

## Activity of Bangun-Bangun Leaves (*Plectranthus Amboinicus* (Lour.)) as Antibacterial Against ESBL-Producing *Escherichia Coli*

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### Abstract

Herbal medicine is increasingly being used as a safe and effective alternative treatment. One plant that has antibacterial potential against antibiotic-resistant *Extended Spectrum Beta-Lactamase* (ESBL)-producing *Escherichia coli* bacteria is the leaves of bangun-bangun (*Plectranthus amboinicus* (Lour.)). Research shows that this leaves extract effectively inhibits and kills the growth of ESBL-producing *Escherichia coli*. This study aims to explore more deeply the antibacterial activity of bangun-bangun leaves, with the hope of supporting the development of herbal medicine and improving public health. This research is an *in vitro* descriptive experimental research in the laboratory. The test groups used are several concentrations of ethanol extract of bangun-bangun leaves, namely, 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL with 4 repetitions. This study was conducted using the dilution method to determine the minimum inhibitory concentration (MIC) and minimum kill concentration (MBC). The results of this study showed that bangun-bangun leaves extract with a concentration of 1000 µg/mL as the minimum kill concentration (MBC) and 500 µg/mL as the minimum inhibitory concentration (MIC). The extract of bangun-bangun leaves has antibacterial activity against ESBL-producing *Escherichia coli* bacteria.

**Keywords:** Antibacterial, *Plectranthus Amboinicus*, *Escherichia Coli* ESBL Producers, Bangun-Bangun Leaves Extract

### 1. Introduction

Currently, herbal medicine is widely used in various public health practices around the world because it is safe, cost-effective, effective against multiple deadly diseases, and helps maintain health. World Health Organization (WHO) estimates that around 80% of the world's population still relies on traditional herbal medicine used in treatment, especially in understanding traditional medical practices to cure diseases.<sup>1,2</sup>

Traditional herbal medicine can originate from various natural resources in the form of plants and animals. One of the herbal medicines derived from leaves rich in antibacterial properties is the leaves of *Plectranthus amboinicus*.<sup>1,3</sup> *Plectranthus amboinicus* (Lour.), locally known as "Tor-Bangun" or "Bangun-Bangun," belongs to the Lamiaceae family and is commonly found in tropical regions, including Indonesia.<sup>4</sup> The

leaves of *Plectranthus amboinicus* (Lour.), previously known as *Coleus amboinicus*, are known to contain various secondary metabolite compounds with potential antibacterial properties.<sup>3,5</sup> *E. coli* bacteria are one of the gram-negative rod-shaped bacteria, which belong to the Enterobacteriaceae family.<sup>6,7</sup> *Escherichia coli* can respond to antibiotic selection pressure by developing resistance mechanisms, one of which is the production of extended-spectrum beta-lactamase (ESBL) enzymes.<sup>8</sup>

Extended-Spectrum Beta-Lactamase (ESBL) is an enzyme that can hydrolyze and inactivate beta-lactam antibiotics, such as third and fourth generation cephalosporins and monobactams.<sup>8</sup> *Escherichia coli* can become ESBL producers through horizontal gene transfer from other bacteria, using genetic elements such as plasmids or transposons. Additionally, *E. coli* can also

undergo mutations in genes encoding beta-lactam hydrolyzing enzymes, such as AmpC-BL, which increase resistance to beta-lactam antibiotics.<sup>9</sup>

The incidence of infections caused by ESBL-producing *E. coli* is continuously increasing worldwide, especially in developing countries. Epidemiological studies show a high prevalence of *ESBL-producing E. coli* among hospitalized and outpatient patients, reaching more than 50% in 50,000-700,000 cases.<sup>10</sup> In Indonesia, data from the Ministry of Health indicate an increase in ESBL-producing *E. coli* infections in hospitals, from around 30% in 2018 to 40% in 2022, with a total of 45,000 cases per year.<sup>11</sup>

Antimicrobial resistance is an increasingly concerning global health issue, with microorganisms such as bacteria, viruses, fungi, and parasites becoming resistant to antimicrobial drugs (e.g., antibiotics, antivirals, and antifungals).<sup>12</sup> Infections caused by multidrug-resistant bacteria are on the rise. The discovery of innovative treatment approaches and antimicrobial therapies is necessary.<sup>13</sup>

