

## Antibacterial Activity of Mango Gedong Gincu Peel Fraction in Inhibiting the Growth of *Staphylococcus aureus*

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### ABSTRACT

Gedong Gincu Mango peel (*Mangifera indica* L.) contains a variety of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and terpenoids. These compounds play a crucial role in self-defence and exhibit significant pharmacological activity, particularly as antibacterial agents. The purpose of this study is to identify the content of secondary metabolite compounds of each fraction based on its polarity properties, and to determine the inhibitory power of *Staphylococcus aureus* ATCC 25293 bacteria. This study is experimental research with a post-test only control group. This study used 2 control groups and 12 treatment groups consisting of n-hexane, ethyl acetate, and butanol fractions with concentrations of 6.25%, 12.5%, 25%, 50% respectively and repeated 3 times. The antibacterial test was carried out by the well diffusion method, which was repeated 3 times on the MHA medium, and the inhibition zone was measured. The study showed that the n-hexane fraction contained alkaloids, steroids, and saponins, while the ethyl acetate and butanol fractions contained flavonoids, alkaloids, tannins, triterpenoids, and phenolics. A 50% concentration produced the greatest inhibition zone in all fractions, with mean values of 8.05 mm (moderate) for n-hexane, 15.41 mm (strong) for ethyl acetate, and 12.16 mm (strong) for butanol. The 50% ethyl acetate fraction was the most effective in inhibiting the growth of *Staphylococcus aureus* ATCC 25923.



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## 1. Introduction

*Staphylococcus aureus* is a pathogen that is often associated with skin infections, pneumonia, sepsis and endocarditis in humans. (Wu et al., 2022). Antibiotic treatment, whether bacteriostatic or bacteriocidal, has become a therapeutic option for treating infections caused by *Staphylococcus aureus* bacteria and is known to provide a high success rate (Rossiter et al., 2017). In fact, antibiotic misuse in society is widespread and has resulted in an increased prevalence of antibiotic-resistant bacterial strains, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), which then causes a significant global health problem. According to data from the Centres for Disease Control and Prevention (CDC), resistance due to inappropriate antibiotic use causes 2.8 million more complex infections and in America, as many as 35.000 deaths are reported annually (Jain et al., 2022). *Staphylococcus aureus* resistance data in Indonesia increased by 77.20% in 2023 compared to previous years (Fitrandi et al., 2023).

Incidence of *Staphylococcus aureus* bacterial resistance is partly due to the extension of biofilm formation that determines its pathogenesis (Chen et al., 2022). Antibiofilm phytochemical compounds have been proven to affect the formation of bacterial biofilms, and it is believed that these compounds have a lower rate of resistance. Therefore, one of the strategies to reduce antibiotic resistance is to use herbal ingredients that have a variety of compounds and activities (Newman & Cragg, 2020). Earlier studies have shown that secondary metabolites, including flavonoids, tannins, steroids, triterpenoids, alkaloids, and phenols, possess significant antibacterial activity by inhibiting bacterial growth (Lobiuc et al., 2023).

Previous studies have reported that mango plants contain micronutrients, vitamins, and various phytochemicals that contribute to human health, with notable effects such as lowering uric acid in the blood, having anti-inflammatory properties, and as antibacterial activity. (Brajawikalpa et al., 2024; Ifmaily et al., 2023). Mango peel, especially gedong gincu mango peel, based on previous research, indicates that mango peel extract contains secondary metabolites with antibacterial, including against gram-positive (Adawiyah et al., 2025) and gram-negative bacteria (Siskanti et al., 2025) as well as being anti-fungal (Safitri et al., 2025).

Based on the previously known background regarding the potential of mango peel as an antibacterial. The novelty of this study evaluates the antibacterial activity of the fractions of Gedong Gincu mango peel (*Mangifera indica* L.) var. Gedong Gincu against the growth of *Staphylococcus aureus*. Previous studies have only examined ethanolic extracts of Gedong Gincu mango peel against *S. aureus* without fractionation, or assessed peel fractions of Gedong Gincu mango against other bacteria such as *Escherichia coli*. By separating the extract into several solvent fractions and comparing their inhibitory effects, this study identifies the most active fraction and links it to its phytochemical profile, thereby providing a stronger scientific basis for the development of Gedong Gincu mango peel as a candidate raw material for antibacterial agents.

