption, distribution, metabolism, excretion and toxicity (ADMET) studies which were the most important part of pharmacological studies. These studies can be predicted insilico by Molinspiration and OSIRIS software. Hence, all compounds which were used for docking were tested for their drug-likeliness, ADME profile and toxicity analysis.

Invitro studies

The data generated by computer aided studies should be tested experimentally for further confirmation. Based on the pharmacological properties studied by ADMET and docking studies, five compounds were identified for in vitro testing. The leaf extracts were prepared by standard solvent extraction method and the antimicrobial activity of each extract was tested in different concentrations. The pure culture of MRSA was swabbed on Muller Hinton agar plates which have many wells. The extracts were added in different concentration to each well (well diffusion method) and the plates were incubated at 37^{o}C for 24 hours. The antimicrobial activity was determined by measuring the zone of inhibition. The efficiency of herbal extracts was also compared with known antibiotics. The antibiotic susceptibility patterns were carried out by disc diffusion method. The sensitivity patterns of each antibiotic were confirmed by measuring the zone of inhibition around the disc and compared with standard antibiotic susceptibility chart [23-29].

3. RESULTS AND DISCUSSION

After collecting the crystal model, the possible binding sites of Penicillin Binding Protein 2a was searched with CASTP server and shown in Figure 3. From the binding site analysis of Penicillin Binding Protein 2a we identified that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies. The crystal structures of Penicillin Binding Protein 2a are similar and we have therefore taken 1VQQ (chain A) as representative structure for docking studies. The docking of compounds into the active site of Penicillin Binding Protein 2a was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function [30,31].

Molecular docking study

The selected docked conformations of in the Penicillin Binding Protein 2a binding site are shown in Figure 1A-1E. The docked conformations revealed that all compounds were located in the hydrophobic binding pocket surrounding the binuclear copper active site. In this study, all docked compounds were found to have some interaction between an oxygen atom of the Compound and

Penicillin Binding Protein 2a . Moreover, these docked conformations also formed an H-bonding interaction with in the active site. **In the binding pocket, common h-bonding interactions were formed between all docked compounds and GLY 135, GLN 137, GLN 140, HIS 143, GLU 145, GLN 145, GLN 207, ASP 209, HIS 232, THR 300, and HIS 311.** In order to explain the binding of these compounds, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated (4A-4E).

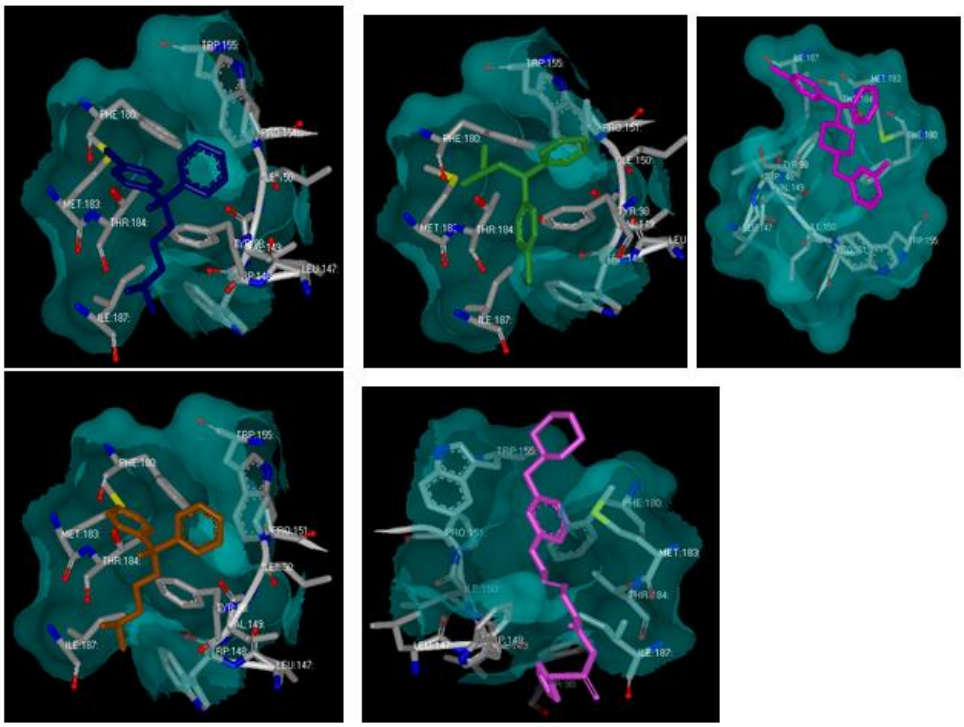

Fig 4A-4E: docking studies of the compounds

The docking results agreed well with the observed *in vitro* data, which showed that the Penicillin Binding Protein 2a inhibitory activity of pepataline was higher than those of other compounds (Table 1).

