



Holothurin Compound from Sea Cucumber (*Holothuria* sp.) as Antifungal Alternative against *Candida* Infections

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Abstract

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BACKGROUND: The previous studies have identified chemical compounds in sea cucumbers that have antifungal properties. However, further information on the underlying antifungal needed to be updated.

AIM: This study aimed to discover efficient antifungal treatments against candidiasis disease.

MATERIALS AND METHODS: This study analyzed the antifungal activity from Holothurin against *Candida albicans* *in silico* using molecular docking and minimum inhibitory concentration (MIC).

RESULTS: The results revealed that holothurin has a binding affinity of -7.9 kcal/mol and MIC value of 1.5 mg/ml.

CONCLUSION: Holothurin may inhibit the infection of *C. albicans*. Furthermore, additional research is required to validate the activity of this compound.

Introduction

Candidiasis is an infectious disease caused by *Candida albicans* that has a high mortality. In general, it is found in elderly patients, hospitalized patients, and immunocompromised patients. Candidiasis can be treated by antifungal properties [1], [2]. However, antifungal resistance leads to increased cases of the disease.

Candida albicans involves as virulence factors in the critical for growth, infections, and survival, including Secreted Aspartic Proteinases (SAPs); surface adhesion such as the family of agglutinins (agglutinin-like sequence, ALS); phospholipases; and the ability to form hyphae and biofilms [3].

The subfamily of SAPs is SAP5 which has been highlighted in invasion process mediated E-cadherin degradation. High adhesion capacity is related to biofilm formation which most of these pathogen [4]. Therefore, drugs of anti-adhesion prevent that biofilm formation represent a potential alternative therapy to prevent or control infection of fungal.

Antifungal properties for Candidiasis treatment should have minimum toxicity to human cells [5]. One of the most commonly used antifungal drugs is Caspofungin, which has a specific target to repress *C. albicans*. Caspofungin has fungicidal activity against several fungal species. It has a low level of toxicity to human cells, which is properly required for chronic Candidiasis treatment [6], [7], [8].

Several natural products of marine biota have been reported as potential sources with ideal properties for antifungal development [9]. Sea cucumbers (*Holothuria* sp) in one of the marine organisms discovered in Indonesia [10]. The previous studies have confirmed that sea cucumber extract contains active chemical properties that possess anticancer, anti-coagulant, antimicrobial, antioxidant, antiviral, and anti-inflammatory ability [11], [12], [13], [14], [15]. The dominant active compounds discovered in sea cucumbers are triterpenoid glycosides. [16], [17].

There are still limited reports about the discovery of antifungal properties in holothurin. Therefore, this study aimed to discover the potential of holothurin chemical properties as an antifungal

against *C. albicans*, to aims reveal novel insight into the mechanisms of antifungal activity.

Materials and Methods

In this study, the holothurin samples were obtained from China.cn. The samples came with a concentration of 10mg/10ml in a bottle. Caspofungin is an antifungal medicine that was obtained from Sigma Aldrich. Furthermore, the isolation of fungal *C. albicans* (Code: 4506547065307370) was collected from the laboratory-grown bacterial colony obtained from Microbiology Laboratory, Faculty of Medicine, Brawijaya University.

Prediction antifungal activity

The antifungal activity against *C. albicans* was studied *in silico* using molecular docking. The molecular docking was performed with AutoDock Vina integrated into Pyrex (active site residue 32 and 218) [18].

For the specific target, there were several studies came as consideration to define possible targets against *C. albicans*. The selected protein as ligands were SAP5 (PDB ID: 2QZX), holothurin (CID: 56842184), and Caspofungin (CID:2826718). Results of the molecular docking interaction were analyzed by BIOVIA Discovery Studio.

Inoculum preparation

The fungi *C. albicans* was inoculated on Sabouraud Dextrose Agar (SDA). After incubation, the colonies were transferred using a sterile loop to test tubes containing 9 mL NaCl solution. Then, the turbidity was equalized to the standard procedure using spectrophotometry with absorbance 520 nm.

Determination of minimum inhibitory concentration (MIC)

The determination of the MIC on the *C. albicans* was performed by the Agar Dilution method [19], [20]. Samples in several solution densities were prepared, with the following concentration: Holothurin was prepared in the concentration of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 mg/ml, and Caspofungin was prepared with concentrations 0, 20, 40, 60, 80, 100, 120, 140, and 160 $\mu\text{g}/\mu\text{l}$, respectively

Furthermore, each compound was added into the isolation tube which contains Sabouraud Dextrose Broth (SDB). The solutions were incubated for 24 h at 37°C.

