Isolation and Antimicrobial Activity of Flavonoid Compounds from Mahagony Seeds (*Swietenia macrophylla*, King)

S Mursiti and Supartono
Semarang State University, Semarang, Indonesia

Email: kumalasari_berliana@yahoo.com

**Abstract.** Flavonoid is one of the secondary metabolites compounds in mahogany seeds. Mahogany seeds can be used as an antimicrobial. This study aims to determine the antimicrobial activity of flavonoid compounds from mahogany seeds against *Escherichia coli* (*E. coli*) and *Bacillus cereus* (*B. cereus*). Isolation of flavonoid compounds done step by step. First, the maceration using n-hexane, then with methanol. The methanol extract was dissolved in ethyl acetate and aquadest, then separated. Ethyl acetate extract evaporated Flavonoid compounds were. The testing of antimicrobial activity of flavonoid compounds using the absorption method. The results showed that the antimicrobial activity of flavonoid compounds from mahogany seeds shows the inhibitory activity and provide clear zone against bacteria *E. coli* with value Inhibitory Regional Diameter 18.50 mm respectively, and 14.50 mm to the bacteria. Based on the results of the study, it can be concluded that flavonoid compounds from mahogany seeds have antimicrobial activity against *E. coli* and *B. cereus*.

1. Introduction
Existing research shows that mahogany seeds contain compounds that have antidiabetic activity, usually as well as insecticides, larvicides, nematicides, antipyretic, fungicides, antimicrobials and antioxidants [1]. Chemical constituents in mahogany seeds are alkaloids, saponins, flavonoids [2][3]. Research on antimicrobial activity test phase basil leaf extract n-hexane, ethyl acetate phase and the phase of 70 % ethanol and 70 % ethanol phase that shows antimicrobial activity *S. aureus* and *C. albicans* with agar diffusion method and dilution liquid [4]. However, the study did not report the active compounds that have antimicrobial activity. Results of other studies say that basil chloroform extract can inhibit the bacteria *Shigella dysenteriae* and methanol extract can inhibit microbe *Klebsiella pneumoniae*, *Salmonella paratyphy* and *S. aureus* with Inhibitory Regional Diameter (DDH) respectively 10 mm, 9 mm, and 7 mm, but does not mention the concentration used to test the antimicrobial activity [5].

These studies concluded that the part of the basil plant that often tested the activity of antimicrobial is part of basil leaves, whereas according to [6], not only the basil leaves just that contain secondary metabolites such as essential oils, but part of the basil plant such as trunks also contain secondary metabolites that may also have antimicrobial activity. So far have not found a research report stating about power test the antimicrobial activity on the stem of flavonoid from mahogany seeds against *B. cereus* and *E. coli* bacteria.
2. Research Methods
Flavonoid compounds isolation done step by step. First, the maceration using n-hexane, then with methanol. A total of 50 g of the methanol extract put in a separating funnel, then added each 50 mL of distilled water and ethyl acetate, then shaken, allowed to stand, and separated. The ethyl acetate phase was taken, evaporated to obtain a dry extract flavonoids. The extract was detected by TLC and KCV fractionated by using silica gel GF254 G60 with enhanced polarity eluent (n-hexane-EtOAc, EtOAc, EtOAc-MeOH). The fraction which still contains impurities further fractionated in a row using radial chromatography to obtain pure compounds. The testing of antimicrobial activity of flavonoid compounds using the absorption method.

Antimicrobial activity test using the absorption method is a modification of the order and that has been done [7] [8]. Nutrient medium so as to be used as a medium for bacterial growth is provided by heating NA back, then poured into a sterile petri dish aseptically. The bacteria are grown on medium NA by entering 1 mL of bacterial culture medium in NA then averaged over the surface of the agar medium. Paper discs with a diameter of 6 mm are dipped into isolated compounds were each concentration 10 mg/mL, 25 mg/mL, 50 μg/mL, 100 μg/mL, then allowed to stand for 1 hour, then placed on a saucer solder containing bacteria sterile. Negative controls using paper discs were dipped in distilled water, whereas the positive control using a paper disc dipped in wipol. The entire cup solder containing seeding bacteria were incubated for 12 hours at a temperature of 37 °C in reverse, then observed and measured the inhibition of bacterial growth in the area around the paper disc, followed by calculating the area.