## 2. Methods

### 2.1. Tools and Materials

Material used in the extraction process until fractionation is gedong gincu mango peel, which comes from Cirebon regency, West Java, Indonesia and has been identified as a taxon in the Plant Taxonomy Research laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Semarang State University, Indonesia. The test bacteria used were *Staphylococcus aureus* ATCC 25923 (bioMérieux), and Mueller-Hinton Agar (Himedia) media was used for antibacterial testing.

A rotary evaporator (DLAB RE100-Pro) is used to separate the ethanol solution and its

filtrate, then the filtrate is thickened using a water bath (Memmert). The fractionation process uses a separatory funnel (Pyrex). The incubator (ESCO Isotherm) is used for bacterial incubation, and the calliper (Tricle Brand) is used to measure the inhibition zone on the bacterial growth medium.

## 2.2. Types and Design of Research

A post-test only with control group design was applied in this experimental research, involving two control groups and twelve treatment groups. The treatments consisted of butanol, ethyl acetate, and n-hexane fractions at concentrations of 6.25%, 12.5%, 25%, and 50%, each replicated three times.

## 2.3. Mango Peel Extract Manufacturing

A total of 581 grams of mango peel simplicia was obtained from 26 kg of mango fruits. An extract was made using 70% ethanol solvent for a maceration process with a ratio of 1:5 for 3 days, and stirring for 10 minutes every day. After the maceration process is complete (3 days), the solution is then filtered using 40-gauge filter paper on a Buchner funnel. The resulting filtrate is placed in a container to remove the solvent using a rotary evaporator until it thickens at a temperature of 60°C, and then continued using a water bath at a temperature of 60°C (Handayani et al., 2023).

## 2.4. Making Mango Peel Fraction

Fractionation is carried out by the liquid-liquid extraction method using a separatory funnel. The thick extract of mango peel obtained was dissolved using aquades in a ratio of 1:10, then put into a separatory funnel and added n-hexane solvent in a ratio of 1:1 and homogenised. Wait until you can see the difference in colour layer between the n-hexane fraction and the water fraction, both fractions are expelled through the separatory funnel faucet. The n-hexane fraction is thickened using a water bath at a temperature of 40°C, then the water fraction is added with ethyl acetate and butanol solvents alternately. The fractionation process is carried out sequentially, starting from n-hexane, followed by ethyl acetate and finally butanol, with the same procedure for each fraction.

### 2.4.1. Phytochemical Screening Test

#### 2.4.1.1. Flavonoid

The n-hexane, ethyl acetate, and butanol fractions of Gedong Gincu mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 0.5 g and dissolved in 2 mL of methanol p.a. The mixtures were heated, allowed to cool, and then filtered. The resulting filtrates were added to the H<sub>2</sub>SO<sub>4</sub> reagent, vortexed, and placed in a test tube rack until the reaction was complete. The appearance of a red, yellow, or orange colour indicated the presence of flavonoid compounds (Khan et al., 2011).

#### 2.4.1.2. Alkaloid

The n-hexane, ethyl acetate, and butanol fractions of mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 0.5 g, then mixed with 1 mL of 2 N HCl and 9 mL of hot distilled water that had been allowed to cool. The mixture was then filtered, and the filtrate was divided equally into three test tubes (tubes 1, 2, and 3) (Khan et al., 2011).

- Tube 1: Two drops of Bouchardat reagent were added; the formation of a brown-black precipitate indicated a positive alkaloid reaction.
- Tube 2: Two drops of Dragendorff reagent were added; a brick-red precipitate signified a positive result for alkaloids.
- Tube 3: Two drops of Mayer reagent were added; the presence of a yellow or white precipitate confirmed a positive alkaloid reaction.

#### 2.4.1.3. *Tanin*

The n-hexane, ethyl acetate, and butanol fractions of Gedong Gincu mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 1 g, then 10 mL of hot water was added and the mixture was allowed to cool. The solution was filtered, and the filtrate was treated with two drops of 1% FeCl<sub>3</sub>. The presence of tannins was indicated by the formation of a dark blue or greenish-black colour (Khan et al., 2011).

#### 2.4.1.4. *Steroids/Triterpenoids*

The n-hexane, ethyl acetate, and butanol fractions of Gedong Gincu mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 0.5 g, then 2 mL of ethanol was added, followed by vortex mixing. Warm distilled water was added, the mixture was allowed to cool, and then filtered. The filtrate was evaporated to a thick residue, to which ether, 3 drops of acetic anhydride, and 1 drop of concentrated H<sub>2</sub>SO<sub>4</sub> were added. The appearance of a reddish colouration indicated a positive result for triterpenoids, while a green colouration indicated the presence of steroids (Khan et al., 2011).

