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ANTI-FUNGAL EFFECTS OF AMBON BANANA SKIN EXTRACT (*MUSA PARADISIACA* LINN. *VAR.SAPIENTUM*) ON *CANDIDA ALBICANS* ATCC® 10231 ™ (IN VITRO)

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ABSTRACT

Oral Introduction disease is a global problem for health. One of the most common infectious diseases is a disease caused by fungi (mycosis), a species of *Candida* (candidiasis) caused by *Candida albicans.* These bananas are generally often consumed daily by Indonesians. Ambon banana skin extract (*Musa Paradisiaca* Linn. *Var.Sapientum*) is a nutritious medicinal plant, because Ambon banana skin waste has active antifungal compounds, namely tannins, flavonoids, quinones, phenols, and steroids. which can damage fungal cell wall membrane proteins, damage the DNA chain causing brittle cell walls resulting in fungal cell death. **objectives** Research to determine the zone of inhibition, MIC and MBC of Ambon banana skin extract at concentrations of 100%, 90%, 80%, 70%, 60%, and 50% against *Candida albicans* ATCC® 10231™. **Method** laboratory experimental research with a post test only control group research design, the sample used was 18 samples, tested with the Anova test. In determining the inhibition zone using the well diffusion method, the measurement of the inhibition zone uses the calipers, MIC and MBC using the dilution method. Ambon banana skin was extracted by maceration method using 70% ethanol solvent, carried out 6 treatments with various concentrations. Each treatment was repeated 3 times. **The results** of the inhibition zone research at concentrations of 100%, 90%, 80%, 70%, 60%, and 50% with an average of 13.2mm 11.6mm 11.3mm, 10.5mm, 9.9mm, 8.3mm. The MIC of each concentration was clear and equalized according to the standard of 0.5 McFarland (1.5 x 10^8 CFU / ml) with potato dextrose broth medium, and the MBC was emphasized with potato dextrose agar (PDA) medium. Conclusion Inhibition zone concentrations of 100% -70% indicate strong criteria, 60-50% including weak criteria. MIC obtained at a concentration of 50%, MBC at a concentration of 60%. In this study, using a concentration of 70% indicates an average diameter of above 10mm, meaning that it is in accordance with the David and Stout method which states that the criteria are strong at an average of 10-20 mm. a concentration of 70% is used because it is effective in killing *Candida albicans* ATCC® 10231™, 100% -80% has a high toxicity which can cause toxic effects on all organisms such as the body, fungi, plants, etc.

Key words: Ambon banana skin extract, *Candida albicans ATCC® 10231™* zone inhibition, MIC and MBC

Introduction

Ambon banana fruit is a fruit that is often consumed daily, because Ambon banana has a sweet taste so that the banana is a part that is often consumed by Indonesian people. Each type of banana has its own uniqueness and characteristics. One of them is the Ambon banana plant (*Musa paradisiaca* L.) which is effective in healing wounds, anti-diabetes, and anti-hypertension.[1]

Classification of Ambon Banana

Other name : Banana Ambon
Kingdom : Plantae
Divisio : Spermatophyta
Sub divisio : Angiosperms
Class : Dicotyledoneae
Order : Zingibirales
Family : Musaceae
Genus : *Moses*
Species : Musa paradisiaca *Var.Sapientum (L.) Kunt*

Figure 1. Pohon Pisang Ambon

Banana skin is the most common waste from banana fruit and is rarely used by community as a food ingredient and its utilization is still lacking because banana skins are often thrown away by the community so that it piles up as garbage.[2]

Benefits of Banana

skin In previous studies it was proven that the Ambon banana skin extract (Musa paradisiaca Linn. Var.Sapientum) contains tannins, flavonoids, quinones, phenols, and steroids. which has been confirmed to have an antifungal effect against *Candida albicans*.[3]

Tannins are active compounds that act as antifungals. because of its ability to inhibit the synthesis of chitin which is used for the formation of cell walls in fungi and damage cell membranes so that fungal growth is inhibited.[4]

Flavonoids are the largest group of compounds in nature known as antioxidants that have antibacterial and anti-fungal effects because they contain phenol groups, coagulating protein by inhibiting DNA synthesis by metabolites of 5-flurouracil. and reduce the surface tension of the fungal cell walls.[5]

Quinon works by breaking the DNA chain.[3]

