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Inhibition Test of Ethanol from Extract Mangosteen Leaves *(Garcinia mangostana L.)* as an Acne Antibacterial

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Abstract

A prevalent global infection that manifests frequently in Indonesia. An example of an infectious disease that is commonly encountered during adolescence is acne (Acne vulgaris). Antibiotics can be used to treat acne; however, their misuse can lead to the development of resistance; therefore, we are searching for inexpensive, readily available alternatives that are also secure. Mangosteen leaves (Garcinia mangostana L.) are one alternative to synthetic ingredients when it comes to the treatment of acne. Mangosteen leaves comprise xanthone derivative compounds that exhibit significant biological activity, including antioxidant, antibacterial, and antimicrobial properties. Therefore, this this study aimed to ascertain the inhibitory power of an ethanol extract derived from mangosteen leaves in order to determine its antibacterial activity against Staphylococcus epidermidis bacteria. Additionally, the compound content of the ethanol extract of mangosteen leaves was determined. The inhibition test was conducted by utilizing the paper disc technique, while the extraction method employed the maceration method. As indicated by the phytochemical screening test results, simplicia and ethanol extract of mangosteen leaves were found to contain steroids/triterpenoids, alkaloids, flavonoids, saponins, and tannins. The inhibition test outcomes against Staphylococcus epidermidis bacteria revealed the following: a diameter of 3.3 mm for bacteria at a concentration of 20%, 9.4 mm for bacteria at 40%, 10.4 mm for bacteria at 60%, 12.9 mm for bacteria at 80%, and 13.2 mm for bacteria at 100%

Keywords: *Antibacterial, Mangosteen Leaves, Acne, Staphylococcus epidermidis*

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INTRODUCTION

Acne (*Acne vulgaris*) is a skin condition that arises from a combination of genetic, endorphin-related, psychological, climatic, stress-related, dietary, cosmetic, and bacterial variables. The bacteria responsible for acne are Propionibacterium acnes, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Zulfa, 2023). The bacteria present in sebum can gather in the follicular ducts and proliferate within the sebaceous glands (Bungau *et al.*, 2023). In Indonesia, the occurrence rate of individuals with acne is documented to be 80-85% among youths aged 15-18 years, 12% among women under the age of 25, and 3% among women aged 35-44 years (Zulfa, 2023). The reason for this is that Indonesia is situated in a tropical region characterized by elevated humidity and low temperatures, creating favorable conditions for rapid bacterial proliferation and subsequent development of infectious diseases (Nurfiana & Turahman, 2018), such as *Staphylococcus epidermidis*.

Staphylococcus epidermidis is a kind of staphylococcus bacteria that lacks the enzyme coagulase. It is commonly found in the human body, particularly on the skin and mucous membranes. Although typically harmless, it can lead to clinical illnesses like acne (Storz, 2020). *Staphylococcus epidermidis* have the capacity to create biofilms as a means of evading the host's immune system by diminishing the infiltration of phagocytes into the biofilm matrix. Biofilms contribute to the development of antibiotic resistance through two mechanisms: the impermeability of the biofilm matrix to antibiotics and a reduction in cell growth and metabolic activity, leading to the pathogenicity of *Staphylococcus epidermidis* (Siciliano *et al.*, 2023).

Treatments for acne typically involve the use of pharmaceutical formulations that contain antibiotics, such as clindamycin. Non-compliance with antibiotic treatment can lead to the development of resistance (Maftuhah et al., 2016). The overutilization of antibiotics can lead to the development of antibiotic resistance when bacteria undergo genetic alterations that diminish or eliminate the efficacy of antibiotics. Therefore, it is imperative to seek out alternative medications that are readily accessible, cost-effective, and safe (Roanisca & Mahardika, 2021). The mangosteen plant possesses antimicrobial properties (Turahman & Sari, 2018).