Afterward, *C. albicans* was smeared on the SDA growth medium and the discs were put on. Then, the Petri dishes were incubated for 24 h at 37°C (Figure 1).

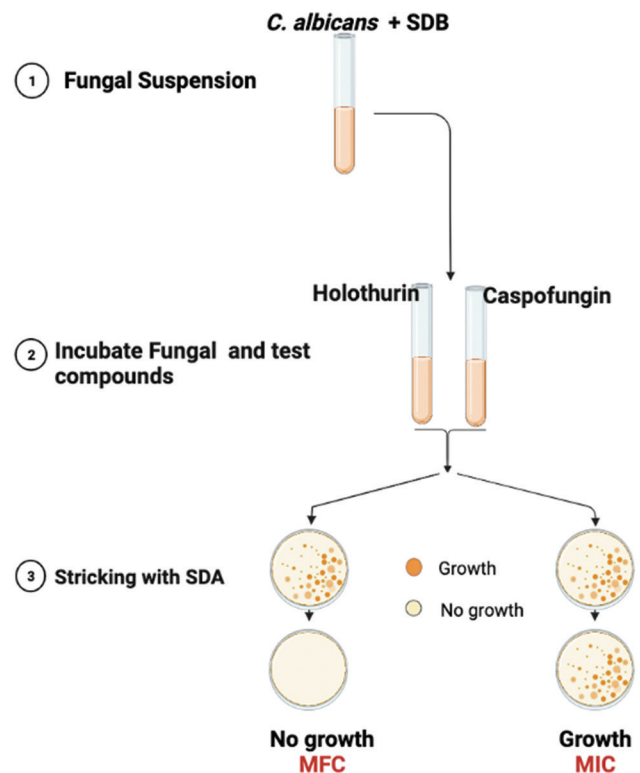


Figure 1: Illustration of MIC test. (MFC) Minimum fungal concentration; (MIC) minimum inhibitor concentration

Results

Antifungal activity

Furthermore, for the *in silico* analysis, we used holothurin as a compound from sea cucumber and SAP5 protein by molecular docking. The results of the docking between SAP5 with holothurin revealed that binding affinity value -7.9 kcal/mol exhibited higher binding affinities.

SAP5 with a known inhibitor Caspofungin with value -7.4 kcal/mol was presented in Figure 2. It was

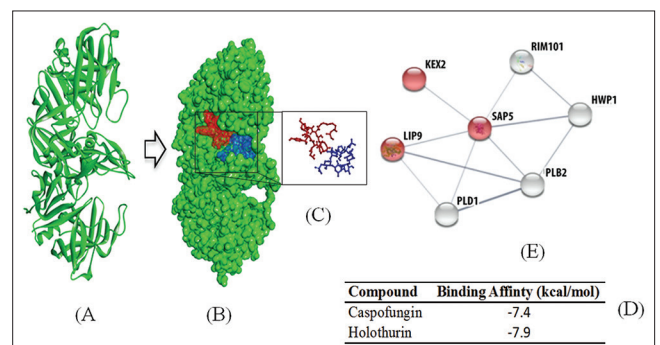


Figure 2: Interaction between protein SAP5 and holothurin (a) 3D structure of protein; (b) binding site between protein-ligand; (c) position of ligands to protein, SAP5 presented in green color, SAP5 inhibitor (caspofungin) presented in red and holothurin in blue; (d) binding affinity of results docking; and (e) network of SAP5 interactions based on the string database which involved protein hydrolase activity (red)

demonstrated that holothurin bound to the active site of SAP5 in a similar position to caspofungin. Based on the *in silico* analysis, these compounds may have potential as SAP5 inhibitors.

