

## [ijhs] Submission Acknowledgement

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Dari: Prof. Dr. M. R. Herrera (editorsciencescholar@gmail.com)

Kepada: dwimurtiastutik@yahoo.co.id

Tanggal: Kamis, 28 Juli 2022 21.13 WIB

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Hello,

Prof. Dr. Maria R. Herrera has submitted the manuscript, "Comparison of antifungal activity of fluconazole and clove leaf essential oil on Candida species isolate in HIV/AIDS patients with oral candidiasis" to International journal of health sciences.

If you have any questions, please contact me. Thank you for considering this press as a venue for your work.

Prof. Dr. M. R. Herrera

**Editor-in-Chief**

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**M. R. Herrera**, Scopus ID: [7202050008](https://orcid.org/0000-0002-7202-0008), Nursing, Universidad de Camagüey, Camagüey, Cuba

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[International journal of health sciences](#)

## [ijhs] Editor's Decision [Accept]

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Dari: Prof. Dr. Maria R. Herrera (editorsciencescholar@gmail.com)

Kepada: dwimurtiastutik@yahoo.co.id

Tanggal: Kamis, 28 Juli 2022 21.13 WIB

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Citra Dwi Harningtyas, Dwi Murtiastutik, Irmadita Citrashanty, Pepy Dwi Endraswari, Retno Widjowati, Evy Ervianti, Sawitri, Rahmadewi:  
Paper ID: **[IJHS-11207]**

We have reached a decision regarding your submission to International journal of health sciences, "Comparison of antifungal activity of fluconazole and clove leaf essential oil on Candida species isolate in HIV/AIDS patients with oral candidiasis".

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It is a pleasure to accept your manuscript for publication in IJHS. The comments of the referee(s) who reviewed your manuscript are included at the bottom of this letter.

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Reviewing #1

Comments to the Author

Acceptable and considerable for publication

*Significance*

- The manuscript represents a new and demonstratively significant advancement in research on international students

*Review of Literature*

- The manuscript includes a well-organized and analytical review of relevant literature
- The manuscript uses a clear and well-developed theoretical/conceptual framework

### Research Design

- The manuscript reflects appropriate design and methodology
- The manuscript reflects high-quality data and analysis

### Discussion

- The discussion and conclusion highlight the relevance of the findings for research, policy, and practice

### Style

- The manuscript clear, logical, and concise

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### Reviewing #2

#### Comments to the Author

#### Recommended to Associate Editor

- The manuscript must follow APA publication guidelines and will be done by Copy Editor.
- Production Editor will arrange all term of APA citation in copy editing, therefore, they have a responsibility regarding the contents of the manuscript
- Acceptable for next copy-editing process and recommended to Associate Editor.

#### Associate Editor Comments to Author:

#### Comments to Author:

- The content of the manuscript is consistent with the scope and aims of the journal
- The manuscript consistently includes articles that are scientifically sound and relevant to an international academic or professional audience in this field by citations in other journals currently covered by Scopus.
- Approve for publication and recommended to Publisher.

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## [ijhs] Submissions with Production Completed

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Dari: Prof. Dr. Maria R. Herrera (editorsciencescholar@gmail.com)

Kepada: dwimurtiastutik@yahoo.co.id

Tanggal: Kamis, 28 Juli 2022 21.15 WIB

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Citra Dwi Harningtyas, Dwi Murtiastutik, Irmadita Citrashanty, Pepy Dwi Endraswari, Retno Widyowati, Evy Erianti, Sawitri, Rahmadewi:

The editing of your submission, "Comparison of antifungal activity of fluconazole and clove leaf essential oil on Candida species isolate in HIV/AIDS patients with oral candidiasis," is complete. We are now sending it to production.

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## Letter of Acceptance

Dear Author(s),

Mr./Mrs. Dwi Murtiastutik

Title: Comparison of Antifungal Activity of Fluconazole and Clove Leaf Essential Oil on Candida Species Isolate in HIV/AIDS Patients with Oral Candidiasis

Reference your article, I would like to inform you that the 1st round evaluation of your manuscript has been completed. Based on the reviewer's recommendations, I am delighted to inform you that your manuscript has been initially accepted. The article as well as has been processed utilizing the peer-review process, and has been **accepted for publication**. The article will be published in *International Journal of Health Sciences (IJHS)* on **2022, Vol. 6, No. S6**.

