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Phytochemical analysis of Indonesian gadung mango leaf (*Mangifera indica* L. var. gadung) and their antibacterial activity

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Abstract. *Sulistyarsi A, Rahayu T, Primiani CN, Pujiati. 2023. Phytochemical analysis of Indonesian gadung mango leaf (*Mangifera indica *L. var. gadung) and their antibacterial activity. Biodiversitas 24: 6295-6304.* The study was carried out to find out the secondary metabolite compounds in mango leaf extract (*Mangifera indica* L. var. gadung) using qualitative, quantitative, LC-MS and antibacterial activity with well diffusion method in inhibiting the growth of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Research used a quantitative approach of randomized block design with variations of concentrations of 60, 80, and 100%, K- (aquadest), and K+ (chloramphenicol) in three times repeated groups. The results of qualitative and quantitative phytochemical tests show that mango leaf extract contains flavonoids (63408.464 mg/kg), saponins 3212.942 mg/kg), tannins (1050.191 mg/kg), alkaloids (605.123 mg/kg) and steroid (1050.191 mg/kg). Test of flavonoid derivat extract using LC-MS shows apigenin, kaempferol, fisetin, catechins, quercetin, ramnetin, astragalin, hirsutrin, isocercitrin, hyperin, rhamnetin 3-glucoside, rhamnetin 3-o-βgalactopyranoside, isorhamnetin 3-galactoside, amentoflavone, routine, hyperin-6-gallate, epigallocycin (4β→8) epigallocycin-3-ogalate ester. Antibacterial testing on *S. aureus* forms an average clear zone diameter of 30.46, 31.43, 32.81 mm furthermor in *E.coli* is 23.23, 23.84, 24.23 mm. Data of clear zone diameter was analyzed using ANOVA two-way and showed a value of sg. 0,001<0,05. It can be concluded that Indonesian gadung mango leaf extract contains secondary metabolites which have the potential to be an antibacterial agent.

Keywords: Antibacterial, *Escherichia coli*, *Mangifera indica* L var. gadung, phytochemicals, *Staphylococcus aureus*, well-diffusion

INTRODUCTION

Phytochemicals are classic secondary metabolites of plants that have many pharmacological benefits (Jalalvand et al. 2019; Prasathkumar et al. 2021). The content of phytochemical compounds in plants depends significantly on the type of dry sample and the extraction process (Hossen et al. 2022). Extraction with a methanol solvent produces the highest phytochemical compounds (Truong et al. 2019). The type of solvent in the extraction process will affect the amount of phytochemical content (Hossen et al. 2022). The benefits of phytochemical compounds in the field of pharmacology are very diverse. Phytochemicals have antimicrobial properties and are natural ingredients whose effects have been proven through biological experiments (Afata et al. 2022). These compounds can regulate the growth, cell cycle, proliferation, apoptosis, adhesion, migration, and angiogenesis of various cancer cells (Zhai et al. 2022). Phytochemicals have been widely used as admixtures to improve results and reduce side effects of chemotherapy (Kong et al. 2020). Potential compounds as antibacterials are called phenolics, including saponins, tannins, flavonoids, and steroids (Afata et al. 2022). These compounds are able to inhibit the growth of *Escherichia coli* bacteria. Secondary metabolites are increasingly attracting the interest of researchers because they have resulted in the discovery of ultra-modern methods for isolating pure compounds from plants that can solve human health problems (Tonga et al. 2022).

Antibacterial can be obtained from various herbal plants. In recent years, the food, pharmaceutical and cosmetic industries have focused on natural compounds with antimicrobial and antioxidant properties; generally, these compounds are obtained from Kingdom Plantae (Shankar et al. 2018; Aguilar-Villalva et al. 2021). Just as the Chinese people use traditional medicines, which are often called Traditional Chinese Medicine (TMC) and have been extensively validated based on the content of chemical components for antibiotic and antibacterial drugs (Zheng et al. 2017; Li et al. 2018; Liu et al. 2019). Active phytoconstituents isolated from plant parts have been used to eliminate pathogenic bacteria due to their developed resistance to antibiotics (Jain and Parihar 2018; Shankar et al. 2018). The potential of plants as antibacterial agents is because they develop phytochemical compounds such as polyphenols and flavonoids with strong antibacterial potential and significantly inhibiting Gram positive bacteria (Asfaw et al. 2023).

