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Effectivity Test of n-Hexane, Ethyl Acetate, and Butanol Fractions of Mango Peel (*Mangifera indica* L.) Gedong Gincu Variety on the Growth of *Escherichia coli*

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ABSTRACT

Background: *Escherichia coli* was the most common cause of diarrhea. Diarrhea reached a high prevalent in West Java. The cause of antibiotic resistance to *Escherichia coli* was noncompliance medication. Mango peel (*Mangifera indica* L.) of the Gedong Gincu variety had secondary metabolites compound which act as antibacterial that can be used as alternative medication.

Aims: To analyze phytochemicals and determine effectiveness of n-hexane, ethyl acetate, and butanol fractions of mango peel Gedong Gincu variety in the growth of *Escherichia coli.*

Methods: This study used laboratory true experimental research with post-test only control group design. This research consisted 14 groups, divided into 2 control groups, namely control (+) given ciprofloxacin and control (-) given 10% DMSO, and 12 treatment groups given n-hexane, ethyl acetate, and butanol fractions concentration of Gedong Gincu 6.25%v/v, 12.5% v/v, 25% v/v, and 50% v/v. The test used well diffusion method on *Mueller-Hinton Agar* media.

Results: The secondary metabolites in the n-hexane fraction included alkaloids, steroids, and saponins, while ethyl acetate and butanol fractions contained flavonoids, phenols, alkaloids, triterpenoids, and tannins. The results of the *oneway ANOVA* test obtained $p<0.001$ which indicated n-hexane, ethyl acetate, and butanol fractions were significantly inhibit the growth of *Escherichia coli* with the greatest mean inhibitory zones of 6.86 mm, 12.08 mm, and 9.35 mm, respectively.

Conclusion: The most effective fraction of mango peel (*Mangifera indica* L.) Gedong Gincu variety in inhibit the growth of *Escherichia coli* was ethyl acetate fraction at a 50% concentration (12.08 mm).

Keywords: *Escherichia coli; Fractions; Mango peel gedong gincu.*
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1. Introduction

Based on The Ministry Health of Indonesia, diarrhea is defined by more frequent and watery stools that occur more than three times in a day. World Health Organization (WHO) was estimated that 1.7 million cases worldwide affected from diarrhea every year. In West Java, diarrhea is an endemic disease that impacts 270 individuals among a population of 1000 according to the report from Riset Kesehatan Dasar 2018. (Ministry Health, 2018) The causes of diarrhea are the involve of bacteria, contaminated food and drink, allergies, and inadequate nutrition (Hutasoit, 2020). From 50 samples of people with acute diarrhea, significant correlations have been identified between diarrhea and Escherichia coli infection with 25 samples (or simply 50%) showed Escherichia coli infection, 6 samples showed Gram-negative coccus, and 6 samples showed Klebsiella sp. Infection (Halim et al, 2017).

The growth of *Escherichia coli* can be prevented through the use of antibiotics. The use of antibiotics to *Escherichia coli* leads to resistance that caused by noncompliance medication. *Escherichia coli* has been reported resistant to ß-lactams, fosfomycin, phenicol, and quinolones (Handayani et al, 2017). According to a research by Antimicrobial Resistance in Indonesia (AMRIN), *Escherichia coli* were resistant to various antibiotics, such as chloramphenicol (25%), cotrimoxazole (29%), and ampicillin (34%) (Maulawan, 2022). To reduce this problem, it necessitating alternative medication specifically mango peel (*Mangifera indica* L.) of gedong gincu variety.

According to the research from Ginting (2022), extract of mango peel Harum Manis variety showed high antibacterial activity toward *Salmonella typhi* and *Escherichia coli*. Mango peel of honey variety contains flavonoids that can inhibit the growth of *Escherichia coli* (Jais, 2019). Morelloflavon compounds, a family of flavonoids, found in the ethyl acetate fraction of Mango peel (*Mangifera indica* L.) that analyzed by phytochemical screening (Manullang, 2019). Flavonoids are secondary metabolite compounds that typically found in plants and have been identified as microbial or antibacterial agents, therefore could be helpful to reduce diarrheal symptoms (Amyati, 2019).