#### 2.4.1.5. *Saponin*

The n-hexane, ethyl acetate, and butanol fractions of Gedong Gincu mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 0.5 g and mixed with 10 mL of hot water. The mixture was allowed to cool and shaken until foam formed. After standing for 2 minutes, 1 drop of 2 N HCl was added, and the solution was shaken again. The formation of stable foam that persisted for 10 minutes indicated a positive result for saponins (Khan et al., 2011).

#### 2.4.1.6. *Phenolic*

The n-hexane, ethyl acetate, and butanol fractions of Gedong Gincu mango peel (*Mangifera indica* L. var. Gedong Gincu) were each weighed at 0.5 g, then 1 mL of ethanol was added to homogenise the sample. Subsequently, 3 drops of FeCl<sub>3</sub> solution were added, the mixture was vortexed, and the tubes were placed on a rack until the reaction was complete. A positive result was indicated by a colour change to blackish-blue (Khan et al., 2011).

### 2.5. Creation of Fractional Concentration

Each fraction was taken as much as 1 gram, then dissolved in 10% DMSO solvent to make a stock solution (100%), and then diluted to obtain concentrations of 6.25%, 12.5%, 25%, and 50%. Dilution was carried out using the formula:

$$V1 \times M1 = V2 \times M2$$

### 2.6. Antibacterial Testing and Calculation of Inhibition Zone Diameter

The suspension of test bacteria as much as 50µL was inoculated on MHA medium. The antibacterial testing in this study used the well method, as much as 30µL each of the concentrations of mango peel fractions (n-hexane, ethyl acetate, and butanol) were put into a well and then incubated for 24 hours at a temperature of 37°C. The inhibition zone was measured and calculated based on the surface area of the clear zone formed on the growth medium (Lewis et al., 2024).

### 3. Results and Discussion

#### 3.1. Results

##### 3.1.1. Phytochemical Screening

Phytochemical screening is carried out to determine the content of secondary metabolite compounds contained in each mango peel fraction, which can be seen in Table 1 below.

Table 1 Result of Screening Phytochemicals in Mango Peel Fraction (*Mangifera indica* L.) var. Gedong Gincu

Secondary Metabolite	Reagents	Fraction			Analysis Techniques
		n-Hexane	Ethyl Acetate	Butanol	
Flavonoids	HCL Concentrate + Mg	-	+	+	Colour Visualization
Alkaloids	Dragendrouf, Mayer, Wagner, Bhaucardat	+	+	+	
Tannins	FeCl3	-	+	+	
Steroids	Ether + As. Anhydrous	+	-	-	
Triterpenoids	acetate + H2SO4	-	+	+	
Saponins	HCL2N	+	-	-	
Phenolic	FeCl3	-	+	+	

Qualitative phytochemical screening of the n-hexane, ethyl acetate, and butanol fractions of mango peel (*Mangifera indica* L.) var. Gedong Gincu is summarised in Table 1 above. Secondary metabolites contained in the n-hexane fraction include alkaloids, steroids, and saponins. The ethyl acetate and butanol fractions contain the same secondary metabolite compounds, namely flavonoids, alkaloids, tannins, triterpenoids and phenolics.

##### 3.1.2 Effectiveness of Gedong Gincu Mango Peel Fractions (Butanol, Ethyl Acetate, and n-Hexane) in Inhibiting Growth of *Staphylococcus aureus* ATCC 25923

Measuring the inhibition zone is carried out after 24 hours of the incubation period. The resistance area is calculated by subtracting the diameter of the resistance zone from the diameter of the wellbore. The results of each quadrant are averaged so that the diameter of the inhibition zone at each concentration in each fraction is obtained.

Table 2 Average Inhibition Zone of Each Fraction

Group/Concentration of Fraction	Average Inhibitor Zone (mm)				P Value
	6,25%	12,5%	25%	50%	
Control (-)	0	0	0	0	0.000
Control (+)	39,38	39,38	39,38	39,38	
n-Heksane	3,53	5,30	6,51	8,05	
Ethyl acetate	8,13	9,93	13,21	15,41	
Butanol	5,88	9,00	10,63	12,16	

Table 2 shows the antibacterial activity of the three fractions of Gedong Gincu mango peel as demonstrated by inhibition zones against *Staphylococcus aureus* ATCC 25923. The inhibition zone of the n-hexane fraction is in the weak to moderate category. Meanwhile, the ethyl acetate and butanol fractions of mango peel have inhibition zones in the moderate to strong category.

