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Inhibitory Potency of Ethyl Acetate Extract of Jasmine Flower (*Jasminum sambac* Ait.) Against the Growth of *Streptococcus pyogenes* and *Escherichia coli*

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Abstract: **Jasmine (*Jasminum sambac* Ait.) is one of the plants which widely cultivated in Indonesia and used as a traditional medicine to cure diarrhea, fever, influenza, shortness of breath, and also act as an antibacteria. The objective of this study is to determine the antibacterial activity of ethyl acetate extract of jasmine flower against the growth of *Streptococcus pyogenes* and *Escherichia coli*. This study is a laboratory experimental study with a *Randomized Post-Test Only Control Group* design. Ethyl acetate extract of jasmine flower is diluted into four different concentration, such as 25%, 50%, 75% and 100%. The inhibitory potency test against the colony of *S. pyogenes* ATCC 19615 and *E. coli* ATCC 25922 was conducted in Laboratorium Kesehatan Provinsi Bali. The inhibition zone is measured by calipers. Data result were analysed by descriptive statistic. The ethyl acetate extract of jasmine flower inhibit the growth of *S. pyogenes* with the smallest inhibition zone at the 25% concentration (12,75 ± 0,500 mm) and the largest inhibition zone at 100% concentration (15,75 ± 0,500 mm). Ethyl acetate extract of jasmine flower inhibit the growth of *E. coli* with the smallest inhibition zone at 75% concentration (12,25 ± 0,500 mm) and the largest inhibition zone at the 100% concentration (19,00 ± 1,154 mm). The increased inhibition zone of the bacteria is directly proportional to the increase in extract concentration.**

Keywords: **ethyl acetate extract, jasmine flower, antibacterial activity, Streptococcus pyogenes, Escherichia coli.**

I. INTRODUCTION

Streptococcus pyogenes and *Escherichia coli* are the two species of bacteria that often cause disease in the human body. *S. pyogenes* is found in the respiratory tract, but it does not cause symptoms or disease when the host's body defense is in a good condition.(1) Unlike *S. pyogenes* who lives in the respiratory tract, *E. coli* lives as a normal flora in the digestive tract. Generally *E. coli* is not dangerous, but there are several strains that can cause serious food poisoning and diarrhea.(2)

Bacterial infections normally can be treated with antibiotics. However, due to its extensive and excessive use, the bacteria become able to adapt, which result in reduced antibiotic effectiveness and even side effects in its usage.(3) The 2006 report states that more than 70% of infections are caused by bacteria that are resistant to one or more treatments that is usually used to fight these bacteria.(4) Therefore, research is needed to find alternative antibiotics from herbal plants. The use of herbs in the treatment of various diseases has various advantages, such as the side effects are relatively smaller than the use of chemical drugs. (5)

Jasmine is one of the herbal plants that have a lot of beneficial compounds in them. This flowering plant is widely cultivated in Indonesia. In addition to its fragrant aroma, every part of the plant such as leaves, stems, flowers and roots

has benefits in the pharmaceutical field. These various properties of jasmine are due to the content of active compounds such as alkaloids, flavonoids, saponins and tannins which obtained from the process of extraction.[6]

Based on a research conducted by Reema and Adel in 2011 regarding the antibacterial and antifungal activity of jasmine extracts, it was found that the inhibitory zone of the flowers extract was larger than the extract made of the leaves.[7] Maghfiroh also conducted a similar study in 2014 using several different polarity of solvents to extract the flower. The study showed that the flower extract of jasmine with ethyl acetate solvents had a larger inhibition zone than other solvents.[8] In connection with these indications, further research is needed to find out the inhibitory potency of jasmine flower extract as an antibacterial. In this study, the extraction extraction was made using ethyl acetate, and testing of its inhibitory strength was performed on *S. pyogenes* as a representative of Gram positive bacteria and *E. coli* as a representative of Gram negative bacteria. The results obtained are expected to be a reference for the use of jasmine flowers as an alternative antibacterial drug.

