Jurnal Biodjati 9(1): 26-40, May 2024

e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

In Silico Studies of Bioactive Compounds from *Pseudomonas azotoformans* UICC B-91 in Inhibiting *Candida albicans*

Rina Hidayati Pratiwi[*1], Endang Sulistyaniningsih[2], Jumadi Jepri[3]

Received: December 22, 2023
Revise from: February 05, 2024
Accepted: April 05, 2024

DOI: 10.15575/biodjati.v9i1.32147

[1]Department of Mathematics and Natural Sciences Education, Faculty of Post Graduated, Universitas Indraprasta PGRI, South Jakarta 12530, Indonesia. [1]Department of Biological Education, Faculty of Mathematics and Natural Sciences, Universitas Indraprasta PGRI, East Jakarta 13760, Indonesia. [2,3]Department of Informatic Engineering, Faculty of Engineering and Computer Science, Universitas Indraprasta PGRI, East Jakarta 13760, Indonesia

e-mail:
[1]rina.hp2012@gmail.com*
[2]esulistyaniningsih@gmail.com
[3]jumadi.jepri@gmail.com

**Corresponding author*

Abstract. *Futoamide, Gentialutine, Gentiabetine, 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine, Lycopodine, Dihydro-lycopodine are bioactive compounds that can be obtained Pseudomonas azotoformans UICC B-91. Previous research results indicate that P. azotoformans extracted with dichloromethane, chloroform, and ethyl acetate have anticandidal activity against Candida albicans ATCC 10231. C. albicans is considered to become an opportunistic pathogen and well-known as the main cause of candidiasis. This study aims to determine the mechanism of inhibition of bioactive compounds from P. azotoformans UICC B-91 on the growth of C. albicans using the molecular docking method. Docking was carried out using the targeted (Lanosterol 14 alpha demethylase and Glucan endo-1,3-beta-D-glucosidase) docking method with an exhausted parameter of 50. The size of the gridbox was adjusted to the position of the amino acid residues based on predictions of binding sites using PrankWeb. The docking results were obtained in the form of binding affinity resulting from the interaction of the compound with the protein. Results showed that the three sample compounds had the potential to form strong and stable bonds with both protein targets with only two ligands show a binding energy value of less than -7 kcal/mol. In addition, the speed and stability of the bond between the sample and the target protein cannot exceed control ligands, thus it can be predicted the structure of ligand. Futoamide, 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine, Lycopodine, and Dihydrolycopodine had binding potential with Lanosterol 14 alpha demethylase. For Glucan endo-1,3-beta-D-glucosidase, only Futoamide had the potential to form stable and strong bonds that similar to the control. It can be concluded that the futoamide, one of ligand from P. azotoformans compounds has the potential as a multitarget inhibitor of the two C. albicans proteins, because it has various affinities and interaction stability for Lanosterol 14 alpha demethylase and Glucan endo-1,3-beta-D-glucosidase.*

Keywords: *Candida, glucosidase, lanosterol, molecular docking, Pseudomonas azotoformans.*

Citation
Pratiwi, R. H., Sulistyaniningsih, E., & Jepri, J. (2024). In Silico Studies of Bioactive Compounds from *Pseudomonas azotoformans* UICC B-91 in Inhibiting *Candida albicans*. *Jurnal Biodjati*, 9(1), 26-40.

Copyright © 2024 Jurnal Biodjati
This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Jurnal Biodjati 9(1):26-40, May 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

INTRODUCTION

Candida albicans is a fungal species that can cause disease in humans. This fungus is a commensal organism that can be naturally found in the gastrointestinal tract, urinary tract, oral mucosa, vagina, and on the surface of skin. The fungus is one of normal flora because plays a role in the balance of microorganisms in the body. However, *C. albicans* is considered to become an opportunistic pathogen because if the balance between host defense systems, microbiota, and *C. albicans* is disturbed, *C. albicans* can cause both local and disseminated infections. It well-known as the main cause of candidiasis (Caetano et al., 2023; Lemberg et al., 2022; Talapko et al., 2021).

Candidiasis is an acute or subacute disease caused by *C. albicans* or sometimes by other species that can attack various body tissues (Guntur et al., 2014). Candidiasis is commonly found in the axillary region, thigh crease, inter-breast notch, and intergluteal, as well as between the fingers and umbilicus. In the United States, as many as 75% of women in the reproductive period have endured vulvavaginous candidiasis. Between 40-50% have recurrent infections while 5-8% suffer from chronic Candida infections. Candida infection often results in fatal complications in cases of organ transplant. In London, 40.5% of patient suffered from fungal infections after liver transplantation with 90% of the cases caused by *Candida* spp. and 66% specifically by *C. albicans*. Among 345 cases of Candidemia studied in a hospital in Spain, the mortality rate was 44%, of which 51% were caused by *C. albicans* infection. Meanwhile, in Germany, the mortality rate due to necrosectomy followed by fungal infections including Candida, reached 62%. In Indonesia, it was recorded that of, 84% of candidiasis occurred inpatients with AIDS and some also had diabetes mellitus (Getas et al., 2014). Laboratory diagnosis and treatment of diseases caused by *Candida* sp. especially *Candida albicans* have not given satisfactory results. Resistance to antifungals is also common (Kusumaningtyas et al., 2008).

It is worth mentioning that worldwide, many people favor herbal medicine over synthetic medicine. Research on the antibacterial properties of herbal plants has been prompted due to the acceptance of traditional medicine as an alternative form of healthcare and the emergence of microbial resistance to existing antibiotics (Eve et al., 2020). The present study found that a total of 125 isolates were isolated from surface-sterilized root and stem tissues of the endangered medicinal plant, *F. sinkiangensis* (Liu et al., 2017). These results also provide a preliminary framework for exploring endophytic bacteria associated with endangered medicinal plant as potential bioindicator for antimicrobial.

Neesia altissima Bl. is a large tree (grows up to ± 40 m) and distributed primarily in the rainforest of Malaysia and Indonesia (Sumatera, Borneo, and Java islands). In Indonesia, this endangered endemic plant is used medically for treatment of gonorrhea, diuretic, and diarrhea (Rahayu et al., 2002). Although having such an important medicinal value, studies on microbial endophytes from *N. altissima* in relation to discovery of alternative secondary metabolites are lacking. Based on phylogenetic analysis of nucleotide sequence generated from 16S rRNA region, two endophytic bacteria isolates isolated from *N. altissima* determined as P. aeruginosa and one isolate belongs to *P. azotoformans* (Pratiwi et al., 2016). *Pseudomonas azotoformans* UICC B-91 when extracted with dichloromethane, chloroform, and ethyl acetate exhibits antimicrobial activity against *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6583, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 25241, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 19659, and *Candida albicans* ATCC 10231 (Oktarina et al., 2021). Previous fractionation and identification of the extract by thin-layer chromatography (TLC) and liquid chromatography-mass spectrophotometry (LCMS-MS) detected several compounds of

P. azotoformans UICC B-91, including futoamide ($C_{18}H_{23}NO_3$), gentialutine ($C_9H_{11}NO$), gentiabetine ($C_9H_{11}NO_2$),1-[(2E,4E)-2,4-decadienoyl]pyrrolidine ($C_{14}H_{23}NO$), lycopodine ($C_{16}H_{25}NO$) and dihydrolycopodine ($C_{16}H_{27}NO$) (Pratiwi et al., 2022).

