



Potential Antifungal of Merang Padi Extract (*Oryza sativa*) Against Fungal Growth

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<p>Track Record Article</p> <p>Accepted: 03 February 2024 Revised: 03 March 2024 Published: 20 March 2024</p> <p>How to cite : Masniah, M., & Faisal, A. P. (2024). Potential Antifungal of Merang Padi Extract (<i>Oryza sativa</i>) Against Fungal Growth. <i>Contagion : Scientific Periodical of Public Health and Coastal Health</i>, 6(1), 387–394.</p>	<p style="text-align: center;">Abstract</p> <p><i>Merang padi is a traditional medicine that comes from natural ingredients, namely plants. However, there has not been much research on the activity of rice straw extract (<i>Oryza sativa</i>) being explored and researched. The aim of this research was to determine the yield of extracts and the number of antifungal isolates in inhibiting fungal growth and their potential as antifungals against <i>Candida albicans</i> and <i>Aspergillus fumigatus</i> fungi. This research uses experimental research with a quantitative approach carried out through laboratory tests. The design used was a Completely Randomized Design (CRD) which was grouped into 6 treatment groups, namely 4 groups of rice straw extract and 2 control groups which were repeated for each treatment 4 times. The treatment group consisted of P1 with rice straw extract with a concentration of 20,000 ppm, P2 with rice straw extract with a concentration of 40,000 ppm, P3 with rice straw extract with a concentration of 60,000 ppm and P4 with rice straw extract with a concentration of 80,000 ppm. Meanwhile, the control group, namely P0 as a negative control, namely NaCl 0.9% and P5 as a positive control, namely Nystatin 100,000 IU. The research sample was rice straw from rice plants that had been identified and then weighed. Data analysis used one way ANOVA SPSS version 20. If there were differences then a further test was carried out, namely the Honest Significant Difference Test (BNJ) to see the differences between each treatment based on the coefficient of diversity (KK) value obtained at the 5% test level. The results of tests that have been carried out show that the n-hexane fraction of rice straw has no antifungal activity, while the ethyl acetate and n-butanol fractions have antifungal activity. The concentration of ethyl acetate in this study was greater than the other compounds.</i></p> <p>Keywords : <i>Candida albicus, merang padi, mushrooms, oryza sativa</i></p>
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INTRODUCTION

Fungi can cause serious infectious diseases. Increased infections result in excessive morbidity and mortality. In addition, the population at risk of infection due to fungi will increase significantly (Aly, 2011; Hamidah, 2013). However, not all of the population will immediately feel the effects or symptoms of the infection, until the infection becomes more serious. Based on reports, the increasing variety of pathogens will also increase serious and dangerous infections such as opportunistic *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (Mirza et al., 2003).

Candida albicans is a pathogenic species and is the main cause of candidiasis. *Candida albicans* grows in various human bodies (Salmiati et al., 2021). *Candida albicans* under normal circumstances can live in balance with various other microbes in the intestine. In individuals with a suppressed immune system, the body's normal fungi can cause disease (Kumamoto et al., 2021). *Candida albicans* is part of the normal microbial flora that is well adapted to living

in humans, especially in the gastrointestinal, urogenital and skin tracts (Mutiawati, 2016; Suraini, 2023).

There are *Aspergillus* species that are pathogenic to humans and animals, and the disease they cause is called aspergillosis (Hasanah, 2017). One example of a pathogenic *Aspergillus* is *Aspergillus fumigatus*. *Aspergillus fumigatus* is the cause of pulmonary aspergillosis as an opportunistic pathogen in humans with an incidence of 80-90%. *Aspergillus fumigatus* is more likely to cause infection and death than *Candida albicans* (Indriani et al., 2020). *Aspergillus fumigatus* is more likely to cause infection and death than *Candida albicans* (Kumamoto et al., 2021).

