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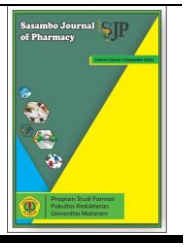

Molecular docking study of natural compounds from red betel (*Piper crocatum* Ruiz & Pav) as inhibitor of secreted aspartic proteinase 5 (Sap 5) in *Candida albicans*

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Abstract: *Candida albicans* can cause adverse infections in humans. The targeting of Sap 5 is due to its virulence factor in *C. albicans*. The method used is molecular docking using YASARA structure and BIOVIA Discovery Studio. The purpose of this study was to investigate the molecular interaction between red betel and Sap 5 as a potential inhibitor of *C. albicans* in infecting humans. The results showed that CHEMBL216163 (9,644 kcal/mol) and MLS000557666 (9,525 kcal/mol) have Binding energy above Pepstatin (9,484 kcal/mol) and affect the active site of Sap 5 so that the two test ligands could be further analyzed.

Keywords: *Candida albicans*, Sap 5, Molecular docking, YASARA.

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Introduction

In general, *Candida albicans* (*C. albicans*) reside on the intestinal mucosa, oral cavity, or vaginal canal. Efforts have been made to balance the immune system in the body so that the fungus remains in a commensal state, thus preventing invasion, epithelial damage, and mucosal infection. However, it is known that immunosuppression antibiotic treatment is one of the predisposing factors (Westman et al., 2022).

The Secreted aspartic proteinase (Sap) families from Sap 1 to Sap 7 secrete protease enzymes in *C. albicans*. All *C. albicans* Sap have their respective roles as virulence factors (Monod, Hube, Hess, & Sanglard, 1998). Sap 5 is susceptible to causing virulence. Sap attempts to steal nutrients, disrupt host cell membranes, and damage the tissue (Meenambiga, Venkataraghavan, & Abhishek Biswal, 2018).

Indonesia is known to have various kinds of herbal plants that have various benefits. Hence, one of its uses is serving as a source of traditional medicine for several diseases (Zahra et al., 2022). Red betel (*Piper crocatum* Ruiz & Pav) is one of the widely known medicinal plants in Indonesia (Lister et al., 2020). This study aims to investigate the molecular interaction potential of red betel against Sap 5 as a strong drug candidate by doing molecular docking, and analyzing the Binding energy and amino acid residue interaction.

Materials and Methods

This study was designed using a computer device with Windows 10 Professional 64-bit operating system, x64-based processor, and an Intel ® Core TM i5-6400T @ 2.20GHz 2.21 GHz processor specification. The software used was *Yet Another Scientific Artificial Reality Application* (YASARA) structure and BIOVIA Discovery Studio. All test ligand materials were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and receptor from RCSB PDB (https://www.rcsb.org/).

Receptor preparation

Secreted aspartic proteinase 5 (Sap 5) is the receptor of choice in this study. Sap 5 was downloaded

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from RCSB PDB With PDB ID "2QZX" (Borelli et al 2008). Sap 5, which has been downloaded in (.pdb) format, was prepared using YASARA structure to remove water molecules, add hydrogen atoms and remove unnecessary chains. Other parameters are set in their default state (Wang et al., 2019). Then, the Chain A complex and the crystallographic ligands were used in this study to assist in the validation and standardization of the inhibitors (Gonzales et al., 2019).

Table 1. Compounds of red betel (*Piper crocatum* Ruiz & Pav) used as test ligand (Fatima et al., 2021)

CID	Test Ligand
44257338	Glabrescione
73160	Catechin
5281515	Caryophyllene
5317570	Germacrene
10248	Elemicin
1032	Propionic acid
10446	Neophytadiene
31272	Butyl ethanoate
82227	Alfa pinene
22311	Limonene
2758	Cineole-1,8
11230	Terpinene-4-ol
75019	6XO32ZSP1D
2729185	Ethyl L-serinate hydrochloride (1:1)
108130	Schisandrin B
188289	Columbin
6070252	ZINC8756459
1077234	MLS000557666
2865476	Oprea1_462146
44418672	CHEMBL216163
7127	Methyl eugenol
138363	4-methoxyindole
16219591	Leucylleucinamide hydrochloride (1:1)
61440504	5-isopropyl-3-pyrazolidinecarbohydrazide hydrochloride (1:1)
2734672	1H-pyrazole-1-carboximidamidmidhyrochloride
72	Protocatechuic acid
2805645	N1-(5-methylisoxazole-3-yl)ethanediamide
90665169	CHEMBL3217136
45595316	2-(4-morpholinylmethyl)aniline sulfate hydrate
14839452	SCHEMBL569003
66250	L-Arginine hydrochloride
116510220	1-(1,4-Dithian-2-ylmethyl)-3-(3-methoxypropyl)thiourea
1511955	ALBB-026042

