

The antifungal potential of rice straw (*Oryza sativa*) extract against the growth of pathogenic fungi

Potensi Antifungal Ekstrak Merang Padi (*Oryza sativa* L.) terhadap Pertumbuhan Jamur

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ABSTRACT

Background: Opportunistic fungal infections such as *Candida albicans* and *Aspergillus fumigatus* are increasing, particularly among immunocompromised patients. The use of synthetic antifungal agents is often limited by side effects, resistance, and high costs, thus alternatives from natural sources are needed. Rice straw (*Oryza sativa* L.), an agricultural by-product rich in bioactive compounds, has been reported to possess antibacterial activity; however, its potential as an antifungal agent has not been extensively investigated.

Objective: This study aimed to evaluate the antifungal potential of rice straw (*Oryza sativa* L.) extract against the growth of two pathogenic fungi, *Candida albicans* and *Aspergillus fumigatus*.

Methods: The research was conducted experimentally in a laboratory with a quantitative approach. Extraction of rice straw was carried out using maceration with 96% ethanol, followed by fractionation with *n*-hexane, ethyl acetate, and *n*-butanol solvents based on polarity differences. Antifungal activity was tested using the well diffusion method, with inhibition zone measurements as an indicator of effectiveness.

Results: The results showed that ethyl acetate and *n*-butanol fractions exhibited significant antifungal activity against *Candida albicans* and *Aspergillus fumigatus*, as evidenced by the largest inhibition zones at a concentration of 7%. The ethyl acetate fraction demonstrated an average inhibition zone of 18.25 mm against *Candida albicans* and 12.83 mm against *Aspergillus fumigatus*. Conversely, the *n*-hexane fraction did not show antifungal activity. Statistical analysis using one-way ANOVA revealed significant differences among concentrations and fraction types.

Conclusion: This study concludes that rice straw extract, particularly the ethyl acetate fraction, possesses potential as a natural antifungal agent effective against pathogenic fungi, and may be further developed for traditional plant-based therapy.

Keywords: antifungal, fractionation, rice straw

ABSTRAK

Latar Belakang: Infeksi jamur oportunistik seperti *Candida albicans* dan *Aspergillus fumigatus* semakin meningkat, terutama pada pasien imunokompromais. Penggunaan antifungal sintesis sering terkendala oleh efek samping, resistensi, dan biaya tinggi, sehingga diperlukan alternatif dari sumber alam. Merang padi (*Oryza sativa* L.), limbah pertanian yang kaya senyawa bioaktif, telah dikenal memiliki aktivitas antibakteri, namun potensinya sebagai antifungal masih belum banyak diteliti.

Tujuan: Penelitian ini bertujuan untuk mengevaluasi potensi antifungal dari ekstrak merang padi (*Oryza sativa* L.) terhadap pertumbuhan dua jenis jamur patogen, yaitu *Candida albicans* dan *Aspergillus fumigatus*.

Metode: Penelitian dilakukan melalui metode eksperimental di laboratorium dengan pendekatan kuantitatif. Ekstraksi merang padi dilakukan dengan metode maserasi menggunakan etanol 96%, kemudian difraksinasi dengan pelarut *n*-heksana, etil asetat,

dan n-butanol yaitu metode fraksinasi berdasarkan tingkat kepolarnya. Aktivitas antifungal diuji menggunakan metode sumuran dengan pengukuran zona hambat sebagai indikator efektivitas.

Hasil: Hasil penelitian menunjukkan bahwa fraksi etil asetat dan n-butanol memiliki aktivitas antifungal yang signifikan terhadap *Candida albicans* dan *Aspergillus fumigatus*, ditunjukkan oleh terbentuknya zona hambat terbesar pada konsentrasi 7%. Fraksi etil asetat menunjukkan zona hambat rata-rata 18,25 mm terhadap *Candida albicans* dan 12,83 mm terhadap *Aspergillus fumigatus*. Sebaliknya, fraksi n-heksana tidak menunjukkan aktivitas antifungal. Analisis statistik dengan ANOVA satu arah menunjukkan perbedaan signifikan antar konsentrasi dan jenis fraksi.

