Screening of Antibacterial Activity of Ethyl Acetate Fraction of

Eleutherine palmifolia (L.) Merr Bulbs against Salmonella typhi

Penapisan Aktivitas Antibakteri Fraksi Etil Asetat Bawang Dayak

(Eleutherine palmifolia (L.) Merr) terhadap Salmonella typhi

Siti Rofida*, Ahmad Shobrun Jamil, Nurul Amalia Azhari

Department of Pharmacy, University of Muhammadiyah Malang
Jl. Bendungan Sutami 188A Malang, Jawa Timur, Indonesia

*Corresponding author email: rofida79@umm.ac.id

Received 10-11-2020 Accepted 14-01-2021 Available online 28-02-2021

ABSTRACT

Typhoid fever is an infection caused by the bacterium Salmonella typhi, spread through contaminated food or water. Typhoid fever can be treated with antibiotics, but at this time some pathogen microbes have been resistant to the available antibiotics. Antimicrobials derived from natural product might be the alternative to overcome antibiotic resistance. Eleutherine palmifolia (L.) Merr is empirically used to treat infectious diseases. E. palmifolia has chemical compounds of alkaloids, glycosides, flavonoids, phenolics, steroids, and tannins. This study aims to evaluate the antibacterial activity of ethyl acetate fraction against S. typhi. E. palmifolia bulbs were subsequently extracted using n-hexane solvents and followed by ethyl acetate solvents. The ethyl acetate fraction was tested for antibacterial activity using the disc diffusion method. The ethyl acetate fraction of E. palmifolia at the concentrations of 8, 6, and 8 mg/paper disc showed the diameters of the inhibition zone of 15.1±3.6, 15.3±3.3, and 16.9±1.9 mm, respectively. The conclusion of this study was the ethyl acetate fraction of E. palmifolia has a strong antibacterial activity against S. typhi.

Key words: antibacterial, Eleutherine palmifolia (L.) Merr, ethyl acetate fraction, Salmonella thyphi

Introduction

Typhoid fever is an infection caused by the Salmonella typhi and commonly spread through contaminated food or water. According to WHO, the incidence of typhoid fever is estimated at 11-20 million cases each year resulting in 128-161 thousand deaths each year (WHO Situation Report, 2018). Typhoid fever can be treated with
antibiotics, but at this time some antibiotics have been resistant. According to the study antibiotic sensitivity against S. thypi, suggests that some antibiotics such as amoxicillin, sefazolin, ampicillin, trimethoprim-sulfamethoxazole, amikacin, gentamicin, and ampicillin-sulbactam have experienced resistance (Juwita, Hartoyo and Budiarti, 2013; Kelanit, Runtuboi and Gunaedi, 2016).

In the drug discovery process, natural products are valued as the alternatives to overcome antibiotic resistance. Eleutherine palmifolia (L.) Merr is empirically used to treat infectious diseases. It has been scientifically proven active against Pseudomonas fluorescens (Fransira, Yanuhar and Maftuch, 2019), Shigella dysenteriae (Wicaksono, Runadi and Firmansyah, 2018), and Escherichia coli (Mahmudah, Muntaha and Muhlisisn, 2019). E. palmifolia contains alkaloids, glycosides, flavonoids, phenolics, steroids, and tannins (Harlita, Oedjijono and Asnani, 2018; Mutiah et al., 2019; Limantara et al., 2020).

This study aims to evaluate the antibacterial activity of the ethyl acetate fraction of E. palmifolia against Salmonella typhi. E. palmifolia bulbs were stratifiedly extracted using n-hexane solvents and followed by ethyl acetate solvents. The ethyl acetate fraction was tested for antibacterial activity using the disk diffusion method.

Method

Materials and Equipments

E. palmifolia bulbs used in this study were obtained from Palangkaraya and the identity had been determined in UPT. Balai Materia Medika. Salmonella typhi as the tested microorganism had been identified in the Biomedical and Microbiology Laboratory, Faculty of Medicine, University of Muhammadiyah Malang.

