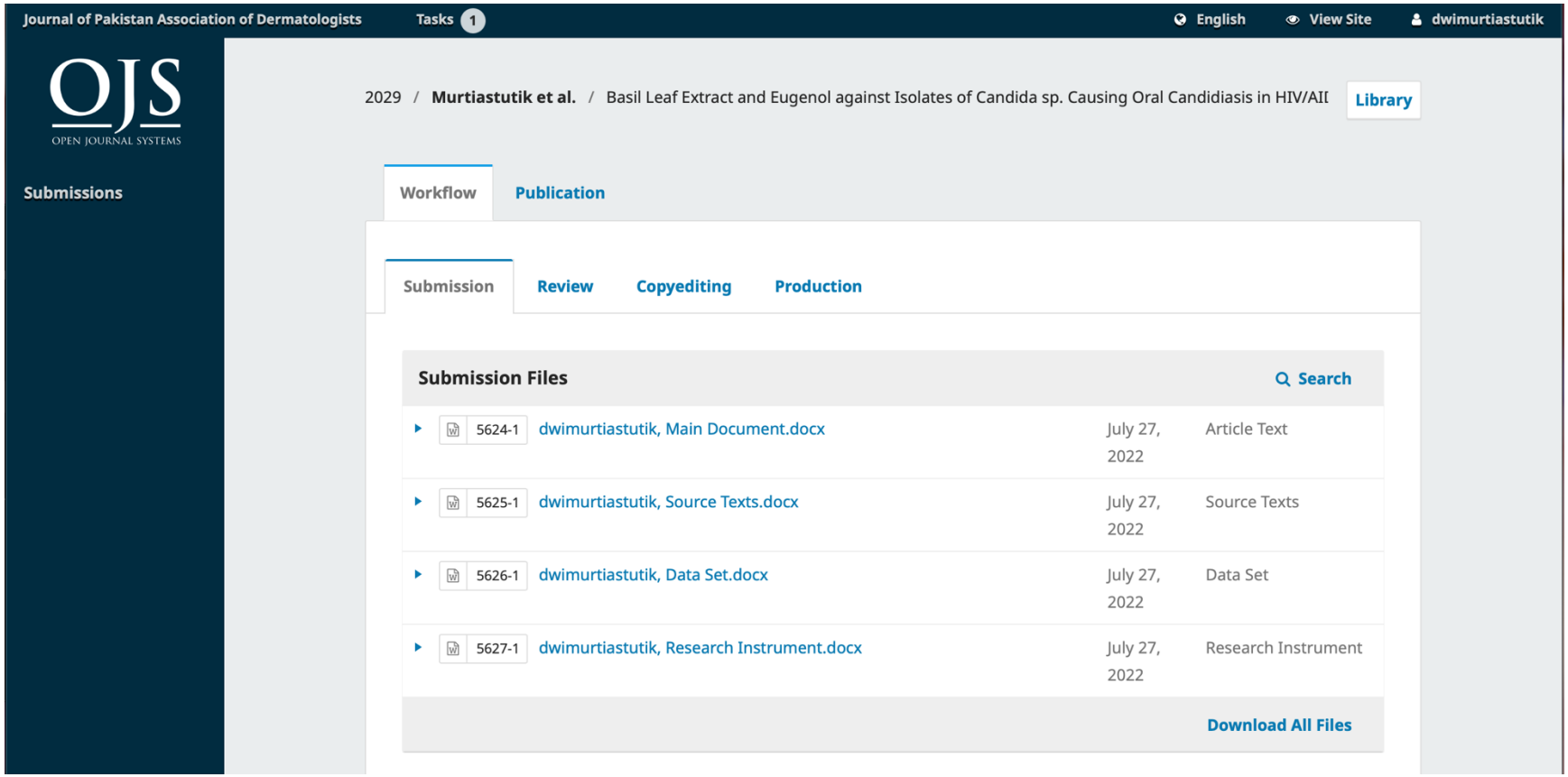
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