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Antimicrobial Activity of Moringa Leaf Infusion on *Escherichia coli* Isolate from *Musca domestica* L.

Maria Kanan [1]*

[1]Department of Public Health, Tompotika Luwuk Banggai University, Jl. Dewi Sartika, 94715, Indonesia.

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Abstract

The *Moringa oleifera* can be used as a medium for the prevention and treatment of infectious diseases such as diarrhea. Diarrhea can be caused by *Escherichia coli*, which house flies can transmit. *Moringa oleifera* leaves have antimicrobial substances such as flavonoids, triterpenoids, steroids, saponins and tannins. This aim of the study was to determine the minimum inhibitory concentration of *M. oleifera* infusion on the growth of *E. coli*. This research is a laboratory experiment with Well Diffusion Agar method and dilution technique. The materials used in the study were *M. oleifera* leaf infusion with concentrations of 50%, 40%, 30%, 20%, and 10%, while 0% was a negative control (Aquadest). *E. coli* isolates were obtained from *M. domestica*. The results showed that the inhibition zone formed at concentrations of 50%, 40%, 30%, 20%, 10%, and 0% was 15.55 mm, 13.80 mm, 13.10 mm, 10.10 mm, and 0 mm, respectively. The minimum inhibitory concentration of leaf infusion against *E. coli* isolates was 25%, and the minimum bactericidal concentration could not be determined because all concentrations tested showed bacterial growth. Instead, the lowest inhibitory concentration was 25%. In concentrations of 50%, 40%, and 30%, *M. oleifera* leaf infusion has potent antibacterial action; at concentrations of 20%, it has moderate antimicrobial activity; and at concentrations of 10%, it is unable to inhibit the growth of *E. coli*. Further research is needed to determine the value of the minimum bactericidal concentration at concentrations above 25%, and it is necessary to test it on other digestive tract pathogenic bacteria.

INTRODUCTION

Infectious diseases are one of the major public health problems in the world (Nii-Trebi, 2017), including Indonesia (Ear, 2012; Burgos & Ear, 2015). Infectious disease is a state of entry of microorganisms into the body, then multiply and cause disease (Fierer *et al*. 2010). In general, infectious diseases such as diarrhea, pneumonia, measles, malaria and malnutrition are the main causes of death in children (Caulfield *et al*. 2004; Strong *et al*. 2021). The diarrheal disease can be caused by *Escherichia coli* (Zhou *et al*. 2021; Khan *et al*. 2022); these bacteria are normal in the intestine but are not normally pathogenic (Sarowska *et al*. 2019).

Flies are endophilic synanthropic insects that dwell in human settlements and live in human habitation (Yin *et al*. 2022). Synanthropic flies can carry pathogens that cause serious human diseases (Khamesipour *et al*. 2018), one of them is the *Musca domestica* (Issa, 2019). The activity of *M. domestica*, which lands on dirty substrates, causes the fly's body to carry various types of bacteria and transfer them to food (Rofieq *et al*. 2020). *M. domestica* flies are

*Coresponding Author: mariakanan829@gmail.com
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known to act as mechanical vectors of pathogenic bacteria, including *E. coli* (Lindeberg *et al*. 2018; Chandrakar *et al*. 2022). The prevalence of diarrhea may rise if *M. domestica* fly populations rise (Khamesipour *et al*. 2018). Nazari *et al*. (2017) showed that there were 394 bacterial strains isolated from *M. domestica*, which were dominated by Bacillus sp. (31.1%), Staphylococcus spp. (22.9%), and *E. coli* (11.6%). One of the efforts to control the diarrheal disease is by controlling the population of *E. coli* (Karimi *et al*. 2018).