The chemicals and bacterial culture media used in this study were n-hexane (technical grade, Bratachem), ethyl acetate (technical grade, Bratachem), DMSO (Merck), Dragendorf’s reagent (Bratachem), anisaldehyd-sulfuric acid (Bratachem), FeCl3 (Merck), KOH (Merck), 10% H2SO4 (Merck), Chloramfenicol (Oxoid), Aquadest, silica gel F254 plate (Merck), Mueller Hinton broth, and Mueller Hinton Agar.

Experiments

1. Extraction of plant material

Plant materials (3200 g) was extracted using n-hexane (5x10 L). The residue was further extracted using ethyl acetate (5x10 L). Filtrates were combined and evaporated to dryness with the total yield of 43,72 g.

2. Secondary metabolite profiling of E. palmifolia

Profile of the secondary metabolite of ethyl acetate fraction of E. palmifolia was carried out using thin layer chromatography (TLC) method. The stationary phase used was silica gel F254 and mobile phase of ethyl acetate – chloroform, 3:7 (v/v). Chromatogram profile were
observed under UV lamp at the wavelength of 254 and 365 nm. The group of the metabolites were detected by Dragendorf’s, anisaldehyde-sulfuric acid, FeCl3, KOH, and H2SO4 10% reagent.

3. Antibacterial activity assay

*S. typhi* were grown on Mueller Hinton Agar. Solution of the tested samples at concentrations of 80, 120, and 160 mg/mL, each of 50 µl, was placed onto the paper disc. Samples on paper discs were diffused on bacterial growth medium and incubated at 37°C for 24 hours. The clear zone around the paper disc indicates inhibition of bacterial growth. The inhibition activity and the effectiveness of ethyl acetate fraction of *E. palmifolia* as antibacterial on *S. typhi* were calculated by equation 1 and 2:

\[
\text{Inhibition activity (\%)} = \frac{(d2-d1)}{d1} \times 100\% \\
\text{Antibacterial effectiveness (\%)} = \frac{d2}{d3} \times 100\%
\]

Where \(d1\) = diameter of paper disc (6 mm), \(d2\) = diameter of inhibition zone of ethyl acetate fraction of *E. palmifolia* (mm), and \(d3\) = diameter of inhibition of positive control chloramphenicol (mm).

Results and Discussion

Extraction of *E. palmifolia* bulbs using maceration method. Extraction was carried out in stages using solvents with different polarity index, i.e., n-hexane and ethyl acetate. The obtained ethyl acetate fraction was 43.72 g. Results of organoleptic identification of the fraction showed that it was brownish black, and aromatic.

Secondary metabolites extracted by ethyl acetate were identified. The TLC chromatogram showed that ethyl acetate fraction of *E. palmifolia* contained terpenoids, alkaloids, polyphenols, anthraquinones and flavonoids (Table 1, Figure 1).

According to Harborne (Harborne, Padmawinata and Soediro, 1987), Figure 1 showed the presence of terpenoids, alkaloids, polyphenols, anthraquinones dan flavonoids. The compounds that have been isolated from this plant were including eleutherine, eleutherol, eleutherinol-8-O-β-glucoside, isoeleutherine dan eleutherinol (Almeida, 2014), eleubosa A, eluobosa B, eleubosa C, karwinaphthol A, germacrene dan senkyunone (Jiang et al., 2020).

