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INHIBITORY TEST OF DAYAK ONION (*ELEUTHERINE BULBOSA* MILL.) ESSENTIAL OIL TO THE GROWTH OF *MALASSEZIA FURFUR*

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ABSTRACT

Background: *Certain fungi are normal flora on human mucosa and skin, and they require lipids for growth. One of them is Malassezia furfur, which can develop into an opportunistic infection and will take a long time to treat. The fungus may cause pityriasis versicolor, which cause scaly and discoloration of the skin. It is estimated that 40-50% from population in the tropical country is infected. Dayak onion (Eleutherine bulbosa Mill.) essential oil have the potential to treat fungal infections. This research aims to see the inhibitory potential of dayak onion essential oil (Eleutherine bulbosa Mill.) in inhibiting the growth of Malassezia furfur.*
Methods: *This is an experimental study with a post-test control group design. The samples grouped into 7 groups. The first five treatment (P1 to P5) was given dayak onion (Eleutherine bulbosa Mill.) essential oil with a concentration of 3.125%,6.25%, 12.5%, 25%, and 50%, P6 (positive control) was given ketoconazole, and P7 (negative control) was given 10% DMSO. The inhibitory effect is measured from the diameter of the inhibitory zone, then analyzed using Kruskal-Wallis and Mann-Whitney tests.*
Result: *The result showed that average diameter on 50% concentration is 9.25mm, 25% concentration is 7.5mm, 12.5% concentration have diameter of 4.5mm, 6.25% concentration have diameter of 3mm, 3.125% concentration with diameter of 1.75mm, positive control group with diameter of 20mm, and negative control group with diameter 0mm.*
Conclusions: *Essential oil of dayak onion (Eleutherine bulbosa Mill.) shows inhibition of the growth of Malassezia furfur. The smallest inhibition zone diameter was 1.75 mm with concentration of 3.125%. The largest inhibition zone at a concentration of 50% with diameter of 9.25 mm. This finding showed potential effects towards antifungi treatment.*

Keywords: *Eleutherine bulbosa Mill, Malassezia fufur, pityriasis versicolor.*

INTRODUCTION

Malassezia furfur is normal flora on human mucosa and skin [1]. *Malassezia furfur* such as superficial fungus containing liporphilic dimorphic, requires lipids for growth [2]. *Malassezia furfur* can develop to an opportunist change from the yeast phase to the mycelia phase, attack the skin stratum corneum and can take a long time in treatment. [3]. Those tend to form *pityriasis versicolor*, percentage in Indonesia can be estimated 40-50% from population in tropical country. The percentage of the incidence of *pityriasis versicolor* in Jakarta and Bandung ranks second after dermatitis [4.5].

Treatment for a disease that caused by *Malassezia furfur* can use Ketoconazole medicine. But this medicine has side effects such as irritation to the skin, itching and burning sensation [6]. Because of that we need alternative treatment with herbs, because people believe in alternative or traditional

medicine. One of them is dayak onion (*Eleutherine bulbosa* Mill.) essential oil as a traditional medicine for fungal that originating from Kalimantan. Metabolic compounds contained such as alkaloids, glycosides, flavonoids, steroids, tannins, and metabolic compounds like naftokuinon has potential as antifungi interferes with the synthesis of the constituent components of the fungal cell wall. Even dayak onion (*Eleutherine bulbosa* Mill.) essential oil potentially against a MDR (*Multi Drug Resistant*) bacteria [7.8.9].

Research that has been done on essential oil of dayak onion (*Eleutherine bulbosa* Mill.), the result is that no inhibition zone is formed around the disc paper which indicates that there is no inhibition of the growth of *Malassezia furfur* fungi by the ethanol extract of dayak onion (*Eleutherine bulbosa* Mill.). Therefore, this research aims to see the inhibition of essential oil of dayak onion (*Eleutherine bulbosa* Mill.) in inhibiting the growth of *Malassezia furfur*.

METHODS

Experimental research by using *post test control group design*. Taxonomic test in biological laboratory of University of Semarang. The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon. This research has obtained *Ethical clearance* with No.33/EC/FK/XI/2018 on 9 November 2018 by the Ethics Commission of the Faculty of Medicine, Swadaya Gunung Jati University.

The sample used in this study is pure isolates of *Malassezia furfur* which were bred in the microbiology laboratory of the Faculty of Medicine, Swadaya Gunung Jati University, Cirebon. The inclusion criteria of *Malassezia furfur* colony which growth on SDA (*Sabouraud Dextrose Agar*) media in the form of yeast, white to cream. Exclusion criteria are *Malassezia furfur* fungus colonies contaminated with other microorganisme. How to take samples using *Purposive Random Sampling*. The sample group was divided into 7 groups. The number of repetitions is determined by the *Federer* formula and the results of the repetition are 4 repetitions. The independent variable of this research is dayak onion (*Eleutherine bulbosa* Mill.) essential oil. The dependent variable of this study is the diameter of the growth area of *Malassezia furfur*.

