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Article

Ligand Based Pharmacophore Modelling, Virtual Screening, Molecular Docking, and ADMETOX of Natural Compounds as Antibiotic Candidates against Urinary Tract Infections (UTI)

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Abstract. The use of antibiotic drugs requires close supervision that patients take antibiotics according to the rules. Irregular antibiotic use led to increased ADR cases (Antibiotic Drug-resistant). ADR is when an individual becomes resistant to an antibiotic drug that cannot kill bacteria. The high number of ADR cases prompted drug discovery to be implemented in analysis for Antibiotic candidates with good effectiveness through the Molecular Docking approach. The search for candidate test compounds as antibiotics were performed using the pharmacophore modelling method and molecular docking. And piperine, withaferin, has some of the same amino acids Ala101, Val103, Glu166, Trp165, and Leu102. Based on the prediction of the promising potential test ligand compound is Corosolic acid. In addition to assessing drug-likeness, pharmacokinetic and toxicity parameters, corosolic acid also has the lowest binding energy among other compounds. Through a textual bioinformatics approach, molecular docking simulations can be used as a first step in the search for new drug candidates in silico by considering various aspects, starting from the physicochemical properties of protein-ligand compounds and the environment. Analysis during the docking process to ADMETOX is an analysis to see the effectiveness and in silico compound safety.

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1. Introduction

Urinary tract infection (UTI) is a bacterial infection affecting the quality of life of human individuals. UTI is one of the most common diseases that is found more often in women than men, with a ratio of 8:1. UTI is the most severe global health problem in the 21st Century, which is the cause of morbidity in outpatients with UTI cases. It is frequently involved in causing nosocomial infections in many hospitals. UTI can infect anyone, both men and women, of all ages, namely adolescents, adults, to old age. The leading causes of UTIs are bacteria, but 80% of UTIs in the hospital are caused by improper catheter installation. Most UTIs are caused by gram-negative aerobic bacilli found in the gastrointestinal tract. Included in this family are Escherichia coli (E. coli), Klebsiella, Enterobacter, Citrobacter, Proteus, and Serratia [1-2].

Antimicrobial therapy is the cornerstone of treatment for any bacterial infection, including UTIs. The type and duration of antimicrobial therapy depend on the site and severity of the infection and host/bacterial factors. Antibiotic therapy in urinary tract infections is used for 7 to 14 days [3-4]. Some examples of the use of antibiotics in the treatment of urinary tract infections are ampicillin, gentamicin, cephalosporins, ceftriaxone, and sulbactam [5]. Therapeutic decisions are taken according to anatomical location and risk factors. Depending on the clinical scenario, the available options are fluoroquinolones and beta-lactams. In the early 2000s, fluoroquinolones became the most prescribed antibiotics in the United States, contributing to the increasing resistance rate of E. coli to this class of antibiotics. The third-generation class of cephalosporins is the most prescribed group in UTIs. The class includes ceftriaxone, cefdinir, cefditoren, cefpodoxime, ceftazidime, ceftizoxime, ceftibuten, cefixime and others. They are broad-spectrum antimicrobial agents with activity against gram-negative and gram-positive organisms [6-7].

Adherence to the use of antibiotics as the first line of therapy for infectious diseases caused by microbes is closely related to cases of antibiotic drug resistance (ADR) which are increasing every year. Based on studies conducted in Ethiopia, most of the bacteria isolated were multidrug-resistant. The recommended antibiotics for the treatment of UTIs are ciprofloxacin (CIP), ceftriaxone (CRO), cefotaxime (CTX), clindamycin (DA), cefuroxime (CXM), and ceftazidime (CAZ) [7-8]. According to WHO, Antibiotics are becoming increasingly ineffective as drug resistance spreads globally, leading to more difficult-to-treat infections and deaths. In addition, the high number of ADR cases will affect a country's national economy and health system because hospitalization will take longer to treat infectious diseases with antibiotic resistance [9].