As is the case with mango plants containing phytochemicals in the form of flavonoids, saponins, alkaloids, and tannins, which have potential as antibacterial agents (Ouf et al. 2021; Omotayo et al. 2022; Tacias-Pascacio et al. 2022). Mango (*Mangifera indica* L.) is a local plant with an abundant presence in Indonesia. Mango variants are very diverse, one of which is gadung mango. Gadung mango is an endemic mango from Indonesia, found mostly in East Java. The gadung mango has a dark green skin and a wax-like coating on the outside. Mango

leaves are less optimized for utilization at this time. Based on previous research, besides containing flavonoids, mango leaves also contain phenols, tannins, saponins, and cytosine, which both have anti-inflammatory and anti-bacterial properties (Salem et al. 2013). Despite having a lot of potential, gadung mango leaves have not been used to their full potential.

Pathogenic bacteria such as *Staphylococcus aureus* can cause several disorders like skin infections, boils, and even acne (Al Bshabshe et al. 2020; Byrd et al. 2018; Pollitt et al. 2018). *Staphylococcus aureus* is a cocci-shaped gram-positive bacterium with golden, grape-like colonies. Based on the above characteristics, the type of bacteria that causes infection and inflammation in acne is *Staphylococcus aureus* (Adetutu et al. 2017). *Escherichia coli* is a pathogenic bacterium that can cause gastrointestinal infections, urinary tract infections, local tissue and organ infections (Govindarajan et al. 2020; Wang et al. 2020). The present study was conducted to determine the potential of the extract of Indonesian gadung mango leaf extract to inhibit the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Study area

The research was conducted at the Pharmaceutical Biology Laboratory, Pharmacy Study Program, University of PGRI Madiun, Madiun, Indonesia from March to June 2023. Gadung mango leaves were obtained from Jogorogo Sub-district, Ngawi District, East Java Province, Indonesia as much as 3 kg and dried with the air dry method without being exposed to direct sunlight. The simplicia as much as 300 g and then extracted.

Materials

The materials used in the study were 96% ethanol, distilled water, chloramphenicol, pure bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli ATCC 25922*, Mueller Hinton agar, mango gadung leaf extract, H2SO4, HCl, Mayer, acetic anhydrous, and FeCl3. Research equipment included autoclaves, maceration vessels, rotary evaporator, vacuum, Erlenmayer vacuum, Erlenmayer, beaker, spatula, hot plate, petri dish, cork drill, pipette, cotton swab, ose, test tube (Al Bshabshe et al. 2020).

Procedures

Preparation of leaf extract

The first leaf extract was made by drying the raw material of gadung mango leaves for two weeks without being exposed to sunlight (Sánchez-Gomar et al. 2022). The dried leaves were then blended to form simplicia powder. Simplicia powder was extracted by maceration method with a ratio of 1:10. A total of 300 g of simplicia was soaked in 3000 mg ethanol for 3 days using the diplo technique. The triplo technique is dividing the solvent into three parts, so that during immersion the solvent is replaced every day (Al Bshabshe et al. 2020). After soaking, the extract was concentrated using a rotary evaporator. Condensed extracts are stored in tight containers free of light and placed in the refrigerator to prevent the growth of fungi or other microbes. Extract yield was calculated using the following formula (Dhanani et al. 2017): %Extract Yield=(extract weight)/(simplicia weight) x 100%

Phytochemicals analysis of gadung mango leaf extract qualitative phytochemical test

Gadung mango leaf extract was then tested for the qualitative analysis of phytochemical contents. The secondary metabolites or phytochemicals in the tested gadung mango leaves was compounds with antibacterial potential including flavonoids, steroids, tannins, alkaloids, and saponins. Flavonoids were tested by adding 3 drops of concentrated HCl into the sample and then heating it. A positive reaction containing flavonoids was indicated by the appearance of a reddish-orange precipitate (Dahanayake et al. 2019). The tannin compound was tested by adding 1% FeCl3 and the dark blue color will be an indicator of the presence of the compound (Mumtaz et al. 2014). Steroids were seen from the change in the color of the extract to green-blue after being reacted with 3 drops of acetic anhydrous and H2SO4 (Mumtaz et al. 2014). Saponins were tested by adding distilled water and then shaking and if it formed 1-10 cm foam, the reaction was positive. While the alkaloids can be seen from the formation of a reddish-brown precipitate after being given concentrated HCl, distilled water, and Mayer's reagent (Kancherla et al. 2019).