Pure isolates from *Malassezia furfur* were taken from one ose, transferred into a test tube and 0.9% NaCl was added until the turbidity was the same as the *Mc Farland standard 0.5* (concentration of 10^8 CFU/mL). Dilutions produced are then planted on SDA (*Sabouraud Dextrose Agar*) media by the pour plate method by taking 1 mL of the desired dilution material and poured on a petri dish then add the SDA (*Sabouraud Dextrose Agar*) liquefied at 45° C as much as 2 mL and homogenized. After that, it was incubated at 37° C for 4 days.

Making essential oil using the ethanol solvent method was carried out in the Lansida Yogyakarta laboratory in Indonesia, and carried out by the laboratory. Dayak onion (*Eleutherine bulbosa* Mill.) was checked for correct species in Universitas Negeri Semarang,. Then the bulbs was sorted, peeled and weighed in 1 kg. The bulbs then Then put it in the extractor container, added ethanol solvent, rotated with ultra turax at 1.000 rpm for 1 hour, then macerated for 24 hours. Then filtrate it with a Buchner funnel, sucked with a vacum machine. The residue is re-extracted as above. The first and second filtrate was evaporated with a rotary evaporator vacum at 35^o C vacum pressure. Pour the extract into a porcelain dish. Steam the remaining solvent with the waterbath. Weigh the extract obtained. There are seven groups, positive control group using Ketoconazole, negative control group using 10% DMSO (*Dimethyl sulfoxide*) and 5 treatment groups dayak onion (*Eleutherine bulbosa* Mill.) with concentration 50%; 25%; 12.5%; 6.25%; and 3.125%.

Determination of inhibitory zones in this study with the well method. Each well is dripped with dayak onion (*Eleutherine bulbosa* Mill.) essential oil. Next observe the results, antifungal potential is characterized by a clear zone around the well and measured the zone. The data obtained is processed

using a computer program. The statistical test used is *saphiro wilk* to see data distribution because the sample used is less than 50. The results of the data distribution were not normal so that it was continued with the *kruskal wallis* test and *mann whitney* test to see the comparison of the average of two groups from each group.

RESULTS

The dayak onion plants was checked for the correct taxonomy in University of Semarang to proof that the dayak onion used in this study is a correct species of *Eleutherine bulbosa* Mill.. The results of the average inhibition zone of *Malassezia furfur* growth after being given essential oil of dayak onion (*Eleutherine bulbosa* Mill.), presented as follows:

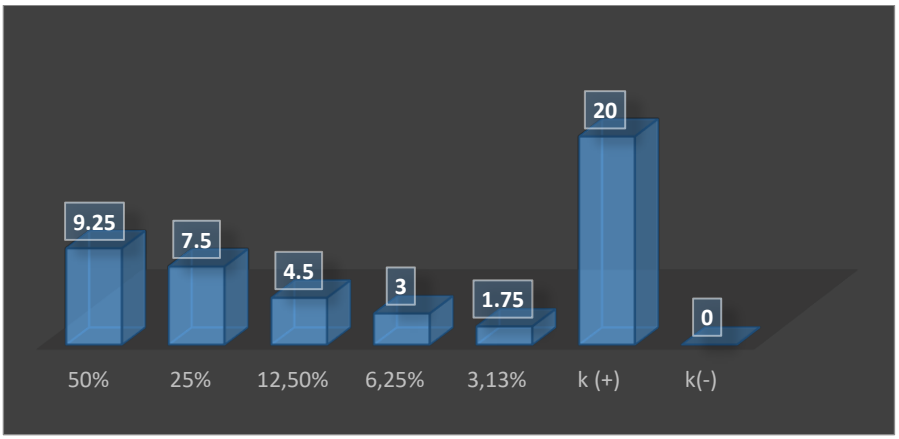

Figure 1. Average Means Diagram of *Malassezia furfur* Inhibitory Zone

The results of the analysis shows that the average diameter at a concentration of 50% was 9.25 mm have moderate barriers, 25% concentration as wide as 7.5 mm have moderate barriers, 12.5% concentration with diameter 4.5 mm have moderate barriers, 6.25% concentration with diameter 3 mm have weak barriers, 3.125% concentration with diameter 1.75 mm have weak barriers, positive control group with diameter 20 mm have strong barriers and negative control group with diameter 0 mm which means it does not inhibit. The highest concentration of dayak onion (*Eleutherine bulbosa* Mill.) can inhibit the growth of *Malassezia furfur* fungi with an average diameter of inhibition zone of 9.25 mm. Of all treatment groups the largest inhibition zone was obtained by positive control with a diameter of 20 mm.

The result of *saphiro wilk* test show the value $p<0,05$ which means that data is abnormally distributed, so *kruskal wallis* test was conducted instead. The test brings p 0.001 ($p<0.05$), that shows that there are significant differences between all groups of treatment of dayak onion (*Eleutherine bulbosa* Mill.) to fungi test.

