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Activity of *Moringa oleifera* seed ethanolic extract against *E. coli*

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Abstract. Bacterial contamination in our environment is worrying, mainly contamination at drinking waters, vegetables, foods, soil which close to our daily activity. The main contamination in environment is caused by *E. coli* which simply found easily surround us. So, it leads to bigger problem if not immediately solve. One of possible yet safe compounds to overcome this problem is the use of natural product such as *M. oleifera* seeds as antibacterial agents. This study want to find out the ability of *M. oleifera* seed ethanolic extract as antibacterial agent against *E. coli*. Ethanolic extract of *M. oleifera* seeds are concentrated into 25%, 50%, and 75%, then treated to *E. coli* culture under laboratory condition. The inhibitory zone diameter which formed after 24 hours incubation was measured and compared to control with no extract treatment. The result of this study showed that there is inhibitory zone formed in three groups of treatmen (T1-T3), but there is no inhibitory zone formed at control group. The 75% ethanolic extract of *M. oleifera* seeds (T3) has the wides inhibitory zone diameter among four groups, followed by the 50% extract (T2) and 25% extract (T1) with diameter 15,03 ± 0,55 mm; 11,00 ± 1,32 mm; 7,03 ± 0,90 mm, respectively. All inhibitory zone diameter among groups in this research statistically different with strong inhibitory status at T2 as well as T3, and moderate inhibitory status at T1.

Keywords: antibacterial, *Escherichia coli*, *Moringa oleifera*

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1. Introduction

Escherichia coli is one of fecal-oral pathogens transmitted from feces or contaminated water into new host, through complex environmentally mediated pathways [1]. Those species has been implicated as diarrheagenic bacteria which outbreaks worldwide through foodborne and associated with childhood stunting [2]. With complex phylogenetic substructure, many *E. coli* are harmless but some are infectious cause intestinal and extra-intestinal infections. *E. coli* can cause serious infections via contaminated food and water occurs in healthcare unit, at home, dan during travel [3]. Contaminated water in Indonesia are one of community problem which drive to serious health problem. The present of *E. coli* in water bank such as wells, river, etc can cause serious infectious diseases in Indonesia, especially in rural area with low hygiene level. Whereas, health and productivity of community system hinge on water quality and hygiene status of society environmen [4].

The habitat of *E. coli* are primary (gastrointestinal tract) and secondary (water, sediment, soil, and flora), with negative growth rate in secondary habitat implies short-term host persistence [5]. Gastrointestinal disease which also can provided by *E. coli* contamination cause an estimated 500.000-

700.000 deaths in children under 5 years old, annualy [6]. Water sources, especially in rural area, such as wells or rivers are one of primary *E. coli* contamination sources [7]. Moreover, contamination of *E. coli* also can be found in leafy greens vegetables, such as spinach, lettuce, water spinach, etc [8]. Those fact provide more possibility *E. coli* contaminated vegetables be consumed by Indonesian society and cause an foodborne diseases. So, the control of *E. coli* contamination is necessary.

Indonesian biodiversity provide many natural product bioactive compounds in a role as antibacteria. The extraction of bioactive compounds from natural material such as plants, fungus, bacteria itself, as antibacteria has recently expanded. Plants natural compounds has many functional values for health and has been widely studied in Indonesia [9][10][11]. One of Indonesian plant bioactive compounds which purposes as antibactial is *Moringa oleifera*. It has been shown as an antimiceobial agent against different bacteria and fungi in various water studies. It also been considered as an ideal application for water contamination in developing countries [12].

Moringa oleifera with *Jatropha curcas*, *Hibiscus sabdariffa*, *Clidemia angustifolia*, and others have been known and used for water purification. The bioactive compounds of *M. oleifera* has been found as coagulation factors and acts as natural cationic polyelectrolyte causes coagulation in turbid water [13]. Moringa species have been known as antibacteria, antihelminthic, detoxifiers, immune builders [14], antipyretic, antiepileptic, antiinflamatory, antiulcerative, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, and hepatoprotective [15] in folk medicine. It provides zeatin, quercetin, and many others phytochemicals properties. Based on its bioactive compounds potential, *M. oleifera* widely studied as antibacterial properties, include for *E. coli* in contaminated water due to its possibility to enhance the coagulation activity [16]. The objective of this study is to know the activity of ethanolic extract of *M. oleifera* seed as anti-*E. coli*.