The antibacterial activity of ethyl acetate fraction of *E. palmifolia* was evaluated by using the disc diffusion method. The results showed there was a clear around the disc. The ethyl acetate fraction of *E. palmifolia* at a concentration of 4, 6, and 8 mg / paper disc against *S. typhi* showed inhibitory activity of 152%, 155% dan 182% respectively (Table 2, Figure 2).
Tabel 1. The result of the secondary metabolites screening of the ethyl acetate fraction of \textit{E. palmifolia}.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Reagent</th>
<th>Rf</th>
<th>Color of stains</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>Anisaldehyde-sulfuric acid</td>
<td>0.63</td>
<td>Purple</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>0.88</td>
<td>Orange</td>
<td>Present</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>FeCl3</td>
<td>0.00</td>
<td>Black</td>
<td>Present</td>
</tr>
<tr>
<td>Antraquinones</td>
<td>KOH 10%</td>
<td>0.20</td>
<td>Purple red</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Sulfuric acid 10%</td>
<td>0.93</td>
<td>Intensive yellow</td>
<td>Present</td>
</tr>
</tbody>
</table>

Figure 1. Chromatogram profile of ethyl acetate fraction of \textit{E. palmifolia} separated over stationary phase of silica gel F$_{254}$ by the mobile phase of ethyl acetate – chloroform, 3:7 (v/v), and visualized under (a) UV 254, (b) UV 365, as well as visible light after derivatization with (c) anisaldehyde-sulfuric acid, (d) Dragendorf’s reagent, (e) FeCl3, (f) KOH, and (g) 10% H2SO4.

Antibacterial activity of the ethyl acetate fraction of \textit{E. palmifolia}. Compared to the positive control chloramphenicol, it showed an effectiveness of 55-62%. The antibacterial activity in the ethyl acetate fraction of \textit{E. palmifolia} is mediated via several mechanisms. The terpenoids compounds is thought to have a mechanism by damaging the membrane function of bacteria (Cowan, 1999) and inhibition the enzymatic protein (Bajpai, Shukla and Sharma, 2013). Alkaloids had mechanisms such as inhibition of pyruvate kinase, Quorum quenching effect, alteration in efflux pump in MRSA and intercalating of bacterial DNA (Pervaiz et al., 2016). The mechanism of action of anthraquinones as antibacterials by producing changes in
physical structure and increase cell membrane permeability (Li et al., 2016). Flavonoids are phenolic compounds that are widely in plant. The mechanism of flavonoids compounds as antibacterials is to damage or disrupt the function of the bacterial membrane, inhibiting the formation of biofilms, inhibition of cell envelope synthesis, inhibition of nucleic acid synthesis, inhibition of electron transport chain and ATP synthesis, antibacterial action of flavonoid-metal complexes, inhibition of bacterial toxins (Górniak, Bartoszewski and Króliczewski, 2019). In addition, eleubosa A and B showed a moderate antibacterial activity against Escherichia coli with an MIC value of 12.5 µg/mL. Eleubosa A, eluobosa B and karwinaphthol A were also reported to show a mild activity against Staphylococcus aureus and Pseudomonas aeruginosa with the respective MIC value 25.0 µg/mL (Jiang et al., 2020).

Table 2. Antibacterial activity of ethyl acetate fraction of E. palmifolia against S. thypi by the disc diffusion method

<table>
<thead>
<tr>
<th>Sample concentration/paper disc</th>
<th>Inhibition zone (mm)</th>
<th>Inhibition activity (%)</th>
<th>Antibacterial effectiveness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate fraction of E. palmifolia</td>
<td>Chloramphenicol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg</td>
<td>15.1 ± 3.6</td>
<td>152</td>
<td>55</td>
</tr>
<tr>
<td>6 mg</td>
<td>15.3 ± 3.3</td>
<td>155</td>
<td>56</td>
</tr>
<tr>
<td>8 mg</td>
<td>16.9 ± 1.9</td>
<td>182</td>
<td>62</td>
</tr>
<tr>
<td>15 µg</td>
<td>-</td>
<td>27.27 ±1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 2. Inhibition activity of ethyl acetate fraction of E. palmifolia against the growth of S. typhi
Conclusion

The ethyl acetate fraction of *E. palmifolia* has a strong antibacterial activity against *S. typhi*, with an efficacy about a half of the antibacterial effect of chloramphenicol.

Reference