Phytochemicals analysis of gadung mango leaf extracts using LC-MS method

The supernatant was added to a Sep-Pak C18 Cartridge (1 mL, 100 mg) that had been preconditioned with 1 mL of an 80:20 (acetonitrile: water) solution, and 1 mL of the sample was added to the column along with around 0.5 mL of the solution. Additionally, 0.5 mL of an acetonitrile/water solution in an 80:20 ratio was introduced to the Sep-Pak column, and 0.5 mL of the solution that emerged was taken. Then, 0.25 mL of a 50:50 acetonitrile/methanol solution containing 200 mm ammonium formate was added to the Sep-Pak column. The obtained 0.5 mL was combined with 0.2 mL of buffer (25 mm ammonium formate, pH 4.5) in a 25:75 acetonitrile: buffer solution before being injected into an instrument of LCMS. Furthermore, a membrane filter was used to carry out the filtration (Ismail et al. 2020; Primiani et al. 2022).

Antibacterial assay

Antibacterial testing was carried out using the Mueller Hinton agar media well diffusion method. Wells were made using a crock drill with a diameter of ± 6 mm (Kumaria et al. 2019). The extract sample was first diluted to form variations of 60, 80, and 100% then put in the wells, then the media was incubated for 18 to 24 hours at 35°C. MHA with mass 13 g were dissolved in 300 mL of distilled water and then heated using a hotplate stirrer. The media was then sterilized using an autoclave at 121°C for 15 minutes. Sterile media with a temperature of about 45°C was poured into a petri dish with a media height of 4 mm.

The wells were made with a cork drill with a diameter of 6 mm and then filled with extract. In each cup, there are 5 wells that will be filled with 3 treatment concentrations: 60, 80, and 100%; control + (chloramphenicol); and control - (aquadest). The Staphylococcus aureus and Escherichia coli test microbe made with standard Mc Farland 0.5 standard (Lozano et al. 2018; De Zoysa et al. 2019) was spread with a sterile cotton swab, then the media was incubated for 18 to 24 hours at 35°C (Kowalska-Krochmal and Dudek-Wicher 2021; Das et al. 2021). The clear zone around the wells was measured with a caliper to see the growth of the microbes (Cheng et al. 2022).

Data analysis

Data analysis was carried out to prove the hypothesis that mango gadung leaf extract was able to inhibit the growth of *S. aureus* and *E. coli* bacteria. Each variation of extract concentration was repeated three times. Analysis used two way Anova through IBM Statistics 26 software. The previous data must meet the normality test using Kolmogorov Smirnov and Levene homogeneity analysis.

RESULTS AND DISCUSSION

The results of the phytochemical test qualitatively and quantitative

Furthermore, the extract was tested for the content of phytochemical compounds with antibacterial potential, namely tannins, flavonoids, alkaloids, saponins, and steroids. The results of the qualitative phytochemical test of gadung mango leaf extract are presented in Table 1. Results of phytochemical tests using LCMS are shown in Figure 1.

LCMS has many advantages due to its compatibility with chromatographic techniques, its speed, specificity and sensitivity. Reported the method using LC-MS has a longer analysis time with minimal accuracy. UPLC-ESI-QqQLIT−MS/MS (Ultra High Performance liquid chromatograph/triple-four-pole linear ion-trapping mass spectrometer) enables the determination of the target analyses at very low concentrations (Shukla et al. 2021). Based on a quantitative analysis of the most phytochemical life that has antibacterial potential in mango leaf extract are flavonoids. The results of the LC-MS analysis showed that gadung mango leaf extract contained flavonoid group compounds namely apigenin, kaempferol, fisetin, catechin, quercetin, rhamnetin, astragalin, hirsutrin, isoquercitrin, hyperin, rhamnetin 3-glucoside, rhamnetin 3-o-βgalactopyranoside, isorhamnetin 3-galactoside, amentoflavone, rutin, hyperin-6"-gallate, epigallocatechin (4β→8) epigallocatechin-3-gallate ester. Flavonoid compounds can inhibit bacterial growth by attacking cells and shrinking and then destroying their cell membranes. Flavonoids work to damage the bacterial cell wall because the reaction between the phosphate groups and H+ ions so that the phospholipids in the bacterial cell membrane will dissolve (Chen et al. 2021). Based on LC-MS analysis, the type of flavonoid with the highest amount was hirsutrin with a composition of about 1.87%. Figure 2 shows the hirsutrin compound which has a chemical structure of C22H22O11 and a molecular weight of 462.4070.

