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Antibacterial Test of Bangle Rhizome Essential Oil (*Zingiber Purpureum* Roscoe) Originated from East Kalimantan Against *Cutibacterium acnes* and *Staphylococcus epidermidis in Vitro*

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Abstract: Two bacteria, *Cutibacterium acnes* and *Staphylococcus epidermidis*, contribute to the pathophysiology of acne vulgaris and have led to resistance. Therefore, a new antibacterial agent is needed that is not resistant to both bacteria, namely the rhizome of *Zingiber purpureum* Roscoe (Bangle) from East Kalimantan. The purpose of this study is to examine the antibacterial activity of *Zingiber purpureum* Roscoe rhizome essential oil from East Kalimantan and the optimal concentration that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing *in vitro*. This research used a laboratory experimental study and Kirby-Bauer disc diffusion method. A posttest only control group as a study design in this study. This study used four concentrations of *Zingiber purpureum* Roscoe rhizome essential oil (25%; 50%: 75%; and 100%), Chloramphenicol 30µg/disk as a positive control, and tween 80 as a negative control. The result of the inhibition zone on *Cutibacterium acnes* was 5.6 mm (25% concentration), 5.8 mm (50% concentration), 7.5 mm (75% concentration), and 7.8 mm (100% concentration). Meanwhile, the inhibition zone on *Staphylococcus epidermidis* was 4.8 mm (25% concentration), 7.8 mm (50% concentration), 8.5 mm (75% concentration), and 9.1 mm (100% concentration). The results of the data analysis were $p>0.05$. The conclusion is that *Zingiber purpureum* Roscoe rhizome essential oil from East Kalimantan has an antibacterial activity that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing *in vitro* and optimal concentration at a concentration of 100%.

Keywords: Antibacterial, *Cutibacterium acnes*, *Staphylococcus epidermidis*, Essential Oils, *Zingiber purpureum* Roscoe, Bangle

1. Introduction

Cutibacterium acnes live in the pilosebaceous unit and contribute to the development of acne vulgaris (Kim *et al.,* 2022). In addition, many *Staphylococcus epidermidis* bacteria are also found in acne vulgaris (Dreno *et al.,* 2024). An inflammation of the skin that affects the pilosebaceous unit is called acne vulgaris (Reynolds *et al.,* 2024). Acne vulgaris can cause physical and psychological discomfort for years (Balik *et al.,* 2023). Therefore, acne vulgaris treatment is essential. One of the treatments for acne vulgaris is using antibiotics (Leung *et al.,* 2020). However, antibiotics have caused resistance to *Cutibacterium acnes* by up to 40%, thus

risking failure in treatment (Alkhawaja *et al.,* 2021; Mayslich *et al.,* 2021). *Staphylococcus epidermidis* has also been reported to cause resistance to almost all classes of antibiotics (Severn and Horswill, 2023). Therefore, acne vulgaris treatment requires new antibacterial agents known not to cause resistance to *Cutibacterium acnes* and *Staphylococcus epidermidis* bacteria.

Zingiber purpureum Roscoe (Bangle) is a plant that grows in East Kalimantan and is used as a traditional medicine (Supiandi *et al.,* 2023). Based on previous studies, it was explained that *Zingiber purpureum* Roscoe has various bioactivities, one of which is as an antibacterial (Mukti and Andriani, 2021). *Zingiber purpureum* Roscoe rhizome essential oil shows high antibacterial activity. *Zingiber purpureum* Roscoe rhizome essential oil can fight 8 different Gram-negative bacteria and 5 different Gram-positive bacteria at concentrations of 2.5-10 vol% (Tandirogang *et al.,* 2022). The isolate content in *Zingiber purpureum* Roscoe rhizome essential oil that has antibacterial activity is terpinen-4-ol, sabinene, and γ-terpinene (Cordeiro *et al.,* 2020; Pardosi *et al.,* 2022).