The secondary metabolites compound in mango peel have potential as antibacterial agents and a shortage of reported health advantages found in mango peel of gedong gincu variety formed the background of this study. Therefore, this study analyze phytochemicals and determine effectiveness of n-hexane, ethyl acetate, and butanol fractions of mango peel (*Mangifera indica* L.) gedong gincu variety in the growth of *Escherichia coli.*

2. Methods

Study design

This research was conducted laboratory true experimental research with a post-test only control group design. This study used 2 control groups consisting of positive control (Ciprofloxacin 500 mg) and negative control (10% DMSO). There were 12 treatment groups consisting of n-hexane, ethyl acetate, and butanol fractions with each fraction having 4 concentrations (6.25%, 12,5%, 25%, and 50%) tested. This study has been ethically approved by the Ethic Committee of Faculty of Medicine, University of Swadaya Gunung Jati with number No.12/EC/FKUGJ/IV/2024 on 28th April 2024.

Tools and Materials

This research used the instrument are knife, blender from National (National Super Company, Indonesia), beaker glass, measuring cup from Pyrex (Iwaki Glass Company, Indonesia), spatula, separatory funnel, maceration container, vacuum rotary evaporator, oven, analytical balance, dropper pipette, aluminum foil, Erlenmeyer from Pyrex (Iwaki Glass Company, Indonesia), waterbath, sieve, micro pipette, tube, filter paper, vortex, sterile petri dish, Bunsen burner, autoclave, incubator, HVS paper, hot plate stirrer, digital scale, ose needle, object glass, deck glass, label, microscope from Olympus (Olympus Medical Company, Indonesia), and caliper.

The materials used include mango peel gedong gincu variety, 70% ethanol, distilled water, n-Hexane solvent, ethyl acetate solvent, butanol solvent, Bauchardat reagent, Dragendorff reagent, Hager reagent, Meyer reagent, 2 N HCl, technical methanol, 10% NaOH, concentrated H_2SO4, Mg, Zn, petroleum benzene, 1% $FeCl_3$, suspension of *Escherichia coli* ATCC 25922 was taken from the Faculty of Pharmacy, STIKES Muhammadiyah Cirebon, *Mueller-Hinton Agar* (MHA), cotton, gauze, 10% Dimethyl Sulfoxide (DMSO), Ciprofloxacin, and 0.5 *McFarland* standard (1.5 x 10^8 CFU/mL).

Research Procedure

1. Plant Determination
   Determination of mango plants was sent to the Laboratory of Plant Taxonomy Research, Department of Biology - FMIPA, State University of Semarang. Pants are determined morphological characteristics according to plant taxonomy.
2. Preparation of Mango Peel Extract
   Mango (*Mangifera indica* L.) gedong gincu variety was peeled, separated from the fruit, and dried for 72 hours in the sun. After that, the mango peel was blended and sieved until simplisia was obtained. The fine mango peel was macerated using ethanol 70% for 72 hours while being periodically stirred in a flat vessel. After 72 hours, the mixture was filtered and evaporated at a temperature of 40°C with vacuum rotary evaporator, then kept on waterbath at 60°C until a thick extract was obtained.
3. Preparation of Mango Peel Fraction
   This study using fractions method that carried out liquid - liquid fractionation using a separatory funnel tool. Mango peel extract weighed 115 grams in total was dissolved with distilled water, put into a separatory funnel and fractionated in a 1:1 ratio used n-hexane solvent, shaken until homogeneous. After the appearance of a distinct layer of solution, the n-hexane fraction was separated from the water fraction and gathered in an Erlenmeyer with opening the separating funnel tap. The n-hexane fraction collected in rotary evaporator at a temperature of 60°C and with a waterbath at 60°C until a thick fraction was formed. The process of fractionation ethyl acetate solvent and butanol solvent used the same method.
4. Phytochemical Screening Test
   a. Flavonoid Screening
      The 0.5 grams of each fractions of mango peel (*Mangifera indica* L.) gedong gincu variety were dissolved in technical methanol 10 mL, brought to a boil, and filtered hot into a separating funnel. The solution was added to Mg powder and concentrated HCl (the reaction that occurred there were bubbles) then vortexed and placed in a tube rag until the reaction is complete. The formation of a yellow or orange red color indicates of flavonoid compounds.
   b. Alkaloid Screening
      The n-hexane, ethyl acetate, and butanol fractions of mango peel gedong gincu variety in 0.5 grams were added to 2 N HCl in 1 mL and 9 mL of boiled distilled water. Then, the filtrate divided into 4 tubes A, B, C, and D equally.
      Tube A is added Bauchardat reagent 2 drops, positive results of alkaloids will form a brown - black precipitate.
      Tube B is added Dragendrauf reagent 2 drops, positive results of alkaloids will form a brick red precipitate.
      Tube C is added 2 drops of Mayer reagent, positive results of alkaloids will form a yellow or white precipitate.
      Tube D is added Hager reagent + methanol + Bauchardat reagent, positive results of alkaloids formed a brown - black precipitate.
   c. Tannin Screening
      A total of 1 gram from each fractions of mango peel gedong gincu variety were added to 10 mL of boiled water and cooled. Filter the filtrate, $FeCl_3$ 1% as much as 2 drops. Positive tannin results if a dark blue or greenish-black color is formed.
   d. Steroid/Triterpenoid Screening
      The 0.5 grams of mango peel gedong gincu variety fractions added 2 mL of ethanol and then vortexed and soaked in warmed distilled water then cooled and filtered. The filtrate was evaporated until thick and added eter, 3 drops of anhydrous acid, and 1 drop of concentrated H_2SO_4, observe until there is a red-purple color tinge that indicates positive triterpenoid. Meanwhile, the positive results of steroids formed a green-blue color.
   e. Saponin Screening
      Mango peel gedong gincu variety in 0.5 grams of n-hexane, ethyl acetate, and butanol fractions were added to 10 mL of hot water, cooled and homogenized until foam appeared. After 2 minutes then, add 1 drop of HCl 2 N, homogenize again until foam stays for 10 minutes showing positive results for saponins.
   f. Phenol Screening
      The butanol, ethyl acetate, and n-hexane fractions of mango peel gedong gincu variety as much as 0.5 grams were added to 1 mL of methanol and homogenized, then added 3 drops of $FeCl_3$. After that, vortexed and placed in a test tube rack. Positive results will form a blue-black color.