Table 1. The phytochemical of gadung mango leaf using qualitative methods

Phytochemicals compounds	Reactor	Identification
Tannins	$FeCl_3$ 1%	+
Alkaloids	HCl, aquadest, Mayer	+
Saponins	Aquadest	+
Flavonoids	HCL	+
Steroids	Asetat anhidrat, H_2SO_4	+

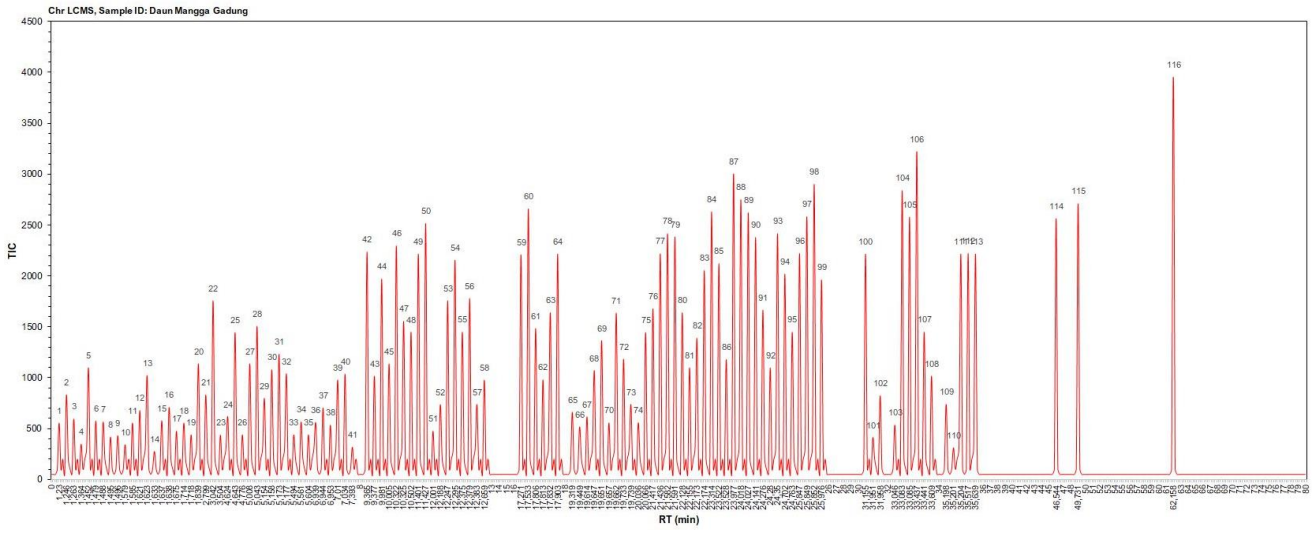


Figure 1. LCMS chromatogram result of gadung mango leaf extract

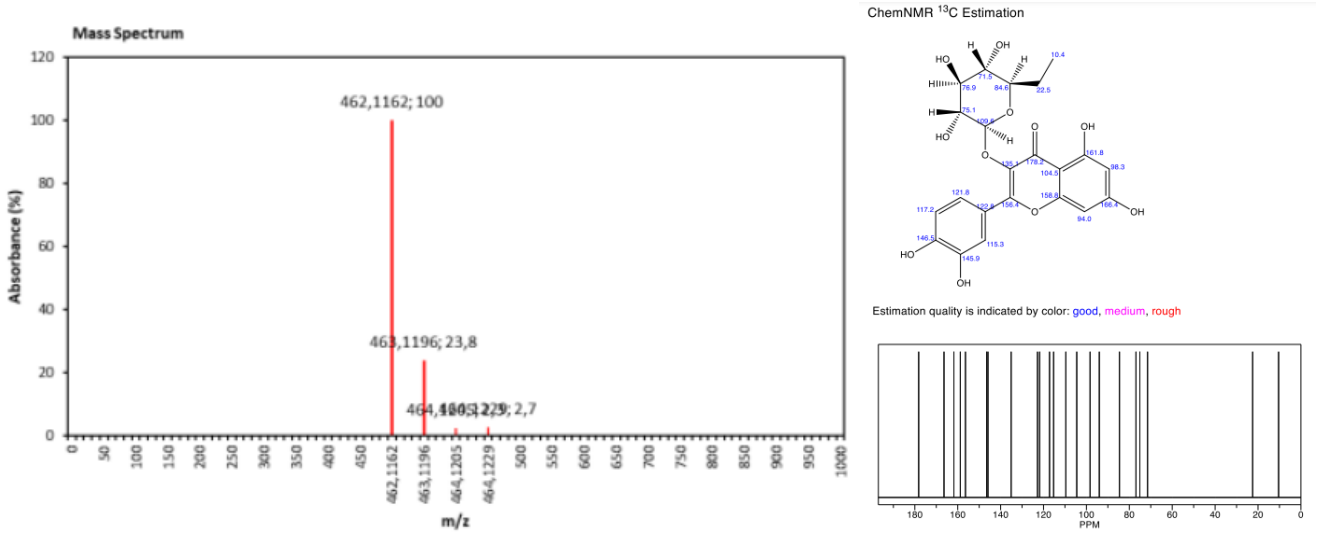


Figure 2. Hirsutrin, one of flavonoid compounds in gadung mango leaf, indicated by the adsorption value and chemical structure

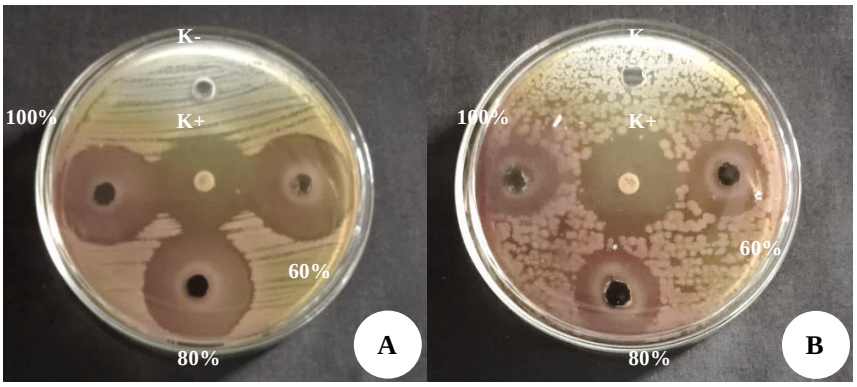


Figure 3. Results of the well-diffusion method of antibacterial test: A. *Staphylococcus aureus* ATCC 25923, and B. *Escherichia coli* ATCC 25922

Antibacterial test results

Antibacterial activity was determined by well diffusion method (Figure 3). The results of measuring the average diameter of the inhibition zone are presented in Table 4. The extract was diluted into several concentration variants, which would become the treatment groups for the test bacteria. Diluted extract concentrations include 60, 80, and 100%. Furthermore, the extract was tested for the content of phytochemical compounds with antibacterial potential, namely tannins, flavonoids, alkaloids, saponins, and steroids.