Ligand preparation

The ligand 2D and 3D structures were obtained from the PubChem database in (SDF) format. All test ligands were prepared before screening by adding hydrogen atoms and energy minimization (Options > Choose experiment > energy minimization) using YASARA structure (Bilal et al., 2021; Patel et al., 2021; Gholam 2022a; Yadav et al., 2017; Gholam et al., 2022). Then, the ligands are stored in a (*_ligands.sdf) file format. (Cortes-Benitez et al., 2021). All test ligands are listed in **Table 1**.

Validation and molecular docking

Redocking was done to get the most suitable Grid box size. The measure is continued for use in screening. YASARA structure with dock_run.mcr.macro runs=100, Amber14 is used for validation (Patel et al., 2020; Ali et al., 2020; Gholam et al., 2022).

The dock_runscreening macro file YASARA structure written by Elmar Krieger is used to attach an unlimited number of ligands to the target receptor using the VINA or AutoDock methods; the binding energy results are sorted respectively (http://www.yasara.org/dock_runscreening.mcr).

Screening uses YASARA structure with the macro dock_runscreening.mcr set in the VINA method, runs=100, and Amber14 (Patel et al., 2020; Ali et al., 2020; Cojocaru and clima 2020; Venkatachalam and Ettrich 2021; Krieger and Vriend 2015; Gholam 2022b).

Residue interaction analysis

Analysis of the screening data and visualization in two dimensions (2D) and three dimensions (3D) was done using BIOVIA Discovery Studio software which can be downloaded from the website (https://discover.3ds.com/discovery-studio-visualizer-download). The (.yob) files or objects that contain complexes are converted into (.pdb) format using YASARA structure to help 2D and 3D visualization using BIOVIA Discovery Studio (Srivastava et al., 2018).

Result and Discussion

The Grid box size used for the screening is 1 Å because this size had an RMSD value of 0Å with Binding energy of 9.034 kcal/mol when redocking was done. This research provides screening results in the form of Binding energy, residual contact, and the types of interactions formed. Sap 5 has catalytic residue located on Asp32 and Asp218 (Borelli et al., 2008). Pepstatin is a crystallographic ligand bound in Sap 5 PDB structure. We used pepstatin as a Binding energy control so that the test ligand that has Binding energy above pepstatin can be considered as a potential ligand to be developed. We visualized and analyzed the type of interaction formed with the potential test ligands.

Based on **Table 2**, the ligand used as control, pepstatin (CID_5478883), has Binding energy of 9.484 kcal/mol. The test ligands with Binding energy above pepstatin are CHEMBL216163 (CID_44418672) and MLS000557666 (CID_1077234). The binding energies of the two test ligands were 9,644 kcal/mol (CID_44418672) and 9,525 kcal/mol (CID_1077234). YASARA structure identifies the best score by sorting the best receptor-ligand complex. A positive score indicates the best binding energy in YASARA structure. Thus, a score with a more positive result indicates the better binding of the ligand to the receptor (Patel et al., 2020; Krieger et al., 2015). In addition, a small Kd value indicates a stronger ligand binding to the receptor (**Table 2**) (Aamir et al., 2018; Forlemu et al., 2017; Masomian et al., 2018). The ligand efficiencies of CHEMBL216163 and MLS000557666 were 0.2922 and 0.3528, respectively, while that of pepstatin was 0.1976 (a crystallographic ligand), indicating that the binding energy contributed by per atom of the compounds was almost similar to the need to develop a key contacts with Sap 5 target (Patel et al., 2020). The range of ligand efficiency under pepstatin (based on binding energy) is 0.2506 to 0.7214.

