



ANTIFUNGAL ACTIVITY TEST OF ETHANOL EXTRACT OF PANDAN WANGI (*Pandanus ammaryllifolius.R*) LEAVES AGAINST THE GROWTH OF THE FUNGUS *Aspergillus sp*

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ABSTRACT

Aspergillosis or Brooder Pneumonia, Mycotic pneumonia or pneumonycosis, is a disease of the respiratory system caused by infection with the fungus genus *Aspergillus*. The purpose of this study was to determine the potential of ethanol extract of pandan leaves (*Pandanus ammaryllifolius. R*) against the growth of *Aspergillus sp. fungi*. The research method was an experiment using a completely randomized design. The data used in this study were primary data obtained from experiments in the laboratory. The samples used were ethanol extracts of pandan leaves with concentrations of 20%, 30%, 40%, and 50%, each repeated three times. Laboratory testing used the disc diffusion method with PDA (potato dextrose agar) antimicrobial test media with the following average results: 20% concentration of fragrant pandan leaf ethanol extract (5.99 nm), 30% (7.26 nm), 40% (9.75 nm), 50% (12.1 nm) and 2% ketoconazole (38.56 nm) as a positive control. Statistical testing used ANOVA (Analysis of Variance) and Tukey HSD as a follow-up test. *Pandanus ammaryllifolius R. ethanol leaf extract* affected the growth of *Aspergillus sp. (P = 0.000)*.

Keywords: *pandanus ammaryllifolius. R; Aspergillus sp*



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INTRODUCTION

Aspergillus fungi are cosmopolitan contaminants that spread easily through the air because their spores can survive in various environments. These spores easily grow on foodstuffs and agricultural products. Aspergillus infection can cause various health problems, including fever, inflammation, shortness of breath, coughing, chest and joint pain, chills, and headaches (Hasanah, 21017). In addition, Aspergillus produces aflatoxins, which are toxic compounds that are carcinogenic and hepatotoxic. Human exposure to aflatoxins generally occurs through the consumption of contaminated food, and prevention is difficult because the fungus easily grows on foodstuffs (Mizana & Arni, 2016; Jirna, 2019).

Aspergillosis, also known as Brooder Pneumonia, Mycotic Pneumonia, or Pneumomycosis, is a respiratory disease caused by infection with the fungus Aspergillus sp. This fungus reproduces through the formation of hyphae and conidiophores that produce spores. These spores are widely dispersed in the air and are easily inhaled into the lungs (Hasanah, 2017). Allergic bronchopulmonary aspergillosis (ABPA) is reported to affect 1–15% of cystic fibrosis patients. In addition, approximately 2.5% of asthma sufferers, or about 4.8 million people worldwide, also experience ABPA. Of these, about 400,000 people suffer from chronic pulmonary aspergillosis (CPA). Furthermore, an estimated 1.2 million people experience post-tuberculosis CPA, and about 70,000 cases occur as a complication of sarcoidosis (CDC, 2020).

Study titled Antioxidant Anti-Cancer and Antimicrobial Activities of Ethanol Pandanus amaryllifolius Roxb. Leaf Extract (In Vitro) shows that 70% ethanol extract of pandan leaves (Pandanus amaryllifolius R.) has antimicrobial activity against Streptococcus sanguinis, Streptococcus mutans, Streptococcus salivarius, and Porphyromonas gingivalis. This activity is mainly due to the high phenolic and flavonoid content. Flavonoids work by forming complexes with extracellular proteins, thereby disrupting the integrity of bacterial and fungal cell membranes (Suwannakul, Chaibenjwong, & Suttasinee, 2018).

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Tested a combination of fragrant pandan leaf extract and noni fruit (*Morinda citrifolia*) against *Aspergillus* sp., *Paecylomyces varotii*, *Candida* sp. (non-*albicans*), and one yeast isolate. The results showed that 82.4% of fungal isolates experienced growth inhibition with an inhibition zone diameter of 15–35 mm. The active compounds in pandan leaf extract include phenols (9.42% w/w) and flavonoids (4.39% w/w), while noni fruit extract contains alkaloids (1.97% w/w), phenols (12.50% w/w), and flavonoids (8.61% w/w) (CDC, 2020).

