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RESEARCH ARTICLE

Molecular Docking of *Hygrophila auriculata* (Schum.) Heine Compounds Targeting PBP2 Protein in *Neisseria gonorrhoeae*

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ABSTRACT

Antibiotic resistance in gonorrhoea cases and patients is detrimental. Amidst the rising cases of gonorrhoea caused by *Neisseria gonorrhoeae*, *Hygrophila auriculata* (Schum.) Heine has activity as an antibiotic for the disease. The penicillin-binding protein 2 (PBP2) is a transpeptidase that catalyses the formation of cross-bridges on bacterial cell wall peptidoglycan, which will be the target of this plant. The purpose of molecular docking study is to see the binding affinity, compounds in *Hygrophila auriculata* (Schum.) Heine and ceftriaxone which is used as a comparison drug, will be targeted at PBP protein. Discovery studio visualizer v21.1.0.20298 was used for PBP2 protein preparation and visualisation. DoG Site Scorer was used to predict ligand binding sites on PBP2 proteins. PyRx 0.8 was used for virtual screening, validating of the docking method, and ligand preparation. Compounds in *Hygrophila auriculata* (Schum.) Heine as ligands were derived from MPDB 2.0 and the following PubChem codes; apigenin CID 5280443, luteolin CID 5280445, allagic acid CID 5281855, gallic acid CID 370, quercetin CID 5280343, lupeol CID 259846, lupenone CID 92158, betulin CID 72326, stigmasterol CID 5280794, and comparator drug ceftriaxone CID 447043. The binding affinity of ellagic acid -9,8 from *Hygrophila auriculata* (Schum.) Heine was lower than ceftriaxone -9,4 on the target PBP2 protein. Some of the amino acid residues that appear in protein-ligand docking include: ALA A:310, THR A:500 and 347, LYS A:313, and SER A:362. These amino acid residues owned by the PBP2 protein serve as the bonding bridge. Ellagic acid, the compound has potential as an antibiotic in gonorrhoea. Further testing and studies are needed to strengthen the evidence of the findings in this study.

Keywords: *Hygrophila auriculata*, lupeol, *Neisseria gonorrhoeae*

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Introduction

Adults aged 15 to 49 years in 2020, an estimated 82.4 million people of that age were infected with *Neisseria gonorrhoeae* (*N. gonorrhoeae*) [1]. Currently, a third-generation cephalosporin (ceftriaxone), along with azithromycin, is used as combined antimicrobial therapy for the treatment of gonorrhea in most countries around the world [2]. But, World Health Organization (WHO) recommends stopping first-line treatment if failure or non-susceptible isolates are above 5%. Data from 2015 to 2016 showed that 100% of countries reported ciprofloxacin resistance, 80% reported resistance to azithromycin, 45% reported resistance to cefixime, and 24% reported resistance to ceftriaxone. Ceftriaxone resistance was rare in Euro-GASP countries when compared to other regions, while azithromycin resistance was highly prevalent also in the European region, although the spread of multiple drug resistance (MDR) lineages associated with travel was observed [3]. Considering that the percentage of ceftriaxone is lower than the above data, the drug was used as a comparison.

Hygrophila auriculata (Schum.) Heine is indigenous to southern and tropical Africa, as well as south and southeast Asia. In South Africa it occurs in the lowveld of Mpumalanga and Limpopo, and in the coastal flats east of the Lebombo Mountains of KwaZulu-Natal, where the climate conditions are hot and humid most of the year [4]. This plant has properties as an antbiotic, gram-positive bacterial strains have a greater susceptibility to *Hygrophila auriculata* (Schum.) Heine plant extracts than gram-negative bacteria, where the sensitivity is due to the permeability of the peptidoglycan layer, differences in the composition and thickness of the cell wall [5]. However, the antibiotic activity of *Hygrophila auriculata* (Schum.) Heine plant extracts on *N. gonorrhoeae* (gram-negative) bacteria needs to be study through in silico.