Kesimpulan: Penelitian ini menyimpulkan bahwa ekstrak merang padi, khususnya fraksi etil asetat, memiliki potensi sebagai agen antifungal alami yang efektif terhadap jamur patogen, dan dapat dikembangkan lebih lanjut dalam pengobatan tradisional berbasis tanaman.

Kata kunci: antifungal, fraksinasi, merang padi

INTRODUCTION

Fungal skin infections, or dermatophytosis, are common in tropical countries. The cause is humid air, which favors the growth of fungal skin infections. High humidity makes it easier for fungi to infect and spread. Skin diseases in Indonesia are generally caused by bacterial, viral, fungal, and parasitic infections. Other contributing factors include social habits and an unclean environment[1]. This infection can be transmitted through various media, such as the use of bed sheets that are rarely changed, wearing clothes for a long time and repeatedly, body parts that are often covered for a long time by clothing, using shared towels, rarely washing towels, and leaving towels wet in a closed room. Moisture in towels that are rarely exposed to sunlight can become a medium for fungal growth[2]. Fungal infections are known to be indicators of compromised immunity. Many microorganisms with diverse characteristics form microscopic fungi. Some of them, particularly in immunocompromised patients, can infect humans and cause disease. Due to their high mortality rate and relatively low treatment costs, invasive opportunistic fungal infections have attracted the attention of public health systems worldwide[3]

Meanwhile, *Aspergillus fumigatus* is the main causative agent of invasive aspergillosis, with a high mortality rate in patients with systemic disease. *Aspergillus fumigatus* causes a wide range of infections, from allergies and colonization to acute, life-threatening invasive infections[4]. The fungus *Aspergillus fumigatus* is the most common cause of human infections, accounting for more than 90% of cases of invasive and non-invasive aspergillosis. This fungus can be found in soil, water, and decaying plants, particularly manure and humus. Aspergillosis is quite widespread and can cause disease in both humans and animals[5].

Infectious diseases are a group of diseases that easily affect children and are caused by viral, bacterial, or parasitic infections. Infectious diseases are one of the most common health problems in developing countries, including Indonesia. The body fights infection through the innate immune system, which works through an inflammatory process followed by an adaptive response. To combat infectious diseases, drugs can be used. Fungal infections in Indonesia are not only influenced by climate factors, but are also exacerbated by low hygiene awareness, population density, non-sterile medical procedures, and inappropriate use of antifungals that can trigger resistance[6].

The use of medicinal plants in traditional medicine has long been known in Indonesia. The World Health Organization reports that 80% of the world's population uses herbal medicine. Of the 194 countries worldwide, 179 still use traditional medicine[7] One plant

with potential for development is rice straw (*Oryza sativa*). It contains secondary metabolites that act as defense agents, providing disease resistance. Its diverse biological activities, such as antibacterial, antioxidant, cytotoxic, and anti-inflammatory properties, are associated with health-promoting and disease-preventing effects. Its metabolites include flavonoids, terpenoids, steroids, alkaloids, saponin, and tannins.

Straw waste or rice residue can also be used as a preservative because it contains sodium and potassium, antimicrobial substances that can inhibit decay[8]. However, studies on its antifungal potential against major pathogenic fungi are still limited. Therefore, this study aims to evaluate the antifungal potential of rice straw extract against *Candida albicans* and *Aspergillus fumigatus* through an in vitro approach. The results of this study are expected to contribute scientifically to the development of safe and effective natural antifungal agents based on local plants.

METHODS

Study Design

This research is a laboratory experimental study with a quantitative approach. Rice straw comes from rice plants (*Oryza sativa* L.) that have been identified morphologically. This research was conducted from May to October 2023 at the Medan Ministry of Health Polytechnic.

