Bioactive Compound of Syzygium zeylanicum Leaves Asthe Escherichia coli and Staphylococcus aureus Antibacterial

Hamidah1, Salni2, Nina Tanzerina2
1* Student of Environmental Management Pascasarjana Sriwijaya University
2Lecturer of Biology department FMIPA Sriwijaya University
*email: fennyhamidah@student.pps.unsri.ac.id
Received on 07th September 2017 and Accepted on 11th April 2017

ABSTRACT

Escherichia coli is one of the bacteria that cause infections of the human digestive tract, such as diarrhea, while Staphylococcus aureus is one of the bacteria that cause infections of the skin injury such as boils and pimples. This study used Syzygium zeylanicum leaves because it has potential as an antibacterial because it contains active compounds. This study aimed was determined the antibacterial activity of the fraction and the active compound in Syzygium zeylanicum leaves against E. coli and S. aureus. Research conducted in November 2015 to January 2016. The method used in this research was extracted by maceration, fractionation by liquid fractionation, antibacterial activity test, and determination of minimum inhibitory concentration with the diffusion method and isolation of active compounds by column chromatography method. The bacteria used in this test are E. coli and S. aureus. The results of this research showed the water methanol active fraction against the bacteria that used in this test. The methanol water fraction had obtained one antibacterial compound in bottle 1,3,5 which shows the value of tannin Rf 0,416. The minimum inhibitory concentration of water methanol of water apple leaves is 1000 µg/mL for E. coli and 500 µg/mL for S. aureus. The minimum inhibitory concentration of the active compound to E. coli and S.aureus in 500 µg/mL. The fraction and the active compound of Syzygium zeylanicum leaves have an antibacterial activity with E. coli and S. aureus and the active compound is tannin.

Keywords: Myrtaeaceae, Syzygium zeylanicum, Anibacterial activity, tannin
INTRODUCTION

The infectious disease was caused by the bacterium *E. coli* and *S. aureus* can be cured with antibiotics. The commonly used antibiotic was sulfonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline and aminoglycosides (Ganiswarna, 1995). Treatment with antibiotics will bring the side effects if it used for a long time, Mulyadi and Sulistiyani (2013) because it causes bacteria to become resistant.

*Syzygium zeylanicum* plant or Betti’s plant in Tebedak village area of OganIlir regency, this plant is utilized its leaves to treat the wound caused by the scratch. Anoop (2014), *Syzygium zeylanicum* leaves had used for joint sore and the oils had obtained from the leaves of *Syzygium zeylanicum* and used as an arthritis drug medicine. The *Syzygium zeylanicum* reported as a stimulant, antimicrobial and antiinflammatory.

The research can be done by using the proactive ingredient from natural antibacterial compounds contained in the plant. One of the plants with antibacterial potential is *Syzygium zeylanicum* leaves. Based on phytochemical screening Anoop (2014), wounds healing can be done by the presence of secondary metabolites in plants such as alkaloids, flavonoids, phenols, glycosides, plant sterols, terpenoids, saponins and tannins. Salni et al., (2011) stated, in order to obtain proactive antibacterial compounds can be done by several stages of the extraction, fractionation and isolation.

MATERIAL AND METHODS

This study was conducted in November 2015-January 2016. Located in the Laboratory of Genetics and Biotechnology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, Inderalaya.

Formulation of Simplisia

At first, *Syzygium zeylanicum* leaves with good morphological was chose and then dried it with indirectly light. Then crushed the leaves using a blender until it powdered. Making NA medium (*Nutrient Agar*) and NB (*Nutrient broth*) refers to Bridson (1998).

Extraction

The simplicia of *Syzygium zeylanicum* leaves were weighed as much as 250 grams, which was inserted into erlenmeyer and added with methanol to 1000 mL. The mixture of these solvent was stirred until homogen and covered with aluminum foil. These solvent was left for two days than it was filtered with filter paper. The extract was evaporated and was obtained until the viscous extract by *Rotary evaporator*. The extract was dried with the condensed hair dryer to obtain a dry extract (Mulyaniet al., 2013).

