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ACTIVITY OF ETHANOL EXTRACT A*systasia gangetica* LEAVES AGAINTS *Escherichia coli*

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ABSTRACT

The leaves *of Asystasia gangetica* have various secondary metabolite compounds. This metabolite compound has the potential to inhibit the growth of *Escherichia coli* bacteria. The purpose of this study was to test the antibacterial activity of *Asystasia gangetica* leaf extract against *Escherichia coli* bacteria. This study used maceration method with ethanol solvent pro analysis. Secondary metabolite assays are performed using color reagents. The antibacterial activity test was carried out by disc diffusion method with 4 repetitions consisting of concentrations of 15%, 25%, 35%, 45%, 55% and 65%. The positive control used in this study was ciprofloxacin and the negative control was the pro-analysis ethanol solvent. Secondary metabolite tests show that *Asystasia gangetica* leaves contain alkaloid compounds, flavonoids, saponins, steroids, and tannins. Antibacterial activity tests show that ethanol extract of *Asystasia gangetica* leaves has the potential to inhibit *Escherichia coli bacteria*.

Introduction

The most common problem today is infectious disorders which trigger high morbidity and mortality rates. Infectious disorders are disorders caused by microbes that are parasitic. Cases of infectious diseases in Indonesia such as diarrhea have a large prevalence rate (Konoralma, 2019). Diarrhea is a digestive system disorder that causes individuals to become frequent bowel movements with a mushy or liquid consistency. Diarrhea can last for several days and cause a person to experience severe dehydration and fluid loss so that it can cause death (Tuang, 2021).

Based on data from Riskesdas in 2018, a number of regions on the island of Sumatra have a diarrhea prevalence value greater than the national value of 8%, including Aceh (9.1%), West Sumatra (9.3%), and North Sumatra (9.1%) (Ministry of Health, 2018). Every year there are approximately 1.7 billion occurrences of diarrhea with a mortality value of 760,000 occurring in individuals under 5 years of age. According to evidence from the Indonesian Health Profile (2019), the age group with the largest prevalence of diarrhea is aged 1-4 years with a percentage of 11.5% and in individuals under 1 year with a percentage of 9% (Ministry of Health, 2020).

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Diarrhea can be caused by bacterial infections that occur due to contamination from beverages, food, water, unclean environments and sanitation (Tuang, 2021). Types of bacteria that can cause diarrhea are: *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella, Shigella, Campylobacter,* and *Vibrio cholera* (Susilawaty *et al*., 2022). One of the microorganisms that cause diarrhea is *Escherichia coli.* Bacteria *Escherichia coli* can parasitize if present outside the intestine with increasing numbers (Zaunit *et al*., 2019).

Prevention of diarrheal diseases can be done with antibiotic therapy. But prolonged use of antibiotics can cause resistant bacteria so that new sources of antibiotics are needed that are expected to reduce resistance. New sources of antibiotics can be obtained from bioactive substances contained in one plant (Pusporini, 2019). One of the plants that can be used is plants *Gangetic asystasia.*

Asystasia gangetica often referred to as breech fig, israeli grass and china violet (Kumalasari *et al*., 2020). *A. Gangetica* It is a weed plant that is often used as a source of forage for animal feed and is easily found in the yard of houses, roadsides, gardens, vacant land and open fields. *A. Gangetica* is a type of grass that is often suspected to be a nuisance grass in oil palm and rubber farms in Indonesia, but grass *A. gangetica* has a number of benefits, namely healing wounds, muscle spasms, and coughing (Karyati &; Adhi, 2018). Leaf *Asystasia gangetica* has greater nutrient levels than stem organs and other organs. Based on previous research, phytochemical test of leaf ethanol extract *A. gangetica* obtained the content of metabolite compounds in the form of alkaloids, flavonoids, steroids and saponins (Yulaikah, 2020). The secondary metabolite compounds obtained are bioactive components that have the potential to be antidiarrheal and antibacterial (Mutmainah &; Ni, 2022). Based on research by Hamid (2011), showed extracts of whole plants *A. gangetica* Using solvents ethyl acesat, methanol, and hexane showed an influence on growth *Escherichia coli* which means that plants *A. gangetica* can be used as an antibacterial ingredient to inhibit bacteria *Escherichia coli.* Based on this description, further tests are needed on the antibacterial activity of leaf extract *Asystasia gangetica* in inhibiting bacteria *Escherichia coli* by using ethanol solvent. The basis for determining ethanol to be a solvent is because ethanol is a polar solvent that is able to attract secondary metabolite compounds found in a plant that has semipolar, polar, and nonpolar properties with a level of safety and ease when evaporated (Afifah &; Niken, 2022).