PBP2 is an essential peptidoglycan transpeptidase (TPase) that crosslinks the peptide chains from adjacent peptidoglycan strands during cell-wall synthesis [6]. β-lactam antibiotics, including the extended-spectrum cephalosporin (ESC) ceftriaxone, are analogs of the d-Ala-d-Ala C terminus of the peptidoglycan substrate and as such target PBP2 by binding to and reacting with the active-site serine nucleophile (Ser310 in *N. gonorrhoeae* PBP2) to form a covalently acylated complex [7], [8].

Through in silico, more precisely molecular docking. Ceftriaxone obtained from PubChem and *Hygrophila auriculata* (Schum.) Heine plant compound obtained from MPDP 2.0 will be tethered to PBP2 protein. Thus, it will bring out the binding value in the form of the lowest binding affinity between ceftriaxone and compounds from *Hygrophila auriculata* (Schum.) Heine which has the potential as an antibiotic.

Methods

Protein Preparation

Penicillin-binding protein 2 was obtained from https://www.rcsb.org/ PDB ID 6XQV, then prepared with Discovery Studio Visualizer v21.1.0.20298 in pdb format [9]. Protein preparation aims to remove water molecules, ligands, and other molecules. This leaves the PBP2 protein structure as a molecular target for docking with compounds from *Hygrophila auriculata* (Schum.) Heine. This preparation was done using Discovery Studio Visualizer v21.1.0.20298 [10].

Bond Point Prediction

Difference of Gaussian (DoG) Site Scorer https://proteins.plus/ is a grid-based method that uses a DoG filter to detect potential binding pockets [11] solely based on the 3D structure of the protein and splits them into sub pockets. Global properties, describing the size, shape and chemical features of the predicted (sub) pockets are calculated. Per default, a simple drug ability score is provided for each (sub) pocket, based on a linear combination of the three descriptors describing volume, hydrophobicity and enclosure. Furthermore, a subset of meaningful descriptors is incorporated in a support vector machine (libsvm) to predict the (sub) pocket drug ability score (values are between zero and one). The higher the score the more druggable the pocket is estimated to be [12]. The PBP2 protein was input to DoG Site Scorer in pdb file format.

Validation of The Docking Method

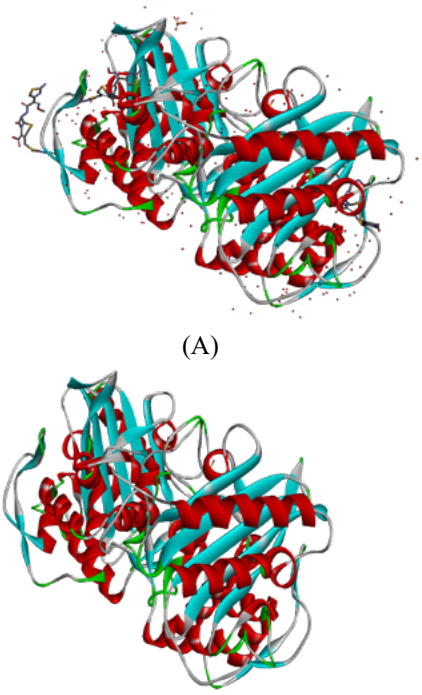
Validation of the docking method against the original ligand was carried out to find the conformation of the original ligand. The docking result conformation obtained is then aligned with the conformation of the original ligand crystallography expressed in root mean square deviation (RMSD) value. The parameter on validation is the RMSD value. Root mean square deviation is the value used to determine whether the prediction of the bonding mode is successful and important for validation of the docking programme. In general, the value of RMSD is said to be good if ≤ 2 Å. The greater the deviation, the greater the error in the prediction of ligand interaction with protein [13]. The grid box in the research used is centre X:0.5931, Y:34.2413, Z:-7.5875 and dimention X:38.4320, Y:37.5138, Z:36.7095.