3.1.3. Assessment of the Inhibitory Potential of Gedong Gincu mango peel fractions (Butanol, Ethyl Acetate, and n-Hexane) on the growth of *Staphylococcus aureus* ATCC 25923.

All fractions tested have the potential to inhibit *Staphylococcus aureus* ATCC 25923 bacteria. The average of the largest inhibition zones was obtained from each fraction at a concentration of 50%. The diameter of the resulting inhibition zone serves as a measure of the effectiveness of the inhibition mechanism. The following is a table of analysis of the differences of each treatment group based on the inhibition zones obtained:

Table 3 Average Difference in Inhibition Zones between Three Fraction Groups

Group	Treatment	Mean diff.	p
n-Hexane concentration 50%	Ethyl Acetate concentration 50%	-7.36667*	0.000*
	Butanol concentration 50%	-4.11667*	
	Control +	-31.33333*	
	Control -	8.05000*	
Ethyl Acetate concentration 50%	Butanol concentration 50%	3.25000*	
	Control +	-23.96667*	
	Control -	15.41667*	
Butanol concentration 50%	Control +	-27.21667*	
	Control -	12.16667*	
Positive Control	Control -	39.38333*	

Based on the LSD test (Table 3) shown in the table above, there was a significant difference in the average inhibition zone in each fraction with a P value <0.05, and it was seen that the concentration of the 50% ethyl acetate fraction had a larger inhibition zone when compared to the other fractions based on the mean difference value.

### 3.2. Discussion

In this study, anti-bacterial activity is seen based on the inhibition zone generated from each fraction. Fractionation is aimed at classifying secondary metabolite compounds contained in gedong gincu mango peels based on their polarity properties in inhibiting *Staphylococcus aureus* ATCC 25293. The n-hexane fraction of *Mangifera indica* L. var. Gedong Gincu peel is characterised predominantly by alkaloids, steroids, and saponins, indicating the presence of relatively non-polar secondary metabolites. In contrast, the ethyl acetate and butanol fractions share a similar phytochemical profile, containing flavonoids, alkaloids, tannins, triterpenoids, and phenolic compounds, which reflects a higher enrichment of semi-polar to polar constituents that are often associated with notable biological activities.

The results of the research showed that the ethyl acetate fraction showed a larger inhibition zone compared to the other two fractions. This stronger antibacterial activity is due to the difference in solvent polarity, which allows for efficient extraction of semi-polar secondary metabolites such as flavonoids, phenolic acids, tannins, and certain alkaloids (Singapurwa et al., 2024). These compounds have several mechanisms of action, such as inhibiting nucleic acid synthesis, inhibiting cell membrane permeability (Octaviani et al., 2022), inhibiting the formation of peptidoglycan (Ligina & Sudarmin, 2022), inhibiting DNA synthesis, inhibiting iron uptake from the bacterial environment (Farha et al., 2020), and affecting gene expression and degrading bacterial biofilms (Cosmo Andrade et al., 2019). In addition, the semi-polar nature of these metabolites facilitates better diffusion in agar media and increases their ability to penetrate bacterial cell walls, unlike the highly nonpolar constituents extracted with n-hexane or the overly polar compounds obtained from the butanol fraction, which often show limited antimicrobial effects (Hadji et al., 2025).

Based on the literature, it was found that all mango peel extracts from the gedong gincu contain a variety of flavonoid compounds. Previous research flavonoids can damage the cell membranes of *Staphylococcus aureus* by impairing essential physiological processes, including osmoregulation, respiration, and the transport mechanisms required for peptidoglycan biosynthesis, through both direct and indirect pathways. In addition, flavonoids are able to inhibit the synthesis of intracellular and extracellular enzymes, which prevent the formation of lipid layers in bacteria (Górniak et al., 2019). Inhibition of the enzyme DNA Gyrase and the formation of biofilms by means of cell aggregation leading to decreased absorption of active nutrients are other roles of flavonoids (Fang et al., 2016), as well as flavonoids can interfere with the interaction between acyl-homoserine lactones (AHLs) and their receptors (Roy et al., 2018). This explanation is in accordance with research conducted by (Thebti et al., 2023), with the results that flavonoids have antimicrobial activity on gram-positive, especially *Staphylococcus aureus*, which is better than as an antifungal. Antibacterial activity derived from flavonoid derivatives has a different mechanism compared to conventional treatment.