Table 2. Red betel molecular docking results

CID	Ligand Efficiency [kcal/(mol*Atom)]	Binding energy (kcal/mol)	Dissoc. Constant (pM)
44418672	0.2922	9.644	85281.39
1077234	0.3528	9.525	104251.523
5478883 (a crystallographic ligand)	0.1976	9.484	111721.257
6070252	0.2506	8.771	372196.031
44257338	0.2573	8.492	596048.312
2729185	0.2937	8.224	936975.125
73160	0.3754	7.884	1663226.375
2865476	0.2621	7.863	1723235.25
1511955	0.2929	7.616	2614556.25
188289	0.2929	7.616	2614556.25
14839452	0.3120	7.488	3245063.75
61440504	0.3526	7.052	6773536.5
49862728	0.3537	6.721	11842453
5317570	0.4331	6.497	17283666
75019	0.4291	6.436	19157968
108130	0.2207	6.399	20392516
4162211	0.3752	6.378	21128274
72	0.5738	6.312	23617974
2805645	0.4982	5.978	41501892
63221	0.4955	5.946	43805052
138363	0.5398	5.938	44400544
7127	0.4361	5.669	69914608
10446	0.2829	5.657	71345080
11230	0.5095	5.604	78021360
10248	0.3670	5.505	92210736
22311	0.5505	5.505	92210736
2758	0.4967	5.464	98817736
82227	0.5415	5.415	107337720
116510220	0.3168	5.069	192474736
2734673	0.5851	4.681	370494272
31272	0.5219	4.175	870330944
1032	0.7214	3.607	2270042368

CHEMBL216163 (CID_44418672) forms bonds with Sap 5 of which are Hydrogen Bonds, Salt Bridge type Electrostatic bond, and Attractive Charge on amino acid residues Asp303. Electrostatic bonds are formed at the amino acid residues Asp86, Asp218, and Tyr225. Hydrogen bonds are formed at the amino acid residues Gly85, Asp86, Tyr225, and Gly34. Each bond distances are 2.31, 2.96, 2.42, and 2.28 Å. The hydrogen bond is the most formed bond in the CHEMBL21613 Sap 5 complex (**Figure 1**).

MLS000557666 (CID_1077234) with Sap 5 forms hydrogen bonds at the amino acid residue Gly34. The

hydrogen bond distance is 2.86 Å. Two Electrostatic bonds formed on the amino acid residues Asp86 with a distance of 3.53 and 3.86 Å. Hydrophobic interactions formed on the amino acid residues Tyr84, Ile305, Lys193, and Tyr225 (**Figure 2**).

Pepstatin is the crystallographic ligand that acts as an inhibitor standard for this study. Most bonds formed in the pepstatin-receptor complex are hydrogen bonds at the amino acid residues Gly85, Gly86, Phe128, Leu194, Thr222, Asp86, Gly220, Asp32, Gly34, Gly127, Lys193, Thr221, Gly220, and Lys192. The bond distance formed varies from 2.00 to 3.04 Å. Pepstatin has some hydrophobic interactions at the amino acid residues Ile12 and Ile123 (**Figure 3**).

The presence of hydrogen bonds and hydrophobic interactions helps strengthen and stabilize bonds in the receptor-ligand complex (Krisnamurti et al., 2020). Hydrogen bond has an important role in receptor-ligand complexes (Meyer et al., 1996). A good hydrogen bond has a distance of less than 2.3 Å (Uzzaman et al., 2021).

The formation of electrostatic bonds can help increase the efficiency of the ligand (Novoseletsky et al., 2010; Krisnamurti et al., 2020; de Freitas and Schapira 2017). Other records show that high-efficiency ligand has a lot of hydrophobic interactions (de Freitas and Schapira 2017).

