Antioxidant and antibacterial activities of secondary metabolite endophytic bacteria from papaya leaf (Carica papaya L.)

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Abstract. Endophytic bacteria are capable of producing secondary metabolite compounds similar to their host plants that can act as antioxidants and antibacterial. Previous research on endophytic bacterial isolation from papaya leaf (Carica Papaya L.) obtained 5 consortia of endophytic bacteria symbiotic with papaya leaf. One of them is EC-3 isolate which are bacillus and gram positive. From this research, antioxidant activity test, antibacterial and extracellular content of EC-3 isolate metabolite compounds were conducted. The isolates of endophytic bacteria that symbiotes with the papaya leaves are able to produce alkaloid, flavonoids, saponins, tannins and triterpenoids compounds that act as the antioxidant and antibacterial. These isolates were selected from carica papaya leaves have antioxidant and antibacterial potential of secondary metabolite. EC-3 of secondary metabolite has the free radical scavenging activity of 68.5% and total phenolic as much as 69 mg greq gallic acid/gram sample. This value indicates that in 1 gram of metabolite extract, there is a phenolic compound equivalent to 69 mg of gallic acid. EC-3 secondary metabolites enhances the antibacterial potential to inhibited S. aureus, S.Typhi and E. coli.

Keywords: endophytic bacteria, secondary metabolites, antioxidant, antibacterial.

1. Introduction

Endophytic bacteria are bacteria that live in plant tissues for a certain period of their life cycle. Endophytic bacteria can be isolated from plant tissues and grown on certain fermentation mediums [1, 2]. In the fermentation medium endophytic bacteria generally can produce similar compounds contained in host plants with the help of an enzyme activity [3]. According to Stierle et al. [4] the use of endophytic microbes in producing active compounds has several advantages, including faster production of uniform quality compounds, can be produced on a large scale and the possibility of obtaining new bioactive components by providing different conditions.

Plants that contain bioactive compounds usually have endophytic microbes with similar abilities to their hosts in producing metabolites [5, 6]. According to Marshall et al. [7], papaya leaves contain secondary metabolite compounds such as alkaloids, flavonoids, tannins, saponins and steroids. The content of secondary metabolites in papaya leaves can act as antioxidants [8]. Water extract from papaya leaves also has antioxidant activity with IC50 value of 198 mg/mL in DPPH test [9] and is able to inhibit gram-positive bacteria namely B. subtilis, P. aeruginosa and S. aureus and gram-negative namely E. coli, S. typhi and K. pneumonia.
Secondary metabolites can also be found in endophytic bacteria that grow on papaya leaves and are able to produce secondary metabolites similar to or even the same as compounds produced by their hosts [10]. Previous research, Ramadhan [11] managed to get 5 isolates of endophytic bacteria from papaya leaves. The five isolates are EC (Endophyte Carica)1, EC-2, EC-3, EC-4 and EC-5.

In this study, bioactivity exploration of one of the 5 isolates was carried out, namely EC-3, by testing the antioxidant ability, antibacterial metabolites of endophytic EC-3 and the potential of extracellular enzymes from EC-3 isolates. Based on this information, this study was conducted to obtain phenotypic characteristics, growth curve data, phytochemical qualitative data, antioxidant activity data, total phenol levels, antibacterial endophytic EC3 bacterial metabolites from papaya leaves and extra cellular EC-3 potential. The other isolates will be explored in future studies.

2. Experimental

2.1. Equipment and Materials

equipment used in this research were standard research glassware, balance sheets, ose needles, micro pipettes, shaker incubators, laminar air flow, autoclaves, vortex, freeze dryers, microscopes, centrifuges, UV-Vis spectrophotometers. The materials used in this study, namely EC3 endophytic bacteria that had been isolated by Ramadhan [11] from papaya leaves (Carica papaya L.), Zobell media (peptone and yeast extract), 70% ethanol (Brataco Chemika), gram colouring, phytochemical test reagents (ethanol pa, ammonia (Merck), chloroform (Merck), distilled water, ferric chloride 1% (Merck), concentrated sulphuric acid (Merck), anhydrous acetic acid (Merck), hydrochloric acid (Merck), dragendorff reagent (Merck), mayer (Merck), Mg powder), DPPH (Merck), ascorbic acid (Merck) and methanol pa (Merck) test solutions.

