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Lemongrass (*Cymbopogon Citratus*) Essential Oil Inhibits *Candida Albicans* Growth in Vitro

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ABSTRACT

Candidiasis is an important nosocomial infection with high morbidity value, high mortality rate, and expensive clinical cost. However, public access to well-developed treatment is not acquired. Current medicines mostly used like -azol drugs had been showing the resistant effects because of the long period use of the same medicines. Alternative medicines like herb medicine are discussed to reduce multi-resist infections, such as lemongrass *Cymbopogon citratus* essential oil. This study aims to know the effect of lemongrass essential oil on the growth of *Candida albicans* in vitro. Laboratory experimental (in vitro) was conducted in this research. *Candida albicans* strains were being used as the objects, which were picked by random sampling. *Candida albicans* were divided into 12 groups of treatment, the group I was treated with ethanol 96% as the negative control, group II with fluconazole 25µg as the positive control, and group III-XII with *Cymbopogon citratus* essential oil with 10%, 20% to 100% concentrated. The diameter of inhibition zones was measured after 2x24 hours incubation. The data was analyzed by post-hoc Mann Whitney test with SPSS 18.0 ($p<0.05$ considered as significant). *Cymbopogon citratus* essential oil showed antifungal activity to the *Candida albicans* began in the 10% to 100% concentration ($p<0.05$). Inhibition zones with the 50% to 100% concentration had similar results to the positive control ($p>0.05$). The *Cymbopogon citratus* essential oil has an antifungi effect toward *Candida albicans* in vitro significantly by the negative control.

Keywords:
lemongrass essential oil; *Cymbopogon citratus; Candida albicans*

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INTRODUCTION

Candidiasis is one of the most important nosocomial infections worldwide, with significant morbidity, mortality, and health cost (Li et al., 2018). The incidence of candidiasis is still very high in developing countries such as Indonesia (Nelwan & Wisaksana, 2010). The high incidence of candidiasis in Indonesia may be caused by the lack of public access to good treatments in Indonesia. This is because drug prices are relatively costly for some people (Riyarto et al., 2010).

Chronic candidiasis can manifest through the spread of candida through blood vessels or candidaemia. Candidemia has been resulted to cause death by 30-40% despite intensive care (Salazar et al., 2020). The most commonly administered synthetic treatment for candida is the *-azole* group, such as fluconazole after 2020 (Denison et al., 2020). The use of antifungal drugs, such as the *-azole* group, will eventually lead to increased drug resistance. Several species of *Candida albicans* have also shown resistance to many antifungal drugs (Ramírez-Amador et al., 2020).

Use of alternative treatments is being developed, especially herbal medicine to overcome this problem. Herbal medicine generally uses ingredients that are relatively easy to obtain and the plants are easy to breed so that people can easily get them (Rohilla, Bhatt, & Gupta, 2018). Herbal medicine that have the potential as an alternative treatment for candidiasis include essential oil from *Cymbopogon citratus*. Lemongrass in the form of essential oil has also been shown to be effective against several species of bacteria, fungi, and yeast, one of which is *Candida albicans* (Abe et al., 2003; Taguchi et al., 2010). The use of lemongrass essential oil in inhibiting the growth of *Candida albicans* needs to be investigated to determine the most effective dosage form and confirm previous research evidence so that the potential of lemongrass can be optimized for use by the wide community.

MATERIALS AND METHODS

This research is experimental laboratory research conducted at the Parasitology and Mycology Laboratory of Sebelas Maret University Surakarta. This research was conducted in March-April 2012. The research subjects used were clinical samples of *Candida albicans* obtained from the installation of the Laboratory of Parasitology and Mycology at Dr. Moewardi Hospital, Surakarta, which was then cultured on Sabouraud Dextrose Agar (SDA) media. The colonies of *Candida albicans* were taken first by random sampling then diluted with 0.9% NaCl until the turbidity was equivalent to the standardization of 0.5 Mc Farland.

This study used 12 treatment groups, namely group I which was the *Candida albicans* group with ethanol as a negative control, group II with 25μg fluconazole as a positive control, while group III-XII with *Cymbopogon citratus* extract with a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. The study was conducted in three repetitions to avoid bias. The *Candida albicans* plates were stored at room temperature for 2x24 hours then the diameter of the inhibition zone was measured. The data obtained were analyzed using the Mann Whitney post-hoc test using SPSS 18.0 ($p < 0.05$ was considered significant).

