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Differences in Antibacterial Activity of Petroleum Ether and Ethanol Extracts of Bangle Rhizome (Zingiber Montanun (J. Koenig)) Against Staphylococcus aureus and Escherichia coli Bacteria

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ABSTRACT

Disease-causing bacteria are divided into two groups: Gram-positive and Gram-negative. These bacteria have different cell wall characteristics, which affect treatment options. The aim of this study was to determine the differences in antibacterial activity of petroleum ether and 96% ethanol extracts against S. aureus and E. coli. The extraction method used was reflux. Antibacterial activity was measured using the well diffusion method with Mannitol Salt Agar and Eosin Methylene Blue Agar media. The results showed that PE extract 5.25% and ethanol 11.09%. The inhibition zone (cm) of antibacterial activity of PE extract on S. aureus bacteria at concentrations of 1%, 5%, and 10% was 1.38±0.155; 1.58±0.138; 1.72±0.129, respectively, while E. coli bacteria were 1.16±0.123; 1.37±0.212; 1.52±0.238. The inhibition zone of ethanol extract on S. aureus bacteria at concentrations of 1%, 5%, and 10% were 0; 1.39±0.136; 1.63±0.150, respectively, while E. coli bacteria were 1.30±0.191; 1.51±0.145; 1.54±0.159. Based on SPSS analysis, non-parametric test showed that the activity of PE and 96% ethanol extracts on S. aureus bacteria was significantly different from E. coli bacteria with a sig. value <0.05 and between PE and ethanol extracts provided significantly different activity against one type of bacteria (sig. <0.05).

Keywords: Antibacterial, PE Extract, Ethanol, S.Aureus, E.Coli

INTRODUCTION

Bacteria are microorganisms with a single-celled structure and no cell membrane (Artati & Oman, 2019). The presence of these microorganisms in the human body can cause various diseases, ranging from mild to severe and even death. Bacteria can be classified into two types: Gram-positive and Gram-negative. Gram-positive bacteria have a thicker cell wall structure containing peptidoglycan, while Gram-negative bacteria have a thinner peptidoglycan layer. Therefore, this classification is important for identifying and determining antibiotic treatment (Nurhidayati et al., 2015)

Staphylococcus aureus is a Gram-positive bacterium whose cell wall is coated with peptidoglycan, polysaccharides, and phospholipids. These components protect the bacterial cell from external attacks, enabling the bacteria to survive (Rajagopal & Walker, 2018). Staphylococcus aureus bacteria can cause various infectious diseases such as skin infections, bacteremia, and pneumonia. This bacterium can also become more resistant to antibiotic abuse, making treatment more difficult (Guo et al., 2020). Staphylococcus aureus that has developed resistance to the drug class methicillin, often referred to as Methicillin-Resistant Staphylococcus aureus (MRSA), is a leading cause of death from bacterial infections (Chambers & Fowler, 2024). Escherichia coli is a group of Gram-negative bacteria. These bacteria commonly grow in the digestive tract and commonly cause urinary tract infections and mastitis. They can also cause nosocomial infections associated with urinary catheters in

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hospital patients. These bacteria are capable of forming biofilms as a defense mechanism, enabling them to resist external attacks (Giannattasio-Ferraz et al., 2022).

Based on the information on the dangers that can be caused by the presence of these bacteria, it is necessary to take measures to prevent or overcome them. One way to overcome this is to seek sources of treatment from natural ingredients. The natural ingredient used in this study is bangle rhizome. Bangle (Zingiber montanun (J. Koenig)) is a rhizome that contains various secondary metabolites, including triterpenoids, alkaloids, flavonoids, phenolics, and tannins (Daryanti et al., 2023). These compounds have various mechanisms against bacteria. Research conducted by (Khairunnisa; et al., 2025) stated that the ethanol extract of bangle rhizome at a concentration of 25% was able to provide antibacterial activity against Shigella dysenteriae of 8.8 mm. Research using other parts of the bangle, namely ethanol and n-hexane leaf extracts, was able to provide antibacterial activity against Escherichia coli of 0.648 cm and Staphylococcus aureus of 0.559 cm (Sari et al., 2016). Therefore, this study was conducted with the aim of determining the differences in the activity of petroleum ether and ethanol extracts on the growth of Gram-positive bacteria, represented by Staphylococcus aureus bacteria, and Gram-negative bacteria represented by Escherichia coli bacteria.

