Minimum inhibitory concentration of leaf and fruit extract
*Ficus lyrata* Warb against *Salmonella thypi* bacteria

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**Abstract.** *Ficus lyrata* Warb is a part of the genus *Ficus*, which in its utilization is only limited as a shade plant on the roadside. The *Ficus lyrata* Warb leaves and fruits contain bioactive compounds, namely flavonoids, phenolic compounds, and tannins, which have the potential to be an antimicrobial. The antimicrobial activity of leaf extracts of *Ficus lyrata* Warb against *Escherichia coli* has already known in previous studies. However, the study about antimicrobial activity of leaf and fruit extracts of *Ficus lyrata* Warb tree against *Salmonella typhi* is still not known until now. This research aims to obtain the antimicrobial activity of leaf and fruit extracts of *Ficus lyrata* Warb and to determine Minimum Inhibitory Concentration (MIC) against *Salmonella typhi*. The method used for antibacterial activity testing was the disc diffusion method, and the determination of MIC was carried out by the microdilution method using spectrophotometric UV-Vis analysis. The inhibition zone data were analyzed using one way ANOVA and Least Significant Differences (LSD). An independent *t*-test was used for pairwise comparison. The results showed that there were effects of fruit and leaf extracts on the growth of *Salmonella typhi* bacteria and each concentration of the extracts have a significant difference, compared to the positive control. The MIC test results using spectrophotometry with two replications showed that the fruit MIC value of 1.56% and the leaf MIC value was 6.25%.

**Keywords:** Antimicrobial activity, *Ficus lyrata* Warb, Minimum inhibitory concentration (MIC), *Salmonella typhi*

1. **Introduction**

Indonesia is one of the developing countries with a poor level of sanitation. Diseases that can be caused due to poor sanitation and hygiene behaviors are typhoid fever. Typhoid fever is a disease of acute fever caused by a bacterial infection of *Salmonella enterica* Alba *et al* [1]. Based on the results of the WHO study, sufferers of typhoid fever in Indonesia reached 81% per 100,000 inhabitants Departemen Kesehatan [2].

The spread of disease by microorganisms needs to be controlled to suppress the spread and infection of infected products. Based on this it is needed an antibacterial as an attempt to inhibit bacteria. However, bacterial resistance to antibiotics is increasing causing many researchers to develop antimicrobial agents derived from plants Tkachenko *et al* [3].
Ficus lyrata Warb is one of the plants of the genus Ficus containing bioactive compounds. The Ficus lyrata Warb plant is generally only used as a shade tree on the roadside because its branches have similarities to the umbrella which is arranged and supported by wide and dense leaves. The provision of the Ficus lyrata Warb leaf extract by Wira et al. [4] shows the antibacterial activity of bacteria, Escherichia coli, and Bacillus subtilis. The resulting antibacterial is expected to have selective toxicity, where selective toxicity can be discovered by determining the minimum inhibitory concentration of active microbial substances to inhibit bacterial growth. Research of Wira et al. [4] demonstrates the presence of antibacterial activity on the leaves of E. coli, but is not yet known to have antibacterial activity of the fruit and leaves against the bacteria of S. typhi and not yet identified what is the value of the concentration Minimum extracts of fruit and leaves Ficus lyrata Warb against bacteria S. typhi.

This research aims to determine the antibacterial activity of fruit extracts and leaves of the Ficus lyrata Warb and determine the minimum concentration of the fruit and leaf of Ficus lyrata Warb in the Salmonella typhi bacteria.

2. Research methodology

2.1. Type of research
This research aims to obtain the antimicrobial activity of leaf and fruit extracts of Ficus lyrata Warb and to determine Minimum Inhibitory Concentration (MIC) against Salmonella typhi. The type of methods that were used in this research was laboratory experiments with five different concentrations (100%, 50%, 25%, 12.5%, dan 6.25%), one negative control group (DMSO), and one positive control group (amoxicillin).

