RESEARCH ARTICLE

Editorial Process: Submission:02/15/2023 Acceptance:10/16/2023

The Antifungal Activities of *Syzygium aromaticum* and *Alpinia purpurata* Extracts Against *Candida krusei*: Bioactivity Tests, Molecular Modeling, and Toxicity Tests

Dzia Ulhaq Rohadatul Aisy[1], Robiatul Adawiyah[1,2,3,4], Anna Rozaliyani[2], Ari Estuningtyas[5], Fadilah Fadilah[1,6,7]*

Abstract

Background: *Candida krusei* is the cause of the fungal infection candidiasis, which has a high mortality rate. Intrinsic resistance to fluconazole can cause the failure of Krusei candidiasis treatment. Therefore, it is necessary to find alternative drugs to eliminate the fungus. Extracts of *Syzygium aromaticum* and *Alpinia purpurata* have been proven to be alternative solutions for treating *Candida krusei* resistance. **Objective:** This study aims to explore the active compounds *Syzygium aromaticum* and *Alpinia purpurata* as treatments against *Candida krusei* through bioactivity tests, molecular modeling, and toxicity tests. **Methods:** Determination of antifungal activity with the agar well diffusion and microbroth dilution method. Molecular modeling was conducted using the following software: Marvin Sketch, LigandScout 4.4.5, AutoDock ver 4.2.6, PyMOL, LigPlus, MOE ver 2008. **Result:** Bioactivity test results of the two natural extracts against C. krusei ATCC 6258, it was found that the S. aromaticum and A. purpurata extracts have MIC50 values of 0.031 µg/mL and $1.435x10^5$ µg/mL. The molecular modeling found that the compounds Benzotriazole, 1-(4-methyl-3-nitrobenzoyl)-, 1,3,4-Eugenol Acetate, Stigmasta-5,22-dien-3-ol, acetate (3 beta)- and Farnesyl acetate from the two natural extracts, interacts with the active site of the enzyme lanosterol-14-α-demethylase with a binding energy of -8.91, -6.04, -13.53, and -7.15 kcal/mol. The oral acute toxicity test of S. aromaticum and A. purpurata extracts proved that the LD50 was >6000 mg/kg BW and >8000 mg/kg BW. The acute dermal toxicity test of the two extracts showed that the LD50 was >6000 mg/kg BW. **Conclusion:** S. a*romaticum* and A. *purpurata* extracts have been proven to be alternative solutions for treating *Candida krusei* resistance.

Keywords: *Candida krusei*- *Syzygium aromaticum*- *Alpinia purpurata*- lanosterol-14-α demethylase

Asian Pac J Cancer Prev, **24 (10)**, 3403-3409

Introduction

The rise in the number of immunocompromised patients, such as premature babies, HIV (Human Immunodeficiency Virus) patients, cancer patients, and recipients of immunosuppressive drug therapy, has led to an increase in the number of fungal infections in recent years (Denning & Bromley, 2015). More than 300 million people worldwide suffer from serious fungal infections, causing more than 1.5 million deaths each year, with the highest cause of death being Candida spp (Schmiedel & Zimmerli, 2016). As many as 35% -65% of Candida spp infections are caused by non-Candida albicans (Tan et al., 2010). Candida glabrata and *Candida krusei* are common causes of candidiasis in the bloodstream. Treatment failure due to *Candida krusei*'s intrinsic resistance to fluconazole is one of the causes of the high mortality rate from *Candida krusei* infection, with a prevalence of 20-40% (Krcmery & Barnes, 2002)(Quindós et al., 2008)Whaley et al., 2017).

Candida krusei's resistance mechanism to fluconazole is attributed to several factors: the drug's lower affinity towards lanosterol-14α-demethylase, the overexpression of lanosterol-14α-demethylase, the decreased effective concentration of antifungal drugs by efflux pump activity, and the formation of biofilms that hinder the antifungal diffusion process (Berkow & Lockhart, 2017; Jamiu et al., 2021; Cuéllar-Cruz et al., 2012). An alternative solution to handling *Candida krusei* resistance is the development

*[1]Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia. [2]Department of Parasitology, Faculty of Medicine, Universitas Indonesia. [3]Study Program of Clinical Parasitology, Faculty of Medicine, Universitas Indonesia. [4]Infectious Diseases and Immunology Research Center (IDIRC), Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. [5]Farmacology department, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. [6]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. [7]Bioinformatics Core Facilities, IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. *For Correspondence: fadilah.msi@ui.ac.id. Dzia Ulhaq Rohadatul Aisy and Robiatul Adawiyah have equal contribution in this study.*

of natural products, provided that they have low toxicity to humans (Cuéllar-Cruz et al., 2012; Khoddami et al., 2013). Phenolic compounds in many vegetable sources can be an alternative to antifungal drugs (Khoddami et al., 2013). This is because the hydrophobic properties of phenolic compounds allow them to enter the lipid membrane of the fungus, thereby affecting the function of the cell membrane and causing homeostasis disturbances, resulting in the disruption of the integrity of the fungal cell (Rao et al., 2010).