Materials and Methods

Location and Time of Research

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

Tools and Materials

The tools in this study are erlenmeyer, analytical scales, measuring cups, stirring rods, isopeds, blenders, autoclaves, petri dishes, aluminum foil, drip pipettes, test tubes, filter paper, incubators, *rotary vacuum evaporators*, *laminar air flow*, vortex, bunsen, paper disks, tweezers, calipers, plastic wrab, cotton swabs, ose needles, cotton, scissors, and label paper.

The ingredients in this study were *Asystasia gangetica* leaves, ethanol pro analysis (p.a), aquades, 70% alcohol, BaCl2, H2SO4, 0.9% physiological NaCl, ciprofloxacin 500 mg, *Mueller Hinton Agar (*MHA) media, *Nutrient Agar (*NA) media, spritus, and *Escherichia coli* ATTC 25922 bacterial cultures.

Asystasia gangetica Leaf Retrieval

A. gangetica leaves are obtained from vacant land on Jl. Nilam 21, Prumnas Simalingkar A, Medan Tuntungan District, Medan, North Sumatra. A. *gangetica* leaf

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sampling by picking fresh green leaves from sequence number 3 from leaf shoots to sequence number 7. According to Manguntungi (2017), leaf number 3 from the top of the leaf has gone through a physiological maturation process and has maximum metabolite compounds.

Asystasia gangetica leaf preparation

Leaf *A. gangetica* What has been collected is then cleaned with running water until clean and then dried in a room that is not exposed to sunlight within ±7 days. Leaf *A. gangetica* which has been dried, dried again using an oven at 50ºC with a time of 120 minutes. Oven drying is done to reduce the moisture content of the leaves with the aim of avoiding the presence of pathogenic microbes or fungi when stored (Dharma, M.A *et al*., 2020). The initial parameter of stopping the engeringan stage is kneadable leaves (Luliana *et al*., 2016). Leaf samples inPuree with a blender until it is simplisia and then sieving is done. The purpose of sieving is to obtain smaller simplisia particles so as to facilitate the extraction process and to make the size of the simplisia used uniform (Jayani &; Helena, 2018). Fine simplisia can cause the surface area of the powder to increase so that there are also many components contained in the simplisia that can be withdrawn by the solvent. The simplisia obtained must meet the moisture content standardization. Simplisia standardization based on the Indonesian Herbal Pharmacopoeia Edition III that the standardization of water content is not more than 10% (<10%) (Ministry of Health, 2017).

Determination of Water Percentage Standardization

Standardization of the percentage of water is applied using the way porcelain dishes are heated in the oven within 30 minutes at 105 ° C, after heating the porcelain is cooled on a desiccator and then measure the weight. A total of 2 gr of simplisia leaves of A. *gangetica* are poured into porcelain dishes and then dried in the oven for ± 3 hours at 105°C. A porcelain dish containing simplisia, cooled on a desiccator and then measured in weight. The requirement for determining the standardization of leaf moisture content is not less than 10% (≤10%). The determination of the percentage of water is carried out according to the determination of the volume of dry weight of simplisia and the formula for calculating the percentage of water is used, namely:

$$\text{Water percentage (\%)} = \frac{W-(W1-W2)}{W}$$

Information:
W = Weight of simplisia before drying (gr)
W1 = Weight of cup and simplisia after drying (gr)
W2 = Weight of empty cup dried (gr) (Sumiati *et al*., 2019)

Manufacture of Ethanol Extract of Asystasia gangetica Leaves

The extract is obtained by maceration. Simplisia leaves *of A. gangetica* a total of 300 gr are poured into erlenmeyer, then put in 600 ml of ethanol p.a at a ratio of the amount of solvent which is 1: 2. The extraction process is carried out within 5 days and stirred every 6 hours for 5 minutes. The extract is then filtered with filter paper to separate from the pulp, then soaked again at the same ratio and amount of ethanol within 3 days. The residue resulting from the second maceration is macerated again in a ratio of 1:1 solvent within 3 days. The extract results obtained, combined and evaporated with the use of *a rotary vacuum evaporator* at a temperature of 50ºC until it is in the form of concentrated extract.