DOI/URL: <https://doi.org/10.53730/ijhs.v6nS6.11207>

Thank you very much for submitting your article to "International Journal of Health Sciences."  
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Thank you for your contribution to the Journal and we are looking forward to your future

Best Regards,



Prof. María Rodríguez Gámez, Ph.D.  
Chief Executive Editor



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## **Comparison of antifungal activity of fluconazole and clove leaf essential oil on *Candida* species isolate in HIV/AIDS patients with oral candidiasis**

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**Abstract**--Current treatment guidelines include fluconazole as the main therapeutic option for treating oral candidiasis infection in HIV/AIDS patients, but resistance increases. Several in vitro studies have shown that clove leaf essential oil with the main content of eugenol can be an alternative therapy for oral candidiasis that is resistant to fluconazole. This study was conducted to determine the zone of inhibition of clove leaf essential oil and fluconazole against *Candida* isolates species that cause oral candidiasis in HIV/AIDS patients. This study showed that the average fluconazole disk 25 µg/mL inhibition zone in 20 isolates of *Candida albicans* species was larger than *non-albicans Candida* species. The zone of inhibition of clove leaf essential oil was significantly lower than that of fluconazole ( $p < 0.05$ ), except at a concentration of 15% against *non-albicans Candida* isolates. Clove leaf essential oil has an antifungal effect that can inhibit the growth of *Candida* species, so it can be considered an alternative antifungal therapy. Clove leaf essential oil concentration of 15% showed no significant difference in antifungal activity with the standard drug fluconazole in isolates of *non-albicans Candida* species.

**Keywords**--oral candidiasis, fluconazole, clove leaf, antifungal, HIV/AIDS.

**Introduction**

Oral candidiasis is one of the most common skin and mucosal manifestations found in patients with HIV/AIDS. Nearly 90% of patients with HIV/AIDS have experienced oral candidiasis during their illness (Murtiastutik & Maharani, 2019). The average prevalence in Europe and the United States is 28% and 30%, respectively. The prevalence of oral candidiasis has also been reported in Africa at 51% and in Asia at 39% (Tappuni, 2020). Oral candidiasis can be a morbidity and can interfere in the quality of life in HIV/AIDS patients because it often lasts longer, has a high recurrence rate, and has more severe clinical symptoms (Murtiastutik et al., 2021; Sakkas et al., 2016).

Among various species, *C. albicans* accounts for more than 90% of cases associated with severe fungal infections and causes candidiasis in immunocompetent patients (Alshaik & Kahkashan, 2017). The proportion of *Candida* infections caused by *C. albicans* in HIV/AIDS patients has shifted to *non-albicans Candida* species, and it may be due to high rates of antifungal drug resistance (Berkow & Lockhart, 2017; Murtiastutik et al., 2021). Research by Moges showed that the resistance rate of several antifungal agents by disc diffusion test in HIV/AIDS patients with oral candidiasis in Ethiopia in 2013-2014 showed that all isolates of *Candida* spp. found that 12.3% were resistant to

fluconazole, 8.4% to ketoconazole, and 1.3% to nystatin (Mulu et al., 2013). Current treatment guidelines include fluconazole as the main therapeutic option for treating oral candidiasis infection in HIV/AIDS patients (Berkow & Lockhart, 2017; Dar et al., 2015).

The existence of resistance to the main antifungal drugs urges research to find natural ingredients that can be attractive alternatives to antifungals. Most essential oils obtained from many tropical and subtropical plants contain eugenol as the main antifungal component (Schmidt et al., 2007). The concentration of eugenol in several plants is different. In the literature, it is stated that clove (*Syzygium aromaticum* L.) is considered the highest source of eugenol (45–90%) (Gupta & Prakash, 2021). Research conducted by Bhuiyan and colleagues compared the essential oil content of clove leaves and flowers, showing that the eugenol content was higher in clove leaves, namely 74.28%, while in flowers, as much as 49.71% (Bhuiyan et al., 2010). The most common methods for the isolation of eugenol are steam and water distillation (Khalil et al., 2017).

