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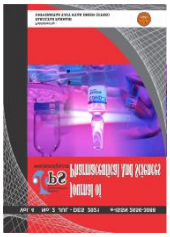

ANTI-BACTERIAL ACTIVITY OF ETHANOL EXTRACT FROM THE FLOWER OF KECOMBRANG (*Etlingera elatior Jack.*) IN VITRO

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ABSTRACT
Traditionally, kecombrang flowers have been used to treat various diseases, including colds, earaches, blood purifiers, treat festering wounds and eliminate body odor. Various bacteria may cause ear diseases, purulent wounds, and body odor, so the kecombrang flower, which has been used traditionally to treat this disease, maybe due to its anti-bacterial activity against both Gram-positive and Gram-negative, so it is necessary to do research with the aim of ensuring the presence of anti-bacterial activity. This research includes collecting kecombrang flowers, plant identification, making Simplicia, phytochemicals, making kecombrang flower extracts by percolation with 96% ethanol extract. Testing anti-bacterial activity *in vitro* by diffusion method with punch *holes on* Mueller Hinton Agar medium, and used Ampicillin sulfate 20 g/ml as a control against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli,* and *Pseudomonas aeruginosa bacteria*. Phytochemical screening showed the presence of the same group of compounds, namely alkaloids, flavonoids, steroids, glycosides, and essential oils. The results of the anti-bacterial activity test of the ethanol extract of the excellent kecombrang flower against *Staphylococcus aureus, Streptococcus pneumoniae*, *Escherichia coli*, and less suitable for *Pseudomonas aeruginosa*.

Keywords : Phytochemical screening, anti-bacterial activity test, Gram-positive, Gram-negative, ethanol extract, kecombrang flower

INTRODUCTION

Various plants have recently investigated the development of medicinal plants in Indonesia for various benefits. One of the plants used as ingredients in traditional medicine is the kecombrang flower (*Etlingera elatior Jack*.) of the Zingiberaceae family. Traditionally, this kecombrang flower has been widely used to treat various diseases, including colds, earache, blood purifiers, treating festering wounds, and eliminating body odor.

The use of kecombrang flowers for natural treatment is not liked by the public because it is considered less practical and difficult to supply and distribute, so it needs to be made into a more practical preparation. The initial stage is for the preparation of more practical preparations; the kecombrang flower is made into simplicia and then made into an extract form to obtain a more practical form with a smaller volume. However, it is not known whether the kecombrang flower that has been made into simplicia or in the form of an extract still contains the same class of chemical compounds as the unprocessed kecombrang flower; for this, it is necessary to carry out a phytochemical screening of the simplicia and the resulting extract.

Based on this, the researchers are interested in conducting a phytochemical screening study of fresh kecombrang flowers, Simplicia and their ethanol extracts and testing the anti-bacterial activity of the ethanolic extract of kecombrang flowers against several bacteria. For this purpose, Gram positive bacteria were selected, namely *Staphylococcus aureus* and *Streptococcus pneumoniae*, and as Gram bacteria. negative selected *Escherichia coli* and *Pseudomonas aeruginosa.* The method used in

this study was the agar diffusion method with a punch *hole*, and the broad-spectrum antibiotic ampicillin was used as a control.

MATERIALS AND METHODS

This study was carried out experimentally in the laboratory. The research was conducted at the Research Laboratory and Microbiology Laboratory, Tjut Nyak Dhien University, Medan. The ingredients used are kecombrang flowers, MHA (Mueller Hinton Agar), NA (Nutrient Agar), distilled water, Ampicillin sulfate, *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 6303), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853). Pro-analytical quality chemicals, namely acetic acid, hydrochloric acid, nitric acid, sulfuric acid, barium chloride, iron (II) chloride, bismuth nitrate, 96% ethanol, iodine, potassium iodide, mercury (II) chloride, or-naphthol, lead (II) acetate, and toluene.

Plant identification was carried out at Herbarium Medanense (MEDAN), University of North Sumatra, Medan.

Phytochemical screening was carried out on fresh flowers, simplicia, and chemical class tests for the ethanolic extract of the kecombrang flower (Etlingera Flos), including examination of alkaloids, tannins, flavonoids, steroids/triterpenoids, saponins, glycosides, and essential oils.

The simplicia powder was put into a closed vessel and moistened with 96% ethanol as a solvent, macerated for at least 3 hours. The mass is transferred little by little into the percolator, then the filtered fluid is poured sufficiently, and above the simplicia, there is still a layer of the filtered fluid; the percolator is closed and left for 24 hours. The liquid is allowed to drip at a rate of 1 ml per menu; the filtered fluid is added repeatedly so that there is always a layer of filter fluid on top of the simplicia until the liquid that comes out is colorless, or the last liquid that comes out leaves no residue. The perchlorate is pronounced or distilled under low pressure at a temperature of not more than 50°C until a thick extract is obtained. Then it was dried with a freeze dryer at a temperature of -40°C, pressure of 2 atm for 24 hours, then the ethanol extract of kecombrang flower was obtained, hereinafter referred to as EEBK, then weighed and stored in a brown container and tightly closed (Ditjen POM, 1974).