5. Antibacterial Test

   a. Tools sterilization
   Autoclaves loaded with distilled water up to the nesting limit were used to carry out the physical tool sterilization procedure and set at high temperatures of 160°C for 120 minutes. The instruments were sealed in HVS paper before sterilization. Over a bunsen flame, the tweezers and loop needle were sterilised.

   b. Preparation of media
   Mueller-Hinton Agar (MHA) in 38 grams was dissolved in a glass beaker containing 1000 mL of distilled water and stir until evenly. The media heated carefully on a hot plate stireer until mixed homogeneously. The media was autoclaved for 15 minutes at 121°C to sterilize it and chilled at between 45°-50°C. Then, the media was divided by 20 mL in one petri dish and allowed to solidify.

   c. Cultivation of *Escherichia coli*
   All control and treatment groups used *Escherichia coli* ATCC 25922 colonies on *Mueller-Hinton* Agar media taken from pour plate method.

   d. Suspension of *Escherichia coli*
   After the bacteria were cultured on agar plates, one ose of the *Escherichia coli* ATCC 25922 isolates was put into a tube containing 10 mL of sterile physiological NaCl solution (0.9% sterility). Then, shaken until homogeneous and equalized turbidity with 0,5 *Mc Farland* standard solution (1.5 x10^8 CFU/mL).

   e. Preparation of Positive and Negative Controls
   The positive control used Ciprofloxacin 500 mg tablets which were crushed and then weighed. Ciprofloxacin powder was dissolved with 10% Dimethyl Sulfoxide (DMSO) and homogenized, so that a concentration of 100% was obtained. Therefore, the negative control used in this study was 10% DMSO.

   f. Preparation of Mango peel Fraction Concentration
   The 100% concentration (stock) mango peel (*Mangifera indica* L.) gedong gincu variety fraction will be diluted with 10% Dimethyl Sulfoxide (DMSO) to create concentrations of 6.25%, 12.5%, 25%, and 50%.