2.2. Research focus
Extract fruit and leaves of Ficus lyrata Warb is produced by using the maceration method using ethyl acetate solvents with twice replications. Fruit extracts will be diluted with DMSO solvent to produce five variations of concentration, namely 6.25%, 12.5%, 25%, 50% and 100%. This research aimed to obtain the antimicrobial activity of leaf and fruit extracts of Ficus lyrata Warb and its Minimum Inhibitory Concentration (MIC) against Salmonella typhi. The method used for antibacterial activity testing was the disc diffusion method, using calipers for the measurement, and the determination of MIC was carried out by the microdilution method using spectrophotometric analysis.

2.3. Data analysis
The analytical method that is used for the inhibition zone data was analyzed using one way ANOVA, Least Significant Differences (LSD) and independent t-test was used for pairwise comparison. The analytical method that is used for the determination of MIC was descriptive analysis data.

3. Results and discussion

3.1. Antimicrobial activity of leaf and fruit extracts of Ficus lyrata Warb
The bioactive compounds in the fruit and the leaves of Ficus lyrata Warb were produced from the maceration extraction process using ethyl acetate solvents with twice replications. Based on the research of Wira et al [5] the optimal solvent for producing Ficus lyrata Warb leaf extract that has better antibacterial activity is ethyl acetate when compared to ethanol and water solvents. Antimicrobial activity of fruit and leaf extracts of Ficus lyrata Warb against Salmonella typhi bacteria tested for any concentration of 100%, 50%, 25%, 12.5%, and 6.25%. Testing uses a disk diffusion method with Duplo testing and double repetition. The result of the measuring inhibition zone of the fruit and leaf extracts of Ficus lyrata Warb against Salmonella typhi bacteria could be seen in table 1.
Table 1. Inhibition zone of *Ficus lyrata* Warb fruit and leaf Extract to *Salmonella typhi* bacteria.

| Concentration of Extract | Fruit Inhibition Zone (mm) | Leaf Inhibition Zone (mm) |
|--------------------------|---------------------------|---------------------------|
| 100%                     | 13 ± 0.70                 | 6 ± 0.70                  |
| 50%                      | 6.5 ± 0.00                | 2.75 ± 1.06               |
| 25%                      | 1.5 ± 0.70                | 1 ± 0.70                  |
| 12.5%                    | 1.375 ± 0.88              | 0.875 ± 0.53              |
| 6.25%                    | 1.375 ± 0.88              | 0.75 ± 0.70               |
| Positive Control          | 31.25 ± 0.35              | 29.75 ± 1.06              |
| Negative Control          | -                         | -                         |

The data that has been obtained from the observed results are then analyzed in the statistic. Statistical testing conducted is a parametric test, One Way ANOVA. The requirement of testing One Way ANOVA is the data should be of normal distribution and have the same variance (homogeneous). Testing normality using a one-sample Kolmogorov-Smirnov test and the test of homogeneity using SPSS version 25 indicates that the data spread is a normal distribution with a value of sig. of 0.200 where the value of \( p > 0.05 \). The data is said to be normal if it has a value of sig. > 0.05. However, a homogeneity test cannot be performed because the standard deviation between the samples is approaching zero, which if the data has the same variance does not require a test of homogeneity. Some researchers found that the ANOVA test robust against the unhomogenization of data with the condition that the data group compared was equivalent.

The significance value of fruit and leaf extracts with One Way ANOVA is 0.000, where the significance value is 0.000 < 0.005. This indicates that there is an influence on the concentration of fruit extracts as well as the leaves of *Ficus lyrata* Warb to the growth of *Salmonella typhi* bacteria. The One Way ANOVA test only shows whether there is an influence on the concentration of extracts against the test bacteria, but it is unknown whether there was a meaningful difference between the concentrations of bacterial growth in each extract. Therefore, an advanced test of LSD (Least Significant Differences) was performed on the fruit and leaf extracts showed in table 2 and 3.