So far *P. azotoformans* is known for its benefits in agriculture as a soil fertility enhancer. It act as biocontrol agents because it has been identified as inhibiting *Fusarium solani* growth, causing chitin degradation, producing siderophores and solubilizing phosphate in vitro (Banerjee et al., 2020). Therefore, it is important to explore the anticandidal mechanisms from bioactive compound of endophytic bacteria, *P. azotoformans* UICC B-91.

The discovery of new drugs recently relies on in silico study, specifically molecular docking, to simplify the overall process. However, computer-aided drug design (CADD) could save time as well as the cost of synthesis of molecules and would ultimately curtail the cost of research (Abdullahi & Adeniji, 2020). In molecular modeling, sequence analysis platforms, and clinical training management, molecular docking has been identified as a useful technique (Ekins et al., 2007). In silico molecular docking is one such CADD technique that would virtually predict the binding efficacy as well as the structure-based drug design (Abdullahi & Adeniji, 2020). Moreover, the molecular docking provides successful insights into the structure-activity relationships, mode of activity, and further analysis from protein-ligand interaction (Abdullahi et al., 2020). Such studies would culminate in the development of novel drug molecules at a faster pace against infectious pathogens. Additionally, the physicochemical properties of the molecule would provide vital information on the initial phase of drug development (Abdullahi & Adeniji, 2020; Abdullahi et al., 2020).

This study aims to determine the mechanism of inhibition of bioactive compounds from P. azotoformans UICC B-91 on the growth of C. albicans in silico using the molecular docking method with the protein targets are Lanosterol 14 alpha demethylase and Glucan endo-1,3-beta-D-glucosidase. Lanosterol 14 α-demethylase is a part of the cytochrome P450 superfamily. This heme thiolate enzyme transforms lanosterol to 4,4'-dimethyl cholesta-8,14,24-triene-3-beta-ol (Bard et al., 1993). Azole medicines action is attributed to the coordinated binding of the heterocyclic nitrogen atom (N-3 in imidazole and N-4 in triazole) to the heme iron atom in the CYP51 enzyme binding site. Inhibition of CYP51 and ergosterol depletion, together with the built up of 14 methyl sterols, can cause fungal growth reduction (Ji et al., 2000). The importance of CYP51 in fungus metabolism makes it a potential target for antifungal medication development (Lamb et al., 1999). Numerous classes of drugs have been designed to target the ergosterol biosynthesis pathway (Aoyama et al., 1989). Meanwhile, glucan 1,3-beta-glucosidase involved in cell wall biosynthesis and virulence of candida. It was very crucial for delivery of beta-1,3-glucan to the biofilm matrix and for accumulation of mature matrix biomass. The enzyme has played a role as a major antigen in human systemic candidiasis patients (Mitchell et al., 2013).

Previous in vitro research on the antimicrobial activity of bioactive compounds from *P. azotoformans* UICC B-91 has demonstrated that these bioactive compounds can inhibit the growth of *C. albicans*. The current in silico research aims to investigate the anticandida mechanism of the bioactive compounds from *P. azotoformans* UICC B-91. It seeks to determine whether these bioactive compounds bind to either lanosterol 14 alpha-demethylase or endo-1,3-beta-D-glucosidase proteins of *C. albicans* through molecular docking. Understanding the binding preferences is crucial for developing targeted antifungal therapies and elucidating the mechanisms of action of existing antifungal drugs (Sheng et al., 2004; Guan et al., 2010). Understanding the difference between the two is crucial for developing targeted antifungal therapy and explaining the mechanisms of action of existing antifungal drugs.

Jurnal Biodjati 9(1):26-40, May 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

MATERIALS AND METHODS

Sample Preparation

SMILEs (simplified molecular-input line-entry system) and the 3D structure of the ligand to be analyzed were obtained from the PubChem database(https://pubchem.ncbi.nlm.nih.gov/) (Table 1). SMILEs is a unique code that describes the structure and profile of the ligand being analyzed. The selected bioactive compound (Table 1) based on previous fractionation and identification of the extract by thin-layer chromatography (TLC) and liquid chromatography-mass spectrophotometry (LCMS-MS). They detected several compounds of *P. azotoformans* UICC B-91, including futoamide ($C_{18}H_{23}NO_3$), gentialutine ($C_9H_{11}NO$), gentiabetine ($C_9H_{11}NO_2$), 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine ($C_{14}H_{23}NO$), lycopodine ($C_{16}H_{25}NO$) and dihydrolycopodine ($C_{16}H_{27}NO$) (Pratiwi et al., 2022).

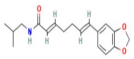
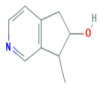
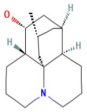
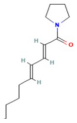
Bioactivity Prediction with SAR

The SMILEs obtained from PubChem were then analyzed using the Structure Analysis Relationship approach to predict their potential as antifungals. SAR prediction was performed using the Way2Drug webserver (http://www.way2drug.com/passonline/). After accessing to PASS Online service, to obtain the predicted biological activity profile for the compound, only structural formula is necessary. Thus, prediction is possible even for virtual structure designed in computer but not synthesized yet.

Analysis using the SAR approach showed that all compounds possess potential as antifungals. SAR is an approach that compares the profile of the input compound with the database. The more similar the structures, the greater confidence in the resulting value (Yang et al., 2022). The parameter employed is Pa Score Prediction with a value range of 0-1. If the prediction score has a value > 0.7 then the prediction is considered strong, because the profile similarity between the input compound and the database is high (Filimonov et al., 2014).The SMILEs obtained from PubChem were then analyzed using the Structure Analysis Relationship approach to predict their potential as antifungals. SAR prediction was performed using the Way2Drug webserver (http://www.way2drug.com/passonline/). After accessing to PASS Online service, to obtain the predicted biological activity profile for the compound, only structural formula is necessary. Thus, prediction is possible even for virtual structure designed in computer but not synthesized yet.

