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Anti-Fungal Activity Test of Sengkuang (*Dracontomelon dao*) Leaf Ethanol Extract on The Growth of *Candida albicans*

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Abstract: *Candida albicans* is an important opportunistic fungal pathogen that is responsible for the majority of fungal infections in humans. In healthy individuals, Candida colonizes mainly on the mucosal surfaces of the oral cavity, digestive tract, skin and genitals. *Dracontomelon dao* or known as Sengkuang has been used as a traditional medicine for various diseases. Based on various studies, *Dracontomelon dao* extract is reported to exhibit antimicrobial properties. This study aimed to analyze the antifungal activity of the ethanol extract of sengkuang (*Dracontomelon dao*) leaves on the growth of *Candida albicans*. The study used 96% ethanol extract by macerating the simplicia leaves of *Dracontomelon dao* originating from South Kalimantan, Indonesia. The antifungal activity was tested by well diffusion method at various concentrations of the test extract (10%, 20%, 30%, 40% and 50%). The results showed that the average diameter of the inhibition zones at concentrations of 10%, 20%, 30%, 40% 50% were 19.2 mm, 21.8 mm, 23.2 mm, 24.8 mm, 27 mm, respectively. Based on the results of the study, it was found that the ethanolic extract of sengkuang (*Dracontomelon dao*) leaves had antifungal properties against the growth of *Candida albicans* with the most effective concentration at a concentration of 50% with an average zone diameter of 27 mm. The content of phytochemical compounds from sengkuang leaf extract (*Dracontomelon dao*) are flavonoids, alkaloids, saponins, tannins, and triterpenoids. This secondary metabolite is thought to be able to inhibit the growth of the fungus *Candida albicans*. The ethanol extract of sengkuang leaves (*Dracontomelon dao*) has been shown to have antifungal activity in vitro so that it has the potential to be developed as an antifungal agent and can be a new solution in handling cases of *Candida albicans* infection.
Keywords: Antifungal; *Dracontomelon dao* Leaf; diffusion method; *Candida albicans*

INTRODUCTION

Fungi are one of the causes of infectious diseases, especially in tropical countries. The incidence of fungal infections in humans has increased in the last decade[1]. Candida species are commensal species and are part of the normal human flora and are localized to the skin, digestive tract, reproductive tract and skin, especially *Candida albicans* species. However, Candida can also cause various infections in susceptible patients including elderly, hospitalized, or immunosuppressed patients[2,3]. Candida spp.

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become the main nosocomial agent[1]. *Candida albicans* is part of the normal microbiota in about 50% of individuals. Candida infection has various clinical manifestations, from superficial mucocutaneous, invasive infectious disorders affecting multiple organ[4]. *Candida albicans* is an opportunistic fungus that causes candidiasis, thrush, skin lesions, vulvovaginitis, candiduria, gastric ulcers, and can even be a complication of cancer[1,4]. Treatment for *Candida albicans* infection can use drugs from natural ingredients. The use of these natural ingredients in an effort to find new and more effective antifungals against infection[3]. One of the natural ingredients that functions as an antimicrobial is *Dracontomelon dao*[5].

Dracontomelon dao (*Blanco*) *Merr et Rolf* belongs to the Anacardiaceae family and is widely distributed throughout South and Southeast Asia[5]. D. dao is known locally as Pacific Walnut, in Malay it is known as Sengkuang and in Indonesia it is known as Dahu. D. dao is a large tree with a height of 45-55 meters. D dao can be found in Cambodia, China, India, Indonesia, Malaysia, Myanmar, Papua New Guinea, the Philippines, Solomon Islands, and Thailand[6]. *Dracontomelon dao* bark is used for medicine. The ripe fruit from the seeds can be eaten, while the flowers and the young leaves are eaten as vegetables[7].

Dracontomelon dao has been used in traditional Chinese medicine for 1000 years to treat various infectious diseases, such as pressure sores and skin ulcers[6,8]. The bark of the D. dao tree is also used in traditional Filipino medicine to relieve sore throats, toothaches, gum problems, skin infections, dermatitis, and also for childbirth[7]. In addition, the bark of D. dao is used for diarrhea medicine by the Dayak tribe in East Kalimantan, Indonesia and dysentery by local people in the Philippines. In addition to the bark used as medicine, the leaves and flowers are also used in traditional medicine[9,10].