New antibacterials are urgently needed – for example, to treat gram-negative carbapenems as identified on the WHO list of priority pathogens. However, if people do not change how antibiotics are used, these new antibiotics will suffer the same fate as the current ones and become ineffective. Antimicrobial resistance has become one of the top ten global public health concerns globally. Resistance to antimicrobials is not only hindering the health sector but is also an economic burden for developed and developing countries. The antimicrobial resistance crisis has been ascribed to the misuse of these agents and the unavailability of newer drugs attributable to exigent regulatory requirements and reduced financial inducements[10-11].

Computer-aided drug design (CADD) methodologies are used in drug discovery which is critical in the cost-effective identification of promising drug candidates. These computational methods are relevant in limiting the use of animal models in pharmacological research, assisting the rational design of new and safe drug candidates, and repositioning drugs on the market. Depending on how the pharmacophore features are derived, 3D pharmacophore elucidation methods can be classified as feature-based, substructure pattern-based, or molecular field-based. Feature-based methods derive pharmacophore features by filtering for geometric descriptors that match the characteristics of molecular interactions. Pattern-based methods, such as those implemented in PHASE, LigandScout, and Catalyst, detect substructures for chemical features in molecules [12-13].

Bioinformatics and computational tools offer an in-silico approach to reducing costs and times in the specific field of drug development, limiting the possibilities of fighting more pathologies. To date,

in vitro screening is expensive and time-consuming, and alternatives are highly desirable. Virtual Screening (VS) is a CADD method involving in silico screening of a library of chemical compounds to identify those most likely to bind to a specific target. A compound's biological activity can be evaluated whenever the compound binds with the targeted macromolecule and triggers a specific response. Calculation of the binding capacity of a compound was time-consuming and costly in conventional drug development due to requiring a large-scale in-vitro and in-vivo experiment, in that case, molecular docking approach makes it easier within a short time. Pharmacokinetics and pharmacology properties like absorption, distribution, metabolism, and excretion (ADME) and even toxicity of a compound can predict by using a computer aided drug design process [14-15].

This study aimed to understand the interactions in inhibiting the various proteins listed in the Protein Data Bank (PDB) using molecular docking. The ADMETOX properties of compounds, which indicate their efficacy and toxicity, can be easily predicted using computer-aided methods. Computational approaches are valuable tools to interpret and guide experiments to expedite the antibiotic drug design process. Ligand-based drug design (LBDD) methods focus on known antibiotic ligands for a target to establish a relationship between their physiochemical properties and antibiotic activities, referred to as a structure-activity relationship (SAR), information that can be used for the optimization of known drugs or guide the design of new drugs with improved activity[16-17].

2. Experimental Section

2.1. Materials

The search for candidate test compounds was performed using the Ligand Based Drug Design Pharmacophore modelling method using Liganscout software. The description of the analysis executed is a flowchart in Figure 1.

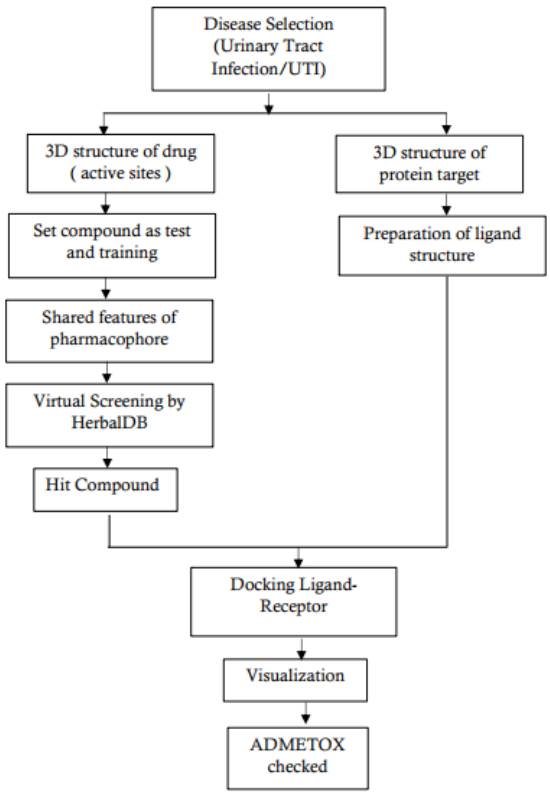


Figure 1. Schematic/flowchart of pharmacophore modelling and molecular docking [18]