II. MATERIAL AND METHOD

A. Experimental Procedure

This study is a laboratory experimental study with a randomized post-test only control group design. It was approved by the Committee of Research Ethics, Udayana University. The antibacterial effect of jasmine flowers was tested using the disc diffusion method by measuring the diameter of the inhibition zone on each bacteria. The research was started from the preparation of jasmine plant flowers as the extract material, then extracted using a maceration method with ethyl acetate solvents to produce ethyl acetate extracts of jasmine flowers which would then be tested for its antibacterial activity.

The plant material used in this study is the jasmine flowers cultivated in Cirebon, West Java with consideration of the ease of getting flowers compared to other regions. This study was conducted at Clinical Microbiology Laboratory, Bali Provincial Health Laboratory Center. The sample used was *S. pyogenes* ATCC 19615 isolate obtained from the Laboratory of Microbiology at the Faculty of Medicine, Udayana University, *E. coli* isolate ATCC 25922 obtained from Bali Provincial Health Laboratory Center.

Each colony culture of *S. pyogenes* and *E. coli* was given six treatments as follows: positive control (cefotaxime 30 µg), negative control (ethyl acetate solution), and ethyl acetate extract of jasmine flower with concentrations of 25%, 50%, 75% and 100 %. The amount of repetition needed in the study is calculated using the Federer formula. These formula result in four times repetititon for each bacteria. Ethyl acetate extract of jasmine flower is said to have an antibacterial effect if the extract can inhibit the growth of *S. pyogenes* and *E. coli*. In contrast, the ethyl acetate extract of jasmine flowers is said to have no antibacterial effect if the extract is not able to inhibit the growth of *S. pyogenes* and *E. coli* (negative inhibition zone). The inhibitory potency was assessed by measuring the diameter of the inhibition zone, a clear colored area that arose around the disc that had been previously extracted with various concentrations. Furthermore, the diameter is calculated by caliper in units of millimeters (mm).

B. Statistical Analysis

Data were analyzed using the SPSS software. The results were presented as mean ± SD (standard deviation). The normality of data distribution was confirmed using the Shapiro-Wilk test. The homogeneity of the data was confirmed using Levene test. If the data is normally distributed and homogeneous, then proceed with a one-way ANOVA parametric test. If the data is not normally distributed and not homogeneous, then it will be followed by the Kruskal Wallis non-parametric statistical test. Results were considered statistically significant if $p<0,05$.

III. RESULT AND DISCUSSION

A. Result

Based on the research that has been done, there is an inhibition zone found in *S. pyogenes* colony. It is a beta hemolytic group of bacteria, thus it will cause hemolysis and form a bright zone when grown in a MH blood agar media. The inhibition zone formed can be seen in Figure 1.

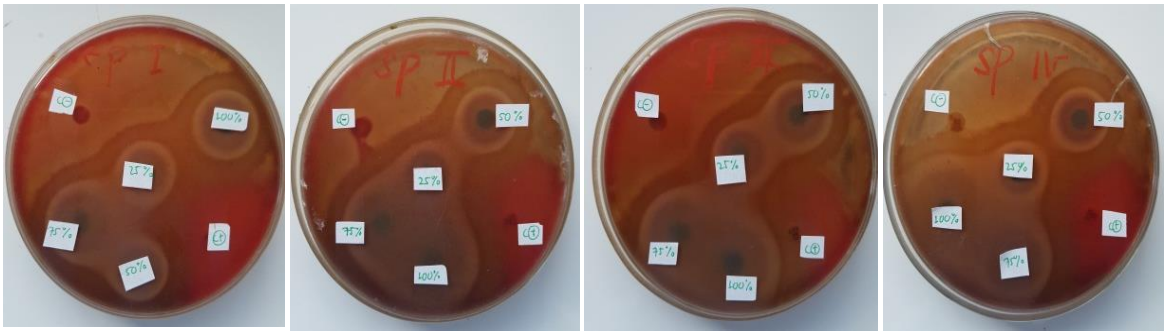

Fig. 1: Inhibition zone found in *S. pyogenes* with four times repetition

The results of inhibition zone measurement on *S. pyogenes* are presented in Table 1 below. When compared with various other concentrations of extracts, the smallest inhibition zone area was found in a concentration of 25% with a mean inhibition zone of 12,75 ± 0,500 mm. Whereas the largest inhibition zone area was found in 100% concentration with a mean inhibition zone at 15,75 ± 0,500 mm.