Dracontomelon dao extract was reported to exhibit antimicrobial properties[5]. The study of Peña et al showed that the ethanolic extract of *Dracontomelon dao* sapwood showed antimicrobial activity against *Salmonella typhimurium, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis*, *Candida albicans*, and *Aspergilus niger*[7]. Research by Khan and Omoloso showed that the ethanolic extract of D. dao leaves showed antibacterial effect against *Staphylococcus aureus* and *Bacillus*[6]. Liu et al demonstrated the antibacterial effect of D. dao leaf ethyl acetate extract against *E. coli*[11]. Zhao et al. also demonstrated the antibacterial effect of D. dao leaf ethyl acetate extract against *Staphylococcus aureus*[12].

There have been studies on the activity of the ethanolic extract of sapwood *Dracontomelon dao* against *Candida albicans*, but the leaf extract of *Dracontomelon dao* was still limited to research on activity tests against *Staphylococcus aureus* and *Bacillus* bacteria. Thus, this study aimed to analyze the antifungal activity of the ethanol extract of Sengkuang (*Dracontomelon dao*) leaf on the growth of *Candida albicans*.

MATERIALS AND METHODS

The research materials in this study were sengkuang leaves (*Dracontomelon dao*) from Hulu Sungai Selatan Area, South Kalimantan Province, Indonesia, 96% ethanol, Muller Hinton Agar (Merck), Sabouraud Dextrose Agar (Merck), Mc Farland standard solution 0.5, DMSO 10% and sodium chloride solution (NaCl) 0.9 %.

The selected *Dracontomelon dao* leaves are whole leaves, both young and old leaves. Cleaned with water and sterilized with 70% alcohol, then aerated. The leaves that have been aerated were dried in an oven (WTC Binder) at 50ºC for 4 hours. The dried sengkuang leaves were mashed using a blender and then filtered using a 30 mesh sieve to obtain a fine powder of sengkuang leaves. Sengkuang leaf extraction process was carried out by maceration method with ratio 1:3, 1 portion leaf powder and 3 portion ethanol 96% solvent. Sengkuang leaf powder was put into a closed container and then soaked with 96% ethanol solvent then stirred and allowed to stand for 24 hours. Then after 1x24 hours, filtering was carried out using filter paper to separate the macerated solvent (filtrate) with the remaining filtering simplicia (debris) interspersed with solvent replacement. The collected filtrate was then evaporated and evaporated with waterbath (Memmert) at a temperature of 60ºC to form a thick extract. The ethanol extract of sengkuang leaves in this study was made in concentrations of 10%, 20%, 30%, 40%, and 50% (v/v). The concentration was made by pipetting the ethanol extract of sengkuang leaves and then each of them was dissolved with 10% DMSO until the volume was 1 mL. Pure cultures of *Candida albicans* were grown on Sabouraud Dextrose Agar media, then incubated for 24 hours at 37ºC. *Candida albicans* colonies were taken using a sterile loop from SDA media and then added to a sterile 0.9% NaCl solution until the turbidity was equivalent to a 0.5 N Mc Farland standard solution.

Phytochemical screening for Sengkuang (*Dracontomelon dao*) leaf extract was carried out to determine the presence of flavonoids, alkaloids, saponins, tannins, and triterpenoids. Alkaloids test was carried out by placing the sample in a porcelain cup then adding 2 M HCl, stirring and then cooling it at room temperature. After the cold sample was added a small amount of NaCl then stirred and filtered. The filtrate obtained was added with a few drops of 2 M HCl, then chloroform was added, and the filtrate was added with Dragendorff's reagent. Formation of an orange precipitate indicates the presence of alkaloids[13].

For flavonoid, a little amount sample was evaporated, washed with hexane until clear. The residue was dissolved in ethanol and then filtered. The filtrate was added with a few drops of HCl and Mg metal and the color changes were observed. The red to orange color is given by flavone compounds[13]. The saponin test was carried out by adding several mL of the sample into a test tube and then adding distilled water and shaking it for thirty seconds, observing the changes that occurred. If a solid foam is formed (not lost for thirty seconds), the identification indicates the presence of saponins[14]. Saponin test was start with extract sample with hot distilled water and then cooled. After that, a few drops of 10% NaCl were added and filtered. The filtrate was added with 3 drops of FeCl3 reagent, then the changes were observed[13]. For triterpenoid test, the sample was extracted with ethanol, then the filtrate was added with chloroform and concentrated sulfuric acid. The observed result is formed. The formation of a red color indicates the presence of triterpenoids in the sample[13].