MATERIALS AND METHODS

Equipment and Materials

The materials used in this study were bangle rhizome powder, 96% ethanol, petroleum ether, Mg powder, hydrochloric acid, amyl alcohol, gelatin, sodium chloride, Wagner, Mayer, Hager, Libermann-Burchad, butanol, acetic acid, distilled water, ammonia vapor, ethyl acetate, methanol, FeCl3, toluene, acetone, formic acid, methanol, Dragendorf, chloroform, anisaldehyde, sulfuric acid, benzene, Staphylococcus aureus, and Escherichia coli bacteria. The equipment used included a series of reflux apparatus, glassware, petri dishes, and vernier calipers.

Methods

Extraction

Bangle rhizome extraction was carried out using the reflux method using petroleum ether and 96% ethanol as solvents. 225 grams of powdered rhizome were weighed and placed in a round-bottom flask with petroleum ether in a 1:5 ratio. The extraction was then carried out using the reflux method for 2 hours. The extraction process was repeated three times to ensure complete extraction. The filtrate was evaporated using a rotary evaporator and then concentrated using a water bath at 60°C until a thick extract was obtained (Indonesian Ministry of Health 2000). The dregs were then dried and re-extracted using 96% ethanol, repeating the extraction process. The filtrates obtained from each solvent were evaporated using a rotary evaporator and a water bath at 50°C until a thick extract was obtained.

% Yield = (Extract Weight (g)) / (Richmond Weight (g)) x 100%

Phytochemical Screening and Thin Layer Chromatography.

The active compounds in the extract were identified by color and TLC using appropriate reagents and eluents. Flavonoids were identified by adding 0.1 g of magnesium powder, 1 mL of HCl, and 2 mL of amyl alcohol to the sample (Rahardhian et al., 2025). The eluent used for flavonoid identification was acetic acid: water: butanol (1:2:6) and ammonia vapor as a spot detector (Firnando et al., 2019). Phenolics were reacted with 1% FeCl3 to form a green or blue-black color. The eluent used was ethyl acetate: methanol: water (100:13.5:10), and 5% FeCl3 as a spot detector. Tannins were identified with 1% gelatin and 10% sodium chloride, eluent

toluene: acetone: formic acid (6:6:1) FeCl3 5% spotting (Hanani, 2015). Alkaloids were identified with 10 mL of 2% HCL, 10 mL of 10% ammonia, 20 mL of chloroform, phosphomolybdic acid, Wagner, Mayer, and Hager. Eluent methanol: ammonia (100:1.5), Dragendorff spotting (Mu'awwanah & Ulfa, 2015)

Saponins were identified by shaking the sample with 10 mL of hot distilled water and 1 drop of hydrochloric acid. The eluent was chloroform: methanol: water (64:50:10) to detect anisaldehyde-sulfuric acid spots and heated on a hot plate at 100°C (Fahrurroji & Riza, 2020). Essential oils were identified with anisaldehyde-sulfuric acid solution, viewed under UV light 366. The eluent used was benzene: chloroform (1:1) to detect anisaldehyde-sulfuric acid spots and was heated on a hot plate at 100°C. Steroids/Triterpenoids were identified using Liebermann-Burchard solution, acetic anhydride, and concentrated sulfuric acid; the eluent used was toluene: chloroform: methanol with a ratio of (40:40:10) to show anisaldehyde-sulfuric acid spots and heated on a hot plate at 100°C (Fajriaty et al., 2018).

Antibacterial Activity Test

The antibacterial activity of bangle rhizome extract was determined using the well method. A well was prepared by adding 10 mL of Mannitol Salt Agar (for Staphylococcus aureus) and Eosin Methylene Blue Agar (for Escherichia coli) to a Petri dish and allowing it to solidify. After the well was filled, 20 mL of the medium was poured into the cylinder cup, followed by 5 µL of bacterial suspension. After the well was formed, samples at concentrations of 1%, 5%, and 10%, along with a positive control, ciprofloxacin, were added. The Petri dish was incubated at 37°C for 24 hours, and the diameter of the inhibition zone was measured using a vernier caliper (Wulandari & Sulistyarini, 2018).