Based on the description above, to consider *Zingiber purpureum* Roscoe rhizome essential oil as a natural antibacterial agent, further studies are needed regarding its antibacterial activity that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing. This study will study the concentration of *Zingiber purpureum* Roscoe rhizome essential oil that has an optimal concentration that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing *in vitro.*

2. Materials and Methods

This research used a laboratory experimental study. The study method used was the Kirby-Bauer disc diffusion method. A posttest only control group as a study design in this research. This study used four concentrations of *Zingiber purpureum* Roscoe rhizome essential oil (25%; 50%: 75%; and 100%), Chloramphenicol 30µg/disk as a positive control, and tween 80 as a negative control. This study was conducted with three repetitions. This study used 6 petri dishes to obtain three repetitions of two different bacteria, namely *Cutibacterium acnes* and *Staphylococcus epidermidis*. There are six discs in one petri dish. Each petri dish was filled with four concentrations of *Zingiber purpureum* Roscoe rhizome essential oil (25%; 50%: 75%; and 100%) and two groups, namely positive control using Chloramphenicol 30µg/disk and negative control using tween 80.

The materials used in this study were *Zingiber purpureum* Roscoe rhizomes that were cut into pieces and processed into essential oil, vials, Chloramphenicol 30µg/disk as a positive control, tween 80 as a solvent and negative control, Mueller-Hinton Agar (MHA), Thioglycol sterile, 0.9% NaCl, 0.5 McFarland standard solution, cultures of *Cutibacterium acnes* and *Staphylococcus epidermidis*.

The tools used in this study were a steam and water distillation apparatus, handscoon, mask, autoclave, tube rack, test tube, sterile cotton swab, ose needle, bunsen, match, petri dish, pipette, beaker glass, incubator, vortex, vernier caliper, label, pen, disk, digital scale. *Zingiber purpureum* Roscoe rhizomes were collected and washed thoroughly. The rhizomes were sliced

3-5 mm thick and put into a distillation device and the distillation process was carried out for 7 hours. The distillation method used a steam and water distillation. The oil products are quite good (Aryani *et al.,* 2020). Then, essential oil was obtained which was still mixed with water. After that, a separating funnel was used to separate the essential oil from the water. Then, anhydrous Na2SO4 was added to the essential oil to purify it by removing any remaining water. The extracted essential oil was kept in a vial bottle covered with aluminum foil at a temperature of 4°C (Pardosi *et al.,* 2022; Astuti *et al.,* 2023).

The concentration of essential oil from *Zingiber purpureum* Roscoe rhizome was made by diluting the essential oil with tween 80 solution (Astuti *et al.,* 2023). Dilution is carried out until four essential oil concentrations are obtained, namely 25%; 50%: 75%; and 100%. Dilution is carried out in stages as follows:

a. 100% essential oil concentration
   The preparation of 100% essential oil concentration is with 5 ml of essential oil solution without tween 80 solution.
b. 75% essential oil concentration
   The preparation of 75% essential oil concentration is by dissolving 3.75 ml of 100% essential oil solution into 1.25 ml of tween 80 solution.
c. 50% essential oil concentration
   The preparation of 50% essential oil concentration is by dissolving 3.3 ml of 75% essential oil solution into 1.6 ml of tween 80 solution.
d. 25% essential oil concentration
   The preparation of 25% essential oil concentration is by dissolving 2.5 ml of 50% essential oil solution into 2.5 ml of tween 80 solution.

Inoculate 1 ose of pure cultures of *Cutibacterium acnes* bacteria into MHA. Then, inoculate 1 ose of pure cultures of *Staphylococcus epidermidis* bacteria into MHA. After that, for 24 hours, the inoculated MHA was kept in an incubator at 37°C (Wina *et al.,* 2024). *Cutibacterium acnes* was diluted by mixing 1 ose of *Cutibacterium acnes* bacterial suspension into a test tube containing sterile Thioglycolate. Then homogenized using a vortex and the turbidity is standardized with a concentration of 0.5 McFarland. The *Cutibacterium acnes* bacterial suspension is then applied to the MHA using a sterile cotton swab. A sterile 0.9% NaCl solution (10 ml) was put into another test tube. Then, *Staphylococcus epidermidis* was put into that test tube and homogenized using a vortex. Its turbidity was standardized to a concentration of 0.5 McFarland. And then, the suspension of *Staphylococcus epidermidis* bacteria was applied to the MHA using a sterile cotton swab (Wina *et al.,* 2024; Deswita *et al.,* 2021).