Table 2. LSD test result *Ficus lyrata* Warb fruit extract against *Salmonella typhi*.

| Concentrations | 100% | 50% | 25% | 12.5% | 6.25% | K (+) | K (-) |
|----------------|------|-----|-----|-------|-------|-------|-------|
| 100%           | -    | 0.00| 0.00| 0.00  | 0.00  | 0.00  | 0.00  |
| 50%            | 0.00 | -   | 0.00| 0.00  | 0.00  | 0.00  | 0.00  |
| 25%            | 0.00 | 0.00| -   | 0.846 | 0.846 | 0.00  | 0.046 |
| 12.5%          | 0.00 | 0.00| 0.846| -     | 1.000 | 0.00  | 0.062 |
| 6.25%          | 0.00 | 0.00| 0.846| 1.000 | -     | 0.00  | 0.062 |
| K (+)          | 0.00 | 0.00| 0.00 | 0.00  | 0.00  | -     | 0.00  |
| K (-)          | 0.00 | 0.00| 0.046| 0.062 | 0.062 | 0.00  | -     |
The LSD test on fruit extract showed the results of the analysis of the concentration of 100% and 50% with a concentration of 25%, 12.5%, 6.25%, positive and negative controls had a significant difference. However, because the LSD test shows that the concentration of 100% has a significant difference with a concentration of 50%, it can be concluded that the concentration of 100% was a concentration that has a significant difference in providing antibacterial activity against Salmonella typhi bacteria. The leaf extract in the analysis of the concentration of 100% is the only concentration that has a significant difference with a concentration of 50%, 25%, 12.5%, 6.25%, while the analysis of the concentration of 50% of fruit extract shows a significant difference with a concentration of 100%, 12.5%, 6.25%, positive and negative controls. To see whether there are significant differences in the comparison of fruit and leaf extracts at each concentration, then it was followed by an independent t-test. The results of the comparison of fruit and leaf extract can be seen in table 4.

Table 3. LSD test result from Ficus lyrata Warb leaf extract against Salmonella typhi.

| Concentrations | 100%   | 50%    | 25%    | 12.5%  | 6.25%  | K (+) | K (-) |
|----------------|--------|--------|--------|--------|--------|-------|-------|
| 100%           | -      | 0.004  | 0.000  | 0.000  | 0.000  | 0.00  | 0.00  |
| 50%            | 0.004  | -      | 0.055  | 0.043  | 0.034  | 0.00  | 0.008 |
| 25%            | 0.000  | 0.055  | -      | 0.874  | 0.751  | 0.00  | 0.229 |
| 12.5%          | 0.000  | 0.043  | 0.874  | -      | 0.874  | 0.00  | 0.287 |
| 6.25%          | 0.000  | 0.034  | 0.751  | 0.874  | -      | 0.00  | 0.356 |
| K (+)          | 0.000  | 0.000  | 0.000  | 0.000  | 0.000  | -     | 0.00  |
| K (-)          | 0.000  | 0.008  | 0.229  | 0.287  | 0.356  | 0.00  | -     |

Based on statistical tests with an independent t-test presented in table 4 shows that the significance value of fruit extract compared to leaf extract at each concentration, except the concentration of 100%, obtained a value of p > 0.05. This shows that there are differences in inhibition zones of fruit and leaf extract of Ficus lyrata Warb at concentrations of 100% and there were no significant differences between other concentrations in fruit and leaf extract. Based on that, it can be concluded that the extract with a concentration of 100% of fruit extract of Ficus lyrata Warb has a significant difference in inhibition zone with the leaf extract of Ficus lyrata Warb while at concentrations of 50%, 25%, 12.5%, 6.25% there is no significant difference. This stated that the extract with a concentration of 100% of fruit extract of Ficus lyrata Warb was more effective than the leaf extract of Ficus lyrata Warb in inhibiting the growth of Salmonella typhi bacteria which was shown from the inhibition zone formed in the fruit extract was wider than in the leaf extract.