Deaths due to fungal infections other than skin diseases are very high, also due to late or wrong diagnosis and non-toxic antibiotics that can be used medically, so the disease spreads and gets worse (Pelczar & Chan, 2007). To prevent or control infectious diseases is to eliminate microbes by administering antibiotics (Ihwana et al., 2022). The choice of therapy and dose of antifungal given depends on the patient's health condition, comorbidities suffered and has been proven to be an effective antifungal (Hsu et al., 2006).

However, designing an effective antifungal is not easy. This is because fungi (fungi) are eukaryotic organisms that have fungal cell membranes that contain sterols. Sterols are an important component in maintaining the life of fungi (Rohmawati & Harahap, 2017). The important sterols in fungal cell membranes are ergosterol and zymosterol, whereas in mammalian cells, the membranes contain cholesterol (Romanos et al., 1992). So antifungal agents not only kill fungi but also harm human tissue. The ideal antifungal drug should target specific pathways or processes for fungal cells, thereby reducing the possibility of tissue damage (Hasim & Coleman, 2019).

Rice straw has various benefits. Then research related to the activity of rice straw extract (*Oryza sativa*) has not been explored and researched further (Suriani, 2018). Sumardiani (2018) stated that Merang Padi is effective as an antibacterial against Tricophyton mentagrophytes bacteria. Therefore, researchers are interested in conducting this research. Based on the description above, researchers are interested in conducting research entitled "Antifungal Potential of Merang Padi (*Oryza sativa*) Extract on Fungal Growth".

METHODS

This research uses experimental research in the laboratory with a quantitative approach. The samples used in this research were merang padi from rice plants that had been identified and then weighed. The design used was a Completely Randomized Design (CRD) which was

divided into 6 treatment groups, namely 4 merang padi extract groups and 2 control groups. The repetitions carried out for each treatment in this study were 4 times. The treatment group consisted of P1, P2, P3, and P4. Each is merang padi extract with concentrations of 20,000 ppm, 40,000 ppm, 60,000 ppm and 80,000 ppm. Determination of concentration is based on preliminary tests that have been carried out previously. Meanwhile, the control group consisted of P0 as a negative control, namely NaCl 0.9%, and P5 as a positive control, namely Nystatin 100,000 IU . Research data were analyzed using one way ANOVA SPSS version 20. If the calculated F value \geq F table then there are real differences between treatments and the alternative hypothesis (H_a) is accepted . Then, if there are real differences, further tests are carried out to see the differences between each treatment based on the diversity coefficient (KK) values obtained. The further test used is the Honestly Significant Difference Test (BNJ) at a test level of 5% .

1. Tools and materials

The tools used in this research include Erlenmeyer flasks, beakers, test tubes, measuring cups, stir sticks, petri dishes, cork borers, digital scales, incubators, laminar *air flow* , sterile cotton swabs, spirit lamps, oses, micropipettes, tips, autoclaves, hot plate stirrers, refrigerators, rotary evaporators, cuvettes, spectrophotometers, ovens, vernier calipers.

Meanwhile, the ingredients used include merang padi extract. Meanwhile, *Candida albicans* and *Aspergillus* isolates were obtained from the Microbiology Laboratory, Pharmacy Department, Health Polytechnic, Ministry of Health, Medan. Other ingredients are filter paper, label paper, Nystatin 100,000 IU, NaCl 0.9%, ethanol 96%, sterile distilled water, tissue, SDA media (*Sabouraud Dextrose Agar*), aluminum foil, masking tape, HCl, H₂SO₄ , FeCl₃.

2. Research procedure

a. Making Merang Padi Extract

Merang padi simplicia was macerated in 96% ethanol with a simplicia and solvent ratio of 1:7.5. Maceration takes 3x24 hours, stirring every 4 hours for 15 minutes, then the ethanol solvent is replaced every 24 hours. The extraction results are filtered with filter paper until the filtrate and residue are obtained. Filtering is carried out 3 times so that no residual residue is included. The extraction results are mixed together and then evaporated using a rotary vacuum evaporator or water bath at a temperature of 60°C to obtain a thick extract..