Analysis using the SAR approach showed that all compounds possess potential as antifungals. SAR is an approach that compares the profile of the input compound with the database. The more similar the structures, the greater confidence in the resulting value (Yang et al., 2022). The parameter employed is Pa Score Prediction with a value range of 0-1. If the prediction score has a value > 0.7 then the prediction is considered strong, because the profile similarity between the input compound and the database is high (Filimonov et al., 2014).

Table 1. Bioactive compound profile

Compound	ID	Structure
(E,E)-Futoamide	15596445	
Gentialutine	5315179	
Lycopodine	5462445	
Dihydrolycopodine	387102991	
1-[(2E,4E)-2,4-deca-dienoyl]pyrrolidine	6440616	
Gentiatibetine	5317559	

Molecular Docking

The 3D structure of the selected target protein was obtained from the RSCB PDB database (https://www.rcsb.org/), namely Lanosterol 14 alpha demethylase (PDB ID: 5TZ1). Meanwhile, the structure of Glucan endo-1,3-beta-D-glucosidase/GG was obtained from the modeling results using alphafold with ID AF-P34742-F1-model_v4. Furthermore, the 3D structure of each ligand was retrieved from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov). The proteins were pretreated by removing water molecules in Discovery Studio 2019 software, while the ligand was energy minimized using Pyrx v.0.9.8 software. Docking was performed using Autodock Vina integrated into Pyrx v.0.9.8 (Trott & Olson, 2010). The size of the grid box was adjusted to the position of amino acid residues based on the prediction of binding sites using PrankWeb (https://prankweb.cz/) (Table 2) (Jakubec et al., 2022).

According to table 2, lanosterol 14 alpha demethylase have probability: 0.984, center coordinates (x, y, z): (68.8143, 67.9798, 3.5902), dimension (x, y, z): (25, 25, 25). This suggests that for Lanosterol 14 alpha demethylase, there's a high probability (0.984) of the ligand binding. The center of the binding box is at coordinates (68.8143, 67.9798, 3.5902) with dimensions of 25 Å x 25 Å x 25 Å. Meanwhile, glucan endo-1,3-beta-D-glucosidase have probability: 0.853, center coordinates (x, y, z): (7.9551, -1.6066, 2.5403), dimension (x, y, z): (35, 35, 35). For glucan endo-1,3-beta-D-glucosidase, the probability is slightly lower (0.853) was compared to the first protein. The center of the binding box was at coordinates (7.9551, -1.6066, 2.5403), and the dimensions of the box were larger, with each side being 35 Å. In summary, this analysis provides insight into the predicted binding affinities and spatial requirements for ligand binding to the specified proteins. The data can help guide further experimental or computational studies related to drug discovery or protein-ligand interactions.

Docking results were obtained in the form of binding affinity or affinity energy as a result of compound interaction with the protein. Furthermore, the interaction between the compound and protein docking results was visualized using BioVia Discovery Studio 2019 software.

Table 2. Gridbox of molecular docking analysis

Protein	probability	center_x	center_y	center_z	dimension_x	dimension_y	dimension_z
Lanosterol 14 alpha demethylase	0,984	68,8143	67,9798	3,5902	25	25	25
Glucan endo-1,3-beta-D-glucosidase	0,853	7,9551	-1,6066	2,5403	35	35	35

Jurnal Biodjati 9(1):26-40, May 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

RESULTS AND DISCUSSION

SAR Analysis

Figure 1 shows the high potential of compounds as Glucan endo-1,3-beta-D-glucosidase inhibitors because the score prediction of its compound is 0.444. It is the highest score of *P. azotoformans* compounds. The parameter utilized is the Pa Score Prediction with a value range of 0 to 1. If the prediction score exceeds 0.7, the prediction is deemed strong, as it indicates a high similarity profile between the input compound and the database (Filimonov et al., 2014). The more similar the structures, the higher the confidence in the resulting value.

Glucan endo-1,3-beta-D-glucosidase (Table 2) is a *C. albicans* protein that plays a role in cell wall biosynthesis, acts as a virulence factor, and serves as a primary antigen in candidiasis. This protein plays a crucial role delivering beta-1,3-glucan to the biofilm matrix and in the accumulation of mature biofilm (Uniprot ID Q5AMT2). This enzyme is involved in the degradation of beta-glucans, which are polysaccharides found in the cell walls of fungi, bacteria, and plants. Glucan endo-1,3-beta-D-glucosidase specifically hydrolyzes the beta-1,3-glucosidic linkages within beta-glucans, contributing to the remodeling and turnover of fungal cell walls. This enzyme may be found associated with the cell membrane or secreted into the extracellular space, where it acts on beta-glucans within the fungal cell wall. Additionally, an essential part of the extracellular matrix that maintaining the biofilm structure are β-1,3-glucans, which significantly contribute to the biofilm's resistance to antifungal drugs because they prevent contact with target cells (Li et al., 2020; Karygianni et al., 2020). Due to the importance of glucan endo-1,3-beta-D-glucosidase to the integrity of *C. albicans*, glucan endo-1,3-beta-D-glucosidase is widely targeted to obtain antifungal drugs.

Meanwhile the compound *P. azotoformans* has low potential as an inhibitor of lanosterol 14 alpha demethylase (Figure 1). Lanosterol 14 alpha demethylase (Table 2) is an enzyme involved in the biosynthesis of ergosterol, a crucial component of fungal cell membranes. It catalyzes the demethylation of lanosterol, an intermediate in the biosynthesis *P. azotoformans* compound pathway, to form ergosterol. In fungi, ergosterol plays a role similar to cholesterol in mammalian cells, contributing to the stability and integrity of the cell membrane. Disruption of lanosterol 14 alpha demethylase activity can lead to the accumulation of toxic sterol intermediates and impaired membrane function, ultimately inhibiting fungal growth (Monk et al., 2020). Lanosterol 14 alpha demethylase only have 0.048 in the score prediction of *P. azotoformans* compound content as antifungal. The score prediction for lanosterol 14 alpha demethylase inhibitor is lower than glucan endo-1,3-beta-D-glucosidase inhibitor.

Gentialutine is the compound with the highest relative prediction score (derived from the average of all prediction scores) (Figure 2). Meanwhile, (E,E)-Futoamide is the compound with the lowest relative prediction score. Binding energy indicates the amount of energy required to form a bond between a ligand and a receptor. A smaller binding energy signifies a more stable bond. The greater the stability of the ligand-receptor bond, the greater its predicted activity (Kesuma et al., 2018).

