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Antibacterial Activity of *Ocimum citriodorum* Leave Extracts Against *Shigella dysenteriae*

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ABSTRACT

Dysentery is a disease caused by infection of Shigella dysenteriae *(S. dysenteriae*). This type of acute diarrhea characterized by the liquid stool mixed with blood and mucus caused by these bacteria. *Ocimum citriodorum* (*O. citriodorum*) leaves contain various chemical compounds that can inhibit the growth of diarrhea-causing bacteria. This study aims to determine the antibacterial activity of *O. citriodorum* leaves extracts obtained from graded extraction method against *S. dysenteriae*. The *O. citriodorum* leaves were extracted using ethanol, ethyl acetate, and n-hexane solvents sequentially. The Minimum Inhibitory Concentration (MIC) test was carried out using dilution method. The positive control used was 10 ppm ciprofloxacin (0.002%). The results showed that the extract of n-hexane, ethyl acetate, and ethanol of *O. citriodorum* leaves all three have antibacterial activity against S. dysenteriae with the same MIC value of 15%. The most effective MIC of n-hexane, ethyl acetate, and ethanol extract of *O. citriodorum l*eaves was found at a concentration of 15% zone inhinbition with ethanol extract of *O. citriodorum* leaves.

Keywords: *Ocimum citriodorum* leaves, *Shigella dysenteriae*, antibacterial

INTRODUCTION

Dysentery is one type of acute diarrhea be seen from the liquid stool mixed with blood and mucus caused by bacteria as *Shigella dysenteriae* or *S. dysenteriae* (Baker & The, 2018). The solution to treat diarrhea can be used by several traditional medicinal plants known in the community, one of which is *Ocimum citriodorum* (*O. citriodorum*) leaves (Radha, 2021). The results of the study at a concentration of 10% showed zone of inhibition 18.88 mm. In total, the *O. citriodorum* samples showed the presence of seven caffeic acid and derivatives (dimers, trimers, and tetramers) and five flavonoids, mainly glycoside derivatives of quercetin (Majdi et al, 2020). According to Tan et al. (2022) that flavonoids could exert antibacterial activity via damaging the cytoplasmic membrane, inhibiting energy metabolism, and inhibiting the synthesis of nucleic acids, so flavonoids are considered constitutive antibacterial substances. Maceration a very simple extraction method with the disadvantage of long extraction time and low extraction efficiency. It could be used for the extraction of thermolabile components (Hidayat & Wulandar, 2021). The use of the graded maceration method aims to extract all compounds based on the polarity of the solvent used in general. According to Ullah, *et al.* (2020), flavonoids content of *O. sanctum* and *O. basilicum* were 201 mg and 203 mg quercetin QE/100g of extract. The graded maceration method can produce higher quality extracts

compared to the non-graded maceration method because all chemical compounds in plant sample can be fractionated based on the polarity of the solvent used. Extraction, is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Extraction itself may be performed by repeated maceration with agitation, perculation or by continuous extraction e.g.in a Soxhlet extraction (Khalaf, 2021). The n-hexane, ethyl acetate, and methanol solvents will attract non-polar, semi-polar, and polar compounds without any interference being extracted from other group compounds. The purpose of this study was to determine the antibacterial activity of n-hexane, ethyl acetate, and ethanol extract of *O. citriodorum* leaves against *S. dysenteriae* bacteria using graded maceration extraction.

METHODS

O. citriodorum Leaf Extracts

Extraction was carried out by graded maceration methods whereas 500 g of *O. citriodorum* leaves powder were soaked in a series of organic solvents with increasing polarity (n-hexane, ethyl acetate, and ethanol 96%) for 3 x 24 hours and in 5000 mL of each solvent. The first maceration was done by soaking the leaf powder with 2000 mL of n-hexane for 24 hours while stirring occasionally, after 24 hours it was separated from the filtrate, then continued the maceration process with 1500 mL of the remaining n-hexane solvent (24 hours). Finally, the last 24 hours were carried out with 1500 mL of solvent. Then the residue was separated from the filtrate. These steps were repeated using ethyl acetate and ethanol 96%. The filtrate of each solvent was collected and evaporated using vacuum dry to obtain a thick extract, then calculated for its yield value. Furthermore, each of the extract was tested qualitatively for alkaloids, flavonoids, saponins and tannins.

Powder and Extract Phytochemical Tests

Alkaloid Test

The sample was weighed as much as 0.5 g, added with 1 mL of 2N HCl and 9 mL of water then heated for 15 minutes, and filtered after it was cold. The solution was dripped on a watch glass, and each added with Dragendorff, Mayer and Buchardatt reagent. The presence of alkaloids is indicated by an orange, brown, and white precipitate in the Dragendorff, Bouchardat, and Mayer reagents (Shaikh & Pati. 2020).