The mangosteen plant (*Garcinia mangostana L.*) is a tropical plant commonly found in Southeast Asian countries like Indonesia, Malaysia, Sri Lanka, the Philippines, and Thailand (Gutierrez-Orozco & Failla, 2013) consisting of 35 genera to 800 species (Rizaldy *et al.*, 2022). Various plant components, including the pericarp (fruit skin), cortex (bark), and radix (roots), have been utilized as medicinal remedies throughout Southeast Asia (namely India, Thailand, and China) for centuries. Fruit peel powder is utilized for its antibacterial, antiparasitic, dysentery-fighting, wound healing, and chronic ulcer-treating properties. The leaves and bark possess potent anti-inflammatory qualities, making them frequently employed in the treatment of eczema, hyperkeratosis, and various skin problems. The bark decoction is employed for alleviating diarrhea, serving as an astringent lotion, and addressing skin problems (Jang *et al.*, 2008). A decoction of the skin and seeds is used to treat urinary tract infections, anti-scourge, laxative, anti-fever, acne, arthritis, obesity, diabetes and cancer (Ovalle-Magallanes et al., 2017). The pharmacological activity of the mangosteen plant is closely related to the bioactive

compounds contained in it such as xanthones, terpenes, anthocyanins, tannins, phenols and several vitamins. Mangosteen leaves contain xanthones (Ansori *et al.*, 2020).

According to Rosalina & Mahendra (2021), the methanol extract of mangosteen leaves at a concentration of 100% had the greatest antibacterial activity with an inhibition zone of 27.01 ± 0.1 mm. According to Sari et al. (2019), Mangosteen leaf extract and fractions have activity against Staphylococcus aureus with an inhibitory zone diameter of <10 mm while the inhibitory zone diameter of the positive control Ciprofloxacin is 25 mm. Based on the description above, research was conducted to test the antibacterial activity of ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) against Staphylococcus epidermidis bacteria.

METHOD

Instruments and materials

Instruments used include Erlenmeyer, beaker glass, measuring cup, petri dish, test tube, autoclave, stirring rod, wool thread, blender, incubator, vernier caliper, cycle needle, camera, analytical balance, glass jar, oven, hotplate, Bunsen and rotary evaporator. The materials were mangosteen leaves, NA, MHA, Dragendroff's reagent, Wagner's reagent, Mayer's reagent, Lieberman-Bouchard's reagent, Mg, HCl, amyl alcohol, FeCl3, disc paper, filter paper, plastic wrapping, 96% ethanol, aluminum foil, spiritus and Staphylococcus epidermidis.

Making Mangosteen Leaf Ethanol Extract

The extract was made by extracting simplicia powder by maceration using 96% ethanol solvent. 500 g of mangosteen leaf simplicia powder was macerated with 75 parts of ethanol solvent until all the powder was submerged, covered and left for 5 days protected from light, stirring occasionally. Then the sample was filtered and the filtrate was obtained, while the residue was extracted again using 25 parts of ethanol, put into a vessel and stored in a place protected from light for 2 days, then filtered (Directorate General of POM RI, 1979). All the macerates were combined and concentrated with the help of a rotary evaporator at a temperature of not more than 40oC until a thick extract is obtained.

Making Concentration Variations

The concentrations of ethanol extract of mangosteen leaves were 20%, 40%, 60%, 80% and 100% (g/ml). The sample solution was made by weighing the thick extract of mangosteen leaves, 2 g each dissolved in 10 mL DMSO for a concentration of 20%, 4 g dissolved in 10 mL DMSO for a concentration of 40%, 6 g dissolved in 10 mL DMSO for a concentration of 60%, 8 g dissolved in 10 mL DMSO for a concentration of 80%, and 10 g dissolved in 10 mL DMSO for a concentration of 100%.

Determination of the Antibacterial Activity of Ethanol Extract of Mangosteen Leaves against *Staphylococcus epidermidis* Bacteria

A total of 0.1 mL of inoculum (CFU/mL) was put into a sterile petri dish, after which 20 mL of melted Meuller Hinton Agar (MHA) media was poured in at a temperature of 45-50^{o}C, homogenized and left until the media solidified. On the solid media, paper discs were placed which had been soaked first in a solution of the ethanol extract of mangosteen leaves at each concentration. Then incubated at 37^{o}C for 18-24 hours. Next, measure the diameter of the inhibitory area around the test material solution using a caliper. The experiment was carried out 3 times in repetition.

Data analysis

The data obtained was presented in tabular form which is the result of measuring the inhibition zone. Furthermore, the data obtained from the research results were processed using statistics, namely the Analysis of Variant (ANOVA) test using SPSS version 23.

