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Research Article

Inhibitory Test Of Citronella Essential Oil (*Cymbopogon Nardus L. Rendle*) Againts *Aspergillus Flavus* Growth

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ABSTRACT

Background: Indonesia is the one of tropical climate country with high air humidity. It becomes the main factor of fungal infection, that is called otomycosis, which is caused by Aspergillus flavus. The treatment of otomycosis is usually done by giving an azole antifungal drugs that can give the beneficial and adverse effects. Treatment of otomycosis can be done with various kinds of herbs. One of herbs that is used, it is citronella. Citronella contains essential oil 0,4% and in citronella essential oil contains citronellal 32-45% and geraniol 12-15% has ability as an antifungal. The aim of this study is to determine the inhibitory of citronella essential oil against Aspergillus flavus growth.
Methods: The type of this research is laboratory experiments with posttest only with control group design. 24-hour Aspergillus flavus is used as the research subjects which had been inoculated on SDA media and given essential oils in various concentrations of 0.5%, 1.0%, 1.5%, 2.0 %. The observation of citronella essential oil inhibitory as an antifungal by measuring the diameter of inhibitory zone, it uses a caliper.
Results: The measuring from average diameter of Aspergillus flavus fungal growth zone in each concentration, they are 0.5% ;1.0%; 1.5%; 2.0% is 9.11 mm; 12.03 mm; 13.62 mm; 16.81 mm. The higher concentration of essential oil, it makes the wider diameter of the inhibition zone. The level of sensitivity is intermediate, sensitive, sensitive, sensitive. The level of effectiveness is less effective, effective enough, effective, very effective. The results of statistical analysis showed that citronella essential oil has the inhibitory against Aspergillus flavus growth.
Conclusion: this study was various concentration of citronella essential oil have a significanteffect on the growth of inhibitory Aspergillus flavus.

Key words: Citronella essential oil, Aspergillus flavus, antifungal

INTRODUCTION

Indonesia is the one of tropical country with high humidity. It becomes the main factor that caused the fungal infection in human skin [1] Fungal that often infects the skin, it is non-dermatophytotic fungi. Non-dermatophytosic fungal infection is a disease that occurs on the outer skin. This disease is caused by a species of fungus that cannot secrete substances that can digest skin keratin, so it can only attack the outermost layer of the skin. Types of non-dermatophytosis infectious diseases are otomycosis, pityriasis versicolor, piedra, and tinea nigra [2].

Otomycosis is a chronic or subacute fungal infection that occurs a lot in tropical and humid climates. Otomycosis occurs in the outer ear canal and the outer ear opening is characterized by inflammation and accompanied by itching [3]. This infection is usually signed by Ears reddened, covered with fine scales and secretes serosanguinous fluid. Patients with otomycosis will experience hearing loss [4]. Otomycosis is a cosmopolitan disease that often occurs in hot and humid areas, for example Indonesia. This disease can attack men and women at an average age of 20-30 years.

Installation of Medical Records RSUP Dr. Sarjito said that there were 902 cases of otomycosis that occurred in Yogyakarta during the period 2014 to December 2018. Otomycosis is caused by a contaminating fungus of Aspergillus genus. The most common infecting species were Aspergillus flavus (42.4%), Aspergillus niger (35.9%) and Aspergillus fumigatus (12.5%) [5].

Aspergillus flavus is a contaminant fungus that occurs in nature as a saprophyte and grows in tropical areas with high humidity. Aspergillus flavus can infect the human by inhalation transmission [6]. Aspergillus flavus infection happened when there are occurs when there are predisposing factors, they are decreased immune system, use of steroids, dermatological diseases, use of broad-spectrum antibiotics, and hearing aids that produce a damp environment and cause otomycosis [7].

Treatment of otomycosis is usually done by giving antifungal drugs or antibiotics. The antifungal drugs most often used by the public are azole class drugs. The drugs of azole class can provide beneficial effects, but can also cause detrimental effects. If it given since pregnancy, it can be teratogenic to the fetus 8].