The discs were dipped into four concentrations of *Zingiber purpureum* Roscoe rhizome essential oil (25%; 50%: 75%; and 100%), positive control using Chloramphenicol 30µg/disc, and negative control using tween 80. Then, for 24 hours, incubated at 37°C in an incubator (Astuti *et al.,* 2023). After incubation for 24 hours, used vernier caliper to measure the inhibition zone diameter. Measurement of the inhibition zone diameter used the formula for the results of subtracting the vertical diameter and the diameter of the disc paper added to the results of

subtracting the horizontal diameter and the diameter of the disc paper and then divided by 2 (Tjiptoningsih, 2020). Data from the antibacterial study results of *Zingiber purpureum* Roscoe rhizome essential oil against *Cutibcterium acnes* and *Staphylococcus epidermidis* were analyzed using SPSS. This analysis aims to see if there are any differences produced by each disc containing various concentrations of *Zingiber purpureum* Roscoe rhizome essential oil can inhibit *Cutibcterium acnes* and *Staphylococcus epidermidis* from growing.

The first statistical test of the data was used Shapiro-Wilk to test the normality of the data with a small sample. The distribution is not normal if $p<0.05$. Conversely, the distribution is considered normal if $p>0.05$. The data in this study contained more than two unpaired groups so a parametric test was used in the form of one way ANOVA if the data is a normal distribution. However, the Kruskall-Wallis test was used as a nonparametric test in cases when the data isn't a normal distribution. If $p>0.05$ then there was no difference in the meaning of each disc. Conversely, if $p<0.05$ then there is a difference in meaning between each disc.

3. Results and Discussion

The research results data showed in the following table.

3.1 Antibacterial Test Result

3.1.1 Cutibacterium acnes

Table 1 Inhibition Zone Diameter of *Zingiber purpureum* Roscoe Rhizome Essential Oil on *Cutibacterium acnes* Growth

Sample	**Concentration**	**Inhibition Zone Diameter (mm)**			**Inhibition Zone Diameter Average (mm)**
		I	**II**	**III**	
Zingiber purpureum Roscoe rhizome essential oil	100%	9	8.6	6	7.8
	75%	9	7	6.6	7.5
	50%	9	0	8.6	5.8
	25%	9	8	0	5.6
Positive control (Chloramphenicol) 30µg/disk	-	22	22	21.6	21.8
Negative control (Tween 80)	0	0	0	0	0

The results of the calculation of *Cutibacterum acnes* are displayed in Table 1. The essential oil of *Zingiber purpureum* Roscoe rhizome at a concentration of 25% is the smallest inhibition zone diameter can be found. The largest inhibition zone diameter was found in the essential oil of *Zingiber purpureum* Roscoe rhizome with a concentration of 100%. The inhibition zone

diameter average of *Cutibacterium acnes* in the *Zingiber purpureum* Roscoe rhizome essential oil is smaller than that of Chloramphenicol.

3.1.2 Staphylococcus epidermidis

Table 2 Inhibition Zone Diameter of *Zingiber purpureum* Roscoe Rhizome Essential Oil on *Staphylococcus epidermidis* Growth

Sample	**Concentra-tion**	**Inhibition Zone Diameter (mm)**			**Inhibition Zone Diameter Average (mm)**
		Repetitions			
		I	**II**	**III**	
Zingiber purpureum Roscoe rhizome essential oil	100%	8.3	10	9	9.1
	75%	9,6	8	8	8.5
	50%	8	8	7.6	7.8
	25%	9	0	5.6	4.8
Positive control (Chloramphenicol) 30µg/disk	-	23	22	20.3	21.7
Negative control (tween 80)	0	0	0	0	0

The results of the calculation of *Staphylococcus epidermidis* are displayed in Table 2. The essential oil of *Zingiber purpureum* Roscoe rhizome at a concentration of 25% is the smallest inhibition zone diameter can be found. The largest inhibition zone diameter was found in the essential oil of *Zingiber purpureum* Roscoe rhizome with a concentration of 100%. The inhibition zone diameter average of *Staphylococcus epidermidis* in the *Zingiber purpureum* Roscoe rhizome essential oil is smaller than that of Chloramphenicol.