2.2. Antibiotic Active Compounds

Analysis of antibiotics used in the treatment of urinary tract infections was fulfilled. The 3D structure of this active compound was obtained from pubchem.org. The following is a list of active antibiotic compounds used in Tabel 1.

Table 1. Antibiotics Compound are used to make pharmacophore models.

No	Antibiotic
1	Amoxicillin
2	Cefixime
3	Imipenem
4	Ciprofloxacin
5	Gentamicin
6	Trimethoprim
7	Ofloxacin
8	Sulbactam
9	Vancomycin
10	Ampicillin
11	Acriflavine
12	Meropenem
13	Tazobactam
14	Cefotaxime
15	Cefadroxil
16	Ceftriaxone

The 16 compounds process test sets, training sets, and decoy sets. The number of test sets and training sets was divided randomly. The decoy set is created by entering compound Chantal smiles into the dude.docking.org web server. The training set compounds are used to make pharmacophore models. The results of the training set obtained ten pharmacophore models and then validated the ROC curve, AUC, specificity, sensitivity, accuracy, and precision. Datasets comprise known operational data and inactive compounds called "decoys." Both active and passive compounds should be selected based on experimental data. The documentation on static data is scarce, and putative inactive compounds are generally used instead. Among the typical metrics used to estimate the performance of virtual screening methods, we find receiver operating characteristics (ROC) curves, the area under the ROC curve (ROC AUC), Enrichment Curves (EC), Enrichment Factors (EF), and predictiveness curves. Of the 10 models, 1 model was selected, which was the best for virtual screening to obtain hit compounds to proceed to the molecular docking stage. All ligand-based pharmacophore modeling processes use Ligan scout software LigandScout4.3 essential advanced molecular design software generated the critical chemical features based on the pharmacophore model[15][19].

2.3 Molecular Docking Method

The research was computationally using protein data bank (pdb) data from 3RXX macromolecules (figure 3) which were downloaded via the rscb.org website, as well as the ligand test data obtained from the results of the ligand-based drug design using the ligandscout software then the test ligand compound data was downloaded from the PubChem website. The method uses molecular docking, a computational approach that is the first step in drug design with the help of specific software. The hardware includes the HP Pavillion laptop with an Intel Core i3- processor. The software consists of Ligand scout 4.4.8, Autodock Vina, Autodock tools, Marvin Sketch, and Ligplot for visualizing results.

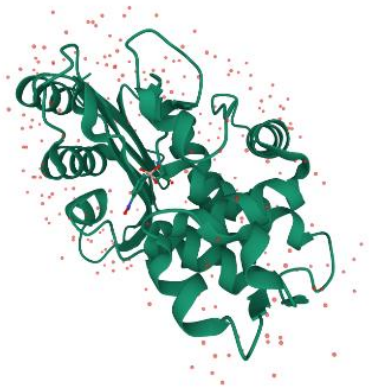


Figure 2. 3D visualization of 3RXX structure
(Source: https://www.rcsb.org/3d-view/3RXX)

Related information includes expression systems, resolution, and quality of ligand structures on the protein databank website.

Figure 3. Summary of the 3RXX structure

2.3.1 Protein and Ligand Preparation

The structure of the 3RXX complex that has been downloaded is then separated from the water molecule and its natural ligands using the Autodock software. This separation was carried out because the presence of water molecules and natural ligands could interfere with the docking process. Water molecules' presence will slow the docking process because more variables need to be resolved. In contrast, natural ligands in proteins can prevent other ligands from binding.