Data source and sampling procedure

The samples used in this study were 400g of rice husks from identified rice plants, which were then weighed. The criteria for healthy and pesticide-free rice plants have been met, and they have gone through a botanical identification process to ensure the species used. After harvest, the straw must be thoroughly dried to a low water content through a heating method using an oven, with a golden brown color and free from contamination such as soil, roots, or grain. For the needs of bioactive compound extraction, the rice straw used must not be mixed with other varieties and must be stored in a dry and dark place to maintain the stability of the active compounds. In its application as a growing medium for water content mushrooms, the straw ideally comes from a fresh harvest (maximum two weeks), has a low water content resulting from drying, and has gone through a natural composting process without harmful chemicals. Fulfillment of these criteria plays an important role in maintaining the quality, effectiveness, and safety of using rice straw in research and biotechnology industrial applications. Rice straw extraction was carried out using the maceration method using 96% ethanol, followed by multi-stage extraction fractionation with repeated solvent replacement according to its polarity using n-hexane, ethyl acetate, and n-butanol solvents.

Variables of the study

Data Collection

The first procedure in this study was that rice straw *simplicia* was macerated in 96% ethanol with a *simplicia* to solvent ratio of 1:7.5. The maceration was carried out for 3 x 24 hours with stirring every 4 hours for 15 minutes; the ethanol solvent was changed every 24 hours. The extraction results were then filtered with filter paper to obtain the filtrate and residue. The extraction results were evaporated using a rotary vacuum evaporator or water bath at a temperature of 60°C to obtain a thick extract.

Next, a suspension of *Candida albicans* and *Candida aspergillus* fungi was made. Take one eye of a 24-hour-old *Candida albicans* fungal culture loop, then mix it into a test tube containing 10 mL of 0.9% NaCl solution. The fungal suspension was homogenized by shaking for approximately 15 seconds, then poured into a cuvette of 7 mL. The cuvette was inserted into a spectrophotometer to measure its turbidity with a wavelength of 530 nm and an absorbance number of 0.5-0.6, which means it is equivalent to the McFarland standard of 0.5 (1x10⁶ - 5x10⁶ cells / mL)[9].

Measurement and instruments

Antifungal activity testing was performed using a 6 mm diameter well method. *Candida albicans* suspension was prepared with turbidity according to McFarland standards. Sterile cotton swabs were dipped into the inoculum suspension, then drained by pressing and rotating the cotton swab tip against the tube wall to remove excess liquid. The inoculum was applied to the surface of Sabouraud Dextrose Agar (SDA) media three times, rotating the plate at a 60° angle each time. Afterward, a sterile cotton swab was used to smear the edges of the agar surface to ensure even distribution. The plates were covered and left at room temperature for several minutes to allow the surface of the media to dry before further treatment[10].

A *Candida albicans* suspension is inoculated onto the surface of Sabouraud Dextrose Agar (SDA) media using a sterile cotton swab. It is then left for 5–15 minutes at room temperature to allow the suspension to penetrate the media. A 6 mm hole is then made in the media using a sterilized cork borer.

50 µL of rice straw extract was added to each well at graded concentrations: 20,000 ppm, 40,000 ppm, 60,000 ppm, and 80,000 ppm. Nystatin 6.7 ppm was used as a positive control, and NaCl 0.9% was used as a negative control. The plates were incubated at 37°C for 24 hours. After incubation, a clear zone (zone of inhibition) formed around the wells was observed as an indicator of antifungal activity. The diameter of the zone was measured vertically and horizontally using a caliper, and the results were expressed in millimeters (mm).

Data Analysis

Testing was also conducted on *Aspergillus fumigatus* in the second year using the agar well diffusion method and paper discs as the distribution medium. Extracts and fractions were tested at concentrations of 3%, 5%, 7%, and 9%, with a negative control (0.9% NaCl) and a positive control (0.025% Nystatin). The measurement data were analyzed statistically using one-way ANOVA, followed by an Honestly Significant Difference (HSD) test at a significance level of 5% to determine differences between treatments.