The rapid development of science and technology has caused the use and utilization of traditional medicine to progress [9]. World Health Organization (WHO) said that around 65-80% of the world's population depends on traditional medicines to fulfil their primary health needs. Safety factors and price considerations are selling points for increasing the use of traditional medicines [10].

Various countries, it includes Indonesia has for years used plants as traditional medicines to overcome various diseases including fungal infections [11].

Fragrant lemongrass (Cymbopogon nardusL. Rendle) is one of the herbal ingredients that can be used to treat fungal infections, sweat decay, sputum thinner, mouthwash and body warmer. Citronella contains 0.4% essential oil [9]. The essential oil components consist of Sitronellal, Geraniol, Citronellol, Geraniol Acetate, Citronellil Acetate and L-Limonene [12]. Citronella essential oil compounds function as anti-fungi, that is inhibiting the synthesis of ergosterol (the main sterol that forms fungal cell membrane), so the membrane protein structure becomes damaged and

membrane permeability increases which will cause fungal cell death [13].

Thus research was done by Oki Saraswati Utomo in 2015, with the title "The effect of citronella extract (Cymbopogon nardus L. Rendle.) as the antifungal to the development of Candida albicans in vitro" known that citronella extract has the ability as the antifungal to the Candida albicans. The results of preliminary tests of this study have been carried out on October 3-4, 2018, citronella essential oil was able to inhibit the growth of Aspergillus flavus fungus with an inhibitory zone diameter of 7 mm and 14 mm at a concentration of 1% and 2%. Therefore, further research is needed on the concentration of citronella essential oil that can effectively inhibit the growth of Aspergillus flavus fungus. The aim of this study was to determine the sensitivity and effectiveness of citronella essential oil against Aspergillus flavus in various concentrations.

METHOD

This type of research is a laboratory experiment with treatment in the form of giving various concentrations of citronella essential oil to the growth of the fungus Aspergillus flavus. The design of this research is Post – test Only Control Group Design, where is two groups, the first one is experiment group and the other group is control group. The concentration of experimental group is the essential oil concentration of 0.5; 1.0; 1.5 and 2.0%. The control group consisted of 1% CMC (Carboxymethyl cellulose) negative control and 1% fluconazole positive control. The number of repetitions used was eight times for each group.

The research was conducted in December 2018 - February 2019 and was conducted at the Mycology Laboratory, Department of Health Analyst, Poltekkes, Ministry of Health, Yogyakarta. Tests for citronella essential oil were carried out at the Traditional Medicine Research and Development Institute (LPPT) Gadjah Mada University. The determination test for citronella ingredients was carried out at the Department of Pharmaceutical Biology Unit II, Gadjah Mada University. Test of Inhibitory Power from Lemongrass Essential Oil (Cymbopogon nardus L. Rendle) uses the disc diffusion method according to Kirby Bauer:

a. SDA media that has been sterilized, it is placed at room temperature until the temperature decreases by about 45 – 50 0C.
b. SDA media is poured as much as 20 ml which has been sterilized into a disposable petri dish.
c. Aspergillus flavus fungus suspension is poured as much as 1 ml in the same petri dish.
d. Both solutions are homogenized by shaking the petri dish to form the number 8, wait until the media harden.
e. Plain disc paper is soaked for 5 minutes in essential oil with a concentration of 0.5%, 1.0%, 1.5% and 2.0%, respectively, and drained. Then it is placed on the media that has been inoculated with the fungus.
f. Plain disc paper is also dipped in the positive control and negative control, then placed on the media that had been inoculated with the fungus.
g. Positive control is made by giving 1% fluconazole and negative control was made by giving 1% CMC.
h. The petri dish is closed tightly, then wrapped with paper and plastic.

i. The media is stored at a room temperature of 22oC – 26oC for 24 hours, then measured the diameter of essential oil inhibition zone of citronella against the growth of Aspergillus flavus fungus.