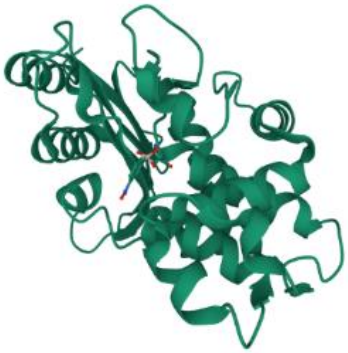


Figure 4. The structure of 3RXX without hydrogen atoms

The preparation of the protein ligands follows the separation of proteins from water molecules and their natural ligands. The trial was performed by adding a polar hydrogen atom, a gesteiger charge on the protein–ligand. In addition, adjustments are made to the use of the grid box at this stage. After analysis, the grid box is 40x40x40. GRID uses an empirical force field to evaluate the probe's energy at each grid point around the target structure and determine the optimal pose at the hot spot (a position that shows a high propensity to be occupied ligand) [20]. Gridbox settings and analysis were operated using the Autodocktool software, which functions as a binding site for proteins and ligands.

2.3.2 Validation

Docking between protein macromolecules is operated in this stage, namely with the 3RXX ligand. The aim is to determine the complex interactions between protein-ligands and to find the deviations that occur by looking at the resulting RMSD parameter values so that the ligand poses resulting from docking will be closer to the crystallographic ligand poses. This analysis uses Autodocktool software.

2.3.3 Redocking

At this stage, redocking is implemented between protein macromolecules and the test ligand compounds obtained from the results of the Ligand scout. Redocking used 37 compounds from scout ligands and 4 from scientific journals. After redocking one by one, 3 test compounds with the best parameter values were taken based on the low-affinity energy values.

2.3.4 Visualization of Results

Visualization of results using Ligplot software. LigPlot was used to study protein–ligand interactions for a given pdb file encrypting the docking. The LigPlot program self-generated schematic 2D representations of the interfaces of protein–ligand complexes from standard pdb file input. The output was an informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic contacts, and atom accessibilities [21]. By using Ligplot we can see the interactions of amino acids and the distance of each hydrogen bond so that it can affect the affinity energy value.

2.3.5 Results Analysis

Data analysis was seen from several parameters reviewed, including affinity energy (ΔG) which can be seen from the docking results of each test ligand against Mpro using Autodocktool inhibition constant (ki), protein-ligand complex, and residue-ligand interaction complex using ligplot software.

3. Results and Discussion

3.1 Pharmacophore Modeling

Pharmacophore models are generated to increase the understanding of ligand–protein interactions. They can identify new molecules that satisfy the pharmacophore requirements and thus are expected to be active. A pharmacophore describes the framework of molecular features that are vital for the biological activity of a compound. The pharmacophore model that performed better in all the validation procedures was considered the best. It was used further as a 3D structural query to search chemical databases like PubChem, ZINC, and DRUGBANK [22-23].

After analysis using the data set (test set, training set) by ligandscout, 10 pharmacophore models were obtained. The validation process was carried out by looking at the ROC, AUC, specificity, and sensitivity values and the number of hits of the best compound. Following are the best curve results used by the virtual screening process. The importance of enrichment factor (EF) and goodness of hit score (GH) refer to the excellent ability of the model to identify active compounds. Results were analyzed in LigandScout by retrieving the receiver operating characteristic curve (ROC) constructed based on the ratio of actives and decoys identified by the pharmacophore model. The area under the curve (ROC-AUC) is used to estimate the detecting power of the model. A pharmacophore model is valid if it exhibits an AUC > 0.5 and will have excellent detective capacity if it is near 1. Early enrichment factors (EF) at 1, 5, 10, and 100 % also reveal the early detection of actives and support model validation [24-25]. The receiver operating characteristic (ROC) curve of the pharmacophore model is shown in figure 5.