The independent variable consists of the concentration of *Cymbopogon citratus* essential oil, on an ordinal scale of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. Meanwhile, the dependent variable consists of the diameter of the growth inhibition zone of *Candida albicans* on a ratio scale. Uncontrolled variables include 1) age and the number of cultures; 2) growth of other germs; 3) dilution volume; and 4) incubation time. The uncontrollable external variable was the growth rate of *Candida albicans*. The data were processed using the LSD Mann Whitney post-hoc nonparametric statistical test. Data were processed using Statistical Product and Services Collection (SPSS) 18.0. The level of significance set was $p < 0.05$.

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RESULT AND DISCUSSION

The preliminary test was carried out in concentrations of 0%, 25%, 50%, 75%, 100%. While the research test starts from a concentration of 10% to 100% with an interval of 10%. It has expected that the shorter the interval, the better results are obtained. The inhibition zone of *Cymbopogon citratus* essential oil against *Candida albicans* in vitro, in each treatment group, were shown in Table 1 and Figure 1. The Mann Whitney post-hoc test was used to compare the average diameter of the inhibition zone between treatment groups so that it can be known which group differed significantly ($p < 0.05$) with other groups.

The results in this study show the negative control has an average inhibition zone diameter of 0.6 cm. Whereas in the positive control with fluconazole, the average inhibition zone diameter was 3.53 cm. The treatment group with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% had an average inhibition zone diameter of 2.08 cm, 1.82 cm, 2.12 cm, 1.95 cm, 3.42 cm, 2.47 cm, 2.50 cm, 2.27 cm, 1.53 cm, and 2.27 cm, respectively. The treatment group, the concentration of 10-100%, had a higher mean inhibition zone diameter than the negative control and lower than the positive control (Table 1).

This study shows lemongrass essential oil can inhibit the growth of *Candida albicans* in vitro as indicated by a significant difference to negative controls ($p < 0.05$). While the optimal inhibitory power was seen at concentrations of 50%, 60%, 70%, 80%, 100% which showed similarities or insignificant differences ($p > 0.05$) with positive control (fluconazole) (Table 2).