Phenols are fungistatic compounds that can denaturate proteins. Protein synthesis of fungal cells is disrupted due to the direct inhibition of DNA synthesis by the metabolite 5-fluurouracil, causing brittleness of the fungal cell walls so that they are easily penetrated by other active substances that are fungistatic.[5]

Steroids can inhibit fungal growth, ɐ cytoplasm or interfere wɪtn tne growtn and development of fungal spores. function as an antifungal because the lipophilic properties of steroids can inhibit spore germination in fungi

Classification of *Candida albicans*

Classification of *Candida albicans* according to the *Integrated Taxonomic Information System* (ITIS) Catalog of Life 2020 is Kingdom : Fungi
Division : Ascomycota
Subdivision : Saccharomycotina
Class : Saccharomycetes
Order : Saccharomycetales
Family : Saccharomycetaceae
Genus : *Candida*
Species : *Candida albicans*

Figure 2. *Candida albicans*

Candida albicans olso contains virulence factor that can contribute to its ability to cause infection. In addition, the phenotife or appearance of microorganisms can change from white and flat to irregular wrinkles, star-shaped, circular, and opaque. *Candida albicans* has a complex cell wall structure, 100 to 400 nm thick.cell walls *Candida* and give shape to cells and protect yeast cells from their environment.[7]

Candida albicans is a small, oval mushroom, measuring 2.5 x 4 x 6 µ. Grow at room temperature and incubator. Is dimorphic and produces true hyphae. In the agar medium at 25^0C or room temperature, *Candida albicans* can form cream-colored colonies and have cell budding.[8]

Candidiasis is found all over the world, can affect all ages, both men and women. Transmission can occur via direct contact or through contact. *Candida albicans* is the most abundant species worldwide, representing a global average of 66% of all *Candida sp*.[9]

This candidiasis can develop in any oral cavity, but the most frequent locations are the buccal mucosa, buccal mucosal folds, oropharynx and tongue. In addition, candidiasis can also develop into a systemic infection through lymph flow that attacks vital organs such as the kidneys, lungs, brain and blood vessel walls which are fatal.[10]

Candida fungi have factors that affect adhesion to epithelial cell walls such as mannose, C3d receptors, mannoprotein and Saccharin. The hydrophobic nature of the fungus as well as its adhesion ability with the host fibronectin also plays an important role in the initiation of this infection.[10]

2. Host

A. Local factors

use of drugs such as inhaled steroids has been shown to increase the risk of oral candidiasis infection. This is due to the suppression of cellular immunity and phagocytosis. The use of dentures is a predisposing factor for oral candidiasis infection. This use results in the formation of a microenvironment that facilitates the development of candida fungi in low PH, low oxygen, and anaerobic environments.[10]

B. Systemic factors

use of drugs such as broad-spectrum antibiotics can affect the local oral flora thereby creating a suitable environment for candida fungi to proliferate. Discontinuation of these drugs will reduce the chances of a candida yeast infection. Other drugs such as antineoplastic agents which are immunosuppressive also affect the development of candida fungi. Several other factors that predispose to oral candidiasis infection are smoking, diabetes, Cushing's syndrome and HIV infection.[10]

Methods of Antifungal Examination for Candida albicans ATCC

Dilution Method

There are two kinds of dilution methods, namely solid dilution and liquid dilution. Both of these methods have the same principles, what distinguishes them is the media used. The concentration of the test solution that has been determined as MIC is re-cultured on new media and incubated for 18-24 hours, if the media does not have microbial growth after incubation, it is determined as MBC.[8]

A. Liquid Dilution

This method measures MIC (*minimum inhibitory concentration*) and MBC (*minimum bactericidal concentration*). The way this is done is by making a series of dilution of the antimicrobial agent in a liquid medium which is added with the test microbes. The test solution for the antimicrobial agent at the smallest level that looks clear without any microbial growth was determined as MIC, then re-cultured in liquid media without the addition of test microbes or antimicrobial agents, and incubated for 18-24 hours. Liquid media that remained clear after incubation was defined as MBC.[8]

B. Solid Dilution

This method is the same as the liquid dilution method but uses solid media. The antifungal agent was diluted in agar medium and then poured into a petri dish. After the agar freezes, the test microbes are inoculated, then incubated at a certain time and temperature. The lowest concentration of antibacterial agent solution which still provides inhibition against germ growth is defined as the minimum inhibitory level (MIC). Where the advantage is that one concentration of antifungal medium can be used to test multiple test fungi.[8]

A. The diffusion method in this study used the cup plate technique method.

The cup-plate technique method.

