

# The Effect of Various Concentrations of Ethanol Extract of the Leaves of *Paederia foetida* L. on the Growth of *Escherichia Coli* Bacteria

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Research Article

## The Effect of Various Concentrations of Ethanol Extract of the Leaves of *Paederia foetida* L. on the Growth of *Escherichia Coli* Bacteria

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### Abstract

Bacterial infection of *Escherichia coli* (E. coli) as the cause of gastrointestinal disorders in humans has increased their prevalence. Treatment using natural ingredients can be a choice of therapy because of the minimal side effects. One of the rare plants believed by the community as an antibacterial is stinking vine known as the 'leaf fart'. The purpose of this research is for knowing the activity of the ethanol extract of *Paederia foetida* L can affect the growth of *E.coli*. The serial diffusion disc method is being used as the antibacterial activity test. The concentration of this extract are 10%, 20%, 40%, 80%, 100% with positive control (*ciprofloxacin*) and negative control (aqua dest). The inhibition zone diameter characterized the effect of Extract on bacterial growth were 6.16 mm of the concentration 10%, 6.667 mm of the concentration 20%, 7.10 mm of the concentration 40 %, 7.78 mm of the concentration 80%, and 10.02 mm of the concentration 100%. As for the negative control has no effect. The study stated that the higher concentration of a bacterial agent used, the greater the inhibition zone formed. Based on the result of the analysis of the data by using the One-Way ANOVA Test showed a probability value (p) = 0.000 or value (p) < 0.05, that H<sub>0</sub> is rejected and H<sub>1</sub> is accepted. The conclusion is that the Extract of stinking vine has an antibacterial effect on the growth of *E.coli*.

**Keywords:** Antibacterial, *E.coli*, Extract of Sembukan leaf

## INTRODUCTION

*E.coli* is a typical microorganism in the human intestine, which is often one of the causes of health problems in humans.<sup>1,2</sup> Bacteria that a veterinarian first discovered from Germany named Theodor Escherich in 1885 turned out to be four different classification groups, namely EPEC (Enteropathogenic *E-Coli*), EIEC (Enterotoxigenic *E-Coli*), EHEC (Enterohemorrhagic *E-Coli*), EIEC (Enteroinvasive *E-Coli*), and EAEC (Enteraggregative *E-Coli*).<sup>4,5,6</sup> Microbiologically, these bacteria are often found in water sources, vegetables, and fruits that humans consume without proper processing. The author understands the habits of Indonesian people who usually consume raw food such as vegetables as fresh vegetables. This phenomenon will be severe because certain strains of *E.coli* can cause diarrhoea in humans.<sup>6</sup>

Human pathogenic *E. coli* O<sub>157</sub>: H<sub>7</sub> infections that are verotoxigenic in nature have caused 16,000 cases of foodborne disease (Food Borne Diseases) and 900 deaths per year in the United States, with an estimated annual cost of \$200,000 to \$600,000.<sup>1</sup> The 2013 Basic Health Research (Rikesdas) results show that the incidence of diarrhoea in children under five in Indonesia was 6.7%. The five provinces with the highest incidence of diarrhoea were Aceh 10.2%, Papua 9.6%, DKI Jakarta 8.9%, South Sulawesi 8.1% and Banten 8.0%.<sup>7</sup> Diarrhoea is an endemic disease in

Indonesia and is also a potential disease of Extraordinary Events (KLB) which is often accompanied by death. The 2016 Indonesian Health Profile shows that there have been three outbreaks of diarrhoea spread across three provinces, three districts, with 198 sufferers and six deaths. The incidence of national diarrhoea from the diarrhoea morbidity survey in 2014 was 270/1,000 population, it is estimated that the number of diarrhoea sufferers in health facilities in 2016 was 6,897,463 people.<sup>8</sup>

The use of antibacterial drugs is often used in handling this phenomenon. However, more and more people are aware of the side effects of antibiotic drugs, so not a few people are using herbal treatment.<sup>9</sup> At this time, the use of traditional medicine is still in demand. It is believed to be the provision of our ancestors to treat diseases because the raw materials are easy to obtain, affordable prices, and can be cultivated by themselves, especially for people who still live in remote areas in Indonesia.