RESULTS

Rice straw is a plant we often encounter, a staple food for humans, and grows in tropical regions. This study aims to determine the yield of the extract. To determine the number of antifungal isolates in inhibiting fungal growth and their potential as antifungals against *Candida albicans* and *Aspergillus fumigatus* fungi.

Antifungal activity testing was conducted using the agar diffusion method using paper discs as a backup, with observations of the formation of clear zones indicating antibacterial activity, either killing or inhibiting the growth of the test bacteria. The data obtained were then analyzed using a one-way ANOVA method to determine the effect of variations in test concentration on killing or inhibiting bacterial growth, followed by further analysis.

Yield

Rice straw extract is an extract resulting from an extraction process using the maceration method, namely, soaking a 400g dry sample in 3 L of ethanol solvent at room temperature. The maceration solution is then concentrated with a rotary evaporator, the concentrated extract is then evaporated in a water bath to obtain an extract with a minimum water content, resulting in 84.4 g of crude rice straw extract.

The resulting extract was then weighed to 20g and then fractionated. The solvents used were n-hexane, ethyl acetate, and n-butanol, respectively. The results yielded 2.91g of the n-hexane fraction, 4.26g of the ethyl acetate fraction, and 6.14g of the n-butanol fraction.

Table 1. Rice Straw Extract Yield Data

Sample	Yield (%) of rice straw 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)
Fresh Sample	-	-	-	-	-	-
Dry sample	12.5	-	-	-	-	-
Crude Extract	2.63	21.1	-	-	-	-
n-hexane fraction	0.09	0.72	3.44	-	-	-
Ethyl acetate fraction	0.13	1.06	5.04	-	-	-
n-butanol fraction	0.19	1.53	7.27	-	-	-

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

Antifungal Activity of Rice Straw Extract on Candida Albicans Fungus

Determination of the antifungal activity test concentration series was performed using the agar diffusion method. The concentration series created were 3%, 5%, 7%, and 9%. Based on the test results, the following was obtained:

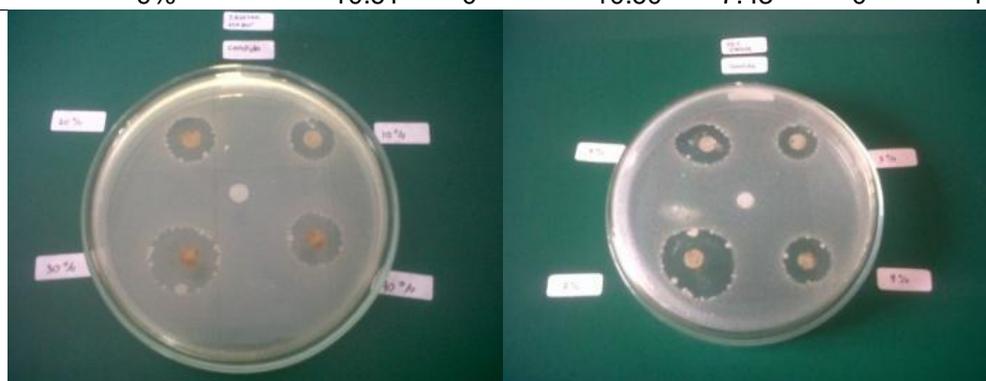
Table 2. Antifungal Activity of Rice Straw Extract on Candida Albicans Fungus