Triterpenoids were identified as the predominant secondary metabolites in the Gedong Gincu mango peel ethyl acetate fraction. This compound has activity in inhibiting the growth of bacteria and fungi (Mahizan et al., 2019). Previous research has shown that the pentacyclic triterpenoids tested can influence gene expression, peptidoglycan structure, and biofilm formation in bacteria. Furthermore, this study also demonstrated that triterpenoids are more potent in inhibiting the growth of gram-positive bacteria due to differences in cell wall structure compared to other bacteria, which have a hydrophilic layer that acts as a permeability barrier to compounds entering the cell (Briers & Lavigne, 2015). Another mechanism of action of triterpenoids is to react with a transmembrane protein called porin, located in the outer membrane of bacterial cell walls. This mechanism causes the polymer bonds and disrupts membrane formation, which then affects the structure of bacterial peptidoglycan, thereby reducing the permeability of the bacterial cell membrane (Rini et al., 2017). Triterpenoids combined with flavonoids can increase antimicrobial potential (Wrońska et al., 2022).

Alkaloids were identified as secondary metabolites in the form of gedong gincu mango peel ethyl acetate fraction, which likely contribute significantly to its antibacterial activity. Recent studies have shown that alkaloids such as isoquinolines, indoles, pyridines, and steroids exhibit potent antibacterial effects through mechanisms that include disruption of bacterial cell membranes, inhibition of DNA or protein synthesis, and alteration of cell wall integrity (Thawabteh et al., 2024). Inhibition of synthesis and permeability of cell membranes caused by the release of large amounts of molecules that increase the permeability of the bacterial cell wall, so that the cell wall defence is lost and there is an intracellular electrolyte leak in the form of Alkaline Phosphatase (AKP), which causes cell lysis (Yan et al., 2021). Alkaloids also have a role in inhibiting the synthesis of nucleic acids and proteins by damaging bacterial DNA and RNA molecules or by inhibiting the DNA replication process, thereby preventing the process of virulence gene expression, which will affect the nature and growth of microorganisms (Othman et al., 2019).

Tannin compounds are found in the gedong gincu mango peel ethyl acetate fraction and have an antibacterial effect. Tannins are simple polyphenol polymerisations that contain phenolic hydroxy groups, so that tannins are polar and can also be extracted by semi-polar ethyl acetate fractions. Recent studies explain that tannins can inhibit bacteria effectively with the iron chelation mechanism of their environment, so that iron availability decreases significantly in bacteria, which causes bacteria to be unable to grow (Chan et al., 2018). Another mechanism of tannin as an antibacterial is that it can activate enzymes involved in the process of forming bacterial cell wall synthesis, and tannic acid compounds, which are the basis of tannins, can strongly bind to the membrane layer that forms peptidoglycan and have an effect on cell wall permeability (Dong et al., 2018). In general, tannins can cause bacteria to be more susceptible

and become lysed (Farha et al., 2020).

The phenolic compounds contained in the gedong gincu mango peel ethyl acetate fraction are known to have the most sensitive role in inhibiting gram-positive bacteria. This mechanism is due to the presence of phenolic compounds such as quercetin, diosmin, rutin, genistein, and hesperetin, which can interact with many other compounds and work synergistically to inhibit bacterial metabolic processes. (Wishart et al., 2018). Inhibition of bacteria by phenolic compounds can be done by inhibiting cell wall synthesis and DNA replication (Lobiuc et al., 2023). In addition, other research explained that phenolic compounds can bind to the bacterial DNA genome so that they can damage the secondary structure of DNA and change its morphology (Guo et al., 2018).

Phenolic acids have a hydroxyl group structure and aromatic rings that allow it to interact with amino groups or carboxylates of proteins. The activity of this compound occurs in the membrane and cytoplasm of bacteria, causing the loss of cell components such as nucleic acids, proteins, and inorganic ions, for example, potassium and phosphate (Sun & Shahrajabian, 2023).

#### 4. Conclusion

Gedong Gincu Mango Peel (*Mangifera indica* L.) contains secondary metabolites with antibacterial properties. The antibacterial activity of each fraction varies according to its polarity, and the ethyl acetate fraction at a concentration of 50% is the most effective in suppressing the growth of *Staphylococcus aureus* ATCC 25923.

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