Fractionation

Fractionation was conducted by the FCC (Fractionation Liquids) with solvent n-hexane, ethyl acetate and methanol. The extract was dissolved into methanol and distilled water in the ratio 1:1 200 mL. Furthermore inserted into the separating funnel, and added 200 mL of N-hexane, then shaken gently and allowed to stand. After the visible layers separate solutions, the solution removed and separated fractions into a jam jar. Fractionation using a solvent ethylacetate followed by the way, and the same volume. This is done to obtain a liquid fraction of n-hexane, ethyl acetate fraction of liquid, and the fraction of the methanol-water is then evaporated with a Rotary evaporator to obtain the thick extract fractions and dried with a hair dryer to obtain the dry fraction. All three fractions obtained tested the activity of the bacteria (Salni et al., 2013).

Fraction Antibacterial Activity Test
The tested fraction was required 2000 µg/mL and was inserted in DMSO. The suspension of E. coli and S. aureus was added 0.1mL petri dish, then added 10 ml of medium NA(NutrientAgar), homogenized and left to freeze. Medium containing bacteria inserted 6 mm paper disc that has been dipped in control, N-hexane fraction, ethyl acetate fraction, methanol water by diffusion in order. Later in the incubation temperature of 37°C for 24 hours.

The Concentration of Fraction Manufacture

The active fraction from the tested activity of antibacterial was weighed by using analytical scale. The highest concentration was made by dilution with solvent DMSO 0.5 mL by using a syringe inserted into the bottle vial that will be used. Then it was taken 0.5 mL of concentration that has made 4000 µg/mL and placed in the bottles containing DMSO concentration of 0.5 mL of the 2000 µg/mL. The same thing was get the next concentration to obtain a concentration of 625 µg/mL (Salnietal., 2011).

Bioautografi Test

Bioautografi test was done by using thin layer chromatography. Fraction Active spotted 3 times on a plate of silica gel GF_{254} (KLT) with capillary pipette, and then developed with a mobile phase corresponding to the separation of the compounds contained infractions, in this study used a mobile phase of methanol water: ethyl acetate with a ratio of 9:1. The chromatograms were traced into a petri dish containing bacterial. The chromatograms were left clinging on agar for ± 60 minutes so that the active compound diffuses into the agar medium, then removed carefully and incubated for 24 hour at 37°C.

Purification of Active Compounds

Active compound purification of the active fraction of gravity is done by column chromatography with an adsorbent of silica gel 60 F_{254} on a 1.7 cm diameter column. Fraction is dripped slowly on the top of the column with a mobile phase solvent element according to the rate of election of 40-50 drops per minute, do illusion until exhausted component. Volume fraction was accommodated 10 mL in which the active isolates were obtained and were searched the minimum inhibitory concentration values in antibacterial test using the agar diffusion method.

Data Analysis

The data were obtained from the experiment that were tabulated in M.Excel, the difference obtained was analyzed using standard deviation (Standard deviation) and presented in descriptive analysis.

RESULTS AND DISCUSSION

Extraction Syzygium zeylanicum Leaves

This research was used the extraction of Syzygium zeylanicum leaves with 250 grams. From the extraction was obtained in pasta around 51.7 grams, with the extraction percentage of 20.68%. Extraction was done by maceration method for extraction by maceration can dissolve the compound as well. Rahmawatiet al., (2013), maceration method chosen as a way of extraction because the process is simple and can produce extracts in large quantities. The results obtained by evaporation of the filtrate condensed extract. Condensed extract from the extraction of red guava fruit (Psidium guajava L.) is then weighed and the result of evaporation of 11,377 grams with a percentage of 45.49%.
Fractionation Syzygium zeylanicum Leaves

Based on the results of fractionation that was used a solvent according to the degree of polarity. N-hexane (non-polar), ethyl acetate (semi-polar) and methanol (polar) is known from 23 grams extract of Syzygium zeylanicum leaves, that was get each fraction as shown on Table 1.