Indonesia has diverse flora and medicinal plants that could potentially work against Candida (Karo et al., 2017). Phenolic compounds of *Syzygium aromaticum* and *Alpinia purpurata*, such as eugenol, flavonoids, hydroxybenzoic acid, hydroxycinnamic acid, hydroxyphenyl propane, saponins, and tannins, are known to have the potential to be used against Candida (Kochuthressia et al., 2010; Yassin et al., 2020; C et al., 2012). Flavonoids work by inhibiting the fungal nucleic acid synthesis process and destabilizing cell membranes by changing the properties of fungal cell membranes. In comparison, saponins work as antifungals by interfering with the permeability of the fungal cell wall (Fakhrurrazi et al., 2012).

The potency of *Syzygium aromaticum* and *Alpinia purpurata* as anti-*Candida krusei* agents was discovered through bioactivity tests, molecular modeling, and toxicity tests. The agar well diffusion and microbroth dilution bioactivity methods were conducted to determine the antifungal activity of *Syzygium aromaticum* and *Alpinia purpurata* based on their fungal growth patterns (Balouiri et al., 2016). For the development of antifungal drugs, molecular modeling using the in-silico method through molecular docking and dynamic simulations was performed to determine the affinity of new compounds toward target enzymes (Bitencourt-Ferreira & de Azevedo, 2019). Overall, it is necessary to study the antifungal activity of extracts of *Syzygium aromaticum* and *Alpinia purpurata* in inhibiting the growth of *Candida krusei* as potential agents that can overcome antifungal drug resistance in *Candida krusei* infections. The antifungal activities of the above-mentioned extracts can be investigated through bioactivity testing, molecular modeling, and toxicity testing.

Materials and Methods

Preparation of Candida krusei ATCC 6258 Fungus Test Samples

The test fungus were grown on Sabouraud Dextrose Agar at room temperature for 24 hours. The fungal suspensions were formed with sterile NaCl 0,9% with a concentration of 0.5 McFarland.

Preparation of Test Solutions

Five concentrations of fluconazole controls (4, 8, 16, 32, and 64 µg/mL) along with five concentrations of ethanol extracts of *Syzygium aromaticum* and *Alpinia purpurata* were made (1600, 800, 400, 200, 100 mg/mL with 10% DMSO solution) (Omar, 2017; Kamoda et al., 2020).

Determination of Antifungal Activity with the Agar Well Diffusion Method

The *Candida krusei* suspension was smeared on Müller Hilton's media, and holes of 6 mm diameter were made aseptically. 50 µL of fluconazole and the ethanol extracts of *Syzygium aromaticum* and Alpinia purpurata were added to the wells. They were then incubated at 37 ℃ for 24 hours. Antifungal activity was observed based on the diameter of the inhibition area (Sahal et al., 2019; Cordeiro et al., 2014). Guidelines for the disk diffusion susceptibility test of Candida species were adapted from the M44 document from the Clinical and Laboratory Standards Institute (CLSI) (Sheehan et al., 2004).

Determination of Antifungal Activity with the Microbroth dilution method

Guidelines for the in vitro susceptibility of Candida species were adapted from the M27-A3 document from the Clinical and Laboratory Standards Institute (CLSI) (Song et al., 2015). RPMI 1640 media was placed into a 96-well round-bottom microplate. Then, the fungal suspensions were tested. The fluconazole and ethanol extracts of *Syzygium aromaticum* and *Alpinia purpurata* were added to the positive control and test columns. Negative controls and growth controls were included in each run. Each test was conducted three times and incubated at 35℃. Antifungal activity was read using an ELISA reader based on optical density (OD) values at a wavelength of 405 nm before and after 24 hours of incubation. The value of antifungal inhibition was calculated by the following formula:

% of inhibition = (1-((ODt24-ODt0)/(ODk24-ODk0))) x 100

Key:

ODt_{24}= The test sample's optical density value after 24 hours of incubation.

ODt_0 = The test sample's optical density value before 24 hours of incubation.

ODk_{24}= The optical density value of the control after 24 hours of incubation.

ODk_0= The optical density value of the control before 24 hours of incubation.

(Kaya & Ozbilge, 2012; Pratiwi et al., 2015)

Molecular modeling

Molecular modeling was conducted using the following software: Marvin Sketch, LigandScout 4.4.5, AutoDock ver 4.2.6, PyMOL, LigPlus, MOE (Molecular Operating Environment) ver 2008, and Notepad++ applications, as well as the NCBI website (https://www.ncbi.nlm.nih.gov/), Protein Data Bank, (https://www.rcsb.org/), SWISS-MODEL (https://swissmodel.expasy.org/), PubChem (https://pubchem. ncbi.nlm.ni.gov), ChemSpider (http://www.chemspider.com/).

Protein and Ligand Preparation

The target protein *Candida krusei* lanosterol-14-α-demethylase was obtained by creating a 3-dimensional structural model on the SWISS-MODEL website (https://swissmodel.expasy.org/) based on target FASTA sequences from the NCBI database (https://www.ncbi.

nlm.nih.gov(Jamiu et al., 2021). The proteins were then optimized into PDBQT files by using AutoDocks. The active compounds in the *Syzygium aromaticum* and *Alpinia purpurata* extracts were selected from secondary data from a gas chromatography–mass spectrometry (GC-MS) analysis at the DKI Jakarta Provincial Health Laboratory. The 3D structures of the active compounds were downloaded via PubChem and ChemSpider and optimized using Marvin Sketch and AutoDocks.