RESULTS AND DISCUSSION

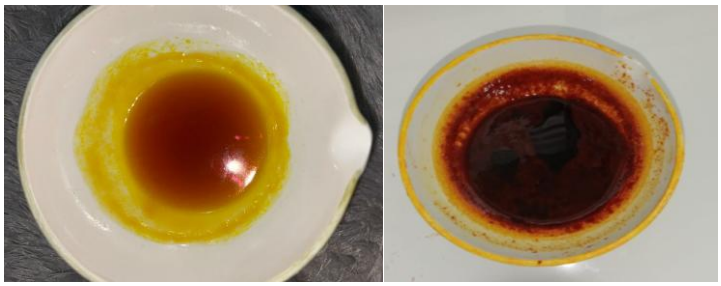
This study aimed to determine the differences in antibacterial activity of bangle rhizome against both Gram-positive and Gram-negative bacteria. The Gram-positive bacteria used were Staphylococcus aureus, while the Gram-negative bacteria were Escherichia coli. The bangle rhizome extract samples used for the antibacterial test were obtained using the reflux extraction method. The reflux method is a heat extraction method because it utilizes temperature during the extraction process. Using temperature in the extraction process can provide the advantage of a faster extraction process because the reaction rate between the solvent and the powder containing the active ingredient is faster with increasing temperature (Kusumo et al., 2020). Bangle rhizome extraction used petroleum ether and 96% ethanol solvents with varying levels of polarity. Bangle rhizomes contain various compounds with varying polarity, such as alkaloids, flavonoids, phenolics, saponins, and triterpenoids (Setyani et al., 2021). Petroleum ether is a non-polar solvent that will dissolve non-polar compounds such as alkaloids, saponins, and triterpenoids. Meanwhile, 96% ethanol can dissolve compounds that tend to be semi-polar and polar, such as flavonoids and phenolics. The yield of bangle rhizome extraction using petroleum ether and 96% ethanol solvents is presented in Table 1 below.

Table 1. Yield of Bangle Rhizome Extract

Petroleum ether	Ethanol 96 %
$\frac{\mathbf{11,8215}}{\mathbf{225,09}}\ \boldsymbol{x}\ \mathbf{100} = \mathbf{5,25}\ \%$	$\frac{24{,}9635}{225{,}09}\ x\ 100 = 11{,}09\ \%$

From Table 1, the yield obtained with 96% ethanol solvent is greater than the extraction yield with ether solvent. The amount of yield indicates the number of compounds extracted

with the solvent. Ethanol consists of an ethyl group (C2H5), which can attract non-polar compounds, and a hydroxyl group (OH), which can attract polar compounds, ethanol is also called a universal solvent because it can attract various secondary metabolite compounds in simples (Noli & Idris, 2023). The image of the results of the extraction of bangle rhizomes with petroleum ether and 96% ethanol solvents is presented in Figure 1.



A **B**

Figure 1. Results of Bangle Rhizome Extraction (A) Petroleum Ether Solvent, (B) 96% Ethanol Solvent

The active compounds of the petroleum ether and 96% ethanol extracts were identified using color reactions and thin-layer chromatography, respectively. The identification results are presented in Table 2.

Table 2. Results of Phytochemical Compound Screening of Petroleum Ether Extract (PEE) and 96% Ethanol Extract (EE) of Bangle Rhizomes

No	Compound	Reagent	Literature Results	Test Results	
				PE Extract	Ethanol Extract
1.	Flavonoids	Mg powder, concentrated HCl, and amyl alcohol	Red and orange-yellow	(+)	(+)
2.	Phenolic	FeCl 1 %	Blue-green to black	(-)	(+)
3.	Tannin	Gelatin 1 % dan NaCl 10 %	White/yellowish-white precipitate	(-)	(+)
4.	Alkaloids	HCL 2%, ammnonia 10%, chloroform, phosphomolybdic acid, Wagner, Mayer, and Hager	Phosphomolybdic acid: insoluble precipitate	(-)	(-)
				(+)	(-)
			Wagner : brown precipitate	(-)	(+)
			Mayer : white precipitate	(+)	(+)
			Hager : blackish-brown precipitate		
5.	Saponin	Hot water and hydrochloric acid	Stable foam forms	(-)	(-)

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No	Compound	Reagent	Literature Results	Test Results PE Extract	Ethanol Extract
6.	Essential oil	Anisaldehyde-sulfuric acid	Forms blue, green, red, and brown colors in visible light, and some compounds fluoresce in UV light. 366	(+)	(+)
7.	Steroids/Triterpenoids	Liebermann-Burchard solution, acetic anhydride, sulfuric acid	Steroids (green-blue), triterpenoids (red-purple)	(+) Steroids	(+) Triterpenoids

The color screening test results for the 96% ethanol extract showed that more compounds were attracted than those for the petroleum ether extract. The difference lies in the phenolic and tannin compounds. Both compounds tend to be polar, making them more attracted to ethanol than ether. In the flavonoid compound test, both extract samples produced a red color in the amyl alcohol layer. The color formed was due to the reduction of magnesium powder by the flavonoid compound. The red color indicates the presence of a flavonoid compound, 2,3-dihydroflavonol. The presence of a hydroxyl group in this compound makes it more polar, allowing it to be attracted to ethanol.