2.2. Purification of Bacteria
Endophytic EC3 bacteria were recultivated on oblique zobell media which were then incubated at 37°C. Separation of EC3 endophytic bacteria colonies was carried out using the streak method, until a single colony was obtained. Single colony results were stored in stock to tilt and replanted into liquid media for characterization.

2.3. Characterization of EC3 Endophytic Bacteria
Observation of bacterial morphology was carried out by gram staining method. The bacterial isolate from the liquid medium was applied to the glass preparation and passed over the bunsen to dry. Then dye crystal violet, lugol, iodine, acetone alcohol, and safranin were dropped. Observations were made using a microscope with 1000x magnification using emersion oil to show the shape and color of the bacterial cell wall. Gram positive bacteria are purple, while gram negative bacteria are red.

2.4. Production of Secondary Metabolites
Secondary metabolite production was carried out by EC3 endophytic bacterial fermentation which takes 1% starter and then inoculated on erlenmeyer containing liquid zobell media. Production was carried out during the time determined from the bacterial growth curve, which were at 20 hours, 32 hours, and 36 hours, each produced as much as 1000 mL. Centrifugation was carried out at 6000 rpm and room temperature for 15 minutes. The supernatant from the culture was concentrated using a freeze dryer to obtain extracts of solid metabolites.
2.5. Phytochemical Screening Test

2.5.1. Alkaloid Test. A total of 0.5 grams of metabolite compound were added 4 drops of ammonia and 10 mL of chloroform. The mixture was then shaken and 10 drops of concentrated sulphuric acid were added. After 2 layers were formed, the acid layer was transferred and divided into 2 parts in a test tube. The first tube was dripped with dragendorff reagent and the second tube was dripped with Mayer’s reagent. The positive test of dragendorff reagent is brown solution, while the positive test of Mayer’s reagent is a white misty solution [12].

2.5.2. Saponin test. As much as 1 mL of water is shaken hard and allowed to stand for 2 minutes. The formation of foam that is stable and does not disappear when added 1 drop of HCl 2 N indicates the presence of saponins [12].

2.5.3. Tanin test. The water layer is dripped in a drop plate, then 1% ferric chloride was added. The blue-purple solution formed shows the presence of tannins [12].

2.5.4. Triterpenoid/Steroid test. The chloroform layer was dripped in a drop plate, then acetic acid anhydride and concentrated sulphuric acid were added. The blue-green stain formed shows the presence of steroids, while the red-purple stains indicate the presence of triterpenoids [13].

2.5.5. Quinone test. Solid metabolites were boiled in 10 mL of distilled water for 5 minutes, then cooled and filtered. The obtained filtrate was added with 1 M NaOH. A positive quinone test shows the change in solution to red [13].

2.5.6. Flavonoid test. A total of 0.5 grams of sample were macerated in 10 mL of hot alcohol and allowed to evaporate, then chloroform and water were added (1:1). The mixture was shaken to form 2 layers (chloroform and water). A layer of water was added with Mg metal and hydrochloric acid and then shaken hard. The presence of flavonoids is characterized by an orange-red solution [13].

2.5.7. Antioxidant test. Metabolite samples were dissolved in methanol p.a with various concentrations. As much as 1 mL of metabolite samples from each concentration variation were added 3 mL of 0.1 mM DPPH left for 30 minutes in the dark. The next step was to measure absorbance at a maximum wavelength of 517 nm [9]. In the ascorbic acid sample, the same thing was done as a comparative antioxidant. Control absorbance and test samples were used to calculate %inhibition using the following formula:

$$\%\text{inhibition} = \frac{A_{\text{control}} - A_{\text{test sample}}}{A_{\text{control}}} \times 100\%$$

The IC$_{50}$ value can be calculated by creating a linear curve between the concentration of the test solution (x-axis) and the %inhibition value (y-axis).

2.6. Preparation of Standard Gallic Acid Calibration Curves

Determination of gallic acid standard calibration curves was based on the method carried out by Lim and Quah [14]. A total of 0.2 mL of gallic acid solution with various concentrations of 300, 400, 500, 600 and 700 mg/L were added with 15.8 mL of distilled water and 1 mL of Folin Ciocalteu. The solution was then shaken and allowed to stand for 8 minutes. Then as much as 3 mL of 20% Na$_2$CO$_3$ solution was added to the solution and shaken back until homogeneous and then allowed to stand for 30 minutes. The solution was then analysed by UV-Vis Spectroscopy at the maximum wavelength. Standard calibration curves were made by plotting each concentration vs. absorbance.