Table 1: The Fractionation Results of the Syzygium zeylanicum Extraction

<table>
<thead>
<tr>
<th>No</th>
<th>Solvent</th>
<th>The Fraction (gram)</th>
<th>The Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>N-hexane</td>
<td>3.6</td>
<td>15.79</td>
</tr>
<tr>
<td>2.</td>
<td>Ethyl acetate</td>
<td>7.8</td>
<td>34.21</td>
</tr>
<tr>
<td>3.</td>
<td>Water methanol</td>
<td>11.4</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>22.8</td>
<td>100</td>
</tr>
</tbody>
</table>

This result showed that the greatest fraction of Syzygium zeylanicum leaves was contained in the methanol fraction with a weight of 11.4 grams and a percentage weight of 50%. The increase of methanol fraction was greater than the fraction of n-hexane and ethyl acetate fraction. In the fractionation process, the compounds in the extract will be bound with a suitable solvent to the level of polarity. In the opinion of Nurdin et al., (2010) that the methanol solvent was capable to dissolve all components of both polarities, semi-polar and non-polar. Harbone (1987) states that the polar nature of the solvent, the greater the number of fractions obtained.

Activity Test Antibacterial Fraction Methanol Syzygium zeylanicum Leaves Against Escherichia coli and Staphylococcus aureus

Activity test fraction of n-hexane, ethyl acetate fraction and a methanol fraction of water from the Syzygium zeylanicum leaves is performed at a concentration of 2000 µg/mL. Fractions were tested for antibacterial activity to find the type of fraction that are active against E. coli and S. aureus. The results of the antibacterial testing of fractions (Table 2 and Figure 1).

Table 2: Results of antibacterial activity test methanol fraction of Syzygium zeylanicum leaves on the growth of E. coli and S. aureus

<table>
<thead>
<tr>
<th>No.</th>
<th>Fraction</th>
<th>Diameter Zone of Inhibition (mm ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>1</td>
<td>N-hexane,</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>6.5 ±0.07</td>
</tr>
<tr>
<td>3</td>
<td>Water methanol</td>
<td>7.0±0.63</td>
</tr>
<tr>
<td>4</td>
<td>Extract</td>
<td>0</td>
</tr>
</tbody>
</table>
Based on Table 2, it can be seen that the result of antibacterial activity from methanol fraction had the inhibition zone against *E. coli* of 7.05 mm and *S. aureus* of 9.75 mm ferrous. Based on the diameter of formed methanol fraction was categorized to the medium of antibacterial. Davis and Stout (1971), the strength of the antibacterial activity is very strong in inhibitor zone of 20 mm, and the inhibition of 10-20 mm is strong. If the resistor has an inhibitor zone of 5-10 mm that was categorized to the medium antibacterial. The inhibitor zone of 5 mm or less was categorized weak antibacterial.

*E. coli* has an inhibitor zone of 7.05 mm whereas inhibition of *S. aureus* has inhibitory of 9.75 mm, it indicates that *S. aureus* more sensitive to antibacterial, that was the reason as *S.aureus* had big inhibitory zone, it can also be caused the differences in cell membrane structure. According to research Aini et al., (2015), the structure of the cell membrane of gram positive bacteria have more peptidoglycan whereas Gram negative bacterial cells slightly peptidoglycan. The difference is what causes the cell membrane antibacterial easily penetrate the cell membrane of gram positive bacteria.