One of the traditional plants that the people trusted in ancient times to remedy digestive disorders such as diarrhoea is the Sembukan leaf (*Paederia foetida* L.). This leaf is known to emit this unpleasant odour when rubbed on the palms of the hands, and if eaten, it will cause a distinctive fart odour. The rotten smell is due to the active compound metal mercaptan in the leaves.<sup>10,11</sup> The public well knows the benefits of healing plants. Some areas, such as Central Java,

still serve *Sembukan* leaves as home food. They cook this plant as pipes along with grated young coconut and rotten tempeh. The mixture will be wrapped in banana leaves and then steamed. This side dish is commonly known as 'gembrot semukan'. *Sembukan* leaves are also known to have health benefits, including laxative, healing ulcers, dysentery, overcoming constipation, anti-inflammatory, detoxifying, and relieving spasms. The healing benefits are also felt by foreign people familiar with *Sembukan* leaves (*Paederia foetida* L.). The leaves of *Sembukan* have good antiarrheal activity.<sup>10</sup> *Sembukan* leaves contain secondary metabolites, such as alkaloids, saponins, tannins, and flavonoids, which in pharmacology have benefits like antioxidants, antibiotics, anticancer, anti-insects, antitumor agents, and immunomodulating agents.<sup>12,13,14</sup>

The scientific evidence is shown through the research above further clarifies that *Sembukan* leaf (*Paederia foetida* L.) has excellent potential in the health sector. However, in Indonesia, research on *Sembukan* leaves is still relatively small. Therefore the authors are interested in conducting research that aims to determine the antibacterial activity of *Sembukan* leaves (*Paederia foetida* L.) against the growth of bacteria that cause digestive disorders in humans, especially *E.coli*. Based on the background of the problem above, the problems answered in this study are a) Does the ethanol extract of *Sembukan* leaves have antibacterial activity against the growth of *Escherichia coli* bacteria?, and b) What is the effect of several concentrations of ethanol extract of *Sembukan* leaves on the growth of *Escherichia coli* bacteria?. The research objectives are a) The activity of ethanol extract of *Sembukan* leaves in influencing the growth of *Escherichia coli* bacteria, and b) The effect of several concentrations of ethanol extract of *Sembukan* leaves on the growth of *Escherichia coli* bacteria.

## LITERATURE REVIEW

*Sembukan* leaves, often known as fart leaves, are among Indonesian medicinal plants originating from East Asia. Scientifically, this leaf is called *Paederia foetida* L. Description foetida indicates that this plant is a type of plant that smells bad.<sup>10,15</sup> Based on history, the term 'Paederia' is a derivative term derived from the Greek, namely Paederos, which means cat's eye. In contrast, the term '*sembukan*' is usually used by native Malays. Unlike the case with the Sundanese people who call this leaf the term 'kahitutan', while in the Ternate area, it is known as '*gumi siki*' and on the island of Sumatra, it is known as 'fart leaf'.<sup>15</sup> The people of Indonesia do not only feel the existence of this plant as a medicine but in China, this plant is known as 'Ji shit eng', while in English, it is known as 'stinking vin'e'.<sup>10</sup>

Morphologically, *Sembukan* leaf is a type of plant that propagates quickly to a height of 5-10 m. Although the leaf smell is unpleasant, the growth of the *Sembukan* leaves is complemented by flowers arranged in a compound with a length of 4-30 mm. This plant has elongated round leaves measuring 6-10 cm, while the width is 3-5 cm and also the base of the leaves is heart-shaped or rounded. The edges of this plant's leaves are flat, and the tip is pointed, the base is grooved, has fine hairs, pinnate bones, rounded petioles, and has a booklet. The *Sembukan* plant also has a 4-6 mm round fruit diameter, shiny and yellow.<sup>10,11,15</sup> *Sembukan* plants can thrive in sandy, loam, and even clay soils. Besides that, they can also grow in acidic, neutral, or alkaline soil conditions. Stem cuttings can propagate this plant. When viewed in terms of maintenance, this plant is relatively easy to maintain. It is enough to maintain watering, humidity, and fertilization.