Flavonoids that reduce magnesium to an orange color include flavones, aurones, and chalcones. Flavonoids generally occur naturally as glycosides bound to sugars. In the petroleum ether extract of bangle, the sugar groups in the flavonoid glycosides likely detached, forming aglycones that are soluble in petroleum ether (Oktavia & Sutoyo, 2021).

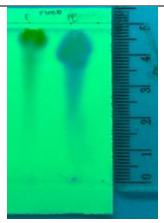
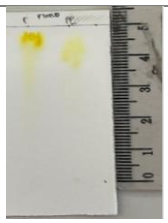
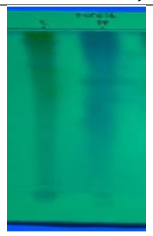
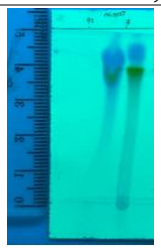
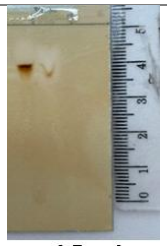
The FeCl3 reagent contains Fe+3 ions that can hybridize hydroxyl groups in phenol, forming colors such as green or blue-black (Nofita et al., 2020). Phenol compounds are generally polar due to the presence of hydroxyl groups. The greater the number of hydroxyl groups, the more polar the compound becomes, making it more attracted to ethanol solvents than petroleum ether (Manongkoa et al., 2020). Tannins are secondary metabolites with chemical properties that can bind or precipitate proteins. Gelatin is a protein used in tannin identification, forming a clump that is insoluble in water. The resulting precipitate is white or yellowish-white. The addition of sodium chloride is intended to bind compounds other than tannins and facilitate further precipitation (Kasih et al., 2022).

Alkaloids are secondary metabolites containing nitrogen. These compounds are generally basic, so in their identification, hydrochloric acid is added to form salts. The basic principle in testing alkaloid compounds involves precipitation reactions caused by ligand replacement. In alkaloid compounds, nitrogen atoms with lone electron pairs bond covalently with K+ metal ions from the reagents used. Alkaloid precipitation reactions are classified into four, group 1 reacts with silicotungstic acid, phosphomolybdic acid, or phosphotungstic acid to form insoluble salts. Group II with Bouchardat and Wagner reagents forms a free complex and then forms a brown to black precipitate. Group III can be reacted with Mayer to form a white or yellow precipitate or with Dragendorff's reagent to form a dark yellow or orange precipitate. Group IV alkaloid precipitation reactions can be added with Hager's reagent to form an insoluble organic acid bond. A sample is said to contain alkaloids if at least two

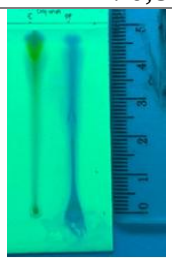
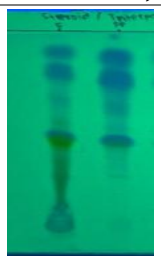
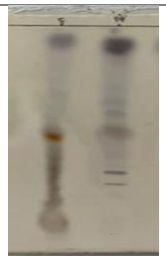
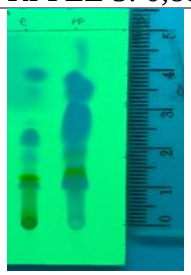
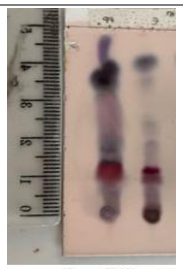
reactions from the four groups produce a precipitate (Depkes RI, 1995). Both extract samples contained alkaloids because each extract sample produced deposits in two groups of reagents.

Saponins are compounds that have hydrophilic groups (water-loving groups) and hydrophobic groups (water-hating groups). These two groups make saponins behave like soap, where the hydrophilic group binds water, and the hydrophobic group binds air, forming a soap-like foam. No positive saponin results were found in the sample (Suleman et al., 2022). The test for triterpenoid/steroid compounds is based on the compound's ability to form a color with concentrated sulfuric acid in acetic anhydride solvent. In the ethanol extract sample, the test produced a red color, while the petroleum ether extract produced a green color. The color difference is due to the difference in groups on the C-4 atom (Habibi et al., 2018). In general, rhizomes contain essential oils that give the rhizome a characteristic odor. Bangle rhizome contains essential oils of no more than 1.16% v/b with the identity compound terpinen-4-ol (Kemenkes RI, 2017), so the identification of essential oils in both extracts gave positive results. Identification of compounds in samples, in addition to using color reactions or precipitation, can also be performed using thin-layer chromatography to separate compounds from their mixtures. The results of the thin-layer chromatography identification of the 96% ethanol extract and the petroleum ether extract are presented in Table 3.

