

## RESEARCH ARTICLE

### Antibacterial activity test of dayak onions (*Eleutherine palmifolia* L. Merr.) ethanolic extract against *Shigella dysenteriae* ATCC 13313

Imam Adi Wicaksono<sup>1,3</sup>, Dudi Runadi<sup>2</sup>, Iman Firmansyah<sup>2</sup>

<sup>1</sup>Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia, <sup>2</sup>Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia, <sup>3</sup>Scientific Consortium of Drug Discovery and Development, Universitas Padjadjaran, Jatinangor, Indonesia

Correspondence to: Imam Adi Wicaksono, E-mail: imam.adi@unpad.ac.id

Received: December 22, 2017; Accepted: January 25, 2018

#### ABSTRACT

**Background:** Acute bloody diarrhea, which is also called dysentery, is damaged of the intestinal mucosa, sepsis, and malnutrition. *Shigella* bacteria cause 50% of cases of dysentery; it is due to its ability to produce shiga toxin that can cause dysentery. In traditional medicine, dayak onions are commonly used as antibacterial agent to various infectious diseases. **Aims and Objectives:** This study aims to determine the antibacterial activities of the ethanolic extract of dayak onions, determine the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against *Shigella dysenteriae*. **Materials and Methods:** The study was conducted through several processes: (i) Extraction of dried dayak onions and phytochemical screening of extract, (ii) the antibacterial activity test of ethanolic extract of dayak onions, and (iii) determination value of MIC and MBC by tube dilution method. **Results:** The result showed that ethanolic extract of dayak onions has antibacterial activity against *S. dysenteriae* bacteria. This extract has MIC and MBC in the concentration range of 12–14% (w/v). The antibacterial activity of this extract derived from alkaloids, monoterpenes and sesquiterpenes, steroids, flavonoids, polyphenols, and quinones. **Conclusion:** The results of this research from the present investigation suggest that the extract of the studied plants can be used as potential leads to discover new drugs to control diarrhea infection.

**KEY WORDS:** Acute Bloody Diarrhea; *Eleutherine palmifolia* L. Merr.; Antibacterial Activity, *Shigella dysenteriae*


#### INTRODUCTION

Diarrhea is a common disease affecting developing countries because it is a major cause of morbidity, mortality, and malnutrition in infants and children. Diarrhea is defined as a liquid or watery stool, increased volume, or frequency at least 3 times in a 24 h period.<sup>[1]</sup>

Acute diarrhea infection is a major cause of outpatient visits, hospitalization, and lost quality of life.<sup>[2]</sup> There are two

clinical types of acute diarrhea: (i) Simple diarrhea without blood and (ii) dysentery or bloody diarrhea. Dysentery or bloody diarrhea one of them caused by *Shigella*. *Shigella dysenteriae* is one of the most important etiologies of acute diarrhea infection and dysentery. *Shigella*-associated diarrhea occurs in 50% of cases of bloody diarrhea.<sup>[3-5]</sup> Etiology of acute diarrhea infection is commonly associated with other clinical features, suggesting enteric involvement including nausea, vomiting, abdominal pain and cramps, bloody stools, and some acute gastrointestinal infections.<sup>[6]</sup>

Antibiotics are usually used to treat infectious diarrhea. Some antibiotics used such as cotrimoxazole, ciprofloxacin, ampicillin, tetracycline, trimethoprim/sulfamethoxazole, nalidixic acid, or ceftriaxone are resistant to *Shigella* bacterial infections.<sup>[7]</sup> Antibiotics have many side effects include kidney problems, abnormal blood clotting, blood

Access this article online	
Website: <a href="http://www.njppp.com">www.njppp.com</a>	Quick Response code
DOI: 10.5455/njppp.2018.8.1248625012018	

National Journal of Physiology, Pharmacy and Pharmacology Online 2018. © 2018 Imam Adi Wicaksono, et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license.

disorders, and defenses.<sup>[4]</sup> Due to serious effects of synthetic drugs, required the development of new drugs to overcome diarrheal disease from medicinal plants.

Empirically, one of the medicinal plants used to treat diarrhea is dayak onions (*Eleutherine palmifolia* L. Merr.). Ethanolic extracts of dayak onions have antibacterial activity against some bacteria causing gastrointestinal infection such as *Escherichia coli* MTCC 1098, *S. dysenteriae*, *Shigella boydii*, *Salmonella typhimurium*, and *Shigella sonnei*.<sup>[8]</sup>

## MATERIALS AND METHODS

### Preparation of Plant Extract

The simplicia of the dayak onion, *E. palmifolia* L. Merr. species, was obtained Tasikmalaya, West Java, Indonesia. Determination of the simplicia was carried out in Taxonomy Laboratory, Biology Department, Faculty of Mathematics and Science, Universitas Padjadjaran.