Research by Musta and Nurliana in 2019 regarding the effectiveness of clove leaf oil as antifungal *Candida albicans* showed antifungal activity for each concentration variation of 100%, 75%, 50%, and 25%, respectively, 14.2 mm, 12.2 mm, 10, 8mm and 10.4mm. The minimum concentration of clove leaf oil as antifungal *Candida albicans* is 17.86%. The clove leaves used were obtained from Bombana Regency, Southeast Sulawesi (Musta & Nurliana, 2019). A similar study was also conducted by Khansa (2019) who used clove flower essential oil from plantations in Yogyakarta. The diameter of the inhibition zone of clove flower essential oil at a concentration of 0.5% was 7.79 mm, a concentration of 1.0% was 12.02 mm, a concentration of 1.5% was 12.80 mm and a concentration of 2.0% was 13.96mm. This shows that the higher the clove flower essential oil concentration, the larger the resulting inhibition zone (Khansa, 2019). Variations in components and composition depend on the variety, agro-ecological conditions, pre-treatment, processing, and extraction method (Nurdjannah & N., 2012).

Clove oil has considerable antifungal activity against human fungal pathogens, including fluconazole-resistant strains. Clove essential oil can inhibit dermatophyte, *Aspergillus* and *Candida*, thus meeting the criteria for broad-spectrum agents (Alshaik & Kahkashan, 2017). Based on these data, this study aimed to compare the inhibitory of clove leaf essential oil (*Syzygium aromaticum*) and fluconazole in vitro.

## **Method**

The research design used in this study was an experimental laboratory to compare the antifungal activity of clove leaf essential oil (*Syzygium aromaticum* L.) with fluconazole 25 µg/mL. There were 20 *Candida albicans* isolates and 20 non-*albicans* *Candida* isolates from the oral cavity of HIV/AIDS patients hospitalized at the Infectious Disease Intermediate Treatment Unit (UPIPI) RSUD Dr. Soetomo Surabaya from April – July 2019, which was reactivated. The clove leaf essential oil concentration based on the former study was 5%, 10%, and 15%. The antifungal activity was evaluated paper discs or blank discs using the disk diffusion method. These data were entered into a data collection sheet and



analyzed 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 (0286/KEPK/X/2021).

## Results

This study showed that the average fluconazole disk 25  $\mu\text{g}/\text{mL}$  inhibition zone in 20 isolates of *Candida albicans* species was  $29.31 \pm 7.847$  mm, while in 20 isolates of non-*albicans* *Candida* species was  $23.97 \pm 8.682$  mm. The average inhibition zone of clove leaf essential oil concentrations of 5%, 10% and 15% in 20 isolates of *Candida albicans* species were  $10.83 \pm 3.135$  mm,  $18.00 \pm 4.141$  mm and  $23.69 \pm 7.062$  mm, respectively. While the 20 isolates of non-*albicans* *Candida* species were  $9.54 \pm 6.942$  mm,  $17.93 \pm 5.543$  mm and  $21.36 \pm 6.313$  mm, respectively (Table 1). Four samples (10%) of isolates showed resistance to the antifungal fluconazole 25  $\text{g}/\text{mL}$ , one from *Candida albicans* species and three from species non-*albicans* *Candida* (one from *C. glabrata* species, two from *C. krusei* species).

Table 1  
Distribution of mean diameter of the inhibition zone of fluconazole and clove leaf essential oil (*Syzygium aromaticum* L.) 5,10 and 15% in isolates *Candida* spp

Antifungal type (n=40)	Average inhibition zone (mm)	
	$\pm$ Standart Deviation (SD)	
	<i>Candida albicans</i> (n=20)	non- <i>albicans</i> <i>Candida</i> (n=20)
Flukonazol 25 $\mu\text{g}/\text{mL}$	$29.31 \pm 7.847$	$23.97 \pm 8.682$
Clove leaf essential oil 5%	$10.83 \pm 3.135$	$9.54 \pm 6.942$
Clove leaf essential oil 10%	$18.00 \pm 4.141$	$17.93 \pm 5.543$
Clove leaf essential oil 15%	$23.69 \pm 7.062$	$21.36 \pm 6.313$

The comparative analysis of the average inhibition zone between fluconazole and clove leaf essential oil concentrations of 5%, 10%, and 15% on 20 isolates of *Candida albicans* species obtained significant differences with p-value, respectively 0.000; 0.000; 0.018. The mean diameter of the fluconazole inhibition zone was greater than that of clove leaf essential oil (Table 2).