The well method is to make a hole in the solid agar that has been inoculated with the fungus. On the agar plate that has been inoculated with the test fungus, a hole is made which is then filled with the test antimicrobial agent. Then each hole is filled with the test substance. Then incubated and assessed the formed inhibition zone.[8]According to David and Stout (1971) clear zone criteria can be seen in

Table 1. Criteria for Clear Zone

No	Inhibition Power	Results
1	> 20 mm	Very Strong
2	10-20 mm	Strong
3	5-10 mm	Medium
4	<5 mm	Weak

Source: David and Stout 1971

Research method

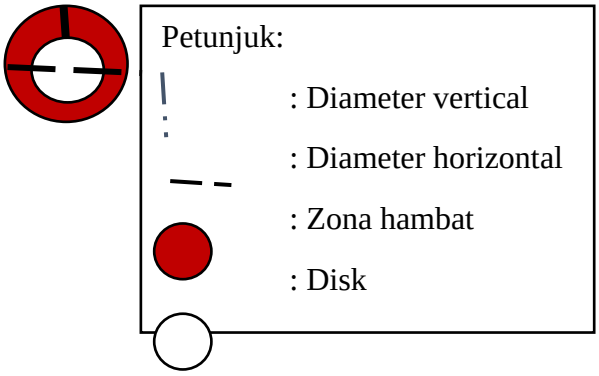
type of research is a laboratory experimental research design with post test only control group design, namely by measuring observations after special treatment. The research period was January to March 2021. The activities were collecting references, exploring thefungus *Candida albicans* available at the USU Pharmaceutical Microbiology Laboratory from pure culture products ATCC® 10231™. from Microbiologics USA with PT. Multi Redjeki Jakarta.

The extract of Ambon banana skin (*Musa paradisiaca Linn. Var.Sapientum*) was carried out at the Laboratory for Research and Development of Medicinal Plants of the Indonesian Herbal Medicine Traditional Medicine Association (ASPETRI), North Sumatra.

The research sample used was the *Candida albicans* ATCC® 10231isolate™ available through PT. multi redjeki Jakarta.

Measurement method

Diameter of inhibition zone = $\frac{horizontal + vertical}{2}$



Federer's sample size formula:

$$(t-1)(r-1) \geq 15$$

Description:
t = number of treatments
r = number of replications

In this study, 8 treatment groups were used:
1. 70% ethanol extract of Ambon banana skin (*Musa paradisiaca* Linn. *Var. Sapientum*) 100%.
2 . 70% ethanol extract of Ambon banana skin (*Musa paradisiaca* Linn. *Var.Sapientum*) 90%.
3. 70% ethanol extract of Ambon banana skin (*Musa paradisiaca* Linn. *Var.Sapientum*) 80%.
4. 70% ethanol extract of Ambon banana skin (*Musa paradisiaca Linn.Var.Sapientum*) 70%.
5 . Ethanol extract 70% Ambon banana skin (*Musa paradisiaca* Linn. *Var.Sapientum*) 60%

6 . Ethanol extract 70% Ambon banana skin (*Musa paradisiaca* Linn. *Var.Sapientum*) 50%
7. Positive control (standard therapy) with nystatin.
8. Negative control using DMSO

$$(t-1)(r-1) \geq 15$$
$$(8-1)(r-1) \geq 15$$
$$(r-1) \geq 2,14$$
$$r \geq 3,14 \approx 3$$

$$(t-1)(r-1) \geq 15$$
$$(8-1)(r-1) \geq 15$$
$$(r-1) \geq 2,14$$

So, the number of treatments (t) = 8, then the number of samples needed is 24, the sample used is the *Candida albicans* ATCC® 10231culture™, with the number of repetitions of 3 times for each concentration of 100% 90% 80 % 70% 60% 50%. this repetition was done to avoid bias in the results of the study. Nystatin positive control and DMSO negative control as a comparison with the respective concentrations of Ambon banana skin extract.[11]

Observation of Inhibition Zones on *Candida albicans* ATCC® 10231™ with the Diffusion Method

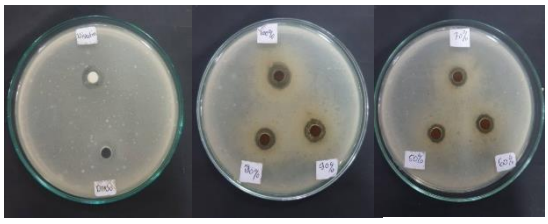


Figure 3. Inhibition zones formed at each concentration, positive control, andcontrol negative.