Each value in Table 3 represents the predicted bioactivity of the respective compounds against the specified targets, with higher values generally indicating stronger activity. For instance, Lycopodine exhibits a higher value in the "Antifungal" column, suggesting a potentially greater antifungal efficacy compared to other compounds. As for (E,E)-Futoamide, it is hypothesized to possess potential as a glucan endo-1,3-beta-D-glucosidase inhibitor, lanosterol 14 alpha-demethylase inhibitor, sterol 24-C-methyltransferase inhibitor, cell wall biosynthesis inhibitor, and peptidoglycan glycosyltransferase inhibitor due to its zero value. Conversely, non-zero values in other columns indicate potential inhibitory effects on the respective enzymes or cellular processes.

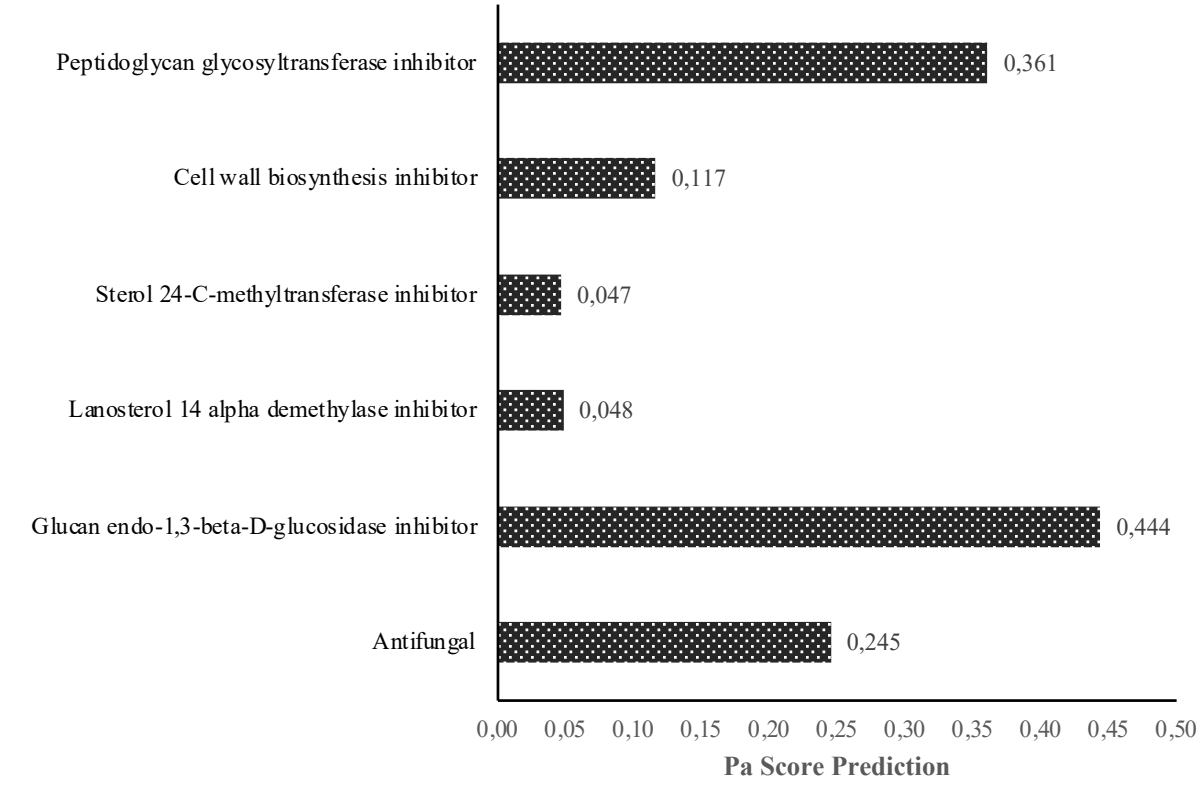


Figure 1. Pa score prediction of *Pseudomonas azotoformans* compound content as antifungal

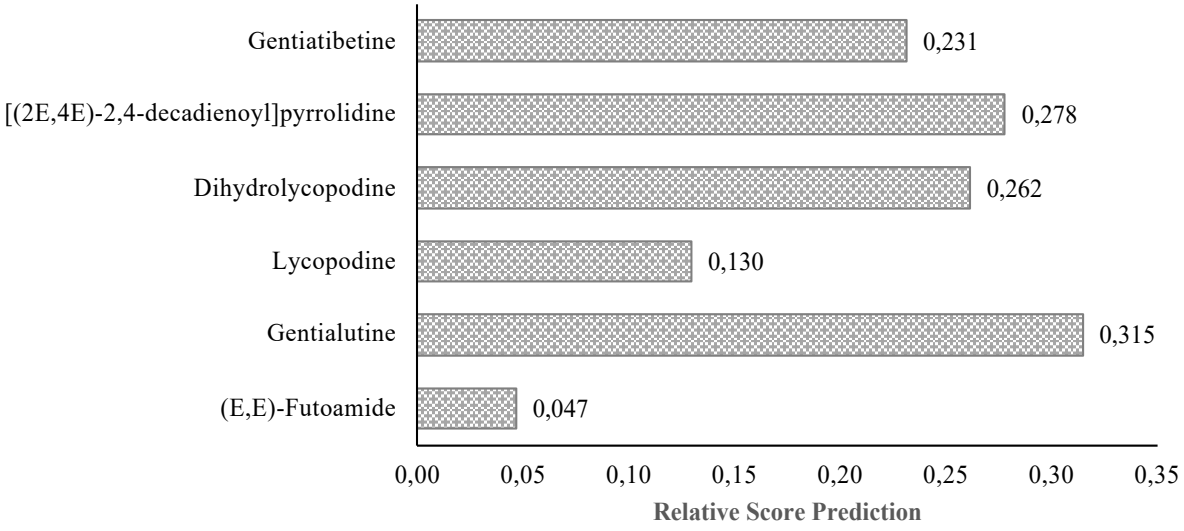


Figure 2. Relative score prediction of each *Pseudomonas azotoformans* compound as antifungal

Table 3. Predicted bioactive potential of *Pseudomonas azotoformans*

Compound	ID	Anti-fungal	Glucan endo-1,3-beta-D-glucosidase inhibitor	Lanosterol 14 alpha demethylase inhibitor	Sterol 24-C-methyltransferase inhibitor	Cell wall biosynthesis inhibitor	Peptidoglycan glycosyltransferase inhibitor
(E,E)-Futoamide	15596445	-0.28	0	0	0	0	0
Gentialutine	5315179	-0.342	-0.545	-0.121	-0.143	-0.277	-0.461
Lycopodine	5462445	0	-0.355	0	0	0	-0.423
Dihydrolycopodine	3.87E+08	-0.277	-0.56	0	-0.138	0	-0.594
1-[(2E,4E)-2,4-decadienoyl] pyrrolidine	6440616	-0.378	-0.627	-0.169	0	-0.101	-0.393
Gentiatibetine	5317559	-0.195	-0.577	0	0	-0.321	-0.295