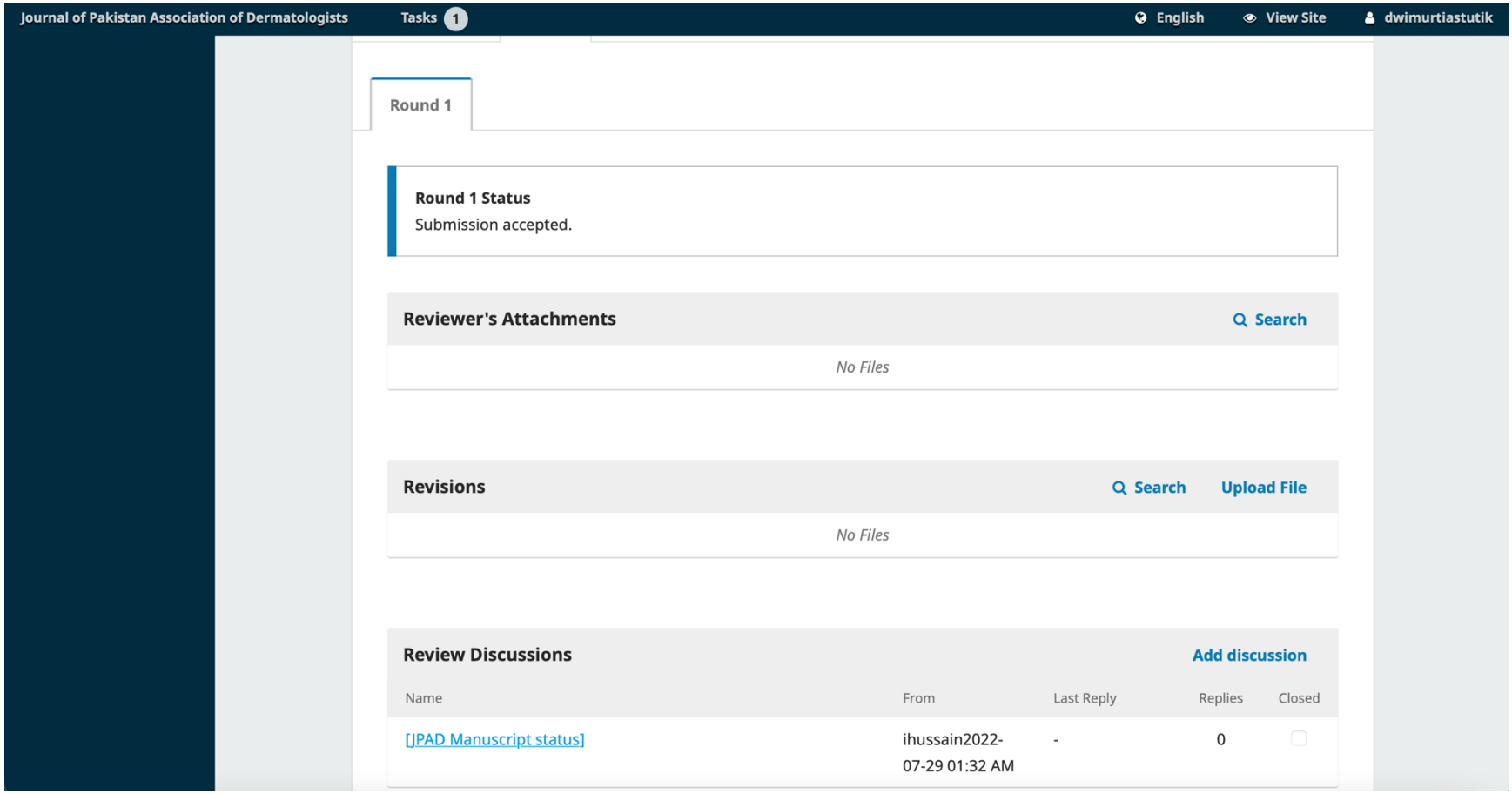
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