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Inhibition Test of Honey Mango Leaf Extract (*Mangivera Indica L.*) against the Growth of *Aspergillus flavus*

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ABSTRACT

Background: Aspergillus flavus is a dangerous pathogenic contaminant in agricultural commodities capable of producing highly carcinogenic aflatoxins. The escalation of resistance against synthetic chemical antifungals necessitates the discovery of safer natural alternatives. Honey mango leaves (Mangifera indica L. var. madu) accumulate potent secondary metabolites including flavonoids, tannins, saponins, alkaloids, and phenolics that manifest high antimicrobial properties. **Objective:** To determine the inhibitory effect of honey mango leaf (Mangifera indica L.) extract at concentrations of 80%, 70%, 60%, 50%, 40%, 30%, and 20% on the growth of Aspergillus flavus. **Research Methods:** This laboratory experimental design employed the disc diffusion technique using seven concentration levels (80%, 70%, 60%, 50%, 40%, 30%, and 20%) with triplicated observations. The primary metric recorded was the diameter of the inhibition zone around the paper discs. **Research Results**: The organic extract significantly restricted the proliferation of Aspergillus flavus across all tested ranges. The mean diameter of inhibition zones for concentrations of 80%, 70%, 60%, 50%, 40%, 30%, and 20% were measured at 39.3 mm, 33.7 mm, 31.3 mm, 26.0 mm, 24.7 mm, 20.7 mm, and 17.0 mm, respectively. Statistical trends confirmed that the inhibition area expands proportionally with the increase of extract dose. **Conclusion**: Honey mango leaf extract exhibits robust antifungal activities. A concentration of 50% represents the optimum threshold, yielding an inhibitory zone of 26.0 mm which demonstrates clinical parity with the commercial positive control group.

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Introduction

Climate conditions in Indonesia, which tend to be warm and humid, naturally support the acceleration of the growth of contaminant molds. *Aspergillus flavus* is one of the main pathogenic microorganisms that often infect post-harvest agricultural commodities, such as cereals and beans when stored in facilities with poor air management.

The main risk of *Aspergillus flavus* contamination is not just physical decay, but its ability to synthesize a dangerous secondary mycotoxin compound known as aflatoxin. Exposure to aflatoxin, especially type B1, has been shown to be strongly carcinogenic, hepatotoxic, and can trigger acute and chronic systemic contamination in the human body that consumes it. Therefore, the level of fungal contamination is an important indicator in national food safety and global agricultural product import and export regulations.

Until now, efforts to eradicate and prevent mold in agricultural commodities are still very dependent on the application of synthetic chemical fungicides such as phosphine and methyl bromide. Although effective in a short duration, the massive use of synthetic chemicals risks leaving toxic residues in food, damaging soil ecosystems, and triggering the emergence of resistant fungal strains. Mitigation measures that are environmentally friendly and safe for human health are very urgent to be researched, one of which is by exploring the antimicrobial potential of local herbal plants.

The honey mango plant (*Mangifera indica L*. var. honey) is one of the most abundant local fruit commodities in Indonesia. So far, its use has only focused on the pulp, while the leaves often end up as organic waste with no economic value. Phytochemically, honey mango leaves accumulate an abundant variety of active secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and phenolic compounds. Special xanton structures such as mangiferin are reported to have strong biological activity as an antioxidant as well as a natural antimicrobial.

Although the efficacy of mango leaves in suppressing pathogenic yeasts such as *Candida albicans* has been proven, the potential of phytochemical intrigue in suppressing the destruction of filament molds of aflatoxin producers, especially *Aspergillus flavus*, has not been comprehensively mapped. On this basis, this in vitro investigation is designed to evaluate the effectiveness of honey mango leaf fraction antibiofilm/antifungal fraction in a measured dose range of 20%–80% to determine the optimal resistance point.

Research Methods

The test design applied a purely laboratory experiment scheme with a *post-test only control group approach*. The series of studies were realized in the Microbiology laboratory of the Department of TLM, Polytechnic of the Ministry of Health, Mataram throughout March-May 2026.

The experimental unit in this study was in the form of a fungal colony of Aspergillus flavus cultured in a petri dish containing *Potato Dextrose Agar* (PDA) media. The determination of the repeat quota refers to Federer's ***criterion (t - 1)(r - 1) ≥ 15***. Through nine treatment combinations (seven dose levels and two standard controls), a total of 27 independent sample units were prepared which were observed in a triple

manner.