2.7. Determination of Total Phenol Extract

A total of 0.2 mL of extract solution was added with 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. The solution was shaken and allowed to stand for 8 minutes. Then as much as 3 mL of 20% Na$_2$CO$_3$ solution was added to the solution and shaken back until homogeneous and then allowed to stand for 30 minutes. Then the solution was analysed by UV-Vis Spectroscopy at the maximum
wavelength. The total phenol content was calculated by plotting absorbance into the linear regression equation of the standard gallic acid curve. The total phenol content was expressed as mgreq gallic acid/g sample.

2.8. Antibacterial Test

2.8.1. Turbidimetry Method. The suspension of test bacteria (S. typhi, E. coli and S. aureus) which were in accordance with the McFarland 0.5 turbidity standard was taken as much as 0.35 mL, then inoculated into a test tube containing 3.15 mL of Nutrient Broth media. Next 0.35 mL of secondary metabolite was added and the solution was incubated for 24 hours. Furthermore, optical density measurement was carried out by UV-Vis spectrophotometer.

2.8.2. Disc Diffusion Method. In this method, 40 μL of test bacterial suspensions (S. typhi, E. coli and S. aureus) which had been synchronized with McFarland 0.5 turbidity standard were inoculated on petri dishes containing Nutrient media in order to be solid. Furthermore, paper discs that have been soaked into secondary metabolites were placed on the media, then incubated for 24 hours. As a positive control, the disc paper was immersed in 0.3 mg/mL of antibiotics (ampicillin), while as a negative control disc paper was only soaked in distilled water.

3. Results and Discussion

3.1. Purification of Endophytic EC3 Bacteria Culture

Based on the purification of Endophytic EC3 Bacteria Culture method, a single colony of endophytic EC3 bacteria was obtained as shown at Fig. 1. This can be seen from the same colonies on petri and separate colony. A single colony is a colony that comes from individual bacteria and is separated from one colony with another [15].

![Figure 1. Single colony of EC3 endophytic bacteria.](image)

3.2. Characterization of EC3 Endophytic Bacteria

Characterization of endophytic bacteria was carried out by observing bacterial morphology through gram staining [16].
Based on Fig. 2, EC3 endophytic bacterial isolates are gram positive because they are purple [15]. The results of the morphology of EC3 isolates are shown in Table 1.

### Table 1. Morphological characteristics of EC3 endophytic bacterial colonies.

| Bacterial isolates | Color | Colony shape | Elevation | Cell shape | Coloring | Gram |
|--------------------|-------|--------------|-----------|------------|----------|------|
| EC3                | White | Round        | Convex    | Monobasil  | Purple   | Positive |

### 3.3. Production of Secondary Metabolites

Based on the previous EC3 endophytic bacterial growth curve, secondary metabolite production was carried out on incubation of 20, 32 and 36 hours with the incubation treatment in the incubator swaying. The crude extract of secondary metabolites was then concentrated by the freeze drying method. The main principle of freeze drying method is drying or concentration where the solvent undergoes crystallization and sublimation at low temperatures [17]. Samples that have been concentrated by the freeze drying method were in the form of paste-shaped brown. The weight of the extract of secondary metabolites obtained from 1000 mL of supernatant after the freeze drying process are shown in Table 2.

### Table 2. Mass of secondary metabolites of EC3 endophytic bacteria from papaya leaves.

| Bacterial isolate | Incubation time(hour) | Metabolite Mass (gram) |
|-------------------|------------------------|------------------------|
| EC3               | 20                     | 1.77                   |
|                   | 32                     | 1.23                   |
|                   | 36                     | 1.05                   |
|                   | 38                     | 1.30                   |
3.4. Endophytic EC3 Bacterial Growth Curve

Observation of bacterial growth curves aims to determine the phases of bacterial growth. In this study, observations were made by measuring the number of bacteria every 2 hours. Fig. 3 shows the growth curve of endophytic bacteria isolated from EC3.

![EC3 growth curve](image)

In this study, observations of growth curves were carried out to determine the production time of secondary metabolites from EC3 endophytic bacterial isolates. According to Madigan et al. [18], the stationary phase to death is the right time to produce bacteria so that the best secondary metabolites are obtained. The growth curve of endophytic EC3 bacteria shows that the stationary phase occurs at the 20th hour until the 36th hour so that the production of secondary metabolites at the stationary start is the 20th hour, the centre is stationary at the 32nd hour and the stationary end is at the 36th hour. Secondary metabolites are produced by bacteria when bacteria undergo certain phases. One of them is when bacterial nutrition starts to run out so there is competition between bacteria to get the nutrients. In the struggle for nutrients, bacteria carry out metabolic processes to produce secondary metabolites that are used to defend themselves [19]. The next research phase is the production of secondary metabolites of EC3 endophytic bacteria from papaya leaves.

