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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*

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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 thypi*

Introduction

Typhoid fever is an infection caused by the *Salmonella typhi* and commonly spreaded 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, trimethroprime-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 thypi*. *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 thypi* 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% H_2SO_4 (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

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observed under UV lamp at the wavelength of 254 and 365 nm. The group of the metabolites were detected by Dragendorf's, anisaldehyd-sulfuric acid, FeCl3, KOH, and H2SO4 10% reagent.

3. Antibacterial activity assay

S. thypi were grown on Mueller Hinton Agar. Solution of the tested samples at concentrations of 80, 120, and 160mg/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 arroud 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:

Inhibition activity (%) = (d2-d1)/d1 x 100% (1)

Antibacterial effectiveness (%) = d2/d3 x 100% (2)

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, antraquinones 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. thypi* 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 *E. palmifolia*

Compounds	Reagent	Rf	Color of stains	Interpretation
Terpenoids	Anisaldehyde-sulfuric acid	0,63	Purple	Present
Alkaloids	Dragendorff	0,88	Orange	Present
Polyphenols	FeCl3	0,00	Black	Present
Antraquinones	KOH 10%	0,20	Purple red	Present
Flavonoids	Sulfuric acid 10%	0,93	Intensive yellow	Present

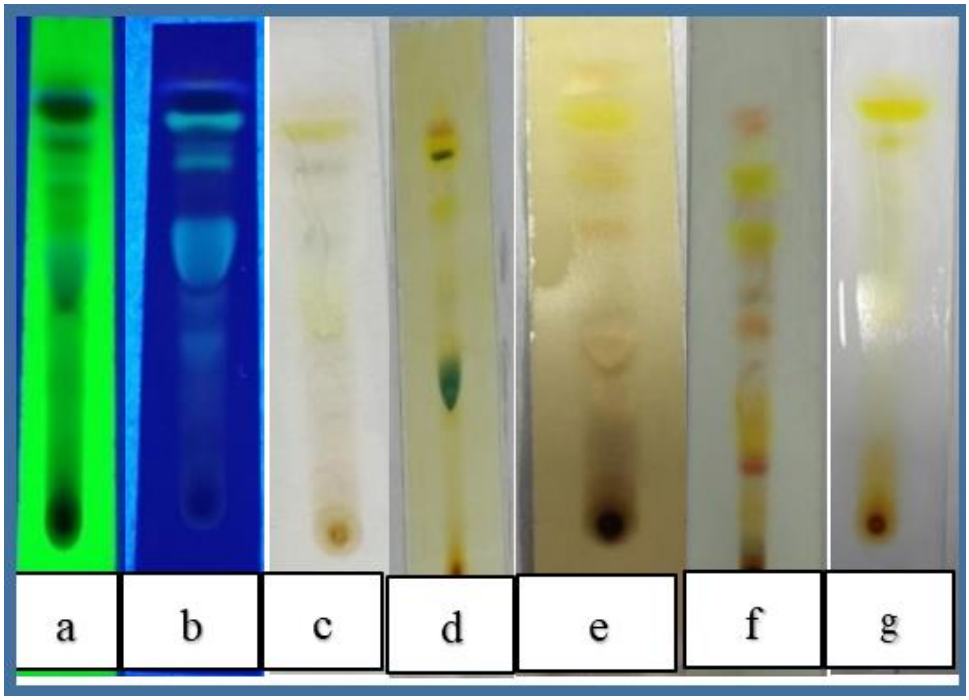

Figure 1. Chromatogram profile of ethyl acetate fraction of *E. palmifolia* separated over stationary phase of silica gel F_{254} by the mobile phase of ethyl acetate – chloroform, 3:7 (v/v), and visualized under (a) UV 254, (b) UV 365, as well as visible light after derivatization with (c) anisaldehyd-sulfuric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

Antibacterial activity of the ethyl acetate fraction of *E. palmifolia.* Compared to the positive control chloramphenicol, it showed an effectiveness of 55-62%. The antibacterial activity in the ethyl acetate fraction of *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

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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 fuction of the bacterial membrane, inhibiting the formation of biofilms, inhibition of cell envelope synthesis, inhibition of nucleic acid synthesis, inhibition of electrone 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

Sample concentration/ paper disc	Inhibition zone (mm)		Inhibition activity (%)	Antibacterial effecticness (%)
	Ethyl acetate fraction of *E. palmifolia*	Chloramphenicol		
4 mg	15.1 ± 3.6	-	152	55
6 mg	15.3 ± 3.3	-	155	56
8 mg	16.9 ± 1.9	-	182	62
15 µg	-	27.27 ±1.0	-	-

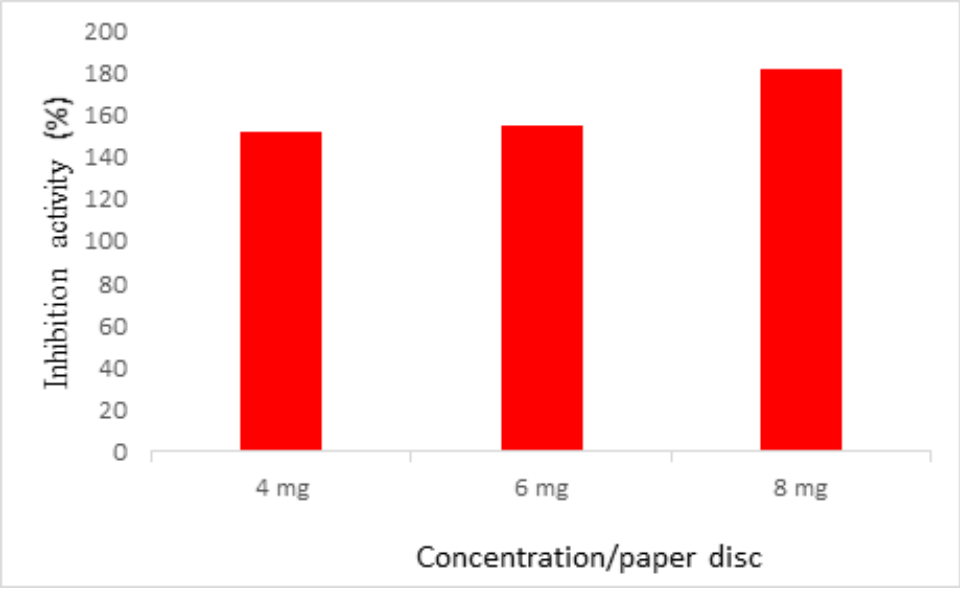

Figure 2. Inhibition activity of ethyl acetate fraction of *E. palmifolia* against the growth of *S. typhi*

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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.

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