Several studies have shown that the extract of bangun-bangun leaves has effective antibacterial activity against ESBL-producing *Escherichia coli* bacteria, including resistant strains. Islami et al. (2019) found that the aqueous extract of bangun-bangun leaves can inhibit the growth of *E. coli* bacteria at a concentration of 50 mg/mL with an inhibition diameter of 12.5 mm.<sup>14</sup> Singarimbun et al. (2022) reported that the n-hexane extract of bangun-bangun leaves against ESBL-producing *Escherichia coli* bacteria at concentrations of 50%, 25%, and 12.5% showed antibacterial potential with inhibition diameters of 10-20 mm.<sup>15</sup> Alves Resende et al. (2022) also found that the methanol extract of bangun-bangun leaves against ESBL-producing *Escherichia coli* bacteria at a concentration of 100 µg/mL could inhibit the

growth of *E. coli* bacteria with inhibition zones between 11-27 mm.<sup>16</sup>

Based on the above background, the antibacterial activity of the ethanol extract of bangun-bangun leaves (*Plectranthus amboinicus* (Lour.)) against ESBL-producing *Escherichia coli* bacteria using the dilution method has not yet been studied, thus this research was conducted to develop broader information and provide a more comprehensive picture. Therefore, the researcher is interested in conducting further studies to determine the potential of bangun-bangun leaves regarding the "Antibacterial Activity of Bangun-bangun Leaves (*Plectranthus amboinicus* (Lour.)) against ESBL-producing *Escherichia coli*." The results of this study are expected to provide useful scientific information for the development of traditional herbal medicine and the improvement of public health.

## 2. Method

This research was a descriptive in vitro experimental study conducted in the Microbiology Laboratory of the Faculty of Medicine, Sriwijaya University, Palembang, with a post-test-only group design. This study uses the dilution method with a total of 24 samples from 6 treatment groups and 4 repetitions, calculated using the Federer formula as follows.<sup>17</sup>

$$(n-1)(t-1) \geq 15$$

n : Number of repetitions  
t : Number of treatments

The independent variables of this study were ethanolic extract of bangun-bangun leaves with graded concentrations of 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL, and dependent variables were the minimum inhibitory concentration

(MIC), minimum bactericidal concentration (MBC), and the compound groups of the ethanol extract of bangun-bangun leaves.

Clinical isolate of ESBL-producing *Escherichia coli* bacteria was used from the Microbiology Laboratory collection of the Faculty of Medicine, Sriwijaya University, Palembang, in 2024. The simplicia of the bangun-bangun leaves was obtained from the Kuningan area, West Java. The negative control of the study consisted of a bacterial growth suspension without treatment, and the extract control consisted of an MHB (Mueller Hinton Broth) media suspension dissolved with each concentration of the extract.

This study uses the maceration method to obtain the extract of bangun-bangun leaves. The simplicia of the bangun-bangun leaves and the solvent were mixed in a 1:4 ratio into an Erlenmeyer flask, which was then filtered after 48 hours and the process was repeated four times. The separation of the solvent and extract was done using a rotary evaporator, resulting in a dry extract that can then be tested. After obtaining the dry extract, the % yield extract value is calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{number of dry simplicia results}}{\text{number of concentrated extract results}} \times 100\%$$

Phytochemical tests are conducted using a qualitative method by adding reagents to the extract. To determine the presence of flavonoid groups, concentrated  $\text{H}_2\text{SO}_4$  is added, which will produce a reaction resulting in yellow, orange, red, or green colors. The reaction when saponins are detected is the formation of stable bubbles. The reaction to tannins is when three drops of 5%  $\text{FeCl}_3$  produce a dark greenish-black or dark blue color. For terpenoids, a reddish-brown color is observed after adding chloroform and  $\text{H}_2\text{SO}_4$  solution.

The liquid dilution or multi-serial method is a testing method that uses turbidity measurement. The treatment tube is said to show bacterial growth inhibition if it is clearer compared to the control tube. The minimum inhibitory concentration (MIC) tube is the concentration that appears clearer compared to the control tube. The determination of the Minimum Bactericidal Concentration (MBC) is the next step by observing the absence of bacterial colony growth from the streak plate on MHA (Mueller Hinton Agar) media from the tube with a clearer appearance.