Table 1. Preparation of Mango peel Fraction Concentration

Concentration	Fraction	DMSO
6.25%	62.5 µL	937.5 µL
12.5%	125 µL	875 µL
25%	250 µL	750 µL
50%	500 µL	500 µL

   Following the addition of 6.25 µL of the 6.25% concentration fractions, 937.5 µL of 10% DMSO is used to dissolve them. To a fraction with a concentration of 12.5%, 125 µL of extract are added with 875 µL of 10% DMSO. The 25% concentration fraction requires the addition of 250 µL of the fraction, which is subsequently dissolved in 750 µL of 10% DMSO. The 50% concentration fraction are made with 500 µL and 500 µL of 10% DMSO.

   g. Determination of Inhibition
   Bacterial suspensions were inoculated on *Mueller-Hinton Agar* (MHA) media, then flattened with a hockey stick and allowed to dry. The wells were made using a cork borer. The fraction of mango peel (*Mangifera indica* L.) gedong gincu variety was inserted into the wells in 20 µL and incubated at 37°C for 24 hours and observed the inbitory zone, which known as the clear zone that formed around the wells, using caliper.

Statistical techniques

The *Shapiro-Wilk* test was used to statistically assess the inhibitory zone of the n-hexane, ethyl acetate, and butanol fractions data. The parametric *One Way ANOVA* test was applied in the subsequent study to determine significant differences. Subsequently, additional assessments were conducted using the *Post Hoc Least Significant Difference* (LSD) test to determine the differences of every concentration in the treatment group.

3. Results

Fractionation

The mango peel (*Mangifera indica* L.) gedong gincu variety used was 26 kilograms and produce 115 grams of extract. The extract was fractioned by liquid-liquid technique using n-hexane, ethyl acetate, and butanol solvents.

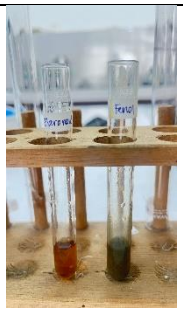
Table 2. Fraction Yield of Mango Peel (*Mangifera indica* L.) Gedong Gincu Variety

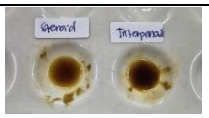
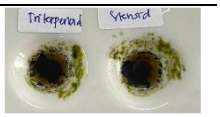
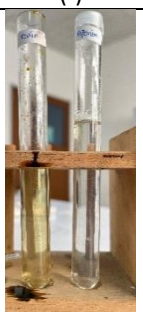
Description	Color	Weight (grams)
n-Hexane fraction	Greenish brown	1.50
Ethyl acetate fraction	Reddish brown	14.80
Butanol fractions	Yellowish brown	2.3

Phytochemical Screening

The qualitative results of phytochemical screening tests are determined by colour changes or the presence of chemicals that precipitate from secondary metabolites.The secondary metabolites tested in this phytochemical screening include flavonoids, phenols, alkaloids, steroids, triterpenoids, tannins, and saponins.

Table 3. Phytochemical Screening Results of Mango Peel Gedong Gincu variety Fraction

Metabolite Compounds		Visualization Reaction	n-Hexane Fraction	Ethyl Acetate Fraction	Butanol Fraction
					
Flavonoid		Orange red colored solution	(-)	(+)	(+)
Phenol		Blue-black colored solution	(-)	(+)	(+)
					
Alkaloid	Bauchardat	Brown-black precipitate	(+)	(+)	(+)
	Dragendorf	Orange to red brown precipitate	(+)	(+)	(+)
	Hager	Brown-black precipitate	(+)	(+)	(+)
	Mayer	Yellowish white precipitate	(+)	(+)	(+)

Metabolite Compounds	Visualization Reaction	n-Hexane Fraction	Ethyl Acetate Fraction	Butanol Fraction
				
Steroid	Green colored solution	(+)	(-)	(-)
Triterpenoid	Red colored solution	(-)	(+)	(+)
				
Tannin	Brown-black solution	(-)	(+)	(+)
Saponin	Foam remains not less than 10 minutes	(+)	(-)	(-)

The secondary metabolites in the n-hexane mango peel (*Mangifera indica* L.) gedong gincu variety fraction included alkaloids, steroids, and saponins, while the ethyl acetate and butanol fractions contained flavonoids, phenols, alkaloids, triterpenoids, and tannins.

Antibacterial Test Results

The inhibition strength is classified as follows according to the inhibitory zone's diameter.