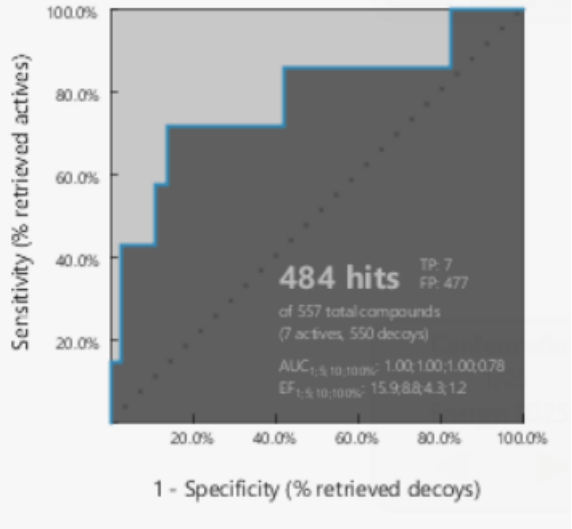


Figure 5. ROC curve model pharmacophore (ligandscout)

The value of the Area shows good curve results Under Curve (AUC) 100% > 0.65 close to 1 (100%) and for the Enrichment factor (EF) 1% above 10. In this result, the AUC value is 0.78%, and the EF value is 15. The lower the Hits value, the better because it shows True Positive (TP) is true Positive and True Negative is negative. After obtaining 1 Pharmacophore model, virtual screening was started to get a list of hits. The following hit compounds were obtained from the virtual screening results with Herbal dB.

Furthermore, protein-ligand docking using the Autodock tool software. AutoDock is a molecular modeling simulation software effective for protein-ligand docking and is among the most accurate docking tools. Moreover, it is open-source software, which makes it publicly available at no charge. On the other hand, AutoDockTools (ADT) is the graphical front-end for setting up and running

AutoDock. AutoDockTool combines accuracy in determining the binding pose of a small-sized chemical in a corresponding receptor pocket and a free, open-source solution available to researchers interested in computational docking [26-27].

3.2 Molecular Docking

3.2.1 Characteristics of The Target Protein 3RXX

Molecular docking is a simulation of proteins and small molecules commonly called protein-ligand interaction by computation procedure. Predicting molecular docking geometry and behaviour of the inner ligand target enzyme binding sites. This technique identifies the correct ligand orientation when bound to proteins and forms a stable complex. Molecular docking can be classified into 3 based on the flexibility of the molecule docking (rigid/rigid), semi-flexible docking (semi-flexible), and flexible docking (flexible). The goal of docking is to achieve conformation optimal protein and ligand. Docking helps study drug/ligand or receptor/protein interactions by identifying the appropriate active site on the protein and getting the best ligand–receptor complex geometry [28-29].

Based on the results of observations of the 3RXX target character from the protein data bank website page, 3RXX binds in a complex with the 3-NPBA ligand. Protein 3 RXX is classified as a hydrolase inhibitor, which can be found in Klebsiella pneumonia organisms. The experimental data results show that the 3RXX analysis resolution is 1.62 Å. The 3RXX protein has a chain A with a sequence length of 264. The binding ligand is 3-NPBA (3-Nitrophenylnoronic acid).

3.2.2 Gridbox Docking Validation

Grid box docking validation was implemented in 3 sizes, namely 40x40x40; 50x50x50; 60x60x60 using autodocktool software. A fair good value for RMSD is < 2 A. RMSD is routinely Utilized to measure the quality of reproduction of a known binding pose by comparing the obtained ligand pose with the experimental crystallographic source [30-31]. The best results are obtained from a grid box size of 40x40x40 which has an RMSD (Root Mean Square Deviation) value of 2.37 with the following results

