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Antifungal Activity of Starfruit Leaf Extract (*Averrhoa bilimbi L.*) Against Aspergillus flavus ATCC 3631 and Trichophyton mentagrophytesATCC 9533

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Abstract

Background: Indonesia is a tropical region with high temperatures and humidity, which makes it prone to skin infections; this condition is very favorable for the growth of fungi on the skin. Starfruit is a plant that has the potential to be a cure for various diseases and is used as a supplement to maintain health. Starfruit leaves (Averrhoa bilimbi L.) are antimicrobial, anticancer, and antioxidant. This study aimed to see the inhibitory activity of Starfruit leaf extract against the growth of Aspergillus flavus and Trichophyton mentagrophytesfungi. Method: Belimbig leaves are extracted using the maceration method, and phytochemical screening is then carried out. Antibacterial testing using a healthy diffusion method. The concentration of extracts ranges from 10% to 40% for positive control, which is ketoconazole, and negative, using DMSO. Results: Screening results of extracts showed positive for flavonoids, alkaloids, saponins, tannins, and steroids. Ethanol extracts of Starfruit leaves (Averrhoa bilimbi L.) at concentrations of 10, 20, 30, and 40% inhibited the growth of Trichophyton Aspergillus flavus 12.25±1.30, 13.1±0.77, 13.4±0.68 and 14±3.61 and mentagrophytes13.1±1.79, 14.7±1.64, 15.4±2.16, and 18.3±2.89. The inhibitory power of ketoconazole against Aspergillus flavus and Trichophyton mentagrophytesis 23±0.877 and 30.1±0.45. Conclusion: Starfruit leave ethanol extract (Averrhoa bilimbi L.) has antifungal activity against Aspergillus flavus ATCC 3631 and Trichophyton mentagrophytesATCC 9533 with strong category.

Keywords: Antifungal; Averrhoa bilimbi L.; Aspergillus flavus; Trichophyton mentagrophytes.

Introduction

Cases of "superbug" fungal infections increased sharply during the Covid-19 pandemic to the point where side effects persist. Recorded cases of fungal infections in the United States after COVID-19 have affected more than 2,000 people (Laras, 2023). Fungal infections, especially those caused by Aspergillus, Candida, and Mucormycetes, are known to occur in critically ill patients with pre-existing comorbidities (Darmadi et al., 2023). Fungal infections are the most important cause of morbidity and mortality in post-liver transplant patients receiving multiple immunosuppressants. *Candida* and *Aspergillus sp.* are the most invasive fungal infections (Karyanti et al., 2020).

Aspergillus fungus can cause an infectious disease called aspergillosis. Aspergillosis generally affects the respiratory system but can also spread to other body parts, such as the skin, eyes, or brain. Aspergillosis, a complication of severe influenza infection, is increasingly being detected worldwide (Marr et al., 2021). It is generally found that irregular lifestyle patterns and poor sanitation or densely populated environments encourage fungal growth (Melinda et al., 2019).

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©2024 by authors. Lisensi Bioeduscience, UHAMKA, Jakarta. This article is openaccess distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license. Another fungal infectious disease is dermatophytosis, caused by the fungus *Trichophyton mentagrophytes*, often found in coastal areas and tropical countries with low levels of hygiene, such as fishermen and home fish processors (Triana et al., 2020). The pathogenesis of dermatophytosis affects heat temperature, a person's cleanliness level, immunogenicity, infection location, and the patient's immune response (Wenas et al., 2021).

Indonesia has various types of plants that function as medicines for infectious diseases. Indonesian people have known about plants as a treatment for disease for quite a long time (Agustina et al., 2016). One is starfruit, another name for sour starfruit, which is included in the Oxalidaceae family and is closely related to starfruit (*Averrhoa carambola*). Starfruit is a plant that has the potential to be used as a medicine for various diseases and as a supplement to maintain health (Alhassan & Ahmed, 2016).

Various biological activities have proven the potential of starfruit as an antioxidant, antimicrobial, antidiabetic, hypotensive, anti-inflammatory, and hepatoprotective, which shows how valuable this plant is as a complementary and alternative medicine (Garg et al., 2022). Besides being used as an herbal medicine, the leaves have been scientifically tested for antibacterial properties (Panjaitan et al., 2017). *Escherichia coli, Staphylococcus aureus,* and *Salmonella Enteritidis* are all susceptible to leaf and fruit extracts (Swedha, 2022).