b. Preparation of *Candida albicans* and *Aspergillus* Fungus Suspension

Candida albicans fungus culture was taken , then mixed into a test tube containing 10 mL of 0.9% NaCl. The mushroom suspension was homogenized by shaking for approximately 15

seconds, then poured into a cuvette as much as 7 mL. The cuvette is inserted into a spectrophotometer to measure turbidity with a wavelength of 530 nm and an absorbance number of 0.5 - 0.6, which means it is equivalent to the Mc Farland standard of 0.5 (1×10^6 - 5×10^6 cells/mL) (WHO, 2009).

c. Antifungal activity test

Antifungal activity was tested using the well hole method with a hole diameter of 6 mm (Balouiri et al., 2016). After turbidity measurements were carried out according to standards, *Candida albicans* was inoculated into SDA media by dipping a sterile cotton swab into the inoculum, then drained. The inoculum is smeared over the entire surface of the media 3 times, rotating the cup at a 60° angle with each application. Then apply a sterile cotton swab around the edge of the surface. Let the inoculum dry for a few minutes at room temperature, making sure the cup is covered (WHO, 2009).

The SDA media that has been inoculated with the *C. albicans suspension* is left for 5-15 minutes so that the fungal suspension absorbs into the media. Next, a hole was made in the SDA media with a diameter of 6 mm using a sterilized *cork borer*. Each hole was dripped with 50µl of merang padi extract with concentrations of 20,000 ppm, 40,000 ppm, 60,000 ppm and 80,000 ppm. Nystatin 100,000 IU as a positive control and NaCl 0.9% as a negative control. Then incubated at 37°C for 1x24 hours and the clear zone formed was measured. The parameter observed in this study was the diameter of the clear zone formed around the well hole and was measured using a caliper vertically and horizontally. Measurement results are expressed in millimeters (mm).

RESULTS

Rendement

Merang padi extract is obtained from the extract resulting from the extraction process using the maceration method, namely soaking 400g of dry sample using 3 liters of ethanol solvent at room temperature. This solution was then concentrated using a rotary evaporator, the concentrated extract was then evaporated in a water bath to obtain an extract with a low water content to produce 84.4 g of crude rice straw extract.

Then 20g of the extract was weighed and liquid-solid fractionation was carried out. The solvents used were n-hexane, ethyl acetate, and n-butanol. From the results obtained 2.91 g of n-hexane fraction, 4.26 g of ethyl acetate fraction, and 6.14 g of n-butanol fraction.

Table 1. Merang Padi Extract Yield Data

No.	Sample	Yield (%) Strawberry rice against					
		Fresh Sample (3200g)	Dry Sample (400g)	Crude Extract (84.4g)	n-hexane fraction (2.91g)	Ethyl acetate fraction (4.26g)	n-butanol fraction (6.14g)
1.	Fresh Samples						
2.	Dry sample	12.5					
3.	Crude Extract	2.63	21.1				
4.	N-hexane fraction	0.09	0.72	3.44			
5.	Ethyl acetate fraction	0.13	1.06	5.04			
6.	N-butanol fraction	0.19	1.53	7.27			

Based on table 1, these results show that the crude rice straw extract contains more polar compounds than non-polar ones.

Antifungal Activity of Merang Padi Extract on *Candida Albicans* fungus

In determining the concentration series for antifungal activity tests, the agar diffusion method was used by researchers. The concentration series designed are 3%, 5%, 7%, and 9%. The test results can be seen in the following table:

Table 2. Research result

Extract	Concentration	n1	n2	n3
Ethanol	3%	8.76	8.46	7.66
	5%	10.63	9.52	9.03
	7%	15.53	15.51	14.91
	9%	10.98	10.66	10.19
n-hexane		not tested		
ethyl acetate	3%	9.19	8.18	8.39
	5%	12.87	12.64	11.74
	7%	18.71	18.46	17.58
	9%	11.38	10.22	10.19
n-butanol	3%	5.72	4.23	4.20
	5%	7.46	7.22	6.02
	7%	13.43	13.42	12,19
	9%	7.89	7.66	6.74
ketoconazole	3%	8.85	-	-
	5%	9.54	-	-
	7%	9.88	-	-
	9%	10.59	-	-