Docking Analysis

The results of molecular docking show that not all sample compounds have the potential to form strong and stable bonds with both target proteins (Table 4). It is because only a few ligands show binding energies smaller than -7 kcal/mol (Trott & Olson, 2010). A binding energy smaller than -7 kcal/mol generally indicates a strong binding affinity between the ligand and the protein target. If the mean binding energies are relatively low (close to or below -7 kcal/mol) and a significant proportion of ligands have binding energies below -7 kcal/mol, it suggests that the compounds have the potential for strong and stable interactions with the proteins. In addition, the binding strength and stability between the samples and the target proteins do not surpass any control ligands of each target protein. Thus, it can be predicted that Futoamide, 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine, Lycopodine, and Dihydrolycopodine compounds have the potential to bind with Lanosterol 14 alpha demethylase protein. Meanwhile, for the Glucan endo-1,3-beta-D-glucosidase protein, Futoamide is the only potential compound to form a stable and strong bond.

Residues in bold fonts represent active site amino acid residues of the control retained by the sample with the same type of binding. While the italicized font indicates active site amino acid residues retained by the sample, but changes its bond type. Furthermore, since the Glucan endo-1,3-beta-D-glucosidase protein is the modeling result, it does not yet have native ligands, so its active site is predicted using PrankWeb. Amino acid residues that are retained according to PrankWeb prediction results are marked in red (Appendix 1).

On average, the sample compound ligands retain 2-3 amino acid residues of Oteseconazole (control) (Hargrove et al., 2017), especially TYR64, PHE233, and PRO230. However, in line with the binding affinity results, Futoamide has the most potential because it not only retains bonds of the hydrophobic type but also conventional hydrogen type at residues TYR64 and TYR132. In addition, the Gentialutine sample ligand is predicted to have the weakest potency ligand as it forms unfavorable binding that can decrease the stability of the bond due to bindings formed in inappropriate locations. The result of amino acid residue binding analysis to the Glucan endo-1,3-beta-D-glucosidase protein showed that although the sample ligand have much lower binding affinities compare to the antifungal FK-463 control (Carbery et al., 2024), its binding accuracy was better than the control when compared with the predicted amino acid residues from PrankWeb which enabling users to easily carry out the prediction and visually inspect the predicted binding sites via an integrated sequence-structure view (Jendele et al., 2019).

Table 4. Binding affinity between target proteins with compound and control ligands

Protein	Ligand	Binding Affinity (kcal/mol)
Lanosterol 14 alpha demethylase	Oteseconazole (Control)	-10,5
	Futoamide	-8,8
	1-[(2E,4E)-2,4-decadienoyl] pyrrolidine	-7,6
	Lycopodine	-7,6
	Dihydrolycopodine	-7,3
	Gentialutine	-5,9
	Gentiatibetine	-5,6
Glucan endo-1,3-beta-D-glucosidase	FK-463 (Control)	-9
	Futoamide	-7,3
	Lycopodine	-6,8
	Dihydrolycopodine	-6,4
	1-[(2E,4E)-2,4-decadienoyl] pyrrolidine	-6,1
	Gentiatibetine	-5,7
	Gentialutine	-5,2

Figures 3 and 4 show the 3D visualization results of the complexes of each of the 3 marker proteins with the compound ligands and the control. The binding position and each ligand are the same as the control, especially for Lanosterol 14 alpha demethylase protein, because the gridbox has been adjusted by control redocking which results in Root Mean Square Deviation (RMSD) below 2 Å (Trott & Olson, 2010). RMSD is a measure of the average distance between atoms in two structures, indicating the degree to which the structures are similar (Kuzmanic & Zagrovic, 2010). With a RMSD value of less than 2 Å it indicates that the difference between the predicted binding positions and the observed binding positions from the control redocking is relatively small (Carugo, 2003). This indicates that the predicted binding positions of these ligands have a high degree of accuracy, as the difference from the observed binding positions from the control is relatively small (RMSD below 2 Å). A RMSD value below 2Å is taken to indicate the model is of high quality. However, this global measure does not provide an assessment of the local quality of the binding site in this predicted structure (Carbery et al., 2024). Based on the visualization results, it shows that the cause of the lower binding affinity of the sample ligand than the control is most likely influenced by the size comparison of the ligands and the functional groups possessed by the sample ligand. The control ligand appears much larger, thus it has more functional groups.