### 3. Results

The dried simplicia of bangun-bangun leaves weighing 250 grams produced a thick extract of 11.32 grams with a yield of 4.52%. The thick extract was then made into concentrations of 2000  $\mu\text{g/mL}$ , 1000  $\mu\text{g/mL}$ , 500  $\mu\text{g/mL}$ , 250  $\mu\text{g/mL}$ , and 125  $\mu\text{g/mL}$ .

#### 3.1. Results of metabolite compound group testing

Phytochemical testing of the concentrated extract of bangun-bangun leaves was conducted to identify secondary bioactive compounds qualitatively. The results of the secondary metabolite compound group test, which functions as an antibacterial agent in this research extract, include flavonoids, terpenoids, alkaloids, saponins, tannins, and steroids (Table 1, Figure 1).

#### 3.2. Antibacterial activity

##### 3.2.1. Minimum Inhibitory Concentration (MIC)

Table 1 shows that in the presence of turbidity between the treatment groups and the untreated control, the treatment concentrations of 2000  $\mu\text{g/mL}$ , 1000  $\mu\text{g/mL}$ , and 500  $\mu\text{g/mL}$  groups were found in this study. On the other hand, the 250  $\mu\text{g/mL}$  and 125  $\mu\text{g/mL}$  groups showed turbidity. In this

study, the concentration of 500 µg/mL visually showed clarity (Figure 1). The clear groups, compared to the untreated control, extract control, and concentrations below, showed inhibition of bacterial growth. Conversely, turbid tubes, compared to the untreated control, extract control, and the concentration below, showed no inhibition against bacterial growth.

### 3.2.2. Minimum Bactericidal Concentration (MBC)

MBC was determined by plating the incubated inoculum from MIC testing into agar plates to observe microbial growth (Figure 2). the concentration of bangun-bangun leaves extract which showed no colony growth (0 CFU/mL were 2000 µg/mL and 1000 µg/mL treatment groups had no bacterial growth (Table 3).

“No growth” indicates that no colonies developed (0 CFU), while “growth” indicates the presence of bacterial colonies. The MBC results in this study were obtained at a concentration of 1000 µg/mL.

Table 1. Results of metabolite compound group testing

| Compound             | Result                                     | Remarks |
|----------------------|--|---------|
| Alkaloid             |  |         |
| Dragendroff          | Orange-red precipitate                     | +       |
| Mayer                | Yellowish-white precipitate                | +       |
| Wagner               | Brown precipitate                          | +       |
| Flavonoid            | Reddish-brown                              | +       |
| Saponin              | Foam with a height of 1 cm for 10 minutes. | +       |
| Tannin               | Bluish-black                               | +       |
| Terpenoid            | Reddish-brown                              | +       |
| Quinone              | Dark green                                 | -       |
| Steroid/triterpenoid | Bluish-green ring                          | +       |

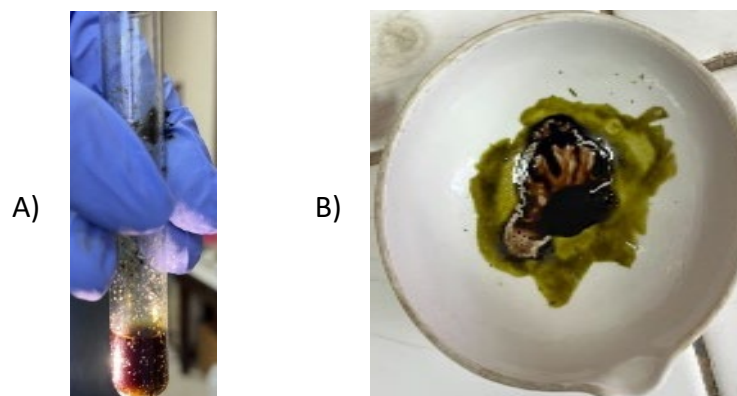


Figure 1. Results of metabolite compound group testing. (A) Flavonoid, (B) Terpenoid