Table 3. Thin-Layer Chromatography Identification Results of Petroleum Ether Extract (PEE) and 96% Ethanol Extract (EE) of Bangle Rhizome

No	Compound	Eluent and Spot Appearance	Test Results	
1.	Flavonoids	Acetic acid: water: butanol (1:2:6) Spot Appearance: ammonia vapor (Yellow stain)	 Rf EE : 0,93 Rf PEE : 0,87	 Rf EE : 0,93 Rf PEE : 0,87
2.	Phenolics	Ethyl acetate: methanol: water (100:13.5:10) Spot Appearance = FeCl3 5% (Blue-black stain)	 Rf EE : 0,94 Rf PEE : 0,92	 Rf EE : 0,96 Rf PEE : -
3.	Tannins	Toluene: acetone: formic acid (6:6:1) Spot Appearance = FeCl3 5% (Blue-black green stain)	 Rf EE 1 : 0,78 Rf EE 2 : 0,88 Rf PEE 1 : 0,72 Rf PEE 2 : 0,84	 Rf EE : 0,78 Rf PEE : 0,72

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No	Compound	Eluent and Spot Appearance	Test Results	
4.	Alkaloids	Methanol : ammonia (100:1.5) Spot Appearance = Dragendorff (red or brown stain)	 Rf EE : 0,86 Rf PEE : 0,84	 Rf EE : 0,84 Rf PEE : -
5.	Saponin	Chloroform: methanol: water (64:50:10) Spot Appearance = anisaldehyde-sulfuric acid for detection and heated on a hot plate at 100℃ (yellow stain)	 Rf EE 1 : 0,88 Rf EE 2 : 0,94 Rf PEE : 0,92	 Rf EE 1 : 0,88 Rf EE 2 : 0,98 Rf PEE : 0,96
6.	Steroid/Triterpenoid	Toluene: chloroform: methanol in a ratio of (40:40:10) Spot Appearance = anisaldehyde-sulfuric acid and heated on a hot plate at 100℃ (green, blue, red, and purple stains)	 Rf EE1: 0,42 Rf EE2: 0,48 Rf EE3: 0,66 Rf EE4: 0,80 Rf EE5: 0,90 Rf PEE 1: 0,42 Rf PEE 2 : 0,48 Rf PEE 3: 0,62 Rf PEE 4: 0,76 Rf PEE 5: 0,88	 Rf EE1: 0,42 Rf EE2: 0,48 Rf EE3: 0,92 Rf PEE 1: 0,18 Rf PEE 2: 0,24 Rf PEE 3: 0,46 Rf PEE 4: 0,54 Rf PEE 5: 0,60 Rf PEE 6: 0,90
7.	Essential Oil	Benzene : chloroform (1:1) Spot Appearance = anisaldehyde-sulfuric acid and heated on a hot plate at 100℃ (blue, green, red, and brown stains)	 Rf EE1: 0,22 Rf EE2: 0,26 Rf EE3: 0,36 Rf EE4: 0,46	 Rf EE1: 0,20 Rf EE2: 0,22 Rf EE3: 0,26 Rf EE4: 0,36

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No	Compound	Eluent and Spot Appearance	Test Results	
			Rf EE5: 0,78 Rf PEE 1: 0,22 Rf PEE 2: 0,26 Rf PEE 3: 0,54 Rf PEE 4: 0,70	Rf EE5: 0,46 Rf EE6: 0,70 Rf EE7: 0,80 Rf PEE 1: 0,22 Rf PEE 2 : 0,36 Rf PEE 3: 0,76

Thin-layer chromatography (TLC) results on the bangle extract sample revealed that almost all compounds exhibited multiple spots. The large number of spots produced by the TLC test indicates the number of compounds eluted in the eluent with varying polarities. Furthermore, the extract sample is complex and contains a variety of compounds, resulting in a greater number of spots eluted by TLC.

The compounds found in the bangle extract were tested against S. aureus and E. coli to determine the ability of the secondary metabolites in the bangle rhizome to inhibit bacterial growth. The antibacterial activity test used the well diffusion method. This method utilizes wells as sample holders, allowing for a larger sample volume, resulting in higher sensitivity. Furthermore, well diffusion allows compounds to diffuse laterally and downward, rather than solely to the surface of the medium, resulting in a more symmetrical zone of inhibition, making it easier to measure (Alouw et al., 2022). The results of the antibacterial activity test for the bangle rhizome extract sample are presented in Table 4 below.

