



**Antibacterial Activity Tests of N-Hexane, Ethyl Acetate and Methanol Extracts of Breadfruit Leaves on *escherichia coli* and *staphylococcus aureus***

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**ABSTRACT**

Traditional medicine has been widely used by the community as alternative medicine in treating a disease. Traditional medicine is more easily accepted by the community due to its familiarity, cheaper price, and easier to obtain. One of the medicinal plants that have potential is *Artocarpus altilis* [Parkins.] Fosberg or widely known as breadfruit. Breadfruit leaves are effective to treat diseases such as liver, hepatitis, enlarged spleen, heart, kidneys, and high blood pressure. They can also be utilized as ingredients for skin healing medicines. Young breadfruit leaves can be chewed to neutralize the toxic content in food. Based on previous studies, the ethanol extract of breadfruit leaves was proven to inhibit the growth of gram-negative bacteria *Escherichia coli*. Most *Escherichia coli* are harmless, but some types of *Escherichia coli* can cause serious food poisoning. The results of phytochemical screening showed that the breadfruit leaves contained flavonoids, tannins, polyphenols, saponins, steroids/terpenoids, monoterpenes, and sesquiterpenoids. This current study aims to determine the activity of antibacterial inhibition by graded extraction of breadfruit leaves using non-polar solvent n-hexane, semi-polar solvent ethyl acetate, and polar solvent methanol by the maceration method. Then,

the obtained extract was tested for antibacterial activity with gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* using the agar diffusion method.

**Keywords:** *Artocarpus altilis* [Parkins.] Fosberg, antibacterial, traditional medicine

**I. INTRODUCTION**

Breadfruit is a type of plant growing in tropical areas. Breadfruit can alternative foods and even the leaves can be utilized to make drinks for treating diseases. Further, young breadfruit leaves are often used to neutralize the toxic content in food by chewing them. Indeed, all parts of this plant are beneficial. 3,4 The results of phytochemical screening show that breadfruit leaves contain flavonoids, tannins, polyphenols, saponins, steroids / terpenoids, monoterpenes, and sesquiterpenoids. 5 Antibacterials can control the growth of harmful bacteria including *Escherichia coli* which is typically found in the human colon. This current study aims to identify the antibacterial inhibitory

activity of n-hexane, ethyl acetate, and methanol extracts of breadfruit leaves on gram-negative bacteria of *Escherichia coli* and gram-positive bacteria of *Staphylococcus aureus*. It used graded extraction in which it utilized solvents such as water, methanol, ethanol, ethyl acetate, and n-hexane that can separate important compounds in materials. In principle, a material will easily dissolve in a solvent with the same polarity<sup>1</sup>. Graded extraction will produce certain compounds specifically extracted from each solvent used. Meanwhile, non-graded extraction produces a total extracted compound that can be extracted with the solvents<sup>2</sup>. Considering the benefits of breadfruit properties that can be used to treat various diseases and its chemical content of antibacterial properties, this study aims to identify the antibacterial inhibitory activity of methanol, ethyl acetate, and n-hexane extracts of breadfruit leaves on gram-negative bacteria of *Escherichia coli* and gram-positive bacteria of *Staphylococcus aureus*.

## II. RESEARCH METHODS

**Tools.** The study used rotary evaporator (maceration), aluminum foil, autoclave analytical scale, filter paper (Whatman No.42), oven, petri dish, micropipette, test tube, sterile gauze, Water bath, Laminar Air Flow (LAF), Erlenmeyer, cotton, stirring rod, tweezers, alcohol burner, Ose needle, disc paper, and ruler.

**Materials.** The study used Mueller-Hinton Oxoid agar, 1% DMSO, *Escherichia coli* and *Staphylococcus aureus*, breadfruit leaves, methanol, ethyl acetate, n-hexane, 0.9% NaCl, and chloramphenicol antibiotics

### Creating Breadfruit Leaf Extract

A total of 1 kg of breadfruit leaves was washed under running water, drained, and dried for 7 days. Then the leaves were chopped and mashed to get the dry weight. A total of 1 kg of fresh breadfruit leaves produced 250 g of dried breadfruit leaves. The dry breadfruit leaf powder

was soaked with n-hexane solvent, covered, and left for 3 days. It was stirred occasionally and placed in a space protected from light. The obtained result was re-soaked with ethyl acetate for 3 days with 1 repetition then it was filtered and soaked with methanol solvent. The obtained result was put into a rotary evaporator to get the thick extract. Then, the phytochemical test was performed followed by antibacterial activity tests.