Study entitled "Testing the Antifungal Activity of Pandan Wangi Leaf Ethanol Extract in Inhibiting the Growth of *Pityrosporum ovale*" found that pandan wangi leaf ethanol extract at concentrations of 40%, 50%, 60%, and 70% was able to inhibit the growth of the fungus that causes dandruff. The test was conducted using DMSO as a negative control and 2% ketoconazole as a positive control. The results showed the formation of inhibition zones with an average of 5.99 mm; 7.26 mm; 9.75 mm; 12.1 mm; and 39.16 mm. Analysis using the ANOVA test showed a significant effect of pandan wangi ethanol extract on the growth of *Pityrosporum ovale* ($p = 0.000$) (Ambarwati & Sintowati, 2017).

Current treatment for aspergillosis uses antifungal drugs such as itraconazole, voriconazole, lipid amphotericin, and posaconazole (CDC, 2022). However, these drugs carry the risk of serious side effects. Therefore, there is a need to develop herbal therapies based on plants with antifungal potential to minimize the side effects of long-term use of chemical drugs. This study focuses on testing the antifungal activity of pandan leaf ethanol extract against the growth of *Aspergillus* sp., with concentrations varying from 20%, 30%, 40%, and 50% (Gustiananda, 2019). The selection of these concentrations refers to research, which proved that concentrations of 40–70% were effective in inhibiting *Pityrosporum ovale*, so this study used lower concentrations to evaluate the potential for inhibition from the early stages (CDC, 2022).

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MATERIALS AND METHODS

This study is an experimental study. According an experimental study is a research method used to find the effect of a particular treatment on another under controlled conditions. This study used a completely randomized design (Sugiyono, 2017).

The design used can be seen in the following table:

Table 1. Completely Randomized Design

<u>Treatment</u>	<u>Repetition</u>		
(P)	1	2	3
P0	P01	P02	P03
P1	P11	P12	P13
P2	P21	P22	P23
P3	P31	P32	P33
P4	P41	P42	P43
P5	P51	P52	P53

The sample used in this study was fragrant pandan leaf extract (*Pandanus ammarylifollius* R.) that had undergone evaporation and was then made into several concentrations, namely 20%, 30%, 40%, and 50%. The fragrant pandan leaf samples were obtained from Banua Village, Kintamani, with the criteria of dark green leaves and no damage. The pandan leaf extract used 96% ethanol, which was concentrated with a rotary evaporator to obtain a thick extract of fragrant pandan leaves.

Two kilograms of fragrant pandan leaves were used for wet samples, which were then sorted, dried, and sieved to obtain 200 grams. In this study, the samples tested were fragrant pandan leaf extracts with concentrations of 20%, 30%, 40%, and 50%, which were made by diluting the concentrated fragrant pandan leaf extract using 96% ethanol solvent. The positive control used Ketoconazole and the negative control used 96% ethanol. Thus, the total number of treatments used was six. For each treatment, repetitions were performed on each concentration variation was calculated using the Federer formula as follows:

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

Description:

t: Treatment

r : Replication

Where:

$$t = 6$$

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

$$(r - 1)(6 - 1) \geq 15$$

$$(r - 1)(5) \geq 15$$

$$r - 1 \geq \frac{15}{5}$$

$$r - 1 \geq 3$$

Based on the above calculations, three repetitions were obtained. According the minimum number of repetitions in laboratory testing is three times (Hanafiyah, 2016).

The unit of analysis used in this study was the inhibition zone of fragrant pandan leaf extract (*Pandanus ammarylifolius* R.) at various concentrations in inhibiting the growth of *Aspergillus* sp. at concentrations of 20%, 30%, 40%, and 50%.

The data collected was quantitative data, namely primary data obtained through laboratory experiments. Primary data was obtained from measurements of the diameter of the inhibition zone

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on the growth of *Aspergillus* sp. fungi produced by extracts of pandan leaves (*Pandanus ammarylifollius* R.).