Table 4. Results of Antibacterial Activity Testing of Bangle Rhizome Extract

	Inhibition Zone (cm)							
Sample	Petroleum ether extract				Ethanol 96 % extract			
Bacteria	1 %	5%	10%	K+	1%	5%	10%	K+
Staphylococcus aureus	1,38±0,155	1,58±0,138	1,72±0,129	1,84±0,067	0	1,39±0,136	1,63±0,150	1,79±0,066
Eshericia coli	1,16±0,123	1,37±0,212	1,52±0,238	1,96±0,533	1,30±0,191	1,51±0,145	1,54±0,159	2,62±0,386

Based on SPSS analysis, the non-parametric test from Table 4 shows that the activity of 96% ethanol extract and petroleum ether on S. aureus bacteria is significantly different compared to the activity on E. coli bacteria with a sig. <0.05. Between ethanol and petroleum ether extracts provide significantly different activity against one type of bacteria (sig. <0.05). This shows that different types of bacteria from the Gram group and types of solvents with different polarities can affect the ability of the extract to inhibit bacteria. This significant difference can occur due to differences in dissolved compounds in petroleum ether and ethanol. Petroleum ether solvents are solvents that do not have polarity, which are able to attract non-polar compounds such as alkaloids, essential oils, steroids, and flavonoids that have been

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released from sugar as aglycones. Polar solvents will attract polar compounds such as phenols, tannins (Lingga et al., 2015).

The activity test of petroleum ether extract of bangle rhizome on S.aureus is larger than the inhibition zone on E.coli bacteria, this can occur because petroleum ether is non-polar, which will attract nonpolar compounds. These compounds more easily penetrate the cytoplasmic lipid layer in S.aureus because it is not blocked by the outer membrane like lipopolysaccharide. Lipopolysaccharide in E.coli is able to protect bacteria, making them more resistant to antibiotics. This is in line with a similar study conducted (Buldani et al., 2017) using bangle rhizome extract using Gram-positive S.aureus bacteria and Gram-negative V. cholerae, which provided greater inhibitory activity on Gram-positive bacteria. Research (Lingga et al., 2015) on torch ginger stem extract, which has almost the same active compound characteristics as bangle rhizome, showed greater inhibitory activity on S. aureus compared to E. coli. Testing the activity of ethanol extract on S. aureus yielded a smaller inhibition zone than on E. coli. This may be because S. aureus is a Gram-positive bacterium with a cell wall containing a relatively thick peptidoglycan content, making it more difficult for compounds found in the bangle rhizome extracted with ethanol to pass through it (Sida & Kasmawati, 2024). E. coli is a Gram-negative bacterium with a cell wall layer consisting of an outer membrane containing porins and lipopolysaccharides (LPS). Porins in the outer membrane allow polar compounds to penetrate the bacterial cell wall (Choi & Lee, 2019).

Flavonoids are responsible for antibacterial activity; these compounds inhibit bacterial growth by inactivating enzymes in the cell membrane, causing damage to the membrane. When the bacterial cell wall and cytoplasmic membrane become unstable, the selective permeation function of the bacterial cell, active transport function, and structural control of proteins are destroyed, macromolecules and ions are lost from the cell, and the bacterial cell's shape collapses. Flavonoid compounds can damage the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA (Liu et al., 2025).

Essential oils play a crucial role in inhibiting membrane and cell wall formation in bacteria, resulting in imperfect bacterial cell formation (Sefriyanti et al., 2020). Steroids/Triterpenoids can cause bacterial cell walls to form strong polymer bonds and lead to liposome leakage, which can lead to bacterial cell wall lysis and death (Widowati, 2022). Alkaloids can inhibit peptidoglycan formation in bacterial cell walls, resulting in suboptimal cell wall formation (Tjandra et al., 2020).

CONCLUSION

Petroleum ether and 96% ethanol extracts of bangle rhizomes exhibited significantly different antibacterial activity against the Gram-positive S. aureus and Gram-negative E. coli bacteria. Further research can be conducted using bangle rhizome samples in the form of fractions or compound isolates.

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REFERENCES

Alouw, G. E. c, Fatimawali, & Lebang, J. S. (2022). *Antibacterial Activity Test Of Ethanol Extraction From Jamaican Cherry Leaves (Muntingia Calabura L.) On Staphylococcus Aureus And Pseudomonas Aeruginosa Bacteria Using Well Diffusion Method*. *5*(1), 36–

44.

Artati, D., & Oman, M. (2019). Identifikasi bakteri melalui penggunaan Kit Analytical Profile Index (API) 20E. *Buletin Teknik Litkayasa Akuakultur*, *17*(2), 149–153.

Buldani, A., Yulianti, R., & Soedomo, P. (2017). Uji efektivitas ekstrak Rimpang Bangle (Zingiber Cassumunar Roxb .). *Prosiding Seminar Nasional IPTEK Terapan (SENIT) 2017*, 229–238.