Flavonoid Test

The sample is weighed as much as 0.5 g and dissolved in 5 mL of distilled water and then evaporated to dryness, then added 2-3 drops of ethanol. Then add Mg powder and a few drops of 5 M HCl. The red to violet red color that appears indicates the presence of flavonones, flavonols, flavanonols, and dihydroflavonols. Then the test is carried out as above but Zn is added, the appearance of a red color indicates the presence of dihydroflavonol (Shaikh & Pati, 2020).

Saponin Test

The sample is weighed as much as 0.5 g and then shaken with 10 mL of water to produce stable foam with the addition of HCl (Shaikh & Pati, 2020).

Tannin Test

The sample is weighed as much as 2 g and dissolved with 10 mL of hot water and then shaken until homogeneous. There are two ways to test for the presence of tannins, the first is after cold plus 3% $FeCl_3$ a positive result is shown if a green-blue-black color is formed. The second is the addition of a 10% gelatin solution, a positive result if a white precipitate appears (Shaikh & Pati, 2020).

Determination of Minimum Inhibitory Concentration (MIC)

Determination of MIC against *S. dysenteriae* was performed using the dilution method. In this study, 1 mL of extract at the test concentration (2.5%; 5%; 10%; and 15%), 15 mL of Nutrient

Agar (NA) medium, and 0.2 mL of bacteria culture were added into Petri dishes, after which the samples were incubated at 37ºC. After 24 hours of incubation, bacterial growth was observed. The smallest concentration that was not overgrown with the bacteria was interpreted as the MIC (Farha *et al.*, 2019).

Determination of zone of inhibition

The determination of zone of inhibition was carried out by the diffusion method using Disk Diffusion Antibiotic Sensitivity test (The Kirby-Bauer test). The sterilized media was poured into a petri dish aseptically, then 0.2 mL of *S. dysentriae* bacteria were inoculated into a medium equivalent to Mc. Farland 0.5. The sterilized disc paper was filled with extracts at various concentration series (15, 25, and 30%), the positive control 0.002% (10 ppm) of ciprofloxacin, and negative control (10% DMSO). Then each was incubated at 37ºC for 24 hours. The determination of zone of inhibition was repeated 3 times. After 24 hours, the clear area formed in the petri dish (Balouiri *et al.*, 2016) was measured using a caliper with an accuracy of 0.05 mm and compared between the clear area of the *O. citriodorum* leaf extract test disc, positive control ciprofloxacin, and negative control 10% DMSO which aims to determine the sensitivity of microbes to *O. citriodorum* leaf extract.

Data Analysis

The resulted data of inhibition zone diameter was analyzed was to determine the differences between treatments, and the observation data were processed statistically with a Completely Randomized Design method of Factorial Patterns in the IBM® SPSS® Statistic 24 for Windows program. The treatment of 3 extracts with various concentrations were replicated 3 times.

RESULTS AND DISCUSSION

As much as 5 kg of wet *O. citriodorum* leaves was sorted and dried. The dry plant obtained was 1.2 kg. The dried *O. citriodorum* leaves were mashed using blender and sieved using a 40 mesh. The total dry powder of *O. citriodorum* leaves obtained was 530 g.

Figure 1. *O. citriodorum* Leaf Powder

The dried *O. citriodorum* leaves were extracted with graded maceration using three different solvent. The characteristic of *O. citriodorum* leaves extract was shown in Table 1.

Table 1. The Result of Ash and Moisture Content of *O. citriodorum* Leaves Extracts From Different Solvent

Extract	Ash content (%)	Moisture Content (%)	Yield (%)
Ethanol 96%	3.8541	5.9776	14.07
Ethyl Acetate	2.9309	2.8462	13.61
n-hexane	2.7653	4.6731	11.90

Based on the research results, ethanol and ethyl acetate extract of *O. citriodorum* leaves contain compounds such as alkaloids, flavonoids, tannins, and saponins. Phytochemical test results of plant sample and extracts can be seen in Table 2.

