Antibacterial Activity Test of Ethanol Extract of Matoa Leaf (Pometia pinnata) against Salmonella typhi

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Abstract
Typhoid fever known as a disease which is caused by pathogenic bacteria of Salmonella-typhi. It is usually curable with antibiotics including synthetic treatments that kill bacteria that cause typhoid fever. The study was conducted to identify traditional treatments that could be used as a cure for typhoid fever. The leaf of the matoa is natural medicine containing a secondary metabolic compound that can impede the growth of bacteria. The study aims to know toxic effect and antibacterial ethanol extract of matoa leaves on Salmonella typhi bacteria. The study used a 96% ethanol maceration method. The extract of matoa leaf ethanol was further done by phytogenic screening and FT-IR characterization. The extract of matoa leaves could be nuisance Salmonella typhi bacteria growth on a category which was being marked by clear zone around the disk at 35% concentration with 3 mm resistance zone.

INTRODUCTION
Salmonella typhi bacteria are pathogenic microorganisms that can cause typhoid or typhoid fever accompanied by intestinal infections (gastroenteritis) or digestive problems that occur more than 18 hours after the bacteria enter the human body, gastroenteritis symptoms can be fever, vomiting, diarrhoea and headache (Cita, 2011).

Salmonella typhi is a germ sticks negative, which does not have the spores, move with the spartan flagellum., It is intracellular and Anerob faculty. They range from 0.7 to 1.5 x 2-5 µm, have somatic antigen (O), flagel antigen (H) with the 2 phases and antigen capsules (Vi) (Cita, 2011). Based on the results of the study, typhoid fever is more prone to occur in men because they generally work and eat in an environment that is not guaranteed to be clean. Meanwhile, according to the immune system, women have a high chance of being severely affected by typhoid fever caused by bacteria because when Salmonella typhi enters the liver cells, their estrogen hormone will work harder (Ardiaria, 2019).

Based on the results of the Basic Health Research (Riskesdas) in 2007, typhoid fever in Indonesia reached 1.7%. The highest number of cases occurred in the age range of 5-14 years with a percentage of 1.9%, the age range of 1 -4 years with a percentage of 1.6%, the age range of 15-24 years with a percentage of 1.5% and at the age of 1 year with percentage 0.8% (Fulka, 2018).

According to the World Health Organization (WHO) cases of typhoid fever occur as many as 17 million people per year worldwide, typhoid fever causes 600,000 and 70% of deaths in Asian countries. The country of Indonesia has cases of typhoid fever which reached 81% per 100.000 population (Keddy et.al, 2011). The high cases of typhoid fever and the mortality rate caused by this disease requires...
pharmacological treatment. In the medical world, several infectious diseases caused by these bacteria are usually treated using antibiotics (Rois, 2017).

The use of synthetic antibiotics for a long time can cause some side effects that can be harmful to the human body. Bacteria or germs have become resistant to antibiotics, so the disease is more difficult to cure (Huda, 2016). The use of antibiotics that are too frequent or not in accordance with the dose can cause germs to experience resistance or immunity. This is one of the most worrying side effects of antibiotics. When the germs that cause infection are resistant to antibiotics, then the bacterial infection disease will be difficult to cure. Because of its immunity, germs are also at high risk of causing severe infections. Therefore, traditional treatment is needed from natural ingredients such as plants. Plants that can be used as a medicine for infections caused by bacteria are matoa plants (Pometia pinnata). The parts of the matoa plant that can be used are leaves, stems and fruit. In this study, the part that was taken as a bacterial inhibitor was matoa leaves which contain secondary metabolites.

Matoa plants are known to contain secondary metabolites, namely tannins, flavonoids, alkaloids, steroids and saponins. This shows that matoa leaves can be used as natural medicine (Wowor, 2015). Secondary metabolites contained in matoa leaves are able to inhibit and even kill Salmonella typhi bacteria, secondary metabolites present in matoa leaves will damage the cell membrane in bacteria so that it can reduce the permeability of bacterial cell walls and can damage bacterial DNA to cause bacterial growth to be inhibited and can even cause bacterial death.

Based on research (Nuryanti & Pursitasari, 2014), Regarding antioxidants, it was stated that ethanol extract from matoa leaves had the highest antioxidant capacity at a concentration of 100 g/mL with ethanol as a solvent, yielding a yield of 90.38%. Other research states that the efficacy of the matoa plant (Pometia pinnata) includes digestive complaints, burns, diabetes, flu and pain (chest, headaches, bones, muscles and joints).