Extract	Concentration	n1 (mm)	n2 (mm)	n3 (mm)	±SD
Ethanol	3%	8.76	8.46	7.66	0.57
	5%	10.63	9.52	9.03	0.83
	7%	15.53	15.51	14.91	0.35
	9%	10.98	10.66	10.19	0.32
n-hexane	not tested				
ethyl acetate	3%	9.19	8.18	8.39	0.42
	5%	12.87	12.64	11.74	0.61
	7%	18.71	18.46	17.58	0.63
	9%	11.38	10.22	10.19	0.67
n-butanol	3%	5.72	4.23	4.20	0.89
	5%	7.46	7.22	6.02	0.65
	7%	13.43	13.42	12.19	0.57
	9%	7.89	7.66	6.74	0.49
ketoconazole	3%	8.85	-	-	0
	5%	9.54	-	-	0
	7%	9.88	-	-	0
	9%	10.59	-	-	0

Based on Table 2, the results obtained show that the n-hexane fraction of rice straw has no antifungal activity, while the ethyl acetate and n-butanol fractions have antifungal activity. Based on Table 3, the results of the clear inhibition zone of the ethyl acetate and n-butanol fractions of rice straw have antifungal activity in inhibiting or killing Candida Albicans fungi and the n-hexane fraction has no antifungal activity.

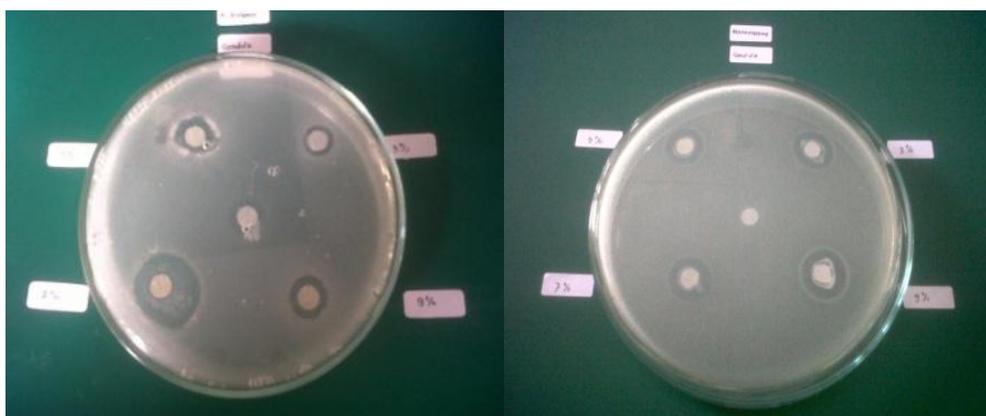
Table 3. Extract clear zone data

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



a. Ethanol Extract

b. Ethyl Acetate Fraction



c.n-butanol fraction d.Positive Control (Ketoconazole)

Figure 1. Antifungal Testing of Rice Straw Extract on Candida Albicans Fungus

Figure 1 a. Ethanol extract shows a clear zone scale of ethanol extract at concentrations of 3% and 5%, which is categorized as moderate, and at concentrations of 7% and 9%, which is categorized as strong in the classification of antibacterial material strength, b. Ethyl acetate fraction shows a clear zone scale of ethyl acetate fraction at concentrations of 3% which is categorized as moderate and at concentrations of 5%, 7% and 9% which is categorized as strong in the classification of antibacterial material strength, c. n-butanol fraction shows a clear zone scale of n-butanol fraction at concentrations of 3%, 5% and 9% which is categorized as moderate and at concentrations of 7% which is categorized as strong in the classification of antibacterial material strength and d. Positive Control (Ketoconazole) shows a clear zone scale of positive control (ketoconazole) at concentrations of 3%, 5%, and 7%, which is categorized as moderate, and at concentrations of 9%, which is categorized as strong in the classification of antibacterial material strength.