Table 2. Results of MIC testing of bangun-bangun leaves extract

| Extract Concentration | Repetition |        |        |        | MIC |
|-----------------------|------------|--------|--------|--------|-----|
|                       | 1          | 2      | 3      | 4      |     |
| 2000 µg/mL            | Clear      | Clear  | Clear  | Clear  |     |
| 1000 µg/mL            | Clear      | Clear  | Clear  | Clear  |     |
| 500 µg/mL             | Clear      | Clear  | Clear  | Clear  | *   |
| 250 µg/mL             | Cloudy     | Cloudy | Cloudy | Cloudy |     |
| 125 µg/mL             | Cloudy     | Cloudy | Cloudy | Cloudy |     |
| Control               | Cloudy     | Cloudy | Cloudy | Cloudy |     |

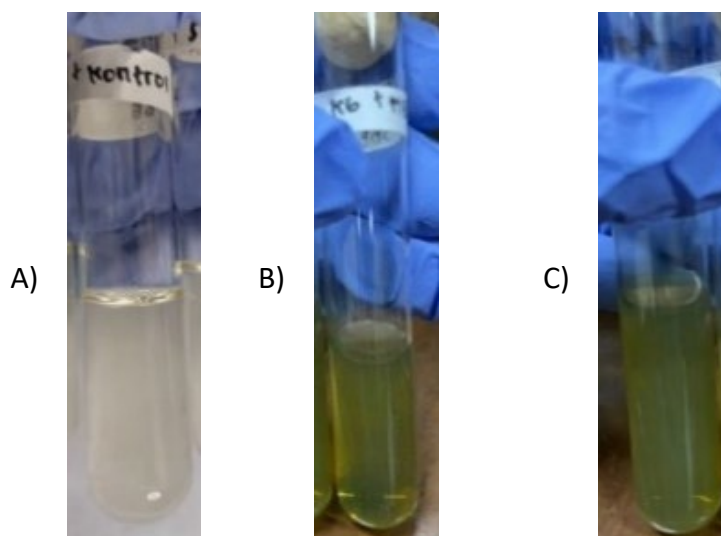


Figure 2. Minimum Inhibitory Concentration (MIC) testing of bangun-bangun extract. (A) Untreated control; (B) MIC 500 µg/mL; (C) Control extract concentration 500 µg/mL

#### 4. Discussion

##### 4.1. Metabolite compound group testing

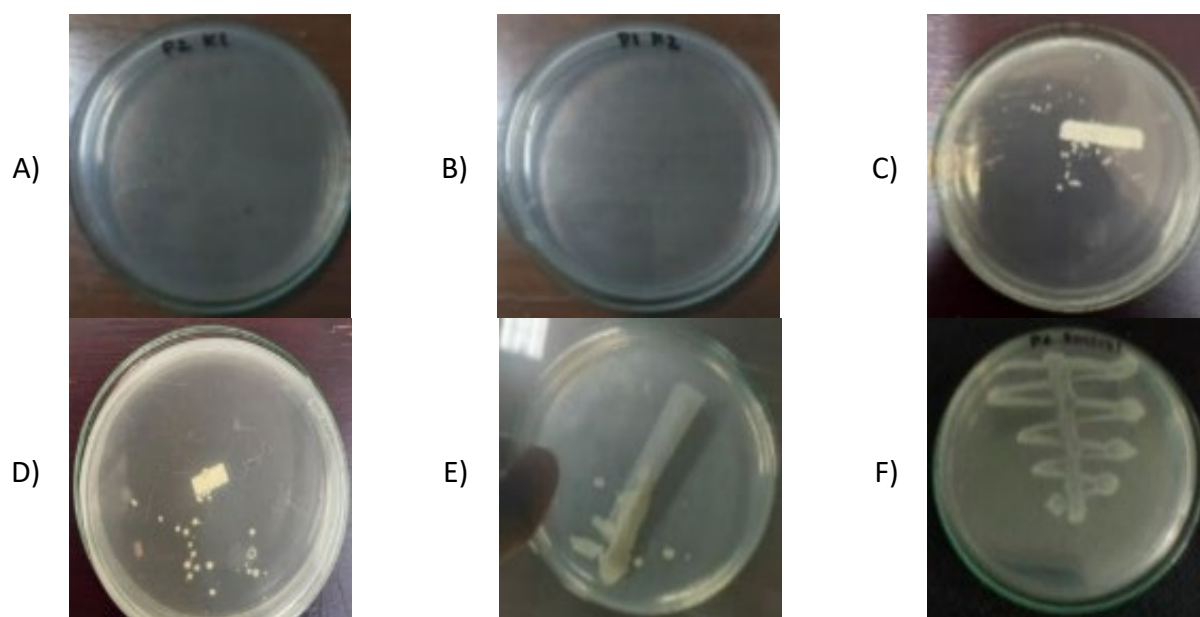
Phytochemical test results on ethanol extracts of bangun-bangun leaves showed the presence of secondary metabolite compounds, including flavonoids, terpenoids, alkaloids, steroids, saponins, and tannins, while quinones were not detected. Previous research by Sihombing *et al.* (2024) and Roslianizar *et al.* (2021) also found similar compounds in 96% ethanol extract, confirming the presence of saponins, flavonoids, alkaloids, tannins, and steroids.