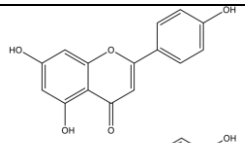
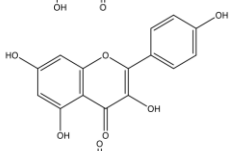
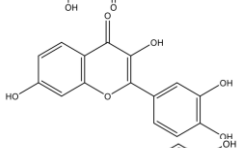
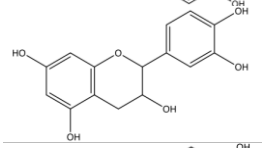
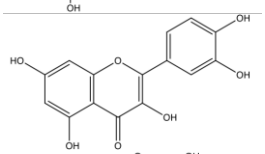
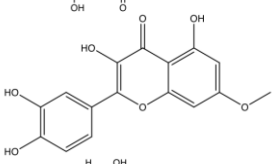
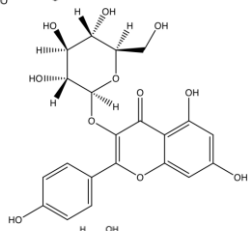
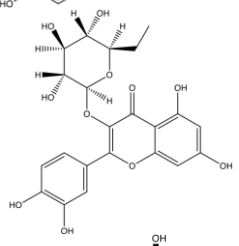
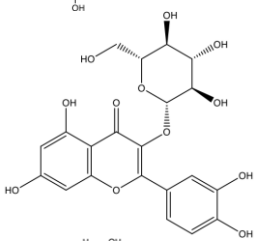
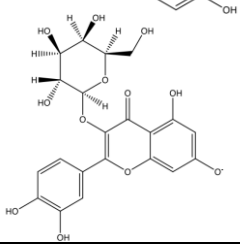
Antibacterial activity was determined by the well diffusion method. All equipment is sterilized by autoclave. Bacterial suspension based on standard McFarlands 0.5-13 g of MHA media were dissolved in 300 mL of distilled water and then heated using a hotplate stirrer. The media was then sterilized using an autoclave at 121°C for 15 minutes. Sterile media with a temperature of about 45°C was poured into a petri dish with a media height of 4 mm. The wells were made with a cork drill with a diameter of 6 mm and then filled with extract. In each cup, 5 wells will be filled with 3 treatment concentrations: 60, 80, and 100%; control + (chloramphenicol); and control - (aquadest).