### Chemical Materials

The chemical materials used consist of solvents and reagents. The solvents and reagents used are ethanol (product number: 159010, Merck, Germany), ammonia (product number: 10523, Merck, Germany), chloroform (Product number: 10245, Merck, Germany), hydrochloric acid (product number: 100317, Merck, Germany), iodide mercury (product number: 104428, Merck, Germany), potassium iodide (product number: 105043, Merck, Germany), magnesium powder (product number: 105815, Merck, Germany), amyl alcohol (product number: 100975, Merck, Germany), ferric chloride (product number: 103943, Merck, Germany), gelatin solution (product number: 104078, Merck, Germany), diethyl ether (product number: 100921, Merck, Germany), sodium hydroxide (product number: 106498, Merck, Germany), dimethyl sulfoxide (DMSO) (product number: 41640, Sigma-Aldrich, Germany), Mueller-Hinton Agar (CM0337, Oxoid, Basingstoke, UK), Mueller-Hinton Broth (CM0405, Oxoid, Basingstoke, UK), and distilled water.

### Extraction of Dayak Onions

The simplicia of dayak onions was extracted by maceration method using ethanol 96% for 24 h thrice. Then, the macerat was concentrated using rotary evaporator at 60°C and reassembled over the water bath at 50°C until a thick extract was obtained. The percentage of crude dry extract was determined as follows:

$$R \text{ extract (\%)} = \frac{w \text{ Crude Extract (g)}}{w \text{ Simplicia (g)}} \times 100\%$$

Where, R extract is the extraction yield, w crude extract is the crude extract mass, and then, w simplicia is the simplicia mass.

## Phytochemical Screening

Phytochemical qualitative screening of plants was conducted using phytochemical screening of plant method.<sup>[9]</sup> The crude ethanolic extract of dayak onions was tested for the presence and absence of various secondary metabolites.

## Antibacterial Activity Tested

Antibacterial activity tested by agar diffusion and perforation techniques. Clinical and laboratory standards Institute (CLSI) for the guideline this antibacterial activity method with some modification. The turbidity of each bacterial suspension was adjusted to 0.5 McFarland standards ( $1.5 \times 10^8$  CFU/mL). The extract (10 mg/mL) was dissolved with DMSO:water (1:9). 20 mL of the molten agar (45°C) was poured into sterile Petri dishes (diameter 90 mm). Bacteria suspensions overnight culture was prepared and 20 µL was evenly spread onto the surface of the agar plates of Mueller-Hinton agar (MHA) medium with sterile swab and left a few minutes to allow the bacteria to adapt with the medium. Once the plates were aseptically dried, the agar plates have been perforated using a perforator 9 mm. The soluble extracts (100 µL) were placed into the wells and the plates were incubated at 37 °C for 24 h. Then, measured the diameter of bacterial inhibition.<sup>[10]</sup>

## Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC test of extract was analyzed through broth dilution assay using a test tube. Sample solution for MIC made with some variation of the smallest concentration which still has activity. After dilution of the extract, added an overnight culture of perijunctional actomyosin ring using a micropipette, and then incubated at 37°C for 18–24 h. Then, from each MIC tube taken 10 µL, then put in MHA plates and spread over the surface of the MHA plate using sterile swab and incubated at 37°C for 18–24 h. MIC value obtained from the turbid tube leading to the clear tube, followed MBC value obtained from the MHA plates was no colony growth with a clear culture of the whole tube and incubated at 37°C for 18–24 h on MHA plates.<sup>[10]</sup>

## RESULTS

### Extraction of Dayak Onions

Maceration extraction from 1000.14 g of fresh dayak onions was obtained 55.33 g thick extract with rendement value 5.53%. The thick extract has viscous liquid and rubbery, brownish black, typical smell, and bitter taste.

### Phytochemical Analysis

Phytochemical analysis screening from the crude ethanolic extracts of dayak onions was tested for the presence of

alkaloids, flavonoids, polyphenols, monoterpenes and sesquiterpenes, steroids and triterpenoids, and quinones, as results shown in Table 1.

### Antibacterial Activity Tested

The result showed that the ethanolic extract of dayak onion had antibacterial activity against *S. dysenteriae* with concentration of 10% w/v, 20% w/v, 40% w/v, and 60% w/v as shown in Table 2.

### MIC and MBC

Determination of MIC and MBC values was performed using macrodilution or tube dilution method with various concentrations: 10% w/v, 12% w/v, 14% w/v, 16% w/v, 18% w/v, and 20% w/v. The result showed that this extract has MIC and MBC value of the extract in the concentration range of 12–14% (w/v) as shown in Table 3.