Table 2. Result of Grid box size 40x40x40 included RMSD values

Rank	Sub-Rank	Run	Binding energy (kcal/mol)	Cluster RMSD	Reference RMSD
1	1	1	-5.12	0.00	6.36 A
2	1	6	-4.97	0.00	3.02 A
2	2	10	-4.97	0.32	3.05 A
2	3	3	-4.86	0.94	3.05 A
2	4	8	-4.82	1.14	3.05 A
2	5	4	-4.81	1.29	2.84 A
2	6	9	-4.79	0.91	3.08 A
2	7	5	-4.73	1.21	3.09 A
2	8	7	-4.73	1.22	3.10 A
2	9	2	-4.71	1.67	2.37 A
Grid Center	-5.12 kcal/mol				
X=	-7.985				
Y=	1.717				
Z=	-3.554				
RMSD	2.37				
Inhibition Constan	176.24 micromolar				

3.2.3 Molecular Docking of Test Compounds

The docking process on the test Ligand compound was obtained from the results of the pharmacophore modeling with ligand scout. The docking process was performed using 37 test compounds. From these results, the best 4 compounds are used.

Table 3. The best 4 of test compound results

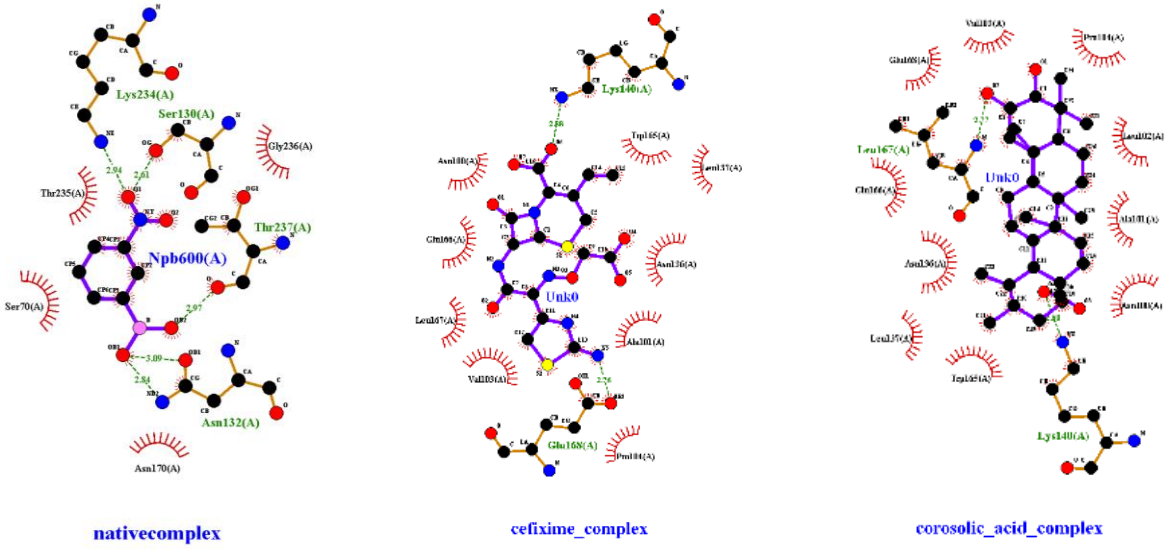
No	Compound	Binding Energy	Inhibition Constant
1	Native	-4.73 kcal/mol	341.26 micromolar
2	Cefixime (positive control)	-4.45 kcal/mol	549.90 micromolar
3	Corosolic acid	-7.12 kcal/mol	6.02 micromolar
4	Piperin	-6.49 kcal/mol	17.48 micromolar
5	Sanguinarine	-6.36 kcal/mol	21.70 micromolar
6	Withaferin	-5.11 kcal/mol	179.53 micromolar

The binding energy value is an essential consideration in selecting the test compound. The smaller the binding energy value. the better the prediction of the combination. Docking verification was performed based on the RSMD value of lowest interaction energy between backbone atoms and active site. Of the 37 tested compounds. corosolic acid. piperine. and sanguinarine had low binding energy with binding energy values of -7.12 -kcal/mol. -6.49 kcal/mol. and -6.36 kcal/mol. Bonding Affinity determines the strength of the ligand-receptor interaction. More negative the binding affinity. the stronger the ligand-receptor interaction and the better the prediction of molecular docking [28][32].