The honey mango leaf extraction procedure begins with the collection of fresh leaves on the 3rd and 4th strands of the plant shoots. The leaf samples are cleaned, dried through the room temperature passive method, and mashed into a fine simplicia powder weighing 500 grams. Extraction is carried out by cold maceration method using 96% ethanol solvent accompanied by periodic stirring. The maserrate is filtered and evaporated using a special oven at a stable temperature until a thick extract of 50 mL is obtained. The test concentration variations (80%, 70%, 60%, 50%, 40%, 30%, and 20%) are precisely made using the standard dilution formula of the solution with sterile aquadest dilution.

The pure isolate *of Aspergillus flavus* was pre-rejuvenated in a PDA cup for 3–5 days at a temperature of 28–30°C. The suspension density of the mushroom culture was regulated in a physiological salt solution until it reached a turbidity level equivalent to a McFarland suspension of 0.5. Measurements of the area of resistance (clear zone) formed around the disc paper were carefully carried out after the incubation period was completed using a millimeter-precision measuring instrument.

Research and Discussion Results

Based on post-incubation macroscopic observations, the antifungal activity of honey mango leaf extract is visually indicated by the appearance of an inhibition zone surrounding the disc paper on agar media. Quantitative data from the measurement of the diameter of the inhibition zone from three repetitions are presented in detail in Table 4.1.

Table 4.1. Suppression Response of Honey Mango Leaf Extract to A. flavusflavus Culture

No.	Treatment Groups	Diameter P1 (mm)	Diameter P2 (mm)	Diameter P3 (mm)	Average (mm)
1	Control (+) Ketoconazole	26	25	27	26
2	Control (-) Aquadest	0	0	0	0
3	Extract Dosage 20%	19	17	15	17
4	Extract Dosage 30%	20	21	21	20.7
5	Extract Dosage 40%	28	21	25	24.7
6	Extract Dosage 50%	28	25	25	26
7	Extract Dosage 60%	32	30	32	31.3
8	Extract Dosage 70%	35	33	33	33.7
9	Extract Dosage 80%	39	37	42	39.3

The test results showed a linear mechanistic gradation phenomenon: the higher the concentration of

the extracts exposed, the wider the area of the clear zone formed. This indicates that the diffusion rate and accumulation of the quantity of phytochemical active compounds in the growth medium are directly proportional to the dose of the extract used. The lowest concentration (20%) provides an inhibition diameter of 17.0 mm, while the peak concentration (80%) produces maximum efficacy with an average inhibition zone of 39.3 mm.

The biochemical mechanisms underlying the suppression ability of honey mango leaf extract are supported by the synergy of secondary metabolites contained in it. The fungicidal properties of this extract are thought to be derived from the collective work of its bioactive constituents. Flavonoid compounds disrupt the integrity of proteins on the cell membrane, while saponins lower the surface tension of the membrane thereby triggering an internal osmotic crisis of the mold cell. On the other hand, the precipitation of proteins by tannins stops vital enzymatic activity, and alkaloids intervene in the nucleic acid replication of the target mold.

Comparative analysis of the control group provided significant scientific findings. The negative control group using aquadestilata did not trigger the formation of clear zones (0 mm) at all, proving the validity that the pure solvent did not have inherently antifungal properties. The positive control group (Ketoconazole) showed stability in the inhibition zone diameter at an average value of 26.0 mm. Interestingly, efficacy equivalent to the positive control was consistently found in the 50% (26.0 mm) extract concentration treatment group. Furthermore, exposure to concentrations of 60%, 70%, and 80% triggered mechanistic performance that exceeded the capabilities of commercial positive control active substances.

The difference in the diameter of the inhibition zone at each concentration showed that the response *of Aspergillus flavus* to the honey mango leaf extract was concentration-dependent. At a concentration of 20%, the number of active compounds diffusing into the medium was relatively lower so that the inhibition zones formed were the smallest compared to other concentrations. The increase in concentration to 30% and 40% was followed by an increase in the diameter of the inhibition zone. This condition suggests that the addition of the concentration of the extract increases the number of active compounds available to reach the fungal growth area.

At a concentration of 50%, the diameter of the inhibition zone reached 26.0 mm and had the same value as the positive control of ketoconazole. The similarity of diameters showed that under the test conditions, honey mango leaf extract at a concentration of 50% was able to produce an inhibition zone of descriptively equivalent size with positive control. However, the similarity in the size of the inhibition zone cannot be used as the only basis for stating that the mechanism and strength of antifungal activity of the two ingredients is the same, since ketoconazole and plant extracts have different characteristics of active compounds and diffusion capabilities.