Many chemical compounds are found in the *Sembukan* plant (*Paederia foetida* L.), including asperuloside, deacetylasperuloside, paederoside, and iridoid glycosides, paderosidic acid, arbutin, gamma sitosterol, and essential oils.<sup>15,16,17</sup> The ethanol extract of *Sembukan* leaves is potentially an anti-inflammatory with a 20mg/kg BW [17]. In addition, *Sembukan* plants also contain indole and methyl mercaptan compounds. The content of this methyl mercaptan compound is what causes the rotten smell of healed plants. Meanwhile, chemically active compounds that are thought to have antibacterial properties are flavonoids, saponins and tannins.<sup>18</sup>

*Sembukan* leaves contain secondary metabolite compounds that have many benefits. This study uses the maceration method. The *Sembukan* leaves were immersed in 96% ethanol and methanol for five days, then the ethanol extract and methanol Extract on each leaf and stem were analyzed using chemical reagents to identify alkaloids and saponins tannins and flavonoids. The results of phytochemical screening showed that the ethanol and methanol extracts of *Sembukan* stems contained alkaloids, saponins, tannins and flavonoids. In contrast, the ethanol and methanol extracts of *Sembukan* leaves contain alkaloids, tannins, and flavonoids.<sup>12</sup> In pharmacology, secondary metabolite compounds are used as medicinal ingredients such as antioxidants, antibiotics, anticancer, anti-insects, antitumor agents, and immunomodulating agents.<sup>12,13,14</sup> Alkaloid secondary metabolite compounds contained in the leaves of the *Sembukan* contain nitrogen and are alkaline. Alkaloids have pharmacological effects, including a trigger for the nervous system, antibacterial and anti-fungal, treat heart disease, raise blood pressure and others.<sup>12</sup>

Flavonoids are one of the secondary metabolites contained in plants. Flavonoids for plants function to protect themselves from disease and the surrounding environment. The function of flavonoids for the human body is to prevent cardiovascular disease because flavonoids are phenolic compounds with antioxidant properties that prevent cell damage by reactive free radicals.<sup>19</sup> Flavonoids in plants function as growth regulators, photosynthetic regulation, antimicrobial and antiviral. The function of flavonoids as an antibacterial is to form complex compounds with extracellular and dissolved proteins to damage bacterial cell membranes and are followed by the release of intracellular compounds.<sup>9,18</sup> These compounds will interfere with energy metabolism similarly to inhibiting the respiratory system because sufficient energy is required for the active absorption of various metabolites and the biosynthesis of macromolecules. Saponins as antibacterial are lowering the surface tension, resulting in increased permeability cell leakage and the release of intracellular compounds. These compounds diffuse through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane and reduce the stability of the membrane. It causes the cytoplasm to leak out of the cell resulting in cell death. Antimicrobial agents that disrupt the cytoplasmic membrane are bactericidal.<sup>6,18</sup>

Tannins are polyphenolic compounds that have large molecular weights consisting of hydroxy and carboxyl groups. Tannin compounds consist of two types, namely condensed tannins and hydrolyzed tannins.<sup>9</sup> Tannins work by forming hydrophobic complexes with proteins, inactivating adhesins, enzymes, and cell wall transport proteins, thereby interfering with the growth of microorganisms. Tannins are suspected as antimicrobial compounds due to their spasmolytic effect. The spasmolytic effect can shrink the bacterial cell wall so that the permeability of the bacterial cell is disrupted. Tannins also

have antibacterial power by precipitation of protein because it is suspected that tannins have the same effect as phenolic compounds.<sup>18</sup> Based on research conducted by Ummah in 2010, tannin compounds in starfruit leaf extract (*Averrhoa bilimbi* L.) are antibacterial against *Staphylococcus aureus* and *Escherichia coli*.<sup>20</sup>