3.2 Data Analysis Result

3.2.1 Cutibacterium acnes

Table 3 Shapiro-Wilk Test Results *Cutibacterium acnes*

Measurement	Group	Normality Test
Inhibition Zone Diameter	100% Concentration	0.235
	75% Concentration	0.298
	50% Concentration	0.075
	25% Concentration	0.194

Table 3 displays the normality test result, which indicate a p value > 0.05. This means that the data distribution is normal. Because the data distribution is normal, then we use the one-way ANOVA parametric test on the *Cutibacterium acnes* inhibition zone diameter data.

Table 4 One Way ANOVA Test Results for *Cutibacterium acnes*

Measurement	Group	P value	Description
Inhibition Zone Diameter	100% Concentration	0.839	Not significantly different
	75% Concentration		
	50% Concentration		
	25% Concentration		

The one-way ANOVA test result in Table 4 show a p value> 0.05, this means that the inhibition zone diameter at each concentration of Zingiber purpureum Roscoe rhizome essential oil (25%; 50%: 75%; and 100%) has no significant difference in inhibits *Cutibacterium acnes* from growing. From the p value, it can be concluded that, although the concentration of *Zingiber purpureum* Roscoe rhizome essential oil is increased, the diameter of the resulting inhibition zone does not significantly different.

3.2.2 Staphylococcus epidermidis

Table 5 Shapiro-Wilk Test Results *Staphylococcus epidermidis*

Measurement	Group	Normality Test
Inhibition Zone Diameter	100% Concentration	0.806
	75% Concentration	0.000
	50% Concentration	0.000
	25% Concentration	0.732

The normality test in Table 5 show a p value > 0.05 at concentrations of 100% and 25%, this means that the data distribution is normal. However, at concentrations of 75% and 50%, the p value <0.05 means that the data distribution is not normal. Because the data distribution is not normal, then we use the Kruskal-Wallis non-parametric test on the *Staphylococcus epidermidis* inhibition zone diameter data.

Table 6 *Kruskal-Wallis* Test Results *Staphylococcus epidermidis*

Measurement	Group	P value	Description
Inhibition Zone Diameter	100% Concentration	0.176	Not significantly different
	75% Concentration		
	50% Concentration		
	25% Concentration		

The results of the Kruskal-Wallis test in Table 6 show a p value > 0.05, this means that the inhibition zone diameter at each concentration of Zingiber purpureum Roscoe rhizome essential oil (25%; 50%: 75%; and 100%) has no significant difference in inhibits *Staphylococcus epidermidis* from growing.. From the p value, it can be concluded that, although the concentration of *Zingiber purpureum* Roscoe rhizome essential oil is increased, the diameter of the resulting inhibition zone does not significantly different.

3.3 Interpretation of Results

This study shows antibacterial activity that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing by finding the inhibition zone diameter after treatment. This is indicated by an inhibition zone formed around the disc paper. The statement is in line with the explanation of Mukti and Andriani (2021), that the *Zingiber purpureum* Roscoe rhizome has bioactivities such as anti-inflammatory, antibacterial, and antifungal. The study of Pardosi *et al* (2022) also showed that the *Zingiber purpureum* Roscoe rhizome essential oil can inhibit *Streptococcus mutans* bacteria. This is supported by Astuti *et al* (2023), that the *Zingiber purpureum* Roscoe rhizome essential oil has antibacterial bioactivity against *Porphyromonas gingivalis* bacteria.

Thus, Zingiber purpureum Roscoe rhizome essential oil from East Kalimantan shows antibacterial activity that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing in vitro. This study is in line with Tandirogang *et al* (2022), that the Zingiber purpureum Roscoe rhizome essential oil can fight *Staphylococcus epidermidis* bacteria with a diameter of 8.3 mm at a concentration of 10% which has maximum inhibitory power.

The inhibition zone diameter of *Zingiber purpureum* Roscoe rhizome essential oil increased with increasing concentration of the tested essential oil. This was proven at a concentration of 100% which had the largest inhibition zone diameter average, while a concentration of 25% had the smallest average inhibition zone in this study. Therefore, antibacterial effectiveness of *Zingiber purpureum* Roscoe rhizome essential oil increases with its concentration. This is because the more Zingiber purpureum Roscoe rhizome essential oil present, the higher the amount of antibacterial isolate. This is supported by Pardosi *et al* (2022), who stated that the essential oil of the *Zingiber purpureum* Roscoe rhizome can inhibit the bacteria from growing with a minimum inhibitory concentration of 3.12%, which is the smallest concentration, and the optimal inhibitory concentration at a concentration of 50%, which is the largest concentration.