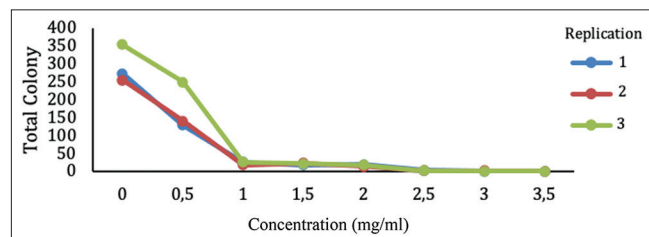


Figure 3: Minimum inhibitory concentration (MIC) of holothurin against *C. albicans*

Minimum inhibitory concentration (MIC)

The antifungal activity of holothurin and Caspofungin was evaluated against *C. albicans* with a minimum inhibitory concentration (MIC) assay. Tests were performed in triplicate, and the mean value of MICs for the holothurin has confirmed that the strong activities against *C. albicans* were obtained by MICs

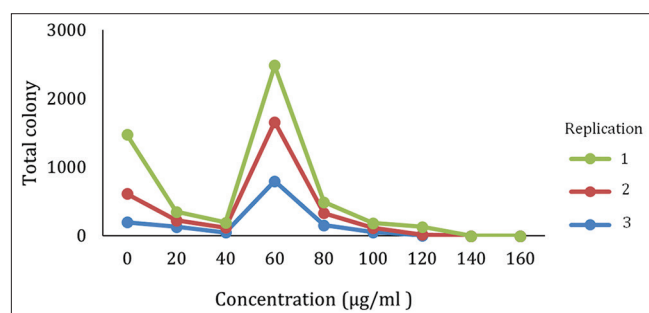


Figure 4: Minimum inhibitory concentration (MIC) of Caspofungin against *C. albicans*. Concentration 0 µg/ml was dilution 10000 folds, 20 µg/ml was dilution 1000 folds, and 40 µg/ml was dilution 100 folds

of 1.5 mg/ml and 3.5 mg/ml to minimum fungicidal concentrations (MFCs) (Figure 3).

However, the MICs result of the Caspofungin was 120 µg/ml and 140 µg/ml as potential MFCs (Figures 4 and 5).

Discussion

The binding of proteins to SAP5 was investigated. The proteins are involved in pathways associated with a fungal infection, including the KEX2 and LIP9 that take part in the hydrolase mechanism (Figure 2). Therefore, the *in silico* result indicated that the inhibition of *C. albicans* by the active compound occurs through inference with the protein that produces hydrolase. However, this discovery still requires further studies to validate these results.

MIC has been used the determination the susceptibility of an antifungal compound of *C. albicans*. The narrow clear zone on the Petri dish indicated that lower antifungal activity and the wide clear zone indicated potent antifungal activities [21]. Holothurin demonstrated that it has potential as alternative antifungal agent against *C. albicans*. As presented in Figure 3, the fungal colonies that grew were very slight, indicating the antifungal concentrations which could inhibit the growth of *C. albicans*.

The inhibition of *C. albicans* growth was initiated by saponin (triterpene glycoside), this compound has been various activities such as antimicrobial, antioxidant, anticancer, and anti-inflammatory [11], [13], [15], [22], [23]. In addition, saponin may inhibit the synthesis of chitin which is produced by CHS3 protein, which leads to the lysis mechanism of *C. albicans* membrane cell [24], [25].