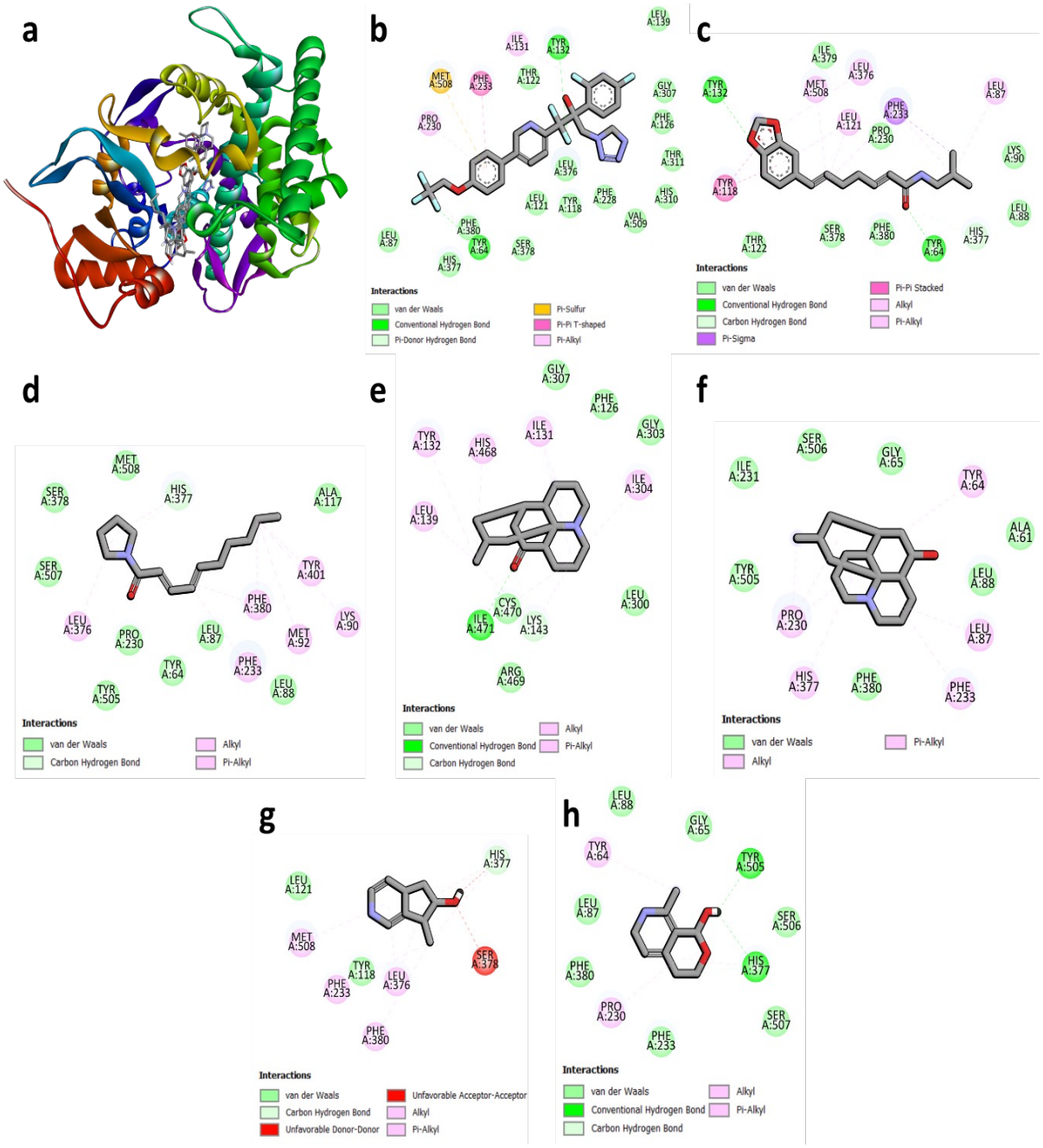


Figure 3. Visualization of docking results of Lanosterol 14 alpha demethylase protein with, a) Ligand binding position, b)control, c) Futoamide, d) 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine, e) Lycopodine, f) Dihydrolcopodine, g) Gentialutine, and h) Gentiatibetine

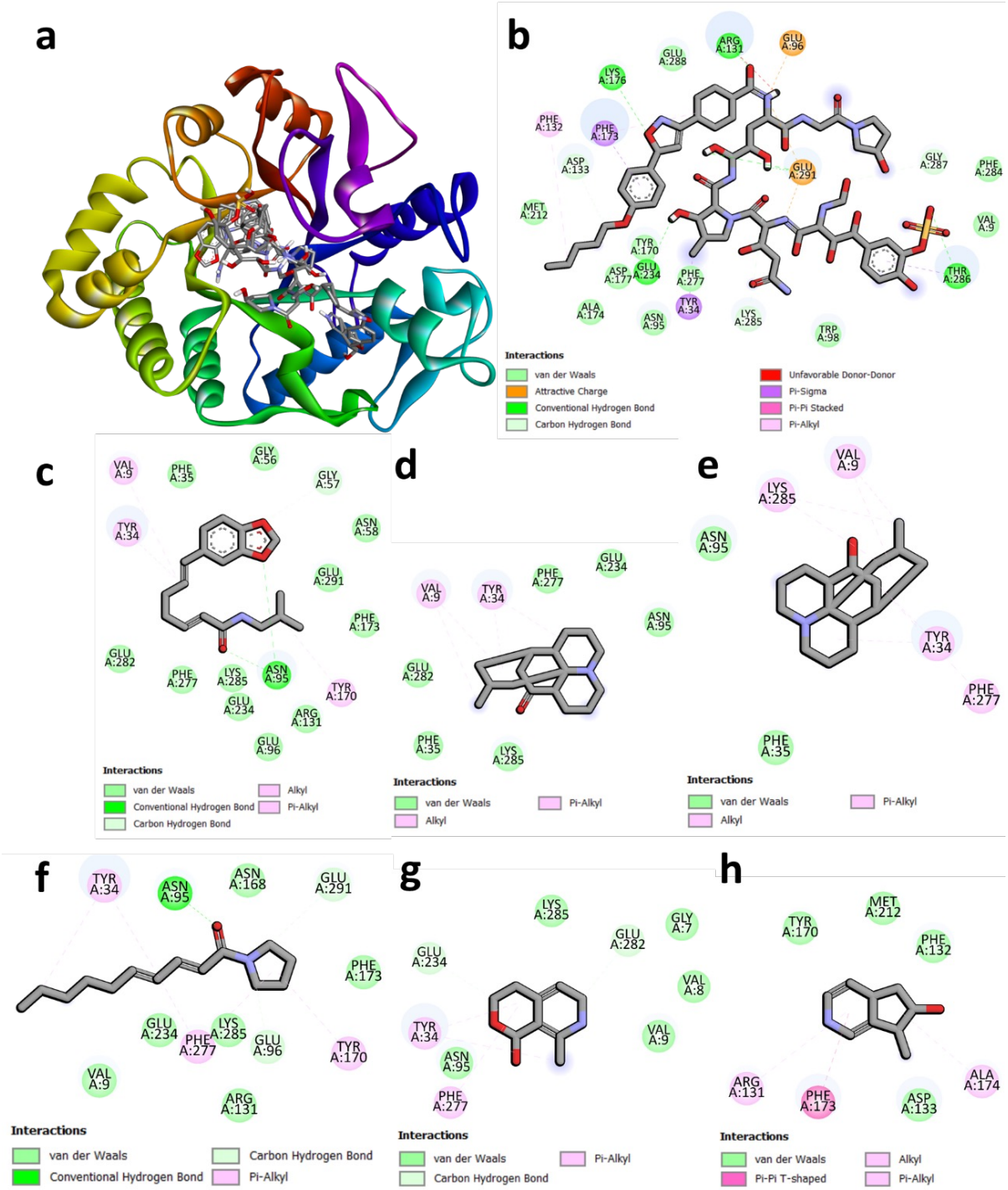


Figure 4. Visualization of docking results of Glucan endo-1,3-beta-D-glucosidase protein with, a) Ligand binding position, b) Control, c) Futoamide, d) Lycopodine, e) Dihydrolycopodine, f) 1-[(2E,4E)-2,4-decadienoyl] pyrrolidine, g) Gentiatibetine, and h) Gentialutine

CONCLUSION

SAR and Docking results showed that the bioactive compounds of *Pseudomonas azotoformans* have potential as antifungals. Based on comparisons among sample compounds, Futoamide shows the greatest potential as a multitarget inhibitor against of both *Candida albicans* ATCC 10231 proteins. The test ligands showed affinity and stability with Lanosterol 14 alpha demethylase and endo-1,3-beta-D-glucosidase that were close to control. However, the test ligands exhibit diverse affinities and stabilites with Lanosterol 14 alpha demethylase and Glucan endo-1,3-beta-D-glucosidase.

