

Activities test of Mahkota Dewa (*Phaleria macrocarpa*) leaves extract against *Candida albicans* of HIV/AIDS patients

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ABSTRACT

Introduction: *Candida albicans* is a local commensal flora of the oral cavity, with opportunistic nature and often causes oral candidiasis in HIV/AIDS patients. Since long time, *Mahkota Dewa (Phaleria macrocarpa)* known of having efficacy to treat various disease traditionally. The purpose of this study was to determine the activity test of *Phaleria macrocarpa* leaves extract against *Candida albicans* from HIV/AIDS patients. **Methods:** Experimental laboratory with samples colonies of the *Candida albicans* fungus obtained from patients with HIV/AIDS at Dr. M. Djamil General Hospital Padang. Research conducted during January-March 2016 in Microbiology and Chemistry Laboratory of Kopertis Region X, Microbiology Laboratory of Dr. M. Djamil General Hospital, and Microbiology Laboratory of Siti Rahmah Islamic Hospital, Padang, West Sumatra. Data was analyzed using the Kruskal-Wallis test. **Results:** The concentration of the *Phaleria Macrocarpa* leaves extract used in this study was 10, 20, 40 and 80%. Inhibition zone average value obtained 0,00 mm, means no inhibition zone, 9.217 mm and 18.017 mm with sig = 0.000 <0.05. **Conclusion:** The higher level of the *Phaleria macrocarpa* leaves extract concentration, the higher inhibition zone diameter against *Candida albicans*, with the highest in concentration 80%.

Keywords: Mahkota Dewa (*Phaleria macrocarpa*) leaves extract, *Candida albicans*, HIV/AIDS

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INTRODUCTION

Indonesia has many traditional medicine that can be used as an alternative treatment, but unexamined scientifically. One of them is *Mahkota dewa (Phaleria Marcocarpa)*.¹ *Phaleria macrocarpa* is a traditional medicinal plant that has been known for long and increasingly enthused, with efficacy healing sores, diabetes, influenza, allergies, asphyxia, dysentery, cancer,

and also skin, cardiac, and kidney diseases. The therapeutic effect of *Phaleria macrocarpa* closely related to the chemical compounds contained inside. It is known that only *Phaleria macrocarpa* grains are toxic, not including it's pulp. The highest inhibitory potentiation of *Phaleria macrocarpa* contained in its pulp, rather than the roots, barks, and leaves.^{1,2}

Phaleria macrocarpa leaves contains anti histamines, alkaloids, saponins, and polyphenols

(lignans).³ Saponin is a glycosides found from natural sources and can be detected by their ability to form a foam once whipped with a liquid solvent. In plants, saponin functionate as a reserved form of carbohydrates, waste product of metabolism, as well as protection against insect attack. Saponin is an antimicrobial agent against bacteria, viruses, fungi, and yeast.⁴ The active ingredient of *Phaleria macrocarpa* pulp is tannins, flavonoids, saponins, and alkaloids.²

The prior literature has mentioned that genera *Phaleria* plantation generally has an antimicrobial activity due to compounds inside. Yunanto⁵ has done research on infusum inhibition zone test of *Phaleria macrocarpa* leaves against *Streptococcus mutans* growth. The results showed that *Phaleria macrocarpa* leaves infusum have the ability to inhibit the growth with the highest inhibition at concentrations of 50%. Suryani and Selly⁶ research showed that *Phaleria macrocarpa* leaves infusum has antibacterial power against *Staphylococcus aureus* with Minimum Inhibitory Concentration (MIC) 3.125 gram% and Minimum Killing Concentration (MKC) 6.25 gram%, also, had not antibacterial power against *Eschericia coli* with MIC greater than 25 gram%.

From Wulandari⁷ research obtained that antibacterial substances contained in the *Phaleria macrocarpa* pulp are tannin, saponins, flavonoids, alkaloids, sterols, and terpenoid. Rostinawati⁸ research showed that *Phaleria macrocarpa* has an antibacterial effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus sp.*, *Escheria coli*, and *Salmonella thypi*, whilst against *Candida albicans* showed no antifungal activity. *Candida albicans* is one of the microorganisms that act as normal flora in the human body and also not harmful, but causes most infections in human, mostly localized infection such as oral and vaginal infections.⁹

Many fungal infection caused by *Candida* species, especially *Candida albicans*. There are about 30-40% in healthy adults oral cavity, 45% in neonates, 45-65% in healthy children, 50-65% in patients using removable denture, 65-88% in patients with long-term medication, 90% in patients with acute leukemia whose undergo chemotherapy, and 95% in HIV/AIDS patients.¹⁰

