

## Bioactivity Test Crude Fruit of Citrus Lime (*Citrus aurantifolia*) on Bacteria *Escherichia coli* in Vitro

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**Abstract** : *The antimicrobial activity of citrus fruit crude (*Citrus aurantifolia*) was proven in this experiment with different concentration treatments. The purpose of this study was to determine the antibacterial power of citrus fruit crude against *Escherichia coli* bacteria tested in vitro. This research uses experimental method with laboratory test for citrus fruit crude using Kirby-baurier of susceptibility test method, with data obtained by using statistical test of Analysis of Variance (Anova) with complete randomized data retrieval. The results showed that of citrus fruit crude has antibacterial power to the growth of *Escherichia coli* bacteria. At concentrations of 25%, 50%, 75%, 100% formed inhibition zone with an average diameter of 12.6 mm, 15.4 mm, 16.6 mm, 17.3 mm. So, the higher the concentration of citrus fruit crude the better of inhibitory power.*

**Keyword** : *Crude; Citrus fruit (*Citrus aurantifolia*); *Escherichia coli*; Inhibition zone*

### I. Introduction

The disease caused by the development of pathogenic microbes is called infection. Infection is one of the serious health problems affecting humans. Various kinds of infections that occur in humans today can be caused by some bacteria derived from the human body itself. Such infections can cause various diseases such as diarrhea and lower respiratory tract disease or pneumonia. This disease is a disease that is often caused by pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*.

*Escherichia coli* bacteria is one of the normal flora in the human colon. In addition to the intestines of these bacteria can also be found in small amounts as part of normal flora in the respiratory system. This bacteria can infect the body if it reaches beyond the normal intestinal. Bacteria *Escherichia coli* is the most common bacteria causing diarrhea worldwide. The effects of diarrheal diseases can cause dehydration, especially in children and the elderly and in more severe conditions can cause acute kidney failure and changes in mental status such as confusion and headaches. Data from the Ministry of Health in 2014 shows that the prevalence of diarrhea in Indonesia 97.45% with the number of cases as many as 8,490,976 cases. The high incidence of infection and resistance to antibiotics are a major problem in the treatment of infections and a new strategy is needed in establishing alternative therapies for the treatment of infections.

Traditional medicine is a collection of beliefs, knowledge and experiences of indigenous peoples used to safeguard health, prevent, diagnose and treat physical and mental diseases. One of the traditional medicine known in Indonesia is Antibiotics. The use of natural ingredients as traditional medicine in Indonesia has also been done by our ancestors since many centuries ago. The trend of traditional drug use in the world is due to the increasing side effects of using chemical drugs. The presence of bioactive components in plants is known to have antibacterial effects. So now many tests of antibacterial effects using natural ingredients. Natural ingredients have lower side effects compared to chemical drugs, other than that cheap and easy to obtain.

## II. Material and Method

### 2.1 Materials and Media

The material used for crude extract samples (*Citrus aurantifolia*), pure culture of bacteria *Escherichia coli* from Endo Agar media, Ciprofloxacin 500 mg antibiotics as positive control, aquadest, Nutrient Broth (NB) media, Nutrient Agar medium (NA), and Alcohol 70%. The tools used in this research are petridish plate, reaction tube, ose needle, measuring cup, micropipet, suction pipette, oven, autoclave, incubator, Erlenmeyer flask, bunsen, stick watten, tweezers, filter paper whattman no .41, slide .

### 2.2 Research Method

The method used in this research is experimental method. Laboratory test for Crude lemon fruit using Kirby-baurier of Suspectibility test method with disc diffusion.

### 2.3 Bioactivity Test

The sterile cotton lidi is immersed in the germ of NB, then the cotton swab is applied on the surface of the NA medium, the procedure is repeated twice by rotating the 60o plate. The 6 mm diameter disc paper was immersed in a tube containing lime juice with concentrations of 25%, 50%, 75%, 100%, positive controls using 500 mg ciprofloxacin antibiotics and negative control using sterile aquades. Then a 6 mm disc paper is placed on the surface of the NA medium with the help of sterile tweezers with little emphasis to keep the disc paper firmly attached, then incubated at 37°C for 24 hours. Calculated the resistor zone on each of the disc paper by using the sliding term. The bioactivity test is done with 3 repetitions.

