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In vitro and In silico Studies of Kayu Raja (*Cassia fistula* L.) Leaves Extract as Potential Antifungal Agent Against *Candida albicans*

Tan David Christian [1], Kasiyati [1], Ariyanti Saputri [2], Tri Yuliani [2], Dadang Priyoatmojo [2], Marissa Angelina [2,*]

[1] Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Jawa Tengah, Indonesia
[2] Research Centre for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), KST BJ. Habibie, Kawasan PUSPIPTEK Serpong, Tangerang Selatan Banten 15314, Indonesia

* Corresponding author: mari011@brin.go.id

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Abstract

Candida albicans is one of the most common fungal species causing opportunistic infections, such as candidiasis. *Candida albicans* has the ability to produce and secrete hydrolytic enzymes, namely secrete aspartate protease (SAP). SAP3 is a virulence factor for mucosal or disseminated infections. *Cassia fistula* leaves have bioactive compounds that can be used as antimicrobial agents, such as hyperoside, delphin, epicatechin, isoquercitrin, and astragalin. This study aims to determine the potential of bioactive compounds in *Cassia fistula* L. as an antifungal by in silico method molecular docking and Minimum Inhibitory Concentration (MIC) analysis with FE-SEM test for microscopical visual results. The results showed that the delphin compound had the best binding energy of -7.73 and an inhibition constant of 2.17 M, almost equivalent to ketoconazole as a positive control. MIC test of ethanol and ethyl acetate extracts of *Cassia fistula* leaves resulted in 100 ppm in the ethanol extract and 50 ppm in the ethyl acetate fraction. This result was supported by FE-SEM results, which showed that the ethanol extract at 100 ppm could damage the structure of *Candida albicans* colony.

1. Introduction

Indonesia has abundant natural plant resources, many of which have properties as herbal plants. Herbal plants have properties and advantages that are superior to synthetic drugs, which are safer for consumption, relatively affordable, and efficient [1]. The therapeutic properties of herbal plants for several diseases, for example, antimicrobials, have been studied by many practitioners. The antimicrobial properties of medicinal plants are increasingly being reported from various parts of the world. Treatment-based research on microbial diseases has become the cornerstone of developing resistant microbes, creating a worrying clinical situation. Based on this, interest in natural ingredients contained in herbal plants has increased over time [2].

Cassia fistula (Leguminosae) is an herbal plant distributed worldwide in Asia, South Africa, Mexico, China, East Africa, and Brazil. This plant is an ornamental tree with unique yellow flower clusters. *Cassia fistula* has herbal properties against several diseases, such as skin infections, liver disorders, and tuberculosis, to treat rheumatism, hematemesis, pruritus, leucoderma, and diabetes. *Cassia fistula* has also been studied for its antimicrobial properties. Due to this property, this plant is used as a broad-spectrum antimicrobial agent to treat several infectious diseases [3].

Candida albicans is a fungus isolated from human mucosal surfaces. *Candida albicans* can cause superficial and systemic infections in human hosts under optimal environmental conditions [4]. *Candida albicans* is also one of the most common fungal species causing opportunistic infections, such as candidiasis, which range from superficially invasive infections to life-threatening in debilitated patients. Invasive candidiasis is associated with a high mortality rate ranging from 20% to 49%. *Candida albicans* can colonize various mucosal surfaces such as the skin, mouth, and vagina; most of the digestive tract is the main entry point for *Candida albicans* to enter the bloodstream [5].

Candida albicans can produce and secrete hydrolytic enzymes, namely secrete aspartate protease (SAP) [6]. SAP is synthesized as a pre-proenzyme containing more than 60 amino acids. Increased SAP production correlates with increased virulence [7]. The secreted protein SAP has several special functions during infection, such as digesting molecules to acquire nutrients, distorting the host cell membrane for tissue invasion, and attacking the host's immune system to withstand host antimicrobial attacks [8]. The SAP protein in *Candida albicans* is divided into ten types (SAP1–SAP10), which are expressed differently depending on the strain and varying environmental conditions according to the type and stage of the disease [9]. Secreted aspartic proteinase 3 (SAP3) is a virulence factor for mucosal or disseminated *Candida albicans* infections. SAP3 is found in large quantities in hyphal cell walls and epithelial cells and contributes greatly to tissue damage. SAP3 is correlated with oral and vaginal diseases. It can induce the secretion of proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNFa) by human monocytes [10].