Secondary Metabolite Compound Test

Metabolite compound tests were carried out using qualitative scales with color reagents to find metabolites in the form of alkaloids, saponins, flavonoids, steroids and tannins.

Alkaloid Identification

A total of 9 ml of aquadest, 1 ml of HCl 2N and 0.5 gr of simplisia leaves of A. *gangetica* are put in a glass container, heated

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within 2 minutes, then cooled and filtered. The screening results are used in subsequent tests.

1) Liquid is taken in the amount of 0.5 ml then put 2 drops of mayer solvent, if it contains a positive white residue containing alkaloids.
2) The liquid is taken in the amount of 0.5 ml and inserted 2 drops of dragondroff reagent solvent, if the filtrate contains orange or reddish deposits after being perected by dragendroff, therefore the extract contains alkaloids (Handayani *et al*., 2019).

Saponin Identification

Liquid extract is made by boiling 0.5 grams of simplisia leaves of *A. gangetica* in 10 ml of aquadest for 5 minutes and cool, then shaken vertically for 10 minutes then added 1 drop of HCl with 2N molar, if foam forms therefore the extract contains saponins (Handayani *et al*., 2019).

Flavonoid Identification

The liquid extract is made by boiling 1 gr of simplisia leaves *of A. gangetica* in 10 ml of aquades within 5 minutes then filtration. the liquid obtained is put as much as 5 ml into the test tube then put 0.1 gr Mg, 1 ml HCl 2N, and 2 ml solvent. The test tube containing the sample is shaken strongly, if orange appears, the positive extract contains flavonoids (Handayani *et al*., 2019).

Steroid Identification

The liquid extract is obtained by soaking 1 gr of simplisia leaves *of A. gangetica* in 20 ml of ether within 120 minutes then filtration, the liquid obtained is filtered and then evaporation. The filtrate is dripped with Lieberman-Burchard solution, if a dark brown color is formed indicating that the extract contains steroids (Handayani *et al*., 2017).

Tannin identification

The liquid extract is obtained by weighing 1 gr of leaf simplisia *A. gangetica* Then heated for 3 minutes in 100 ml of aquades and then cooled and filtered. Liquid is put as much as 2 ml and then dripped 1-2 drops of $FeCl_3$ 1%. If there is a change in color to blackish green, it indicates that there are tannin compounds (Sari &; Melfin, 2018).

Concentration Creation

To determine antibacterial activity, several variations in concentration were used, namely 15%, 25%, 35%, 45%, 55%, and 65% (Hamid et al., 2011). Making concentration can be done using the equation below:

$$M1/V1 = M2/V2$$

Information:
M1 : Mole solution before dilution
V1 : Vol of solution before dilution
M2 : Mole solution after dilution
V2 : Vol solution after dilution

Control Creation (+) and Control (-)

Control (+) obtained from the antibiotic ciprofloxacin 500 mg using 1 tablet and then mashed, then weigh 0.1 gr and mixed with 100 ml of distilled water and for the control solution (-) using ethanol solvent pro analysis (p.a).

Rejuvenation of Test Bacteria

Nutrient Agar (NA) media is made by dissolving 2 grams of NA media put in 100 ml of distilled water in erlenmeyer and then made aluminum foil on the erlenmeyer lid. The media is brought to a boil with isopad until hot, then put into a test tube sterilely. Each test tube is filled with 6 ml of NA media. Test tubes containing NA are sterilized in an autoclave for 15 minutes with a temperature of 121ºC. Then NA is placed at room temperature in an inclined plane for 1x24 hours. Isolates *of E. coli* and S. aureus *were taken one ose then inoculated by scratching in NA media, and incubated for 1x24 hours with a temperature of 37ºC (Panaungi, 2022).*

Mc Farland Standard Suspension

Mc Farland is used to measure the turbidity of bacterial suspensions. Mc Farland solution was obtained by combining with 9.95 ml of $H_{2SO4\ 1}$ % and 0.05 ml of BaCl2 1%. Both solutions are homogenized until a cloudy

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solution is formed and closed. Mc Farland's standard solution works in counting the number of cells used in antibacterial tests. (Nor, 2018).