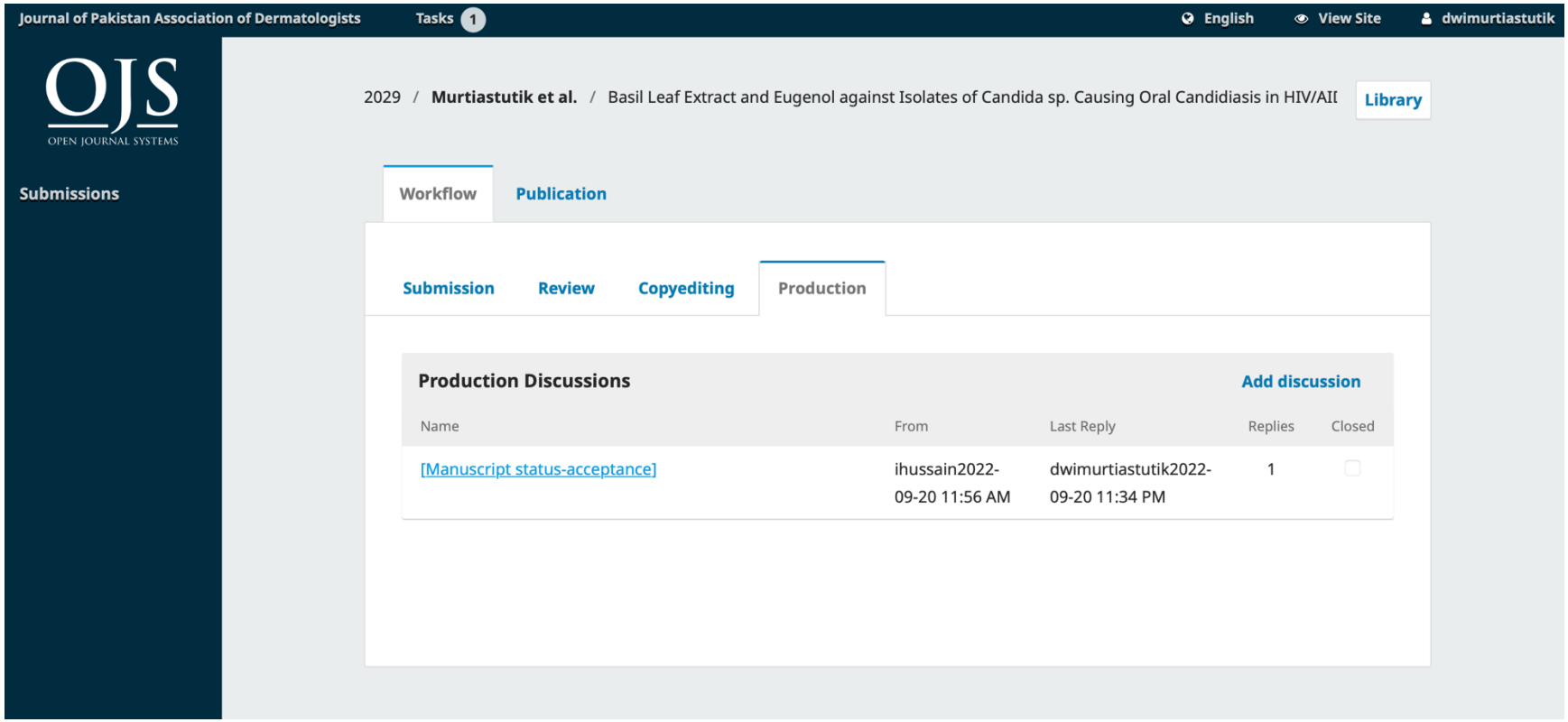
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Original Article