The active center of the malignant SAP enzyme can be inhibited by natural plant phytochemicals. Plant phytochemicals act as potential inhibitors to inhibit the enzyme's active site by competing with the substrate, thus neutralizing the virulence effect of the enzyme [11]. The bioactive compounds contained in the extract of *Cassia fistula* are reported to have antimicrobial properties. Evaluation of the antagonistic effect of the hydroalcoholic extract of *Cassia fistula* leaves on striae [2]. Bacteria and fungi exhibit significant inhibitory effects on growth. Studies on aqueous, alcohol, chloroform, and ethyl acetate extracts from flowers showed positive results. Other experiments revealed that the minimum inhibitory concentration (MIC) of *Cassia fistula* seed methanol extract ranged from 1.563-50.00 mg/mL for the strains tested [12].

This study used an in silico test as an initial step in predicting sample compounds as alternative candidates for synthetic drugs against the test microbes. The molecular docking method functions to predict the drug potency of the test compound against disease. By comparing the similarities of the structure of the test compound to a known target group of compounds in one or more databases, it is possible to predict any potential target macromolecule. The results of this prediction can be further tested with molecular docking to determine the interaction model [8].

In silico tests, it can be determined that the sample can potentially be antimicrobial, and then a MIC test is carried out. MIC is the lowest inhibitory concentration of an antibacterial agent specified in mg/L (µg/mL) whose results are visible visually. Scanning electron microscopy (SEM) is one type of electron microscopy that draws specimens by modifying them using high-energy electron beams in raster pattern scans. The electron has a higher resolution than light. The SEM showed images showing changes in the morphological structure of the microbes from the sample treatment impact [13].

The study tested the content of bioactive compounds in *Cassia fistula* leaf extract as a potential antimicrobial for *Candida albicans*. The initial phase of testing was conducted using the molecular docking method. A MIC test was performed to determine the minimum barrier concentration in ethanol extract and ethyl acetate fraction compared to a positive control using ketoconazole as a synthetic drug for *Candida albicans*. The MIC test results were followed by morphological characterization of *Candida albicans* under an FE-SEM microscope to compare the morphological changes in the test results.

2. Experimental

Cassia fistula leaves were collected from the *Cassia fistula* tree planted around the National Research and Innovation Agency (BRIN). Subsequently, these leaves underwent a drying process before being subjected to extraction. The extract was used for MIC and FE-SEM tests. The in silico method used was experimental research by conducting physicochemical screening through drug-likeness analysis based on Lipinski's Rule of Five, molecular docking, and prediction of pharmacokinetics of the bioactive compounds in the leaves of *Cassia fistula* L., which has the potential to inhibit *Candida albicans* protein SAP3 receptor.

2.1. Materials

The materials used were kayu raja (*Cassia fistula* L.) leaf in ethanol extract and ethyl acetate fraction, ketoconazole, distilled water, *Candida albicans* isolate, NaCl 0.9% sterile, glutaraldehyde, PBS (phosphate-buffered saline), flame lighter, alcohol 70%, alcohol 96%, DMSO (dimethyl sulfoxide), SDB (Sabouraud Dextrose Broth), parafilm, aluminum foil, carbon tape, Biovia Discovery Studio software, Autodock Tools, SAP3 *Candida albicans* protein receptor from Protein Data Bank (PDB), test ligan of the bioactive compounds of the *Cassia fistula* leaves (hyperoside, delphindin 3,5-diglucoside (delphin), epicatechin, isoquercitrin, kaempferol-3-glucosides (astragalin), and ketocazole as a reference ligand were collected from PubChem.