The mushroom suspension was dropped into 0.1 ml of petri dishes, then 15 ml of Potato Dextrose Agar (PDA) was poured into a petri dish, then mixed by stirring to resemble number 8 so that the suspension and *Candida albicans ATCC® 10231™* well blended then allowed to solidify for 2 minutes.[12] Then do the making of three wells for each concentration on the mixed agar medium. Drop each extract concentration into the well, then incubateand observe to see the inhibition zone formed after 24-48 hours.[13] Each test material is done 3 times and observes all the repetitions of the tested material at the same time.

Nystatin is a drug used as a positive control that provides a clear zone, shown to inhibit the growth of *Candida albicans* ATCC® 10231™. In this study. each concentration of Ambon banana skin extract was proven to be able to inhibit the growth of *Candida albicans* ATCC® 10231™, in the presence of a clear zone in the wells area.

Table 2.The measurement results for the diameter of thezone inhibition of various concentrations of Ambon banana skin extract on the growth of the fungus *Candida albicans* ATCC® 10231 ™ (diameter of inhibition zone (mm)

concentration	I	II	III	Mean	Standard Deviation
100%	13.7	13.1	12.9	13.2	0.4163
90%	11.9	11.5	11.4	11.6	0.2646
80%	11.5	11.2	11.3	11.3	0.1528
70%	11.	10.	10.	10.	0.450

	0	1	5	5	9
60%	9.9	9.6	10.2	9.9	0.3000
50 %	8.2	8.5	8.4	8.3	0.1528
Nystatin	11.5	11.8	12.2	11.8	0.3512
DMSO	-	-	-	-	0.0000

Next,used *oneway* ANOVA wasto test whether there was a significant difference in inhibition diameter and number of *Candida albicans* ATCC® 10231colonies™ among Ambon banana skin extracts of 100%, 90%, 80%, 70%, 60%, 50%, positive control (*nystatine*), and negative control (DMSO). Based on the results of the normality test, it was found that each variable had a significance above 0.05, which means that the data was normally distributed. Based on the homogeneity test, a significance value of 0.196 (p> 0.05) was found. shows homogeneous data then One Way ANOVA has a significance value of 0.000 (p <0.05), which means that there is a significant difference in the variation in the concentration of Ambon banana skin extract (Musa paradisiaca Linn. Var.Sapientum) in inhibiting the growth of the fungus *Candida albicans*. Based on the Post Hoc Test Tukey showed that there were significant mean differences between the inhibition zone concentrations of 100%, 50%, and DMSO, and found a significant mean equation between concentrations of 60% and 70%, concentrations of 70% and 80%, concentrations of 80%, 90% and nystatin.

Observation of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) on *Candida albicans* ATCC® 10231™ with the Dilution method for

Determination of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) were carried out t[80%A]m[K+A] smallest extract concentration capable of inhibiting [70%] [60%] [50%A] [K-A] ter the incubation period, by observing the level of turbidity at each concentration in each tube. Observations were made on all repetitions of the experimental material at the same time.

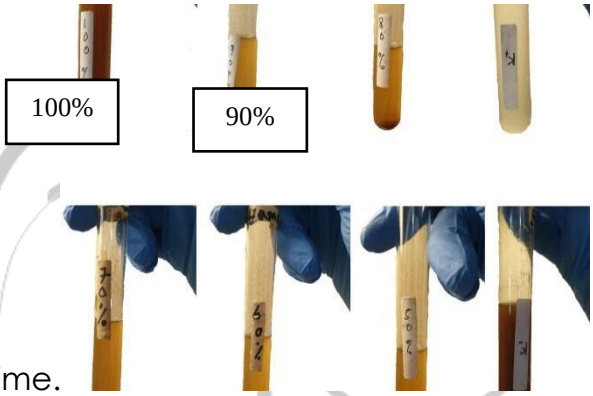


Figure 4. Tube of banana skin extract with Ambon several concentrations with the addition of *Candida albicans* ATCC® 10231™.