Basil leaf extract and eugenol against isolates of *Candida* sp. causing oral candidiasis in HIV/AIDS

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Abstract

Background Oral candidiasis is an opportunistic infection of the oral mucosa caused by *Candida* sp., frequently found in HIV/AIDS patients. Basil leaf extract (*Ocimum sanctum* Linn.), which contains eugenol, a compound believed to inhibit the growth of *Candida* sp.

Objective To evaluate the antifungal effect of basil leaf extract (*Ocimum sanctum* Linn.) and eugenol compared to fluconazole against *Candida* sp. isolates.

Methods Basil leaf extract (*Ocimum sanctum* Linn.) with doses equivalent to eugenol 800 µg/mL and 400 µg/mL, eugenol doses at 800 µg/mL and 400 µg/mL, which was then compared with fluconazole 25 µg/mL against 40 *Candida* sp. isolated stored from the oral cavity of HIV/AIDS patients.

Results The average inhibition zone of fluconazole was 21.81 mm, the mean inhibition zone of eugenol with doses of 800 µg/mL and 400 µg/mL were 17.07 mm and 15.89 mm, and the mean inhibition zone of basil leaf extract (*Ocimum sanctum* Linn.) with doses equivalent to eugenol 800 µg/mL and 400 µg/mL were 14.87 mm and 14.01 mm ($p = 0.001$ and $p < 0.05$).

Conclusion Fluconazole had a significantly higher inhibition zone against *Candida albicans* and *Candida* non-*albicans* isolates than basil leaf extract (*Ocimum sanctum* Linn.) and eugenol.

Key words
Basil leaf extract (*Ocimum sanctum* Linn.), eugenol, fluconazole, antifungal susceptibility testing, oral candidiasis, HIV, AIDS.

Introduction

Oral candidiasis is a fungal infection of the oral mucosa caused by *Candida* sp.[1] *Candida* sp. is a commensal fungus in healthy people, but under certain conditions it can cause an opportunistic infection. Opportunistic infections can occur as a consequence of infection with the human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS). During the course of their illness, nearly 90% of HIV/AIDS patients develop oral candidiasis.[2,3]

Oral candidiasis can be treated with the antifungal drug nystatin oral suspension and/ or fluconazole as a systemic antifungal.[4,5] In a test of *Candida* sp. resistance to fluconazole, isolates resistant to fluconazole were found to be 48.6%. *Candida* non-*albicans* was the most resistant isolate, accounting for 72.2% of the total.[2] Antifungal drug biofilm formation and resistance are two issues that can be addressed by seeking for alternative antifungal drugs.[6]

Basil (*Ocimum sanctum* Linn.) is a tropical plant that can be found in abundance in Indonesia.[7] The main component of basil leaf (*Ocimum sanctum* Linn.) is eugenol. This compound is effective against the adaptive mechanism of *Candida albicans* biofilm resistance to fluconazole.[8] Based on these findings, the researchers proposed to hold an in vitro test of basil leaf extract (*Ocimum sanctum* Linn.) and eugenol against stored isolates of *Candida* sp. and compare them to standard oral candidiasis treatment fluconazole, which was now showing signs of resistance.

Methods

The research design used in this study was an experimental laboratory with the aim of evaluating the comparison of the antifungal activity of basil leaf extract (*Ocimum sanctum* Linn.) with doses equivalent to eugenol 800 µg/mL and 400 µg/mL, eugenol at doses 800 µg/mL and 400 µg/mL was then compared with fluconazole 25 µg/mL against 40 isolates stored *Candida* sp. which consisted of 20 *Candida albicans* and 20 *Candida* non-*albicans* isolated from the oral cavity of HIV/AIDS patients who were hospitalized at the Infectious Disease Intermediate Treatment Unit (UPIPI) RSUD Dr. Soetomo Surabaya for the period April 2019 to July 2019 which was reactivated.

The antifungal activity was evaluated using the disk diffusion method with paper discs or blank discs. These data were entered into a data collection sheet and analysed with SPSS (Statistical Package for Social Sciences). This research has obtained ethical approval from the Ethics Committee of Dr. Soetomo General Academic Teaching Hospital Surabaya (0522/LOE/301.4.2/VII/2021).