We found similarities in the test ligand CHEMBL216163 (CID_44418672) with pepstatin; they both have dominant (more) hydrogen bonds. Pepstatin, as the standard inhibitor in this study, has a binding energy below CHEMBL216163 and MLS000557666, so we predict that these two ligands are potential test ligands to be candidates for inhibitor development. In addition, the interactions formed in the complex of the two test ligands are classified as strong bonds. However, in our analysis, Pepstatin formed hydrogen bonds with the catalytic residue of Asp32, whereas CHEMBL216163 had contact with the catalytic residue of Asp218 by forming electrostatic bonds on the aromatic ring of the ligand. However, we predict that the presence of such a bond indicates a good bond. MLS000557666 is considered a potential test ligand based on bond energy; the interaction formed did not have contact with the catalytic residue of Sap 5. It should be noted that of the three ligands (Pepstatin, MLS000557666, and CHEMBL216163), only MLS000557666 had an Unfavorable interaction. Of the three ligands, it was also known that only CHEMBL216163 did not form hydrophobic interactions.

We also found unfavorable binding in the ligand-receptor complex CHEMBL216163 (CID_44418672) at the amino acid residues Arg120 and Arg299 with a distance of 2.43 and 5.50 Å, respectively. whereas unfavorable binding in the pepstatin complex occurred at the amino acid residue Ser36 with a distance of 2.72 Å. Unfavorable bonds can disrupt the stability of the complex due to the repulsive forces (Dhorajiwala et al., 2019).

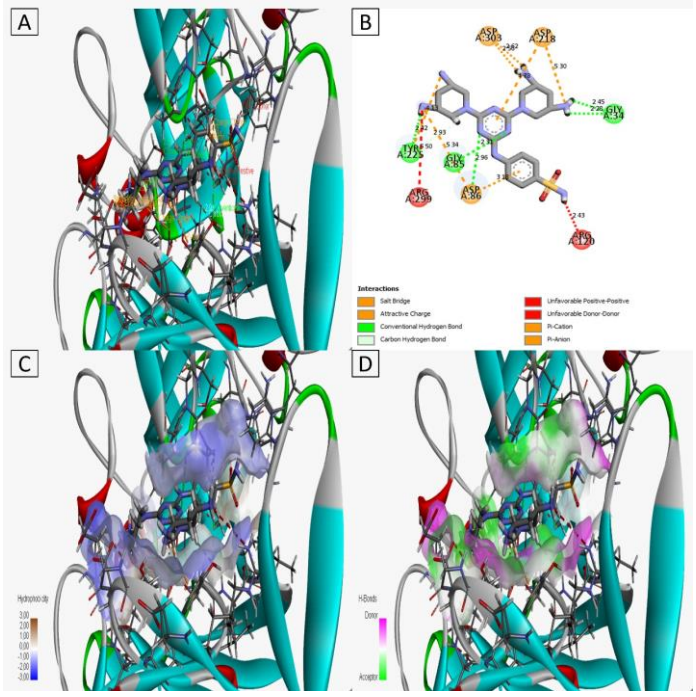

Figure 1. Visualization of 3D interactions between ligand CHEMBL216163 (CID_44418672) and Sap 5 of *Candida albicans*. **(A)** 3D visualization of the resulting interaction, **(B)** 2D visualization of the resulting interaction, **(C)** Hydrophobicity area of the receptor-ligand complex, **(D)** H-Bonds area of the receptor-ligand complex