Based on the results of previous research on the content of secondary metabolite compounds in starfruit leaves, it is proven that there are flavonoid compounds, sterols (Prabhu et al., 2021), alkaloids, saponins, tannins (Aryantini et al., 2017), and triterpenoids (Soleha et al., 2019). Tannins are antifungal because they inhibit protease activity and direct inactivation of fungal cell walls. Damaged fungal cell walls will not be able to interact with host cells (Sari & Cicik Suryani, 2014). The flavonoids in starfruit leaves are polar compounds and will dissolve in solvents such as ethanol, acetone, and butanol. Flavonoids are the largest phenols group and have antiviral, antibacterial, and antifungal properties (Anggraeni & Ardiansyah, 2017).

Based on previous research, a concentration of 20% has an inhibition zone of 6 mm, and the highest inhibition zone is 24.60 mm, with a concentration of 80% (Sari & Cicik Suryani, 2014). Subsequent research conducted by George & Dhivya (2019) using ethanol solvent had an inhibitory zone of 14 mm against the fungus Aspergillus niger at a concentration of 40 μ l followed by a concentration of 30 μ l with an inhibitory zone of 13.5 mm (George & Dhivya, 2019). Extracted samples with known total flavonoid content (10–200 μ g/mL) were used against E. coli, S. aureus, and *B. subtilis*, the MIC results showed respectively 55.6 ± 6, 31 ± 3 and 28 ± 2 μ g/mL (Chau et al., 2023).

Method

The research was a laboratory experimental study to observe the magnitude of the inhibitory power of starfruit leaf extract with several types of concentrations on the growth of *Aspergillus flavus* and *Trichophyton mentagrophytes*using the Well Diffusion Method. The research was conducted at the Microbiology Laboratory, University of North Sumatra. This research was divided into several stages, namely: sample preparation, preparation of microorganisms, work on making simplicia and preparation of ethanol extract of starfruit leaves, and testing the antifungal activity of ethanol extract of starfruit leaves against *Aspergillus flavus* and *Trichophyton mentagrophytes*. The samples from this research were starfruit leaves (*Averrhoa bilimbi L*.) originating from Medan City.

Research procedure

Making Simplicia

The initial process is to prepare 5 kg of fresh starfruit leaves (*Averrhoa blimbi L.*), then clean them of dirt that is not visible to the naked eye and sticks to the leaves, and discard the unused parts of the leaves, then enter the washing process with using running water until clean, after that, the leaves are drained to free the leaves from remaining water particles, then dried by air-drying, after the simplicial is dry, proceed with the sorting stage. This sorting stage is carried out by sorting the parts that could not be cleaned during the previous

cleaning. Simplicia is completely dry and ready to enter the grinding process. After the dried simplicia has been ground, it will enter the sieving process using a 40-mesh sieve and then be stored in a tightly closed container (Sarifudin et al., 2018).

Characteristics of Simplicia

Macroscopic test

Macroscopic examination of leaves can be done in two ways: using or without a magnifying glass. This macroscopic examination can be used to look for the specific morphology, size, and color of the Simplicia being studied. Before carrying out a macroscopic examination, the Simplicia is first subjected to an organoleptic test, which includes smell, taste, and color (Depkes RI, 1989).

Determination of Water Content

A total of two grams of Simplicia is weighed. Then, the Simplicia is put into a porcelain crucible, which was previously heated at 105oC for 30 minutes in a closed state and tarred or calibrated. The simplicia is then flattened in a porcelain crucible by shaking the crucible until the simplicia is evenly distributed. Then, put the simplicia in the oven, open the crucible lid, and heat at 100°C to 105°C. The simplicity is weighed, and the heating is repeated until a constant weight is obtained (Depkes RI, 1989).

Determination of Soluble Essence Content in Water

After turning it into simple starfruit leaf powder, we then take 5 grams to macerate for 24 hours in 100 ml of water-chloroform (with a ratio of 2.5 ml of chloroform dissolved in 1 liter of distilled water) in a clogged flask while shaking several times. Or stirred for the first 6 hours, then left for 18 hours, and then the results were filtered. Then, the first 20 ml of the filtrate is evaporated until dry in a flat evaporator cup that has been heated, balanced, or calibrated. The remainder is heated at 105 °C until it reaches a constant weight. Water-soluble essence content can be calculated using dried material (Depkes, 1989).