Oral candidiasis is co-infection that is most common in patients with HIV/AIDS. Although the

pathogenesis always associated with decrease in patient's immune system, but the real mechanism has not clear yet. *Candida albicans* is commensal flora of the oral cavity with the opportunistic nature. The formation of hyphae is a transformation sign of microorganism nature from commensal becoming pathogenic.¹¹

AIDS (*Acquired Immunodeficiency Syndrome*) is a set of symptoms or infections caused by HIV (*Human Immunodeficiency Virus*). HIV attacks the immune system, causing a decrease in patient endurance. In patients with AIDS, a decrease in the immune system is closely related to the occurrence of opportunistic infections. Cumulatively, AIDS cases in Indonesia since April 1, 1987 until December 31, 2011 were as much as 29.879 cases, with death rate was 5430 patients. Based on gender classification, AIDS cases found mostly in men with 20.333 cases, whilst in women found only 8.122 cases.¹² Based on references from previous research, further researchers have done about activities test of *Phaleria macrocarpa* leaves extract against *Candida albicans* of HIV/AIDS patients.

METHODS

Tools used in this study were scissors, petri dish, large bottle, rotary funnel, porcelain bowl, bath autoclave, test tubes, paper disc, Whatman filter paper, glass rods, incubator, measuring cup, Erlenmeyer flask, digital scales, glass funnel, aluminum foil, plastic wrap, sterile cotton bud, test tubes, rubbing alcohol, inoculating loop, glass slides, microscope, ruler, rotary evaporator, tongue spatel, mask, handscoon, and tissue. Materials used in this study were *Phaleria macrocarpa* leaves extract, ethanol 96%, aquades, Ketoconazole, physiological solution (NaCl 0.9%), Alcohol 70%, coloring materials, and Dimethyl Sulfoxide (DMSO).

Phaleria macrocarpa leaves that obtained from Naniang River Village, Bukit Barisan District, was extracted. The fresh leaves washed with clean water, then being wind dried for 10 days. After that, it was inserted into a dark bottle with the size of 2.5 liters, and poured with 2 liters of ethanol 96% by using a glass funnel. Then, it was tightly sealed by a bottle cap. It was stirred every single day. A week after, *Phaleria macrocarpa*

Table 1. The making of the *Phaleria macrocarpa* leaves extract solution concentration

DMSO (Ml)	Extract (Gr)	Final volume (Ml)	Concentration (%)
9	1	10	10
8	2	10	20
6	4	10	40
2	8	10	80

maceration immersion filtered using a cotton coated glass funnel to be inserted into Erlenmeyer flask so ethanol extract filtrate can be obtained.

In order to obtain a thick extract then evaporated using a rotary evaporator. After obtained a viscous extract, then poured into a porcelain bowl and evaporated again with bath. Aerated at room temperature afterwards. The extraction process was completed and obtained a viscous extract of *Phaleria macrocarpa* leaves. *Phaleria macrocarpa* leaves solution concentration used in this study were 10,20,40 and 80% (Table 1).

The medium (Sabouraud Dextrose Agar) making done by adding 6.5 gr of Sabouraud Dextrose Agar powder with 100 ml aquadest. Both were mixed and then heated until boiled and then cover with gauze containing cotton. Medium and tools that will be used sterilized in the autoclave for 15 minutes at 121°C. Liquid Sabouraud Dextrose Agar as a medium poured into a sterilized petri dish as high as 5-6 mm and let stand until solidified. *Candida albicans* proliferation was done by collecting specimens on HIV/AIDS patients tongue by using sterile *cotton bud*. The basting was done by using a cotton bud in Sabouraud Dextrose Agar medium, incubated for 24 hours to getting a perfect accretion of *Candida albicans*.

This fungus specimen collected using inoculating loop, then basted on Sabouraud medium tester. The tip of inoculating loop then burned by using methylated spirit burner, chilled in Sabouraud Dextrose Agar medium afterwards. Scratched into four parts and do gram stain to ensure the growing mold. Then burn again the inoculating loop tip until the color turned red.

The planted specimens then inserted into an incubator for 48 hours at 37°C. If the visible colonies on Sabouraud Dextrose Agar shaped round, ellipse, or oval, with a slightly convex and smooth

surface and sometimes slightly overlapped, and had the smell of yeast, then the colonies were identified as *Candida albicans*.