Testing the antibacterial activity of matoa leaf extract can be done by diffusion. Disc diffusion is a method intended for testing the inhibition of Salmonella typhi bacteria. The method is most often used for testing antibacterial activity, this method is often referred to as Kirby Bauer. The results of the antibacterial test are seen by calculating the diameter of the clear zone or the inhibition that appears around the media using the calculation of the inhibition zone (Toemon, et al, 2018).

In this study to determine the antibacterial activity of the ethanolic extract of matoa (Pometia pinnata) leaves against Salmonella typhi bacteria with varying concentrations of 0%, 25%, 35%, 45% and 55%.

**METHOD**

Antibacterial testing was carried out at the Chemical Laboratory of Raden Fatah State Islamic University Palembang and antibacterial testing was carried out at Balai Besar Laboratorium Kesehatan (BBLK) Palembang City. The following equipments were used including test tubes, test tube racks, erlenmeyer, spatula, petri cup, dropper, measuring cup, flask measuring, oven, micropipette, incubator, Ohaus analytical balance, separating funnel, autoclave, aerator, sterile tube, blender, beaker, Bunsen, Rotary Evaporator RE301 Yamato and filter paper. Meanwhile, the materials needed included matoa (Pometia pinnata) leaves, 96% ethanol, Mueller Hinton agar, Brain Heart Infusion (BHI) liquid media, sterile distilled water, Salmonella typhi bacteria, Sodium chloride (NaCl), Barium chloride (BaCl2 1%), Sulfuric acid (H2SO4 1%), Hydrochloric acid (concentrated HCl), Magnesium (Mg), Dragendorff's reagent, Wagner's reagent, Meyer's reagent, hot water, lieberman-burchad, Sulfuric acid (H2SO4 50%), Ferric chloride (FeCl3 1%), dimethylsulfoxide (DMSO).

1. **Preparation Matoa Leaf Ethanol Extract**

The matoa leaf samples obtained from Saba Village, Musi Banyuasin were taken on land that has many matoa plants. matoa leaves have secondary metabolites that are able to act as antibacterials and antioxidant (Nuryanti & Pursitasari, 2014).

The method for making ethanol extract of matoa leaves was remaceration. The matoa leaves that have been picked, washed and then cut into pieces and dried in the sun. Dried matoa leaves were mashed with a blender until a powder was formed. The leaves of the fine matoa...
were then soaked with a 96% ethanol concentration which aimed to attract all the chemical components contained in the matoa leaves. Ethanol solvents are universal solvents capable of attracting both polar and non-polar compounds in the sample. Matoa leaves were soaked for 3 x 24 hours so that all chemical components were involved in the solvent (Noviyanti, 2016).

Macerate matoa leaves were used as much as 250 gr. The results of filtrate matoa leaf extract were then rotated in a rotary evaporator for evaporation process, and then a phytochemical screening test was carried out.

2. Phytochemical Screening Test of Matoa Leaf Extract

Secondary metabolites in matoa leaves are known by means of phytochemical tests using several reagents (Baud et.al, 2014):

a. Compound Test_Flavonoids

Test-flavonoids using 1 ml-sample plus powder_Mg to taste and add 2 drops of concentrated HCl reagent.

b. Testing of Alkaloid Compounds

The alkaloid compound test used 1 ml sample and added 3 drops of each reagent, namely Dragendorff, Wagner's reagent, and Mayer.

c. Saponin Compound Test

Test for saponin compounds using 1 ml of sample plus 1 ml of hot water and 2. HCl-drops that produce foam when the test is positive.

d. Steroid Compound Test

Steroid compounds tested using acetic acid-glacial and 3-drops of concentrated H₂SO₄ on 1ml sample.

e. Tannin Compound Test

The tannin compound test used 1 ml of sample plus 2 drops of 1% FeCl₃ reagent with a positive test a greenish black color was formed.

3. Fourier Transform Infrared (FTIR) Testing

A measured substance is an atom or molecule. Infrared light was divided into two beams, one passed through a sample and the other through a comparison. Then the chopper was passed. After passing through the prism, the beam entered the detector and be converted into an electrical signal which was then recorded by the recorder so as to produce a signal in the form of a spectrum.

4. Bacterial Breeding

Sterile ose was used to collect bacterial colonies, then put into 2 mL of Brain Heart Infusion (BHI) liquid media was pun into. Then it was incubated at 37°C for 24 hours. After the incubation process, the bacterial suspension was added using NaCl according to the provisions of MC Farland. NaCl is a comparison solution for bacterial culture turbidity in liquid media. The provisions of the MC Farland solution used were 0.5, 0.5 ml of 1% Barium Chloride (BaCl₂) was added to distilled water and 1% sulfuric acid (H₂SO₄) was added. After that the storage in a place that is not exposed to sunlight was conducted.