2.2. Preparation of Ligand Structures and Target Proteins

Receptors and ligands were obtained through library studies on topics related to the analysis of in silico receptor proteins SAP3 *Candida albicans* by Gholam *et al.* [14] and the analysis of the levels of bioactive compounds in the leaves of *Cassia fistula*. The test compounds from *Cassia fistula* leaves were determined using references from the study by Omer *et al.* [15] that tested the compound with the highest content in the methanol extract of *Cassia fistula* leaves, such as epicatechin (23%), hyperoside (21%), isoquercitrin (17%), delphin (13%), and astragalin (9%). The receptors used were the protein SAP3 of *Candida albicans* obtained from the Data Bank Protein (Code: 2H6T) format .pdb with a native ligand such as pepstatin A. The test ligands of bioactive compounds in *Cassia fistula* leaves were hyperoside (PubChem CID 5281643), delphinidin 3,5-diglucoside (delphin) (PubChem CID 10100906), epicatechin

(PubChem CID 72276), isoquercitrin (PubChem CID 5280804), kaempferol-3-glucoside (astragalin) (PubChem CID 5282102). The reference ligand was a drug synthetic ketoconazole with PubChem CID 456201. The receptors downloaded through the PDB were prepared using Biovia Discovery Studio software to clean up water molecules and separate native ligands from their protein chains. The test ligands were downloaded in 3D format through PubChem in .sdf format, and the ligan in 2D format was prepared into 3D via Avogadro software and then saved in Biovia Discovery Studio in .pdb format.

2.3. Validation of Molecular Docking Parameters

Validation was initially performed by redocking or restarting natural ligands with SAP3 macromolecular proteins from *Candida albicans*. The objective was to validate the docking results of the test by comparing the docking results with the native ligand. The redocking process used the already installed Autodock 4 Tools software. Proteins separated by native ligand were backdocked between native ligand (pepstatin A) and SAP3 *Candida albicans* protein receptor. The general principle of docking validation was by redocking the original ligan to its target protein, where the ligand and protein were prepared in the way and with the docking parameters that would be used for docking the test ligand [8].

The software used to redocking samples was Autodock Tools. Receptors and native ligands were prepared in .pdbqt format. The grid box was set with the native ligand as the center coordinate, i.e., with the coordinates of x (-1.661), y (20.748), and z (13.191). The size of the grid box adjusted the native ligand to 40×40×56. Docking was done using the docking methods of Razzaghi-Asl *et al.* [16] with modifications. The settings were the genetic algorithm run 100 times, the population size of 300, the evaluation figure of 2,500,000, and the maximum generation number of 27,000. Subsequently, Autogrid and Autodock were employed, and the results were analyzed for affinity binding, root mean square deviation (RMSD), and inhibition constants.

2.4. Molecular Docking

Test receptors and ligands (ketoconazole, hyperoside, delphindin 3,5-diglucoside (delphin), epicatechin, isoquercitrin, and kaempferol-3-glucoside/astragalin) were prepared. The docking process used a series of processes described by Nkungli *et al.* [17]. The grid box was done using the native ligand grid box as a comparison. Autogrid and Autodock were run until completed, showing binding affinity, RMSD, and inhibition constant.

2.5. Molecular Docking Visualization

The molecular docking results were visualized using the Pymol software, where the macromolecules were loaded as receptors, and the docking outputs were subsequently loaded. The 3D structures could be shown in cartoons, wire, and licorice. The use of Pymol software followed the methods of Saputri *et al.* [18].

2.6. Molecular Docking Results Analysis

The docking results were analyzed by examining receptor-ligand interactions using the Biovia Discovery Studio software. These interactions were observed by analyzing the chemical bonds between ligands and receptors, identifying the residues involved, and categorizing the types of bonds formed. The outcomes of these interactions were visually represented in a 2D format. The use of the Biovia Discovery Studio software followed the procedure of Fakih *et al.* [19].

