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TESTING THE ACTIVITY OF KETUL LEAF (*Bidens pilosa* L.) ETHANOL EXTRACT IN INHIBITING THE GROWTH OF *Escherihia coli* BACTERIA

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ABSTRACT

Ketul leaves (*Bidens pilosa* L.) have secondary metabolite compounds which are used by the community to treat diarrhea. Ketul leaves contain secondary metabolite compounds including terpenoids, alkaloids, flavonoids and phenools which have the potential to act as antibacterials. This study aims to determine the antibacterial activity of ethanol extract of ketul leaves (B. pilosa L.) in inhibiting the growth of *Escherichia coli* bacteria. This research used the disc diffusion method which consisted of 5 concentration variations, namely 30%, 40%, 50%, 60%, 70%, with 4 repetitions. Data from the inhibition zone results were analyzed using the One Way Anova statistical test. The results showed that ketul leaf extract (*Bidens pilosa* L.) had antibacterial activity against *Escherichia coli* bacteria. The wider the clear zone formed, the stronger the active compound is in inhibiting bacterial growth. Based on the results of Duncan's follow-up test, it was found that there was no significant difference between each concentration treatment in inhibiting *Escherichia coli* bacteria.

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Introduction

Pathogenic bacteria are one of the microorganisms that cause infectious diseases in humans. Infectious diseases can be caused by the entry of pathogenic bacteria into the human body through air, food, drink, or through blood transfusions (Kusumaningrum & Sepvianti, 2020). Diarrhea is an infectious disease of the digestive tract which is characterized by symptoms such as the release of feces with a liquid consistency, which can be accompanied by blood or mucus, resulting in an increase in the frequency of defecation which is more frequent than normal. In this situation, the body will experience a lack of fluids or dehydration so that if you do not get immediate help it can cause death in the sufferer (World Health Organization, 2019). Diarrhea is still one of the causes of increasing deaths in developing countries like Indonesia. In Indonesia, diarrhea is still a public health

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problem, especially in children (Bakri et al., 2015).

Based on data from the Ministry of Health in 2019, it was reported that the service coverage for diarrhea sufferers at all ages was 61.7% and for toddlers it was 40% of the target set (Ministry of Health of the Republic of Indonesia, 2020). In 2020, it was reported that service coverage for diarrhea sufferers at all ages was 44.4% and for children under five was 28.9% of the target set (Ministry of Health of the Republic of Indonesia, 2021). In 2021, service coverage for diarrhea sufferers at all ages will be 33.6% and for children under five will be 23.8% of the target set (Ministry of Health of the Republic of Indonesia, 2022).

Based on data reported every year, it shows that diarrheal diseases in Indonesia, especially in children, still require more optimal treatment. Treatment that has been carried out for diarrheal disease infections is still through antibiotics. When using antibiotics, it must be done rationally, appropriately and safely. If antibiotics are used incorrectly, it can result in bacteria becoming resistant to various drugs, causing side effects and increasing the risk of complications and even death (Pratiwi, 2017).

Resistance from bacteria encourages the continued development of alternative compounds, namely antibacterial compounds. One of the plants that people use as herbal medicine is ketul leaves (*Bidens pilosa* L.). The use of ketul leaves is traditionally used as an alternative medicine to cure diarrhea and heal wound infections. The Ketul plant (*Bidens pilosa* L.) contains secondary metabolite compounds including tannins, saponins, alkaloids, bitter substances, tannic substances, and essential oils, terpenoids, phenols, phenylpropanoids, flavonoid glycosides, fats and benzo. Secondary metabolite compounds found in plants can be used as antibacterials (Seko et al., 2021).

Based on research conducted by Seran*et al*., (2021) on the antibacterial activity of Ketul leaf ethanol extract reported that Ketul leaf extract has antibacterial ability against *Escherichia coli* and Staphylococcus aureus bacteria (Seran et al., 2021). Research on ketul leaves (*Bidens pilosa* L.) as an antibacterial is still minimal and the high level of bacterial resistance to antibiotics has encouraged the development of an antibacterial compound as the basis for research testing the activity of ethanol extract of ketul leaves (*Bidens pilosa* L.) as an antibacterial for *Escherichia coli*.