### 2.4 Measurement of Inhibition Zones

Measurement of lime-limiting inhibition zone against *Escherichia coli* bacteria is by looking at the inhibit zone found on each disc at each concentration. The diameter of the inhibit zone formed can be measured by the sliding range by measuring the vertical and horizontal diameters according to the Kirby-baurier of Suspension test. The zones calculated at each concentration are compared with the classification of the inhibit zone based on Greenwood.

## III. Result

Based on the results of research on bioactivity test of crude citrus fruit (*Citrus aurantifolia*) to *Escherichia coli* bacteria in vitro showed results that vary according to the concentration used. The drag zone results can be seen in table below.

**Table 1.** Measurement Result of Crude Lime Zone Lime Citrus (*Citrus aurantifolia*)  
 Against *Escherichia coli* Bacteria

<i>Escherichia Coli</i> Replicated	Diameter Inhibitor Zone (mm)			
	Lime Citrus Concentration			
	25%	50%	75%	100%
<b>I</b>	12,5	15,4	16,8	17,0
<b>II</b>	12,8	15,2	16,4	17,2
<b>III</b>	12,6	15,6	16,6	17,8
<b>Average Diameter</b>	12,6	15,4	16,6	17,3

Based on the research results obtained that crude citrus fruit (*Citrus aurantifolia*) has bioactivity against bacteria *Escherichia coli* which tested in vitro. The antibacterial effectiveness of lime fruit crude is indicated by the formation of inhibit zone around the disc paper. This inhibit zone is then measured in diameter by using the sliding term to determine the magnitude of its antibacterial power. The concentration of lemon juice used is 100%, 75%, 50%, and 25%. The concentration shown on the concentration of lime (*Citrus aurantifolia*) at concentration 75% yielding the average inhibitory diameter can inhibit bacteria is 16.6 mm, then compared with the classification of greenwood table is classified as a moderate growth inhibitory response. Also shown in the 100% concentration of lime (*Citrus aurantifolia*) that the average inhibitory zone can inhibit bacteria is 17.3 mm, the concentration has significance after being compared with the greenwood table classification that the growth inhibitory response is moderate. At concentrations of 75% and 100% are moderate because of the greenwood table classification between 16-20 mm. Of the four concentrations mentioned above that 100% concentration is the largest concentration of the average inhibit zone produced.

Based on research data shown in table 4 shows that, the concentration of citrus fruit crude (*Citrus aurantifolia*) has an influence on the activity of *Escherichia coli* bacteria. The concentration shown from 25% to 100% has increased inhibit zone.

Based on the above table, it is found that the average concentration of lime (*Citrus aurantifolia*) at 25% concentration has 12.6 mm inhibit zone, the area after comparison with greenwood table classification shows that the growth inhibitory response is weak. At a concentration of 50% showed that the limiting zone of lime concentration (*Citrus aurantifolia*) had a 15.4 mm inhibitory zone, the area compared to the drag zone classification table based on greenwood table means that the growth inhibitory response is weak. At concentrations of 25% and 50% have a weak growth inhibitory response because it is still classified between 11-15 mm.

**Table 2.** Clasification Inhibitor Zona Greenwood

Average Diameter Inhibitor Zona	Respon Inhibitor Growed
> 20 mm	Strong Hight
16-20 mm	Medium
11-15 mm	Low
≤ 10 mm	Nothing

#### IV. Conclusion

Based on the results of research that has been done, it can be concluded that crude citrus fruit (*Citrus aurantifolia*) has bioactivity against bacteria *Escherichia coli* which tested in vitro. Of the concentrations tested against *Escherichia coli* bacteria have varied results depending on their concentration. The concentrations tested in this study were 25%, 50%, 75%, and 100%, with an average yield diameter of 12.6 mm - 17.3 mm. From the diameter obtained after comparison with greenwood table that is classified as being.

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