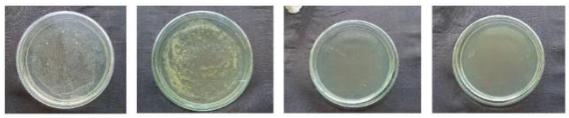
Table 2. The Phytochemical Compounds of *O. citriodorum* Leaves Extracts

Compounds		Extract		
		Ethanol	Ethyl acetate	n-hexane
Alkaloids	+	+	+	+
Flavonoids	+	+	+	-
Tannins	+	+	+	-
Saponins	+	+	+	-

Note : (+) Contains compounds , (-) Does not contain compounds

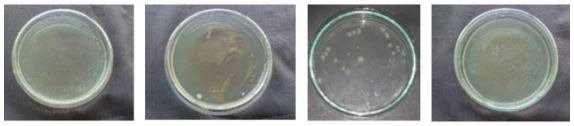
The Minimum Inhibitory Concentration

The dilution method to perform the Minimum Inhibitory Concentration (MIC) was carried out in order to determine the lowest level that could inhibit the growth of certain microorganisms (Flanagan and Steck, 2017). The results of the research on the minimum inhibitory concentration of ethanol 96%, ethyl acetate, n-hexane extract can be seen in Figures 2, 3, and 4.



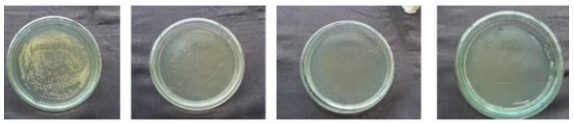
2,5% 5% 10% 15%

Figure 2. The MIC of ethanol 96% extract of O. citriodorum leaves against *S. dysentriae* was present at a concentration of 15%



2,5% 5% 10% 15%

Figure 3. The MIC of ethyl acetate extract of *O. citriodorum* leaves against *S.dysentriae* was present at a concentration of 15%



2,5% 5% 10% 15%

Figure 4. The MIC of n-hexane extract of *O. citriodorum* leaves against *S.dysentriae* was present at concentration of 15%

Based on these results, it can be shown that ethanol extract, ethyl acetate, and n-hexan at a concentration of 15% can inhibit the growth of *S. dysentriae*, this is supported by the previous reserach of Tortora, *et al.* (2010).

The Diameter of Inhibition Zone

The diameter of inhibition zone is a circular area around the disk which contain O. citriodorum extract in which the *S. dysentriae* colonies do not grow. The zone of inhibition diameter can be used to measure the susceptibility of the bacteria (Balouiri, et al., 2016). The concentrations of extract used in each disk were 15%, 25%, and 30% and the positive control used was ciprofloxacin.

Table 3. The Diameter of Inhibition Zone of *O. citriodorum* Leaf Extracts Against *S. dysentriae*

Extract	Concentration (%)	Zone of inhibition (mm)	Category
ethanol 96%	15	8,50±0,05^{f}	medium
	25	9,16±0,51^{g}	medium
	30	9,83±1,00^{h}	medium
	K+	14,00±0,19^{j}	strong
	K-	0	-
ethyl Acetate	15	6.66±0.19^{d}	medium
	25	7.00±0.19^{e}	medium
	30	9.00±0.05^{f}	medium
	K+	13.66±0.19^{j}	strong
	K-	0	-
n-hexane	15	5.66±0.51^{b}	medium
	25	5.83±0.51^{b}	medium
	30	6.33±0.05^{c}	medium
	K+	13.16±0.05^{i}	strong
	K-	0	-

Remarks: (Bhargav *et al.*, 2016)

The results showed that the higher concentration of *O. citriodorum* leaf extract, the greater inhibition zone that would form around the disk. There are the differences in the inhibition zone diameter value resulting from each extract of different solvents (Table 3 and Figure 5,6, & 7). The most effective extracts that inhibit bacteria was extracts using ethanol 96% as a solvent compared to ethyl acetate and n-hexane solvents. The inhibition diameter indicates the in each concentration indicated the inhibitory power of a active compounds contained in the extracs. The inhibition can be seen from the clear zone formed around the disc paper which contains plant extract at various concentrations. If the antibiotic is stated to be effective against the bacteria at a particular concentration, the bacteria will not grow when the concentration of the agar at that point is more than the effective concentration. This region of no bacterial growth is called the Zone of Inhibition (Bhargav et. al., 2016).

According to WHO (2016) infections caused by *S. dysenteriae* can be cured by the antibiotic ciprofloxacin which shortens the duration of symptoms of 96%. This is in accordance with research according to Lankeshwar and Bagde (2013) in the present investigation mechanism of ciprofloxacin (Cp) resistance in *Shigella dysenteriae* was studied.

Flavonoid is a secondary metabolite that inhibit the growth of bacteria. After the phytochemical screening, the *O. citriodorum* leaf extract contains flavonoids. This compound can inhibit the growth of *S. dysentriae* bacteria causing by damage of membrane cell and inhibiting the synthesis of macro molecules cell in *S. dysentriae* bacteria. Flavonoids contain a phenol compound which is acidic alcohol that is also called carbolic acid. The presence of phenol in acidic conditions can affect the growth of microorganisms (Yahiaoui et al., 2016).