**Table 1: Phytochemical screening result**

Compound	Ethanolic extract
Alkaloids	+
Polyphenols	+
Flavonoids	+
Tannins	–
Monoterpenes and Sesquiterpenes	+
Steroid and Triterpenoid	+
Quinones	+
Saponins	–

(+): Detected, (–): Not detected

**Table 2: Antibacterial activity result**

Extract concentration (%w/v)	Inhibition diameter (cm)
60	1.539
40	1.166
20	0.935
10	–

(–): No inhibition

**Table 3: MIC and MBC results**

Extract concentration (% w/v)	Bacterial growth
20	–
18	–
16	–
14	–
12	+
10	+

+: Presence, –: Absence. MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

## DISCUSSION

In this research, extraction was performed using maceration method in an attempt to prevent loss of thermolabile compounds contained in dayak onion that usually being lost due to high temperatures during the process of extraction. Phytochemical qualitative screening from ethanolic extract contains such as flavonoid and polyphenols as shown in Table 1, which are phenolic substances in plants that have antimicrobial activity.<sup>[11]</sup> Related studies of antibacterial activity from crude extract containing flavonoids, triterpenes, and steroids against various bacteria.<sup>[12]</sup>

Antibacterial activity from the extract was used to determine the antibacterial ability of the sample qualitatively. The test was performed by diffusion method to use the perforation technique. The antibacterial activity of the extract can be observed from the diameter of the inhibition zone at around the whole perforation in the test and measured using calipers. The inhibition zone indicated that the extract can inhibit bacterial growth of *S. dysenteriae*.

MIC and MBC results can be seen from turbidity level of solution in tube. Determination of MIC aimed to find the lowest concentration of the extract that can inhibit bacterial growth, while MBC has no bacterial growth.

## CONCLUSION

The results of this study showed that the ethanolic extract of dayak onion (*E. palmifolia* L. Merr.) has antibacterial activity against *S. dysenteriae* starting at concentration 14% w/v. The extract of the studied plants can be used as potential leads to discover new drugs to control some diarrhea infection probably derive from flavonoids, polyphenols, steroids, and triterpenoids.

## REFERENCES

1. WHO. WHO Recommendations on the Management of Diarrhoea and Pneumonia in HIV-Infected Infants and Children: Integrated Management of Childhood Illness (IMCI). Geneva, Switzerland: World Health Organization Department of Child and Adolescent Health and Development (CAH) and HIV/AIDS; 2010. p. 3-6.
2. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States-undefined agents. *Emerg Infect Dis* 2011;17:16-22.
3. WHO. Guidelines for the Control of Shigellosis, Including Epidemics Due to *Shigella dysenteriae* I. Geneva, Switzerland: World Health Organization; 2005. p. 3-5.
4. Rajan S, Thirunalasundari T, Jeeva S. Anti-enteric bacterial activity and phytochemical analysis of the seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysenteriae* (Shiga, corrig.) Castellani and Chalmers. *Asian Pac J Trop Med* 2011;4:294-300.
5. Grouzard V, Rigal J, Sutton M. Clinical Guidelines-Diagnosis and Treatment Manual: For Curative Programmes in Hospitals

- and Dispensaries, Guidance for Prescribing. Paris, France: Medecins Sans Frontieres; 2016. p. 89-93.
6. Riddle MS, DuPont HL, Connor BA. ACG clinical guideline: Diagnosis, treatment, and prevention of acute diarrheal infections in adults. *Am J Gastroenterol* 2016;111:602-22.
  7. Poramathikul K, Bodhidatta L, Chiek S, Oransathid W, Ruekit S, Nobthai P, *et al.* Multidrug-resistant shigella infections in patients with Diarrhea, Cambodia, 2014-2015. *Emerg Infect Dis* 2016;22:1640-3.
  8. Padhi L, Panda SK. Antibacterial activity of *Eleutherine bulbosa* against multidrug-resistant bacteria. *J Acute Med* 2015;5:53-61.
  9. Farnsworth NR. Biological and phytochemical screening of plants. *J Pharm Sci* 1986;55:262-3.
  10. Dezsi S, Badarau AS, Bischin C, Vodnar DC, Silaghi-Dumitrescu R, Gheldiu AM, *et al.* Antimicrobial and antioxidant activities and phenolic profile of *Eucalyptus globulus* Labill. and *Corymbia ficifolia* (F. Muell.) K.D. Hill & L.A.S. Johnson Leaves. *J Mol* 2015;20:4720-34.
  11. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2006;26:343-56.
  12. Chattopadhyay D, Maiti K, Kundu AP, Chakraborty MS, Bhadra R, Maudal SC, *et al.* Antimicrobial activity of *Alstonia macrophylla*: A folklore of bay islands. *J Ethnopharmacol* 2001;77:9-55.

**How to cite this article:** Wicaksono IA, Runadi D, Firmansyah I. Antibacterial activity test of dayak onions (*Eleutherine palmifolia* L. Merr.) ethanolic extract against *Shigella dysenteriae* ATCC 13313. *Natl J Physiol Pharm Pharmacol* 2018;8(5):741-744.

**Source of Support:** Nil, **Conflict of Interest:** None declared