Table 2  
Comparison of the mean diameter of the inhibition zone of fluconazole with clove leaf essential oil (*Syzygium aromaticum* L.) in *Candida albicans* isolates

Antifungal type (n=20)	Average inhibition zone (mm) $\pm$ Standart Deviation (SD)	p-value
Flukonazol 25 $\mu\text{g}/\text{mL}$	$29.31 \pm 7.847$	-
Clove leaf essential oil 5%	$10.83 \pm 3.135$	0.000
Clove leaf essential oil 10%	$18.00 \pm 4.141$	0.000
Clove leaf essential oil 15%	$23.69 \pm 7.062$	0.018

The results of the comparative analysis of the average inhibition zone between fluconazole and clove leaf essential oil on non-albicans *Candida* isolates showed significant differences in clove leaf essential oil concentrations of 5% and 10% ( $p$  values 0.000 and 0.021), where the mean diameter of the fluconazole inhibition zone was more prominent than clove leaf essential oil. Meanwhile, clove leaf essential oil with a concentration of 15% did not show a significant difference compared to fluconazole ( $p=0.330$ ), where the mean diameter of the fluconazole inhibition zone was greater (Table 3).

Table 3  
Comparison of the mean diameter of the inhibition zone of fluconazole with clove leaf essential oil (*Syzygium aromaticum* L.) in non-albicans *Candida* isolates

Antifungal type (n=20)	Average inhibition zone (mm) $\pm$ Standard Deviation (SD)	<i>p</i> -value
Flukonazol 25 $\mu$ g/mL	23.97 $\pm$ 8.682	-
Clove leaf essential oil 5%	9.54 $\pm$ 6.942	0.000
Clove leaf essential oil 10%	17.93 $\pm$ 5.543	0.021
Clove leaf essential oil 15%	21.36 $\pm$ 6.313	0.330

## Discussion

Fluconazole is a systemic antifungal used in oral candidiasis in HIV/AIDS patients. Changes in species that cause oral candidiasis lead to the emergence of resistance to fluconazole (William et al., 2011). The existence of resistance to antifungal drugs, especially fluconazole, urges research to be carried out to find natural ingredients that can be an antifungal alternative. Many essential oils are obtained from several tropical and subtropical plants, containing eugenol as the main antifungal component (Schmidt et al., 2007). The concentration of eugenol in several plants is different. In the literature, it is stated that clove (*Syzygium aromaticum* L.) is considered the highest source of eugenol (45–90%) (Gupta & Prakash, 2021).

The average fluconazole inhibition zone in 20 isolates of *Candida albicans* species was 29.31 $\pm$ 7.847 mm, while in 20 isolates of non-albicans *Candida* species was 23.97 $\pm$ 8.682 mm. This indicates that fluconazole 25  $\mu$ g/mL still has good antifungal activity in this study. When compared to the mean zone of inhibition between *Candida albicans* and non-albicans *Candida* species, it was found that fluconazole 25  $\mu$ g/mL gave a larger zone of inhibition in *Candida albicans* isolates than in non-albicans *Candida* isolates. This is consistent with the theory which states that immunocompromised conditions, comorbidities, use of broad-spectrum antibiotics, and practical use of antimycotic drugs such as fluconazole use in HIV patients are reported to be associated with an increase in non-albicans *Candida* strains and an increase in fluconazole-resistant *Candida albicans* strains (Pudjiati et al., 2018). Combined overexpression of CDR2 and ERG11 and mutations in the ERG11 gene were the genetic mechanism of fluconazole resistance in *Candida* isolated from HIV patients (Rosana et al., 2015).