2.7. Minimum Inhibitory Concentration (MIC) Preparation

The antimicrobial test was initially prepared by dissolving 1.6 mg of ethanol and ethyl-acetate extracts in a solution comprising 100 µL of DMSO and 900 µL of NaCl, resulting in a concentration of 1600 ppm for each extract. The growth medium for the fungi consisted of Sabouraud Dextrose Broth (SDB), wherein 1.5 g of SDB was dissolved in 50 mL of distilled water in an Erlenmeyer flask. The mixture was sterilized using an autoclave at 121°C and 1 atm for 15 minutes. As for the positive control, 20 mg of ketoconazole was dissolved in a solution composed of 100 µL of DMSO and 900 µL of NaCl, resulting in a concentration of 2%. Suspension of *Candida albicans* was prepared by taking one ounce of fungal isolate and then suspending it in a 0.9% NaCl solution. The suspensions were adjusted to match the thickness of the 0.5 McFarland standard, equivalent to 108 CFU/mL.

2.8. MIC Test Microdilution Method

Wells A, B, and C were used for the ethanol sample from the first row to the seventh row triple down. In contrast, wells E, F, and G were for the ethyl acetate sample from the first line to the seventh row (400, 200, 100, 50, 25, 12.5, and 6.25 ppm). Wells D and H were used to control ethanol and ethyl acetate samples without adding *Candida albicans*. The well line 10 was for positive control in columns A, B, and C (triplo). Well 11 was for negative control lines A, B, and C (triplo).

The procedure used the methods of Blaskovich *et al.* [20] with modifications. The process began by introducing 100 µL of medium into all the test wells. The sample was added to the sample well in the first row (100 µL, 1600 ppm) and then homogenized up and down five times. Microdilution was performed by transferring 100 µL from the first line to the second line, with a repeated up-and-down motion five times. This process was then iterated, taking 100 µL from the second to the third row and continuing in a similar pattern.

Candida albicans suspended in 0.9% NaCl were added to each 100 µL sample well. Well D and H (sampling control) applied the same procedure; however, fungi were omitted and replaced with NaCl 100 µL DMSO test wells with the highest and lowest concentrations, positive controls of ketoconazole, and negative controls performed the same procedure (NaCl excluded from negative control). The microplate was closed and incubated at 37°C for 48 hours. MIC value represented the lowest concentration of antimicrobial agents that entirely inhibit the growth of microorganisms in tubes and wells

that can be observed with the naked eye and were visible from their roughness.

2.9. Field Emission Scanning Electron Microscopes (FE-SEM) Analysis

Samples from the antimicrobial test were prepared for FE-SEM analysis, employing the SEM preparation method by Shehadat *et al.* [21]. Ensuring absolute dryness was imperative, prompting the need for chemical fixation in the microbial sample to stabilize its structure steadfastly. This fixation was usually done using a solution of aldehyde-fixing agents such as glutaraldehyde. Positive and negative controls were inserted into the microtube for each sample concentration. The microtube was centrifuged for 2 hours at 13,000 rpm, yielding a supernatant and a fungal pellet. The supernatant was taken slowly to avoid any contact with the fungal pellets. The pellets in the microtube were given 100 µL of PBS. Then, the PBS was carefully removed to prevent contact with the pellet. Supernatants were removed until only the pellets remained. The microtube was opened at room temperature and left to dry.

The dried pellets, encompassing positive control (ketoconazole), negative control (ethyl acetate sample), and ethanol at the minimum inhibitory concentration, were chosen for the FE-SEM analysis. The FE-SEM microscope used was the JIB-4610F, a type of external lens with Schottky electron guns, and the FIB (focused ion beam) column was capable of processing large currents (maximum 90 nA ion beam) installed in a single room. JIB-4610F allowed high-resolution FE-SEM observations after high-speed intersection milling with FIB and high-speed analysis with various analytical instruments.

3. Results and Discussion

3.1. Preparation of Redocking and Visualization

The samples were collected by downloading a 3D structure model of the SAP3 *Candida albicans* protein from the Protein Data Bank (RCSB PDB) with code 2H6T, then prepared by cleaning the residues using the Biovia Discovery Studio software. Removing water molecules aims to avoid complexity during the clamping process. The separation between original ligands and SAP3 *Candida albicans* proteins aims to create available cavities for redocking. The protein receptors were prepared by removing the water molecules, which were then separated by the native or native ligands and stored in the .pdbf format. The redocking result is depicted in Figure 1.