RESULTS AND DISCUSSION

Results of Phytochemical Screening of Simplicia Powder and Mangosteen Leaf Extract

The results of phytochemical screening of simplicia powder and thick extract of mangosteen leaves can be seen in table 1. The phytochemical screening revealed the existence of secondary metabolite chemicals, specifically alkaloid, flavonoid, saponin, steroid/triterpenoid, and tannins. Phytochemical screening experiments were conducted to identify the specific class of chemicals present in simplicia and extracts utilized as herbal remedies. According to Pangow et al. (2018), Mangosteen leaf ethanol extract contains triterpenoid, flavonoid, tannin and saponin. According to Ansori et al. (2020), Mangosteen leaves contain bioactive compounds in the form of xanthones. Xanthones are compounds that are closely related to flavonoids. Xanthones and their derivatives have biological activities such as antioxidant, antibacterial, cytotoxic and antiproliferative.

Table 1. Phytochemical screening of simplicia powder and thick extract of mangosteen leaves

No	Compound Classes	Simplicity	Extract	Information
1	Flavonoid	+	+	An orange-yellow color occurs in the amyl alcohol layer
2	Saponin	+	+	A stable foam is formed
3	Tannin	+	+	Blackish green color
4	Alkaloid	+	+	A yellow precipitate in Mayer's reagent, an orange precipitate in Dragendroff's reagent, and a blackish brown precipitate in Bouchardat's reagent
5	Steroid/Triterpenoid	+	+	A purple/red color forms which turns greenish

Inhibitory Power Test Results

The effect of ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) on the growth of Staphylococcus epidermidis bacteria can be seen in table 2.

Table 2. Test results for the inhibitory power of ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) on the growth of *Staphylococcus epidermidis* bacteria

Extract Variation (%)	Resistance (mm) U1	U2	U3	Average (mm)	Criteria
20	2,1	3,6	4,2	3,3	Low
40	9,1	9,8	9,4	9,4	Medium
60	10,3	10,8	10,1	10,4	Strong
80	12,8	12,9	13,1	12,9	Strong
100	13,0	13,2	13,5	13,2	Strong
Clindamycin	20,1	26,1	27,7	24,6	Very strong
DMSO	0	0	0	0	-

Based on table 2, it can be seen that the antibacterial activity of the ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) against the growth of Staphylococcus epidermidis bacteria at varying concentrations of 20%, 40%, 60%, 80%, and 100% has an inhibitory power of 3.3 mm. 9.4 mm, 10.4 mm, 12.9 mm, and 3.2 mm. The positive control activity used, namely clindamycin, produced an inhibitory power of 24.6 mm, and the negative control activity used, namely DMSO, did not show any antibacterial activity. The results of the research at each concentration variation of 20%, 40%, 60%, 80%, and 100% had antibacterial activity to inhibit the growth of Staphylococcus epidermidis bacteria with an inhibitory power of 3.3 mm, 9.4 mm, and 10 mm, respectively. 4 mm, 12.9 mm, and 13.2 mm, which can be seen around the disc paper. Mangosteen leaf extracts and fractions have antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa, with an inhibition zone diameter of <10 mm. Mangosteen leaf extracts and fractions contain flavonoids, steroids, and saponins (Sari *et al.*, 2019). Methanol extract of mangosteen leaves at a concentration of 100% has the greatest antibacterial activity with an inhibition zone of 27.01 mm (Rosalina & Mahendra, 2021). A comparison of the inhibitory power produced by each concentration of mangosteen leaf extract on the growth of *Staphylococcus epidermidis* bacteria can be seen in Figure 1.