The fluconazole sensitivity criteria or inhibition zone category used was the size of the inhibition zone according to the Clinical and Laboratory Standards Institute

(CLSI) standards for fluconazole. Sensitive results in the sensitivity test showed that the diameter of the inhibition zone was more than and equal to 19 mm. The zone of inhibition between 14 – 18 mm indicated a susceptible-dose-dependent (SDD), and the zone of inhibition less than 14 mm indicated resistance to the growth of *Candida* spp. in vitro (Sumalapao et al., 2018). In the in vitro inhibition zone test of 40 isolates of research subjects, four samples (10%) of isolates showed resistance to the antifungal fluconazole 25 µg/mL because it had an inhibitory zone diameter of 14 mm. Research conducted by Murtiastik et al. (2019) in Surabaya with the number of research subjects of HIV/AIDS patients, as many as 20 patients with 37 isolates of *Candida* species growing in culture, in the test for resistance of *Candida* species to fluconazole showed that 18 (48.6%) isolates were resistant to fluconazole. The most resistant isolates were non-albicans, with as many as 13 isolates (72.2%) (Murtiastutik & Maharani, 2019).

Isolate of *Candida* species that showed resistance to fluconazole were derived from one sample of *Candida albicans* species and three samples of non-albicans *Candida* species (1 from *C. glabrata* species, two from *C. krusei* species). In a laboratory-based population study, the resistance to fluconazole is more excellent in isolates of non-albicans *Candida* species than in *C. albicans*. During the last ten years, there has been documentation of intrinsic resistance to azole antifungals, especially fluconazole, in several non-albicans *Candida* species, especially isolates of *C. glabrata* and *C. krusei*. A study conducted by Sadeghi et al. (2018) evaluated the antifungal susceptibility of non-albicans *Candida* species isolated from various clinical samples in vitro to fluconazole. Although *C. krusei* exhibits intrinsic resistance to fluconazole, it is controversial whether the increased resistance rate is related to fluconazole prophylaxis or prior treatment (Sadeghi et al., 2018). The results of the study by Houshmandzad et al. (2022), which aimed to analyze the anticandidal activity of fluconazole (FLZ) against FLZ-resistant *C. krusei* strains showed that 16 (76.2%) *C. krusei* strains were resistant to fluconazole, except for five (23, 8%) clinical isolate which showed susceptible-dose-dependent (SDD) results (Houshmandzad et al., 2022).

The average inhibition zone of clove leaf essential oil (*Syzygium aromaticum* L.) concentrations of 5%, 10% and 15% in 20 isolates of *Candida albicans* species alone were 10.83±3.135 mm, 18.00±4.141 mm and 23.69±7.062 mm. While the 20 isolates of non-albicans *Candida* species were 9.54±6.942 mm, 17.93±5.543 mm and 21.36±6.313 mm. The formation of inhibition zones in the study showed that clove leaf essential oil had an antifungal activity that could inhibit the growth of *Candida* species in both *Candida albicans* and non-albicans *Candida*. Following the research conducted by Musta and Nurliana in 2019 regarding the kinetics study of the effectiveness of clove leaf oil as an antifungal *Candida albicans* using clove leaves taken from Southeast Sulawesi. This study's minimum concentration of clove leaf oil as an antifungal *Candida albicans* was 17.86% (Musta & Nurliana, 2019). A similar study was also conducted by Khansa (2019) using clove flower essential oil from plantations in Yogyakarta. The measurement of the diameter of the inhibition zone of clove flower essential oil concentration of 0.5% is 7.79 mm, concentration of 1.0% is 12.02 mm, concentration of 1.5% is 12.80 mm and concentration of 2.0% is 13.96 mm. This shows that the higher the clove flower essential oil concentration, the larger the inhibition zone produced (Khansa, 2019).

The sensitivity values of clove leaf essential oil (*Syzygium aromaticum* L.) cannot be compared with fluconazole because they do not have a sensitivity standard according to CLSI, so what can be compared in this study is the average diameter of the inhibition zone between clove leaf essential oil and fluconazole 25  $\mu\text{g}/\text{mL}$  as standard therapy (positive control) in mm. Fluconazole 25  $\mu\text{g}/\text{mL}$  showed the largest mean diameter of the inhibition zone in all *Candida* spp isolates. This shows that the standard antifungal drug fluconazole 25  $\mu\text{g}/\text{mL}$  still provides antifungal activity in a larger zone of inhibition compared to clove leaf essential oil concentrations of 5%, 10% and 15%. In another way, the inhibition zone formed from clove leaf essential oil in fluconazole-resistant isolates in this study gave better results.