Determination of Soluble Essence Content in Ethanol

Five grams of dried powder was taken and placed in air to macerate for 24 hours in 100 ml of 96% ethanol in a stoppered flask while shaking or stirring several times for the first 6 hours, then left for 18 hours and filtered. The first 20 ml of filtrate is evaporated until dry in a shallow cup based on a level that has been measured or calibrated. The remainder was heated in an oven at 105 oC until a constant weight was obtained. The concentration of essence that dissolves in ethanol can be calculated using material dried in the air (Depkes, 1989).

Determination of Total Ash Content

A total of 2 grams of powder is crushed or crushed and put into a porcelain crucible that has been burned and tared, then leveled. The porcelain crucible is burned slowly until the charcoal runs out, then the glow is carried out at a temperature of 500-600 °C for 6 hours, then cooled and weighed until a constant weight is obtained. Ash content can be calculated using dried material (Depkes RI, 1989).

Determination of Insoluble Ash Content in Acid

After obtaining the ash by determining the ash content, the ash is boiled with 25 ml of dilute hydrochloric acid P for 5 minutes. The part that is not soluble in acid can be collected, filtered through ash-free filter paper, washed with hot water, and ignited until the weight remains constan, then weighed. The content of ash that is insoluble in acid can be calculated from the dried material (Depkes, 1989).

Preparation of starfruit leaf extract (Averrhoa blimbi L.)

Making ethanol extract of Starfruit leaves (*Averrhoa blimbi L.*) was carried out using the maceration method using a ratio of 1 10 (300 grams of simplicia in 3 liters of 96% ethanol)

for seven days, where for the first five days, the simplicia was soaked in 75 parts of ethanol, then filtered using filter paper, to obtain macerate one and dregs. Then, the dregs are soaked in 25 parts of ethanol for two days and filtered using filter paper until macerate two and the dregs can be obtained. Macerate one and two are combined and then evaporated using a rotary evaporator at a temperature of 50 oC to evaporate the solvent in the macerate, thus obtaining a thick extract (Idroes et al., 2019).

Antibacterial Activity Test

Antibacterial activity testing can be done using potato dextrose agar (PDA). The first step is to make a base layer by pouring 10 ml of PDA from the base medium into a petri dish and then leaving it until it solidifies. After that, the media is perforated with a steel driller with a diameter of 6 mm, totaling six holes. The six holes are used: one hole for negative control, one for positive control, and four for concentration levels. Next, 1 ml of the fungal suspension was mixed into the PDA seeding medium, and then 20 ml of the mixture of suspension and seeding medium was poured into each petri dish until it became solid. Then, drill holes with a drill aseptically to form wells that will be used in the antifungal test; then put 50 μ l into the wells that have been formed; the media is incubated in an inverted manner, and the diameter of the inhibition zone is calculated, using a caliper. The negative control was (DMSO), and the positive control was Chloramphenicol. The test fungi used were *Aspergillus flavus* and *Trichophyton mentagrophytes* (Sari, 2014).

Data processing

This research obtains results from data received and processed using statistical tests, namely the Analysis of Variance (ANOVA) test.

Result and Discussion

The test results for the characteristics of Simplicia powder can be seen in the table below.

Parameter Test	Result	Parameter (MMI)
Water content	9,6 %	<10%
Ash content	7,1 %	<7,5%
Acid insoluble ash content	0,55 %	<1%
Water soluble essence content	19,1%	>18%
Ethanol soluble essence content	13,9%	>11%

Table 1. Characteristics of Wuluh Starfruit Leaf Simplicia

Determination of water content in simplicia is carried out to determine the amount of water contained in the simplicia used. The water content of Simplicia is determined to maintain its quality because the water content is related to the possibility of mold or fungus growth. The results of determining the water content obtained were less than 10%, namely 9.6%, indicating that Simplicia has a water content that is difficult for fungi to grow and meets the MMI requirements. Water content values above 10% make it easy for mold to grow. The maceration method, which uses water as a solvent, is a suitable medium for microbial contamination so that it will reduce the quality of the simplicial; therefore, in calculating the juice content, it is best to add chloroform to avoid microbes. Meanwhile, when determining the soluble essence content of ethanol, there is no need to add chloroform because ethanol already has antibacterial properties.

