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Phytochemical Identification and Antibacterial Activity Test of *Leucas lavandulifolia* Against Escherichia *coli*

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Abstract

Diarrhea is a condition where the stool changes or becomes softer or more liquid with a frequency of 3 or 4 times a day. Based on its etiology, diarrhea is caused by several microorganisms, one of which is bacteria. The bacteria that cause diarrhea most often found in Indonesia is *Escherichia coli.* One type of plant that has the potential as an antibacterial is the *Leucas lavandulifolia* or Lenglengan plant. Based on ethnopharmaceutical studies, lenglengan leaves are commonly used to treat insomnia, pinworms, coughs with phlegm, epilepsy and digestive disorders. This research was carried out using lenglengan leaf ethanol extract with concentrations of 2.5%, 5%, 10%, 20% and 40%. In this study, phytochemical screening was also carried out using the TLC method. Antibacterial testing was carried out using the well diffusion method. The results obtained were the formation of a clear zone around the well, which indicated activity inhibiting bacterial growth. The results of testing antibacterial activity against *E. coli* showed that the ethanol extract of langlengan leaves at concentrations of 2.5%, 5%, 10%, 20% and 40% had antibacterial activity with clear zone results of 1.04 ± 0.33 , 12.71 ± 0.34, 13.90 ± 0.60, 15.23 ± 0.36, and 19.32 ± 0.79 mm. The results of the phytochemical screening showed that ethanol extract of lenglengan leaves contained alkaloids, saponins, flavonoids, terpenoids/steroids and polyphenols. It can be concluded, the ethanol extract of lenglengan leaves has antibacterial activity against *E. coli* where the greater sample concentration, the greater activity.

Keywords: antibacterial activity, *E. coli,* Lenglengan

Introduction

Diarrhea is defined as a condition where the stool changes or the stool becomes softer or more liquid with a frequency of 3 or 4 times a day (WHO, 2017). According to the Ministry of Health of the Republic of Indonesia (2018), the prevalence of diarrhea sufferers in 2017 in health facilities was 7,077,299 with 40% of them dying. Based on its etiology, diarrhea can be caused by several microorganisms, one of which is bacteria (Bankougo *et al*, 2013). The most common bacteria that causes diarrhea in Indonesia is *Escherichia coli* bacteria (Bakri *et al*, 2015). E. coli reportedly accounts for 24% of diarrhea cases in Indonesia (Halim *et al,* 2017).

Treatment of diarrhea caused by bacteria generally uses antibiotics (Knecht *et al,* 2014). Currently, the irrationality of antibiotic therapy in society has led to cases of antibiotic resistance, making diarrhea caused by bacteria more difficult to treat (Mustika, *et al* 2015). In addition, oral antibiotics such as amoxicillin and penicillin cause side effects for diarrhea sufferers such as abdominal pain, nausea, vomiting, drowsiness, dry mouth and dizziness. Therefore, alternative treatments for diarrhea caused by bacteria need to be explored further. Treatment of diarrhea caused by bacteria can be done by using medicinal plants that have antibacterial properties (Isnawati, *et al* 2019).

The Indonesian medicinal plants include the diversity of locations, cultivation and use of these plants. There are 1,000 recorded types of Indonesian medicinal plants and 350 species are generally used in society (Salim and Munadi, 2017(. Indonesian people's knowledge of medicinal plants comes from ethnopharmacological heritage (Gailea, *et al* 2016). Apart from relatively lower side effects, each medicinal plant can have various benefit (Suryaningsih, *et al* 2015). Lenglengan (*Leucas lavanduvolia*) is an Indonesian medicinal plant with various

properties, one of which is antibacterial (Das, *et al* 2015).

Based on ethnopharmacological studies, lenglengan is commonly used as a medicine to treat insomnia, pinworms, cough with phlegm, febrile seizures, epilepsy, diabetes mellitus, wound antiseptic, scabs, scabies, and digestive disorder (Makhija, *et al* 2011). The use of lenglengan has been reported in the traditional treatment of diarrhea carried out by the village community of Luo District in Kenya (Johns, *et al* 1990).