Before being given the extract, the media was inoculated with the test bacteria using the spread technique and then given the extract. After that, it was incubated for 24 hours at 35°C (Kowalska-Krochmal and Dudek-Wicher 2021). This temperature is the optimal condition for bacteria to grow properly. After incubation, the diameter of the inhibition zone, which is an indicator of the antibacterial activity of the extract, was measured using a caliper. The results of measuring the average diameter of the inhibition zone from 3 repetitions are presented in Tables 2 and 3.

Table 2. The phytochemical of gadung mango leaf using quantitative methods

Phytochemical compounds	Total (mg/kg)
Alkaloids	605,123
Saponins	3212,942
Flavonoids	63408,464
Steroids	1050,191
Tannins	1050,191

Table 3. Results of LC-MS analysis compound of flavonoids groups from gadung mango leaf extract

Peak number	RT (min)	Similarity index %	Curve area	Composition (%)	Compound result	
					Analysis	Structure
42	9,365	92	2236,03558	1,39844	Apigenin	
46	10,322	92	2295,99031	1,43594	Kaempferol	
47	10,325	92	1553,56337	0,97162	Fisetin	
48	10,502	92	1447,56257	0,90532	Cathecin	
50	11,427	92	2515,21202	1,57304	Quercetin	
51	12,001	92	474,66497	0,2966	Rhamnetin	
82	22,173	92	1387,86150	0,86798	Astragalin	
87	23,977	92	3001,96350	1,87746	Hirsutrin	
88	24,018	92	2750,66170	1,72030	Isoquercitrin	
89	24,027	92	2621,59638	1,63958	Hyperin	

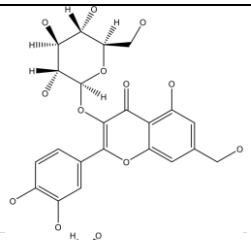
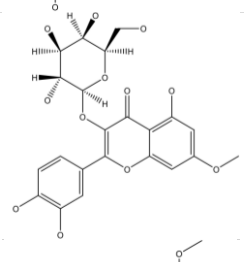
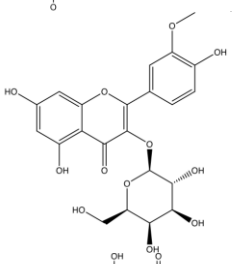
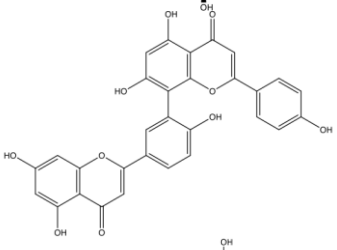
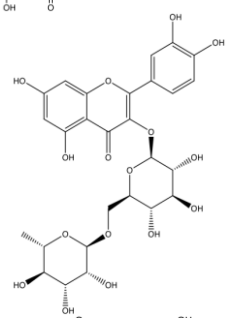
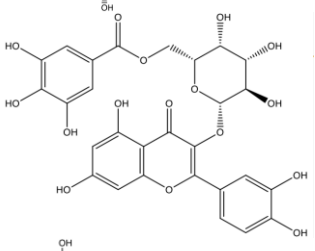
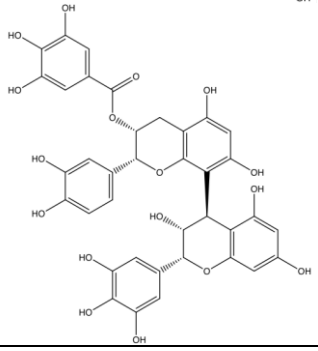
96	25,847	92	2219,86145	1,38833	Rhamnetin 3-glucoside	
97	25,846	92	2582,66150	1,61523	Rhamnetin 3-o-βgalactopyranoside	
98	25,852	92	2901,7292	1,81478	Isorhamnetin 3-galactoside	
100	31,155	92	2215,96034	1,38589	Amentoflavone	
112	35,517	92	2221,42563	1,38931	Rutin	
113	35,639	92	2217,32987	1,38674	Hyperin-6"-gallate	
114	46,544	92	2562,53507	1,60264	Epigallocatechin (4β→8)epigallocatechin-3-gallate ester	