Table 3. Extract clear zone data

Test Bacteria	Concentration	Mean Clear Zone Diameter (mm)					
		ethanol	n-hexane	ethyl acetate	n-butanol	control (-)	control (+)
Candida albicans	3%	8.29	0	8.59	4.72	0	8.85
	5%	9.73	0	12.42	6.90	0	9.54
	7%	15.32	0	18.25	13.01	0	9.88
	9%	10.61	0	10.60	7.43	0	10.59

Based on the results of the tests that have been carried out, it was found that the n-hexane fraction of rice straw does not have antifungal activity because there is no inhibition zone or clear zone, which is indicated by the fraction being good at low concentrations. The concentration of ethyl acetate in this study was greater than that of other compounds. The secondary metabolite compounds in rice straw are alkaloids, saponins and phenols. This class of secondary metabolite compounds can inhibit the growth or kill the bacteria.

DISCUSSION

Rice straw is a plant that grows in tropical areas and is most often found. Rice straw is used as a staple food (Siregar et al., 2022). This research aims to determine the extract yield, determine the number of antifungal isolates in inhibiting fungal growth and its potential as an antifungal against *Candida albicans* and *Aspergillus fumigatus* fungi .

Antifungal activity testing using the agar diffusion method. Paper Disc as a backup by observing the formation of a clear zone which indicates antibacterial activity. The data obtained was then analyzed using the one-way ANOVA method to determine the effect of variations in test concentration in killing or inhibiting bacterial growth, then continued with analysis.

Merang padi ethyl acetate extract has antifungal activity in inhibiting or killing the *Candida albicans* fungus. Water as a negative control had no inhibitory effect (Septiadi et al., 2013). Based on the test results, it was found that the n-hexane fraction of rice straw did not have antifungal activity. This is because there is no inhibition zone or clear zone shown by this fraction even at low concentrations. Research by Triana (2016) states that merang padi extract has high polarity, while the ethyl acetate and n-butanol fractions have demonstrated antifungal activity due to the presence of an inhibition zone.

Then it was also found that the concentration of ethyl acetate was greater than that of other compounds. This is because in the ethanol extract the compound is still in a complex state rather than a single fraction based on its polarity and solubility properties. Thus, the fraction

has better antifungal activity at low concentrations compared to the ethanol extract in a complex state.

The secondary metabolite compounds in rice straw are alkaloids, saponins and phenols. This group can inhibit the growth or kill the bacteria (Julianto, 2019). The antifungal mechanism of alkaloid compounds is by interfering with the peptidoglycan components in bacterial cells, so that the cell wall layer is not fully formed and the bacterial cells will die, for example berberine (Maisarah et al., 2023). Then, saponin is a strong surface tension lowering compound, so it works as an antifungal by damaging the cytoplasmic membrane (disturbing the stability of the cell membrane) and can then cause lysis of microbial cells such as protodioscin (Anggraeni et al., 2023). The mechanism of phenol as an antifungal is by interfering with the permeability of the cytoplasmic membrane. This can cause leakage of intracellular materials that inactivate microbial enzyme activity and synthesis (Hidayah et al., 2017).

CONCLUSIONS

Merang padi ethyl acetate extract has antifungal activity in inhibiting or killing the fungus *Candida albicans*, while water as a negative control does not provide an inhibitory effect. *the concentrations of 3%, 5%, 7%, and 9% were 8.59, 12.42, 18.25, and 10.60, respectively.* The results of the n-hexane fraction of merang padi do not have antifungal activity because there is no inhibition zone or clear zone shown. The concentration of ethyl acetate in this study was greater than that of other compounds. Because in the ethanol extract the compounds are still in a complex state compared to the fraction where the compounds in the fraction are already in a single state based on their polarity and solubility values, so the fraction already has better antifungal activity at low concentrations compared to the ethanol extract which is still in the complex state.

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