Observation:

The diameter inhibitory zone that is resulted, then assessed for its fungal growth inhibition response, whether it includes sensitive, intermediate or resistant. Descriptive results from data on measuring the diameter of inhibition zone of citronella essential oil on the growth of Aspergillus flavus fungus are shown in Table 1.

Table 1. Mean Diameter of Growth Inhibition Zone of Aspergillus flavus Fungus

Citronella Essential Oil Concentration (%)	Number of repetitions	Average
0,5	8	9,11
1,0	8	12,03
1,5	8	13,62
2,0	8	16,81
Fluconazole	8	14,22

Source : Processed Primary Data, 2019.

From Table 1, the average diameter of inhibition zone for the growth of *Aspergillus flavus*, it is then compared to 1% fluconazole which is describe in the form of a bar chart shown in Figure 1.

The Comparison of Average from the Growth Inhibition Zone Diameter of Aspergillus flavus Fungus

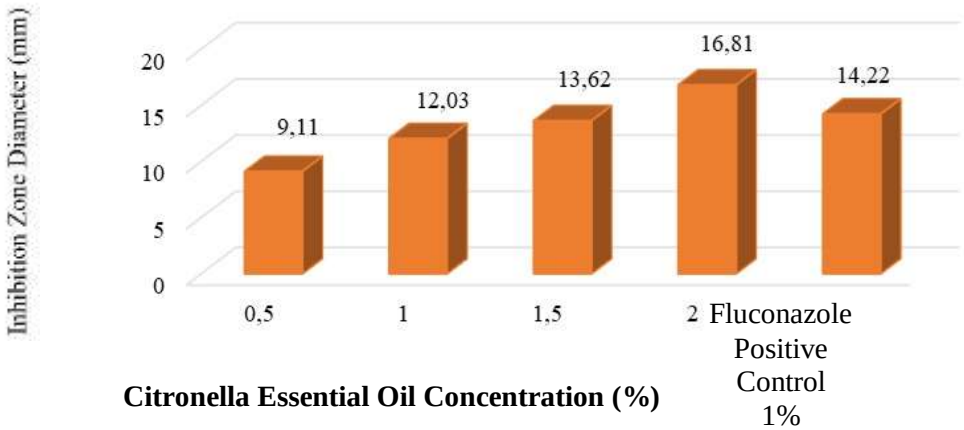

Figure 1. The Comparison of Average from the Growth Inhibition Zone Diameter of *Aspergillus flavus* Fungus

The results of anti-fungi sensitivity criteria to the diameter of inhibitory zone are shown in Table 2.

Table 2. Results of Antifungal Sensitivity Criteria for Inhibition Zone Diameter

Cinnamon Essential Oil Concentration	Inhibition Zone Diameter	Sensitivity Criteria
0,5 %	9,11 mm	Intermediate
1,0 %	12,03 mm	Sensitive
1,5 %	13,62 mm	Sensitive
2,0 %	16,81 mm	Sensitive

Source: Processed Primary Data 2019.

The results of criteria for the level of effectiveness are carried out by calculating the percentage value of effectiveness then compared with the criteria for the level of effectiveness shown in Table 3.

Table 3. Criterion Results on the Level of Effectiveness of Citronella Essential Oil Compared to Fluconazole 1%

Concentration of Essential Oil of Citronella	Effectiveness Percentage (%)	Effectiveness Level Criteria
0,5 %	64,06	Less effective
1,0 %	84,60	Effective enough
1,5 %	95,78	Effective
2,0 %	118,21	Very effective

Source: Processed Primary Data 2019.

The results of data normality test show that the value is significant (p>0,05), it means that the research data is normally distributed. The results of homogeneity test from the research data obtained a significant value, it is 0.422 (p>0,05), which it means homogeneous research data. *One Way* Anova test results show that a significant value is 0.000, that is means p<0,05 up to H_0 is rejected and H_a is received, that shows if there is a significant effect from the average diameter of inhibition zone that comes from various concentrations of citronella essential oil to the inhibition of growth of *Aspergillus flavus* fungus.