**Determination of Minimum Inhibitory Concentration (MIC) Fraction Methanol water against Escherichia coli and Staphylococcus aureus**

Based on the results of the activity test (Figure 2 and Table 3) Indicates the air methanol fraction is the most effective fraction of the n-hexane fraction and the ethyl acetate fraction. Antibacterial activity from open air methanol fraction to *E.coli* and *S.aureus* to obtain the result of minimum inhibitory concentration (MIC).

Based on Table 3 The of inhibition zone is at a concentration of 4000 µg/mL with a inhibitor zone of 10.35 mm on test bacteria *E. coli* and 12.0 mm on the test bacteria *S. aureus*, while the smallest of the inhibitory zone at a concentration of 500 µg/mL for *S.aureus* with a inhibitor zzone of 7.15 mm while the resistor 1000 µg/mL for *E. coli* with an inhibitor zone of 7.1 mm. The smaller the value of the concentration, it will be the smaller inhibition zone is formed. In accordance with the opinion of Salni et al., (2013), the power fraction activity decreased along with the decrease in the value of concentration, so that the inhibitor zone is formed will be smaller.
Table 3 Minimum Inhibitory Concentration (MIC) Fraction Methanol Water Against E. coli and S. aureus

<table>
<thead>
<tr>
<th>No.</th>
<th>Bacteria</th>
<th>Fraction Concentration (µg/mL)</th>
<th>Average Diameter Inhibition (mm ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>10,35 ± 0,21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>7,25 ± 0,35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td><strong>7,1± 0,07</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62,5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>12,0± 0,70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>8,5 ± 0,28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>8,1 ± 0,14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td><strong>7,15 ± 0,21</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62,5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 Minimum Inhibitory Concentration (MIC) of water Methanol fraction Syzygium zeylanicum leaves on Growth E. coli and S. aureus

Caption: 1. The concentration of 4000 µg/mL, 2. 2000 µg/mL, 3. 1000 µg/mL 4. 500 µg/mL, 5. 250 µg/mL, 6. 125 µg/mL, 7. 62.5 µg/mL. The figure of 2A shows E.coli and the figure of 2B shows S.aureus (showing inhibition zone)

On the Table 3 was shown the inhibitory concentrations of an E. coli on 1000 µg/mL that was included as the weak inhibition. While in S. aureus have inhibitory of 250 µg/mL, it is included in the category that is strong enough. In the opinion of Holetzetal.,(2002) states that, the value of the minimum inhibitory concentration (MIC) of the active antibacterial compound has three categories of establishments which have a concentration of less than 100 µg/mL is very strong, if the concentration is between 100-500 µg/mL it does show that of concentration is strong enough, if the concentration is between 500-1000 µg/mL is weak and if the concentration of more than 1000 µg/mL means that the compounds have no antibacterial activity.

According to research Rosaidah and Mahita (2012), states that, with the use of guava leaf extract an average diameter of bacterial inhibition zone ranges from 6,5 mm-
11.5 mm. This shows that the greater the concentration used, the greater the inhibition zone diameters obtained, meaning that the antibacterial activity of guava leaf extract increases with increasing concentration of the extract. Jawetz et al., (2005), the antibacterial activity is influenced by several factors such as the concentration of the extract, contains antibacterial compounds, extracts diffusion power, and type of bacteria is inhibited.

**Bioautografi Test and Determination of Active Compound**

Methanol fraction bioautografi water test using silica gel Fplate254 to determine the class of the active compound with the appropriate eluent ratio 8:2 (ethyl acetate: methanol) as the mobile phase with the appearance of patches of H2SO4. Bioautografi test results (Table 4).