Devkota *et al* (2021) found that by using GC-MS analysis, the essential oil of Zingiber purpureum Roscoe rhizomes contains terpinen-4-ol; sabinene; γ-terpinene; triquinacene 1,4-bis

(methoxy); α-terpinene; β- phellandrene; and (Z)-ocimene. The isolate content in *Zingiber purpureum* Roscoe rhizome essential oil that has antibacterial activity is terpinen-4-ol, sabinene, and γ-terpinene (Cordeiro *et al.*, 2020; Pardosi *et al.,* 2022). According to Devkota *et al*. (2021), sabinene is the most abundant isolate in the essential oil of *Zingiber purpureum* Roscoe rhizome, reaching 27-34%. Sabinene has antibacterial activity because it can inhibit growth, biofilm formation, and bacterial adhesion by inhibiting bacterial virulence factors. This substance possesses anti-inflammatory qualities that preventing bacterial lipopolysaccharides (LPS) from producing nitric oxide (Pardosi *et al.,* 2022).

Terpinen-4-ol is the second most abundant isolate content in *Zingiber purpureum* Roscoe rhizome essential oil, reaching 30-5% (Devkota *et al.,* 2021). Terpinen-4-ol is a monoterpene and has extraordinary antibiofilm activity because it can inhibit the formation of bacterial biofilms even at subinhibitory concentrations. Terpinen-4-ol isolate is bactericidal and has lipophilic properties that can inhibit peptidoglycan synthesis, thereby damaging the structure of phospholipid cell membranes and inhibiting cell respiration. In addition, terpinen-4-ol can increase the fluidity and permeability of cell membranes. This can cause ion leakage and weaken the role of the cell membrane to act as a selective barrier. Terpinen-4-ol also works by inhibiting bacterial DNA and protein synthesis, affecting cell metabolism, disrupting enzymatic systems, inducing coagulation of cytoplasmic components, disrupting genetic synthesis, forming toxic compounds, disrupting cell communication, and reducing the pH inside cells so that it can cause bacterial death (Cordeiro *et al.*, 2020).

Isolate γ-terpinene in essential oil of *Zingiber purpureum* Roscoe rhizome has a content of 6-8% (Devkota *et al.,* 2021). γ-terpinene has antibacterial properties because it can cause cellular protein leakage in bacterial. Protein leakage can be used as an indicator of bacterial membrane damage caused by chemical and physical agents. In addition, γ-terpinene can damage the lipid layer of the outer membrane of cells due to its phenolic structure, which can cause bacterial death (Pardosi *et al.,* 2022).

Staphylococcus epidermidis and *Cutibacterium acnes* are Gram-positive bacteria. They have thick peptidoglycan in their cell walls. *Cutibacterium acnes* is an aerotolerant anaerobic bacteria that can protect itself from surrounding oxygen molecules. Oxygen can survive on the surface of *Cutibacterium acnes* skin because it has enzymes that can detoxify oxygen (Mayslich *et al.*, 2021). Meanwhile, *Staphylococcus epidermidis* is a facultative anaerobic bacteria. They can live in both aerobic and anaerobic conditions (Kaplan, 2023). The cell wall structure of *Staphylococcus epidermidis* and *Cutibacterium acnes* does not have an outer membrane, allows hydrophobic molecules such as essential oils from *Zingiber purpureum* Roscoe rhizome can penetrate cells. Then the essential oil works on the cell wall and in the bacterial cytoplasm. Meanwhile, Gram-negative bacteria have an outer membrane on the outer layer and a thinner peptidoglycan. The outer membrane contains various proteins and lipopolysaccharides. Lipopolysaccharides make Gram-negative bacteria resistant to essential oil. Thus, Gram-negative bacteria are more able to survive than Gram-positive bacteria (Tandirogang *et al.,* 2022; Kim *et al.,* 2023).

4. Conclusion

The conclusion is that the essential oil of *Zingiber purpureum* Roscoe rhizome originated from East Kalimantan has an antibacterial activity that inhibits *Cutibacterium acnes* and *Staphylococcus epidermidis* from growing in vitro and optimal concentration at a concentration of 100%.

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Conflict of Interest

The content of the manuscript is approved by each author.

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