Table 4. Inhibitory Zone Categories

Diameter of Inhibitory Zone	Inhibition Strength
≤5 mm	Weak
6 – 10 mm	Medium
11 – 20 mm	Strong
≥21 mm	Very Strong

Table 5. Antibacterial activity of n-hexane, ethyl acetate, and butanol fractions mango peel of Gedong Gincu variety

Treatment	Inhibitory zone (mm) repetion to-			Mean (mm)	Inhibition Strength
	I	II	II		
Control (-)	0.00	0.00	0.00	0.00	None
Control (+)	28.50	28.60	29.30	28.80	Very strong
n-Hexane 6.25%	3.60	3.20	3.30	3.36	Weak
n-Hexane 12.5%	4.35	4.10	4.50	4.31	Weak
n-Hexane 25%	5.60	5.30	5.45	5.45	Weak
n-Hexane 50%	6.75	7.05	6.80	6.86	Medium
Ethyl acetate 6.25%	8.60	8.80	8.50	8.63	Medium
Ethyl acetate 12.5%	9.65	10.20	9.80	9.80	Medium
Ethyl acetate 25%	10.55	10.70	10.25	10.50	Medium
Ethyl acetate 50%	11.85	12.15	12.25	12.08	Strong
Butanol 6.25%	5.40	5.75	5.80	5.65	Weak
Butanol 12.5%	6.25	6.80	6.40	6.48	Medium
Butanol 25%	8.20	8.50	8.35	8.35	Medium
Butanol 50%	9.30	9.50	9.25	9.35	Medium

The largest mean inhibitory zone of the n-hexane, ethyl acetate, and butanol fractions mango peel (*Mangifera indica* L.) of the gedong gincu variety in inhibit the growth of *Escherichia coli* was found at 50% concentration with a mean inhibitory zone of 6.86 mm, 12.08 mm, and 9.35 mm, respectively. The positive control group showed a very strong inhibitory zone of 28.80 mm and there was no inhibitory zone in the negative control group.

Multivariate analysis

The diameter of inhibition showed that the inhibition increased with the concentration of fractions. Figure 1 shows that each fraction with a concentration of 50% has the highest inhibition compared to other fraction concentrations that have been tested.

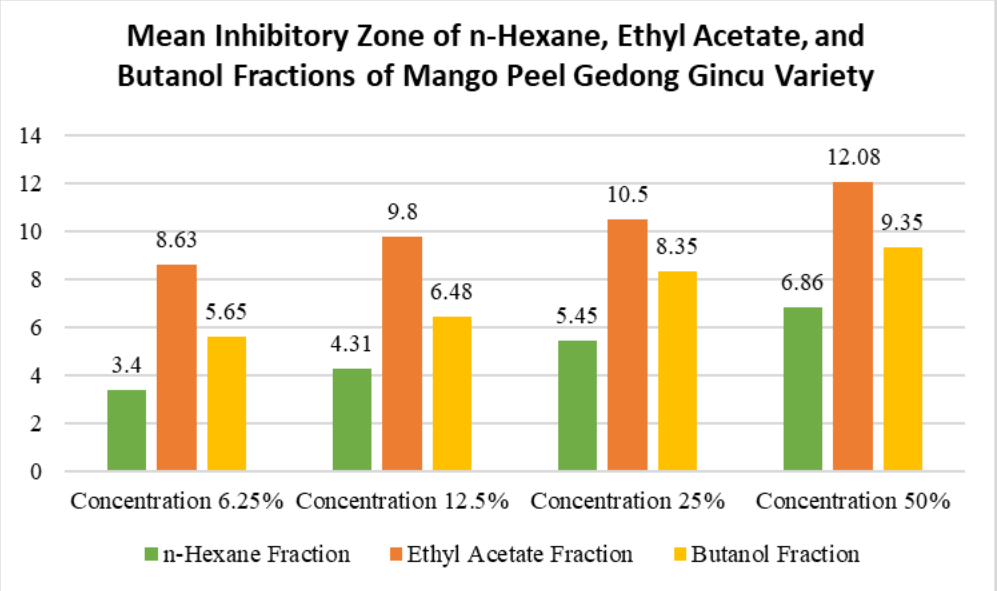


Figure 1. Inhibitory Zone of Fractions of Mango Peel Gedong Gincu Variety

The results of *one way ANOVA* analysis obtained significant results in difference with *p-value* <0.05. Furthermore, the data analyzed with *Post Hoc Least Significant Difference* (LSD) test with the aim determine differences between variables based on their significance and the most effective concentration in inhibiting the growth of *Escherichia coli* with *p-value* <0.001.