Test Bacteria Suspension Setup

Culture of *E. coli* bacteria as much as one ose and then suspended into a test tube containing 10 ml of 0.9% NaCl. Furthermore, homogenized using vortex until appropriate turbidity is obtained on the Mc Farland standard (Nor, 2018).

Antibacterial Activity Test

Activity test of leaf ethanol extract *A. gangetica* against bacteria *E. coli* performed by disc diffusion method on MHA media. MHA is a developmental medium that becomes an organic substance for the development of anaerobic and aerobic bacteria, and is a superior medium for sensitivity testing, namely in the disc diffusion diffusion method (Rahman *et al*., 2022). MHA media is made by combining as much as 38 gr of media with 1,000 ml of aquades in a glass or erlenmeyer container then with aluminum foil made as a cover. The medium is brought to a boil using an isopad. MHA media is sterilized in an autoclave with a temperature of 121oC within 15 minutes then the media is put into petri as much as ±15 ml and left to solidify. The suspension of the test bacteria was etched on the existing petri MHA with *cotton swab* Evenly. Disc paper with a size of 6 mm dripped 0.5 ml of leaf ethanol extract *A. gangetica* with various concentrations. The disc paper inserted into the antibiotic ciprofloxacin serves as a control (+) and the disc paper dipped in ethanol solvent p.a serves as a control (-). Disc paper that has been treated, placed on the media using tweezers in the treatment of each concentration. Then petri is incubated in a 1x24 hour wakatu with a temperature of 37ºC in an inverted position with the aim of giving bacteria time to develop and react with the test material. After incubation, the inhibitory zone is observed by looking at the clear zone area around the paper disc and measured by calipers (Rizki *et al*., 2021).

Data Analysis

The results of antibacterial activity test research data in the form of the diameter of the inhibitory zone *of ethanol extract of A. gangetica* leaves were shown by the formation of clear zones attached in the form of tables and figures. Antibacterial inhibitory power categories can be determined based on the following categories:

Table 1. Antibacterial Inhibitory Power Category (Kumowal, 2019)

No.	Clear Zone Diameter (mm)	Category
1.	> 20 mm	Very Powerful
2.	10-20 mm	Strong
3.	5-10 mm	Keep
4.	<5 mm	Weak

Results and Discussion

Test of secondary metabolites of leaves *A. gangetica* carried out with a qualitative scale using color reagents. In alkaloid testing using Mayer reagent is characterized by the presence of white residue while with dragendroff reagent there is no orange residue. Alkaloid testing is carried out by giving HCl which functions in forming alkaloid salts. The administration of Mayer reagents causes nitrogen to react with K+ metal ions, so alkaloid complex deposits are formed. Dragendroff's reagent consists of bismuth nitrate which reacts with potassium iodide to form a precipitate of bismuth(III) iodide, and a precipitated potassium tetraiodobismutate complex. The resulting sediment formed is orange-red (Dewi, 2021). Results from secondary metabolite assays show that leaves *A. gangetica* Positively contains alkaloids using Mayer's reagent and shows negative results using Dragendroff's reagent.

Test saponins are characterized by the

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formation of foam. The addition of HCl 2N causes the level of polarity to increase, so that hydrophilic groups contain stronger bonds and form a stable foam. The foam formed is caused by polar bonds that are outside and nonpolar bonds that are inside in the micelle structure so that they can form foam (Dewi, 2021). Results from secondary metabolite tests proved leaves *A. gangetica* There are saponin compounds. Testing Flavonoids are recognized by the formation of orange color on the test tube. The application of Mg powder and HCl solution causes the reduction of benzopiron formed in the flavonoid arrangement to produce flavylium salts that have an orange color (Dewi, 2021). Results from secondary metabolite tests proved leaves *A. gangetica* There are flavonoid compounds.

Test steroids are recognized by the presence of a dark brown color that occurs due to the ability of steroid compounds and dripping Lieberman-Burchard solution on solution. Results from secondary metabolite assays show that leaves *A. gangetica* positively contains steroids. The tannin test is carried out with the addition of FeCl3 solution so that it produces a blackish-green color. FeCl3 1% reacts with hydroxyl bonds contained in tannin compounds so that color forms (Dewi, 2021). Results from secondary metabolite assays show that leaves *A. gangetica* positively contains tannins.