Molecular Docking Analysis and Visualization

The target protein and ligand were entered into AutoDock ver 4.2.6. Gridbox was added at the protein receptor site with the coordinates X:18,701 Y:8,328 Z:23,177 and dimensions X:40 Y:40 Z:40. The Lamarckian Genetic Algorithm method was used to calculate the protein and ligand interactions. The affinity value of the protein bond with the ligand compound determines the strength of the conformation. The lower the binding energy value (delta G) is the stronger the interaction between the ligand and the protein. The visualization and analysis of the interactions between the target protein and ligands were done using the PyMOL and LigPlus software.

Dynamic Simulation Analysis

The molecular dynamic simulation analysis was simulated using MOE (Molecular Operating Environment) version 2008. The simulation consisted of three main steps: initialization, equilibration, and production. The simulation was performed using the ensemble isobaric-isothermal (NPT) and ensemble canonical algorithm (NVT). In the initialization and equilibration stages, the simulation was conducted at 100 ps at 300 K, whereas the production stage was performed at 1000 ps (1 ns) at 300 K. The simulation results were then analyzed to determine the stability of the lanosterol-14-α-demethylase-ligand complex.

Acute Oral Toxicity

The acute oral toxicity test was performed per the OECD-423 guidelines (Acute toxic class method) and used male Wistar strain white rats aged 3-4 weeks.

Acute Dermal Toxicity

The acute oral toxicity test was performed per the OECD-402 guidelines (Acute dermal toxicity) and used male Wistar strain white rats aged 3-4 weeks.

Results

Antifungal activity test

The antifungal activity test with the agar well diffusion method found that *Syzygium aromaticum* and *Alpinia purpurata* extracts could inhibit the growth of *Candida krusei* ATCC 6258 with an inhibition zone diameter of 19.67-34.33 mm and 7.33-11.0 mm (Figure 1). The antifungal activity test with the microbroth dilution method found that the MIC50 and MIC90 value were defined as the lowest concentration of the antibiotic at which 90 and 50% of *Candida krusei* were inhibited, respectively. The MIC50 and MIC90 of *Syzygium aromaticum* extract (MIC50 : 0,031 µg/mL, MIC90: 2,15 x103 µg/mL) was higher than the *Alpinia purpurata* extract (MIC50 : 1,435x105 µg/mL, MIC90: 12,03 x105 µg/mL) (Figure 2).

Molecular modeling test

The modeling results from the AutoDock program found two compounds with the lowest binding energy from the extract of *Syzygium aromaticum*, namely, Benzotriazole,1-(4-methyl-3-nitrobenzoyl)- and 1,3,4-Eugenol acetate. The benzotriazole compound, 1-(4-methyl-3-nitrobenzoyl), with a binding value of -8.91 kcal/mol, binds to amino acid residues at the active heme binding site at the amino acid position Val306 and Cys475 (Figure 3). Whereas 1,3,4-Eugenol Acetate, with a binding value of -6.04 kcal/mol, binds to amino acid residues on

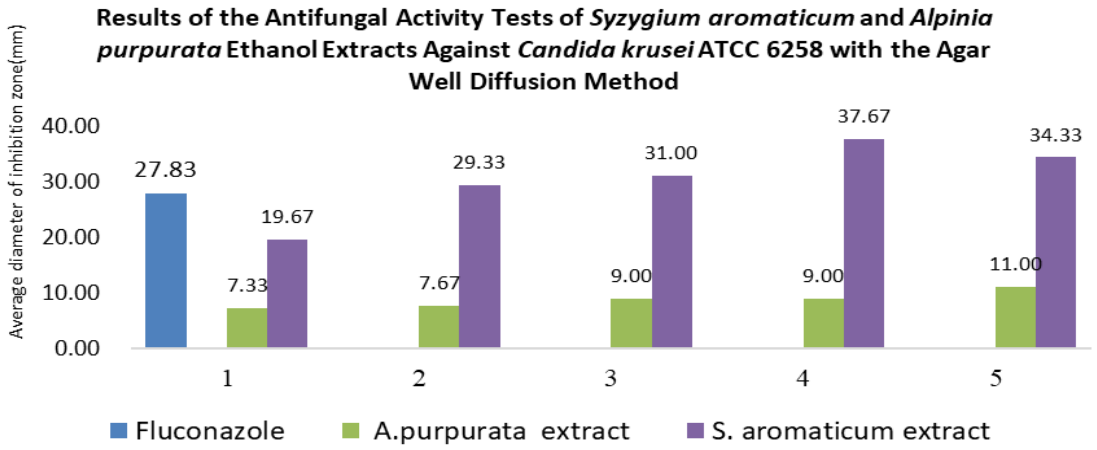


Figure 1. Results of the Antifungal Activity Tests of *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts Against Candida *krusei* ATCC 6258 with the Agar Well Diffusion Method, (1) *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts 100 mg/mL, (2) *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts 200 mg/mL, (3) *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts 400 mg/mL, (4) *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts 800 mg/mL, (5) *Syzygium aromaticum* and *Alpinia purpurata* Ethanol Extracts 1600 mg/mL