Table 4. The average diameter of the extract inhibition zone against *S. aureus* and *E. coli* bacteria

Bacterial	Treatment	Inhibition zone diameter Repetitions			Mean (mm) ± SD	Category of inhibition (Ouchari et al. 2019)
		1	2	3		
Staphylococcus	K +	28.39	29.28	28.04	$28.57^{b} \pm 6.39$	Very strong
aureus	K –	0	0	0	$0^{a} \pm 0.00$	None
ATCC 25922	60%	33.65	31.45	33.33	$32.81^{c} \pm 11.88$	Very strong
	80%	32.43	30.0	31.85	$31.43^{c} \pm 12.69$	Very strong
	100%	32.11	27.47	31.79	$30.46^{bc} \pm 25.91$	Very strong
Escherichia coli	K +	26.71	28.18	28.90	$27.93^{c} \pm 11.1$	Very strong
ATCC 25922	K –	0	0	0	$0^{a} \pm 0.00$	None
	60%	22.98	23.77	22.94	$23.23^{b} \pm 4.68$	Very strong
	80%	23.58	24.68	23.25	$23.84^{b} \pm 7.48$	Very strong
	100%	24.38	23.43	24.89	$24.23^{b} \pm 7.40$	Very strong

Note: The mean with a different alphabetical code shows a significant difference or a P value >0.05. K+ (chloramphenicol 30 mg), K- (aquadest), SD (standard deviation). Category of inhibition indicate by diameter of clear zone (weak activity (<5 mm), moderate (5-10 mm), strong (>10-20 mm), very strong (>20-30 mm) (Ouchari et al. 2019)

The mean with a different alphabetic code in Table 4 shows the results of Duncan's test that the treatment has a significant difference or P>0.05. The diameter of the inhibition zone is a sign that the extract has the ability to inhibit the growth of *S. aureus* and *E. coli* bacteria. The average range of inhibition zone diameters of extract concentrations of 60, 80, and 100% against *S. aureus* bacteria were 30.46, 31.43, and 32.81 mm with values were >21 mm. The diameter of the inhibition zone formed on *S. aureus* bacteria was included in the very strong inhibition category. In *E. coli* bacteria, the diameter of the inhibition zone formed was 23.23-24.23 mm and was included in the category of very strong inhibition. Comparison of the diameter of the inhibition zone for each treatment in inhibiting the growth of *S. aureus* and *E. coli* bacteria can also be seen in Figure 4.

From Figure 4, it can be seen that the higher the concentration of the extract, the larger the diameter of the inhibition zone. This is because the concentration of the extract has an effect on inhibiting bacteria. The best concentration for inhibiting the growth of *S. aureus* bacteria was 60%, with an average inhibition zone diameter of 32.81 mm ± 11.88. The three extract concentrations produced a higher inhibition zone compared to the control plus chloramphenicol, which resulted in an average inhibition zone diameter of 28.57 mm ± 6.39. whereas in *E. coli* the concentration of the extract that formed the largest diameter of the inhibition zone was 100% with a diameter of $24.23^{b} \pm 7.40$ mm.

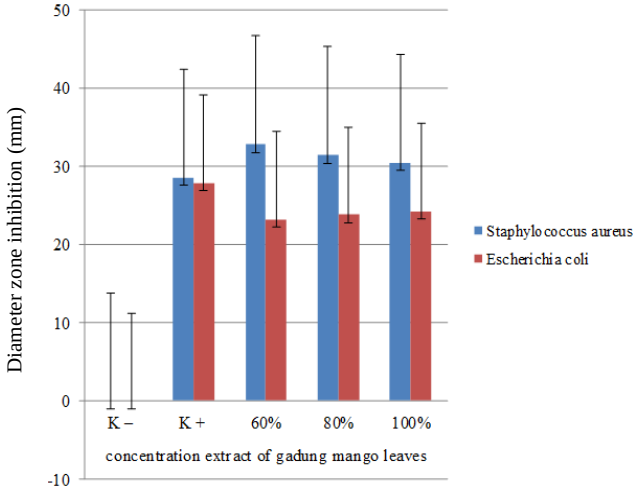


Figure 4. Diameter zone inhibition

Discussion

The resulting gadung mango leaf extract is 46.94 g from a weight of 300 g of simplicia. The resulting extract yield value was 15.64%; this value was higher than previous studies, which were similar to extract yields of 10.55% (Dhanani et al. 2017). The yield value is influenced by several factors, one of which is the water content of the sample. High water content can reduce the weight of the resulting extract. Dried mango gadung leaves, before being mashed, have a water content of 11.82%. The characteristics of the extract are blackish green in color with a distinctive aroma of ethanol extract.