M. oleifera leaves can be used as an antibacterial because they contain various compounds that can inhibit bacterial growth (Mangundayao & Yasurin, 2017; Sharma *et al*. 2020; Khan *et al*. 2021). Additionally, *M. oleifera* leaf infusion can be act as an antioxidant (Yuliani & Dienina, 2015) because the leaves contain active components such as flavonoids, saponins, tannins and triterpenoids (Sopandani *et al*. 2020; Arifan *et al*. 2021). The study by Moyo *et al*. (2014) showed that the acetone extract of *M. oleifera* leaves at a concentration of 5 mg/ml showed antibacterial activity against *E. coli*. In addition, Bukar *et al*. (2010) showed that the ethanol extract of *M. oleifera* leaves exhibited broad-spectrum activity against *E. coli*, with minimum inhibitory concentration values ranging between 2.0 and >4.0 mg/ml.

Study on the ability of *M. oleifera* leaves in the form of infusion to inhibit the growth of *E. coli* is still limited. Research using *M. oleifera* leaves mostly done only in the form of extracts using the maceration method. Therefore, the aim of this study was to determine the antimicrobial activity of *M. oleifera* leaf infusion on the growth of *E. coli* isolates from the body surface of *M. domestica*.

MATERIALS AND METHODS

This study was used *E. coli* isolates from *M. domestica* flies, physiological NaCl (0.9%), Eosin Methylene Blue (Oxoid CM0069) media, Nutrient Agar, and Aquadest.

Isolation and Purification of *E. coli*

E. coli were isolated from *M. domestica* that collected from landfills using Eosin Methylene Blue Agar medium. The growth of *E. coli* colonies was shown in metallic green with a metallic lustre and a black dot in the colony's center. Then it was purified several times until a pure culture was obtained and grown on NA media in a test tube. Furthermore, Gram staining was performed.

Preparation of *M. oleifera* infusion

The extraction method used was the infusion method with water as a solvent. Simplisia leaves of *M. oleifera* were mashed and weighed as much as 100 grams, diluted with 200 ml of distilled water, and then heated over a water bath at 90°C for 15 minutes while stirring occasionally. The infusion was sprinkled hot with a flannel cloth using a funnel. The infusion obtained was a concentration of 50%. Furthermore, dilution was carried out with concentrations of 50%, 40%, 30%, 20%, and 10%.

Testing of *M. oleifera* infusion against *E. coli*

a. Well Diffusion agar Method
   Testing the inhibition power of *M. oleifera* leaf infusion was carried out by diffusion on NA media. The *E. coli* isolate was diluted with 0.9% NaCl until it had a standard McFarland turbidity of 0.5. Then 1 ml was put into a petri dish, homogenized with NA media by pour plate method, then 6 wells were made with a diameter of 8 mm. *M. oleifera* leaf infusion was filled into the wellbore according to the concentration tested (6 well bodies), there are 50%, 40%, 30%, 20%, 10%, and aquadest as a negative control. Furthermore, the media was incubated at 37 °C for 24 hours (Valgas *et al*. 2007). The diameter of the inhibition zone was analyzed following the formula from Warbung *et al*. (2013).
b. Stratified Dilution Method
   Dilution was carried out until the following concentrations were obtained: 50%, 25%, 12.5%, 6.25%. Then 1 ml of the test bacterial suspension was added using a 0.5 Mc Farland standard and incubated at 37°C for 24 hours. The final concentration were: 25%, 12.5%, 6.25%, and 3.125%.

A cloudy-looking tube indicates bacterial growth and a clear-looking tube indicates no bacterial growth. The last tube that looks clear is the MIC value. The MIC values are defined as concentrations that exhibit a reduction in bacterial growth, whereas the MBC values are defined as concentrations that exhibit no bacterial growth.

RESULTS AND DISCUSSION

Isolation and identification of *E. coli* from *M. domestica*

Isolation of *E. coli* from the body surface of flies was carried out by the isolation method using EMBA media as a selective medium. The EMBA media showed the growth of metallic green bacterial colonies (Figure 1), characteristic of *E. coli*. From the gram staining results, the bacteria morphology in

the form of short rods was obtained with a red gram staining reaction, indicating gram-negative bacterial colonies.