Antifungal Activity of Rice Straw Extract on *Aspergillus fumigatus* Fungus

Table 4. Research Results of Antifungal Activity of Rice Straw Extract on *Aspergillus fumigatus* Fungus

Extract	Concentration	n1(mm)	n2(mm)	n3(mm)	SD±
Ethanol	3%	5.82	5.81	5.83	0.01
	5%	8.33	8.30	8.36	0.03
	7%	13.90	13.80	13.70	0.10
	9%	8.71	8.72	8.70	0.01
n-hexane	not tested				
ethyl acetate	3%	5.39	5.40	5.38	0.01
	5%	8.09	8.09	8.09	0.00
	7%	12.83	12.83	12.83	0.00
	9%	5.50	5.52	5.51	0.01
n-butanol	3%	1.74	1.74	1.74	0.00
	5%	3.98	3.97	3.99	0.01
	7%	7.88	7.87	7.86	0.01
	9%	4.75	4.75	4.75	0.00
ketoconazole	3%	8.85	-	-	-
	5%	9.54	-	-	-
	7%	9.88	-	-	-
	9%	10.59	-	-	-

Based on Table 4, the results obtained from the n-hexane fraction of rice straw were not tested, while the ethyl acetate and n-butanol fractions had antifungal activity.

Table 5. Extract clear zone data

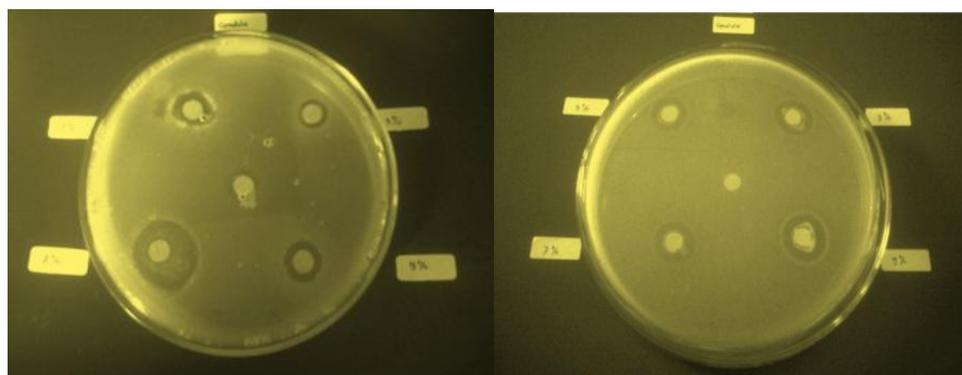
Test Fungi	Concentration	Mean Clear Zone Diameter (mm)					
		ethanol	n-hexane	ethyl acetate	n-butanol	control (-)	control (+)
Aspergillus	3%	5.82	0	5.39	1.74	0	8.85
	5%	8.33	0	8.09	3.98	0	9.54
	7%	13.80	0	12.83	7.86	0	9.88
	9%	8.71	0	5.51	4.75	0	10.59

Based on Table 5, the results of the clear inhibition zone of the n-hexane fraction of rice straw did not have antifungal activity, while the ethyl acetate and n-butanol fractions had antifungal activity.



a. Ethanol Extract

b. Ethyl Acetate Fraction



c. n-butanol fraction

e. Positive Control (Ketoconazole)

Figure 2. Antifungal Testing of Rice Straw Extract on Aspergillus Fungus

Figure 2a ethanol extract shows clear zone scale. ethanol extract at concentrations of 3% 5% and 9% is categorized as medium, and at a concentration of 7% is categorized as strong in the classification of antibacterial material strength, b. Ethyl acetate fraction Shows Clear Zone Scale Ethyl acetate fraction at Concentrations of 3%, 5% and 9% is categorized as medium and at a concentration of 7% is categorized as strong in the classification of antibacterial material strength, c. n-butanol Fraction Shows Clear Zone Scale n-butanol Fraction at Concentrations of 3%, 5% and 9% is categorized as weak and at a concentration of 7% is categorized as medium in the classification of antibacterial material strength and e. Positive control (Ketoconazole) shows a Clear Zone Scale. Positive control (Ketoconazole) at concentrations of 3%, 5%, and 7% is categorized as medium, and at a concentration of 9% is categorized as strong in the classification of antibacterial material strength.