Based on the analysis of two way anova data, where the average significant value of the diameter of the inhibition zone was 0.000 and the value was sig <0.05, it can be concluded that mango leaf extract affects and inhibits the growth of *S. aureus* and *E. coli* bacteria. The diameter of the inhibition zone is formed due to the influence of several factors, such as the sensitivity of the test microbe, the rate of diffusion, and the concentration of antibacterial compounds (Parisa et al. 2019). Antibacterial compounds from mango leaf extract have different reactions and effects when inhibiting bacteria. The mechanism of bacterial inhibition of phytochemical compounds is sensitive to the bacterial cell membrane and results in lysis. Bacterial cells undergo lysis because phenolic compounds change the permeability of cell membranes. Damage to the permeability of the membrane can cause intracellular components such as amino acids, nucleic acids, and proteins to exit, and cell death occurs (Suhendar et al. 2019; Wang et al. 2014). Flavonoid compounds attack bacterial cells by shrinking and destroying their cell membranes (Chen et al. 2021). The phospholipids in the bacterial cell membrane will dissolve as a result of the

reaction between the phosphate groups and H+ ions in flavonoids, which also can inhibit ATP synthase and disrupt bioenergetic status (Oktavia et al. 2013; Veiko et al. 2023). According to earlier research, the interaction features of the membrane are closely related to the antibacterial activity of plant tannins (Liu et al. 2020). Tannin chemicals can disrupt the metabolism of bacterial cells by denatureing proteins and inactivating enzymes. A group of naturally occurring substances known as saponins typically consist of non-sugar components (glycosides) and sugar chains that are joined together by glycosidic linkages. Steroids (C-27) and triterpenoids (C-30) are examples of saponins. At various doses and when coupled to one or more glycone molecules, these compounds have been demonstrated to have specific antibacterial action against marine biofilm bacteria (Dong et al. 2020). Saponins have the ability to create micelles and lower surface tension in aqueous solutions. Biological macromolecules exhibit certain structural changes when the concentration of micelles reaches a crucial level. In the surface sterols of eukaryotic cell membranes (such as those of bacteria and fungus), saponins can bind. The reaction damages the biofilm system by causing the cell membrane to rupture and perforate. When employed against pathogenic germs that are found in food, this substance has antibacterial properties. Saponin substances that resemble detergents will stop the permeability of the cell wall and lead to the lysis of bacteria (Donga et al. 2020).

The metabolites in the extract and their concentrations had an impact on the differences in the inhibition zones. Metabolites will work depending on the morphological properties of the test bacteria. *Staphylococcus aureus* bacteria is a positive strain with thicker peptidoglycan but is polar, making it easier for metabolites in the extract to dissolve. Whereas the cell walls of gram-negative bacteria (*E. coli*) contain lipopolysaccharide containing hydrophobic lipids on the outer layer (Mulangsri and Zulfa 2020). Antibacterial mango gadung leaf extract is more effective against gram positive bacteria, namely *Staphylococcus aureus*, compared to *Escherichia coli*. This is because gram-negative bacteria are more resistant to antibacterial (Semeniuc et al. 2017). Differences in the composition of the cell walls in gram-positive and gram-negative bacteria can cause differences in the inhibition zones formed. The cell wall of gram-positive bacteria is single-layered with a lipid content of 1-4% so that the antibacterial inhibitory zone formed is larger. The cell wall in the gram-negative bacteria has three layers consisting of lipoproteins, outer membrane phospholipids, and lipopolysaccharides. The lipid content in the gram-negative cell wall ranges from 11-22%. The phospholipid in the outer membrane makes it difficult for chemical antibacterial components to penetrate the cell walls of gram-negative bacteria (Lencova et al. 2022). Wall teichoic acid in the outer layer of the cell membrane of gram-positive bacteria which helps the penetration of flavonoids easily into the membrane and then affects RNA. Meanwhile, gram-negative bacteria do not have a wall teichoic acid, this is assumed to be the reason the antibacterial activity of mango leaf extract is better on *S. aureus* than *E. coli* (Wang et al. 2021).

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