Extracts are dry, viscous or liquid preparations made by extracting vegetable or animal simplicia according to a suitable method, outside the influence of direct sunlight.<sup>18</sup> The extraction process is the separation of materials from the mixture by using a suitable solvent. The extraction process was stopped when equilibrium was reached between the compound concentration in the solvent and the plant cells.<sup>21</sup> The quality of the Extract obtained depends on the solvent used. Selective solvents can produce extracts containing only the desired compound. The factors that need to be considered in selecting solvents are selectivity, safety, economy, ease of operation, and environmental friendliness. The quality that is allowed is that which meets the pharmaceutical requirements or known as pharmaceutical grade specifications. Several types of solvents are available: a) Aliphatic hydrocarbons are hexane and cyclohexane; b) Aromatic hydrocarbons are toluene, benzene; c) Chlorinated hydrocarbons are dichloromethane, carbon tetrachloride (chloroform); d) Alcohols such as methanol, ethanol, propanol, and butanol; e) Ketones such as acetone, methyl ethyl ketone; f) Carboxylic acids such as acetic acid; g) Esters such as ethyl acetate; h) Ether such as diethyl ether; i) Water that is transparent, colourless, tasteless, free of pathogenic germs and compounds that are harmful to health, and does not contain iron and other organic compounds; and j) Oil (rarely used).

Based on the water content, also known as liquid, viscous, and dry extracts. The liquid Extract still contains most of the solvent. Some solvents have been evaporated in the thick Extract, while the dry Extract contains no solvent. The extraction method is divided into several types, namely maceration method, percolation method, Soxhlet method, Reflux method or Steam Distillation Method, and Modified Maceration method (Ultrasound-Assisted Solvent Extraction Method).

*E. coli*, also called 'Bacterium coli commune', was first identified from the faeces of a baby in 1885 by a paediatrician from <sup>10</sup>many named Theodor Escherich.<sup>4,5</sup> *E. coli* is a normal flora in the intestines of humans and animals. *E. coli* is also considered a non-pathogenic bacterium in the digestive tract and can be considered a pathogenic bacterium if not in the digestive tract.<sup>3,4</sup>

*E. coli* is a gram-negative bacteria with a temporary structure with a length of 2.0  $\mu$ m, a diameter of 0.25-1.0  $\mu$ m and a cell volume of 0.6-0.7  $\mu$ m. The cell wall consists of peptidoglycan and an outer membrane. The cell wall of *E. coli* functions as self-protection from internal osmotic pressure that reaches 5-20 atmospheres and is also helpful in bacterial self-division. These bacteria do not have a nucleus. *E. coli* has villi which are thin filaments for capturing specific substrates and long peritrichous flagella as a means of locomotion when swimming.<sup>3,4</sup>

*E. coli* is a facultative anaerobic bacterium that can give positive results for indole, lysine decarboxylation, gas production from glucose, and its ability to ferment lactose. These bacteria can grow at a temperature of 15-48°C. The maximum growth rate of bacteria is in the temperature range of 37 - 42°C. In addition, *E. coli* can also grow at pH

conditions of 5.5 - 8.0 and optimal growth in neutral pH conditions. However, some strains of *E. coli* that cause diarrheal disease can survive in poor pH conditions (pH 2.0). *E. coli* in urine can be identified quickly by looking at its hemolytic ability on blood agar, and the characteristic feature of *Escherichia coli* bacteria is the appearance of a "sheen" (shiny) colour on Eosin Methylene Blue (EMB) agar.<sup>3,12</sup>