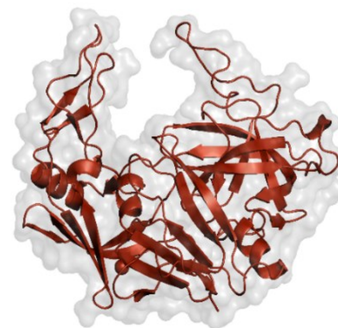

Figure 1. 3D visualization of redocking result

3.2. Validation Molecular Docking of Native Ligand

Test ligand models were collected by downloading the test compounds from the PubChem database page. The docking test performed on the *Cassia fistula* sample test compound as a test ligand was compared to the redocking result of the native ligand with the SAP3 protein receptor. The process of redocking native ligands and pre-prepared protein receptors through Autodock Tools is aimed at determining whether there are any abnormalities in the positioning of the native ligand within the SAP3 protein. The redocking results can also be compared with the retardation of the bioactive compound test ligand from *Cassia fistula* on the SAP3 protein of *Candida albicans*. The redocking outcomes reveal information regarding binding affinity, RMSD, and inhibition constant.

The 3D conformity value is expressed by the root mean square deviation (RMSD). A value closer to zero signifies a better and more acceptable result. Binding affinity is a value that indicates the ability of a ligand to bind to a receptor in kcal/mol. A smaller binding affinity value shows a stronger association between the receptor and ligand. On the contrary, larger binding affinities correspond to smaller associations between receptors and the ligand. A lower bonding energy value leads to more favorable results. This suggests that the docking method is valid and acceptable for further docking procedures [22].

Table 1. Redocking results of native ligand (Pepstatin A) with SAP3 receptor

Ligand	Binding affinity	RMSD	Inhibition constant (µM)
Native ligand (Pepstatin A)	-5.91	0.00	46.74
	-5.48	0.00	95.79
	-5.32	0.00	125.46
	-5.27	0.00	136.07
	-5.08	0.00	190.34
	-5.04	0.00	202.56
	-5.02	0.00	207.45
	-4.99	0.00	218.51
	-4.82	0.00	293.58

Table 1 shows that redocking native ligands (pepstatins) with SAP3 protein receptors of *Candida albicans* yields best at binding affinity -5.91, RMSD 0.00, and inhibition constant of 46.74 μM. The redocking result serves as a benchmark, allowing for a comparison with the docking process of test compounds. The decrease in binding value indicates bioactive ease in interacting on the binding site of target proteins compared to the reference ligand (ketoconazole). A docking test was conducted on a positive control test compound (ketoconazole), and the compounds in the *Cassia fistula* leaves sample.

3.3. Docking of Test Ligands

A docking test was conducted on a synthetic/control-positive drug, ketoconazole. In contrast, the test compounds from a sample of *Cassia fistula* leaves included delphin, isoquercitrin, astragalin, hyperoside, and epicatechin. The best affinity docking result was achieved by ketoconazole compounds, yielding a value of -7.91 with an inhibition constant of 1.56 M. In contrast, the most favorable docking outcome for the bioactive compounds in *Cassia fistula* leaves was observed with delphin compounds, attaining a value of -7.73 with an inhibition constant of 2.17 M. The sequence after the delphin compound is followed by isoquercitrin (-6.80), astragalin (-6.77), hyperoside (-6.65), and epicatechin (- 6.10) (Table 2).

Binding affinity is an index that shows the strength of the binding energy interaction between a biomolecule and its partner's ligan molecule. Bond affinity increases as the bond energy decreases, and vice versa. The inhibition constant is a parameter that shows the interaction between the ligand and its receptor; the smaller the value of the inhibiting constant, the more stable the interactions that occur [23]. The results showed that the delphin test compound had a low binding energy of -7.73 and an inhibition constant of 2.17 M, almost equivalent to ketoconazole as a positive control so that the delphin trial compounds could be the basis for the development of a potential inhibitor of the SAP3 protein in *Candida albicans*. Analysis of ligand-receptor chemical binding interactions in native ligands, ketoconazole, and delphin test compounds were selected as the most potential compounds.