Chambers, H. F., & Fowler, V. G. (2024). Intertwining clonality and resistance: Staphylococcus aureus in the antibiotic era. *Journal of Clinical Investigation*, *134*(19). https://doi.org/10.1172/JCI185824

Choi, U., & Lee, C. (2019). *Distinct Roles of Outer Membrane Porins in Antibiotic Resistance and Membrane Integrity in Escherichia coli*. *10*(April), 1–9. https://doi.org/10.3389/fmicb.2019.00953

Daryanti, E. P., Alfiah, F. B., & Melatiara, D. A. (2023). Perbandingan Skrining Fitokimia Esktrak Etanol Rimpang Bangle (Zingiber purpureum) Metode Maserasi dan Refluks. *Borneo Journal of Pharmascientech*, *7*(2), 52–58. https://doi.org/10.51817/bjp.v7i2.479

Depkes RI. (1995). Materia Medika Jilid VI. In . *Jakarta: Departemen Kesehatan Republik Indonesia.*

Fahrurroji, A., & Riza, H. (2020). Karakterisasi Ekstrak Etanol Buah Citrus amblycarpa (L), Citrus aurantifolia (S.), dan Citrus sinensis (O.). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *7*(2), 100. https://doi.org/10.20473/jfiki.v7i22020.100-113

Fajriaty, I., I H, H., Andres, & Setyaningrum, R. (2018). Skrining Fitokimia Lapis Titpis Dari Ekstrak Etanol Daun Bintangur (Calophyllum soulattri Burm . F .). *Jurnal Pendidikan Informatika dan Sains*, *7*(1), 54–67.

Firnando, H., Purwanti, N. U., & Susanti, R. (2019). Penetapan Kadar Total Fenolik dan Flavonoid Fraksi Butanol dan Fraksi Air Dari Ekstrak Etanol Rimpang Acorus Sp. *Jurnal Mahsiswa Farmasi Fakultas Kedokteran*, *4*(1), 1–13.

Giannattasio-Ferraz, S., Ene, A., Gomes, V. J., Queiroz, C. O., Maskeri, L., Oliveira, A. P., Putonti, C., & Barbosa-Stancioli, E. F. (2022). Escherichia coli and Pseudomonas aeruginosa Isolated From Urine of Healthy Bovine Have Potential as Emerging Human and Bovine Pathogens. *Frontiers in Microbiology*, *13*(March), 1–11. https://doi.org/10.3389/fmicb.2022.764760

Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. *Frontiers in Cellular and Infection Microbiology*, *10*(March), 1–11. https://doi.org/10.3389/fcimb.2020.00107

Habibi, A. I., Firmansyah, R. A., & Setyawati, S. M. (2018). Skrining Fitokimia Ekstrak n-Heksan Korteks Batang Salam (Syzygium polyanthum). *Indonesian Journal of Chemical Science*, *7*(1), 1–4.

Hanani, E. (2015). *Analisis Fitokimia*. Buku Kedokteran EGC.

Kasih, D., Salsabila, I., Aulia, S., Wulan, E., & Yumareta. (2022). *Identifikasi Tanin pada Tumbuh-tumbuhan di Indonesia*. *03*, 11–24.

Kemenkes RI. (2017). Farmakope Herbal Indonesia Edisi II, Departemen Kesehatan Republik Indonesia, Jakarta. In *Kemenkes RI*. https://doi.org/10.1201/b12934-13

Khairunnisa;, Ramadhani, Y., & Sapada, I. E. (2025). *Studi Efektivitas Antibakteri Ekstrak Etanol Rimpang Bangle (Zingiber Purpureum Roxb) Terhadap Patogen Shigella Dysenteriae*. 74–85.

Kusumo, P., Biyono, S., & Tegar, S. (2020). *Isolasi Lignin dari Serbuk Grajen Kayu Jati (Tectona Grandis) dengan Metode Klasson*. *19*(02), 130–139.

Lingga, A. R., Pato, U., & Rossi, and E. (2015). Uji Antibakteri Ekstrak Batang Kecombrang

(Nicolaia speciosa Horan) Terhadap Staphylococcus aureus dan Escherichia coli. *JOM Faperta*, *2*. http://www.tjyybjb.ac.cn/CN/article/downloadArticleFile.do?attachType=PDF&id=9987

Liu, Y., Zhu, J., Liu, Z., Zhi, Y., & Mei, C. (2025). *Flavonoids as Promising Natural Compounds for Combating Bacterial Infections*. 1–20.