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Results

In this study, using the disc diffusion method, the average results of the inhibition zone test of basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to 800 µg/mL eugenol, basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to 400 µg/mL eugenol, eugenol 800 µg/mL, and eugenol 400 µg/mL will be tested and compared with the standard antifungal treatment fluconazole against *Candida* sp.

The average fluconazole zone for all *Candida* sp. was 21.81 mm, eugenol 800 µg/mL was 17.07 mm, eugenol 400 µg/mL was 15.89 mm, basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to eugenol 800 µg/mL was 14.87 mm, and basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to eugenol 400 µg/mL was 14.01 mm. **Table 1** shows a comparison of the average inhibition zone results for all *Candida* sp.

The average fluconazole inhibition zone in *Candida albicans* species was 22.75 mm, eugenol 800 µg/mL was 16.76 mm, eugenol 400 µg/mL was 15.52 mm, basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to

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Table 1 Comparison of the mean diameter of the inhibition zone of basil leaf extract (Ocimum sanctum Linn.), eugenol and fluconazole in all species.

No.	*Antifungal type*	*Number of Isolates*	*Average Inhibition Zone Diameter (mm)*	*P value*
1	Fluconazole 25 µg/mL	40	21.81	
2	Eugenol 800 µg/mL	40	17.07	
3	Eugenol 400 µg/mL	40	15.89	0.001
4	Basil leaf extract equivalent to eugenol 800 µg/mL	40	14.87	
5	Basil leaf extract equivalent to eugenol 400 µg/mL	40	14.01	

Table 2 Comparison of the mean diameter of the inhibition zone of basil leaf extract (*Ocimum sanctum* Linn.), eugenol and fluconazole in *Candida albicans*.

No.	*Antifungal type*	*Number of Isolates*	*Average Inhibition Zone Diameter (mm)*	*P value*
1	Fluconazole 25 µg/mL	40	22.75	
2	Eugenol 800 µg/mL	40	16.76	
3	Eugenol 400 µg/mL	40	15.52	0.001
4	Basil leaf extract equivalent to eugenol 800 µg/mL	40	14.41	
5	Basil leaf extract equivalent to eugenol 400 µg/mL	40	13.62	

Table 3 Comparison of the mean diameter of the inhibition zone of basil leaf extract (*Ocimum sanctum* Linn.), eugenol and fluconazole in *Candida* non-*albicans*.

No.	*Antifungal type*	*Number of Isolates*	*Average Inhibition Zone Diameter (mm)*	*P value*
1	Fluconazole 25 µg/mL	40	20.86	
2	Eugenol 800 µg/mL	40	17.38	
3	Eugenol 400 µg/mL	40	16.25	0.001
4	Basil leaf extract equivalent to eugenol 800 µg/mL	40	15.34	
5	Basil leaf extract equivalent to eugenol 400 µg/mL	40	14.39	

eugenol 800 µg/mL was 14.41 mm, and basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to eugenol 400 µg/mL was 13.62 mm. **Table 2** shows a comparison of the average inhibition zone results against *Candida albicans*.

In *Candida* non-*albicans*, the average fluconazole inhibition zone was 20.86 mm, eugenol 800 µg/mL was 17.38 mm, eugenol 400 µg/mL was 16.25 mm, basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to eugenol 800 µg/mL was 15.34 mm, and basil leaf extract (*Ocimum sanctum* Linn.) was equivalent to eugenol 400 g/mL was 14.39 mm. The comparison of the average inhibition zone results against *Candida* non-*albicans* could be observed in **Table 3**.