This can be the basis that clove leaf essential oil with the main content of eugenol can be an alternative therapy for oral candidiasis that is resistant to fluconazole. These results are supported by a similar study conducted by Pinto et al. (2009) to evaluate the antifungal activity of clove essential oil and its main component, eugenol, against *Candida* isolates. This study stated that clove essential oil and eugenol had considerable antifungal activity against *Candida*, including fluconazole-resistant strains, namely *C. krusei*, which was intrinsically resistant to fluconazole, and *C. glabrata*, whose resistance was easily induced. These results strengthen further investigation of clove essential oil and eugenol for clinical application to treat fungal infections (Pinto et al., 2009).

The test results of the comparative analysis of the mean fluconazole inhibition zone to clove leaf essential oil concentrations of 5%, 10%, and 15% in 20 isolates of *Candida albicans* obtained a significant difference (p-value <0.05), where the diameter of the fluconazole inhibition zone bigger. There was no significant difference (p value=0.330) when an analysis of the mean inhibition zone of fluconazole was performed against clove leaf essential oil with a concentration of 15% in 20 isolates of non-*albicans* *Candida*. This shows that the inhibition zone of clove leaf essential oil concentrations of 5%, 10%, and 15% is not better than fluconazole 25  $\mu\text{g}/\text{mL}$  as the standard drug. However, clove leaf essential oil has an antifungal effect that can inhibit the growth of *Candida* species, so it can be considered an alternative antifungal therapy, especially clove leaf essential oil concentration of 15% which shows good activity, not significantly different from the standard drug fluconazole in isolates of non-*albicans* *Candida*.

A similar study by Fristiyanti (2019) was conducted to determine whether or not the difference between two concentrations of clove leaf extract was significant. It was found that the concentration groups of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% had significant differences in reducing colony thickness of *Candida albicans*. However, the concentration of 0.6% extract and 0.6% fluconazole is not significantly different in reducing the thickness of *Candida albicans* colonies. This shows an inverse relationship, in which the higher the concentration of clove leaf ethanol extract given, the thinner the *Candida albicans* colonies that grow. In addition, it can be seen that the effective concentration that can reduce the thickness of *Candida albicans* colonies starts from a concentration of 0.1% in this study (Fristiyanti, 2019).

Several other factors can affect the antifungal activity, including the concentration of the extract, the content of antifungal compounds, the type of fungus inhibited, and the diffusion power. The inhibition zone formed can also be influenced by the concentration of the extract, where the higher the concentration, the larger the clear zone will be formed. The more concentrated the concentration, the more active compounds are contained, thus affecting the diameter of the inhibition zone formed on fungal growth (Andayani et al., 2014). The antifungal activity test method used can also have an effect. This study uses the diffusion method which is one method that is often used to test antifungal activity. The advantage of the diffusion method is that it is a fast and straightforward method to see the antifungal effect by looking at the diameter of the inhibition zone formed, but there are drawbacks to the disc diffusion method, namely that not all substances may be absorbed in the paper disc so that it can affect the inhibition zone formed (Jawetz & Adelberg, 2007).

Clove leaf oil has the most vigorous activity against clinical isolates of *Candida albicans*, which is characterized by the highest eugenol content that can inhibit pre-formed biofilm and *Candida albicans* biofilm formation. This compound is effective against the adaptive mechanism of *Candida albicans* biofilm resistance to fluconazole (Khan & Ahmad, 2012). Biofilm production was slightly higher in non-*albicans* *Candida*. Biofilm is a collection of microorganisms that adhere to the surface and are encased in an extracellular polysaccharide matrix produced by these microorganisms. Types of *Candida* spp. with a high ability to form biofilms are generally more virulent than others. Biofilms also play a role in resistance to antifungal drugs used, although the primary mechanism is still unknown (Williams et al., 2011).

### **Conclusion**

Clove leaf essential oil has an antifungal effect that can inhibit the growth of *Candida* species. Although the mean inhibition zone formed on fluconazole was significantly more extensive than that of clove leaf essential oil, in isolates that showed resistance to fluconazole, clove leaf essential oil gave a larger diameter of the inhibition zone. In addition, clove leaf essential oil with a concentration of 15% showed no difference in antifungal activity with the standard drug fluconazole on isolates of non-*albicans* *Candida* species. It could be considered an antifungal alternative, especially for fluconazole-resistant strains.

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