DISCUSSION

This research was conducted to determine the inhibition of citronella essential oil on the growth of Aspergillus flavus fungus. The action mechanism of citronella essential oil as an antifungal is that a clear zone, it is formed around the discs which widens as the concentration of citronella essential oil increases. Citronella essential oil contains Sitronellal (32 – 45 %), Geraniol (12 – 18%), Sitronellol (12 – 15 %), Geraniol Asetat (3 – 8 %), SitronellilAsetat (2 – 4 %) and L-Limonene (2 – 5 %) [8]. Sitronellal (C10H16O) and geraniol (C10H18O) is a compound, that is anti-fungal. Citronellal and geraniol are able

to suppress the growth of pathogenic fungi. The mechanism of citronella essential oil compounds as anti-fungi is to inhibit the synthesis of ergosterol (the main sterol that forms fungal cell membranes). Enzim dimetilase 14-α-sterol and cytochrome P450 converts lanosterol into ergosterol. However, there are citronellal and geraniol which will interfere lanosterol that interacts with the dimethylase enzyme 14-α-sterol and cytochrome P450, so the structure of membrane protein becomes damaged and membrane permeability increases which will cause fungal cell death. This fungal cell death will then inhibit the growth of Aspergillus flavus fungus [11].

The selection of Aspergillus flavus as a research subject because Aspergillus flavus is one of fungi that can cause otomycosis disease (Barati, dkk.2011). Where this disease occurs in areas with tropical and humid climates, such as Indonesia [3].

This study uses essential oils rather than extracts. Essential oils are obtained by distillation without using organic solvents, while extracts are obtained by separating substances with solvents suitable for organic solvents. Citronellal and Geraniol are soluble in organic solvents, so it to be used as an antifungal extract, high concentrations must be used.

This study used Kirby Bauer's diffusion method, which is the basis for quantitative testing by measuring the diameter of inhibitory zone formed on the media. This method uses discs where to bind to citronella essential oil which has volatile properties by soaking the disc at each concentration. The weakness of Kirby Bauer's method is that in measuring the inhibitory zone is influenced by incubation, inoculum and media thickness [2].

The action mechanism of fluconazole as an antifungal is by inhibiting the synthesis of ergosterol. Ergosterol is the main sterol which functions to maintain the fungal cell wall. Enzymdimethylase 14-α-sterol and cytochrome P450 change lanosterol becomes ergosterol. However, there is an azole group that will interfere with lanosterol interacting with the enzyme dimethylase 14-α-sterol and cytochrome P450. Reducing the amount of ergosterol can increase the permeability of the fungal cell wall which will inhibit the growth of the fungus. It causes the death of fungal cells [6]

This study used 1% CMC as a solvent because CMC has the property of being able to dissolve substances that are insoluble in water. CMC 1% was also used as a negative control. It was shown in the culture of Aspergillus flavus fungus on the Saboraud Dextrose Agar (SDA) media which was given a disk containing 1% CMC shows even fungal growth on the petri dish and no inhibitory zone formed. CMC 1% is as a negative control and as a solvent, it does not have an anti-fungi effect on the growth of Aspergillus flavus fungus, so Aspergillus flavus can still grow properly.

The media used in this study was Sabouraud Dextrose Agar (SDA) media which is a selective media for the isolation of fungi and yeast. SDA media contains casein, peptone and dextrose which play a role in supplying nutrients for fungal growth. Temperature and humidity are also factors that support the growth of mold, so during the study temperature and humidity were monitored with a room temperature of 23-25 °C with a humidity of 80%. The temperature and humidity did not change extreme during the study.