3.4. Chemical Bonding Interaction Results

The results of the interaction in the 2D diagram (Figure 2A) show the interaction between the amino acid residues of the SAP3 protein with the native ligan (pepstatin A), i.e., ILE123, TYR303, VAL30, TYR84, GLY34, GLU83, ASP32, ASP218, ILE82, GLY85, ASP86, ILE82, GLY220, THR221, VAL12, and THR2222. The Pi-Sigma hydrophobic bond type is formed in the amino acid residues TYR84 and TYR303, the Alkyl bond type forms in the amino acid residues VAL12, VAL30, ILE123, and ILE82, as well as the Pi-Alkyl binding type formed on the amino acid residue TYR84, and MYR303.

Figure 2B illustrates the interaction between amino acid residues of the SAP3 protein and ketoconazole. The involved amino acids include GLY85, ILE82, SER35, Ile305, LEU216, ARG195, GLU193, TYR303, ASP86, ASP218, GLY220, THR222, TYR225, and ILE223. Eight hydrogen bonds are formed by the amino acid residues GLY85, ARG195, SER35, ASP218, GLY220, THR222, TYR225, and GLU193. Additionally, hydrophobic bonds are formed on amino acids TYR303, ILE82, ILE305, LEU216, and ILE223. Other types of bonds form Pi-Anion electrostatic bonds on ASP86 amino acid residues. The T-shaped Pi-Pi hydrophobic bond type is formed in the amino acid residue TYR303, the Alkyl bond type forms in the ILE82 and ILE223 amino acid residues, and the Pi-Alkyl bind type forms in the LEU216 and ILE305 amino acid residue.

Figure 2C shows the interaction between amino acid residues of SAP3 protein and delphin: GLY85, SER301, ASP218, ASP86, TYR225, ILE223, THR222, THR2221, VAL119, ASP120. There are seven hydrogen bonds formed by the amino acid residues: TYR-225, SER-301, THR-221; THR-222, ILE-223; ASP-120, GLY-85, whereas the Pi-Alkyl type of hydrophobic bond is formed on the VAL-119 amino-acid residue.

Table 2. Docking result of ligand tests with SAP3 receptor

Ligand	Binding affinity	RMSD	Inhibition constant (μM)
Ketoconazole	-7.91	0.00	1.56
Delphin	-7.73	0.00	2.17
Isoquercitrin	-6.80	0.00	10.36
Astragalin	-6.77	0.00	10.87
Hyperoside	-6.65	0.00	13.37
Epicatechin	-6.10	0.00	33.69

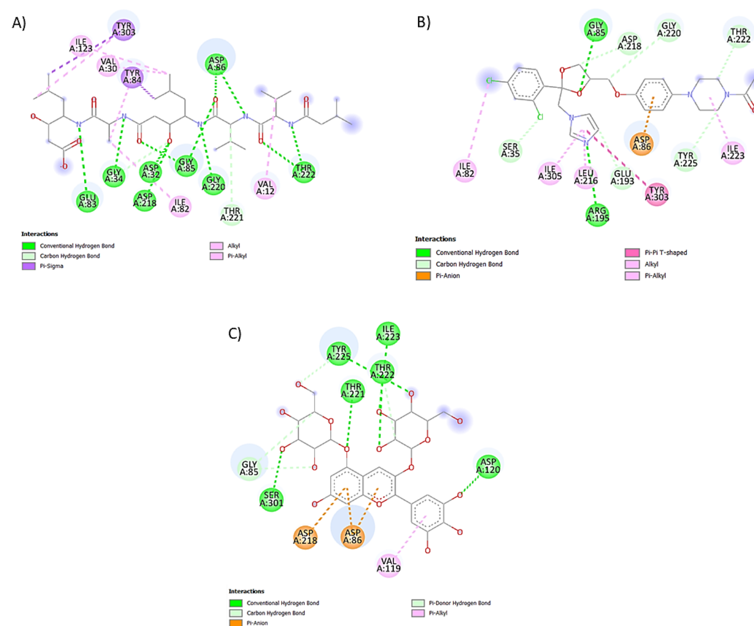

Figure 2. Result of chemical bond interaction between receptor and docking ligands: A) pepstatin A, B) ketoconazole, C) delphin

Observing amino acid residue interactions aims to identify interactions between ligands and receptors. The interactions that occur are hydrogen bonds, hydrophobic interactions, and electrostatic interactions. Hydrogen bonds between test ligands with identical amino acid residues in natural or reference ligands show the similarity of type of interaction, in this case describing similarity activity [24]. The result of the 2D diagram interaction can be seen in Table 3. Chemical binding interactions in the ketoconazole test compounds exhibit some similarity in the amino acid residue binding type, particularly involving hydrogen bonds with residues GLY85, ASP218, GLY220, and THR222.