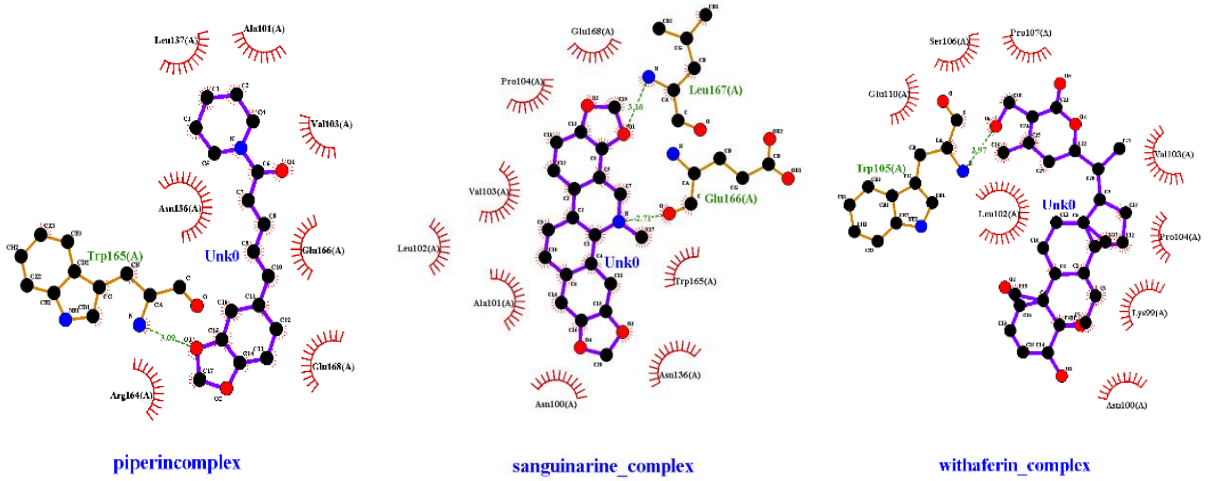
3.2.4 Amino Acid Components

Analysis of the amino acid components of each compound to see types and variations contained in each tested compound. The compounds analyzed for their amino acids were native ligand. Cefixime (positive control). corosolic acid. piperine. sanguinarine. and withaferin. Cefixime is a cephalosporin class of antibiotics. It has good efficacy in treating UTIs but also showed its safety and tolerability in the majority of the patient; therefore can be used as an alternative drug for treating UTIs [33-34].

3.2.5 Visualization



(a) (b) (c)



(d) (e) (f)

Figure 6. 2D visualization docking results (a) Native Ligand, (b) Positive Control (Cefixime), (c) corosolic Acid, (d) Piperin, (e) Sanguinarine, (f) Withaferin

2D visualization using ligplot simplifies the observation of amino acids successfully formed from molecular bonding[35]. Visualization of the results shows that the amino acids in the native ligand are Lys234, Ser 130, Thr237, Asn132, Ser70, and Thr235. In Cefixime which is a positive control namely the amino acids Lys140 and Glu168. Corosolic acid contains Lys140 and Lau167. Piperine compounds show the amino acid Trp165, and sanguinarine are Leu167, Glu166, and withaferin Trp105. The results of the visualization of the compound that has the similarity of amino acids to the positive control are Lys140.

3.2.6 ADMETOX

ADMETOX (Absorption, Distribution, Metabolism, Excretion, Toxicity) prediction was fulfilled for the pharmacokinetic properties of the tested compounds. The ADMETOX profile of bioactive compounds can affect their effectiveness and safety. In addition, efficacy and safety are considered the leading causes of clinical failure in developing new chemical compounds. So it is necessary to carry out ADMETOX predictions to provide complete information on the results of the dosing analysis of a compound.