## RESEARCH METHOD



This research is a laboratory experiment using the serial disc diffusion method known as the Kirby Bauer method. This sensitivity test was carried out by measuring the diameter of the clear zone, which was a sign that there was inhibition of the growth of *E. coli* bacteria by antibacterial compounds contained in the Extract of the *Sembukan* leaf. This research was conducted in September 2018 - November 2018 at the Microbiology Laboratory of the Medical Faculty, Universitas Kristen Indonesia, for bacterial inhibition testing and the Bogor Agricultural Institute's Biopharmaceutical Laboratory for extraction. The research subject was *Escherichia coli* <sup>3</sup>CC 25922, which was obtained from the collection of the Department of Microbiology, Faculty of Medicine, University of Indonesia. The research sample used was *Sembukan* leaf extract. In this study, there were seven treatment groups, namely: distilled water as a negative control, concentrations of 10%, 20%, 40%, 80%, 100%, and the antibiotic ciprofloxacin as a positive control. Based on the Federer formula, the number of repetitions in the seven treatment groups was obtained. So, the number of repetitions that can be done in each treatment group is four times. Data derived from the research results on the effect of *Sembukan* leaf extract on the growth of *E. coli* bacteria were analyzed using SPSS 24.0. The test that can be used on the number of samples < 30 in this study is the One Way Anova test. This analytical test aims to analyze two variables, namely the dependent variable and the independent variable, to determine <sup>2</sup>whether there is an effect of giving *Sembukan* leaf extract on the inhibition of the growth of *E. coli* bacteria. The interpretation of the analysis test, namely: a) If the p-value < (0.05), then  $H_0$  is rejected, and  $H_1$  is accepted. It shows that the sample data support <sup>2</sup>the effect of *Sembukan* leaf extract (*Paederia foetida* L.) on the inhibition of the growth of *E. coli* bacteria, and b) If the p-value > (0.05), then  $H_0$  is accepted and  $H_1$  is rejected. It shows that the sample data do <sup>2</sup>not support the effect of giving *Sembukan* leaf extract on the inhibition of the growth of *E. coli* bacteria.

## RESULT AND DISCUSSION

*Sembukan* leaf extract was made from 3 kg of fresh *Sembukan* leaves, which were then dried and yielded 385 grams of dried simplicia. Then 100 grams of powdered simplicia was extracted by maceration method and <sup>2</sup>using analyzed ethanol solvent (PA), which was carried out at the Microbiology Laboratory of the Faculty of Medicine, Christian University of Indonesia and Phytopharmacy Laboratory, Bogor Agricultural University. The final result obtained is a thick extract of *Sembukan* leaves as much as 2.0 grams.

Bacterial culture was carried out using two different agar plate types: Nutrient Agar (NA) and Eosin Methylene Blue (EMB). Bacterial culture was carried out at the Microbiology Laboratory, Faculty of Medicine, Christian University of Indonesia. The stages of bacterial culture on agar plates can be seen in Appendix 4.

**Table 1: Bacterial Culture Results on Agar Plate**

Agar Plate Type	Colony Results
Nutrient Agar (NA)	<p>Spread according to the zig-zag pattern formed, and the bacterial colonies are round and convex, yellowish-white in colour, smooth, soft inconsistency, and have flat edges.</p>  <p>Figure 1: Escherichia coli Bacteria Culture Results on Nutrient Agar Plates [Source: Personal Documentation]</p>
Eosin Methylene Blue (EMB)	<p>Spread according to a zig-zag pattern formed and looks like a convex round colony, shiny green like metal, smooth, soft consistency, and flat edges.</p>  <p>Figure 2: Bacterial Culture Results of Escherichia coli on Eosin Methylene Blue (EMB) Plate [Source: Personal Documentation]</p>