3.2. Minimum Inhibitory Concentration (MIC) of Ficus lyrata Warb against Salmonella typhi

MIC testing was conducted to determine the minimum concentration of antibacterial substances in inhibiting bacterial growth. The method used in MIC testing is microdilution by observing using a UV-Vis spectrophotometer with duplo testing. The analysis of MIC of Salmonella typhi bacteria was
observed using a UV-Vis spectrophotometer with a wavelength of 600 nm. The test results using a UV-Vis spectrophotometer can be seen in table 5.

Table 5. The absorbance value of fruit and leaf extracts of Ficus lyrata Warb using UV-Vis Spectrophotometer ($\lambda = 600 \text{ nm}$).

| Concentrations | Absorbancy Value of Extract ($\lambda = 600 \text{ nm}$) |
|----------------|--------------------------------------------------------|
|                | Fruit                          | Leaf                          |
| 100%           | Media + Sample + Bacteria | Media + Sample + Bacteria | Description | Media + Sample | Media + Sample + Bacteria | Description |
| 50%            | 0.99                          | 0.97                          | down        | 3.70           | 3.27           | down        |
| 25%            | 0.79                          | 0.73                          | down        | 2.53           | 2.25           | down        |
| 12.50%         | 0.76                          | 0.74                          | down        | 1.43           | 0.87           | down        |
| 6.25%          | 0.83                          | 0.38                          | down        | 1.24           | 1.15           | down        |
| 3.13%          | 0.64                          | 0.28                          | down        | 0.68           | 0.96           | up          |
| 1.56%          | 0.30                          | 0.23                          | down        | 0.51           | 0.65           | up          |
| 0.78%          | 0.24                          | 0.37                          | up          | 0.35           | 0.52           | up          |

The test results on fruit extract using a UV-Vis spectrophotometer showed that inhibition began at a concentration of 1.56% while inhibitory leaf extract occurred at a concentration of 6.25%. If the absorbance values of the media and samples were greater than the values of absorbance of the media, samples, and bacteria, it is concluded that at these concentrations there is an inhibition of bacterial growth. Otherwise, if the absorbance values of the media, samples, and bacteria are greater than the media and samples, it can be concluded that at this concentration the process of bacterial growth still occurs Ramschie et al [6]. The minimum inhibitory concentration (MIC) is determined by looking at the smallest concentration that has begun to show the process of inhibiting the growth of Salmonella typhi bacteria. The decrease in the absorbance value of fruit extract at a concentration of 1.56% and 6.25% in leaf extract of Ficus lyrata Warb showed that the MIC values of fruit and leaf extract of Ficus lyrata Warb against Salmonella typhi bacteria were 1.56% and 6.25% respectively. This shows that Salmonella typhi bacteria are more resistant to the extract of Ficus lyrata Warb leaves compared to the fruit extract of Ficus lyrata Warb. This difference can be caused by the ingredient contained in each extract Purwanto [7].

Based on the research of Wira [5], the content of phenol, tannin, and flavonoids of Ficus lyrata Warb fruit extract with ethyl acetate solvents was 28.5 ppm, 27.5 ppm, and 13.6 ppm respectively. The previous study explained that the content of phenols, tannins, and flavonoids which are compounds that have the function as antibacterials were more contained in fruit extract than leaves so that the inhibition of growth of the bacteria Salmonella typhimurium fruit extract was better than the Ficus lyrata Warb leaf extract.

4. Conclusion

According to the discussion result above, can be concluded:

1. There was an effect of fruit and leaf extracts of Ficus lyrata Warbon the growth of Salmonella typhi bacteria with different inhibitory zones at a concentration of 100% so that they can be used as a natural antibacterial ingredient.

2. The minimum inhibitory concentration (MIC) of Ficus lyrata Warb fruit extract in inhibiting the growth of Salmonella typhi bacteria was smaller than the minimum concentration of Ficus lyrata Warb leaf extract. The minimum inhibitory concentration of fruit extract on the growth of Salmonella typhi bacteria was at 1.56% while the leaf extract MIC was 6.25%.

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