3. Result and Discussion
Extraction of mahogany seed includes several stages of sample preparation and extraction process. The methanol extract of mahogany seeds obtained 10.7 % in the form of a brown powder with a distinctive aroma and taste very bitter. Qualitative test results showed that the methanol extract of mahogany seeds contain secondary metabolites are alkaloids, flavonoids, and saponins, and is consistent with the results of Mursiti’s study (2009). There were containing flavonoid compounds.

Testing the antimicrobial activity of flavonoid compounds of mahogany seeds using two bacterias, E. coli and B. cereus with absorption method. Controls used in this method is a negative control (distilled water) and positive control (wipol). The test results showed that the methanol extract at a concentration of 10 mg/mL, 25 mg/mL, 50 μg/mL, 100 μg/mL showed activity against both bacteria B. cereus and E. coli. It is known by the diameter of the visible area of inhibition around the paper disc. The diameter of inhibitory regions experienced an increase means that the higher the concentration, the greater the concentration of active ingredient that serves as an antibacterial, so the ability to inhibit bacterial growth E. coli also getting bigger. According Dzidic [9] states that one of the mechanisms of bacterial resistance is inaktifikasi antibiotics by producing enzymes. One enzyme that can menginaktifikasi antibiotics are β-glukoronidase. E. coli is a bacteria that is capable of producing β-glukoronidase that allegedly active compound in flavonoid can be described by β-glukoronidase into other compounds that toxic for the bacteria. Higher levels of bioactive compounds that are generally bactericidal (lethal microbes) and a lower level usually is bacteriostatic (inhibits growth, not lethal microbes) [10]. This is consistent with research Khumaisah [11] which states that basil is less susceptible to E. coli and Shigella sonnei, but effectively inhibit Salmonella bacteria sonnei. This is caused by the bacterium Salmonella sonnei thought to have lower metabolic activity so slow to the synthesis of the ribosomal protein that antibacterial agents can freely enter and activity may be hampered. Data inhibitory activity by measuring the diameter of inhibitory regions (DDH) is presented in Table 1.
Table 1. Diameter inhibitory region (mm) mahogany seeds methanol extract against *E. coli* and *B. cereus*

| Sample | *Escherichia coli* | *Bacillus cereus* |
|--------|-------------------|------------------|
|        | 100% | 50% | 25% | 100% | 50% | 25% |
| D1     | 18.50 | 16.50 | 13.50 | 14.00 | 13.00 | 10.00 |
| D2     | 18.00 | 16.50 | 13.50 | 15.00 | 13.00 | 10.50 |
| D3     | 19.00 | 16.50 | 13.50 | 14.50 | 13.00 | 11.00 |
| Average| 18.50 | 16.50 | 13.50 | 14.50 | 13.00 | 10.50 |
| Controle (+) | | | | | | |
| D1     | 35.10 | 35.16 | 35.16 | 51.33 | 51.33 | 51.33 |
| D2     | 35.50 | 35.50 | 35.50 | 51.00 | 51.00 | 51.00 |
| D3     | 35.30 | 35.33 | 35.33 | 52.00 | 52.00 | 52.00 |
| Average| 35.30 | 35.33 | 35.33 | 51.44 | 51.44 | 51.44 |
| Controle (-) | 0 | 0 | 0 | 0 | 0 | 0 |

The results showed that the inhibition of the ethanol extract was higher against *E. coli* bacteria (gram-negative) as compared with *B. cereus* bacteria (gram-positive bacteria) value indicated by inhibition area diameter. This is due to differences in the sensitivity of bacteria to antibacterial influenced by the structure wall cell bacteria. Research of Pramuningtyas [12] concluded that Gram-positive bacteria tend to be more sensitive to the antibacterial because of the structure of the cell wall of gram-positive bacteria is simpler than the structure of the cell wall of gram-negative bacteria, making it easier for antibacterial compounds to enter the cell structure of the cell wall of gram-positive bacteria.

4. Conclusion

Based on the research that has been done, it can be concluded that the antimicrobial activity of flavonoid compounds from mahogany seed gives the largest clear zone where inhibition of *E. coli* bacteria (18.50 mm) higher than *B. cereus* (14.50 mm) with 100% concentration.

5. References

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