3.5. Antioxidant Capacity

Metabolite extract from freeze drying results was used as a sample to test antioxidant activity using DPPH method. The results obtained are shown in Fig. 4.
Figure 4. Percent of inhibition of secondary metabolite production of EC3 endophytic bacteria. EC3-20: incubation time at 20 hours, EC3-32: incubation time at 32 hours, EC3-36: incubation time at 36 hours.

Based on Fig. 4, it can be seen that the inhibition percentages of the three samples experienced a sharp increase from the concentration of 50 ppm to 100 ppm, while at concentrations of 100 ppm to 400 ppm the increase was relatively the same. There is a relationship between increasing concentration and the percentage inhibition value, the higher the concentration, the higher the content of the extract of secondary metabolites so that the percentage inhibition value increases. The highest antioxidant activity was shown by EC3-32 with inhibition of up to 68.507% at a concentration of 400 ppm. This difference can be seen from the graph that the percentage of EC3-32 inhibition is 10% higher than EC3-20 and EC3-36.

Absorbance is used to find the % inhibition value which can be used to calculate IC$_{50}$ as a parameter in determining the capacity of antioxidant activity. The IC$_{50}$ value indicates the need for antioxidant concentration to reduce 50% of DPPH free radical concentration. As IC$_{50}$ gets smaller, the greater the antioxidant capacity to extract its metabolites [20]. IC$_{50}$ results are shown in table 3.

Table 3. IC$_{50}$ values of secondary metabolites of EC3 endophytic bacteria.

| Sample   | IC$_{50}$ (ppm) |
|----------|-----------------|
| Ascorbic acid | 18.85          |
| EC3-20   | 315.39          |
| EC3-32   | 218.90          |
| EC3-36   | 314.90          |

Based on the table 3, the highest antioxidant capacity was at the 32$^{nd}$ hour incubation, namely in the stationary middle phase the growth of EC3 endophytic bacteria with IC50 value of 218.90 ppm which means that with a concentration of 218.90 ppm the sample could inhibit 50% of DPPH free radical work. According to Madigan et al. [18], the stationary phase to death is the best phase in producing secondary metabolites. This is due to the limited amount of nutrients in the phase that causes bacteria to release genes for the synthesis of secondary metabolites that can be used to defend themselves [21].

Blois [22] classified the antioxidant activity where the highly active compounds had IC$_{50}$ values <50 ppm, the active category had IC$_{50}$ values of 50-100 ppm, medium category if they had IC$_{50}$ values of
100-150 ppm, and weak categories if they had IC$_{50}$ 151-200 ppm. It can be concluded that the antioxidants produced are in the weak category. According to Molyneux [20] compounds with antioxidants range from 200-1000 µg/mL less active but still have antioxidant activity.

3.6. Determination of Total Phenolic Content
Measurement of total phenol content was carried out to determine the correlation between antioxidant activity and total phenol content of the sample. Total phenol data is shown in Fig. 5.

![Figure 5. Graph of total phenolic secondary metabolites of EC3 endophytic bacteria from papaya leaves.](image)

Based on Fig. 5, the total phenolic content of EC-20, EC-32, and EC-36 did not change significantly, but it can be said that extracts of secondary metabolites of endophytic EC3 bacteria were comparable to antioxidant capacity. This can be correlated with Fig. 4 that the inhibition percentage experienced a relatively similar increase. The greatest total phenolic content was at the 32nd hour incubation time or when the stationary middle phase of the bacteria was 69 mg gallic acid/g sample. This value shows that in 1 gram of metabolite extract, there are phenolic compounds which are equivalent to 69 mg of gallic acid.

The results of the determination of the total phenol content is proportional to the antioxidant capacity. This proves that one source of antioxidants in endophytic EC3 bacterial isolates derived from phenolic compounds. According to Kinsella et al. [23], phenolic compounds function as antioxidants because of their ability to eliminate free radicals and peroxide radicals so that they are effective in inhibiting lipid oxidation. Phenolic compounds have hydroxyl groups that can reduce radicals by donating hydrogen atoms.