Several plants from the Lamiaceae family have been reported to have antibacterial activity. Kumar *et al*. (2016) reported the antibacterial activity of ethanol extract of L. cephalotes leaves (1 mg/mL) against *E. coli* with an inhibition zone diameter of 25.12 cm. Sarkar *et al*. (2013) reported the antibacterial activity of chloroform extract of L. indica leaves (50 mg/mL) against *E. coli* with an inhibition zone diameter of 14 cm and the results of phytochemical screening showed cardiac glycoside content. John De Britto *et al*. (2012) reported the antibacterial activity of methanol extract of *L. aspera* leaves (50 mg/mL) against *E. coli* with an inhibitory zone diameter of 8.7 cm and the results of phytochemical screening showed the content of alkaloids, phenols, flavonoids, saponins and tannins (John, *et al* 2012). Qureshi et al. (2010) reported the antibacterial activity of ethanol extract of *L. ciliata* leaves (500 µg/mL) against *E. coli* with an inhibitory zone diameter of 10.52 cm and the results of phytochemical screening showed flavonoid and saponin content. Based on the description above, this research will carry out phytochemical screening and test the antibacterial activity of lenglengan plants against *E. coli*.

Methods

The tools used in this research include a rotary evaporator (Heidolph 4000), autoclave (ALP), blender, magnetic stirrer, hot plate (UC-152), ose, porcelain cup, petri dish, maceration jar, Büchner funnel, filter paper, analytical balance (Sartorius), incubator (18-ONE SIC 50L), vortex (Heildolph), oven, metal spatula, tweezers, caliper (TRICLE BRAND), micropipette 10 µL, 100 µL, 1000 µL (Eppendorf), laminar air flow (Airtech), pipettes (drop and volume), tips (blue, yellow, and white), and a set of glassware.

The materials used in this research include lenglengan leaf simplicia obtained from Materia Medika Batu, 96% ethanol, DMSO (Merck), 0.9% NaCl, gentamicin, distilled water, physiological NaCl, *Escherichia coli* ATCC 25922 bacteria, Mueller Hinton Agar media (Merck), F254 silica gel plates. , glacial acetic acid (CH3COOH), iron (III) chloride (FeCl3), chloroform (CHCl3), butanol (C4H9OH), potassium hydroxide (KOH), sulfuric acid (H2SO4) pa, vanillin-sulfuric acid, anisaldehyde-sulfuric acid, dragendorf, and ammonium hydroxide (NH4OH).

Extracts Preparation

100 grams of lenglengan leaf powder was macerated with ethanol solvent (1:5) and remacerated 2 times. The macerate was filtered using a Büchner funnel. Then, the macerate is concentrated using a rotary evaporator. The thick extract is oven at 40ºC until a dry extract is obtained.

Phytochemical Screening

20 mg of the extract was dissolved in 2 mL of ethanol, then phytochemical screening was carried out using the following procedure for alkaloids, flavonoids, polyphenols, terpenoids, steroids, saponins (Harborne, 1973).

Preparation of Test and Control Solutions

10% DMSO as a negative control was made with a certain volume of 100% DMSO diluted using sterile distilled water. Gentamicin 0.005% as a positive control was made with a certain amount of 40 mg/mL gentamicin diluted using sterile distilled water. A certain amount of lenglengan leaf ethanol extract was dissolved in 10% DMSO and diluted to obtain concentrations: 2.5%, 5% 10%, 20% and 40%.

Antibacterial Activity Test Well Diffusion Method

The reference for the antibacterial activity test procedure used in this research is the standard protocol (Balouri, *et al* 2015). MHA media in a petri dish was inoculated with 20 µL of *E. coli* bacterial suspension which had been standardized with Mc Farland and spread evenly with a spreader. The media was perforated with a well with a diameter of 8 mm, then 15 µL of the test solution (extract, negative control, positive control) was pipetted and inserted into the well of the MHA media which already contained bacteria. The diameter of the inhibition zone formed after incubation at 37ºC for 18 hours was measured with a caliper. The antibacterial activity test treatment was carried out 3 times for each test concentration. Data was taken to measure the diameter of the inhibitory zone from 3 sides, namely the diameter of the clear zone which appears to be the largest, the diameter of the clear zone which appears to be the most moderate and the diameter of the clear zone which appears to be the smallest.

Statistical analysis

Statistical analysis was carried out using the One Way ANOVA and LSD test.

Results

Antibacterial testing for all test solutions was carried out using the well diffusion method. The results obtained were the formation of a clear zone around the well hole which indicated the presence of activity to inhibit bacterial growth. The results of the antibacterial activity test against E. coli (Table 1) showed that the ethanol extract of langlengan leaves had antibacterial activity with clear zone results of 1.04 ± 0.33, 12.71 ± 0.34, 13.90 ± 0.60, 15.23 ± 0.36, and 19.32 ± 0.79 mm respectively and the higher the concentration, the greater the activity.