Materials and Methods

Research Location and Time

This research will be carried out at the Microbiology Laboratory and Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Medan State University on Jalan Willem Iskandar, Pasar V Medan Estate, Percut Sei Tuan, Deli Serdang. This research was carried out from May 2023 to September 2023.

Tools and materials

The tools used in this research were incubators, ovens, test tubes, tubes, stirring rods, filter paper, funnels, Erlenmeyer flasks, Petri dishes, dropper pipettes, measuring cups, Bunsen lamps, scissors, autoclaves, digital scales, cotton swabs, vortexes. , laminar air flow, paper disk, tweezers, aluminum foil, caliper, label paper, isopad, rotary vacuum evaporator, blender, sieve. The materials used in this research were ketul leaves (*Bidens pilosa* L.), pure culture of *Escherichia coli* bacteria ATCC 25922, 70% alcohol,

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distilled water, Nutrient Agar (NA) media, Mueller Hinton Agar (MHA) media, spritus, BaCl2, NaCl 0.9%, H2SO4, ethanol pa, amoxicillin.

Collection of Ketul Leaf Samples

Ketul leaves (*Bidens pilosa* L.) were obtained from Brand Village, Brand District, Karo Regency, North Sumatra. Sampling ketul leaves (*Bidens pilosa* L.) by picking leaves that are fresh green and not rotten. Ketul leaves were taken from sequence number 3 from the leaf shoot to sequence number 10, this is because leaf number 3 from the shoot is physiologically mature so it has maximum secondary metabolite content (Manguntungi et al., 2017). Leaves of the same size and color indicate leaves that are of maximum age and level of development (Widiastuti, 2016). The ketul leaves that have been collected are then washed in running water until clean and then air-dried in a room that is not exposed to direct sunlight for ± 3 days (Luliana et al., 2016).

Determination of Water Content of Ketul Leaf Simplicia

Two grams of ketul leaf simplicia powder (*Bidens pilosa* L.) was placed on a cup and then dried in the oven at 105°C for 30 minutes. Cool using a desiccator for 15 minutes, after cooling, weigh the constant weight and calculate the water content (Handayani et al., 2017).

Water content is weighed using the formula (Andarwulan et al., 2011):

$$\text{Water Content} = \frac{b-(c-a)}{b} \times 100\%$$

Information :
a = weight of the cup (gr)
b = sample weight (gr)
c = weight of cup = sample (gr)

Making Ketul Leaf Extract

300 grams of ketul leaf powder (*Bidens pilosa* L.) was macerated with 900 ml of ethanol pa solvent with a solvent ratio of 1: 3 (w/v). The sample was homogenized in a closed container for 3 days with occasional stirring. The liquid extract is then filtered using Whatman No. 1 filter paper, the dregs are macerated again with the same solvent and ratio for 1 day. All maceration filtrates are combined and evaporated using a rotary vacuum evaporator until it becomes a thick extract using a temperature of 50°C. The extract obtained was stored in a refrigerator at 4°C (Handayany, 2016).

Dilution of Ketul Leaf Extract

To determine antibacterial activity, it was made with several variations in concentration, namely 30%, 40%, 50%, 60%, 70%. Extract concentration can be made using the following equation:

$$V1 \cdot M1 = V2 \cdot M2$$

Information :
V1 : Volume of original concentration to be mixed with solvent
V2 :The dilution volume to be used
M1 : 100% original extract concentration
M2 : Concentration to be used

Preparation of Test Bacteria

Before using test bacteria, the bacteria will be rejuvenated first. Making Nutrient Agar (NA) media is done by dissolving 2 grams of NA media with 100 ml of distilled water in a Erlenmeyer flask, then covering it with aluminum foil, then heating the media until it boils and putting it into a test tube aseptically. NA media was sterilized in an autoclave at 121°C for 15 minutes with a pressure of 15 psi. The media was left at room temperature for 1 hour in an inclined position. Take one cycle of *Escherichia coli* bacterial culture and then streak the bacterial colony onto slanted NA media aseptically. The tube is held close to the flame while etching the bacteria. The tube was then covered with cotton and incubated for 24 hours at 37°C.

