

## ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT AND VOLATILE OIL OF LAJA GOWAH RHIZOME (*ALPINIA MALACCENSIS* BURM.F.) ROSCOE) AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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### ABSTRACT

**Objective:** Laja Gowah is one of the herbs that can be used to treat gastrointestinal problems. The aim of this research is characterization, phytochemical screening of simplex and extracts and testing antibacterial activity of ethanol extract and volatile oil of Laja Gowah rhizome against *Staphylococcus aureus* and *Escherichia coli*.

**Methods:** Simplex were characterized, phytochemical screening, and extracted by percolation with ethanol 96% as solvent, and volatile oil was extracted by steam distillation and the antibacterial activity against *S. aureus* and *E. coli* carried out by agar diffusion method.

**Results:** The characteristic of simplex were 7.50% for water content, water-soluble extract 29.69%, ethanol soluble extract 19.30%, total ash 3.97%, and acid insoluble ash 0.38%. The phytochemical screening of simplex and ethanol extract were showed the presence of alkaloids, flavonoids, glycosides, steroids/triterpenoids, and saponins. Antibacterial activity showed that ethanol extract and essential oils effective as antibacterial against *S. aureus* and *E. coli*.

**Conclusion:** Based on the analysis above, that shows that extract and volatile oils rhizome Laja Gowah provide effective activity as anti-bacteria against *S. aureus* and *E. coli*.

**Keywords:** Laja Gowah rhizome, Antibacterial, Ethanol extract, Volatile oil, *Staphylococcus aureus*, *Escherichia coli*.

### INTRODUCTION

Indonesia has a tropical climate and fertile land that can be covered by various types of plants. Some types of these plants have medicinal properties. Most of the plants are still unknown properties that were never well maintained and life as a wild plant [1,2].

The use of medicinal plants can support the need for drugs are increasingly urgent and to get a safe substitute drugs if modern medicine becomes resistance [3].

One of the medicinal plants that can be used for the treatment is Laja Gowah. In Ambon, the Laja Gowah rhizome is used to maintain the throat, so sounds remain excellent. In addition of Laja Gowah rhizomes are also often used to treat colic, ulcers, and sores and medicine for tonic [4]. Laja Gowah plant (*Alpinia malaccensis* (Burm.f.) Roscoe) for treating abdominal pain was also used by the community Namo Rambe, Deli Serdang, North Sumatera Province.

Laja Gowah for abdominal pain medications used  $\pm 10$  g of fresh ginger *A. malaccensis*, washed, shredded, add  $\frac{1}{2}$  cup of boiled water and filtered. Distillate drunk at once [4].

*Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) are pathogenic bacteria that can infect the gastrointestinal tract. *S. aureus* can produce enterotoxins which can cause stomach upsets. *E. coli* is a normal flora found in the large intestine. If these bacteria get into the small intestine are pathogenic and can cause diarrhea [5-7].

Based on the narrative and experience possessed by the community, then the test research on the antibacterial activity of ethanol extract, Laja Gowah rhizome on the growth of *S. aureus* and *E. coli*. This research includes characterization simplex, phytochemical screening, and

extraction of Laja Gowah rhizome by percolation method with ethanol 96%. The ethanol extract and volatile oil of Laja Gowah rhizomes obtained and tested for antibacterial activity.

### METHODS

#### Preparation of ethanol extract

350 g of powder simplex were soaked 3 hrs in a glass container, and then enter it into the percolator for 24 hrs, and then extracted to obtain perfect percolate. Percolate was deposited for 24 hrs, The filtrate was collected, and then evaporated under reduced pressure to give a viscous extract and then freeze dried to give a dried extract [8-10].

#### Characterization and phytochemical screening

Characterization of simplex includes determination of water content, determination of water-soluble content, determination of the ethanol-soluble content, total ash content, and ash-not dissolve in acid content. Determining the phytochemical screening carried out on simplex and extract [8].