The data collection technique in this study was measurement using instruments through laboratory experiments. Measurements were taken on the diameter of the inhibition zone formed on the growth of *Aspergillus* sp. fungi.

The equipment used in this study consisted of vial tubes (4 pieces), a 1000 ml Erlenmeyer flask (1 piece), test tubes (4 pieces), a 250 ml measuring cup (1 piece), a glass funnel (1 piece), a knife (1 piece), a blender (1 piece), round pipette (1), micropipette 20µl-1000µl, tweezers (1), 500 ml glass beaker (1), Bunsen burner (1), test tube rack (1), spatula (1), stirring rod (1), Petri dish (petridish) (10 pieces), vernier caliper (1 piece), McFarland densitometer biosan (1 piece), analytical balance (1 piece), Escos incubator (1 piece), autoclave (1 piece), oven (1 piece), biosafety cabinet (1 piece), evaporator (1 piece),

The materials used in this study were fragrant pandan leaf extract, distilled water, 96% ethanol, *Aspergillus* sp. fungus, Potato Dextrose Agar (PDA) medium, Potato Dextrose Broth (PDB), Ketoconazole, 0.9% NaCl, 0.5% McFarland Standard, cotton swabs, yellow tip, blue tip, cotton, filter paper, aluminum foil, blank disc paper.

The data obtained were the results of measuring the diameter of the inhibition zone of the ethanol extract of fragrant pandan leaves (*Pandanus ammarylifollius* R.) against the growth of *Aspergillus* sp. fungus as one of the contaminant fungi using PDA media.

The data obtained will be analyzed statistically using SPSS, namely the Kolmogorov-Smirnov (KS) test and the , followed by the One Way Anova test and the Turkey HSD test.

RESULTS AND DISCUSSION

The determination test results showed that the fragrant pandan leaf samples used in this study were of the species *Pandanus amaryllifollius* R. From 2 kg of fresh leaves that had been sorted, 250 grams of simplisia powder was obtained with a yield of 12.5%. This value meets the standard for good simplisia yield, which is more than 10%. Furthermore, the maceration process of 250 grams of simplisia powder with 2500 ml of ethanol produced 30

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grams of fragrant pandan leaf extract with a yield of 12%, which also meets the optimal yield criteria (Faras, Wadkar, & Ghosh, 2014)

Qualitative phytochemical analysis showed that the ethanol extract of pandan leaves positively contained secondary metabolite compounds in the form of alkaloids, flavonoids, and tannins, while saponin content was not detected. These three compounds are known to have antifungal activity (Ambarwati & Sintowati, 2017). The mechanism of fungal growth inhibition is thought to occur due to damage to the fungal cell membrane, which is composed of proteins and lipids. This damage causes disruption of compound and ion transport, resulting in nutritional deficiency in the cells (Noer, Pratiwi, & Gresinta, 2016).

Table 2. Results of the Phytochemical test of pandan leaves extract.

<u>No</u>	<u>Uji Fitokimia</u>	<u>Color</u>	<u>Desription</u>
1.	Alkaloid	Mayer Wagner: <u>Orange sediment</u> Dragendrof : <u>Orange sediment</u>	<u>Positive</u> <u>Positive</u>
2.	Saponin	<u>Greenish brown</u>	<u>Negative</u>
3.	<u>Flafonoid</u>	<u>Brownish orange</u>	<u>Positive</u>
4.	Tanin	<u>Blackish green</u>	<u>Positive</u>

Alkaloids in pandan leaves work by inserting themselves between the cell walls and DNA of fungi, thereby inhibiting DNA replication and disrupting fungal growth (Karou, 2006; Lisu, Hartati, & Sufian, 2023). Flavonoids play a role by forming complexes with extracellular proteins that damage cell membrane integrity, as well as inhibiting nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Sulistyowati & Mulyani, 2009; Marjoni, 2016). Tannins work by shrinking cell walls and damaging membrane permeability. In addition, tannins can inhibit chitin synthesis in fungal cell walls, inactivate

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enzymes, and damage fungal genetic material (Wennig et al, 2020; Mardianingsih & Aini, 2014).