Mc Farland Standard Suspension

A solution with a Mc Farland turbidity standard of 0.5 is the equivalent

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of the microbial concentration in a liquid medium with a density of between 1.5 x 108 CFU/ml. Making the Mc Farland solution is by mixing 0.05 ml of 1% BaCl2 solution with 9.95 ml of 1% H2SO4 then storing it in a place protected from direct sunlight.

Making Bacterial Inoculum

1 pure culture of *Escherichia coli* bacteria was taken and then suspended in a tube containing 10 ml of 0.9% NaCl, then homogenized using a vortex, until the same turbidity as Mc Farland 0.5 solution was obtained.

Antibacterial Activity Test

Mueller Hinton Agar (MHA) media was poured into a sterile petri dish ± 15 ml and allowed to solidify. Testing of the antibacterial activity of ketul leaf extract (*Bidens pilosa* L.) was carried out using the disc diffusion method or the Kirby-Bauer method. The bacterial inoculum was smeared evenly on a petri dish containing MHA media using a sterile cotton swab. A sterile cotton swab is inserted into a tube containing a suspension of *Escherichia coli* bacteria and then smeared evenly on the MHA media. Paper discs with a diameter of 6 mm were soaked in 0.1 ml of ketul leaf extract solution with varying concentrations of 30%, 40%, 50%, 60%, 70% and the solvent used as a control extract. The negative control in this study was ethanol pa, which did not have inhibitory power against the test bacteria and as a comparison the solvent did not affect the extract activity test (Utomo et al., 2018). Meanwhile, the positive control used was amoxicillin. After the solution has been completely absorbed, the paper disc is placed on the MHA media using sterile tweezers containing the test bacteria, then incubated at 37°C for 24 hours. The clear zone formed around the disc paper was measured using a caliper to indicate antibacterial activity.

Data analysis

This research is a laboratory experiment using One Way Anova with a significance level of $p<0.05$ and followed by the Post Hoc Duncan follow-up test.

Results and Discussion

Based on the results of research that has been carried out, ketul leaf extract (*Bidens pilosa* L.) has antibacterial activity against *Escherichia coli* bacteria. The research results can be seen in Table 1.

Table 1. Results of measuring the diameter of the inhibitory zone of Ketul leaf extract (*Bidens pilosa*L.)

Bacteria Name	Concentration Treatment	Average Inhibition Zone Diameter (mm)	Category
Escherichia coli	30%	4.76	Currently
	40%	5.08	Weak
	50%	5.41	Currently
	60%	5.53	Currently
	70%	6.07	Currently
	Control (+)	11.42	Strong
	Control (-)	0	Ineffective

Based on Table 4.1, it shows the antibacterial activity of ketul leaves (*Bidens pilosa*L.) against *Escherichia coli* bacteria at a concentration of 30%, the average diameter of the inhibition zone

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was 4.76 mm in the weak category, at a concentration of 40% the average diameter of the inhibition zone was 5.08 mm in the medium category, at a concentration of 50%, the average diameter of the inhibition zone is 5.41 mm in the medium category. At a concentration of 60%, the average diameter of the inhibition zone was 5.53 mm in the medium category. At a concentration of 70% the average diameter of the inhibition zone is 6.07 mm in the medium category. The positive control showed an average inhibitory zone diameter of 11.4 mm in the strong category. The negative control did not show any diameter of the inhibition zone in *Escherichia coli* bacteria.

Based on the inhibition zone diameter data obtained from the research, a One Way Anova statistical test was carried out.

Table 2. Shapiro-Wilk Normality Test of Zone of Inhibition

Shapiro-Wilk Normality Test		
Average Zone of Inhibition	**Treatment**	**Sig.**
Escherichia coli	30%	0.696
	40%	0.366
	50%	0.380
	60%	0.658
	70%	0.983
	Positive Control	0.490

Table 2 is the result of the Shapiro-Wilk normality test which shows that data with a p value > 0.05 means the data is normally distributed. Normally distributed data is a requirement for parametric data so that homogeneity analysis and One Way Anova can be carried out.