5. Production of Mueller Hinton Agar (MH) Medium

Nutrient agar was used as a place for bacterial growth. Mueller Hinton Media. 38 grams of media were taken and boiled using distilled water to 1 L. After dissolving, the agar medium was put into an autoclave for the sterilization process for 15 minutes at a temperature of 121°C. Then the solution was poured into a petri dish to a thickness of 9 mm, closed and allowed to freeze (Adeng, 2014).

6. Positive Control Creation

Amoxilin 500 g was dissolved in 10 ml of distilled water to obtain a concentration of 50 mg/mL. Then 4 ml and add 10 ml of distilled water from the solution, 2 ml and add 10 ml of distilled water were taken to obtain a positive control solution with a concentration of 4 mg/ml.

7. Antibacterial Test Preparation

This disc method was used for bacterial rejuvenation, for culturing bacteria, making paper discs and making 25%, 35%, 45% and 55% concentrations. The first step was inserting a cotton swab or swap into the bacterial suspension as much as 20 µl of the bacterial suspension is applied to the media that has been demarcated in a zig-zag manner.

This procedure is repeated twice with 4 concentrations. The next stage is disc paper with a size of 6 mm which has been containing antibacterial substances, then placed on the surface of the media that already contains bacteria according to the quadrant position. Then the petri dish was closed and entered the incubation stage with a time of 24 hours and a temperature of 37°C. The last step was observing the inhibition of bacteria by looking at the clear zone that appeared around the disc and then
calculating the diameter of the clear zone around the disc. The caliper was used as a measuring instrument for bacterial inhibition. Diameter measurement from Bacterial inhibition was calculated by the formula (see Figure 1).

![Zone block](image)

\[
\text{Zone block:} \quad \frac{(DV - DS) + (DH - DS)}{2}
\]

Note:
- \(DV\) = Diameter-Vertical
- \(DS\) = Diameter-Disc
- \(DH\) = Diameter-Horizontal

Figure 1. Formula Calculated to measure the Diameter of Bacterial Inhibition

**RESULTS AND DISCUSSION**

1. **Preparation and Maceration of Matoa Leaves**
   
The following were the results obtained from the maceration and evaporation processes. The thick extract obtained 27.39 grams with a yield of 10.95%. Yield value aimed to determine the quantity value of an extract of natural ingredients compounds. Maceration with ethanol solvent which produces a high yield value of 10.95% and binds many secondary metabolites because it can dissolve polar compounds. Viewed from Table 1, the condensed exact extract obtained 27.39 grams with a yield of 10.95%. Yield value aimed to determine the quantity value of an extract of natural ingredients compounds.

2. **Test Screening Phytochemicals Matoa Leaf Extract**
   
   Viewed in Table 2, which shows data on the result of phytochemical screening tests. Positive phytochemical test matoa leaf extract contains flavonoid compounds that produce an orange color if positive, a positive test for tannin compounds is green color formation and positive test for saponin compounds with the appearance of foam.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound Group</th>
<th>Results</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flavonoids</td>
<td>Formed color-orange</td>
<td>(+)</td>
</tr>
<tr>
<td>2.</td>
<td>Tannins</td>
<td>Formed color-blackish green</td>
<td>(+)</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>Foamy</td>
<td>(+)</td>
</tr>
<tr>
<td>4.</td>
<td>Steroids</td>
<td>No-ring formed</td>
<td>(-)</td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloids</td>
<td>No yellow precipitate is formed as well as chocolate</td>
<td>(-)</td>
</tr>
</tbody>
</table>

**Table 1. Maceration and Yield Data**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of Samples</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maceration</td>
<td>250 gram/3 Liter</td>
<td>2.102 mL</td>
</tr>
<tr>
<td>Evaporation</td>
<td>2 liters of ethanol extract</td>
<td>10.95%</td>
</tr>
</tbody>
</table>

**Table 2. Screening Results Phytochemicals After Evaporation**
The orange color formed was produced by Mg and HCl powder. The addition of HCl resulted in a hydrolysis reaction. A positive test for tannin compounds was indicated by the presence of color blackish green. The can be seen in Figure 2. The addition of FeCl₃ compound in this reaction made the compound as a compound complex. The oxygen atom on Tannins had a pair of electrons donated free to ion Fe³⁺ which becomes the central atom to form complex compounds. The following in Figure 3, is a tannin reaction with FeCl₃.
3. FT-IR Characterization of Matoa Leaf Ethanol Extract