Table 6. Result of *Post Hoc Least Significant Difference* Test

Concentration of Fraction (I)	**Concentration of Fraction (J)**	***Mean Difference* (I-J)**	***P Value***
n-Hexane fraction at 50%	Ethyl acetate fraction at 50%	-5.21667*	*p*<0.001
	Butanol fraction at 50%	-2.48333*	
	Control (+)	-21.93333*	
	Control (-)	6.86667*	
Ethyl acetate fraction at 50%	Butanol fraction at 50%	2.73333*	
	Control (+)	-16.71667*	
	Control (-)	12.08333*	
Butanol fraction at 50%	Control (+)	-19.45000*	
	Control (-)	9.35000*	
Control (+)	Control (-)	28.80000*	

Based on the mean difference of *Post Hoc Least Significant Difference*, the 50% concentration of ethyl acetate fraction is the most effective fraction of mango peel (*Mangifera indica* L.) to inhibit the growth of *Escherichia coli*.

4. Discussion

The mango peel (*Mangifera indica* L.) gedong gincu variety n-hexane fractions showed weak to moderate of the strength in the antibacterial test considering that this solvent only attracts nonpolar compounds, which are generally less abundant than polar metabolite compounds (Fransiska et al, 2021). The fraction of ethyl acetate showed the highest level of inhibition; it contained 50% concentrated at 12.08 mm and classified to the strong category of inhibitory strength. The ethyl acetate solvent has a higher polarity index than the n-hexane and butanol solvents, and it attracts in an extensive variety of secondary metabolites. The solvent of ethyl acetate dissolves more secondary metabolite compounds—polar or non-polar—than evolves the n-hexane component of the molecule because it is semipolar (Puspa Yani, Nastiti, & Noval, 2023). Ethyl acetate solvent has a high polarity index (4,4), therefore in this study ethyl acetate fractions attracts lots of secondary metabolite compounds in mango peel (Pertala, Tutik, & Nofita, 2022). Furthermore, at concentrations of 6.25% (5.65 mm) and 50% (9.35 mm), the butanol fraction strongly inhibit the growth of *Escherichia coli*. This study show that despite butanol is a polar solvent, its mean inhibitory strength is lower than ethyl acetate fractions because during the fractination process, the polar metabolite molecules were attracted to the ethyl acetate solvent first (Awirana & Sutriningsih, 2018). DMSO should present no significant effect on the growth of *Escherichia coli* bacteria to clarify the function of the mango peel gedong gincu variety fraction. The use of Ciprofloxacin is based on its sensitivity in controlling the growth of *Escherichia coli*.

The phytochemical screening tests carried out for n-hexane fractions mango peel (*Mangifera indica* L.) of gedong gincu variety indicated an existence of secondary metabolite compounds, include alkaloids, steroids, and saponins; while the ethyl acetate and butanol include flavonoids phenols, alkaloids, triterpenoids, and tannins. The result of research by Anggraeni has shown that the arumanis mango peels contain chemicals known to contain secondary metabolites, which consist of flavonoids, phenols, steroids, triterpenoids, alkaloids, and tannins (Anggraeni, 2020).

Flavonoid compounds will be more easily dissolved in polar solvents because of their abundance of groups of hydrogen ions and a significant electronegativity difference, determined by the positive findings of flavonoid in the butanol fraction (polar) and ethyl acetate fraction (semipolar) (Maulana & Nugraha, 2020). Flavonoid molecules have the ability to inhibit the growth of *Escherichia coli* ATCC 11229 through damaging their cell wall, disclosing their essential components, restricting the bacterial production of new cell proteins, and subsequently causing the bacteria to die (Purmaningsih, Kalor, & Atun, 2018).

According to the findings of research by Harahap (2021), phenol compounds have been identified in the semipolar ethyl acetate fraction of mango skin (*Mangifera indica* L.) gedong gincu variety and the polar butanol fraction as they have their ability to bind to hydrogen ions groups in polar or semipolar solvents. Phenol acts as an antibacterial causing the cytoplasmic membrane to become hyperpolarized, decreasing the integrity of membranes, and increasing membrane instability through interactions with lipid and protein membranes that result in membrane malfunction and bacterial cell destruction (Sulaiha et al, 2022). In addition, phenol can activate topoisomerase IV enzymes, inhibit bacterial growth, bound ATP binding sites that reduces ATP generation, and bind bacterial DNA, which prevents bacteria from executing their metabolic processes (Oulahal & Degraeve, 2022).