Table 1: Docking studies of compounds with Penicillin Binding Protein 2a

Fitness	Ligand name
31.62	pepataline
23.23	brachystamide b
32.62	sesamin
24.97	pellitorine
27.77	guneensine

The ADMET studies predicted insilico by Molinspiration and OSIRIS showed that all the compounds can be used orally as they have less toxic. The results were tabulated in table3 and 4. Hence, all compounds which were used for docking were tested for their drug-likeliness, ADME

profile and toxicity analysis (Table 2, 3).

Table 2: Molinspiration Results-Bioactivity Table

COMPOUNDS	Molinspiration bioactivity score	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1. pepataline	v2011.06	0.33	0.05	-0.16	0.78	0.39	0.90
2. brachystamide b	v2011.06	0.14	0.05	-0.51	0.73	0.07	0.51
3 sesamin	v2011.06	-0.06	-0.20	-0.26	0.12	-0.14	0.08
4. pellitorine	v2011.06	-0.85	-0.42	-0.99	-0.62	-1.12	-0.35
5. guneensine	v2011.06	-0.65	-0.28	-0.69	-0.44	-0.82	-0.15

Table 3: OSIRIS ADMET Molecular Prediction Results

COMPOUNDS	MUTAGENIC	TUMORIGENIC	IRRITANT	REPRODUCTIVE EFFECTS	C Log P	SOLUBILITY	MOL. WT	TPSA	DRUG LIKELINESS	DRUG SCORE
1. pepataline	-	-	-	-	-	-	-	-	-	-
2. brachystamide b	-	-	-	-	-	-	-	-	-	-
3 sesamin	-	-	-	+	3.17	-3.35	382.0	93.06	-5.6	0.23
4. pellitorine	+	-	-	-	0.73	-1.35	168.0	66.76	-1.31	0.35
5. guneensine	+	+	-	-	0.66	-1.37	198.0	75.99	1.99	0.44

Invitro studies

MRSA collected from different hospital samples was used to grow on agar media. In invitro studies we used pepataline, Vancomycin and Methicillin discs to find the activity. *Staphylococcus aureus* showed resistance to Methicillin but not to Vancomycin and pepataline. pepataline showed highest zone of inhibition than Vancomycin (Fig 5).

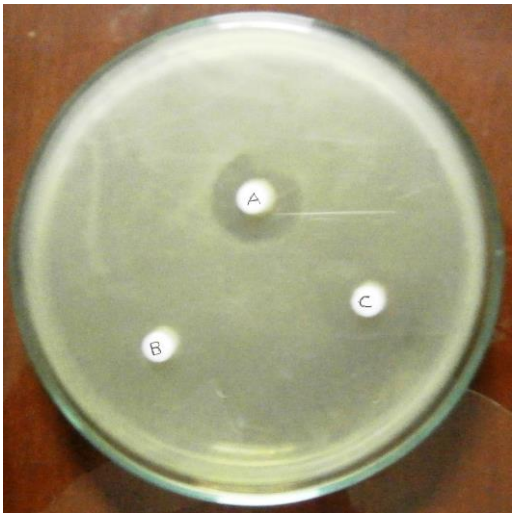

Fig 5: Invitro studies A: pepataline disc; B: Vancomycin; C: Methicillin

4. CONCLUSION

The docking results agreed well with the observed *in vitro* data, in which the anti-Penicillin Binding Protein 2a activity of the pepataline was higher than other compounds. The docking study revealed the binding orientation of compounds in the Penicillin Binding Protein 2a binding pocket surrounding the active site, which resulted in inhibition of enzyme activity. From the results we can conclude that these five plant derived products act as inhibitory compounds of Penicillin Binding Protein 2a .

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CONFLICT OF INTEREST

Authors declare that they don't have any conflict of Interest

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