Table 3. Homogeneity of Variance Test

Homogeneity of Variance Test				
Average zone of inhibition	**Statistical Level**	**df1**	**df2**	**Sig.**
Escherichia coli	2,538	6	21	0.052

Table 3 shows that the p value > 0.05 means that the data contained in the research has homogeneous data variance, so testing can be carried out using*One Way ANOVA.*

Table 4. One Way Anova Test

ANOVA test					
Group Data Variance	**JK**	**DB**	**KR**	**F**	**Sig.**
Escherichia coli	265,615	6	44,269	43,164	0,000

Table 4 shows that the results of the One Way Anova test show that the treatment group has a p value = 0.000. If the p value <0.05, then the average value

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between treatment groups has a significant difference. To find out which treatment groups have real differences, the Duncan test is then carried out as a further test (Post Hoc Test).

Table 5. *Escherichia coli* Post-Hoc Analysis Test

Post-Hoc Test					
Duncana	**Subset for alpha = 0.05**				
Treatment	**N**	**1**	**2**	**3**	**Notation**
Negative Control	4	0.0000			A
30%	4		4.5375		B
40%	4		5,1000		B
50%	4		5.5125		B
60%	4		5.9875		B
70%	4		6.6500		B
Positive Control	4			12,3000	C

Information: numbers followed by the same notation mean they are not significantly different, and

If they are not accompanied by the same letters then they are significantly different.

Table 5 is the result of a Post-Hoc test which shows that the diameter of *Escherichia coli* bacteria in the negative control treatment was significantly different from a concentration of 30%. There is no significant difference between the 30% concentration and the 40%, 50%, 60% and 70% concentrations. The positive control was significantly different with concentrations of 30%, 40%, 50%, 60% and 70%.

Based on the results of the activity of the bacterial inhibition zone, it shows that the growth rate of the bacteria formed is inhibited in proportion to the increase in extract concentration. This is because the more active compounds contained in the extract, the more the concentration of the extract increases, the wider the inhibition zone, so that more microbial cell growth is inhibited or experiences cell death (Ifriana & Kumala, 2018).

Alkaloid active compounds are toxic to humans but can be used as traditional medicine. The mechanism of action of alkaloid compounds as antibacterials is by disrupting the peptidoglycan components in bacterial cells so that the cell wall layer does not form completely and causes cell death. In addition, alkaloid components are known to act as DNA interchelators and inhibit the topoisomerase enzyme in bacterial cells (Sari et al., 2022).

Flavonoids that have been proven to have antibacterial activity are apigenin, galangin, naringenin, epigalocatechin gallate, and their derivatives, flavones and isoflavones (Alfaridz, 2018). Flavonoids are able to inhibit DNA gyrase in bacteria, thereby inhibiting bacterial growth. Apart from that, the flavonoid content causes toxic effects on bacteria due to the presence of flavonoid hydroxyl groups which results in changes in organic components and nutrient transport in bacteria (Ningsih et al., 2023).

The mechanism by which phenol compounds inhibit bacterial growth is by denaturing bacterial proteins so that the protein structure becomes damaged. The hydrogen bonds formed will affect the

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permeability of the cell wall and cytoplasmic membrane. Disturbed permeability of the cell wall and cytoplasmic membrane can cause an imbalance of macromolecules and ions in the cell resulting in lysis (Seko et al., 2021).

The terpenoid content in ketul leaves is a component that has the potential to act as an antibacterial, so an antibacterial agent will be produced which is also able to inhibit the growth of bacteria that are resistant to existing antibiotics. The mechanism of action of terpenoid compounds as antibacterial substances involves membrane damage by lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) on the outer membrane of bacterial cell walls, forming strong polymer bonds and damaging porins, reducing the permeability of bacterial cell walls so that bacterial cells lack nutrition, bacterial growth is inhibited or die (Wulansari et al., 2020).

Gram-negative bacteria contain a higher percentage of fat-like substances and have thinner cell walls than gram-positive bacteria. The structure of gram-negative bacteria has an outer membrane covering a thin layer of peptidoglycan. The outer structure of this peptidoglycan is a double layer containing phospholipids, proteins and lipopolysaccharides (Mengko et al., 2022). The presence of a peptidoglycan layer shows that gram-negative bacteria have better resistance to compounds that leave or enter the cell and cause toxic effects (Septiani, 2017).