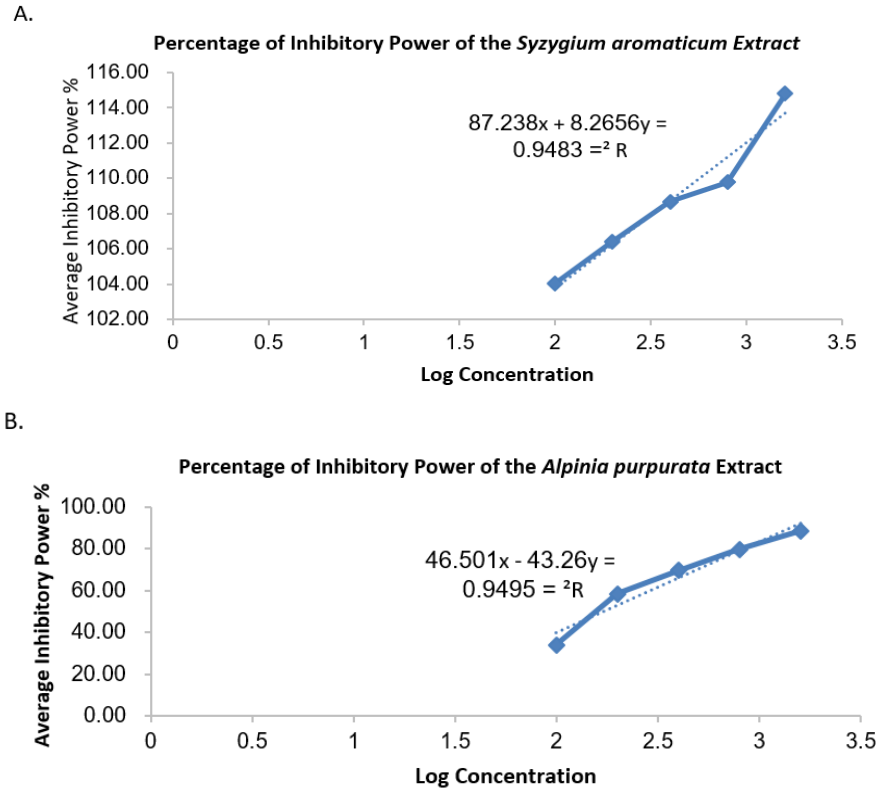
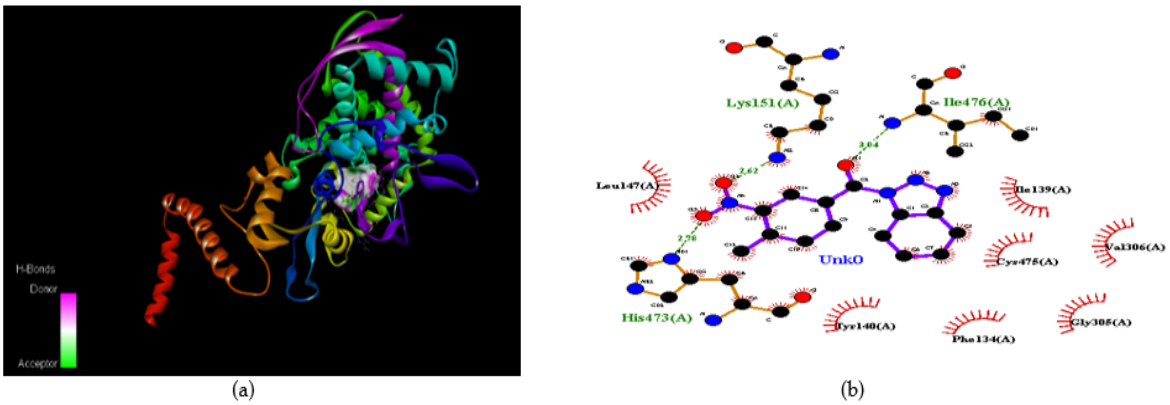


Figure 2. Curve of Percentage Inhibition of Fluconazole Ethanol Extracts of *Syzygium aromaticum* and *Alpinia purpurata* against Candida *krusei* ATCC 6258. The value of antifungal inhibition was calculated by the following formula : % of inhibition = (1-((ODt24-ODt0)/(ODk24-ODk0))) x 100. (A) Concentrations of *Syzygium aromaticum* to Candida *krusei* ATCC 6258 shows a line equation y=8.2656x + 87.238, so that the concentration that can produce an inhibitory power of 50% is 0.031 µg/mL, (B) Concentrations of *Alpinia purpurata* to Candida *krusei* ATCC 6258 shows the line equation y = 43.26x - 46.501, so the concentration that can produce an inhibitory power of 50% is $1.435x10^5$ µg/mL.

the active site of the chemical substrate binding pocket at the amino acid position His377 (Figure 4).