DISCUSSION

Fungi are saprophytic microorganisms found widely on the surface of the human body. *Candida albicans* infection in the oral cavity is known as oral candidiasis[11]. Ethyl acetate extract of rice straw has antifungal activity in inhibiting or killing *Candida albicans* fungus, while water as a negative control, does not provide an inhibitory effect. Based on the results of the tests that have been carried out, the results of the n-hexane fraction of rice straw do not have antifungal activity because there is no inhibition zone or clear zone shown by the fraction at low concentrations. This is because the rice straw extract has high polarity properties, while the ethyl acetate and n-butanol fractions have antifungal activity, indicated by the presence of an inhibition zone. According to the classification criteria for the strength of an antibacterial material, namely the diameter of the inhibition zone of 5 mm or less is categorized as weak, and the inhibition zone of 5-10 mm is categorized as moderate, while the diameter of the inhibition zone of 10-20 mm is categorized as strong and even more than 20 mm is categorized as very strong[12] *Candida albicans* is a normal flora found primarily on the skin, mucous membranes, mouth, digestive tract, and respiratory tract. Initially, *Candida albicans* is non-pathogenic, but when predisposing factors are present, it becomes pathogenic[13].

In the study of the antifungal activity of rice straw extract on *Candida albicans* fungus, the ethanol extract at concentrations of 3% and 5% was classified as moderate, while concentrations of 7% and 9% were classified as strong. For the ethyl acetate fraction extract, a concentration of 3% was classified as moderate, while concentrations of 5%, 7%, and 9% were classified as strong. For the n-butanol extract, concentrations of 3%, 5%, and 9% were classified as moderate, while concentrations of 7% were classified as

strong. Finally, ketoconazole as a positive control at concentrations of 3%, 5%, and 7% were classified as moderate, and concentrations of 9% were classified as strong.

In the study of the antifungal activity test of rice straw extract on *Aspergillus fumigatus* fungus, the ethanol extract at concentrations of 3%, 5%, and 9% was categorized as moderate, and the concentration of 7% was categorized as strong. For the ethyl acetate fraction extract, concentrations of 3%, 5%, and 9% were categorized as moderate, while the concentration of 7% was categorized as strong. For the n-butanol extract, concentrations of 3%, 5%, and 9% were categorized as weak, and at a concentration of 7% it was categorized as moderate. Finally, ketoconazole as a positive control at concentrations of 3%, 5%, and 7%, was categorized as moderate, and the concentration of 9% was categorized as strong.

Rice straw extract is categorized as having strong antifungal activity because at this concentration, the content of active compounds such as phenols, alkaloids, and saponins is in higher amounts, so it is able to work more effectively in inhibiting the growth of *Candida albicans* fungus. Phenolic compounds work by damaging the permeability of the fungal cell membrane, causing leakage of cell contents and disrupting the activity of important enzymes. Alkaloids can inhibit cell wall synthesis and damage the internal structure of fungal cells, while saponins bind to sterols in the cell membrane and cause cell lysis. The higher the concentration of the extract, the greater the opportunity for these compounds to interact with vital components of fungal cells, thereby disrupting overall cell function. Therefore, the effectiveness of the extract in destroying fungal cells increases significantly, so it is included in the category of strong inhibitory power[14].

The concentration of ethyl acetate in this study was greater than that of ethanol, n-hexane, and n-butanol compounds. Because in the ethanol extract, the compounds are still in a complex state compared to the fractions, where the compounds in the fractions are already in a single state based on their polarity and solubility, so that the fractions already have better antifungal activity at low concentrations compared to the ethanol extract, which is still in a complex state. The active compounds work more focused and effectively, causing stronger antifungal activity even at lower concentrations compared to the ethanol extract, which has not been fractionated[15].