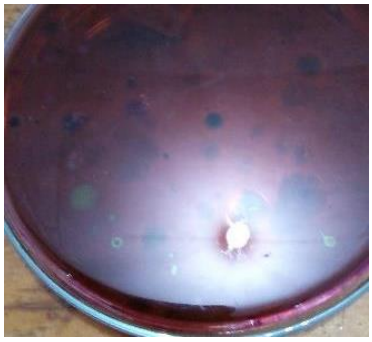

Figure 1. Growth of bacterial colonies on EMBA media

Based on the isolation and identification of bacteria from flies' body surfaces on EMBA media, various colonies were obtained. One has a metallic green colony with a metallic sheen, with a black dot in the centre of the colony. These characteristics are characteristic of *E. coli* colonies on EMBA media. Similar results were also explained by Widodo *et al*. (2022) that *E. coli* isolates on EMBA media showed metallic green colonies with a diameter of 2 - 3 mm with a black dot in the centre of the colony (Hanum *et al*. 2018). Then purification of the colony was carried out until a pure culture was obtained. Based on the Gram staining results, the morphology of cells in the form of short rods was obtained, with a red Gram stain reaction, including Gram-negative bacteria (Figure 2).

According to Leininger *et al*. (2001), EMBA media contains eosin and methylene blue dyes, which are pH indicators and inhibit the growth of Gram-positive bacteria. The high due to lactose fermentation will form a greenish metallic sheen precipitate which *E. coli* can only produce. Other bacteria that can also ferment lactose and cause acidic conditions will produce purplish colonies. Lactose fermenting bacteria will show transparent growth of colonies, or if they produce weak acids, they will show pink colonies.

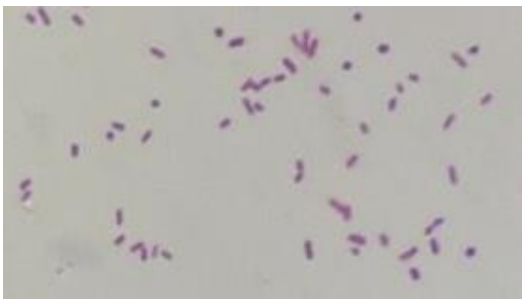

Figure 2. Gram Staining of Bacteria

Testing the infusion on the inhibition zone of *E. coli* using the Agar diffusion technique

The results of the antibacterial activity of *M. oleifera* leaf infusion against *E. coli* showed that the width of the inhibition zone varied depending on the concentration of the infusion (Figure 3 and Figure 4). This study used the well method with a well diameter of 8 mm. The diameter of the wellbore reduces the diameter of the inhibition zone formed.

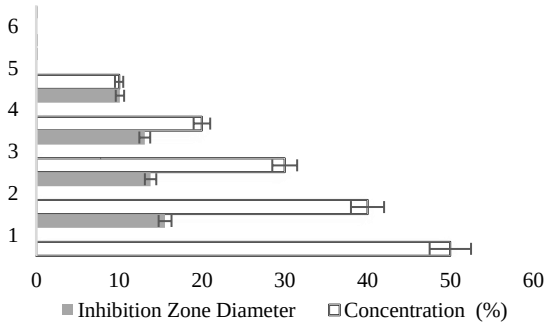

Figure 3. The average diameter of the inhibition area of the *M. oleifera* infusion test using the disc diffusion technique against *E. coli*

In five concentrations, the lowest average diameter of the inhibition zone was shown at a concentration 10% (0 mm). Meanwhile, the highest inhibition zone diameter was demonstrated at a concentration of 50%. According to Panaungi & Sakka (2022), the criteria for the level of antibacterial power places inhibitory zone diameters of 5 mm in the weak category, 5–10 mm in the medium category, 11–20 mm in the strong category, and >20 mm in the very strong category.

The results of this study indicate that the higher the concentration, the greater the inhibition zone formed. No inhibition zones were formed at 10% (Figure 4). The antibacterial inhibition test using the Agar diffusion technique was more effective in producing a larger diameter of the inhibition zone on bacterial growth than the paper disk method. This is due to the osmolality well method occurring more thoroughly and homogeneously to inhibit bacterial growth (Cesar *et al*. 2020).