The method used in this study is the diffusion method with paper discs. E.coli bacteria were inoculated on Mueller-Hinton Agar, and then each agar was planted with seven paper discs consisting of five discs that had been soaked in different concentrations of *Sembukan* leaf extract, namely 10%, 20%, 40%, 80%, 100%, and the other two discs were negative controls using distilled water and positive controls using ciprofloxacin antibiotics. The treatment was repeated at each concentration four times. Mueller-Hinton Agar which had been planted with seven discs, were then incubated at 37°C for 24 hours. The bright zone formed around the paper disc indicates an inhibitory response to bacterial growth by the active compounds found in the Extract of *Sembukan* leaves. The bright zone that is formed can be seen in the image below:

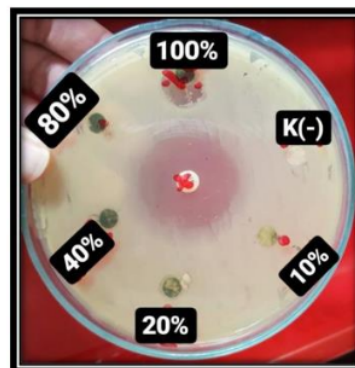


Figure 3: Results of Sensitivity Test Results of Sembukan Leaf Extract Against Escherichia coli Bacteria Inhibition [Source: Personal Documentation]

2  
Based on the results of research conducted using the inhibitory power of *Sembukan* leaves on the growth of *E.coli* conducted at the Microbiology Laboratory of the Medical

Faculty, Universitas Kristen Indonesia, in October, the following results were obtained:

Table 2: Results of Inhibitory Zone Measurements on *E. coli* Growth

No	Concentration	Inhibition Zone Diameter (mm)				Average (mm)
		I	II	III	IV	
1	Control (+) Siprofloksasin	32.025	31.025	34.20	30.45	31.925
2	Control (-) Aquades	0	0	0	0	0
3	10%	6.125	6.30	6.125	6.10	6.1620
4	20%	6.30	7.025	7.15	6.175	6.6625
5	40%	7.475	7.25	7.225	6.475	7.1060
6	80%	8.025	8.425	7.45	7.15	7.7625
7	100%	8.30	9.425	9.125	13.3	10.0375

The results of the above study indicate the presence of antimicrobial activity of *Sembukan* leaf extract on the growth of *E.coli* in the form of the formation of an inhibition zone on Mueller Hinton Agar which can be seen from the clear circle that does not contain *E. coli* bacterial colonies at concentrations of *Sembukan* leaf extract 10%, 20%, 40%, 80%, and 100%. The positive control using the antibiotic ciprofloxacin in this study showed the largest bacterial inhibition zone, while the negative control using distilled water did not get a clear zone.

These results can also determine the inhibitory power of *Sembukan* leaf extract in inhibiting the growth of *Escherichia coli* bacteria. The criteria for the resistance strength can be explained based on the theory proposed by David and Stout in the table below:

Table 3: Criteria for Inhibitory Strength<sup>22</sup>

Inhibition Zone Diameter (mm)	Inhibitory Properties
≥ 20	Very strong
10 - 20	Strong
5 - 10	Currently
≤ 5	Weak

Based on Table 3, the inhibitory properties of the *Sembukan* leaf extract at the available concentrations are moderate, in contrast to the inhibitory properties of ciprofloxacin leaf extract as a positive control with powerful inhibitory properties. Based on the results described in Table 2, an increase in the bacterial inhibition zone was also obtained for each increase in the leaf extract concentration. The increasing graph in the inhibition zone formed by each different concentration can be seen in the image below:

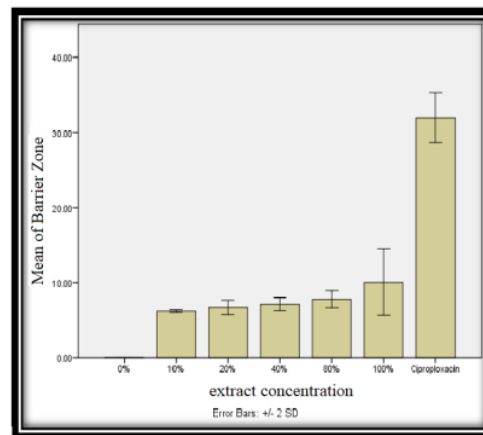


Figure 4: Graph of Increasing Bacterial Inhibition Zone Diameter

Based on Figure 4, it is obtained that the average diameter of the inhibition zone produced starting from a concentration of 10% continues to increase the graph to a concentration of 100%, and the positive control occupies the highest value, while the negative control does not show any value on the graph.