3.7. Phytochemical Screening
Phytochemical screening included qualitative tests on the presence of alkaloids, flavonoids, saponins, tannins, quinones, steroids and terpenoids. Table 4 shows the results of secondary metabolite phytochemical screening.
Table 4. Phytochemical screening results of secondary metabolites of EC3 endophytic bacteria and papaya leaf simplicial.

| Phytochemical       | Result | EC3-20 | EC3-32 | EC3-36 | EC3-38 | Carica leaf |
|---------------------|--------|--------|--------|--------|--------|-------------|
| Alkaloids           | +      | +      | +      | +      | +      | +           |
| Flavonoids          | +      | +      | +      | +      | +      | +           |
| Saponin             | +      | +      | +      | +      | +      | +           |
| Terpenoids          | +      | +      | +      | +      | +      | +           |
| Tannin              | +      | -      | +      | +      | +      | +           |
| Steroids            | -      | -      | -      | -      | -      | +           |
| Quinones            | -      | -      | -      | -      | -      | +           |

Based on table 4, it is known that both ethanol extracts of papaya leaf and extracts of secondary metabolites of endophytic EC3 bacteria isolates from papaya leaves contain alkaloids, flavonoids, saponins, tannins. According to Strobel and Daisy [19], endophytic bacteria that plant on plants that produce bioactive compounds have the potential to produce bioactive compounds similar to or even the same as their host plants. This is thought to occur due to genetic transfers from host plants to endophytic microbes [10]. Genetic transfer occurs because host plants and endophytic bacteria have the same pathway to synthesize secondary metabolites through certain enzymes in which endophytic bacteria and their hosts experience direct contact. Metabolic interactions between endophytes and their hosts can induce the synthesis of secondary metabolites [10].

In the ethanol extract of papaya leaves did not contain triterpenoids while in the secondary metabolite EC3 endophytic bacteria appeared triterpenoid. This can occur because triterpenoids can be produced from the essential components of bacterial cell membranes that stabilize phospholipid interactions such as sterols which are triterpenoid derivatives [24]. In ethanol extract, papaya leaves contain steroids and quinones, but the extract of secondary metabolites does not appear. This is presumably because the bacterial media environment is different from the host plant environment causing the expression of secondary metabolites does not occur, so that the presence of steroids and quinones are not identified in the phytochemical test.

In this study, endophytic bacterial isolates symbiosis with papaya leaves were able to produce alkaloid compounds, flavonoids, saponins, tannins and triterpenoids which have an effect as antioxidants. Flavonoids and tannins are polyphenol compounds that have high antioxidant properties and the ability to reduce free radicals [25]. Gogna et al. [26] added that the highest antioxidant activity in papaya leaves was found in flavonoid compounds. Flavonoid compounds in papaya leaves include kaemferol, hesperidin, quercetin, naringetin, and rutin [26].

Tiong et al. [27] reported that alkaloids could play a role in reducing free radicals. Alkaloids that have the potential as antioxidants are alkaloids that can be extracted in polar solvents namely pseudoalkaloid and protoalkaloid groups [28]. In addition, the presence of saponins makes a plant have natural antioxidants because it can bind free radicals in complex media [24]. The results of antibacterial testing with the turbidimetry method are shown in Fig. 6.
Based on Fig. 6, each metabolite added to the test bacteria has significantly increased percent inhibition as concentration increases. The highest inhibition percentages obtained by EC3-38 compared to EC3-32 and EC3-36 showed that EC3-38 had the highest antibacterial activity. At a concentration of 2 mg/mL against E. coli, 3 mg/mL against S. typhi and 4 mg/mL against S. typhi bacteria, EC3-36 has an almost equal percentage of inhibition with EM38. In Fig. 6 it is also shown that EC3-32, EC3-36 and EC3-38 are more sensitive to S. aureus bacteria compared to S. typhi and E. coli as evidenced by the high percentage of inhibition produced by each secondary metabolite. According to Jawetz et al. [29], the resistance of gram-positive and gram-negative bacteria to antibacterial compounds is relatively different. Differences in the sensitivity of gram-positive and gram-negative bacteria relate to the structure in the cell wall, such as the amount of peptidoglycan (the presence of receptors, pores, and lipids), the nature of cross-linking, and autolytic enzyme activity. These components are factors that determine penetration, binding and the activity of antibacterial compounds.