Alkaloids are a group of secondary plants that can be dissolved and effective in non-polar solvents. Alkaloids have the ability antibacterial. Alkaloids provided the underlying structure for the development of several antibiotics with a diverse range of action (Othman et al., 2019). The mechanism that thought to be able to inhibit bacterial growth by interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed and causes cell death.

Saponins are secondary metabolites that have a bitter taste and foamy in water. Saponin tuber extracts of *Cyclamen* could be used as a moderate to strong antimicrobial substance against gram-negative bacteria (Saboora et al., 2019). The mechanism of action of saponins as antibacterial is by causing leakage of proteins and enzymes in bacterial cells (Madduluri et al, 2013). Saponins can reduce the surface tension of the cell walls to suppress bacterial growth. Saponins will interfere with the surface tension of cells, when the surface tension is disturbed, antibacterial substances will easily enter the cells and interfere with metabolism which causes cell death. Saponin compounds can damage the cytoplasmic membrane of bacteria. Saponins found in *Morinda citrifolia* leaf extract have very important functions that can be used as antibacterials (Pertiwi et al., 2019).

Tannins are secondary metabolites that have chelating properties. Tannins are astringent (substances that can shrink). The results showed that the tannins isolated from the extract of *S. baccatum* can be used as a natural bactericide to control bacterial wilt in tomatoes (Vu et al., 2017).

The highest zone of inhibition test results were shown in the ethanol extract at a concentration of 30% with a result of 9.83mm against *S. dysentriae*. *Escherichia coli*is one of the main causes of diarrhea. Khalil's research (2013) stated that the test result of *O. citriodorum* leaf extracts a stronger inhibitory power against *E. coli* compared to *Staphylococcus aureus*.

The inhibition zone data variable in this study use SPSS 20 analysis with ANOVA test. Based on the test result with some factors, that is concentrations extract and interactions between the two there is a p-value or sig. of 0.00 which is less than 0.05 based on the decision criteria means that reject H0 accept H1, then there is a significant influence between the extract factor, the concentration factor and the interaction

between the two on the width of the inhibitory power. In Duncan's further test namely the difference in solvents for 96% ethanol extract, ethyl acetate, and n-Hexane, it was stated that all extracts gave the same effect on the width inhibition zone, while Duncan's further test with different concentrations stated that all concentration has a different effect on the width of the inhibitory power, this is because of the higher concentration used, the antibacterial will produce greater power. In Duncan's further test on the interactions that it was stated that the interaction of ethanol extract, ethyl acetate, and n-hexane in the negative control gave the same effect on the width of the inhibitory power. In other interactions give a different effect. In the interaction of ethanol 96%, ethyl acetate, and n-hexane with extract concentrations of 15%, 25%, 30% give the same effect on the width inhibition zone. While the other interactions have different effects.

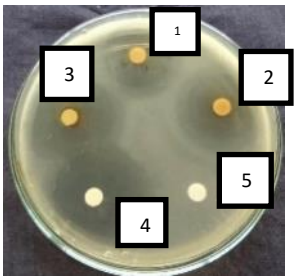


Figure 5. Antibacterial activity of ethanol extract 96% of *O. citriodorum* leaves against *S. dysentriae*. Number 1, 2, 3, 4, and 5 is 15%, 25%, 30% extract concentrations, positive control, and blank (DMSO)

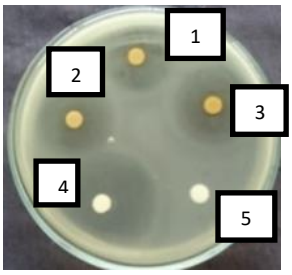


Figure 6. Antibacterial activity of ethyl acetate extract of *O. citriodorum* leaves against *S.dysentriae*. Number 1, 2, 3, 4, and 5 is 15%, 25%, 30% extract concentrations, positive control, and blank

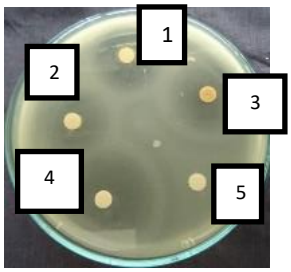


Figure 7. Antibacterial activity of n-hexane extract of *O. citriodorum* leaves against *S. dysentriae*. Number 1, 2, 3, 4, and 5 is 15%, 25%, 30% extract concentrations, positive control, and blank

CONCLUSIONS

1. *O. citriodorum* leaf extracts of ethanol 96%, ethyl acetate, and n-hexane have antibacterial activity against *S. dysenteriae*.
2. The most effective antibacterial activity of *O. citriodoru*m leaves inhibit S. *dysenteriae* 9.83 mm was the ethanol extract with the concentration of 30%.

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