At a concentration of 60%, the diameter of the inhibition zone increased to 31.3 mm, then to 33.7 mm at the 70% concentration, and to 39.3 mm at the 80% concentration. The increase showed that the concentration of 60–80% provided greater inhibition than the positive control based on the size of the inhibition zone formed. The concentration of 80% indicated the widest inhibition zone, so it can be considered as the concentration with the highest inhibition activity in the concentration range used in this

study.

The size of the barrier zone formed is not only determined by the concentration of the extract, but is also influenced by the diffusion process of the active compounds in the agar medium. Compounds that have better diffusion ability will more easily spread from the disc to the medium and form a wider area of inhibition. Therefore, an increase in the inhibition zone at higher concentrations may reflect an increase in the number of active compounds as well as the ability of the compound to reach the growth area *of Aspergillus flavus*. Other factors such as the thickness of the media, the size of the disc, the number of inoculums, the incubation time, the incubation temperature, and the physiological conditions of the fungus can also affect the diameter of the barrier zone.

Differences in response to each repeat can also be found in the results of the study. For example, a concentration of 20% results in diameters of 19 mm, 17 mm, and 15 mm in each repeat. These variations can occur due to the distribution of the fungal inoculum on the surface of the media, the accuracy of the disc placement, the amount of extract absorbed on the disc, and the diffusion process of the active compound that does not always take place identically at each repeat. Although there is variation between repeats, overall there is a tendency to increase the diameter of the barrier zone as the concentration of the extract increases.

The antifungal activity of honey mango leaf extract can also be attributed to the content of phenolic compounds and other secondary metabolites. Phenolic compounds and flavonoids can interact with proteins and mushroom cell membrane components, causing changes in permeability and impaired membrane function. Membrane damage can lead to the release of intracellular components that are necessary to maintain cell metabolism. If the damage is continuous, the growth and development of *Aspergillus flavus* hyphae can be inhibited.

The tannins contained in the extract are also thought to contribute to inhibiting activity. Tannins have the ability to bind to proteins so that they can interfere with the function of proteins and enzymes required by fungal cells. Disruption of enzyme activity can cause disruption of metabolic processes and cell growth. Meanwhile, saponins can interact with cell membranes, increasing membrane permeability and causing instability in cell structure. The combination of the activities of several of these compounds is thought to result in a greater inhibitory effect when the concentration of the extract is increased.

In addition, the alkaloid content in honey mango leaves also has the potential to contribute to antifungal activity. Alkaloids can interfere with metabolic processes and various cellular activities of microorganisms. Thus, the inhibiting activity of honey mango leaf extract may not be caused by one type of compound alone, but is the result of the interaction of several secondary metabolites contained in the extract.

Biologically, inhibition against *Aspergillus flavus* is important because the fungus can grow on various food ingredients and has the potential to produce aflatoxin. Inhibition of fungal growth can be one approach to reduce the development of fungal contamination under certain conditions. However, *the results*

of the in vitro test in this study cannot be directly used to conclude the effectiveness of the extract as a preservative or antifungal in food, as further testing is needed under the actual application conditions.

The results of this study also show that the use of plant extracts as a source of antifungals has the potential to be developed because plant materials are relatively easy to obtain and contain various secondary metabolites. However, the effectiveness of plant extracts can be influenced by several factors, such as the type and age of the leaves, the environmental conditions in which the plants grow, the extraction method, the type of solvent, the duration of extraction, and the temperature during the extraction process. The difference in these factors can cause differences in the levels of active compounds and ultimately affect the antifungal activity produced.

Conclusion

A. Conclusion

Based on the formulation of the problem, the results of quantitative data analysis, as well as in vitro discussions, the following conclusion points were obtained: Ethanolic extract of honey mango leaves significantly suppresses the proliferation *of Aspergillus flavus* in the concentration range of 20% to 80%. Formulations at the level of 50% reveal the most proportional suppression performance (clear zone of 26 mm), showing efficacy equivalent to medical comparators of ketoconazole. Ethanolic extract of honey mango leaves is scientifically proven to have consistent antifungal capabilities in suppressing the colonization of *Aspergillus flavus fungus* in the dose range of 20% to 80%. The 50% concentration was identified as the most optimal, effective, and efficient concentration because it was able to produce a diameter of resistance (26 mm) that was clinically equivalent to a laboratory-standard positive control.

B. Suggestions

Further research is recommended to identify and further analyze the active compounds in honey mango leaf extract that play a role in inhibiting the growth of *Aspergillus flavus*, as well as test different concentrations and extraction methods to obtain more optimal antifungal effectiveness.

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