Based on the results of the determination of MIC and MBC, the turbidity can be compared with positive control and negative control. Turbidity on the media indicates the presence of fungi growth, while the clear media indicates the absence of mold growth. The media is marked as very cloudy (++), cloudy (+), and clear (-) to facilitate observation. The results of the following dilution test were made in the form of a table.

Table 3. Observation results of the dilution turbidity of various concentrations ofskin extract Ambon bananaon the growth of *Candida albicans* ATCC® 10231™

Concentration	Repetition1	Repeat 2	Repetition 3
100%	-	-	-
90%	-	-	-
80%	-	-	-
70%	-	-	-
60%	-	-	-
50%	-	-	-
Positive control	++	++	++
Negative control	-	-	-

Information:

++ : Very cloudy
\+ : Cloudy
\- : Clear

ble 4. Calculation result Of spots number *Candidaalbicans* from the streak plate test confirmation of various concentrations of extract Ambon banana skin growth of *Candida albicans* ATCC® 10231™.

Concentration of	Repetition 1	Repetition 2	Repetition 3
100%	-	-	-
90%	-	-	-
80%	-	-	-
70%	-	-	-
60%	-	-	-
50%	+	+	+
Positive control	+	+	+
Negative control	-	-	-

Remarks:

(+) : there is fungal growth
(-) : there is no fungal growth

Based on the observations, the results of the test results confirm the concentration of 100%, 90%, 80%, and 70% 60% there is no fungal growth in the three replications while the 50% concentration confirmation test is found fungal growth on all three replications. Based on the above results, it can be concluded that the 50% concentration is expressed as MIC, while the 60% concentration is expressed as MBC. The concentration below the MBC indicates the presence of fungal growth again.

Discussion

Based on the research that has been done, the results of the minimum inhibitory

Consentration (MIC) is 50% and the minimum Consentration (MBC) is 60%. This proves that the Ambon banana skin extract has antifungal activity. This effect can occur because Ambon banana skin has potential antifungal compounds, namely tannins, flavonoids, quinones, phenols, and steroids.

Bioactive compounds that have antifungal properties are effective against the growth of *Candida albicans*¸ this can be seen from several previous studies. In previous research, Pranata (2019) proved that Ambon banana skin extract was able to inhibit the growth of *Candida albicans* and had a value of MBC at a concentration of 40%, and in this study, MIC was not found because the results obtained were all turbidity so that it could not be ascertained visually.

From the previous Ambon banana skin extract test results, there were several differences. The difference between this study and the previous research was that the type of solvent used was different, then the different concentrations used, the growth medium, andisolates *Candida albicans*.

The results of the previous research determined the minimum inhibitory level (MIC) and the minimum Bactericidal consentration (MBC) which were found, namely the concentration of 40% was MBC while the MIC could not be determined because there was no affirmation test, from the research results I got in determining the MIC and MBC, namely the concentration. 50% is MIC while 60% MBC, previous studies did not determine the inhibition zone, and the solution used was 96% ethanol, Saboraud Dextrose Agar (SDA) and Saboraud Dextrose Broth (SDB) media, andisolates *Candida albicans* used were not ATCC preparations. , there is a difference from the previous research with the research I used, namely in the form of 70% ethanol solution, Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB)media[14.15],isolates *Candida albicans* ATCC® 10231™

Conclusion

There is an antifungal activity of banana skin extract Ambon (Musa paradisiaca Linn. Var.Sapientum) against the fungus *Candida albicans* at concentrations of 100%, 90%, 80%, 70%, 60%, and 50% were fertilized Note by the formation of an inhibition zone around the well area. Concentrations of 100-70% criteria are strong, 60-50% are weak.

MIC was obtained at a concentration of 50% and MBC at a concentration of 60%.

In this study, the concentration of 70% shows an average above 10mm, which means that according to the method of David and Stout, the average diameter of 10-20mm is categorized as strong, because it is effective in killing *Candida albicans* ATCC® 10231™ with a

concentration of 100%, 90%, 80%. its high toxicity which makes its impact toxic to all organisms such as the body, fungi, plants, etc.

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