The results of *Alpinia purpurata* extract modeling showed two compounds with the lowest binding energy being Stigmasta-5,22-dien-3-ol, acetate (3 beta)- and Farnesol, acetate. The Stigmasta-5,22-dien-3-ol, acetate (3 beta) compound, with a binding energy value of -13.53 kcal/mol, binds to amino acid residues on the active site of the chemical substrate binding pocket at the amino acid position His377 and Phe380 (Figure 5). The Farnesol compound, acetate, with a binding energy value of 7.15 kcal/mol, binds to amino acid residues in the active site of the chemical substrate binding pocket at the amino acid positions His377 and Phe380 (Figure 6).

The results of the oral and dermal acute toxicity tests of *Syzygium aromaticum* extract found no signs of toxicity and death in the test group with doses of > 6000 mg/kg BW (LD50 > 6000 mg/kg BW). Meanwhile, the *Alpinia purpurata* extract was obtained > 8000 mg/kg BW (LD50 > 8000 mg/kg BW). The results of the acute dermal toxicity test of *Alpinia purpurata* and *Syzygium aromaticum* were L > 6000 mg/kg BW (LD50 > 6000



(a) (b)

Figure 3. (a) Three-dimensional (b) and two-dimensional visualizations of the interactions between Benzotriazole,1-(4-methyl-3-nitrobenzoyl)- and lanosterol-14-α-demethylase

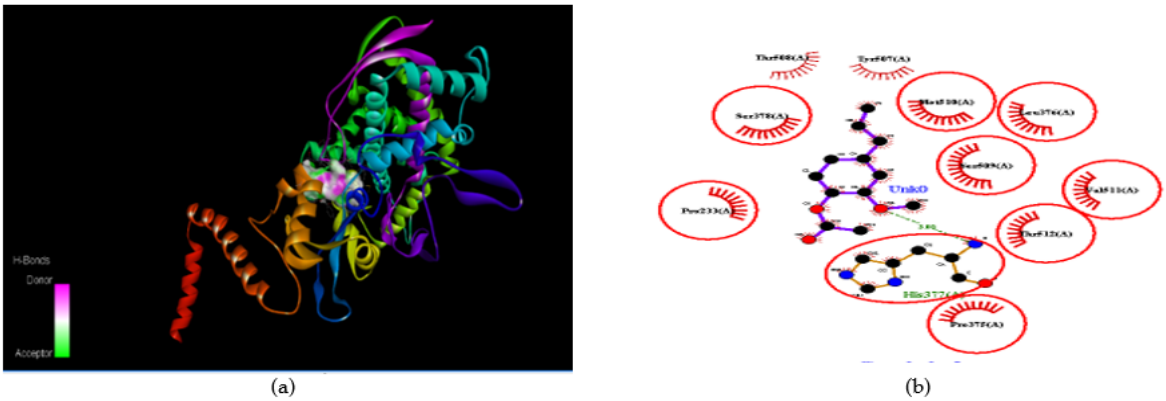


Figure 4. (a) Three-dimensional (b) and two-dimensional visualizations of the interactions between 1,3,4-Eugenol Acetate and lanosterol-14-α-demethylase

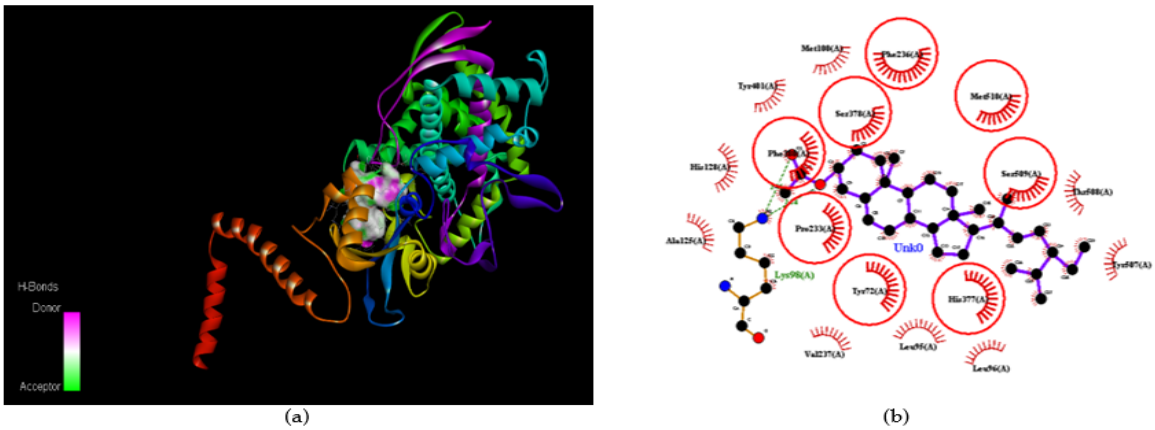


Figure 5. (a) Three-dimensional (b) and two-dimensional visualizations of the interactions between Stigmasta-5,22-dien-3-ol, acetate (3 beta)- with the lanosterol-14-α-demethylase enzyme

mg/kg BW).