Candida albicans identification was done by using gram stain. One inoculating loop contained *Candida albicans* culture placed on the top of a glass object, dripped with 1 drop of normal saline (0.9% NaCl), and poured with gentian violet carbolic dye, then let stand for 1 minute. The dye was then removed and immediately dropped with lugol (without being washed first), let stand again for 1 minute. Afterwards, lugol disposed and the preparation was washed with 96% alcohol until no more color was dissolved. The glass object then washed with flowing water until clean, and poured with fuchsin solution, let stand for 1 minute. Washed again with running water until clean. Pat dry with filter paper. Observed under the microscope with the objective lens. On microscope observation will seen *Candida albicans* having ellipse, small, thin walled yeast, with sprout, and having a violet color (indicating classified to the gram-positive).

The germ tube was done by putting one inoculating loop contained germ into a sterile petri dish that has been filled with egg white. Then incubated for 3 hours in an incubator. Observed under a microscope to see the germ tube. The number of repetitions for antifungal activity test conducted in this study was calculated using the Federer general formula. The number of treatment groups in the study were 4 treatments and repeated 6 times, so the sample numbers became 24 treatment. Thus, to fulfill the requirements of a statistical test, required 6 repetitions using one kind of *Candida albicans* sample.¹³

Testing of antifungal activity using agar diffusion method which was making Sabouraud Dextrose Agar (SDA) media which has been basted with *Candida albicans* in a petri dish. Afterwards, placed paper disc that has been soaked in a concentrated solution, with one petri contained 4 discs with the same concentration, that was the concentration of 10, 20, 40 and 80%. For positive control, 200 mg of ketoconazole 200 mg were placed into one piece of paper disc in a petri. The entire petri incubated at 37°C for 48 hours. Limpid zone seen around the paper disc was then measured using a caliper.

RESULTS

On the research it seen that the visible colonies on Sabouraud Dextrose Agar having the round, ellipse, or oval shape, with a slightly convex and smooth surface and sometimes slightly overlapped, and had the smell of yeast, then the colonies were identified as *Candida albicans* (Fig. 1).

From gram-positive stain results, *Candida albicans* found in the form of yeast, oval shaped, with approximately 5 µm diameter, and reproduced by forming bud (Fig. 2). This kind of fungus often found in the form of mycelium with pseudohyphae.¹⁴ *Germ Tube* formation (sprout) test also can be used to identifying *Candida albicans* species. On germ tube test, the medium used was protein factor contained materials, such as egg white. On this research, medium used was egg white which before used placed in an incubator at a temperature of 37°C

for 2-3 hours.¹⁵ The results confirmed positive if on microscopic examination found cell shape that germinate, racket-like shaped (Germ tube). From colony identification result can be ascertained the mold growing was *Candida albicans*. Activities