Because the data were not normally distributed and homogeneous, non-parametric statistical methods (Mann Whitney Test) was used to analyze it. The results of the non-parametric statistical test showed that the data significance value was 0.001, so <0.05, which means that there was a significant difference between the mean inhibition zone of eugenol 800 µg/mL, eugenol 400 µg/mL, and basil leaf extract (*Ocimum sanctum* Linn.) equivalent to eugenol 800 µg/mL, and basil leaf extract (*Ocimum sanctum* Linn.) equivalent to eugenol 400 µg/mL compared to fluconazole which is the standard antifungal drug against the growth of *Candida* in all species, *Candida albicans* and *Candida* non-*albicans*.

Discussion

In this study, the average inhibition zone of fluconazole was greater than the average inhibition zone of eugenol and basil leaf extract

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(*Ocimum sanctum* Linn.). The inhibition zones of both eugenol and basil leaf extract (*Ocimum sanctum* Linn.) are not better than fluconazole as a standard antifungal drug, but both eugenol and basil leaf extract (*Ocimum sanctum* Linn.) have antifungal effects capable of inhibiting the growth of *Candida* sp., according to the mean comparison.

In a similar study published in 2010, Ahmad and colleagues discovered that fluconazole had a better antifungal effect than eugenol, but some *Candida* sp. Have become resistant to fluconazole. Subsequently a study was conducted with combining fluconazole and eugenol which concluded that a combination of the two were superior to inhibit *Candida* sp. than fluconazole alone.[9] In a similar study published in 2021, Godil and colleagues discovered that basil leaf (*Ocimum sanctum* Linn.) had antifungal activity by inhibiting the growth of *Candida* sp., but in an in vitro test, the fluconazole inhibition zone was larger than that of basil (*Ocimum sanctum* Linn.) against *Candida*.[10]

In a study by Khan and colleagues in 2014, fluconazole revealed an MIC value of 256 µg/mL, capable of inhibiting *Candida* sp., whereas MIC of eugenol were 400µg/mL, showing that MIC of fluconazole were inferior to MIC of eugenol. This indicates that fluconazole is more sensitive in inhibiting *Candida* sp.[11] Basil leaf (*Ocimum sanctum* Linn.) have been shown to inhibit *Candida* sp. in previous studies, but the antifungal activity of basil leaf (*Ocimum sanctum* Linn.) is considered small due to the high MIC. Eugenol is a constituent of basil leaf (*Ocimum sanctum* Linn.) that has been shown to be effective in inhibiting *Candida* sp.[9]

The fluconazole inhibition zone was also significantly higher than the eugenol and basil leaf extract (*Ocimum sanctum* Linn.) inhibition zones. Fluconazole works by inhibiting the enzyme lanosterol 14-demethylase, a microsomal cytochrome P450 enzyme found in fungal cell membranes that prevents lanosterol from being converted to ergosterol. Disturbances in these enzymes cause the integrity of the fungal membrane to be disrupted, causing fungal growth to be inhibited and thus having a fungistatic effect.[4,12]

In a study published in 2020 by Sharifzadeh and Shokri, it was discovered that eugenol can inhibit the growth of *Candida* sp.[13] Eugenol has an antifungal effect by inhibiting the biosynthesis of ergosterol, a key component of fungal cell membranes, causing damage to the membranes and a reduction in function. Damage to cell membranes disrupts the transport of nutrients (compounds and ions) through the cell membrane, preventing fungal cells from growing and resulting in cell lysis. Eugenol also contains lipophilic compounds, which can penetrate the lipid bilayer membrane, which is made up of fatty acid chains, altering the fluidity and permeability of the cell membrane, causing the cell to lose its structure and function, resulting in cell lysis.[14] Eugenol can also prevent the formation of pre-formed biofilms and *Candida albicans* biofilms. This substance inhibits *Candida albicans* biofilm resistance to fluconazole through an adaptive mechanism.[15]