2. Methods

Black seed of *M. oleifera* was separated from its peel then dried at 25-30°C. The dried black seed was mashed and ready for extraction. A 750 g sample powder was maserated with 96% ethanol for 3x24 hours, stirred every 1x24 hours. The extract was ready to filtered after 3x24 hours, then the solvent was evaporated. The concentrated extract was obtained then analyzed for antibacterial activity assay for *E. coli* isolated from water sample in Tlogosari Wetan, Semarang, Indonesia. The isolate was cultured at Mac Conkey Agar (MCA), then at Eosin Methilen Blue (EMB). Separated colony then cultured at indol media, Methyl Red (MR), Voges Proskauer (VP), and Simmon's citrate agar for biochemistry assay.

For antibacterial assay, isolated *E. coli* was inoculated at nutrient agar with pour plate method. The culture then been incubated at 37°C for 24 hours. The cultured *E. coli* was diluted into NaCl 0,9 % to 10^{-5} dilution. The 10^{-5} *E. coli* dilution then poured at sterilized petridish and analyzed for antibacterial assay. The Kirby-Bauer antibacterial assay was performed at 4 group. Group 1 is control (C), group 2 is 25% ethanolic extract of *M. oleifera* seed (T1), group 3 is 50% ethanolic extract of *M. oleifera* seed (T2), and group 4 is 75% ethanolic extract of *M. oleifera* seed (T3). Under the aseptic conditions, saturated filter paper discs were placed on the inoculated solid agar surface. After the 24 hours incubation at 37°C, the inhibition zone was analyzed. The data of inhibition zone were analyzed with SPSS 17.0 version programme. Analysis of variance (ANOVA) were performed using one-way ANOVA. Significant differences among means were determined by Duncan's test and if the P value was less than 0.05, so the datas were considered as statistically significant. The widest inhibition zone is represents the most inhibited colony of *E. coli* by ethanolic extract of *M. oleifera* seed.

3. Result and Discussion

The extraction yield was 21,35% (Table 1) from 160,14 g concentrated ethanolic extract of *M. oleifera* seed. These yield was obtained from 770 g *M. oleifera* seed. The yield percentage of EEMS was measured by ratio of concentrated extract weight (g) and simplicia weight (g) multiplied with 100%. In this study, the yield percentage of EEMS is 21,35%. The extraction was performed with maseration method. Maseration is an extraction process with specific solvent and repeated mixture at room temperature. Maseration was used due to its simple and fast process. The 96% ethanol was used for

faster evaporation of ethanol residue in the extract [17]. Ethanol is known as a efficient solvent for natural product extraction due to its higher eficiency and faster process. Ethanol is considered as universal solvent dissolving natural material compounds, non-polar, semi-polar, or polar [18]. Polarity indext of ethanol is 5,2 so polar or non-polar substances could be extracted and 96% ethanol can be use as solvent for low molecule weight of almost all substances such as saponin and flavonoid.

Table 1. Yield percentage of ethanolic extract of *M. oleifera* seed (EEMS)

Sample Weight (g)	Simplicia Weight (g)	Filtrat Volume (ml)	Concentrated Extract Weight (g)	Yield (%)
770	750	1.500	160,14	21,35

Antibacterial activity assay was performed with Kirby-Bauer method. Saturated filter paper discs was placed on culture surface then the *E. coli* culture was incubated at 37°C for 24 hours. The inhibitory zone then be measured. The result of this study showed thatethanolic extract of *M. oleifera* seed (EEMS) significantly inhibit the growth of *E. coli* under laboratory condition. Culture with 75% EEMS showed the highest inhibitory effect with 15,03 ± 0,55 mm inhibition zone diameter. It is significantly different from culture with 50% EEMS and 25% EEMS which are showed 11,00 ± 1,32 mm and 7,03 ± 0,90 mm inhibition zone diameter, respectively (**Table 2**). All of three group treated with EEMS showed wider inhibition zone diameter than control group because the culture media of control group was only added with aquades.