Flavonoids function as antifungals by forming complex compounds with extracellular proteins that disrupt the integrity of fungal cell membranes (Sulistiyowati & Mulyani, 2009). Tannin compounds can shrink cell walls, thereby disrupting cell wall permeability, resulting in cells being unable to carry out life activities, thus inhibiting growth or even causing death. They also act as antifungals by reacting with cell membranes, inactivating enzymes, and inactivating fungal genetic material (Yulaika, 2009).

Table 3. Average Results of the Inhibitory Zone Diameter of Ethanol Extract of Pandanus Amaryllifolius Leaves (*Pandanus amaryllifolius R*) on the Growth of *Aspergillus sp.* Fungus

<u>Treatment</u>	<u>Average diameter of inhibition zone</u>
Etanol 96%	0,00 mm
Ekstrak 20%	5,99 mm
Ekstrak 30%	7,26 mm
Ekstrak 40%	9,75 mm
Ekstrak 50%	12,1 mm
<u>Ketoconazole 2%</u>	39,16 mm

Based on the results of the study, ethanol extract of pandan leaves (*Pandanus amaryllifolius R.*) was proven to inhibit the growth of *Aspergillus sp.*, as indicated by the formation of a clear zone around the test disc. The average diameter of the inhibition zone at concentrations of 20%, 30%, 40%, and 50% ranged from 5.99 mm to 12.1 mm. The greatest inhibition was obtained at a concentration of 50% with a diameter of 12.1 mm, which was categorized as strong antifungal activity.

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This inhibitory ability is thought to originate from the phytochemical content in the extract, namely alkaloids, flavonoids, and tannins. Flavonoids work through three main mechanisms, namely inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and inhibiting energy metabolism. These compounds can also damage the permeability of fungal cell walls, thereby disrupting their survival (Marjoni, 2016).

Alkaloids act by inserting themselves between the cell walls and DNA of fungi, which then inhibits the DNA replication process, thereby disrupting fungal growth. Meanwhile, tannins work by inhibiting chitin synthesis in fungal cell walls and damaging cell membranes. The lipophilic nature of tannins facilitates their interaction with cell walls, resulting in growth inhibition and even fungal cell death (Wennig et al, 2023).

Table 4. Classification of Growth Inhibition Zone Responses of *Aspergillus sp.*

<u>Treatment</u>	<u>Average diameter of inhibition zone</u>	<u>Category</u>
Etanol 96%	0,00 mm	<u>Weak</u>
Ekstrak 20%	5,99 mm	<u>Moderate</u>
Ekstrak 30%	7,26 mm	<u>Moderate</u>
Ekstrak 40%	9,75 mm	<u>Moderate</u>
Ekstrak 50%	12,1 mm	<u>Strong</u>
<u>Ketoconazole 2%</u>	39,16 mm	<u>Very Strong</u>

The ethanol extract of pandan leaves (*Pandanus amaryllifolius R.*) showed the ability to inhibit the growth of *Aspergillus sp.* with different categories at each concentration. As a comparison, 2% ketoconazole, which was used as a positive control, produced an average inhibition zone of 39.16 mm and was classified as very strong. At extract concentrations of 20%, 30%, and 40%, the inhibition powers produced were 5.00 mm, 7.26 mm, and 9.75 mm, respectively, which were categorized as moderate. Meanwhile, a concentration of 50% showed an

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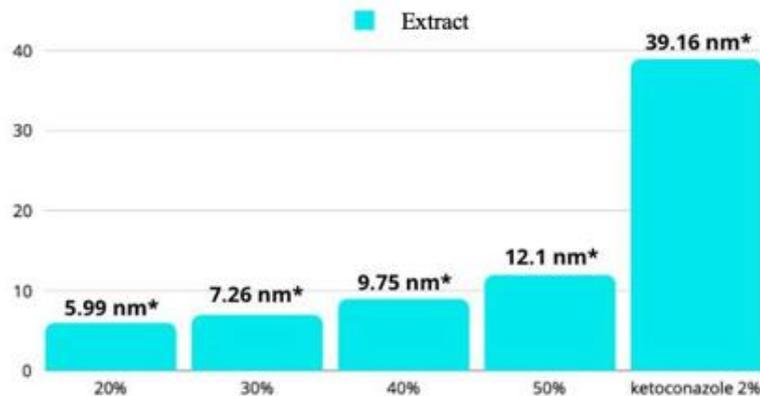
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inhibition power of 12.1 mm and was categorized as strong. This classification is in accordance with the criteria proposed namely inhibition zone diameter < 5 mm is classified as weak, 5–10 mm as moderate, >10–20 mm as strong, and >20– 30 mm as very strong. Thus, the higher the concentration of pandan leaf ethanol extract administered, the larger the inhibition zone produced against the growth of *Aspergillus sp* (Lisu, Hartati, & Sufian, 2023).