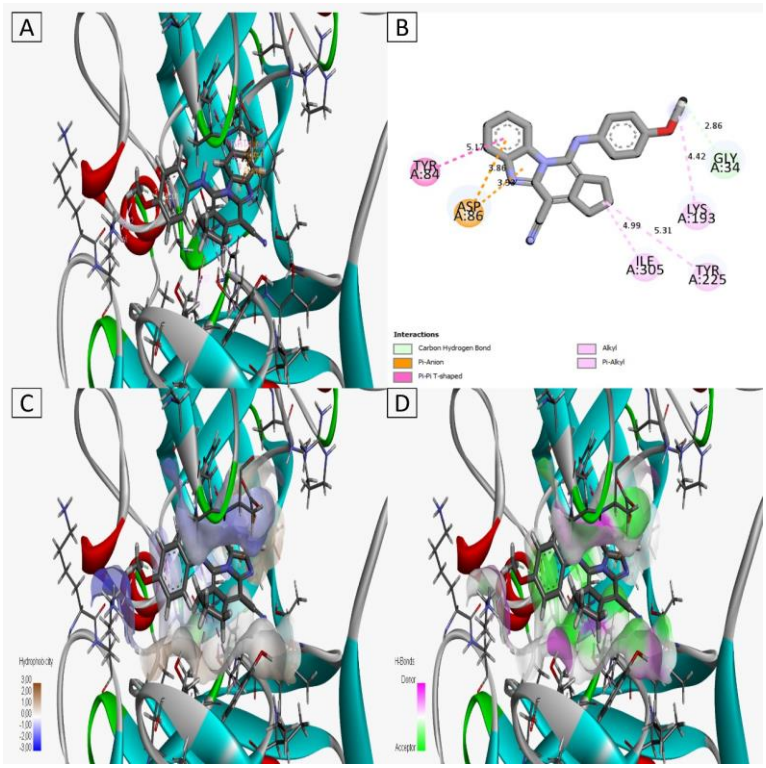

Figure 2. Visualization of 3D interactions between ligand MLS000557666 (CID_1077234) and Sap 5 of *Candida albicans*. **(A)** 3D visualization of the resulting interaction, **(B)** 2D visualization of the resulting interaction, **(C)** Hydrophobicity area of the receptor-ligand complex, **(D)** H-Bonds area of the receptor-ligand complex

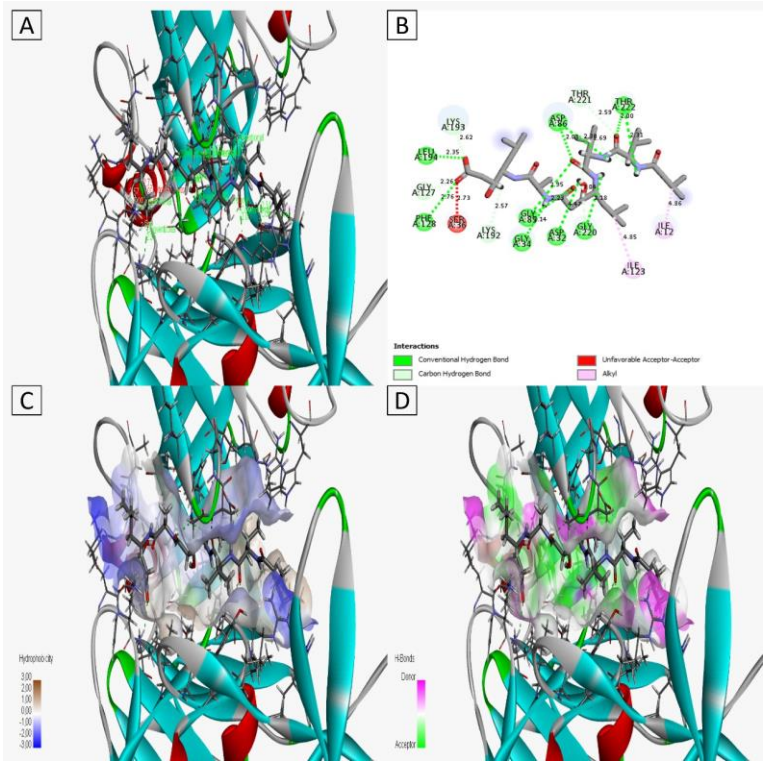

Figure 3. Visualization of 3D interactions between ligand Pepstatin crystallographic ligand (CID_5478883) and Sap 5 of *Candida albicans*. **(A)** 3D visualization of the resulting interaction, **(B)** 2D visualization of the resulting interaction, **(C)** Hydrophobicity area of the receptor-ligand complex, **(D)** H-Bonds area of the receptor-ligand complex

Conclusion

Based on the results of Red Betel (*Piper crocatum* Ruiz & Pav) molecular docking against Sap 5, the assay ligands CHEMBL216163 (9.644 kcal/mol) and MLS000557666 (9.525 kcal/mol) had binding energy above pepstatin (9.484 kcal/mol). In addition, the two test ligands had more interaction types than the crystallographic ligand.

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Author contribution statement

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Competing interest statement

The authors declare no conflict of interest.

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