Table 2. The resistance status of *E. coli* with ethanolic extract of *M. oleifera* seed (EEMS) treatment

Group	Inhibition Zone Diameter (mm)	Resistance Status
C	$0,00 \pm 0,00^{a}$	Low
T1	$7,03 \pm 0,90^{b}$	Moderate
T2	$11,00 \pm 1,32^{c}$	Strong
T3	$15,03 \pm 0,55^{d}$	Strong

Note: The symbol C is control (aquades); T1 represents 25% of EEMS treatment; T2 represents 50% of EEMS treatment; and T3 represents 75% of EEMS treatment

The resistance status of EEMS for the growth of *E. coli* are low at control group, moderate at T1, strong at T2 and T3. This result indicate the ability of EEMS to inhibit the growth of *E. coli* so this natural resources could be utilized as antibacterial agent to overcome the soil, water, or other pollution sourced by *E. coli* contamination. This result is supported by previous research which showed that *M. oleifera* seed oil has 40,17 ± 0,01 mg GAE/g total phenol compound, 18,24 ± 0,01 mg RE/g total flavonoid compound, and 37,94 ± 0,02 mg AAE/g total antioxidant capacity [19]. Fatty acids composition inside *M. oleifera* seeds showed that it is belong to high-oleic acids category. Moringa oleifera is also a source of tocopherols at 98,82-134,42 mg/kg for tocopherol-α, 27,90-93,70 mg/kg for tocopherol-γ, and 48,00-71,16 mg/kg for tocopherol-δ [20].

In this study, ethanolic extract of *M. oleifera* seed showed a good performance as inhibitory agent for *E. coli* growth under laboratory condition. All of three group of treatment showed a significant difference of inhibitory zone diameter compared with control group (**Table 2**). Those result indicate that ethanolic extract of *M. oleifera* seed has bioactive compounds which play a role as antibacterial agent, especially for *E. coli*. Those bioactive compounds such as alkaloid, flavonoid, tannin, and saponin which has ability to inhibit the bacterial growth. Alkaloids are a nitrogen containing naturally occuring compounds that has an antibacterial activity due to their ability to intercalate with DNA of the microbes [21]. Alkaloid interrupt the composition of peptidoglican so that bacterial cell wall is not fully formed and leads to bacterial cell death. Flavonoid play a role as antibiotic, also tannin and saponin is a fenolic

compound which has antibacterial activity. Flavonoids have been reported have antimicrobial activity under laboratory condition through destruction of microbial membrane [22]. So, the bioactive compounds found at *M. oleifera* seeds promote its potency as antibacterial agent.

The widest inhibitory zone diameter was seen at T3, followed by T2 and T1 with 15,03 ± 0,55 mm; 11,00 ± 1,32 mm; and 7,03 ± 0,90 mm, respectively. Those result indicate that the higher concentration of ethanolic extract of *M. oleifera* seed has higher antibacterial activity for *E. coli* growth under laboratory condition. Both concentration of extract at T3 and T2 fall in the category as strong inhibitory activity for *E. coli*, yet concentration of extract at T1 is categorized as moderate (**Table 2**). The previous study indicate that *M. oleifera* has greater antibacterial activity against Gram positive bacteria, such as *S. aureus* and *E. faecalis* than Gram negative bacteria, such as *E. coli*, *Salmonella*, *P. aeruginosa*, *V. parahaemolyticus*, and *A. caviae* [23]. Nevertheless, in this study, ethanolic extract of *M. oleifera* seed still showed a greater inhibitory result to *E. coli* growth under laboratory condition. The ability of ethanolic extract of *M. oleifera* seeds to inhibit bacterial growth leads to its potency as antibacterial agent to overcome health issue about *E. coli* contamination among water, soil, food and other environmental properties. This antibacterial compounds found in *M. oleifera* enable society to utilize this plant as naturally safe antibiotics. Hopefully, the result of this study triggers other advance research to develop *M. oleifera* as natural product which can be used by society as local wisdom to overcome their environmental problem, especially in environment contamination of *E. coli*.

4. Conclusion

From this study, we conclude that ethanolic extract of *Moringa oleifera* seeds has the ability to inhibit *Escherichia coli* growth under laboratory condition. The widest inhibitory zone diameter is showed by 75% ethanolic extract of *M. oleifera* seeds, with strong inhibitory status. Even though the 50% extract is also showed strong inhibitory status, but its inhibitory zone diameter is tighter than 75% extract. The tightest inhibitory zone diameter is showed by 25% ethanolic extract of *M. oleifera* seeds, with moderate inhibitory status. Those result indicate that the higher concentration of ethanolic extract of *M. oleifera* seeds performs the wider inhibitory zone diameter which suggest a better inhibitory status to *E. coli* under laboratory condition.

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