In several studies investigating the antifungal effect of eugenol, the mechanism by which eugenol induces *Candida* cell death has not been fully understood. The inactivation of ergosterol synthesis and the production of free radicals, which can be an antifungal effect of eugenol, are the mechanisms of action of eugenol against *Candida*. However, the mechanism of action of eugenol is probably not related to the degradation of *Candida* fungal cell walls.[15] According to Jawetz and colleagues, the

mechanism by which antifungal active substances inhibit fungal growth is through membrane damage. The integrity of the cellular components will be compromised if the cell membrane is damaged, and the fungal respiration process will be disrupted. In the end, there is insufficient energy for active substance transport, causing fungal growth to be disrupted.[16]

The results of this study showed that by giving basil leaf extract (*Ocimum sanctum* Linn.) to both *Candida albicans* and *Candida* non-*albicans*, all isolates showed inhibition zones for the growth of *Candida* sp. Basil leaf (*Ocimum sanctum* Linn.) are a tropical plant that can be found throughout Indonesia. Research by De Ornay *et al.* in 2017 and Desmara *et al.* in 2017, found antifungal activity from basil leaf extract (*Ocimum sanctum* Linn.) on the in vitro growth of *Candida albicans*.[7,8] Eugenol is the main component of basil leaf (*Ocimum sanctum* Linn.). Several in vitro and in vivo studies show that eugenol inhibits the growth of the *Candida albicans* fungus, which causes candidiasis.[17]

Khan and colleagues found that basil leaf (*Ocimum sanctum* Linn.) inhibited the transition of yeast formation into hyphae, inhibited fungal proteinase enzymes, and inhibited the expression levels of HWP1, SAP1, and PLB2 genes, which are pathogenesis crucially expressed during a *Candida albicans* infection, in a study published in 2014. As a therapeutic effect of the content of basil leaf (*Ocimum sanctum* Linn.), the content of basil leaf (*Ocimum sanctum* Linn.) can cause *Candida* apoptosis by performing programmed cell death (PCD) on fungi.[11]

The main component of eugenol, which is highly volatile, may have caused the inhibition zone of basil leaf extract (*Ocimum sanctum Linn.*) to be inferior to fluconazole in the study.[18] The antifungal activity test method used can also have an impact; in this study, the diffusion method was used to test antifungal activity, which is a common method. The diffusion method has the advantage of being a simple and quick way to see the antifungal effect by measuring the diameter of the inhibition zone formed; however, the disc diffusion method has a disadvantage in that not all substances are absorbed on the paper disc, potentially affecting the inhibition zone formed.[16]

The concentration of the extract, the content of antifungal compounds, the type of fungus inhibited, and the diffusion power all affect the antifungal activity of a compound. The concentration of the extract can also influence the inhibition zone formed, with the higher the concentration, the larger the clear zone. The more active compounds present, the more focused the concentration, affecting the diameter of the inhibition zone formed on fungal growth.[19]

Several other studies produced very encouraging results. According to Ahmad and colleagues 2010 research, eugenol is an antifungal agent with antifungal activity in vitro against *Candida albicans* and *Candida* non-*albicans*, that are intrinsically resistant to fluconazole. The interaction of eugenol and fluconazole was found to have a high level of synergism. In the strains that were tested, no antagonistic interactions were found. Fluconazole in combination with eugenol improves efficacy while lowering the fluconazole minimum effective dose.[9]

In a study conducted by Jafri and colleagues in 2020, it was discovered that administering fluconazole and eugenol to *Candida* isolates worked synergistically, with eugenol disrupting cell membrane integrity and allowing the entry of standard fluconazole drugs into mold cells. Combining antifungal drugs with eugenol has

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several benefits, including increased potency, reduced drug doses, and reduced toxicity, all of which help to inhibit or overcome biofilms and antifungal drug resistance.[20]

Given the growing treatment failure and antifungal resistance in *Candida* sp., more in vivo studies are needed to assess the potential of these compounds for therapeutic applications and suggest ways to treat resistant *Candida* infections using a combination drug approach. The synergistic interactions between eugenol and antifungal drugs must also be evaluated, with additional studies in animal models needed to assess therapeutic efficacy, topical formulation and toxicity.[9,20]

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