The test results in Table 1 show that the water-soluble essence content of Simplicia is 19.1%, and the ethanol-soluble essence content is 13.9%. Based on these results, it meets the requirements for water-soluble essence content below 18% and ethanol-soluble essence content less than 11% (Depkes, 1989).

Results of Extract Making

41.5 g of thick extract was produced from the initial weight of 305 g of simplicia, so an extract yield of 14% was obtained. This yield value is practical, and many factors can influence this, including the size of the simplicia, method, and length of extraction time (Wijaya et al., 2018). Apart from these factors, there are other factors, namely storage conditions and time, the ratio of the amount of solvent to the sample, and the type of solvent (Salamah & Widyasari, 2015).

Results of Phytochemical Screening of Starfruit Leaf Extract

The results of the phytochemical screening of simplicia powder from starfruit leaves can be seen in Table 2.

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Parameter	Preaction	Reaction	Test Result	Information	
	Mayer	White/yellow precipitate	A white precipitate is formed		
Alkaloids	Bouchardat	Brown precipitate	A brown precipitate forms	+ (Alkaloids)	
	Dragendroff	Light brown precipitate	A light brown precipitate is formed	IS	
Saponins	Aquadest + HC1 2 N	Foam 1-10 cm high is formed.	Foam is formed	+ (Saponins)	
Flavonoids	Mg + HCl (p)	Red/yellow color solution	Red color	+(Flavonoids)	
Tannin	FeC13 1%	Blackish blue/green color solution	Blackish green color	+ (Tannin)	
Steroids/ Triterpenoids	n-heksan, (CH3C0)20, H2SO4(P)	Red-orange/Green	Green color	+ (Steroids)	

Information: (+) positive = contains a group of compounds

The results obtained in Table 2 show that starfruit leaf extract contains alkaloid, flavonoid, saponin, steroid, and tannin compounds.

Antifungal Activity Test Results

Tools and materials are sterilized first before antifungal activity tests are carried out. Sterilizing tools and materials aim to prevent contamination by foreign microorganisms when testing antifungal activity. Testing processes involving fungi require an aseptic technique. Aseptic techniques aim to eliminate unwanted microbial contamination (Lestari et al., 2019). The results of the fungal inhibition test can be seen in Table 3 below.

Table 3. Results of testing the antifungal activity of starfruit leaf extract (Averrhoa bilimbi L.)

	Barrier Zone Diameter (mm)/Sd			
Concentration	Aspergillus flavus	Trichophyton mentagrophytes	Catagory	
	Mean ± Sd	Mean ± Sd	Category	
K +	23±0,877	30,1±0,45	Very Strong	
40 %	14±3,61	18,3±2,89	Strong	
30 %	13,4±0,68	15,4±2,16	Strong	
20 %	13,1±0,77	14,7±1,64	Strong	
10 %	12,25±1,30	13,1±1,79	Strong	
K -	0	0	Nothing	

The strength of the inhibition zone is categorized as follows: very strong (>20 mm), Strong (10-20 mm), medium (5-10 mm), and Weak (< 5 mm) (Davis, WW, & Stout, TR, 1971). Table 3 shows that in the activity test of the two fungi against the extract of starfruit leaves, the diameter of the inhibition zone was wider against *Trichophyton mentagrophytes* and in

the test on the fungus *Aspergillus flavus* showed lower results; this means that the ethanol extract of starfruit leaves has antifungal activity. It is more excellent against the fungus *Trichophyton mentagrophytes*than against the fungus *Aspergillus flavus* (seen in figure 1).