Table 2. Results of secondary metabolites of *A. gangetica leaves*

Test	Reagent	Reaction Results	Information
Alkaloids	Mayer	+	There is a white precipitate
	Dragendroff	-	No orange-red deposits
Saponin	Water + HCl concentrated	+	There is foam
Flavonoid	Concentrated HCl + Mg	+	There is an orange color
Steroid	Lieberman Bouchard	+	There is a dark brown color
Tannins	FeCl3 1%	+	There is a blackish-green color

Table 3. Measurement Results of Antibacterial Activity Test of *A. gangetica Leaf Ethanol Extract*

Bacteria	Concentration (%)	Average Diameter of the Inhibition Zone (mm)	Category
Escherichia coli	15	2,53	Weak
	25	3,27	Weak
	35	3,6	Weak
	45	5	Keep
	55	4,73	Weak
	65	3,23	Weak
	Control (+)	30,68	Very powerful
	Control (-)	0,00	Ineffective

Antibacterial activity testing of leaf ethanol extract *A. gangetica* Performed by disc diffusion method at concentrations of 15%, 25%, 35%, 45%, 55%, and 65%. Figure

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1 shows that leaf ethanol extract *A. gangetica* which has the ability to inhibit bacteria *E. coli* and *S. aureus* which is included in the medium and weak categories. The formation of clear zones is influenced by metabolite compounds found in leaves *A. gangetica* namely alkaloid compounds, saponins, flavonoids, steroids, and tannins. Alkaloid compounds that act as antibacterial inhibit the peptidoglycan forming elements of cells, so bacterial cell walls cannot be formed (Amaliah &; Lisa, 2022). Saponin compounds that act as antibacterial act with porins found on the outer surface of the bacterial cell wall and create resistant polymer clusters, causing tansmembrane proteins to be damaged. Transmembrane protein is a way out and a way to enter substances and if transmembrane proteins are damaged, it can reduce the ability of cell membranes to cause cells to experience substance difficulties until cell death occurs (Rahmawati *et al*.,2020).

Substance flavonoids as antibacterial have the ability to denature bacterial cell proteins so that they can disrupt the bacterial cell layer (Rahmawati, A *et al*.,2020). Flavonoids can inhibit cytoplasmic membrane function, aerobic metabolism, nucleic acid synthesis so that cells do not have energy and cause cells to become lysis (Kirtayanasa, 2022). The way steroids work when inhibiting the development of bacteria is that they react with the lipid layer, which can cause leakage in bacterial lysosomes (Kirtayanasa, 2022). The way tannins work that act as antibacterial agents is to inactivate cell wall transport proteins and enzymes to inhibit bacterial growth. Tannin compounds can shrink the bacterial cell wall to be able to damage the ability of the bacterial cell wall and inhibit bacterial growth (Rahmawati, A *et al*.,2020).

Based on the test results, the average data of the largest inhibitory zone was obtained at a concentration of 45% which was included in the medium category. This study is in accordance with Hamid's (2011) research using hexane extract, methanol extract and ethyl acetate leaf extract *A. gangetica* shows the average of the inhibitory zones belonging to the sedan category Research data from concentrations of 15%, 25%, 35%, 45% showed that the average inhibitory zone increased while at concentrations of 55% to 65% experienced an average decrease in the inhibitory zone. These data show that the concentration level of leaf ethanol extract *A. gangetica* does not correspond to the result of the diameter of the inhibitory zone. The results of this study are in line with Dewi's research (2010) proving that with higher concentrations, it is uncertain to have a higher inhibitory ability but has not a large inhibitory power (*Zeniusa* et al., 2019).

Ciprofloxacin is able to inhibit bacteria that include gram-positive and gram-negative bacteria. The way the antibiotic ciprofloxacin works is by interfering with the replication process *Deoxyribose Nucleat Acid* (DNA/Deoxyribose nucleic acid). Ciprofloxacin is an antibiotic that is able to cure several diseases caused by infections such as bacteria *E. coli, S. aureus, Salmonella typhi Klebsiella pneumoniae, S. saprophyticus,* and *Streptococcus pneumonia.* Ciprofloxacin is often used as an antibiotic to cause diarrhea, but long-term use can cause toxicity or side effects, accelerate the occurrence of resistance to the risk of damage to organs. Negative control is carried out so that the effect of the solvent used in dissolving the extract is known. The pro-analysis ethanol solvent is a control (-) in this test. There is no diameter of the inhibitory zone around the paper disc in the negative control, thus proving that the solvent ethanol p.a has no antibacterial properties and does not affect the action of the leaf extract *A. gangetica* in inhibiting test bacteria.