The FT-IR spectra above which was assumed at a length of 3393 cm⁻¹ was the range of regions owned by OH, where each flavonoid, tannin and saponin compounds had C bonds with OH. Spectrum 2923-2853 cm⁻¹ that showed the range of regions that CH bonds have. The spectrum of 1737 cm⁻¹ showed the area owned by C=O which is owned by flavonoid compounds. The spectrum of 1083 cm⁻¹ shows the range of regions owned by the CO bonds contained in the structure of flavonoids, tannins and saponins. The FTIR spectra in Figure 4, showed the presence of secondary metabolites in the ethanolic extract of matoa leaves, namely saponins, tannins and flavonoids.

4. Antibacterial Test

Inhibition test with matoa leaf extract using *Salmonella typhi*. The following was the result of inhibition of the matoa leaf extract formed:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Repetition</th>
<th>Average (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>25%</td>
<td>2.75</td>
<td>3</td>
</tr>
<tr>
<td>35%</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>45%</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>55%</td>
<td>2.75</td>
<td>2</td>
</tr>
<tr>
<td>K+</td>
<td>26.55</td>
<td>27.5</td>
</tr>
<tr>
<td>K-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. K+: Amoxilin K- (0%): Aquadest

Figure 4. Matoa leaf FTIR spectra
Viewed from Table 3, the optimum condition of bacterial inhibition from the ethanolic extract of matoa leaves was categorized as moderate at a concentration of 35% which was the optimum concentration with a diameter of 3 mm. The resulting inhibition is shown in Figure 5, which forms a decreasing pattern with an optimum concentration of 35%. The cause of this was due to the resistance of bacteria or the optimum conditions of bacteria. In addition, the effectiveness of inhibition of matoa leaves against *Staphylococcus epidermidis* bacteria occurred at an optimum concentration of 20% with a diameter of 3 mm which was included in the medium category (Rossalinda et.al, 2021).

Gram negative bacteria can make self-defense against antibacterial compounds, bacterial chromosomes and plasmids are resistant genes that can coordinate the resistance properties of bacteria (Huda, 2016). Gene trigger Resistance is caused by external factors, one of which is antibacterial substances. Plasmids can move to other cells or species, the spread of resistance properties of these bacteria occurs because plasmids can replicate or multiply in a fast time, therefore plasmids can be said to be very effective agents of resistance carriers (Zeniusa et.al, 2019).

The properties of resistance and the spread process carried by bacteria are developed in several ways, namely molecular of antibacterial substances can be destroyed by enzymes produced by bacteria, bacteria are able to have a special efflux pump used to remove antibacterial substances that are in their cells, and regulate certain parts of their cells so that they are more immune and cannot be attacked by antibacterial substances. The inhibition of bacterial growth with ethanolic extract of matoa leaves occurs due to the content of secondary metabolites found in matoa leaves which are able to inhibit bacterial growth in the form of tannins, saponins and flavonoids. The following is an attack of secondary metabolites on the bacterial structure.

![Figure 5. Diagram of the Average Inhibitory Diameter of Matoa Leaf Ethanol Extract](image)

![Figure 6. Mechanism Attack Saponins against Bacterial Cell Wall *Salmonella typhi*](image)
The mechanism of action of saponins in Figure 6, explains that bacterial cell walls composed of peptidoglycan can be damaged by saponins compounds, outer membranes and liposaccharides. The active substance of saponins is similar to detergent, so saponins can reduce voltage surface of the cell wall in bacteria and damage membrane permeability (Fulka, 2018).

Figure 7. explains that the cell membranes of bacteria can be damaged by saponins to form complex compounds. Hydrogen that binds to proteins in flavonoids compounds will damage the permeability of the bacterial cell membrane. The enzymes reverse transcriptase and DNA topoisomerase are inhibited when attacked by tannins. DNA in bacteria which is composed of proteins and amino acids has peptide bonds. Peptide bonds are attacked by tannins by inhibiting the work of enzymes so that the synthesis of peptidoglycan in bacterial DNA is disrupted and causes damage. The reaction can be seen in Figure 8 (Auerkari, 2002).

**CONCLUSION**

Study showed that extract's ability leaf ethanol matoa in inhibiting growth *Salmonella typhi* bacteria at a concentration of 35% with an average diameter of inhibition of 3 mm belonged to the medium category.

**REFERENCES**