Manongkoa, P. S., Sangia, M. S., & Momuat, L. I. (2020). *Uji Senyawa Fitokimia dan Aktivitas Antioksidan Tanaman Patah Tulang (Euphorbia tirucalli L .)*. *9*(2), 64–69.

Mu'awwanah, A., & Ulfa, M. (2015). Uji Aktivitas Antioksida Fraksi N-heksan Ekstrak Etanol Daun Karika (Carica pubescens) Dan Identifikasi Senyawa Alkaloid Dan Flavonoid. *Prosiding Seminar Nasional Peluang Herbal Sebagai Alternatif Medicine*, *2012*, 118–124.

Nofita, D., Sari, S. N., & Mardiah, H. (2020). *Penentuan Fenolik Total dan Flavonoid Ekstrak Etanol Kulit Batang Matoa (Pometia pinnata J.R& G.Forst) secara Spektrofotometri*. *8*(1), 36–41.

Noli, Z. A., & Idris, M. (2023). *Jurnal Biologi Tropis Application of Portulaca oleracea L . Extract as a Biostimulant with Several Types of Solvents on The Growth of Kale (Brassica oleracea var . achepala)*.

Nurhidayati, S., Faturrahman, F., & Ghazali, M. (2015). Deteksi Bakteri Patogen Yang Berasosiasi Dengan Kappaphycus Alvarezii (Doty) Bergejala Penyakit Ice-Ice. *Jurnal Sains Teknologi & Lingkungan*, *1*(2), 24–30. https://doi.org/10.29303/jstl.v1i2.53

Oktavia, F. D., & Sutoyo, S. (2021). *Skrining Fitokimia, Kandungan Flavonoid Total, Dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan Selaginella doederleinii*. *6*(2), 141–153.

Rahardhian, M. R. R., Aryanti, N., Susilawati, Y., Sumiwi, S. A., Putri, C. N., Ramonah, D., & Suharsanti, R. (2025). Exploring the α-Amylase Inhibitory Potential of Peronema canescens Jack: An In Vitro and In Silico Study. *Hayati Journal of Biosciences*, *32*(3), 712–726. https://doi.org/10.4308/hjb.32.3.712-726

Rajagopal, M., & Walker, S. (2018). Envelope Structures of Gram-Positive Bacteria Mithila. *Assessment & Evaluation in Higher Education*, *404: 1–44*.(January), 44. https://doi.org/10.1007/82

Sari, A. P., Rahardjo, B., & Purwijantiningsih, E. (2016). *Aktivitas Antibakteri Ekstrak Etanol Dan Heksan Daun Bangle (Zingiber Cassumunar Roxb.) Terhadap Bakteri Eschericia Coli Dan Staphylococcus aureus*.

Sefriyanti, Jayuska, A., & Alimuddin, A. H. (2020). *Uji Aktivitas Antibakteri Minyak Atsiri Serai Wangi (Cymbopogon bernadus L .) Terhadap Bakteri Escherichia coli Dan Staphylococcus aureus*. *8*(4), 1–4.

Setyani, A. R., Arung, E. T., Biologi, J., Mulawarman, U., Kehutanan, F., Mulawarman, U., & Mulawarman, U. (2021). *Skrining Fitokimia, Antioksidan Dan Aktivitas Antibakteri Ekstrak Etanol Akar Segar Bangle (Zingiber montanum)*. *15*(2), 415–427.

Sida, N. A., & Kasmawati, H. (2024). *Aktivitas Antibakteri Sansevieria trifasciata Prain. Menggunakan KLT Bioautografi*. *2*(1), 22–35. https://doi.org/10.33772/lansau.v2i1.22

Suleman, I. F., Sulistijowati, R., Manteu, S. H., & Nento, W. R. (2022). *Identifikasi Senyawa Saponin Dan Antioksidan Ekstrak Daun Lamun (Thalassia hemprichii)*. *4*(2), 94–102.

Tjandra, R. F., Fatimawali, & Datu, O. S. (2020). *Analisis Senyawa Alkaloid dan Uji Daya Hambat Ekstrak Buah Sirih (Piper betle L) terhadap Bakteri Staphylococcus epidermidis*. *8*(2), 173–179.

Widowati, R. (2022). *Senyawa Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Buah Lerak (Sapindus rarak) terhadap Tiga Bakteri Penyebab Infeksi Nosokomial*. *13*(1), 649–654.

Wulandari, & Sulistyarini, I. (2018). Antibacterial activity test of extract ethanol mango arumanis skin (Mangifera indica L) on Methicillin Resistant Staphylococcus aureus (MRSA). *Media Farmasi Indonesia*, *13*(2), 1347–1353.

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