Phytochemical screening conducted in this study aims to determine the content of secondary metabolites contained in the ethanol extract of lenglengan leaves. The screening results using the TLC test (Table 2). Based on Table 2, the secondary metabolites that were positively detected in the ethanol extract of lenglengan leaves include alkaloids, saponins, flavonoids, terpenoids/steroids and polyphenols.

Table 1. Antibacterial activity results

Groups test (%b/v)	Mean of inhibition zone diameter (mm± SD)
2.5	11,04±0,33[a]
5	12,71±0,34[b]
10	13,90±0,60[c]
20	15,23±0,36[d]
40	19,32±0,79[e]
Control (+)	28,40±0,60[f]
Control (-)	00, 00±0,00

Different letter notations indicate significant differences between test groups based on One Way ANOVA and LSD test ($p<0.05$).

Table 2. Screening phytochemical compounds results

Secondary metabolite	Method	Positive Results	Results
Flavonoid	Ammonia test	intensive yellow stain	(+)
Pholiphenol	Ferric chloride	intensive black stain	(+)
Terpenoide/ Steroid	Anisaldehide-sulfuric acid	Purple stain	(+)
Alkaloid	Dragendorf	Orange stain	(+)
Saponin	Foam test	Stable foam for 3 minutes, 30 cm	(+)

Discussion

Based on data on the diameter of the inhibition zone produced, it is known that the concentration of lenglengan leaf ethanol extract of 40% w/v is the concentration that produces the greatest antibacterial activity. Then, the smaller the concentration of lenglengan leaf ethanol extract used, the smaller the inhibition zone produced. The results of the positive control test provide information that *E. coli* bacteria are still sensitive to the antibiotic gentamicin (CLSA, 2013). Meanwhile, the results of the negative control test showed that there was no antibacterial activity that could affect the ability of the test extract.

After the data results were obtained, statistical analysis was carried out using the One-Way ANOVA test. The results of the normality and homogeneity tests show a significance value of $p>0.05$, meaning that the data used is normally distributed and homogeneous. Apart from that, the results of the One Way ANOVA and LSD tests obtained $p=0.000$, meaning that there was a significant difference in the diameter of the inhibition zone produced by concentrations of 2.5, 5, 10, 20, 40% w/v lenglengan leaf ethanol extract, control negative DMSO 10% v/v, and positive control gentamicin 0.005%. From the test results, it is known that the greater the concentration of lenglengan leaf ethanol extract used, the greater the antibacterial activity produced. Therefore, a hypothesis is obtained that there is the more secondary metabolites contained in the extract.

The ethanol extract of lenglengan leaves showed positive results for alkaloids, saponins, terpenoids, flavonoids and polyphenols. Based on Table 2, positive secondary metabolites were detected in the ethanol extract of lenglengan leaves. These include alkaloids, saponins, flavonoids, terpenoids/steroids and polyphenols. This statement is strengthened by the close chemotaxonomic study of the same leucas genus, namely Leucas glabrata, containing mentone, piperiton, and pulegon which have antibacterial activity against gram-positive, gram-negative bacteria, and fungi (Vagionas, *et al* 2007). So, it can be assumed that lenglengan (*Leucas Lavandulifolia*) contains the same active compounds and has antibacterial activity against *E. coli* bacteria which are gram negative.

The ethanol extract of lenglengan leaves has the potential to have antibacterial activity against *E. coli* bacteria. This activity is thought to be caused by secondary metabolites contained in the ethanol extract of lenglengan leaves, such as flavonoids, terpenoids/free steroids, saponins, polyphenols and alkaloids which can have an antibacterial effect.

The antibacterial mechanism of flavonoid secondary metabolites can effectively restrain the formation of complexes by microorganisms with extracellular proteins and bacterial cell walls. Terpenoids or steroids can freely destroy lipid membranes to form leaks in liposomes (Mujeeb, *et al* 2014). Apart from that, saponins will interact with lipid A in gram-negative bacterial liposaccharides to disrupt the membrane permeability of bacterial cells (Arabski, *et al* 2012), polyphenols can play a role in inhibiting hydrolytic enzymes (protease and carbohydrolase) or other interactions that can inactivate adhesins and interactions. non-specific with carbohydrates (Karaou, *et al* 2005), and alkaloids have antibacterial mechanisms as DNA interchelators that can inhibit the topoisomerase enzyme (Karaou, *et al* 2006).

Conclusion

It can be concluded that the ethanol extract of lenglengan leaves has antibacterial activity against *E.coli.* The phytochemical screening showed that the ethanol extract of lenglengan leaves contained alkaloids, saponins, flavonoids, terpenoids/steroids and polyphenols.

Conflict of Interest

"No potential competing interest was reported by the authors".

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