One Way ANOVA test aims to determine the relationship between the independent variable and the dependent variable. H0 is the null hypothesis which states that there is no relationship between the independent variable and the dependent variable, while H1 is the working hypothesis which states that there is a relationship between the independent variable and the dependent variable. If, in this study, the sig (p) < 0.05 probability value, then H0 is rejected and H1 is accepted, which means that there is an effect of *Sembukan* leaf extract as an antibacterial on the growth of *E.coli* bacteria. The results of the One Way ANOVA test can be seen in the table below.

Table 4: Results of One Way ANOVA Probability Test Score

ANOVA				
Barrier Zone				
Variation	df	Rata-rata	F	Sig.
Between groups	6	413.290	342.	.000
In Group	21	1.207	297	
Total	27			

Based on Table 4, the results show that the probability value (p) = 0.000 or the value (p) <0.05 so that H<sub>0</sub> is rejected and H<sub>1</sub> is accepted. It means that the leaf extract of *Sembukan* has an antibacterial effect on the growth of E.coli bacteria.

Inhibition of microorganisms, one of which is influenced by the presence of active antimicrobial compounds. Ethanol and methanol extracts of *Sembukan* leaves contain alkaloids, tannins, and flavonoids, while the ethanol and methanol extracts of *Sembukan* stems contain alkaloids, saponins, tannins and flavonoids.<sup>11</sup> These active compounds are antimicrobial due to their ability to form complexes with soluble extracellular proteins and microbial cell walls and work as type II topoisomerase inhibitors that inhibit bacterial DNA replication and transcription.

Based on the inhibition zone on four repetitions, the inhibition zone diameter was highest in the positive control using the antibiotic ciprofloxacin with diameters of 32.025 mm, 31.025 mm, 34.20 mm, and 30.45 mm, respectively. At a concentration of 10%, the diameters were 6.125 mm, 6.30 mm, 6.125, and 6.10 mm, respectively. At a concentration of 20%, the diameters were 6.30 mm, 7.025 mm, 7.15 and 6.175 mm, respectively. At a concentration of 40%, the diameters were 7.475 mm, 7.25 mm, 7.22 mm, and 6.475 mm, respectively. At a concentration of 80%, the diameters were 8.025 mm, 8.425 mm, 7.45 mm, and 7.15 mm, respectively. At 100% concentration, the diameters were 8.30 mm, 9.425 mm, 9.125 mm, and 13.3 mm, respectively. Negative control using distilled water did not show any growth inhibition zone for E.coli bacteria.

The low inhibition zone produced may occur because the concentration of the pure Extract is too small and due to other factors, such as E.coli bacteria, which are included in the gram-negative bacteria group, which can also affect the study results. It is due to differences in the cell walls of gram-positive bacteria, which have a more straightforward cell wall structure than gram-negative bacteria, which have a reasonably complex bacterial cell wall. The cell wall of gram-positive bacteria consists only of peptidoglycan and teichoic acid, while gram-negative bacteria consists of peptidoglycan and an outer membrane that contains three essential components outside of peptidoglycan, namely lipoproteins, lipopolysaccharides and the periplasmic membrane. It causes gram-positive bacteria to be more easily inhibited than gram-negative bacteria. In addition, the influence of physical and chemical factors in the form of seed properties, extract diffusion power, extract stabilization, and microbial concentration on the surface of the medium used can also affect the inhibition zone produced by *Sembukan* leaf extract against E.coli bacteria.