The research by Aksara (2018) showed that alkaloid compounds contain an N-H functional group where nitrogen atoms with free electron pairs can combine with metal ions to create coordination covalent bonds. Alkaloids adhere with acidic substances in DNA of bacterial cell to modify the process of protein synthesis and nucleic acid synthesis in cells, which inhibits bacterial growth or induces apoptosis (Wijaya, 2020).

The n-hexane fractions of mango peel (*Mangifera indica* L.) gedong gincu variety shows blue-green discoloration in the steroid evaluation because the fractionation process implies the principle of like dissolve like, which suggests that non-polar compounds tend to dissolve in non-polar solvents (Fransiska, 2021) Steroids may additionally possess antibacterial effect because they produce liposome leakage on lipid membranes (Rizki et al, 2021). Steroids can interact with phospholipid membranes due to their lipophilic compound permeability, which causes bacterial cells to become lysed and rigid, which results in diminished membrane integrity and disrupted the cell membrane (Sudarmi, Darmayasa, & Muksin, 2017).

Triterpenoid chemicals are found in the ethyl acetate and butanol fractions mango peel (*Mangifera indica* L.) of the gedong gincu variety. Non-polar triterpenoids can dissolve in polar butanol fractions and semipolar ethyl acetate fractions caused by intermolecular forces. There will be an electrostatic force between the two, known as the "induced dipole-dipole force," as polar molecules with permanent dipoles would induce non-polar molecules without dipoles due to intermolecular forces (Balafif, Andayani, & Gunawan, 2019). Triterpenoids react the strong polymer bond with porins on the outer membrane cell's bacteria that cause the entry of compounds which can reduce the permeability of membrane. Bacterial cells will lack nutrients and experience death (Bazaka et al, 2015).

The ethyl acetate and butanol fractions of mango peel (*Mangifera indica* L.) gedong gincu variety contain tannins because semipolar and polar solvents tend to contain more hydroxyl groups (Farha et al, 2020). Tannins target the lipopolysaccharide membrane-damaging effects of the outer membrane of Gram-negative, which ultimately results in bacterial cell death (Trentin et al, 2013). This study found that the n-hexane fraction of non-polar mango peel (*Mangifera indica* L.) gedong gincu variety contains saponins which polar compounds. Research has also shown that the explanation for saponins dissolve in non-polar solvents is because of electrostatic forces imposed on by the displacement of dipole moments (Kiswandono, 2017). The mechanism of saponins is that they use hydrogen bonds to build complex compounds on cell membranes, which breaks down the permeability of the cell wall, causing the contents to leak out and ultimately leading to the disintegration of cells (Ocholwik-Grabarek et al, 2020). In this study, there was not measured in quantitative phytochemical screening, thus it is not known which secondary metabolite compounds in mango peel (*Mangifera indica* L.) gedong gincu variety are dominant to inhibiting the growth of *Escherichia coli*.

The diameter of the inhibition zone is not a reliable indicator of antibacterial efficacy test because determined by various factors, which includes the ability of fractions and capacity for diffusion in the media, interactions between media components, and in vitro environmental conditions, which means that it is. The minimum inhibitory concentration (MIC) value is more relevant in determining the strength of antibacterial activity because can demonstrate bactericidal ability at low concentrations of fractions. The fractions can have bactericidal ability which characterized by the absence of bacterial colony growth that known through minimum bactericidal concentration (MBC) (Sandy Siska, & Septiarini, 2021).

Further research needs to be carried out on *Escherichia coli* bacteria with different strains or in a broad area and accompanied by the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and IC_{50} in inhibiting the growth of *Escherichia coli*.

5. Conclusion

Alkaloids, steroids, and saponins are the secondary metabolites identify in the n-hexane fraction, while the ethyl acetate and butanol fractions contain flavonoids, phenols, alkaloids, triterpenoids, and tannins. The 50% concentration of n-hexane, ethyl acetate, and butanol fractions is more effectively against *Escherichia coli* than other concentrations of each groups. Ethyl acetate fraction of mango peel (*Mangifera indica* L.) gedong gincu variety with 50% concentration (12.08 mm) is the most effective potential in inhibit the growth of *Escherichia coli*.

Conflict of Interest

The authors declare no conflict of interest for the results.

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