The inhibition of citronella essential oil began to appear at a concentration of 0,5% which was indicated by the inhibition zone formation with an average is 9.11 mm. The increase in the diameter of inhibition zone

was accompanied by a large increase in the concentration of citronella essential oil given. So, it can be concluded that the higher concentration of citronella essential oil, the better inhibitory effect produced.

The positive control of 1% fluconazole when compared with the average diameter of inhibition zone from citronella essential oil on the growth of Aspergillus flavus fungus was classified as higher than the concentration of 1.5% and smaller than the concentration of 2.0%. It can be caused at concentrations below 1.5%, the content of citronella essential oil is still in the form of a mixture from several ingredients which are not entirely anti-fungal, in contrast to fluconazole, which contains an active substance that has anti-fungal properties.

The diameter of inhibition zone that is resulted, it is included in the intermediate to sensitive category according to the sensitivity criteria of Davis and Stout inhibition zones. Meanwhile, according to the criteria for the level of anti-fungal effectiveness, it is included in the level of effectiveness from less effective to very effective. So, it can be stated that at each concentration the essential oil has an inhibitory power as an antifungal with different abilities.

The One Way Anova test results show a significance value is 0,000, where is $p < 0,05$. It means that, there is a significant effect on the average diameter of inhibition zone from various concentrations of citronella essential oil on the inhibition of growth from the Aspergillus flavus fungus. However, it was not yet known in detail which treatment group had a significant antifungal effect, so the LSD Post Hoc test was carried out. The results of LSD Post Hoc test showed that there was significance between treatment groups. It can be seen from the difference in the average diameter of inhibition zone formed.

The previous research was done by Utomo (2015), the citronella extract can inhibit the growth of Candida albicans fungus by 7 mm at a concentration of 30% which contains essential oil of 0.36%. The research that was done by Lely,et al (2018) citronella essential oil at a concentration of 0.5% could inhibit the growth of Trichophyton rubrum 9.5 mm, Trichophyton mentagrophytes 10.1 mm, and Candida albicans 14.7 mm. In this study citronella essential oil could inhibit the growth of Aspergillus flavus fungus by 9.11 mm. So, it can be said that citronella essential oil with a concentration of 0.5% can be used as an antifungal.

The results of research that has been carried out, are influenced by several factors, they include the manufacture of fungal suspense and the ability to absorb different discs. The manufacture of fungal suspense is carried out by comparing with the standard Mc Farland visually. The fungal suspension made by the researchers may have a different level of turbidity than the existing Mc Farland standard. Meanwhile, the different absorption abilities of the disks can also affect the results of resulting inhibition zone diameter, because researchers cannot sort out which disks have good absorption or which do not. It was controlled by the researchers by immersing the discs at the same time, it is 5 minutes. This study also has a weakness, where is the pH was not measured in the process of making SDA media.

Based on the description above, it is in accordance with the hypothesis proposed, that citronella essential oil (Cymbopogon nardusL. Rendle) has inhibitory power against the growth of Aspergillus flavus fungus. The higher concentration of essential oil is given, the higher content of anti-fungal

substances in the essential oil, so the diameter of the inhibition zone of citronella essential oil against the Aspergillus flavus fungus, it is getting wider.

CONCLUSION

Various concentrations of citronella essential oil have a significant effect on inhibiting the growth of Aspergillus flavus fungus with a large effect is 82.9%.

The average diameter of inhibitory zone from various concentrations of citronella essential oil against to the growth of Aspergillus flavus fungus is 9.11 mm; 12.03 mm; 13.62 mm and 16.81 mm.

The sensitivity of inhibitory power from citronella essential oil to the growth of Aspergillus flavus fungus at a concentration of 0.5%, it is intermediate, while at a concentration of 1.0% - 2.0% that is sensitive.

The percentage and degree of effectiveness of various concentrations from citronella essential oil against the growth of aspergillus flavusse fungus is large 64.06 % (less effective), 84.60% (moderately effective), 95.78 % (effective), and 118.21% (highly effective) compared to fluconazole 1%.

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