In another experiment, namely, the test of the bioactive compound of *Sembukan* leaf extract (*Paederia foetida* L.) against *Staphylococcus aureus* by the bioautography method, it can be concluded that the ether extract of *Sembukan* leaves can provide effectiveness against the

growth of *Staphylococcus aureus* at an Rf value of 0.85 with an inhibition zone of 4.75. mm, the Rf value of 0.82, the inhibition zone was 6.5 mm, the Rf value was 0.84 the inhibition zone was 6.25 mm. It shows that the leaf extract of *Sembukan* in specific concentrations can inhibit a microorganism.<sup>23</sup>

The diameter of the inhibition zone in the positive control using the antibiotic ciprofloxacin resulted in the largest inhibition zone from the inhibition zone of other concentration treatments. Ciprofloxacin belongs to the second-generation fluoroquinolone group, which is very active against gram-negative bacteria and is bactericidal by inhibiting DNA gyrase and topoisomerase II, required for bacterial DNA replication, transcription, repair and recombination of gram-negative bacteria, such as E. coli.<sup>24</sup> Research on The effectiveness of the antibiotic ciprofloxacin against E.coli, which Lisa Kepel carried out in 2015, also showed positive results, and it was found that the diameter of the inhibition zone reached 29 mm with three repetitions, which means that if the inhibition zone is >17 mm it is said that the E.coli bacteria are sensitive against the antibiotic ciprofloxacin.<sup>25</sup> Aquades as a negative control in this study showed no zone of inhibition. It indicates that the control used does not have antibacterial activity.

The choice of solvent in the extraction process will affect the active compounds that can be dissolved and affect the antibacterial test results.<sup>26</sup> Ethanol is a polar solvent that is widely used in the extraction process because it is helpful in the synthesis of chemical compounds. Ethanol also has a low boiling point of 79°C when compared to other alcohols and is higher than methanol but tends to be safe and harmless when used. The ethanol solvent has two sides consisting of a polar -OH group and a non-polar CH<sub>2</sub>CH<sub>3</sub> group, and this non-polar nature allows ethanol to extract the active compounds in *Sembukan* leaves.<sup>26,27</sup> Semi-polar ethanol can dissolve polar and non-polar compounds such as tannins, flavonoids, phenols, and essential oils that function as antibacterial.<sup>28</sup>

However, it must also be noted that the extraction process of antibacterial compounds also affects its activity because the solvent of ethanol is semi-polar, so that the extracted compounds are relatively polar. It causes the antibacterial activity of the compound to work less than optimally. Therefore it is necessary to separate the further compound of the crude Extract so that pure antibacterial compounds have more significant inhibitory activity. The polarity of these compounds causes these compounds to penetrate the bacterial cell wall more easily.

## CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that: a) The ethanol extract of the leaves of *Sembukan* (*Paederia foetida* L.) has the potential to inhibit the growth of E.coli with statistical test results showing a probability value (p) = 0.000 or a value (p) <0.05;

and b) There was an increase in the diameter of the bacterial inhibition zone starting from concentrations of 10%, 20%, 40%, 80%, and 100%. It shows that the higher the concentration of *Sembukan* leaf extract, the greater the bacterial inhibition zone produced. Thus, it is necessary to do: a) Further research, such as fractionation to determine the activity of specific compounds in *Sembukan* leaves as antibacterial; b) It is necessary to conduct further research on the comparison of inhibition zones of *Sembukan* leaf extract against gram-positive and gram-negative bacteria; and c) It is necessary to test for *Escherichia coli* bacteria with different series, such as the Japanese Collection of Research Bioresources (JCRB), Belgian Coordinated Collection of Microorganisms (BCCM), the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ or German Collection of Microorganisms), and Cell Cultures), and the National Collection of Plant Pathogenic Bacteria (NCPBB).

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