Table 1: Inhibition zone diameter produced by each group against *S. pyogenes*

Treatment	Inhibition Zone Diameter (mm)				
	Repetition				Mean ± SD
	I	II	III	IV	
Extract 25%	13	12	13	13	12,75 ± 0,500
Extract 50%	14	14	14	13	13,75 ± 0,500
Extract 75%	15	15	16	16	15,50 ± 0,577
Extract 100%	15	16	16	16	15,75 ± 0,500
C (+)	18	17	17	17	17,25 ± 0,500
C (-)	0	0	0	0	0

After the data is obtained, normality test and homogeneity test are carried out. Significance value of p <0.05 obtained indicating that the data is not normally distributed and not homogeneous. Then the Kruskal-Wallis non parametric test was carried out and p value <0.05 was obtained, so it can be concluded that the ethyl acetate extract of jasmine flowers in various concentrations has a statistically significant inhibitory effect on the growth of *S. pyogenes*.

Similar research was conducted on colonies of *E. coli*. The presence of clear areas around the disc paper indicated an inhibition zone produced by the extract, which can be seen in Figure 2.

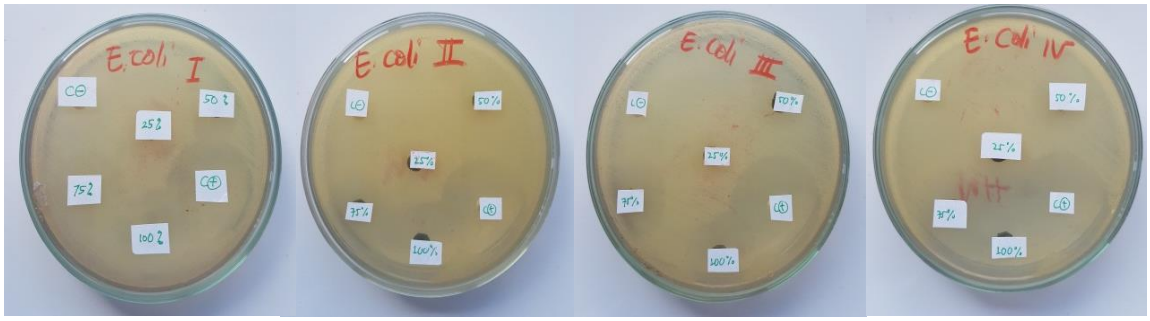

Fig. 2: Inhibition zone found in *E. coli* with four times repetition

The results of inhibition zone measurement on *S. pyogenes* are presented in Table 2 below. Smallest inhibition zone can be found in the concentration of 75% with the mean of 12,25 ± 0,500 mm. Whereas the largest inhibition zone area was found in a concentration of 100% with an mean inhibition zone at 19,00 ± 1,154 mm. The 25% and 50% concentration of the extract did not produce a inhibition zone in the growth of *E. coli*.

Table 2: Inhibition zone diameter produced by each group against *E. coli*

Treatment	Inhibition Zone Diameter (mm)				
	Repetition				Mean ± SD
	I	II	III	IV	
Extract 25%	0	0	0	0	0
Extract 50%	0	0	0	0	0
Extract 75%	12	12	12	13	12,25 ± 0,500
Extract 100%	18	20	18	20	19,00 ± 1,154
C (+)	28	29	29	29	28,75 ± 0,500
C (-)	0	0	0	0	0

After the data is obtained, normality test and homogeneity test are carried out. From the two tests, p value <0.05 was obtained so that it can be concluded that the data is not normally distributed and not homogeneous. Thus, statistical analysis was continued by the Kruskal-Wallis non-parametric test. From the test, the value of p <0.05 was obtained so it can be concluded that the ethyl acetate extract of jasmine flowers in various concentrations has a statistically significant inhibitory effect on the growth of *E. coli*.