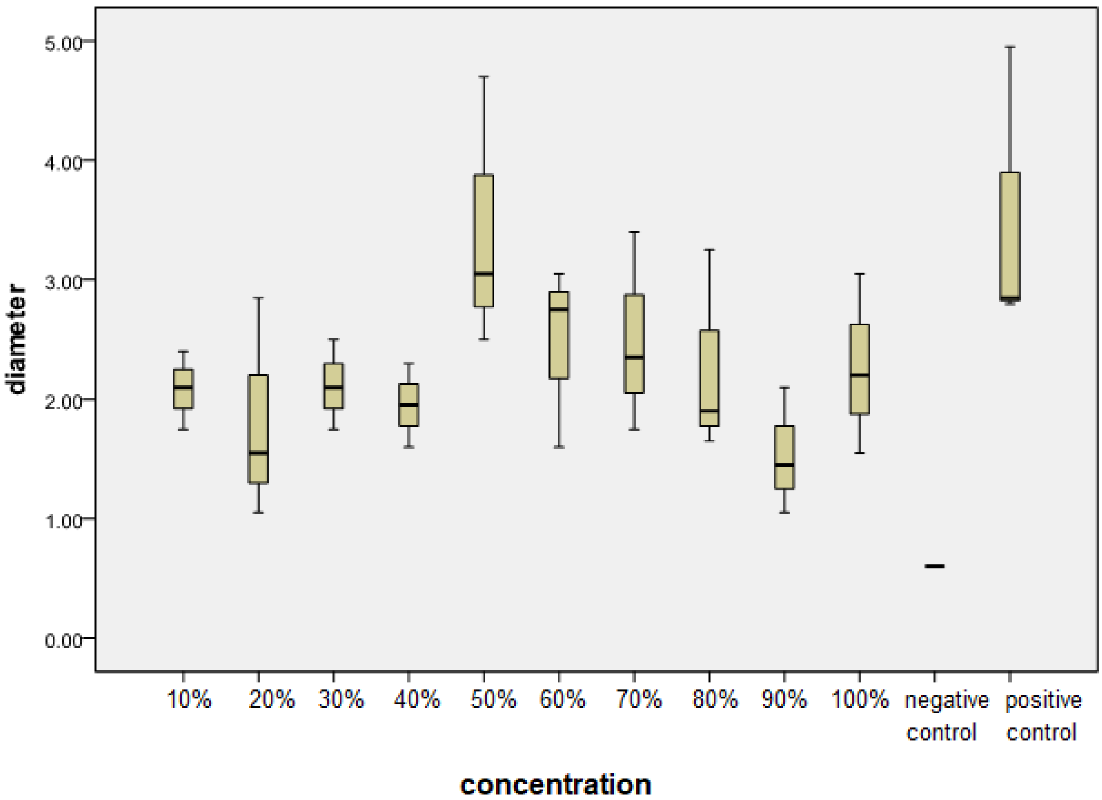

Figure 1. Boxplot graph of the inhibition zone diameter in each group

Table 1. The measurement of the inhibition zone diameter in the research test

Treatment	Inhibition Zone Diameter (cm)*			
	Repetition			Average
	1	2	3	
Negative control	0,60	0,60	0,60	0,60
Positive control	4,95	2,80	2,85	3,53
Concentration 10%	2,10	1,75	2,40	2,08
Concentration 20%	1,55	1,05	2,85	1,82
Concentration 30%	2,50	2,10	1,75	2,12
Concentration 40%	1,95	2,30	1,60	1,95
Concentration 50%	3,05	4,70	2,50	3,42
Concentration 60%	1,60	2,75	3,05	2,47
Concentration 70%	2,35	3,40	1,75	2,50
Concentration 80%	3,25	1,90	1,65	2,27
Concentration 90%	1,05	2,10	1,45	1,53
Concentration 100%	1,55	2,20	3,05	2,27

Note: Measurement of the diameter of the inhibition zone includes a well diameter of 0.6 cm.

Table 2. Mann Whitney post-hoc test results

Treatment	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Negative control	+	-	+	+	+	+	+	+	-	+
Positive control	+	+	+	+	-	-	-	-	+	-

Notes:
(+) : there is a significant difference between treatment groups ($p < 0.05$)
(-) : there is no significant difference between treatment groups ($p > 0.05$)

Fluconazole is the drug of choice for candidiasis which is still used until 2020 (Denison et al., 2020), although it raises some resistance problems in some cases of fungal infection (Ramírez-Amador et al., 2020). Provision of essential oil of *Cymbopogon citratus* start at a concentration of 50% is expected to provide an alternative treatment for candidiasis events. This is in accordance with the results in this study where at concentrations of 10%, 30%, 40%, 50%, 60%, 70%, 80%, and 100% can significantly inhibit Candida albicans ($p < 0.05$) than negative control, whereas at concentrations of 50%, 60%, 70%, 80%, and 100%, inhibitory results were not significantly different from fluconazole ($P > 0.05$).

The *citral* components of the essential oils are contained in several types of plants. *Citral* plays a significant role in antifungal activity (Leite et al., 2014). Lemongrass essential oil contains two key *citral* components, namely *a-citral* (36.2%) and *β-citral* (26.5%) (Silva et al., 2008). Previously, the antifungal mechanism of *citral* may come from the process of *citral* to deplete electron donors from fungi so that it could cause cell death itself (Leite et al., 2014). *Citral* has an antifungal activity by disrupting the activity of the fungi cell membrane (OuYang, Tao, & Zhang, 2018; Tang et al., 2018). The antifungal activity can lead to leakage from the cytoplasm, disruption of membrane synthesis, and inhibition of mycelium growth, and the spread of fungal spores (Leite et al., 2014; Zheng et al., 2015). The inhibitory mechanism may be from the high volume and lipophilicity of *citral*. So, it can adhere to the cell membrane then enters the cell (Lee et al., 2020; Sahal et al., 2020).

The antifungal activity of lemongrass essential oil may be due to the high *citral* component (Silva et al., 2008). Results showed there was an inhibitory activity on the growth of *Candida albicans* start from a concentration of 10%

and at a concentration of 50% showed that the optimum inhibition was slightly lower than the inhibition of the positive control fluconazole 25µg/ml. Further study needs to be conducted on lemongrass essential oil, including research on the *citral* content. Clinical trial research needs to be conducted first before it can be applied directly to the wide community.

CONCLUSION

Lemongrass essential oil *Cymbopogon citratus* showed antifungal activity against the growth of *Candida albicans* in vitro from a concentration of 10% to 100%. The inhibition zone generated at concentrations of 50%, 60%, 70%, 80%, 100% was slightly lower than the 25µg/ml fluconazole inhibition zone.

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