#### Preparation of ethanol extract test solution

The ethanol extract of Laja Gowah rhizomes was weighed 1 g and then reconstituted with the solvent dimethyl sulfoxide (DMSO) up to 2 ml of the extract concentration is 500 mg/ml then made dilution. Furthermore, solution was diluted with solvent DMSO back to get a concentration of 400 mg/ml, 300 mg/ml, 200 mg/ml, and 100 mg/ml [2].

#### Preparation of essential oils test solution

A total of 0.5 ml of the essential oil of rhizomes Laja Gowah was diluted with ethanol up to 10 ml of the extract concentration is 5% then was made further dilution obtain the essential oil with a concentration of 2.0%, 1.5%, 1.0%, and 0.5%. Then was inserted into the paper discs with different concentrations of the extract [2,11].

**Antibacterial activity test**

0.1 ml inoculum was put into a sterile petri dish, then pour the media Muller-Hinton order for as many as 15 ml with a temperature of 45-50°C. Petri dish was rocked on the surface of the table so that the media and the bacterial suspension well mixed and allowed to solidify. Testing the antibacterial activity with a paper disc diffusion method was by putting a paper dish that has been soaked in some concentration of the test solution of ethanol extract/essential oil over solid media inoculated bacteria and allowed 15 minutes, then incubated in an incubator at a temperature of 36 ±1°C for 18-24 hrs, then measured the diameter of the area barriers (clear zone) growth around the disc by using a caliper [2].

**RESULTS AND DISCUSSION**

Characteristic of Laja Gowah rhizome can be seen in Table 1.

Phytochemical screening result of simplex, ethanol extract Laja Gowah rhizomes can be seen in Table 2.

**Extraction results**

The extraction of 350 g Laja Gowah rhizome by means of percolation using ethanol 96%. Ethanol extract of the rhizomes obtained as much as 42.30 g. Ethanol extract, obtained phytochemical screening and then tested antibacterial activity against *S. aureus* and against *E. coli*.

**Result of antibacterial activity test of extract ethanol Laja Gowah rhizome**

The results of antibacterial activity test of ethanol extracts of Laja Gowah rhizome can be seen in Tables 3 and 4.

In Table 3 and 4 appears that the concentration fills the requirements which determined by the Directorate General of POM (1995), with effective border barriers approximately 14-16 mm.

Based on the measurement results showed that the barrier region diameter EERLG provide antibacterial activity, demonstrated the inhibition of the growth of *St. aureus* and *E. coli* at a concentration of 300 mg/ml provide inhibition zone diameter 14.57±0.1 mm [11,12]. Flavonoids are a group of phenolic compounds have a tendency to bind to proteins, thereby disrupting the bacterial metabolism, but it also serves as antibacterial flavonoids by forming complex compounds against extracellular proteins that disrupt the bacterial cell membrane integrity. Polyphenols at high levels can cause coagulation proteins and cause cell membranes undergo lysis [13].

Alkaloid has the ability as an antibacterial. Suspected mechanism is to disrupt peptidoglycan component of the cell bacteria so that the cell wall layers are not fully formed and caused the death of these cells [14]. The results showed that the *S. aureus* bacterium has a zone of inhibition greater than the bacterium *E. coli* in various concentrations of the test solution. According to [15,16], these differences occur because both the test bacteria have the composition and structure of different cell wall. Resulting in Gram-positive bacteria are more susceptible to chemical compounds compared to Gram-negative. The structure of the cell wall of Gram-positive bacteria is more simple, single-layered with a low lipid content (1-4%), making it easier bioactive material into the cell. The structure of the cell wall of Gram-negative bacteria is more complex, which consists of a three-layered outer layer lipoprotein, lipopolysaccharide middle layer which acts as a barrier to the entry of antibacterial bioactive materials and in the form of the peptidoglycan layer with high lipid content (11-12%).

**Result of antibacterial activity test of essential oils Laja Gowah rhizome**

Results of antibacterial activity test of essential oils of Laja Gowah rhizome can be seen in Tables 5 and 6.