The bonds in the remaining amino acids of TYR303 show a different kind of hydrophobic bond, namely the Pi-Pi T-shaped bond in ketoconazole. Another similarity is seen in the type of hydrophobic alkyl bond in the amino acid residue of ILE82. The ASP86 amino acid residues show a different kind of Pi-anion electrostatic bond. According to Nursamsiar *et al.* [25], hydrophobic and electrostatic interactions can improve conformation stability. Electrostatic interaction plays a role in the stability of ligands against receptors, and several electrostatic interactions can significantly contribute to the formation of protein conformations.

The outcomes of chemical binding interactions with the delphin test compounds reveal certain similarities. Notably, there is a resemblance in the hydrogen bonds formed with the amino acid residues GLY85, THR222, and THR221. However, distinctions are observed in the electrostatic bond type, specifically the Pi-Anion bonds in the residues ASP86 and ASP218. The similarity of amino acid residues and type of binding may indicate the potential of the test compound as an inhibitor of the disease-causing target protein activity. Amino acid residues similar to native ligands indicate that ligands can inhibit target protein activity [26]. Residual similarities and chemical interactions of delphin with ketoconazole and native ligands can conclude that delphin can potentially inhibit the activity of SAP3 *Candida albicans* protein and drug alternatives.

3.5. Minimum Inhibitory Concentration (MIC) Results

The results indicate that the ethanol sample exhibited a minimum inhibitory concentration of 100 ppm, evident from its persistent color. Conversely, a concentration of 50 ppm already displayed roughness, suggesting that microbial colony inhibition had not yet been inhibited. A comparison of fungal corrosion can be seen from a negative control. A positive control does not mean the presence of fungi. However, an intact ketoconazole deposit was shown to inhibit the fungal colony. According to Malveira *et al.* [27], the MIC category of plant extracts is divided into strong inhibitors of MIC outputs up to 500 ppm, moderate inhibitors of 600–1500 ppm, and weak inhibitors above 1600 ppm. The results showed an ethanol extract with MIC at 100 ppm and an ethyl acetate fraction at 50 ppm. These results suggested that the ethanol extract and ethyl acetate fraction from *Cassia fistula* leaves are strong inhibitors for the antimicrobial *Candida albicans.*

Figure 3. Minimum inhibitory concentration (MIC) results

Table 3. 2D diagram of chemical bond interaction results

Ligand	Type of ligand	Bond type	Amino acid residue interaction
Native ligand (Pepstatin A)	Hydrogen	Conventional & Carbon	**ASP86**, GLU83, GLY34, ASP32, **ASP218**, **GLY85**, GLY220, **THR222**, **THR221**
	Hydrophobic	Pi-Sigma	TYR84, **TYR303**
		Alkyl	VAL12, VAL30, ILE12, **ILE82**
		Pi-Alkyl	TYR84, **TYR303**
Ketoconazole	Hydrogen	Conventional & Carbon	**GLY85**, SER35, LEU216, ARG195, GLU193, **ASP218**, **GLY220**, **THR222**, TYR225, ILE223
	Hydrophobic	Pi-Pi T -shaped	**TYR303**
		Alkyl	**ILE82**, ILE223
		Pi-Alkyl	LEU216, ILE305
	Electrostatic	Pi-Anion	**ASP86**
Delphin	Hydrogen	Conventional & Carbon	**GLY85**, SER301, TYR225, ILE223, **THR222**, **THR221**, VAL119, ASP120, GLY85
	Hydrophobic	Pi-Alkyl	VAL119
	Electrostatic	Pi-Anion	**ASP86**, **ASP218**

Note: bold print represents the similarity of the amino acid residues of the test ligand to the original ligand

3.6. FE-SEM Analysis Results

The FE-SEM images of the negative control at magnifications of 10,000, 15,000, 20,000, and 30,000 are presented in Figure 4. The images reveal that the *Candida albicans* colony structure in the negative control remains intact. The cells exhibit an undamaged, elongated, oval rod-like shape. Additionally, the blastopore pore structure appears smooth, with cells maintaining interconnections in various round to elongated shapes, and both buds and polar hyphae remain intact [28].