Table 5. ANOVA Test Results for *Aspergillus sp.* Growth

	F	Sig
Zona hambat ekstrak etanol daun pandan	3259.540	.000

Hasil Uji Tukey HSD



The results of the test of homogeneity of variances showed a p-value of 0.138, which is greater than 0.05, meaning that the variances between groups are considered homogeneous and an ANOVA test can be performed. The subsequent ANOVA analysis showed a significant difference between treatment groups with a p-value of 0.000 (less than 0.05). Thus, the alternative hypothesis (H1) is accepted, which means that there is a significant difference in the average diameter of the inhibition zone between concentrations of sweet pandan leaf (*Pandanus amaryllifolius R.*) ethanol

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extract. These results confirm that sweet pandan leaf ethanol extract has a significant effect on the growth of *Aspergillus* sp.

Further testing using Tukey HSD showed significant differences between the positive control (2% ketoconazole) and all treatments with pandan leaf extract at concentrations of 20%, 30%, 40%, and 50%. The average diameter of the largest inhibition zone was obtained at a concentration of 50%, which was 12.1 mm, not much different from the concentration of 40% (9.75 mm). However, both results were significantly different from the concentrations of 30% (7.26 mm) and 20% (5.99 mm). These findings indicate that the higher the concentration of pandan leaf ethanol extract, the larger the inhibition zone produced against the growth of *Aspergillus* sp.

The results of this study are in line with that the effectiveness of an antimicrobial substance is influenced by its concentration. An increase in the concentration of pandan leaf ethanol extract will increase the number of active compounds that act as antifungals, thereby strengthening the inhibitory effect on fungal growth (Ambarwati & Sintowati, 2017).

The limitation of this study is that no moisture content test was conducted on the pandan leaf ethanol extract samples, so information regarding the quality of the extract cannot be fully described.

CONCLUSION

Based on the results and discussion in this study, it can be concluded that: Ethanol extract of pandan leaves (*Pandanus ammarylifollius*. R) contains active compounds such as alkaloids, flavonoids, and tannins. The average diameter of the inhibition zone of fragrant pandan leaf extract (*Pandanus ammarylifollius*. R) at a concentration of 20% was 5.99 nm, 30% was 7.26 nm, 40% was 9.75, and 50% was 12.1 nm. Meanwhile, 2% ketoconazole as a positive control produced an inhibition zone of 39.16 nm, and 96% ethanol as a negative control did not inhibit the growth of *Aspergillus* sp. The ethanol extract of fragrant pandan leaves (*Pandanus ammarylifollius*. R) significantly affected the growth of *Aspergillus* sp. The higher the concentration of pandan leaf ethanol extract (*Pandanus ammarylifollius*. R), the wider the inhibition zone formed in inhibiting the growth of *Aspergillus* sp.

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In the treatment group, the 50% concentration demonstrated the most effective antimicrobial activity compared to concentrations of 40%, 30%, 20%, 10%, and the negative control (96% ethanol). These results indicate a positive relationship between increasing sample concentration and the effectiveness of microbial growth inhibition, indicating a consistent dose-response pattern. Ketoconazole (2 %) was used as a positive control to verify and validate the applied test method. The test results indicated that the test microbes were highly sensitive to ketoconazole, thus confirming that the test procedure and conditions were valid and met standards. Therefore, the comparison of effectiveness between the test samples and the positive control can be interpreted validly to assess the potential antimicrobial activity of the tested samples.

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