AUTHOR CONTRIBUTION

R.H.P. designed the research and supervised all the process, L.A. collected and analyzed the data and wrote the manuscript.

ACKNOWLEDGMENTS

This work was supported in part by a grant of Competitive Research Grant 2021-2022 from the Ministry of Education, Research and Technology with a contract number of 1867/E4/AK.04/2021 and number of 018/E5/PG.02.00/2022, awarded to RHP.

CONFLICT OF INTEREST

The published results of our research do not contain any conflicts of interest.

REFERENCES

Abdullahi, M., & Adeniji, S. E. (2020). In-Silico Molecular Docking and ADME/PharmacokinetiPrediction Studies of Some Novel Carboxamide Derivatives as Anti-Tubercular Agents. *Chemistry Africa*, 3(4), 989–1000.DOI:10.1007/s42250-020-00162-3

Abdullahi, M., Shallangwa, G. A., & Uzairu, A. (2020). In-Silico QSAR and Molecular Docking Simulation of Some Novel Aryl Sulfonamide Derivatives as Inhibitors of H_5N_1 Influenza A Virus Subtype. *Beni-Suef University Journal of Basic and Applied Sciences*, 9(2), 1-12. DOI:10.1186/s43088-019-0023-y

Aoyama, Y., Yoshida, Y., Sonoda, Y., & Sato, Y. (1989). Deformylation of 32-oxo-24, 25-Dihydrolanosterol by The Purified Cytochrome P-45014DM (lanosterol 14 α-demethylase) from Yeast Evidence Confirming the Intermediate Step of Lanosterol 14 α-Demethylation. *Journal of Biological Chemistry*, 264(31), 18502–18505. DOI: 10.1016/S0021-9258(18)51495-2

Banerjee, S., Singh, S., Pandey, S., Bhandari, M. S., Pandey, A., & Giri, K. (2020). Biocontrol Potential of *Pseudomonas azotoformans, Serratia marcescens, and Trichoderma virens* Against Fusarium wilt of *Dalbergia sissoo*. *Forest Pathology*, 50(2), e12581. DOI: 10.1111/efp.12581

Bard, M., Lees, N. D., Turi, T., Craft, D., Cofrin, L., Barbuch, R., Koegel, C., & Loper, J. C. (1993).Sterol Synthesis and Viability of Erg11 (cytochrome P450 lanosterol demethylase) Mutations in *Saccharomyces cerevisiae* and *Candida albicans*. *Lipids*, 28(11), 963–967. DOI: 10.1007/BF02537115

Caetano, C. F., Gaspar, C., Martinez-de-Oliveira, J., Palmeira-de-Oliveira, A., & Rolo, J. (2023). The Role of Yeasts in

Human Health: A Review. *Life*, 13(4), 924. DOI: 10.3390/life13040924

Carbery, A. (2024). Learnt Representations of Proteins Can Be Used for Accurate Prediction of Small Molecule Binding Sites on Experimentally Determined and Predicted Protein Structures. *Journal of Cheminformatics*, 16(1), 1–17. DOI: 10.1186/s13321-024-00821-4.

Carugo, O. (2003). How Root-Mean-Square Distance (r.m.s.d.) Values Depend on The Resolution of Protein Structures That Are Compared. *Journal of Applied Crystallography*, 36(1), 125–128. DOI:10.1107/S0021889802020502

Ekins, S., Mestres, J., & Testa, B. (2007). In Silico Pharmacology for Drug Discovery: Methods for Virtual Ligand Screening and Profiling. *British Journal of Pharmacology*, 152(1), 9–20. DOI: 10.1038/sj.bjp.0707306

Eve, A., Aliero, A. A., Nalubiri, D., Adeyemo, R. O., Akinola, S. A., Pius, T., Nabaasa, S., Nabukeera, S., Alkali, B., & Ntulume, I. (2020). In Vitro Antibacterial Activity of Crude Extracts of Artocarpus Heterophyllus Seeds Against Selected Diarrhoea-Causing Superbug Bacteria. *The Scientific World Journal*, 2020(9813970), 1-11. DOI: 10.1155/2020/9813970

Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V, Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of The Biological Activity Spectra of Organic Compounds Using the PASS Online Web Resource. *Chemistry of Heterocyclic Compounds*, 50, 444–457. DOI: 10.1007/s10593-014-1496-1

Getas, I. W., Wiadnya, I. B. R., & Waguriani, L. A. (2014). Pengaruh Penambahan Glukosa danWaktu Inkubasi Pada Media SDA Terhadap Pertumbuhan Jamur *Candida albicans*. *Jurnal Media Bina Ilmiah*, 8(1), 51-56. ISSN: 1978- 3787.

Guan, Z., Chai, X., Yu, S., Hu, H., Jiang, Y., Meng, Q., & Wu, Q. (2010). Synthesis, Molecular Docking, and Biological Evaluation of Novel Triazole Derivatives as Antifungal Agents. *Chemical Biology & Drug Design*, 76(6), 496–504. DOI: 10.1111/j.1747-0285.2010.01038.x

Guntur, A. H., Setiadi, S., Alwi, I., Sudoyo, A. W., Simadibrata, M. K., Setiyohadi, B., & Syam, A. F. (2014). Sepsis, dalam Buku Ajar Ilmu Penyakit Dalam. FK UI, 3(6), 692–699.

Hargrove, T. Y., Friggeri, L., Wawrzak, Z., Qi, A., Hoekstra, W. J., Schotzinger, R. J., York, J. D., Guengerich, F. P., & Lepesheva, G. I. (2017). Structural Analyses of Candida albicans Sterol 14α-Demethylase Complexed with Azole Drugs Address the Molecular Basis of Azole-Mediated Inhibition of Fungal Sterol Biosynthesis. *Journal of Biological Chemistry*, 292(16), 6728–6743. DOI: 10.1074/jbc.M117.778308

Jakubec, D., Skoda, P., Krivak, R., Novotny, M., & Hoksza, D. (2022). PrankWeb 3: Accelerated Ligand-Binding Site Predictions for Experimental and Modelled Protein Structures. *Nucleic Acids Research*, 50(W1), W593–W597. DOI: 10.1093/nar/gkac389

Jendele, L., Krivak, R., Skoda, P., Novotny, M., & Hoksza, D. (2019). PrankWeb: A Web Server for Ligand Binding Site Prediction and Visualization. *Nucleic acids research*, 47(W1), W345–W349. DOI: 10.1093/nar/gkz424