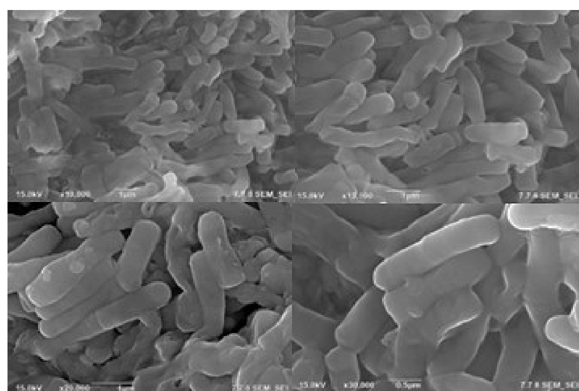

Figure 4. FE-SEM images of negative control *Candida albicans*

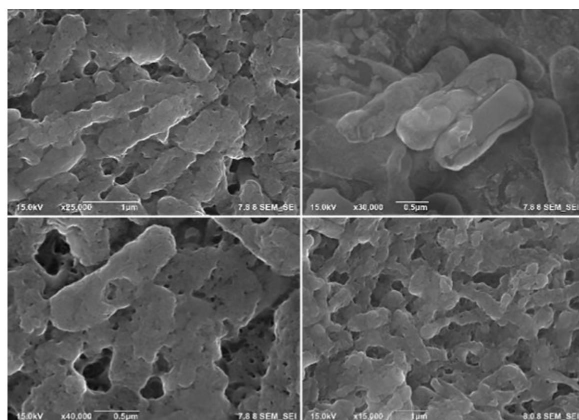

Figure 5. FE-SEM images of 100 ppm of ethanol extract

The FE-SEM results of the 100 ppm ethanol extract, representing the minimum inhibitory concentration for *Candida albicans*, reveal notable structural and morphological changes in the *Candida albicans*. Figure 5 shows extensive cell damage, a change in cell surface morphology characterized by roughness and hollowness, and an overall unclear cellular structure. These findings strongly suggest that the bioactive compounds in the *Cassia fistula* leaf extract exhibit potent antifungal activity against *Candida albicans*. Research by Jothy *et al.* [29] showed that *Cassia fistula* extract actually inhibited the growth of *Candida albicans* and also showed prolonged antifungal activity against *Candida albicans* as determined by the time-kill curve. The phytochemicals in *Cassia fistula* seed extract attack *Candida albicans* cells and cause higher cell damage.

4. Conclusion

Based on the results of in silico tests on compounds in *Cassia fistula* L. leaf extract, specifically delphin, astragalin, hyperoside, isoquercitrin, and epicatechin, it can be concluded that delphin compounds are the leading cause of this disease. Delphin was also the best test compound with a binding affinity and inhibition constant closely resembling those of the synthetic drug ketoconazole. The interaction results showed similarities in chemical interactions and amino acid residues, which were concluded to be potential compounds inhibiting the *Candida albicans* SAP3 protein receptor. The antimicrobial test determining the MIC of the ethanol and ethyl acetate extracts of *Cassia fistula* leaves resulted in a minimum inhibitory concentration of 100 ppm in the ethanol extract and 50 ppm in the ethyl acetate fraction. These findings suggest that both the ethanol extract and ethyl acetate fraction from the leaf samples possess potent antimicrobial inhibitory properties against *Candida albicans*. Furthermore, the FE-SEM analysis of MIC results demonstrated that the ethanol extract at 100 ppm effectively damaged the structure of the *Candida albicans* colony.

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