This study also determined the Minimum Inhibitory Concentration (MIC) with disc diffusion method. In the disc diffusion method secondary metabolites were used with the same concentration as the concentration of positive control but did not give a response in the form of clear zones, so that the extract concentration was increased again to show a clear zone. The test results are shown in Fig. 4.

**Figure 6.** Antibacterial activity test by turbidimetry method.

**Figure 7.** Determination of MIC with disc diffusion method.
Based on Fig. 7, the best MIC obtained from the antibacterial activity of secondary metabolites EC3-32, EC3-36 and EC3-38 was at EC3-38 where that of the *S. aureus* bacteria was 1.3 mg/mL, while those of *S. typhi* and *E. coli* bacteria were 1.4 mg/mL. The difference in MIC values obtained was expected because EC3-38 secondary metabolite content was more than EC3-36 and EC3-32 which was proven by the results of EC3-38 secondary metabolite production having more mass (table 2). Fig. 7 shows that each secondary metabolite produces a 1 mm inhibition zone against the test bacteria. According to [30], the inhibitory zone with a diameter of 20 mm or more has very strong antibacterial potential, the inhibitory zone with a diameter of 10-20 mm has strong antibacterial potential, the inhibitory zone with a diameter of 5-10 mm has moderate antibacterial potential, and the inhibitory zone with a diameter of less than 5 mm has weak antibacterial potential. Overall, the secondary metabolites tested against the test bacteria *S. aureus, S. typhi* and *E. coli* have inhibitory zone diameters between 1-2 mm around the disc. This shows that the metabolites produced by EC3 endophytic bacteria have the potential as antibacterial.

According to Akiyama *et al.* [31], tannins have 3 antibacterial mechanisms namely astringent property that can cause shrinkage of tissue due to complexation with substrates or enzymes in microbes through hydrogen bonds to bacterial proteins; toxicity property to microbial cell membranes where H+ ion from tannin compounds will attack polar groups (phosphate groups) so that the phospholipid molecule will break down into glycerol, carboxylic acid and phosphoric acid; and the complexation of metal ions that can work like siderophore by forming chelate with many metal ions, one of which is iron ions in bacterial cells.

Alkaloids are also identified in EC3 phytochemical screening where the mechanism of inhibition of bacteria by alkaloid compounds is by interfering with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer will not form fully and cause cell death. Nitrogen at the alkaloid will react with amino acids which make up cells and bacterial DNA. This reaction results in changes in the structure and composition of amino acids which causes changes in genetic balance in the DNA chain which causes cell walls of bacterial DNA to be damaged and can promote bacterial cell lysis.

In the phytochemical test flavonoid compounds were also found. According to Cushnie and Lamb [32], flavonoids also have antibacterial activity through several mechanisms, including causing disturbances in cell membranes, inhibition of nucleic acid synthesis, inhibition of energy metabolism and cell wall synthesis. The hydrogen bond formed between phenol and protein causes damage to the protein structure. The hydrogen bond will affect the permeability of cell walls and cell membranes because they are composed of proteins. The permeability of disrupted cell walls and cell membranes can cause an imbalance of macromolecules and ions in cells, so that the cell becomes lysis.

Other compounds found are saponins. Saponins have molecules that can attract water or hydrophilic and molecules that can dissolve fat or lipophilic so that it can interfere with the permeability of bacterial cell membranes, alter the structure and function of membranes which eventually cause the cell membrane to be damaged and lysis [33].

Triterpenoids act as antibacterials through a mechanism of reaction with porin (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond which results in porin damage. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall and cause bacterial cells to be deficient in nutrients, so that bacterial growth is inhibited or dead [34].

Positive control used in this study is 1 mg/mL of ampicillin to inhibit bacterial growth. Ampicillin can inhibit bacterial growth through inhibition of bacterial cell wall synthesis by binding to the enzyme transpeptidase which binds peptidoglycan. Ampicillin can inhibit the action of the transpeptidase enzyme by binding enzymes through covalent bonds so as to prevent the formation of bacterial cell walls (Siswandono, 2000). The use of ampicillin at a smaller concentration compared to the concentration of secondary metabolites so that the results shown are not too much different.
4. Conclusion
EC3 isolate is a potent antioxidant producer, having broad spectrum activity against various free radicals, phenolic total with FCR method is 69 mg gallic acid/g extract. In addition, EC3 isolate has secondary metabolites enhances the antibacterial potential to inhibited S. aureus, S.Typhi and E. coli.

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