Discussion

Alkaloids, saponins, flavonoids, phenols, and tannins from extracts of *Syzygium aromaticum* and *Alpinia purpurata* are known to have curative activity against several pathogenic agents such as C. albicans (Fakhrurrazi et al., 2012; Yassin et al., 2020). Past studies have also shown that tannins, flavonoids, and saponins can act as antifungals by binding to enzymes that play an important role in forming fungal cell membranes. By doing so, they inhibit nucleic acid synthesis, change the properties of fungal cell membranes, and disrupt their permeability (Anani et al., 2016; K.R. et al., 2013).

The average diameter of the zone of inhibition of fungal growth after the administration of *Syzygium aromaticum* extract was greater than that of *Alpinia purpurata* extract. This result is supported by the plant's eugenol content (72-90%) (Mbaveng & Kuete, 2017). Previous studies also found that the zone of inhibition of *Syzygium aromaticum* extracts to C. albicans (25.2 ±

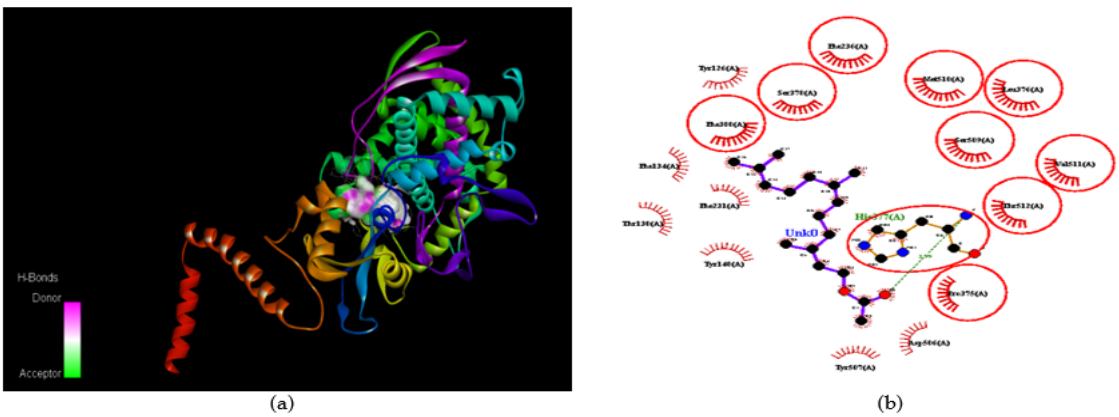


Figure 6. (a) Three-dimensional (b) and two-dimensional visualizations of the interactions between Farnesol, acetate and the lanosterol-14-α-demethylase enzyme

1.4 mm) (Gonelimali et al., 2018) was greater than that of *Alpinia purpurata* (12.4 ± 0.2 mm) (Kochuthressia et al., 2010).

Eugenol from the *Syzygium aromaticum* and *Alpinia purpurata* extracts can inhibit fungal growth by reducing the expression of the Candida tropicalis proteinase by 64.92-87.80%. This reduction disrupts fungal cells from obtaining nutrients, enables invasion and dimorphism, and disturbs biofilm formation (Pandey et al., 2018; Khan & Ahmad, 2013). In addition, the binding of eugenol to the cell surface and penetration onto the target site (the lipid bilayer of the cytoplasmic membrane) causes the depolarization of fungal cells, which results in ion leakage and loss of membrane potential. This loss leads to a disruption of cellular function which will eventually cause cell death (Latifah-Munirah et al., 2015).

Based on the MIC50 value in research it was found that the *Syzygium aromaticum* extract was included in the category of good activity, while the *Alpinia purpurata* extract was included in the inactive category in inhibiting the growth of *Candida krusei* ATCC 6258. (Indrayanto et al., 2021) The results of the MIC50 test of the *Syzygium aromaticum* extract are close to the previous MIC50 tests for C. albicans at 0.0976 mg/mL. This result is likely due to its high eugenol content (45-90%) (Kamatou et al., 2012). Furthermore, other studies have shown a decrease in the Candida virulence factor (Cell Surface Hydrophobicity) after adding *Syzygium aromaticum* extract (R. Goswami et al., 2017; Khan & Ahmad, 2013). According to Afanyibo et al. (2018) and Indrayanto et al. (2021), the *Syzygium aromaticum* L. extract is included in the category of good activity in inhibiting the growth of *Candida krusei* ATCC 6258. Meanwhile, the *Alpinia purpurata* extract results are supported by a previous study where the minimum inhibitory content of C. albicans is 200 mg/ml with an inhibition of 60% (Kamoda et al., 2020).

The binding energy value of each ligand can be seen from the molecular modeling of the compounds extracted from *Syzygium aromaticum* and *Alpinia purpurata*. The ligand with the smallest binding energy has the highest possibility to interact well with the target protein. The enzyme lanosterol-14-α-demethylase was chosen in the design of antifungal drugs as a molecular binding target because of its essential role in ergosterol biosynthesis, which is an important component of fungal cell membranes (Rodrigues, 2018). Based on the NCBI database, there are two binding sites for the enzyme lanosterol-14- α-demethylase *Candida krusei*: the heme binding site and the chemical substrate binding pocket. The heme binding site on the enzyme lanosterol-14-α-demethylase plays an important role in the binding of iron, which is a cofactor (Sutak et al., 2008). A decrease in iron causes the down-regulation of ERG11 and failure in ergosterol biosynthesis, thereby increasing membrane fluidity (Prasad et al., 2006).