The diameter of the inhibition zone formed does not always increase in proportion to increasing concentration, this could also be due to differences in the diffusion speed of antibacterial active compounds on the agar medium (Wahlanto et al, 2020). The higher the extract concentration, the lower the solubility (thickens like a gel), so this can slow down the diffusion of the extract's active ingredients into the medium and ultimately reduce the ability of extracts with high concentrations to inhibit bacterial growth (Zeniusa et al., 2019).

The factor that influences the diameter of the bacterial growth inhibition zone is the turbidity of the bacterial suspension. If the bacterial suspension is less turbid, the diameter of the resulting inhibition zone is larger, and conversely, if the bacterial suspension is too turbid, the resulting inhibition zone is smaller (Zeniusa et al., 2019).

The higher the extract concentration, the lower the solubility (thickens like a gel), so this can slow down the diffusion of the extract's active ingredients into the medium and ultimately reduce the ability of extracts with high concentrations to inhibit bacterial growth (Zeniusa et al., 2019).

The results of the test for the activity of ethanol extract of ketul leaves as an antibacterial for *Escherichia coli* and Staphylococcus aureus are in figures 4.1 and 4.2.

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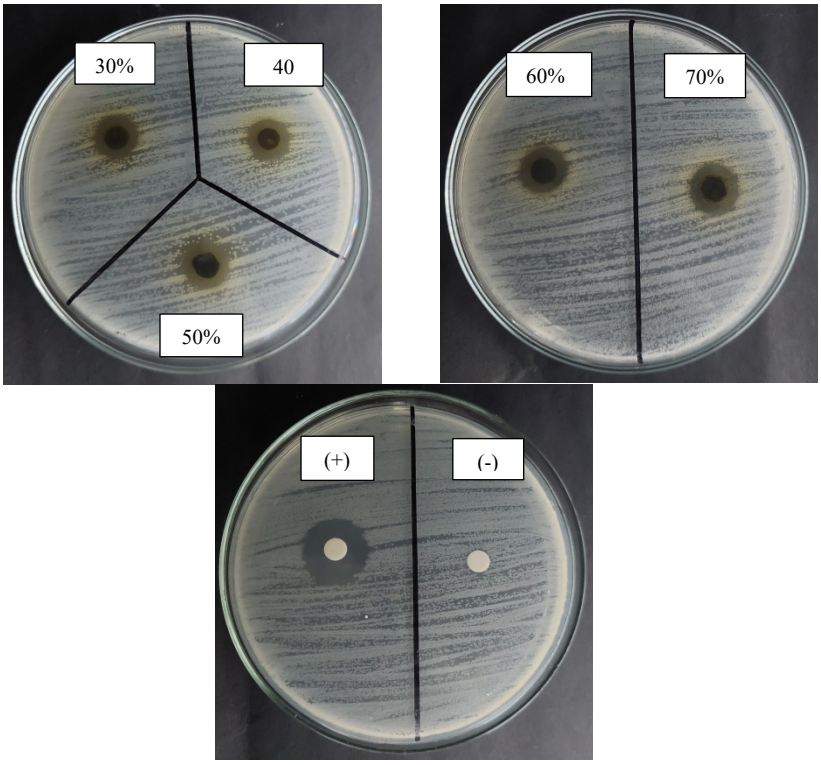

Figure 1. Activity Results of Ethanol Extract of Ketul Leaves (*Bidens pilosa* L.) in bacteria *Escherichia coli*.

Conclusions

The ethanol extract of ketul leaves (*Bidens pilosa* L.) has antibacterial activity against *Escherichia coli* bacteria with bacterial inhibition zones at concentrations of 30%, 40%, 50%, 60%, 70% respectively 4.67 mm, 5.08 mm, 5.41 mm, 5.53 mm, and 6.07 mm. Based on the results of the Duncan test, it shows that there is no significant difference in the concentration treatment in inhibiting *Escherichia coli* bacteria.

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This research could be carried out well thanks to the help of all parties, therefore the author would like to thank all parties who have helped the author in carrying out this research. Further research is needed to determine the inhibitory power of ketul leaf (*Bidens pilosa* L.) ethanol extract against other pathogenic bacteria.

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