Table 4 Test Results Bioautografi water and methanol fraction group Active Compounds against Determination of E. coli and S. aureus

<table>
<thead>
<tr>
<th>Active Fraction</th>
<th>Rf Values</th>
<th>Color Shaped</th>
<th>Group Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>water Methanol</td>
<td>0.41</td>
<td>Brown</td>
<td>tannin</td>
</tr>
</tbody>
</table>

Caption: Comparison eluent 8: 2 (Ethyl acetate: Methanol)

![Inhibition zone](image1.png)

(a) Bioautografi Test

![Tannin (brown)](image2.png)

(b) the TLC plate

**Figure 3** The Results of water and methanol Bioautografi Active Compounds Determination of *Syzygium zeylanicum* leaves toward bacterium *E. coli* and *S. aureus*

Results bioautografi test on the TLC plate visible brown color indicating tannin. According Harbone (1987) states that, tannin yellowish to brown. While the TLC plate is placed in a petri dish looks a clear zone formed in cultured bacteria due to the tannin. According Darwiset al., (2013), tannin is a complex phenolic compounds containing hydroxyl groups. Tannin are phenolic, then tannin have the same mechanism with a phenol compound in inhibiting the growth of bacteria. The mechanism of phenol compounds, According to Dwidjoseputro (1994), phenol compounds enter into bacterial cell pass through the cell membrane of bacteria and cytoplasmic membrane, in bacterial cell of phenol compounds cause the aggregation of protoplasmic proteins so that the circumstances metabolism become inactive and bacteria become obstructed.

According to research Rosaidah and Mahita (2012) found, tannin is the main component in the leaves and seeds because of the amount of tannin content more than the
other compounds. Based on the research that has been done by Widaty (2008) through phytochemical screening test guava leaf extract contains tannins 13.51%.

**Purification and Antibacterial Activity Test Compounds Active Fraction**

Purification of the active compound from the methanol fraction of water to the adsorber column chromatography with silica gel 60 F<sub>254</sub>, eluent used as mobile phase was an elucent ratio of 9:1 (ethyl acetate: methanol water) at a rate of elution 40-50 drops per minute. Fractions testing activity against *S. aureus* is a fraction with odd numbers. Tests using only one test bacteria, this is because the fraction of the test compound is active against *E. coli* together with *S.aureus*, so only have one bacterium alone. Testing is done only on the odd bottle due to the possibility of 61 bottles of compounds obtained from the purification has the same active compound in the adjacent bottles. Diameter of clear zone test results of the antibacterial activity of the methanol fraction of water purification with the odd bottle (Figure 4).

Based on Figure 4 shown the fraction of the number of bottles of 1, 3 and 5 active against *S. aureus* with the formation of inhibition zone around the paper disc. In the bottles 7 to 51 did not show any inhibition zone. But there are also inhibitory zone on the bottle numbers 53, 57 and 51 and the number 55, 61 there is no inhibition zone, this is because it provides 100% methanol as mobile phase. According Tinambunan et al. (2012), giving the extract solution with a concentration of 100% aimed to determine the lowest levels of sample extracts that deliver antibacterial activity against bacteria tested.

![Figure 4](image.png)

**Caption:** Test Results Activities antibacterial compound purification of Fraction Methanol water with a number of bottles of odd against *S. aureus*
Based on the results of active compound activity from the Syzygium zeylanicum leaves against bacterial test, the compounds can inhibit the growth of S. aureus which is a representative gram-positive bacteria. Kusmayati and Agustini (2007) that gram-positive bacteria tend to be more sensitive to antibacterial, because the structure of the cell membrane of gram-positive bacteria was simple because it contains low lipid that made it easier for antibacterial compound to enter the cell of gram-positive bacteria.

CONCLUSIONS

Active fractions of the water methanol extract from Syzygium zeylanicum leaves have antibacterial activity with a diameter of 7.0 mm, inhibition of E. coli and 9.75 mm for S. aureus. Minimum Inhibitory Concentration (MIC) of the methanol fraction of water is 1000 µg/mL for E. coli 500 µg/mL for S. aureus. The methanol compounds of fraction purified water from the Syzygium zeylanicum leaves is tannin with Rf 0.416. Minimum Inhibitory Concentration (MIC) of pure compound is 500 ug / ml to against E. coli and S. aureus.

REFERENCES