The inhibition of *M. oleifera* leaf infusion on the growth of *E. coli* showed different values at each concentration, meaning that there were differences in the effect on the test bacteria given. The results showed that 5 series of *M. oleifera* leaf infusion concentrations, namely 50%, 40%, 30%, 20%, and 10% , showed different antibacterial activity on the growth of *E. coli*. The difference in antibacterial activity at each concentration indicates that the spectrum of inhibition depends on the type and strength of the antibacterial compound from each

extracted component and the amount of active component extracted by the solvent used. According to Egra *et al*. (2019), several factors that might affect antibacterial activity such as the type of bacteria being inhibited, the content of antibacterial compounds, the concentration of the extract and the diffusivity of an extract. In addition, differences in the structure of the bacterial cell wall also determine the activity, penetration and bonding of antibacterial compounds (Nowotarska *et al*. 2014).

R1 **R2** **R3** **R4** **R5**

Figure 4. The results of inhibition zone diameter test, (R: Replication)

The well diffusion agar method showed that the concentrations of 50%, 40% and 30% were included in the category of strong inhibition. In comparison, at a concentration of 20%, it was included in the category of medium inhibition, with the highest inhibition zone at 50% concentration with a diameter of 15.55 mm. *M. oleifera* leaves have active compounds as antibacterial and, at the same time, shows that *M. oleifera* leaves have high potential as antibiotics. Rachmawati & Suriawati, (2019) in their research using the boiling method, stated that *M. oleifera* leaf extract contains flavonoids, triterpenoids, steroids, saponins, and tannins. These are antimicrobial compounds (Seyydnejad *et al*. 2010; Sariwati *et al*. 2019). According to Septiani *et al*. (2017), antibacterial compounds generally work by breaking down cell walls, changing membrane permeability, interfering with protein synthesis and inhibiting enzyme action.

Testing *M. oleifera* infusion with dilution technique on *E. coli*

The minimum inhibitory concentration test aims to determine the minimum concentration that a substance can use to inhibit bacterial growth (Andrews, 2001). Inhibition of *E. coli* activity using the tube dilution method, in which the antibacterial compound is diluted to obtain several concentrations. Then the concentration was added to *E. coli* into Nutrient broth medium. The treatment was incubated for 24 hours at 37°C, and the presence or absence of bacterial growth was observed, which was indicated by turbidity. The minimum inhibitory concentration value is the lowest concentration of antibiotics with culture results that start to appear clear, indicating no bacterial growth (Fitriana *et al*. 2019).

Based on the results of the dilution method, the observations conducted on *E. coli* obtained unsatisfactory results because observations made visually on tubes made it difficult to determine between positive and negative results. This is influenced by the brown color of the resulting infusion, which reduces the purity of the media as a result of bacterial growth.

Table 1. Observations of *M. oleifera* infusion test with dilution technique on *E. coli*

Concentration (%)					
1	**2**	**3**	**4**	**5**	**6**
25	12,5	6,25	3,125	Extract (C)	K Bacteria (C)
+	+	+	+	-	+

Information : + = Growth of Bacteria
- = No bacterial growth
K = Control

Table 1 shows that at a concentration of 3.125% to 25% indicates bacterial growth. However, at a concentration of 25%, the growth of bacteria began to decrease, so it was set as the minimum inhibitory concentration value. The minimum bactericidal concentration was not found because at all concentrations tested using the dilution technique (25%, 12.5%, 6.25%, and 3.125%) indicated the presence of bacterial growth.

CONCLUSION

The antibacterial activity of *M. oleifera* leaf infusion on the growth of *E. coli* was effective at concentrations of 50%, 40%, 30%, and 20% and ineffective at concentrations of 10%. The higher the concentration of *M. oleifera* leaf infusion, the more effectively it inhibits the growth of *E. coli*. The minimum inhibitory concentration of *M. oleifera* leaf infusion is at a concentration of 25%, where the growth of bacterial colonies begins to decrease. There were no minimum bactericidal concentration

value was identified because all concentrations examined by the dilution technique contained bacterial growth.. Further research is needed to find the minimum bactericidal levels at concentrations above 25%.

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