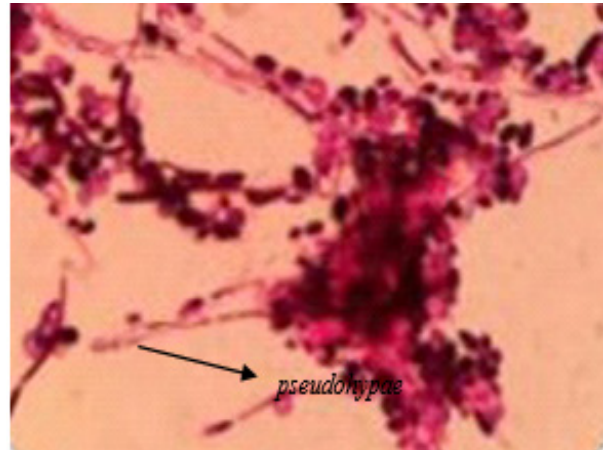


Figure 1. *Candida albicans* culturing after 7 days

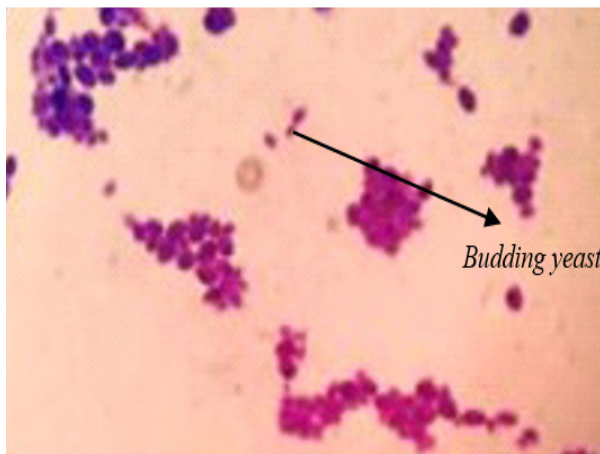


Figure 2. *Candida albicans* gram-positive stain

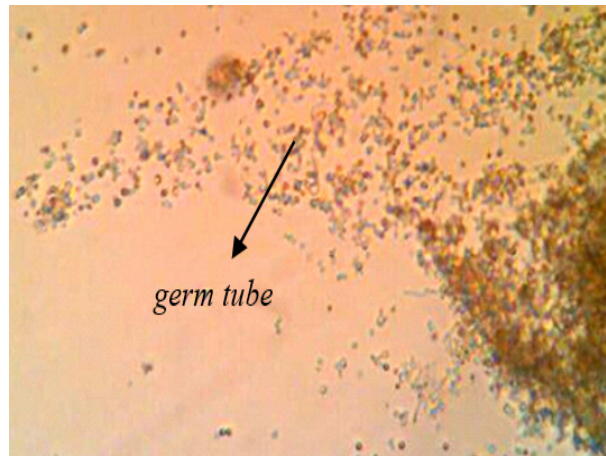


Figure 3. Germ tube test

Table 3. The growth inhibition zone diameter of *Candida albicans* by using *Phaleria macrocarpa* leaves extract

Experiment/ Repetition	Inhibition zone diameter (mm) in various concentration of <i>Phaleria macrocarpa</i> leaves extract				Ketoconazole Control (+)
	10%	20%	40%	80%	
I	-	-	9.3	18.1	
II	-	-	9.2	18.5	
III	-	-	9.0	18.2	
IV	-	-	9.2	17.5	20.1
V	-	-	9.3	17.7	
VI	-	-	9.3	18.1	
Mean			9.217	18.017	20.1

Table 4. Descriptive analysis of *Phaleria macrocarpa* leaves extract activities against *Candida albicans*

Concentration Variable	N	Mean	Deviation Standard	95% CI	
				Lower	Upper
10%	6	0.00	0.000	0.000	0.000
20%	6	0.00	0.000	0.000	0.000
40%	6	9.217	0.1169	9.094	9.339
80%	6	18.017	0.3601	17.639	18.395
Total	24	6.808	7.6486	3.579	10.038

test of *Phaleria macrocarpa* leaves extract against *Candida albicans* of HIV/AIDS patients, on the concentration of 10, 20, 40 and 80% obtained the following results:

The results of the experimental data were analyzed using statistical program in order to knowing the anti-fungal potency of *Phaleria macrocarpa* leaves extract against *Candida albicans* of patients with HIV/AIDS at concentrations of 10, 20, 40 and 80%. Previously, normality test was done by using the Shapiro-Wilk test to determine whether the data was normally distributed or not. Based on the normality test gained that $p=0.000$ where the p -value was less than 0.05 ($p<0.05$), means that the data obtained was not normally distributed. Because the data was not normally distributed then the non-parametric Kruskal-Wallis test used to see the inhibition zone of *Phaleria macrocarpa* leaves extract against *Candida albicans*.

DISCUSSION

On the examination of *Phaleria macrocarpa* leaves extract against *Candida albicans*, at the concentration of 10, 20, 40, and 80%, obtained the average values such as follows: at the concentrations of 10 and 20% found no inhibition zone; at the concentration of 40% obtained the inhibition zone average value of 9.217 mm; and at the concentration of 80% obtained the inhibition zone average value of 18.017 mm. Based on the average value can be concluded that the higher the concentration levels of the *Phaleria macrocarpa* leaves extract the higher the inhibition zone against the growth of the *Candida albicans*, where the highest inhibition zone power obtained at concentration of 80%. From the statistical test results using Kurskall-Wallis test obtained $\text{sig}=0.000<0.05$, and concluded that there were

differences on *Phaleria macrocarpa* leaves extract against *Candida albicans* at concentration of 10, 20, 40 and 80%.

Research on the same extract also has been done by Yunanto⁵ about the infusum inhibition zone test of *Phaleria macrocarpa* leaves against the growth of *Streptococcus mutans*. The results showed that *Phaleria macrocarpa* leaves infusum has the inhibition potency against the growth of *Streptococcus mutans*, with the highest inhibition level at the concentration of 50%. Suryani and Selly⁶ research showed that *Phaleria macrocarpa* leaves infusum having antibacterial potency against *Staphylococcus aureus* with MIC 3.125 gram% and MKC 6.25 gram%, but did not has antibacterial potency against *Eschericia coli* with MIC greater than 25 gram%.