Previous research used a concentration of 20%; the barrier zone formed was 6 mm, whereas, in this study, it was 14.7 ± 1.64 mm and 13.1 ± 0.77 mm for the two test mushrooms with a concentration of 20%. At a concentration of 40%, The inhibition zone formed was 13.2 mm, while in this study, a concentration of 40% already had an inhibitory capacity of 14 ± 3.61 mm and 18.3 ± 2.89 mm (Sari & Cicik Suryani, 2014). Other research conducted by Dewi et al. (2020) stated that the results of the inhibition zone of starfruit extract gel preparations with a concentration of 10% had antibacterial activity against Propionibacterium acnes of 16.67 ± 0.40 mm and a concentration of 20% 28.10 ± 0.36 mm, while for Staphylococcus aureus bacteria the concentration of 20 %, it has the highest inhibition zone of 30.40 ± 0.4 mm. In contrast, in this study, the 10% concentration was only able to inhibit 12.25 ± 1.30 mm on *Aspergillus flavus* and *Trichophyton mentagrophytes*13.1 ± 1.79 mm. At a concentration of 20% it was only able to inhibit 13.1 ± 0.77 mm on *Aspergillus flavus* and *Trichophyton mentagrophytes*14.7 ± 1.64 mm (Dewi et al. 2020).

This difference is due to the activity of each test material against bacteria and fungi, which differences in the morphology of bacteria and fungi can cause. Bacterial cells have a cell wall morphology composed of peptidoglycan, while fungal cell wall morphology comprises chitin. Chitin in fungi is in the form of cellulose microfibrils, the main structure of fungal cell walls consisting of interwoven polysaccharide chains that cross each other to form parallel fibers (Pratiwi, 2008).



Figure 1. (a) *Trichophyton mentagrophytes*inhibition zone, (b) *Aspergillus flavus* inhibition zone

Results of the One-Way ANOVA test on the fungi Aspergillus flavus and Trichophyton mentagrophytes

The data obtained from this research was subjected to statistical testing, namely the One-Way ANOVA test. The conditions required in the One-Way ANOVA test are that the data to be tested is normally distributed and homogeneous. Previously, the data had to be tested for Shapiro-Wilk and homogeneity using SPSS version 20.

The normality test results of both data are normally distributed. This can be seen from the significance value of the inhibition zone for *Aspergillus flavus* $0.606 \ge 0.05$ and the significance value of the inhibition zone for *Trichophyton mentagrophytes* $0.303 \ge 0.05$, so the data is normally distributed so that the One-Way ANOVA test can be carried out.

Next, a homogeneity test was carried out based on Table 4 of the homogeneity test results. The data obtained had the same variance with a significance value of $0.129 \ge 0.05$ for the *Aspergillus flavus* homogeneity data and the significance value for *Trichophyton mentagrophytes* was $0.13 \ge 0.05$, so it could be said that the data were homogeneous. This is in line with previous research; the homogeneity test results obtained a sig value. $0.114 \ge 0.05$ (Sari & Triski, 2024).

Table 4. One-Way Anova Test Results

Trichophyton mentagrophytes		Aspergillus flavus		
	Sum of Squares	Sig.	Sum of Squares	Sig.
Between Groups	1401.611	.000	816.327	.000
Within Groups	38.727		7.193	
Total	1440.338		823.520	

Then, a One-Way ANOVA test was carried out, and a significance value of $0.000 \le 0.05$ was obtained. It can be seen from Figure 2 that the use of starfruit leaf extract had an influence on the growth of *A. flavus* and *T. mentagrophytes* fungi.

So, it can be concluded that the concentration of the extract makes a significant difference in the diameter of the inhibition zone formed. It can be seen in Table 3 that the optimum concentration of starfruit leaf extract against *A. flavus* and *T. mentagrophytes* was found at a concentration of 10%.

A concentration of 10% provides a robust inhibitory effect, so this concentration can be used as a basis for making herbal preparations for treating skin infections.



Figure 2. Treatment graph

Conclusions

Starfruit leaf extract (*Averrhoa bilimbi L.*) can inhibit the growth of the fungi *Aspergillus flavus* ATCC 3631 and *Trichophyton mentagrophytes*ATCC 9533 in the strong category. The antifungal activity of the ethanol extract of starfruit leaves (Averrhoa bilimbi L.) with a more significant inhibition zone on the growth of the fungus *Trichophyton mentagrophytes*ATCC 9533 than on the fungus *Aspergillus flavus* ATCC 3631. The optimum 40% concentration can inhibit the development of the fungus *Aspergillus flavus* ATCC 3631 and *Trichophyton mentagrophytes*ATCC 9533.

Declaration statement

The authors reported no potential conflict of interest.

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