Table 1. Value of Significance Comparison between Two Groups

Kode perlakuan	P1	P2	P3	P4	P5	P(+)	P(-)
P1	#						
P2	0,036	#					
P3	0,019	0,018	#				
P4	0,019	0,019	0,036	#			
P5	0,019	0,019	0,019	0,099	#		
P(+)	0,019	0,019	0,019	0,019	0,019	#	
P(-)	0,013	0,013	0,013	0,013	0,013	0,013	#

**Mann Whitney*

The results of data analysis using *mann whitney* within 21 pairs of groups showed that there were 20 pairs of treatment groups that had a value of $p<0.05$ which means that there were significant differences in inhibitory power in the pairs of treatment groups, and there were 1 pair of treatment groups namely P4 and P5 which has $p>0.05$, which means that there is no significant difference in inhibitory power in the pair of treatment groups.

In the treatment ratio P1 (concentration 50%) to the other treatment groups showed a significant difference between two groups. The treatment ratio P5 (concentration 3.125%) with other treatment groups, showed a significant difference between two groups. However, the treatment ratio between P4 (concentration 6.25%) and P5 (concentration of 3.125%) obtained a p value of 0.099 which indicates there is no significant difference.

DISCUSSION

This study aims to prove the inhibition of essential oil of dayak onion (*Eleutherine bulbosa* Mill.) on the growth of *Malassezia furfur* fungi. The results of the treatment using dayak onion (*Eleutherine bulbosa* Mill.) at a concentration of 50%; 25%; 12.5%; 6.25% and 3.125% to inhibit the *Malassezia furfur* fungi significantly has an antifungal effect which is characterized by the formation of inhibitory zones, namely an area around the well which is not overgrowth with *Malassezia furfur* fungi.

The average calculation of the inhibition zone of dayak onion (*Eleutherine bulbosa* Mill.) essential oil showed that the smallest inhibition zone diameter was 1.75 mm with a concentration of 3.125% and the largest inhibition zone at a concentration of 50% with a diameter of 9.25 mm, but from all treatment groups it was obtained that the largest inhibition zone is produced by positive control with a diameter of 20 mm. This is due to the proven working mechanism of ketoconazol and works by inhibiting fungal ergosterol synthesis which results in defects in fungal cell membranes [10]. The weaker inhibition of dayak onion (*Eleutherine bulbosa* Mill.) from positive control can be caused by the content of secondary metabolite compounds potentially inhibiting test fungi on dayak onion (*Eleutherine bulbosa* Mill.) is lower than the active compounds contained in positive controls. The inhibitory zones that are formed differently indicate that there is an ability of extracts that are different in inhibiting the growth of *Malassezia furfur* fungi which are influenced by the concentration of extracts from each group. The ability of antifungal substances to inhibit the growth of *Malassezia furfur* fungi is directly proportional to the concentration of the substance [7.11].

Comparison of this treatment was carried out to see the difference in significant inhibitory strength between two treatment groups in inhibiting the growth of *Malassezia furfur*. The comparison of treatment between P4 (concentration 6.25%) and P5 (concentration of 3.125%) obtained a p value of 0.099 which showed no significant difference in inhibitory power and no significant difference. This shows that the levels of P4 (concentration 6.25%) and P5 (concentration of 3.125%) have the same strength and are no different in inhibiting the growth of *Malassezia furfur*, and have a weak inhibition zone. This can be caused by several factors such as the type of microbes, the method of nanoparticle synthesis and the concentration of nanoparticles used [12].

The antifungal potential in dayak onion tuber (*Eleutherine bulbosa* Mill.) is also known to have a close relationship with its potential as an antioxidant. However, antifungals do not necessarily have the potential as antioxidants [13]. Dayak onion (*Eleutherine bulbosa* Mill.) in addition to being potential antifungals and antioxidants can also potentially inhibit lipopolysaccharide, which is found in the outer cell membrane of gram negative bacteria and triggers immune system activity. Dayak onion (*Eleutherine bulbosa* Mill.) also has a mechanism to inhibit lipopolysaccharide which stimulates the production of TNF-α cytokines, interleukin 6 (IL-6) and interleukin 12 (IL-12) p40 in bone marrow dendritic cells [9.14]. Even dayak onion (*Eleutherine bulbosa* Mill.) have the potential for a series of MDR (*Multi Drug Resistant*) bacteria [9].

CONCLUSION

Giving essential oil of dayak onion (*Eleutherine bulbosa* Mill.) can inhibit the growth of *Malassezia furfur* with of 9.25 mm inhibition zone at a concentration of 50%, 7.5 mm inhibition zone at a concentration of 25%, 4.5 mm inhibition zone at a concentration of 12.5%, 3 mm inhibition zone at a concentration of 6.25%, and 1.75 mm inhibition zone at a concentration of 3.125%. The smallest inhibition zone diameter was 1.75 mm with concentration of 3.125%. The largest inhibition zone at a concentration of 50% with diameter of 9.25 mm. Further research is needed to see the concentration of dayak onion (*Eleutherine bulbosa* Mill.) which is the most effective in inhibiting the growth of *Malassezia furfur* and the effectiveness of dayak onion (*Eleutherine bulbosa* Mill.) as an antifungal in other fungi.

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