Another research done by Wijaya and Hendra¹ about in vitro test of anti-bacterial effect of *Phaleria macrocarpa* vernal pulp against *Klebsiella pneumoniae*. The results showed that the ethyl acetate and ethanol extract of *Phaleria macrocarpa* vernal pulp capable to inhibit the growth of *Klebsiella pneumoniae* bacteria, with MIC of both extract were at concentration of 1% and the ampicillin antibiotic equality value of ethyl acetate and ethanol extract of *Phaleria macrocarpa* vernal pulp against *Klebsiella pneumoniae* respectively were 0.053 and 0.003%.

Compared with other antifungal research of other plantation, *Phaleria macrocarpa* leaves had more effective antifungal potency compared with Bay leaves extract, as found on Bhaskara¹⁶ research about invitro antifungal potency test of Bay (*Syzygium polianthum*) leaves ethanol extract against *Candida albicans*. The results showed that the ethanol extract of Bay leaves has antifungal potency against *Candida albicans* at concentration of 40, 80, and 100%, with each inhibitory zone diameter respectively 7, 9, and 11. From another

research done by Nahak¹⁷ found that ethanol extract of *Beluntas (Pluchea indica L.)* leaves was capable to inhibit the growth of *Streptococcus mutans* bacteria, at concentration of 25, 50, 75, and 100%, with the inhibitory zone average value respectively 11.2, 14.2, 15.6 and 19.2 mm. Minimum concentration that able to inhibit the bacteria growth was 25%.

Another research done by Kusriani, Khairul, and Bambang¹⁸, about the antimycosis potential of several Indonesian medicinal plantation against *Candida albicans* mycosis, where the ethanol extract of *Annona squamosa L. (Srikaya)*, *Phyllanthus acidus L. (Ceremai)* and *Phaleria macrocarpa (Mahkota Dewa)* had larger antifungicidal potency compared with dichloromethane extract. The highest antimycosis activity against *Candida albicans* as compared to comparator antibiotics showed by the ethanol extract of *Annona squamosa L.*, having the highest activity equal with 11.565,11 ug of Ketoconazole, and *Phaleria macrocarpa* extract equal with 2.344,46 ug of Ketoconazole.

The effectivity of *Phaleria macrocarpa* leaves extract towards the inhibition potency of *Candida albicans* due to so many chemical compounds contained in this plant which was very useful to inhibit the growth of *Candida albicans*. The same thing presented by Hariana³, that the leaves of *Phaleria macrocarpa* contained antihistamines, alkaloids, saponins, and polyphenols (lignans). Saponin as an antimicrobial agent against bacteria, viruses, fungi, and yeast.⁴ The active compositions of *Phaleria macrocarpa* were tannins, flavonoids, saponins and alkaloids.²

On HIV/AIDS patients, *Phaleria macrocarpa* leaves are very useful because it's ability to inhibit the fungus growth. *Candida albicans* is one of the microorganisms that act as normal flora in the human body and also not harmful, but causes most infections in human, mostly localized infection such as oral and vaginal infections.⁹ According to Akpan¹⁰, many fungal infection caused by *Candida* species, especially *Candida albicans*, with the rate of 95% in the oral cavity of HIV/AIDS patients. The most common HIV-related oral cavity lesions is candidiasis, particularly caused by *Candida albicans*. In a study reported that oral candidiasis occurs in 17-43% of patients with HIV infection, and more than 90% of patients with AIDS.¹⁹

From the research data of *Phaleria macrocarpa* leaves can be determined that at concentration of 40% the inhibition zone was 9.217 mm, classified as average criteria, and at concentration of 80% the inhibition zone was 18.017 mm, classified as powerful criteria. This determination based on Davis and Stout in Pratama¹⁹, reported that the provisions of antifungal inhibitory criteria were as follows: inhibitory zone of 20 mm or more includes to very powerful criteria, inhibitory zone of 10-20 mm includes to powerful criteria, inhibitory zone of 5-10 mm includes to average criteria, and inhibitory zone of 5 mm or less includes to weak criteria.

From the research result analysis, there were significant differences of *Phaleria macrocarpa* leaves extract against *Candida albicans* at concentration of 10, 20, 40, and 80%. The highest inhibitory happened at the concentration of 80%, inhibitory average value 18.017 mm. The effectivity of *Phaleria macrocarpa* leaves extract use will be very helpful for HIV patients because it's ability to inhibit fungus growth on patient's oral cavity, so that it can become an alternative natural medication to reduce the *Candida albicans* fungus in patients with HIV.

CONCLUSION

The higher level of the *Phaleria macrocarpa* leaves extract concentration, the higher inhibition zone diameter against *Candida albicans*, with the highest in concentration 80%.

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