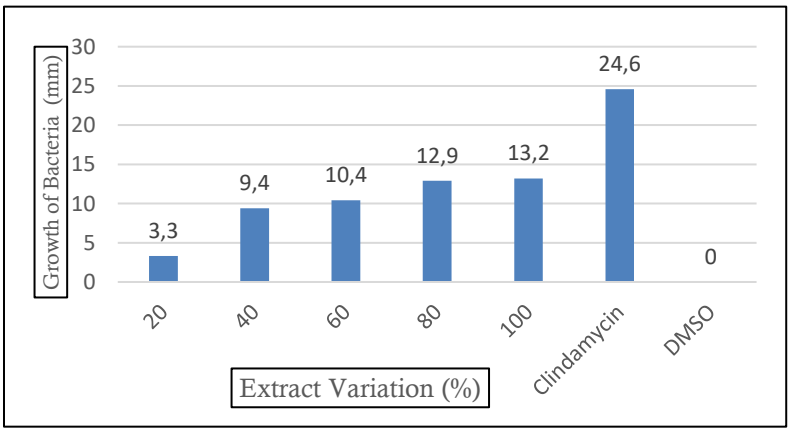

Figure 1. Comparison of Inhibitory Power for each variation

In this research the data obtained was analyzed statistically. The statistical test carried out was the One Way ANOVA Test. The One Way ANOVA test was chosen because there is only one test variable that will be tested, namely the concentration of mangosteen leaf extract. The requirements for the One Way ANOVA test are that the data to be tested must be normally distributed and the data have homogeneous variations. Therefore, before testing with the One Way ANOVA Test, the data must first be tested for Kolmogorov Smirnov normality and homogeneity test first using SPSS which can be seen in the table 3.

Table 3. Normality Test Results

One-Sample Kolmogorov-Smirnov Test

		Test	Obstacle_Zone_ Diameter
N		21	21
Normal Parameters[a,b]	Mean	2.00	10.281
	Std. Deviation	.837	7.5036
Most Extreme Differences	Absolute	.217	.158
	Positive	.217	.158
	Negative	-.217	-.120
Test Statistic		.217	.158
Asymp. Sig. (2-tailed)		.011[c]	.185

Note: a. Test distribution is Normal; b. Calculated from data; c. Lilliefors Significance Correction

Based on the normality test, the inhibition zone data tested is normally distributed. This can be seen from the significance value obtained, 0.0185 > 0.05, so it is proven that the data is normally distributed.

Table 4. Homogeneity Test Data Results

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Obstacle_Zone_Diameter	Based on Mean	.016	2	18	.984
	Based on Median	.021	2	18	.979
	Based on Median and with adjusted df	.021	2	16.969	.979
	Based on trimmed mean	.021	2	18	.979

The results of the homogeneity test are where the data obtained has homogeneous variations with a significance value of 0.979 > 0.05, so the data has the same or homogeneous variance (meets the ANOVA test requirements). This shows that the use of mangosteen leaf extract has an effect on the growth of Staphylococcus epidermidis bacteria.

Table 5. One way ANOVA Test Results

ANOVA

Diameter

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1118.769	18	10.956	0,177	.000
Within Groups	7.304	2	62.154		
Total	1126.072	20			

The next test used is the Duncan test, which aims to see which treatments have the same or different effects, from the smallest effect to the largest effect between one concentration and another. The Duncan test against Staphylococcus epidermidis bacteria for the negative control showed significant differences against the positive control and various extract concentrations. The negative control used was DMSO, which showed the absence of an inhibition zone. This indicates that the negative control used had no effect on the antibacterial test.

The positive control showed a significant difference in the Duncan test because it produced the greatest antibacterial activity (24.633 mm) against the test bacteria compared to the negative control and various extract concentrations. Duncan's test on the diameter of the inhibition zone of Staphylococcus epidermidis bacteria for a 20% concentration was (3,300 mm), for a 40% concentration it was (9,433 mm), for a 60% concentration it was (10,400 mm), for an 80% concentration it was (12,933 mm), and for a 100% concentration it was (13.233 mm), which shows a real difference to various concentrations, which means it shows different effects in inhibiting the growth of Staphylococcus epidermidis bacteria. This shows that the greater the concentration of the ethanol extract of mangosteen leaves, the greater the inhibition zone contained in each concentration, which means that the greater the concentration, the greater the substances contained in the extract, indicating that the greater the ability of the test material to inhibit these bacteria.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that:

1. Ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) with varying concentrations of 20%, 40%, 60%, 80% and 100% shows an inhibitory zone diameter of (3.3 mm), (9.4 mm), (10, 4 mm), (12.9 mm), and (13.2 mm) against Staphylococcus epidermidis bacteria.
2. Secondary metabolite compounds contained in the ethanol extract of mangosteen leaves (*Garcinia mangostana L.*) are alkaloid, flavonoid, saponin, steroid/triterpenoid and tannin

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