In Tables 6 and 7 shows that the essential oil of barriers Laja Gowah rhizome effective zone of approximately 14-16 mm. The test results

**Table 1: Characteristic of rhizome Laja Gowah**

Parameter	Concentration (%)
Water content	7.50
Water soluble extract	29.69
Ethanol soluble extract	19.30
Total ash	3.97
Acid insoluble ash	0.38

**Table 2: Results of phytochemicals screening of simplex and extract Laja Gowah rhizome**

Parameter	Simplex extract	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Anthraquinone glycosides	-	-
Saponins	+	+
Tannins	-	-
Steroids/triterpenoids	+	+

+: Contains a class of compounds, - Not contain groups of compounds

**Table 3: Results of the measurement diameter zone of growth inhibition of *S. aureus***

Concentration (mg/ml)	Diameter inhibition) zone (mm)* ethanol extract Laja Gowah rhizome
500	16.07±0.21
400	15.30±0.13
300	14.57±0.10
200	13.93±0.25
100	13.10±0.16
Blank (DMSO)	-

DMSO: Dimethyl sulfoxide, *S. aureus*: *Staphylococcus aureus*

**Table 4: Results of the measurement diameter zone of growth inhibition of *E. coli***

Concentration (mg/ml)	Diameter inhibition zone (mm)* ethanol extract Laja Gowah rhizome
500	15.87±0.22
400	15.17±0.21
300	14.33±0.17
200	13.27±0.28
100	12.17±0.15
Blank (DMSO)	-

D\*: Diameter of the average zone of bacterial growth inhibition, -: Not available bacterial growth inhibition zone, DMSO: Dimethyl sulfoxide, *E. coli*: *Escherichia coli*

**Table 5: Diameter inhibitory essential oils against *S. aureus***

Concentration (%)	Diameters inhibitory (mm) D*
0.5	13.15±0.20
1.0	13.22±0.23
1.5	14.67±0.19
2.0	17.32±0.21
5.0	23.18±0.17
Blank	-

D\*: Diameter of essential oils inhibits an average of three repetitions, *S. aureus*: *Staphylococcus aureus*

showed that the antibacterial activity of essential oils of Laja Gowah rhizome can inhibit the growth of *S. aureus* at concentrations 1.5% with an effective of the inhibition zone of approximately

**Table 6: Diameters inhibitory essential oils against *E. coli***

Concentration (%)	Diameter inhibitory (mm)
	D*
0.5	12.66±0.19
1.0	13.74±0.25
1.5	13.93±0.18
2.0	16.15±0.23
5.0	17.89±0.28
Blank	-

D\*: Diameter of essential oils inhibits and average of three repetitions, *E. coli*: *Escherichia coli*

14.67±0.19 mm and the bacterium *E. coli* at a concentration of 2.0% with an inhibition zone of approximately 16.15±0.23 mm. The antibacterial activity of a substance to inhibit the growth or kill which requirements determined by the Directorate General of POM (1995), with a limit of 14-16 mm [11]

This study using agar diffusion method and diameter zone of bacterial growth inhibitory were measured that the diameter of the inhibitory zone will increase with increasing concentration of the extract and volatile oil. This proves that the increased concentration of the extract and volatile oil have a positive correlation to the increased diameter of the growth inhibition zone of *S. aureus* and *E. coli* [17,18].

#### CONCLUSION

The results of antibacterial activity test showed that the ethanol extract provides effective antibacterial activity at concentration of 300 mg/ml against *S. aureus* and *E. coli* with inhibition zones diameter 14.33; 14.57 mm, respectively, and antibacterial activity test of volatile oil of Laja Gowah at concentration 1.5% can inhibit the growth of *S. aureus* with an effective diameter inhibitory zones of 14.67 mm and *E. coli* at a concentration of 2.0% with an effective diameter of inhibitory zone of 16.15 mm.

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