A suitable test compound is a compound that meets the Lipinski rule of 5 criteria consisting of Lipiniski stating that most molecules have log P $\leq$ 5, molecular weight $\leq$ 500, The number of hydrogens bond acceptors $\leq$ 10, and the number of hydrogen bond donors $\leq$ 5. The rule is called the "Rule of 5" because the limit values are 5, 500, 2×5, and 5. Molecules that break more of these regimens may have problems with bioavailability. Rule of five (ROF) is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a specific pharmacological or biological activity has properties that would make it a likely orally active drug in humans [36][37]. Three tested ligand compounds. corosolic acid. piperine. and sanguinarine. Meet the requirements of the Linpinski rule. and all compounds fulfill the Linpinski character. so they have good drug-likeness. Pharmacokinetic analysis. including CYP inhibitors. piperine compounds were found to interact with CYP as inhibitors.

Table 4. ADMETOX prediction of bioactive compounds by Druglikeness

Ligands	Druglikeness				
	MW g/mol	HBA	HBD	logP	GI Abs
Corosolic Acid	472.70	4	3	High	3.39
Sanguinarine	332.33	4	0	High	-0.04
Piperine	285.34	3	0	High	3.38
Withaferin	470.60	6	2	High	3.29

Table 5. ADMETOX prediction of bioactive compounds by Pharmacokinetics

Ligands	Pharmacokinetics							
	Inhibitor CYP					Toxicities		
	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4	AM ES	Carsino-genesis	AOT
Corosolic Acid	No	No	No	No	No	-	-	III
Sanguinarine	Yes	Yes	No	No	No	+	-	II
Piperine	Yes	Yes	Yes	No	No	-	-	III
Withaferin	No	No	No	No	No	-	-	I

Table 6. ADMETOX prediction of bioactive compounds by Toxicities

Ligands	Toxicities		
	AM ES	Carsino-genesis	AOT
Corosolic Acid	-	-	III
Sanguinarine	+	-	II
Piperine	-	-	III
Withaferin	-	-	I

Sanguinarine has a poor toxicity value due to a positive AMES value and acute oral toxicity class 2. The Ames test was developed by Dr. Bruce Ames and colleagues in the 1970s and reviewed by Maron and Ames (1983). It is a test performed in vitro in the short term to evaluate possible mutagenic effects caused by chemicals[38]. Withaferin has AOT in grup 2. AOT is Acute oral toxicity data used to satisfy hazard classification and labeling requirements for risk assessment for human health and the environment and when estimating the toxicity of mixtures. To evaluate and optimize the action and efficiency of a bioactive compound. it is necessary to know its pharmacokinetics. Since most bioactive substances are not administered intravenously. the pharmacokinetic predictor that may indicate intestinal absorption level is the jejunum's effective human permeability. Molecules with higher lipophilicity have better permeability through the phospholipid bilayer of enterocytes. so the level of permeability is directly conditioned by the lipophilicity of the molecules[37][39-40].

4. Conclusion

Based on the visualization results of the components of the amino acids Corosolic Acid. Sanguinarine. and piperine. withaferin has the same amino acids Ala101. Val103. Glu166. Trp165. Leu102. Druglikeness analysis. all test compounds met the requirements of the rule of Lipinski of 5. From the results of ADMETOX. sanguinarine has the ability for AMES toxicity. and withaferin AOT is in group 2Therefore. the prediction of the promising potential test ligand compound is Corosoloic acid. In addition to assessing drug-likeness, pharmacokinetic, and toxicity parameters. Corosolic acid has the lowest binding energy value among other compounds. Corosolic acid is usually extracted from the banaba (*Lagerstroemia speciosa*) leaf. It exhibits antihyperlipidemic, antioxidant, anti-inflammatory, antiviral, antineoplastic, osteoblastic, and protein kinase C inhibition activity.

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