This suggests that 96% ethanol solvent is effective in dissolving polar and nonpolar compounds.<sup>18,19</sup>

Ethanol as a universal solvent can extract almost all chemical compounds in bangun-bangun leaves, including compounds that have antibacterial potential such as flavonoids, saponins, and steroids/triterpenoids.<sup>19</sup> Other than the solvent used, variations in extraction methods, including solvent type, temperature, and duration, can affect the content of extracted compounds.<sup>20</sup>

Table 3. Results of MBC testing of bangun-bangun leaves extract

| Extract Concentration | Repetition |           |           |           | MBC |
|-----------------------|------------|-----------|-----------|-----------|-----|
|                       | 1          | 2         | 3         | 4         |     |
| 2000 µg/mL            | No Growth  | No Growth | No Growth | No Growth | *   |
| 1000 µg/mL            | No Growth  | No Growth | No Growth | No Growth |     |
| 500 µg/mL             | Growth     | Growth    | Growth    | Growth    |     |
| 250 µg/mL             | Growth     | Growth    | Growth    | Growth    |     |
| 125 µg/mL             | Growth     | Growth    | Growth    | Growth    |     |
| Control               | Growth     | Growth    | Growth    | Growth    |     |



**Figure 2: Minimum Bactericidal Concentration (MBC) testing. MBC Test Results: (A) Concentration 2000 µg/mL, (B) Concentration 1000 µg/mL (MBC), (C) Concentration 500 µg/mL, (D) Concentration 250 µg/mL, (E) Concentration 125 µg/mL and (F) Bacterial control**

Other factors that affect the content of secondary metabolite compounds include genetic variation, geographical location, harvest time, and environmental conditions. In this study, 96% ethanol was chosen as the solvent due to its ability to attract polar and nonpolar compounds, and has lower toxicity than other organic solvents such as methanol or chloroform. This makes ethanol an appropriate choice for the extraction of bioactive compounds from bangun-bangun leaves.<sup>16,21,22</sup>

Previous research, such as that conducted by Sihombing *et al.* (2024) and Romadhonyah *et al.* (2024), recorded the yield of 96% ethanol extract from bangun-bangun leaves as 13.88% and 8.42%, respectively.<sup>18,21</sup> These results show that the yield obtained is in accordance with the standards set by the Indonesian Ministry of Health, which is at least 4%.<sup>23</sup> This finding indicates that the extraction of bioactive compounds from plant materials is quite effective and has high potential for biological activity, including antibacterial properties.

#### 4.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) test in this study was carried out to determine the smallest extract concentration that could inhibit bacterial growth. The results showed that at a concentration of 500 µg/mL, although there was turbidity, bacterial growth was still detected, but not as much as in the control tube. In contrast, at concentrations between 500 µg/mL and 2000 µg/mL, there was no turbidity, indicating that the bangun-bangun leaf extract was effective in inhibiting bacterial growth. Thus, the MIC was set at 500 µg/mL.