Testing the antifungal activity of the ethanol extract of sengkuang leaves using the well method. Muller Hinton agar media which had been liquid was poured into sterile petri dishes and allowed to solidify. Prepared mushroom cultures aged 24 hours. Mushroom suspension was made by taking colonies from culture using an loop then mixed in a test

tube containing physiological salt (0.9% NaCl). It was made until the cell density in the suspension reached 0.5 McFarland, then a sterile cotton swab was dipped into the fungal suspension by pressing and rotating the cotton swab on the tube wall twice. Wipe a cotton swab containing the fungal suspension evenly on the surface of the Muller Hinton media and allowed to dry for 4-5 minutes. A well was made and sengkuang leaf extract was added according to the variation of concentration. Petri dishes were incubated at 37°C for 24 hours. Last, the inhibition zone formed was measured.

RESULTS AND DISCUSSION

The antifungal activity is a measure of the extract's ability to kill or inhibit the growth of the test fungus. The antifungal activity of sengkuang leaf extract (*Dracontomelon dao*) was indicated by the presence of a inhibition zone where the area was not overgrown by the test microbes. *Candida albicans* colonies that grew in each treatment showed a visible difference in the mean diameter (mm) of the inhibition zone after 24 hours of incubation, like figure 1.

Table 1. Phytochemical Test Result

Parameter	Color Result	Conclusion
Alkaloid	Orange Precipitate	Positive
Flavonoid	Red Colour	Positive
Saponin	Solid Foam formed	Positive
Triterpenoid	Red Colour	Positive
Tanin	Indigo Colour	Positive

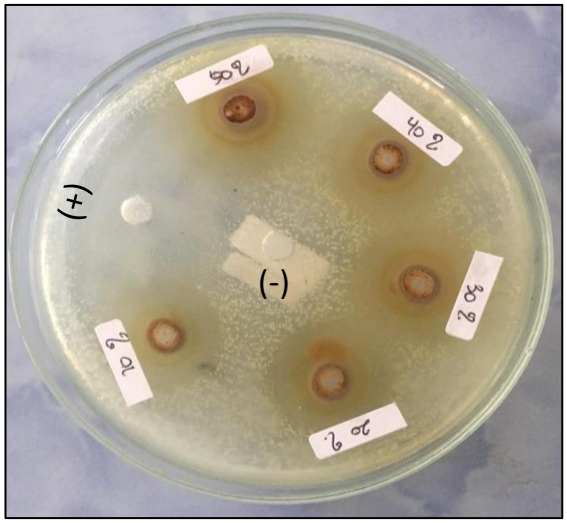

Figure 1. Inbition Zone Test Result

Code	Concentration
A	50%
B	40%
C	30%
D	20%
E	10%
F	Control Positive
G	Control Negative

Table 2. Average Zone Diameter of Each Concentration

Concentration	Average Zone Diameter
10 %	19,2
20 %	21,8
30 %	23,2
40 %	24,8
50 %	27

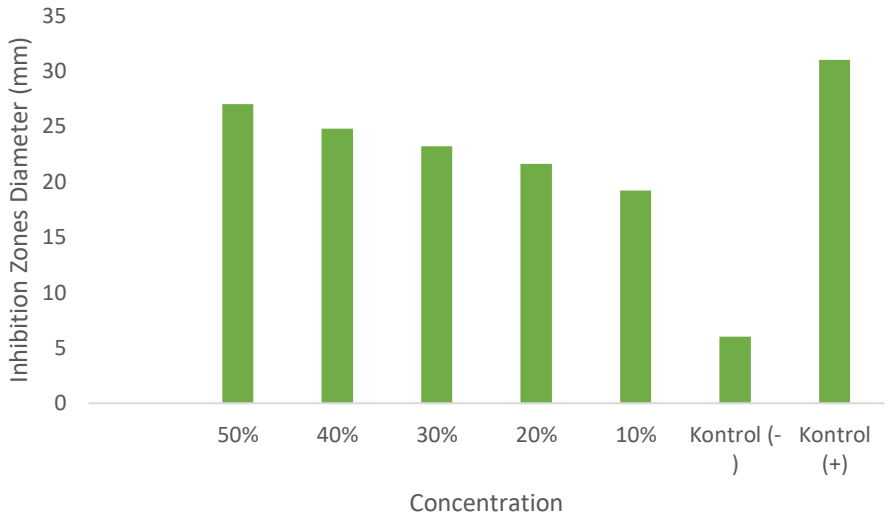

Figure 2. Inhibition Zone Results to Concentration

The statistical test results show normal data with p-value all above 0,05 and homogeneous data distribution with p-value 0,466 for the diameter of *Candida albicans* colony growth. Then the statistical test can be continued using the One-way Anova test.