Docking Protein-Ligand

Virtual molecular screening is used to dock small-molecule libraries to a macromolecule in order to find lead compounds with desired biological function. This in silico method is well known for its application in computer-aided drug design [14]. The test compounds apigenin CID 5280443, luteolin CID 5280445, allagic acid CID 5281855, gallic acid CID 370, quercetin CID 5280343, lupeol CID 259846, lupenone CID 92158, betulin CID 72326, and stigmasterol CID 5280794, comparator compound ceftriaxone CID 447043 were inputed through open babel and energy minimisation was performed and converted to pdbqt. All test compounds were obtained from pubchem [15] and MPDM 2.0 https://www.medicinalplantbd.com/search [16], [17]. Compounds that have the lowest bindig affinity with PBP2 protein are selected compounds (potential as antibiotics in *N. gonorrhoeae*).

Result

Figure 1 below were obtained from a protein data bank and protein preparation was carried out with Biovia Discovery Studio.



(A)

(B)

Figure 1. Three dimensional structure of PBP2 before preparation (A), after preparation (B)

Table 1. Predicted drug score on each bonding site

Sites	Volume A^3	Surface A^2	Drug score
Site 1	761.79	750.14	0.84
Site 2*	1254.87	1595.92	0.8
Site 3	375.28	547.96	0.8
Site 4*	949.43	1030.49	0.79
Site 5	302.28	255.73	0.72

*the site occupied by the compound with the lowest binding

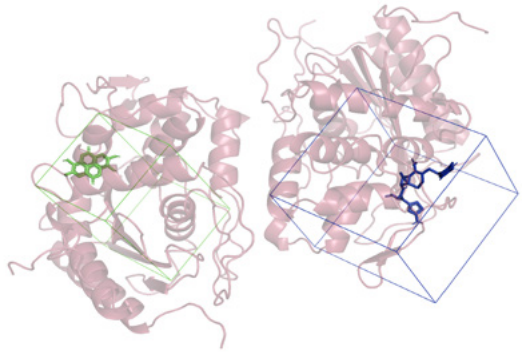


Figure 2. Site 2 (blue box) is occupied by ceftriaxone, while site 4 (green box) by ellagic acid

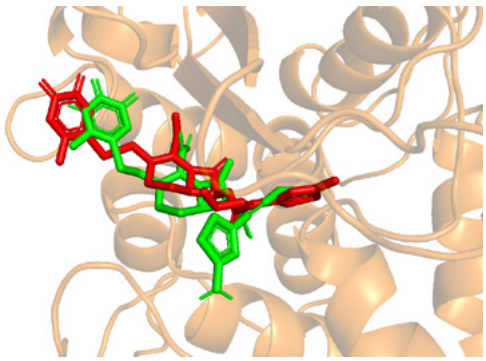
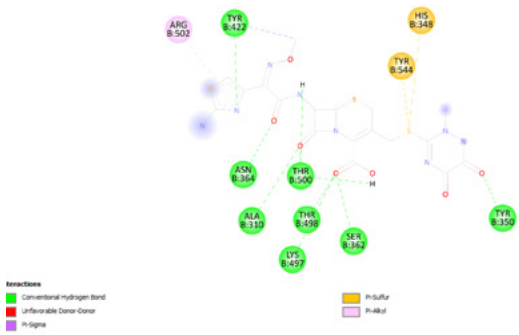


Figure 3. Overlay of ceftriaxone before (red) and after docking (green)

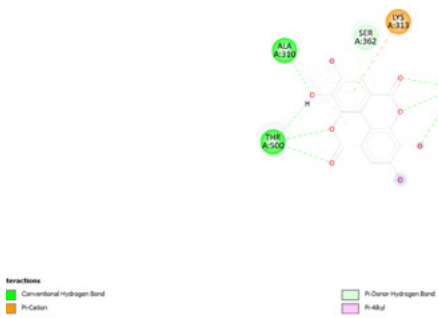
Table 2. The lowest binding affinity from virtual screening of PBP2 with ligand

Ligand	PubChem CID	Binding affinity (kcal/mol)
Ellagic acid*	5281855	-9.8
Ceftriaxone*	-	-9.4
Luteolin	5280445	-8.9
Stigmasterol	5280794	-8.8
Quercetin	5280343	-8.8
Lupeol	259846	-8.7
Lupenone	92158	-8.7
Apigenin	5280443	-8.6
Betulin	72326	-8.3
Gallic acid	370	-6.3