Ji, H., Zhang, W., Zhou, Y., Zhang, M., Zhu, J., Song, Y., Lü, J., & Zhu, J. (2000). A Three-Dimensional Model of Lanosterol 14α-Demethylase of *Candida albicans* and Its Interaction with Azole Antifungals. *Jour-*

nal of medicinal chemistry, 43(13), 2493–2505. DOI: 10.1021/jm990589g

Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. *Trends in microbiology*, 28(8), 668–681. DOI: 10.1016/j.tim.2020.03.016

Kesuma, D., Siswandono, S., Purwanto, B. T., & Hardjono, S. (2018). Uji In silico Aktivitas Sitotoksik dan Toksisitas Senyawa Turunan N-(Benzoil)-N'- feniltiourea Sebagai Calon Obat Antikanker. *JPSCR : Journal of Pharmaceutical Science and Clinical Research*, 3(1), 01-11. DOI: 10.20961/jpscr.v3i1.16266

Kusumaningtyas, E., Astuti, E., & Darmono, D. (2008). Sensitivitas Metode Bioautografi Kontak dan Agar Overlay dalam Penentuan Senyawa Antikapang. *Jurnal Ilmu Kefarmasian Indonesia*,6(2), 75–80. ISSN 2614-6495. Corpus ID: 182117723

Kuzmanic, A., & Zagrovic, B. (2010). Determination of Ensemble-Average Pairwise Root Mean-Square Deviation from Experimental B-Factors. *Biophysical Journal*, 98(5), 861–871. DOI: 10.1016/j.bpj.2009.11.011

Lamb, D. C., Kelly, D. E., Venkateswarlu, K., Manning, N. J., Bligh, H. F. J., Schunck, W. H., & Kelly, S. L. (1999). Generation of A Complete, Soluble, and Catalytically Active Sterol 14α-Demethylase−Reductase Complex. *Biochemistry*, 38(27), 8733–8738. DOI: 10.1021/bi9825089

Lemberg, C., Martinez de San Vicente, K., Fróis-Martins, R., Altmeier, S., Tran, V. D. T., Mertens, S., Amorim-Vaz, S., Rai, L. S., d'Enfert, C., & Pagni, M. (2022). *Candida albicans* Commensalism in The Oral Mucosa is Favoured By Limited Virulence and Metabolic Adaptation. *PLoS Pathogens*, 18(4), e1010012. DOI: 10.1371/journal.ppat.1010012

Li, W., Wang, J. J., Qian, H., Tan, L., Zhang, Z., Liu, H., Pan, Y., & Zhao, Y. (2020). Insights into The Role of Extracellular DNA and Extracellular Proteins in Biofilm Formation of *Vibrio parahaemolyticus*. *Frontiers in Microbiology*, 11, 523806. DOI: 10.3389/fmicb.2020.00813

Liu, Y., Guo, J., Li, L., & Devi, A. M. (2017). Endophytic Bacteria Associated with Endangered Plant *Ferula sinkiangensis* K.M. Shen in An Arid Land: Diversity and Plant Growth-Promoting Traits. *Journal of Arid Land*, 9(3), 432–445. DOI:10.1007/s40333-017-0015-5

Mitchell, K. F., Taff, H. T., Cuevas, M. A., Reinicke, E. L., Sanchez, H., & Andes, D. R. (2013).Role of Matrix β-1, 3 Glucan in Antifungal Resistance of Non-Albicans Candida Biofilms.*Antimicrobial agents and chemotherapy*, 57(4), 1918–1920. DOI: 10.1128/aac.02378-12

Monk, B. C., Sagatova, A. A., Hosseini, P., Ruma, Y. N., Wilson, R. K., & Keniya, M. V. (2020). Fungal Lanosterol 14α-demethylase: A Target for Next-Generation Antifungal Design. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1868(3), 140206. DOI: 10.1016/j.bbapap.2019.02.008

Oktarina, E., Pratiwi, R. H., Mangunwardoyo, W., Hidayat, I., & Saepudin, E. (2021). In Vitro Antimicrobial Activities of Several Extracts Endophytic *Pseudomonas azotoformans* UICC B-91. *IOP Conference Series: Earth and Environmental Science*, 948(1), 12068. DOI: 10.1088/1755-1315/948/1/012068

Pratiwi, R. H., Hidayat, I., Hanafi, M., Mangunwardoyo, W. (2016). Identification and Characterization of Three Endophytic Bacteria from *Neesia altissima* (*Malvaceae*) Antagonistic to Diarrhea-Causing Bacteria. *Malay-*

sian Journal of Microbiology, 12(4), 300–307. DOI:10.21161/mjm.81515

Pratiwi, R. H., Oktarina, E., Mangunwardoyo, W., Hidayat, I., & Saepudin, E. (2022). Antimicrobial Compound from Endophytic *Pseudomonas azotoformans* UICC B-91 of *Neesia altissima* (*Malvaceae*). *Pharmacognosy Journal*, 14(1), 172–181. DOI: 10.5530/pj.2022.14.23

Rahayu, M., Harada, K. and Muzakkir, A. (2002). Kajian pemanfaatan tumbuhan obat olehmasyarakat lokal sekitar Taman Nasional Gunung Halimun, Jawa Barat. *Prosiding SimposiumNasional II Tumbuhan Obat dan Aromatik. Pusat Penelitian Biologi-LIPI, Bogor, Indonesia*,61–72.

Sheng, C., Zhang, W., Zhang, M., Song, Y., Ji, H., Zhu, J., Yao, J., Yu, J., Yang, S., & Zhou, Y. (2004). Homology Modeling of Lanosterol 14α-demethylase of *Candida albicans* and *Aspergillus fumigatus* and Insights into The Enzyme-substrate Interactions. *Journal of Biomolecular Structure and Dynamics*, 22(1), 91–99. DOI: 10.1080/07391102.2004.10506984

Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec, I. (2021). *Candida albicans*—The Virulence Factors and Clinical Manifestations of Infection. *Journal of Fungi*, 7(2), 79. DOI: 10.3390/jof7020079

Trott, O. & Olson, A. J. (2010). AutoDock Vina: Improving The Speed and Accuracy of Docking With a New Scoring Function, Efficient Optimization, and Multithreading. *Journal of Computational Chemistry,* 31(2), 455–461. DOI: 10.1002/jcc.21334

Yang, L., Bo Xu, W., Sun, L., Zhang, C., & Hua Jin, C. (2022). SAR Analysis of Heterocyclic Compounds with Monocyclic and Bicyclic Structures as Antifungal Agents. *ChemMedChem*, 17(12), e202200221. DOI: 10.1002/cmdc.202200221