### A. Antibacterial Activity Test

The extract of n-hexane, ethyl acetate, and methanol from breadfruit leaves was diluted with concentrations of 50%, 25%, 12.5%, and 6.5% respectively using 1% DMSO solvent. A total of 0.5 ml of 10<sup>6</sup> CFU/mL bacterial suspension was piped and poured into solid Mueller-Hinton agar and shake evenly, let it stand tilted. Then, the remaining inoculum was pipetted. Then, a disc paper that has been soaked in the test solution for 5 minutes at a concentration of 50%, 25%, 12.5%, and 6.5% and a comparison of chloramphenicol antibiotics was put on the agar media. The experiment was repeated twice. After the incubation at 37 ° C for 24 hours, it was observed to see whether there was a clear zone around the disc paper or not.

### Minimum Inhibitory Concentration Test (MIC)

The minimum inhibitory concentration (MIC) of the material was tested using the dilution method. For *Escherichia coli*, the solution made from the ethyl acetate extract of breadfruit leaves was diluted at concentrations of 10%, 8%, 6%, 4%, 2%, and 1%. Meanwhile, for *Staphylococcus aureus*, the solution was diluted with concentrations of 5%, 4%, 3%, 2%, 1%, 0.5%, 0.25%, and 0.125% with 1% DMSO solvent. It was incubated at 37 ° C for 24 hours and observed by looking at the bacterial growth.



### III. RESULTS AND DISCUSSION

The antibacterial activity test of n-hexane, ethyl acetate, and methanol extracts of breadfruit leaves at concentrations of 100% and 50% showed that ethyl acetate extract provided better inhibition than n-hexane and methanol extracts. Thus, the ethyl acetate extract of breadfruit leaves was continued with antibacterial activity tests at concentrations of 50%, 25%, 12.5% and 6.5%.

Extract	Bacterial	Concentration	
		100%	50%
Methanol	<i>Escherichia coli</i>	12	10
	<i>Staphylococcus aureus</i>	7	4
Ethyl acetate	<i>Escherichia coli</i>	18	15
	<i>Staphylococcus aureus</i>	20	18
n-hexane	<i>Escherichia coli</i>	-	-
	<i>Staphylococcus aureus</i>	-	-
K + Chloramphenicol	<i>Escherichia coli</i>	29	
	<i>Staphylococcus aureus</i>	28	

Bacterial	Concentration	Repetition		Diameter (mm)
		1	2	
	Control (+) chloramphenicol	29	29	29
	Control (-) DMSO	0	0	0
<i>Staphylococcus aureus</i>	6.5%	10	8	12.5
	12.5%	13	12	9
	25%	16	15	15.5
	50%	19	17	18
<i>Escherichia coli</i>	6.5%	8	7	7.5
	12.5%	9	8	8.5
	25%	13	11	12
	50%	17	15	16

**Figure 2..** The results of the inhibition diameter measurement of the ethyl acetate extract of breadfruit leaves.

### Results of MIC Test

The result of the MIC test of ethyl acetate extract of breadfruit leaves on *Escherichia coli* showed a value of 2%. Meanwhile, it showed a value of 0.25% on *Staphylococcus aureus* considering the absence of bacterial colony growth on Mueller Hinton agar.

### Phytochemical Test

The result of the phytochemical test showed that the breadfruit leaf extract contained flavonoids, alkaloids, phenolics, saponins, tannins, and steroids.

### IV. CONCLUSION

The antibacterial activity test of n-hexane, ethyl acetate, and methanol extracts of breadfruit leaves at concentrations of 100% and 50% show that ethyl acetate extract provides better inhibition than methanol and n-hexane extracts in which the highest concentration is in the ethyl acetate extract on *Staphylococcus aureus* and *Escherichia coli*. The diameter reaches 18 mm and 20 mm so that further test of the ethyl acetate extract of breadfruit leaves can be performed at concentrations of 50%, 25%, 12.5%, and 6.5% with results of 18 mm, 15.5 mm, 12.5 mm, and 9 mm compared to gram-negative bacteria of *Escherichia coli* at concentrations of 50%, 25%, 12.5%, and 6.5% with results of 16 mm, 12 mm, 8.5 mm, and 7.5 mm respectively. However, in the methanol extract, the highest diameter reaches 12 mm and 10 mm. On the other hand, there is no antibacterial inhibition in n-hexane so the antibacterial test cannot be performed.

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