Comparison with previous studies showed variations in MIC values. Gupta *et al.* (2013) found a MIC of 625 µg/mL using ethyl acetate extract, whereas Sreelakshmy *et al.* (2019) recorded a MIC of 800 µg/mL with deionized water extract. This difference may be due to the type of bacteria used, where this study focused on antibiotic-resistant ESBL-producing *E. coli*, as well as the use of 96% ethanol solvent.<sup>22,24</sup>

Research by Pathmavathi et al. (2016) showed a lower MIC of 125 µg/mL with methanol extract.<sup>25</sup> The inhibitory mechanism of the active compounds from wake-up leaf extract involves changes in the permeability of the cytoplasmic membrane, resulting in the escape of food material and denaturation of cell proteins. This contributes to the breakdown of the bacteria's metabolic system, which ultimately inhibits its growth.<sup>20,25</sup>

The bacterial cell wall is the main target of antibacterials in bangun-bangun leaf extract. Compounds such as flavonoids, tannins, and saponins disrupt cell membrane integrity, causing lysis, with flavonoids playing an important role in turning bacterial colonies fragmented.<sup>20</sup> The inhibition of ethanol extract of wake-up leaves in this study was categorized as moderate, with turbidity at a concentration of 500 µg/mL. This suggests the need for higher concentrations for a more significant antibacterial effect.<sup>26</sup>

The activity of an extract is categorized according to Asparinda et al. (2020)<sup>27</sup> (Table 4). This study shows that bangun-bangun leaf extract has moderate antibacterial activity against ESBL-producing *E. coli* bacteria, with a MIC value of 500 µg/mL. This MBC range, which is 100 µg/mL to 625 µg/mL, shows a fairly strong antimicrobial potential, indicating that the extract has the ability to inhibit bacterial growth. The MBC test was carried out by mixing wake-up leaf extract with bacterial suspension and observing bacterial growth on MHA media. The results showed that at concentrations of 500 µg/mL, 250 µg/mL, and 125 µg/mL there were still bacterial colonies, while at concentrations of

1000 µg/mL to 2000 µg/mL there was no colony growth. This indicates that concentrations starting from 1000 µg/mL are effective in killing ESBL-producing *E. coli*.

#### 4.3. Minimum Bactericidal Concentration (MBC)

Antibacterial activity is influenced by several factors, including extract concentration, antibacterial compound content, diffusion ability of the extract, and the type of bacteria inhibited. This study indicates that increasing the concentration of the extract further increases the effectiveness in reducing bacterial growth. Previous studies have also recorded varying MBCs for wake-up leaves, with Gupta et al. (2013) finding a value of 1000 µg/mL, while Bhatt et al. (2012) recording 2000 µg/mL.<sup>24,28</sup>

The difference in results between the studies could be due to variations in the types of bacteria and solvents used, although the methods applied were similar, i.e. liquid dilution. In addition, the extraction method also plays an important role in determining antibacterial activity, where the use of distillation techniques to extract essential oils has been shown to exhibit higher antibacterial activity.<sup>28</sup>

The type of solvent used for extraction affects the ability to solubilize bioactive compounds, which may affect the effectiveness of the extract. This study highlights the importance of solvents in determining MBC, as well as how bioactive compounds such as flavonoids, terpenoids and saponins interact with bacterial cell membranes to produce antibacterial effects.<sup>16</sup>

Table 4. Classification of MIC test values and extract activity

| Strong      | Medium              | Weak       |
|-------------|---------------------|------------|
| < 100 µg/mL | 100 µg/mL-625 µg/mL | >625 µg/mL |

Overall, bangun-bangun leaf extract proved to be effective as an antibacterial agent against ESBL-producing *E. coli*. The bioactive compounds in the extract contributed to the destruction of bacterial cell membrane integrity, increased permeability, and caused cell death. These results demonstrate the potential of bangun-bangun leaf extracts in the development of antibacterial agents.<sup>29</sup>

Research on bangun-bangun leaf extract using the liquid dilution method had its limitation, which was difficulty in visually determining the level of turbidity. This was especially experienced at high concentrations ranging from 2000 µg/mL to higher concentrations. This is due to the color of the extract, which hinders the observation of the turbidity level in the test tube.

## 5. Conclusion

Based on the results of the study, 96% ethanol extract of bangun-bangun leaves has antibacterial activity with a minimum inhibitory concentration (MIC) of 96% ethanol extract of bangun-bangun leaves against ESBL-producing *Escherichia coli* is 500 µg/mL, while the minimum kill concentration (MBC) is 1000 µg/mL. In addition, classes of secondary metabolite compounds that function as antibacterials in this extract include flavonoids, terpenoids, alkaloids, saponins, tannins, and steroids.

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