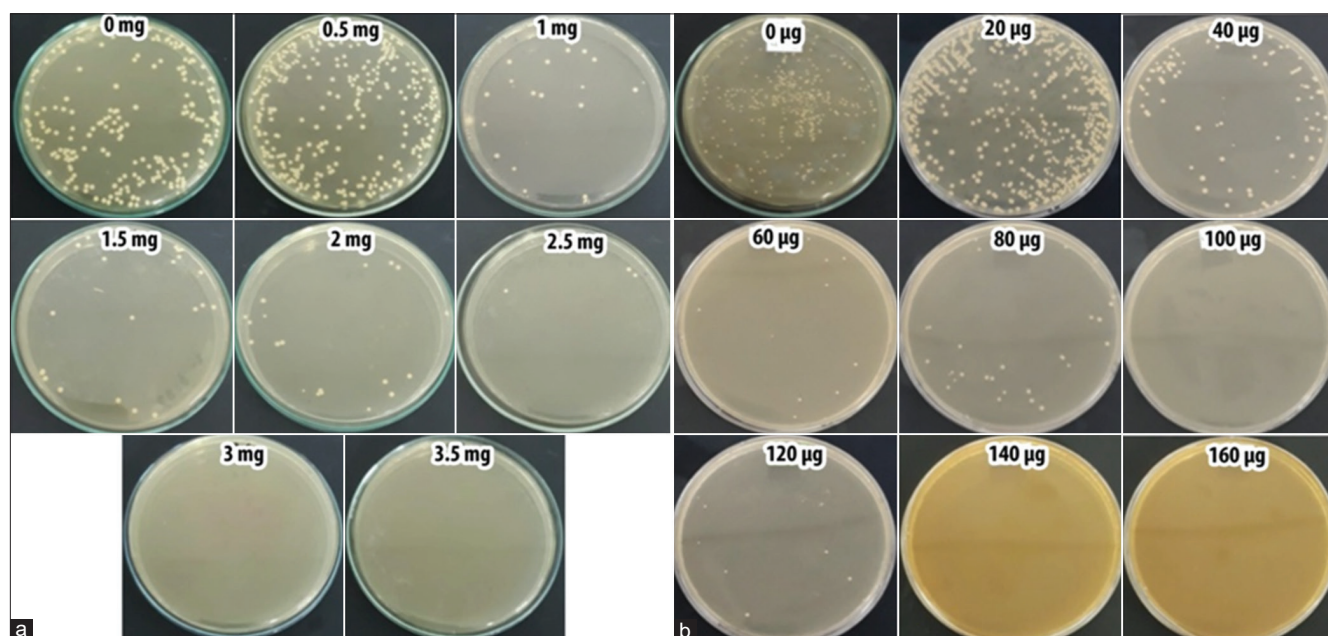


Figure 5: The minimum inhibitory concentration (MIC) of holothurin and Caspofungin against *C. albicans*; (a) holothurin and (b) Caspofungin

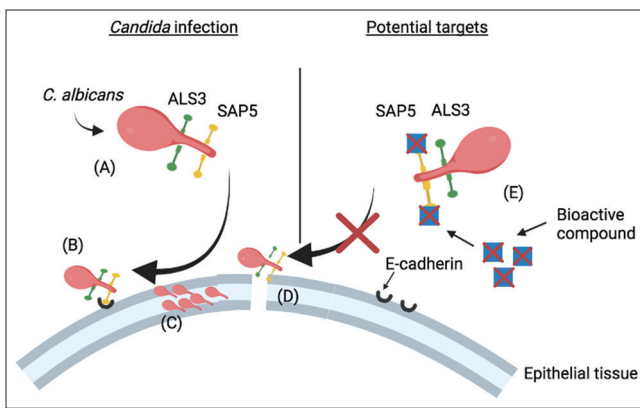


Figure 6: Schematic of the role SAP5 proteins in the progression and establishment of candidiasis. (A) Virulence proteins from *C. albicans* (SAP5); (B) initial adhesion of *C. albicans* to the host tissues mediated by adhesion; (C) biofilm formation; (D) degradation of the intercellular junctions mediated by SAP5 (active invasion); and (E) molecular docking planned to inactivate invasion SAP5 factors to prevent of the infection

Holothurin is one of the saponin compounds that are currently being studied a lot. *C. albicans* invades the host by induced endocytosis [26], this fungal expresses surface proteins E-cadherin and N-cadherin (Figure 6). Segregated aspartic proteases (SAPs) mediate the active further invasion [27], [28], [29]. SAP5 is the subfamily of SAPs compound that has a salient role in the invasion process. Alteration of structural tissue caused by the degradation of this protein may facilitate invasion by *C. albicans* [30].

A previous study discovered that the SAPs family proteins are considered the targets for anti-Candida drug design based on tissue invasion. SAPs reported that it has a salient role in *C. albicans* infection [30]. *C. albicans* produced hydrolytic enzymes, this enzyme may play a menacing role to attack molecules and cells of the host immune system to resist antifungal activity contained in a drug [31].

Conclusion

The present study suggested that the holothurin compound has a 1.5 mg/ml MIC value against *C. albicans*. Holothurin may have potential as antifungal activity as the inhibitor of SAP5. However, this finding required further studies to evaluate holothurin's effectiveness as an antifungal agent.

Authors Contribution

N contributes, oversees directing, and mentoring during MIC and research. AT contributed

to Instrumentation, experimental design, and reagent supplies. SW conducts MIC direction and guidance. SH conducted experiments and experiments. All authors were collected the data and wrote the manuscript. All authors approved the final version of the manuscript.

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