Secondary metabolites in rice straw include alkaloids, saponins, and phenols. These secondary metabolites can inhibit the growth of or kill bacteria. The antifungal mechanism of alkaloids is by disrupting the peptidoglycan component of bacterial cells, preventing the cell wall from forming properly and causing bacterial cell death, for example, berberine. Saponins are potent surface tension-reducing compounds, thus acting as antifungals by damaging the cytoplasmic membrane (disrupting its stability), thus causing lysis of microbial cells, for example, protodioscin. The mechanism of phenols as antifungals is by disrupting the permeability of the cytoplasmic membrane, causing leakage of intracellular materials that inactivate the activity and synthesis of microbial enzymes. These three groups of compounds have been shown to effectively inhibit the growth of pathogenic microbes[15].

This study has several limitations that need to be considered in interpreting the results. First, the antifungal test was only conducted on two types of pathogenic fungi, namely *Candida albicans* and *Aspergillus fumigatus*, so it does not reflect the effectiveness of rice straw extract against a broader spectrum of fungi. Second, the identification of active compounds in the ethyl acetate and n-butanol fractions has not been carried out in depth, so the antifungal mechanism of action cannot be specifically explained. Third, this study was *in vitro* and did not include *in vivo* toxicity or efficacy tests, so the safety and therapeutic potential of the extract in humans cannot be confirmed.

This research has several advantages that make it relevant and valuable in the development of natural-based antifungal agents. The use of rice straw as the main raw material demonstrates an innovative approach to utilizing abundant and underexplored agricultural waste. The methodology used, namely a completely randomized design with positive and negative controls, provides strong validity to the results of the extract effectiveness test. Furthermore, the separation of extract fractions (n-hexane, ethyl acetate, and n-butanol) allows for the early identification of the most biologically active fraction, namely ethyl acetate. This research also contributes to the search for safer and more environmentally friendly antifungal alternatives compared to synthetic compounds, and opens up opportunities for the development of pharmaceutical or cosmetic products based on natural ingredients.

This research has important implications for health and natural resource utilization. The finding that the ethyl acetate fraction of rice straw extract has antifungal activity against *Candida albicans* and *Aspergillus fumigatus* demonstrates the potential for developing natural-based antifungal agents that are safer and more environmentally friendly than synthetic compounds. Practically, these results open up opportunities for utilizing agricultural waste such as rice straw as a source of active ingredients in pharmaceutical, cosmetic, or antiseptic product formulations. Furthermore, this research also encourages an interdisciplinary approach between microbiology, pharmacy, and agricultural technology to develop sustainable therapeutic solutions. These implications reinforce the urgency of exploring local natural materials as alternatives to address the challenges of fungal resistance and the limitations of conventional antifungal agents.

CONCLUSION

Based on the research results, it can be concluded that rice straw extract (*Oryza sativa*) has potential as an antifungal agent against *Candida albicans* and *Aspergillus fumigatus*. The ethyl acetate fraction, at a concentration of 7%, produced an inhibition zone of 18.25 mm against *Candida albicans*, which is included in the strong category. The ethanol fraction, at a concentration of 7%, produced an inhibition zone of 13.80 mm against *Aspergillus fumigatus*, which is included in the strong category. The n-butanol fraction at a concentration of 7% produced an inhibition zone of 7.86 mm, which is included in the moderate category, while the n-hexane fraction did not show an inhibition zone. Further research is recommended to identify the active compounds in the extract, as well as toxicity testing to ensure its safety before implementation as an alternative therapy or active ingredient in pharmaceutical and cosmetic products. Utilizing agricultural waste such as rice husks also aligns with a circular economy approach, thus positively impacting health and supporting efficient resource management.

These results indicate that the active compounds in rice straw are semi-polar and can be fractionated to increase their effectiveness. Therefore, rice straw extract, especially the ethyl acetate fraction, has the potential to be developed as a safe and environmentally friendly alternative natural antifungal base.

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