The in-silico screening test through Admestar (http://lmmd.ecust.edu.cn/admetsar2) based on Lipinski's Rule of Five criteria can be used to evaluate the drug similarity of a chemical compound. These criteria state that the compound should have less than five hydrogen bond donors, less than ten hydrogen bond acceptors, a molecular mass lower than 500 Daltons, and a log P value of less than five (Turner & Agatonovic-Kustrin, 2006). Overall, the drug candidate compounds from the *Syzygium aromaticum* and *Alpinia purpurata* ethanol extracts showed good compatibility and complied with Lipinski's Rules of Five criteria. Therefore, they showed similar bioavailability orally with the active drug, which allows drug-candidate compounds to bind easily to the receptors and cross cell membranes well (Singh et al., 2013).

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

This study was supported by the International Indexed Publication Universitas Indonesia. This data was used by Dzia Ulhaq Rohadatul Aisy for her Master Thesis in the Faculty of Medicine, Universitas Indonesia. This study was approved by the institution ethic committee of the The Faculty of Medicine, University of Indonesia - Cipto Manungkusumo Hospital.

Conflict of Interest

All authors declared no conflicts of interest

References

Afanyibo YG, Anani K, Esseh K, et al (2018). Antimicrobial Activities of *Syzygium aromaticum* (L.) Merr. and L.M. Perry (Myrtaceae) Fruit Extracts on Six Standard Microorganisms and Their Clinical Counterpart. *OALib*, **5**, 1–13.

Anani K, Adjrah Y, Ameyapoh Y, et al (2016). Antimicrobial, Anti-inflammatory and antioxidant activities of Jatropha multifida L. (Euphorbiaceae). *Pharmacognosy Res*, **8**, 142–6.

Balouiri M, Sadiki M, Ibnsouda SK (2016). Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*, **6**, 71–9.

Berkow EL, Lockhart SR (2017). Fluconazole resistance in Candida species: A current perspective. *Infect Drug Resist*, **10**, 237–45.

Bitencourt-Ferreira G, de Azevedo WF (2019). Docking with SwissDock. *Methods Mol Biol*, **2053**, 189-202.

Justi Jovitta C, Sreenivasan A, Suja S (2012). In-Vitro Antioxidant And Phytochemical Screening Of Ethanolic Extract Of *Alpinia Purpurata*. *Int J Pharm Sci Res*, **3**, 2071–4.

Cordeiro R, Lima-Filho JV, Aguiar R de (2014). In Vitro and In Vivo Antibacterial and Antifungal Screening of Natural Plant Products: Prospective Standardization of Basic Methods. In Methods and Techniques in Ethnobiology and Ethnoecology (p. 480).

Cuéllar-Cruz M, Vega-González A, Mendoza-Novelo B, et al (2012). The effect of biomaterials and antifungals on biofilm formation by Candida species: A review. *Eur J Clin Microbiol Infect Dis*, **31**, 2513–27.

Denning DW, Bromley MJ (2015). How to bolster the antifungal pipeline. *Science*, **347**, 1414–6.

Fakhrurrazi F, Hakim RF, Cahya C (2012). Inhibition of 10% Alpinia galanga and *Alpinia purpurata* rhizome extract on Candida albicans growth. *Dent J*, **45**, 84.

Gonelimali FD, Lin J, Miao W, et al (2018). Antimicrobial

properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol*, **9**, 1–9.

Indrayanto G, Putra GS, Suhud F (2021). Validation of in-vitro bioassay methods: Application in herbal drug research. *Profiles Drug Subst Excip Relat*, **46**, 273–307.

Jamiu AT, Albertyn J, Sebolai OM, Pohl CH (2021). Update on *Candida krusei*, a potential multidrug-resistant pathogen. *Med Mycol*, **59**, 14–30.

Subash KR, Muthulakshmi Bhaarathi G, Jagan Rao N, Cheriyan BV (2013). Phytochemical Screening And Acute Toxicity Study Of Ethanolic Extract Of Alpinia Galanga In Rodents. *Int J Parasitol*, **2**, 93–100.

Kamatou GP, Vermaak I, Viljoen AM (2012). Eugenol-From the Remote Maluku Islands to the International Market Place: A Review of a Remarkable and Versatile Molecule. *Molecules*, **17**, 6953–81.

Kamoda H, Lelyana S, Sugiaman VK (2020). Kadar hambat minimum dan kadar bunuh minimum ekstrak etanol lengkuas merah (*Alpinia galanga* L.) terhadap pertumbuhan Candida albicans The minimum inhibitory concentration and a minimum lethal dose of red galangal (*Alpinia galanga* L.) ethanolic extract on. *Jurnal Kedokteran Gigi Universitas Padjadjaran*, **32**, 1.

Karo MB, Tambaip T, Hatta M, et al (2017). A mini review of Indonesian medicinal plants for Vulvovaginal candidiasis. *Rasayan J Chem*, **10**, 1280–8.