Table 3. ANOVA Test Result

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	174.800	4	43.700	26.325	.000
Within Groups	33.200	20	1.660		
Total	208.000	24			

Judging from the results of the ANOVA test in the table 2, a significance value of 0, which means there is a significant difference between the test concentrations. Based on the results of the study (Table 2) a concentration of 50% produced the largest inhibition zone with 27 mm, so that the most effective concentration in inhibiting the growth of *Candida albicans* was a concentration of 50%. According to Pfaller, there are two types of standard methods for antifungal sensitivity testing. These methods are the disk

diffusion method and the microdilution method[15]. The disk diffusion method is an easy and inexpensive method to do. This method is standardized according to the Clinical and Laboratory Standards Institute (CSLI) and the results depend on the calculation of the diameter of the free zone. Fungal isolates could be categorized as sensitive, resistant, dose-sensitive depending on the MIC (Minimum Inhibitory Concentration) limit value.

Based on the results of this study, the greater the concentration of the extract, the greater the inhibition zone formed (Figure 2). The formation of inhibition zones in each treatment with the concentration of ethanol extract of sengkuang (*Dracontomelon dao*) leaves is thought to be due to the presence of active substances or secondary metabolites that can inhibit the growth of the fungus *Candida albicans*. The higher the concentration of the extract, the higher the content of active substances in it so that the antifungal activity will be even greater. On the other hand, the lower the concentration of the extract, the less the active substance in it, so that the antifungal activity will decrease. This is in accordance with Pelezar's statement in Yanti, that the higher the concentration of an antimicrobial substance, the greater the antimicrobial activity[16].

Ethanol solvent is a type of polar solvent used in this study. According to Sudarmadji et al. in Yanti, N., the polarity of the solvent used will also affect the diffusion process of the extract into the test medium[16]. Extracts with polar solvents will more easily enter the media and the process of inhibiting the growth of fungal colonies will be maximized. The solvent used must have the same polarity as the compound to be drawn.

Based on the phytochemical qualitative test results (Table 1), the ethanol extract of Sengkuang (*Dracontomelon dao*) leaves showed the presence of secondary metabolites such as flavonoids, alkaloids, saponins, tannins and triterpenoids. The phytochemical compounds contained in the ethanol extract of Sengkuang (*Dracontomelon dao*) leaves are thought to have antimicrobial properties that inhibit the growth of *Candida albicans*. Antifungal compounds have various inhibitory mechanisms against fungal cells.

Flavonoid compounds, tannins and saponins have a very high potential antimicrobial effect on target cells. This compound works by precipitating proteins and changing cell properties and serves as a good solvent for fatty substances that lyse cell membranes so as to move cellular components from the inside out and kill fungal cells[17,18]. Triterpenoids are bioactive compounds that have antifungal functions. Triterpenoids are toxic which can cause damage to cell organelles thereby inhibiting fungal growth[19]. This compound works by dissolving the cell walls of microorganisms by weakening the membrane tissue. While the mechanism of action of the alkaloid compounds in the extract inhibits the growth of fungal cell respiration and inhibits the synthesis of nucleic acids, proteins, and membrane phospholipids[20].

The ethanolic extract of *Dracontomelon dao* leaves in this study was shown to be able to inhibit the growth of *Candida albicans*, in line with the study of Peña et al, which showed that the ethanolic extract of *Dracontomelon dao* sapwood showed antifungal activity against *Candida albicans*[7]. So this research can prove that apart from *Dracontomelon dao* sapwood effectively inhibiting Candida albican, the leaves of *Dracontomelon dao* also have the same ability.

The limitation in this study is that the fungus used is only focused on *Candida albicans*, none to other types of fungi. This research can be continued by testing this extract on other types of fungi to find out more about its antifungal properties.

CONCLUSION

Based on the results of the study, it was found that the ethanol extract of the leaves of sengkuang (*Dracontomelon dao*) had antifungal abilities against the growth of *Candida albicans*. The results showed that the average diameter of the inhibition zones at concentrations of 10%, 20%, 30%, 40% 50% were 19.2 mm, 21.8 mm, 23.2 mm, 24.8 mm, 27 mm, respectively. The most effective concentration was at a concentration of 50% with an average zone diameter of 27 mm. The content of phytochemical compounds from sengkuang leaf extract (*Dracontomelon dao*) are flavonoids, alkaloids, saponins, tannins, and triterpenoids. The ethanol extract of sengkuang leaves (*Dracontomelon dao*) has been shown to have antifungal activity in vitro so that it has the potential to be developed as an antifungal agent and can be a new solution in handling cases of *Candida albicans* infection.

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CONFLICT OF INTEREST

The author has declared no conflict of interest.

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