*compounds that have the lowest binding affinity values



(A)



(B)

Figure 4. Protein-ligand interaction, PBP2 protein with ceftriaxone (A) and ellagic acid (B)

Discussion

Types of bacteria can be divided into two groups based on bacterial coloring, namely: gram-negative and gram-negative bacteria [18]. Both have differences in the thickness of the peptidoglycan layer, where gram-positive bacteria have thicker peptidoglycan layers than gram-negative [19], [20].

In this study, **Figure 1** (A) was obtained from a protein data bank and protein preparation was carried out with Biovia Discovery Studio. Protein preparation included; hydrogen atoms were assigned to the heavy atoms of the protein and optimized. Water molecules and cofactors were removed. The preparation was done by adding hydrogen atoms, removing water molecules, and other molecules (**Figure 1** (B)). The addition of hydrogen atoms was performed because due to the limitations of X-ray crystallography and NMR spectroscopy, experimentally derived structures often have problems, such as missing hydrogen atoms, incomplete side chains and loops, ambiguous protonation states, and inverted residues. Therefore, it is crucial to prepare a suitable 3D structure to correct these problems before the docking process [21]. Water molecules have important roles in biological systems and interactions, such as stabilizing protein-ligand complexes, biomolecule recognition, and participating in H-bonding networks. Water molecules can participate in ligand-protein interactions by acting as bridging water, and their displacement from the binding site on ligand binding can also contribute to the binding affinity, which plays an important role in the thermodynamics of protein-ligand binding [22]. Nonetheless, researchers chose to remove water molecules and other molecules to help find the most representative binding pose of the protein and ligand.

DoG Site Score which utilizes pattern recognition pattern recognition techniques to identify active sites. The algorithm is inspired by the fact that active sites often consist of invaginations that are large enough to hold at least at least one heavy atom. We identify these regions by filtering representation of the protein lattice with a 3D DoG filter [23].

Table 1 and **Figure 2** show the bonding sites occupied by the ligand are Sites 2 and Site 4 with drug score values of 0.84 and 0.79, respectively. Site 2 is occupied by ceftriaxone and site 4 is occupied by ellagic acid. This proves that a high drug score value will not necessarily be occupied by the ligand, as in site 1. Validation of docking method before and after docking resulted in RMSD values of 0.000 and 1.312 (see **Figure 3**), the value is below 2 Å, so the docking method is said to be valid. Because, the greater the deviation, the greater the error in predicting the interaction of ligand with protein and reversely.

In virtual screening using PyRx 8.0, ellagic acid (-9.8 kcal/mol) had a lower binding affinity value than comparator ceftriaxone (-9.4 kcal/mol) (**Table 2**). The more negative the binding affinity score is, the more stable the bond complex formed between the protein (receptor) and the compound (ligand) [24]. Amino acid residues in PBP2 interaction with ceftriaxone (A) include; conventional hydrogen bond (green) (TYR B:422 and B:350, THR B:500 and B:598, ASN B:364, ALA B:310, LYS B:497, SER B:362), Pi-sigma (ARG B:502), Pi-sulfur (HIS B:348, TYR B:544). Then ellagic acid (B) include; conventional hydrogen bond (green) (ALA A:310, THR A:500 and 347), Pi-cation (orange) (LYS A:313), and Pi-donor hydrogen bond (cyan) (SER A:362) (**Figure 4**). It is possible that ellagic acid compounds from *Hygrophila*

auriculata (Schum.) Heine plants can interact and interfere with the PBP2 protein in *N. gonorrhoeae*.

Conclusion

Ellagic acid compound in *Hygrophila auriculata* (Schum.) Heine plant has antibacterial activity on *N. gonorrhoeae* by molecular docking and has better binding with PBP2 protein compared to ceftriaxone based on the binding affinity value. In vitro testing should be conducted to confirm the results of this in silico study.

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