Anti-bacterial Activity Test of Kecombrang Flower Ethanol Extract Antibacterial

an activity test of kecombrang flower ethanol extract was carried out against Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*, and against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The extract used was first dissolved using 96% ethanol with a concentration variation of 500 mg/m1; 400 mg/ml; 300 mg/ml; 200 mg/ml; and 100 mg/ml. Pipette 0.1 ml of Staphylococcus aureus bacteria suspension with a concentration of 106 CFU/ml, put into a sterile petri dish. Then, 20 ml of liquid MHA medium was poured at a temperature of ± 40°C, then homogenized and allowed to stand until the media solidified. After the media is solid, then it is perforated with seven holes with a diameter of 6 mm, then 0.1 ml of kecombrang flower ethanol extract solution is dripped in each hole with a concentration of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml, 96% ethanol as blank, and Ampicillin sulfate 20 g/ml as control. Left for 15 minutes, then incubated at 37°C for 18-24 hours in an incubator. Then the clear zone around the hole is observed and measured using a caliper, which is the diameter of the inhibition of bacterial growth being tested. So that the diameter of the growth inhibition of each test bacteria with various concentrations of the extract is obtained as anti-bacterial activity. The same was done on *Streptococcus pneumoniae*, *Escherichia coli,* and *Pseudomonas aeruginosa*.

RESULTS AND DISCUSSION

Plant identification sent to Herbarium Medanense (MEDA) the University of North Sumatra showed that the plant used was kecombrang (*Etlingera elatior Jack*.) Zingiberaceae family. The results of phytochemical screening of fresh kecombrang flowers, simplicia, and ethanolic extracts of kecombrang flowers can be seen in Table 1.

Table 1. Results of Phytochemical Screening Tests of Kecombrang Flowers

No.	Group of Compounds of	Flowers Kecombrang Fresh	Simplicia Flowers Kecombrang	Ethanol Extract of Flowers Kecombrang
1	Alkaloids	+	+	+
2	Tannins	_	_	_
3	Flavonoids	+	+	+
4	Steroids	+	+	+
5	Saponins	—	_	_—
6	Glycosides	+	+	+
7	Essential Oils	+	+	+

Table 1 above shows that the chemical compound class in fresh kecombrang flowers is the same as in simplicia and its ethanol extract. Among the groups of chemical compounds resulting from this phytochemical screening, the most likely to have anti-bacterial activity are flavonoids and essential oils.

Testing the anti-bacterial activity of kecombrang flower ethanol extract (EEBK) against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa bacteria* was carried out using the agar diffusion method with a punch *hole*, an area of bacterial growth inhibition was formed, which was characterized by the presence of a clear area around the hole. The diameter of this bacterial growth inhibition was measured using a caliper. The results of the anti-bacterial activity test can be seen in table 2.

Table 2. The results of the anti-bacterial activity of the Kecombrang Flower Ethanol Extract

Test and Control Materials	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*
Ampicillin sulfate 20µg/ml	28.17 ±	26.17 ± 0.425	17 ,17 ± 0.425	14.17 ±
0.425 EEBK 100 mg/ml	14.33 ±	14.25 ± 0.451	13.33 ±	8.17 ±
0.425 EEBK 200 mg/ml	15.25 ± 0.451	15.17 ±	14 ,33 ± 0.425	9.08 ±
0.336 EEBK 300 mg/ml	16.17 ±	16.25 ± 0.451	15.08 ±	EEBK ±
400 mg/m1	17.17 ± 0.425	17.08 ± 0.336	16 ,08 ± 0.336	11.33 ±
0.425 EEBK 500 mg/ml	18.33 ±	18.25 ± 0.451	17.17 ±	0.425 12.17 ±

The diameter of the growth inhibition indicates the anti-bacterial power of the test material, and this depends on the ability of the diffusion of the test material on the media and the sensitivity of the bacteria used as an indicator of the ability of the preparation. The study results were seen by increasing the concentration of the test material, increasing the inhibition of bacterial growth.

This anti-bacterial activity test used Ampicillin sulfate 20 g/ml as a control, showing that ampicillin had an inhibitory effect on all the test bacteria used. This shows that the media and bacteria used are in good condition. These results are also following the literature that ampicillin has a broad spectrum inhibitory power, or also called Gram-positive and Gram-negative bacteria sensitive to ampicillin. Meanwhile, 96% ethanol (the solvent used to dissolve the test material) did not show the inhibitory power of bacteria; this proves that the solvent does not influence the inhibitory power produced by the test material.

The results of the anti-bacterial activity test of the ethanolic extract of kecombrang flowers against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli,* and *Pseudomonas aeruginosa bacteria* showed that the ethanolic extract of kecombrang flowers provided excellent inhibition of bacterial growth against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Escherichia coli bacteria*. A concentration of 100 mg/ml has given diameter of inhibition above 13 mm. At a concentration of 300 mg/ml, it has given a diameter of inhibition above 15 mm. In contrast, *Pseudomonas aeruginosa* gave less good resistance than the other two test

bacteria, namely at a concentration of 100 mg/ml gave the inhibition diameter was below 13 mm, and at a concentration of 300 mg/ml gave the inhibition diameter below 15 mm.

This is following the literature that if the resistance given by the test material gives an obstacle diameter of greater than 15 mm, it is said that the test material is very good (sensitive) in inhibiting bacterial growth if the test material gives an inhibition diameter of 11-14 mm it is said that the test material is not good (intermediate) to inhibit bacterial growth. If the test material gives a diameter smaller than 10 mm, it is said that the test material is not good (resistant) to inhibit bacterial growth (Ich Pujani, 2000).

CONCLUSION

The results of phytochemical screening on fresh kecombrang flowers, simplicia, and ethanol extracts showed the presence of chemical compound groups of alkaloids, flavonoids, steroids, glycosides, and essential oils. The results of the anti-bacterial activity test showed that the ethanolic extract of the kecombrang flower (Etlingera Flos) had excellent anti-bacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Escherichia coli* and was not very good against *Pseudomonas aeruginosa*

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