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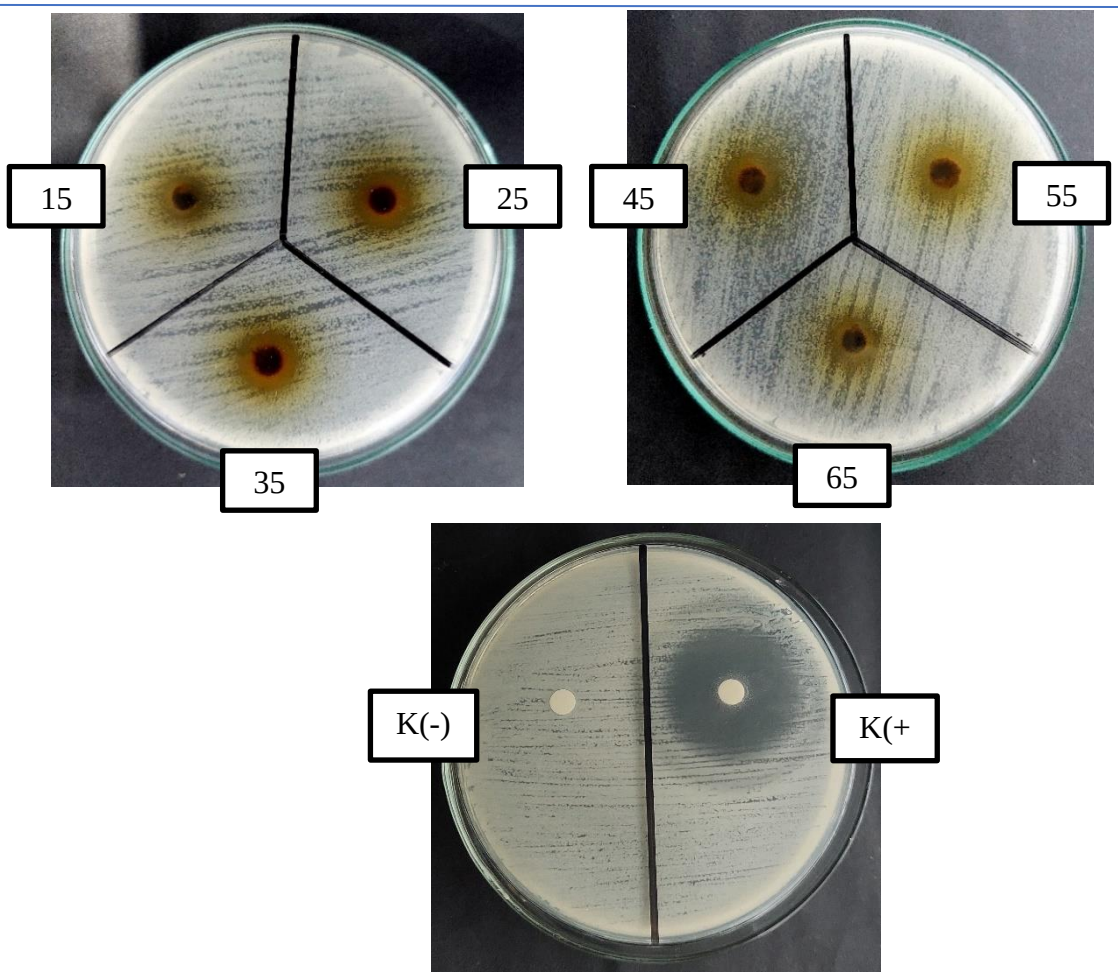

Figure 1. Antibacterial Activity Test Results Against *E. coli Bacteria*

Conclusion

Based on the secondary metabolite test conducted, the content of secondary metabolite compounds found in the leaves *of Asystasia gangetica* are alkaloid compounds, saponins, flavonoids, steroids, and tannins. Ethanol extract of *Asystasia gangetica* leaves has antibacterial activity against *Escherichia coli bacteria.* Ethanol extract of *Asystasia gangetica leaves* produced the highest average inhibitory zone diameter at a concentration of 45% belonging to the medium category.

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