Kaya E, Ozbilge H (2012). Determination of the effect of fluconazole against candida albicans and candida glabrata by using microbroth kinetic assay. *Turk J Med Sci*, **42**, 325–8.

Khan MSA, Ahmad I (2013). In vitro Inhibition of Growth and Virulence Factors Production in Azole-Resistant Strains of Non-albicans Candida by Cinnamomum verum, Cymbopogon citratus, Cymbopogon martini and *Syzygium aromaticum* Essential Oils. *J Biol Act Prod Nat*, **3**, 139–53.

Khoddami A, Wilkes MA, Roberts TH (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, **18**, 2328–75.

Kochuthressia K, Britto S, Jaseentha M, Raj L, Senthilkumar S (2010). Antimicrobial efficacy of extracts from *Alpinia purpurata* (Vieill.) K.Schum. against human pathogenic bacteria and fungi. *Agr Biol J N Am*, **1**, 1249–52.

Krcmery V, Barnes AJ (2002). Non-albicans Candida spp. causing fungaemia: Pathogenicity and antifungal resistance. *J Hosp Infect*, **50**, 243–60.

Latifah-Munirah B, Himratul-Aznita WH, Mohd Zain N (2015). Eugenol, an essential oil of clove, causes disruption to the cell wall of Candida albicans (ATCC 14053). *Front Life Sci*, **8**, 231–40.

Mbaveng AT, Kuete V (2017). *Syzygium aromaticum*. Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases, pp 611–25.

Omar S (2017). Antifungal And Phytochemical Constituents Study of Clove Oil World Journal Of Pharmaceutical Antifungal And Phytochemical Constituents Study of Clove Oil. December.

Pandey N, Gupta MK, Tilak R (2018). Extracellular hydrolytic enzyme activities of the different Candida spp. isolated from the blood of the Intensive Care Unit-admitted patients. *J Lab Physicians*, **10**, 392–6.

Prasad T, Chandra A, Mukhopadhyay CK, Prasad R (2006). Unexpected link between iron and drug resistance of Candida spp.: Iron depletion enhances membrane fluidity and drug diffusion, leading to drug-susceptible cells. *Antimicrob Agents Chemother*, **50**, 3597–606.

Pratiwi SUT, Lagendijk EL, Hertiani T, et al (2015). Antimicrobial effects of indonesian medicinal plants extracts on planktonic and biofilm growth of pseudomonas Aeruginosa and Staphylococcus Aureus. *Int J Pharm Sci*, **7**, 183–91.

Quindós G, Sánchez-Vargas LO, Villar-Vidal M, et al (2008). Activities of fluconazole and voriconazole against bloodstream isolates of Candida glabrata and *Candida krusei*: a 14-year study in a Spanish tertiary medical centre. *Int J Antimicrob Agents*, **31**, 266–71.

Goswami RR, Pohare DS, Raut SJ, Mohan Karuppayil S (2017). Cell Surface Hydrophobicity as a Virulence Factor in Candida albicans. *Biosci Biotechnol Res Asia*, **14**, 1503–11.

Rao A, Zhang Y, Muend S, Rao R (2010). Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. *Antimicrob Agents Chemother*, **54**, 5062–9.

Rodrigues ML (2018). The multifunctional fungal ergosterol. *MBio*, **9**.

Sahal G, Nasseri B, Ebrahimi A, Bilkay IS (2019). Electrospun essential oil-polycaprolactone nanofibers as antibiofilm surfaces against clinical Candida tropicalis isolates. *Biotechnol Lett*, **2019**, 0123456789.

Schmiedel Y, Zimmerli S (2016). Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and Pneumocystis pneumonia. *Swiss Med Wkly*, **146**, w14281.

Sheehan DJ, Brown SD, Pfaller MA, et al (2004). Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. *NCCLS Document MA44-A*, **24**.

Singh S, Gupta AK, Verma A (2013). Molecular properties and bioactivity score of the aloe vera antioxidant compounds - in order to lead finding. *Res J Pharm Biol Chem Sci*, **4**, 876–81.

Song YB, Suh MK, Ha GY, Kim H (2015). Antifungal susceptibility testing with etest for Candida species isolated from patients with oral candidiasis. *Ann Dermatol*, **27**, 715–20.

Sutak R, Lesuisse E, Tachezy J, Richardson DR (2008). Crusade for iron: iron uptake in unicellular eukaryotes and its significance for virulence. *Trends Microbiol*, **16**, 261–8.

Tan TY, Tan AL, Tee N, et al (2010). The increased role of non-albicans species in candidaemia: Results from a 3-year surveillance study. *Mycoses*, **53**, 515–21.

Turner JV, Agatonovic-Kustrin S (2006). In silico prediction of oral bioavailability. *Compr Med ChemI*, **5**, 699–724.

Whaley SG, Berkow EL, Rybak JM, et al (2017). Azole antifungal resistance in Candida albicans and emerging non-albicans Candida Species. *Front Microbiol*, **7**, 2173.

Yassin MT, Mostafa AAF, Al-Askar AA (2020). In vitro anticandidal potency of *Syzygium aromaticum* (clove) extracts against vaginal candidiasis. *BMC Complement Med Ther*, **20**, 1–9.