B. Discussion

In this study, the growth of *S. pyogenes* and *E. coli* was also tested by giving cefotaxime 30 µg as a positive control and ethyl acetate solution as a negative control. Cefotaxime is one of the broad spectrum antibiotics that can be used to eradicate gram positive or negative bacterial infections. It was found in this study that cefotaxime produced a larger inhibition zone compared to jasmine flower extract. The use of ethyl acetate as a negative control in this study was to ensure the inhibitory potency formed is because of the active substance of the jasmine flower itself, not the solvent.

Ethyl acetate extract of jasmine flower inhibit the growth of *S. pyogenes* by producing different diameters of inhibition zone at each concentration. Based on the results of the study, it was found that the ethyl acetate extract of jasmine flowers with various concentrations tested had the potential to inhibit the growth of S. pyogenes bacteria. This is proved by the formed of a brightly colored circle around the disc. According to Davis and Stout, the criteria for antibacterial strength are divided into four categories based on the diameter of the inhibitory zone produced.[9] Ethyl acetate extract of jasmine flower with the concentration of 25%, 50%, 75% and 100% used in this study was categorized into the category of strong inhibition zone. In another words, the four different extract concentrations have the same ability to inhibit the growth of *S. pyogenes*.

The research conducted on *E. coli* found that the ethyl acetate extract of jasmine flowers at the concentration of 75% and 100% had the potential to inhibit the growth of *E. coli* which categorized into strong inhibition zone. While the 25% and 50% concentration of the extract have no potential to inhibit the growth of *E. coli*. The overall results of this study showed a larger inhibition zone diameter produced in *S. pyogenes* compared to *E. coli*. This is due to the differences in bacterial cell wall structure. Gram positive bacteria has a simpler cell wall structure compared to Gram negative bacteria, which made the antibacterial compounds easier to enter the cells of Gram positive bacteria.[10]

The presence of antibacterial activity is due to the active substances contained in plants. Phytochemical screening carried out by Hossain et al. in 2014 on ethyl acetate extract of jasmine flowers showed the presence of saponins, proteins, steroids, glycosides, terpenoids and terpenes.[11] Saponins have surface active substances that resemble detergents, which can reduce bacterial cell wall stress and damage membrane permeability, thus disrupting bacterial survival.[12] While terpenoids will form a strong polymeric bonds with porins on the outer walls of bacterial cells, resulting in damage to proteins. The permeability of the bacterial cell wall will be disrupted so that the bacteria become nutrient deficient and bacterial growth becomes blocked or dead.[13]

There are several other factors that can affect the inhibitory potency of the extract against bacterial growth, including the origin of extract ingredients, climate conditions, storing methods of extract materials, differences in diluents, conditions of the growth media. The process of making extracts and long storage time of extracts also can affect the inhibitory potency of the extract.[14]

IV. CONCLUSION

Ethyl acetate extract of jasmine flower has an antibacterial activity against the growth of *S. pyogenes* and *E. coli*. Various concentrations tested in this study can inhibit the growth of *S. pyogenes* with a strong inhibitory category. Meanwhile, only 75% and 100% concentration of the extract can inhibit the growth of *E. coli* with a strong inhibitory category. The increase in the concentration of ethyl acetate extract of jasmine flowers is directly proportional to the diameter of the inhibitory zone produced. The greater the concentration used, the greater the diameter of the inhibition zone produced by *S. pyogenes* and *E. coli.*

Further research is needed to determine the side effects of administering the extract if used as a therapy for a certain period of time. Minimum inhibitory concentration (MIC) test is also need to be done to determine the minimum concentration of the extract that can inhibit the growth of *S. pyogenes* and *E. coli*.

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