THE ANTIBACTERIAL POTENCY OF *Moringa oleifera* LEAF EXTRACT ON SEMAU ISLAND, EAST NUSA TENGGARA, AGAINST *Escherichia coli*

a Kusmiyati, b Ferry WF Waangsir, c Moses Kopong Tokan, d Apris A Adu, e Anwar Mallongi, f Eryc Zevrily Haba Bunga

**ABSTRACT**

**Objective:** This study was conducted to investigate the phytochemical constituent of *Moringa oleifera* leaf extract and determine the inhibition of these extract against *Escherichia coli* bacteria.

**Methodology:** This was a laboratory experimental study. *Moringa* leaves was collected from Semau Island, East Nusa tenggara, Indonesia. Quantitative testing was carried out to determine the bioactive components of *Moringa* leaf extract. The well diffusion method was used to test the inhibitory activity of *moringa* leaf extract against *E. coli*. The treatment were divided into 10 extract concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%).

**Results:** This study showed the presence of flavonoids, phenols, alkaloids, tannins and saponins in *M. oleifera* leaf extract. *M. oleifera* leaf extract has different inhibitory properties against *E. coli* bacteria. *Moringa* extract concentrations of 10%, 20% and 30% did not show inhibitory activity against *E. coli* while the others concentrations were able to inhibit the growth of *E. coli*. The average inhibition zone diameter for each concentration varied i.e 1.91 mm (40%), 6.27 mm (50%), 8.53 mm (60%), 10.39 mm (70%), 12.80 mm (80%), 14.84 mm (90%) and 19.04 mm (100%). The most effective for inhibiting the growth of *E. coli* bacteria was 100%. The results of statistical analysis using repeated ANOVA tests obtained p value 0.001 (p<0.05) that means the *M. oleifera* leaf extract able to inhibit *E. coli*.

**Contributions:** We conclude that *M. oleifera* leaves have the potential as an antibacterial, so further studies related to the use of *M. oleifera* in overcoming various environmental health problems is needed.

**Keywords:** inhibitory power, *M. oleifera*, *Escherichia coli*, semau.
A POTÊNCIA ANTIBACTERIANA DO EXTRATO DE FOLHA DE Moringa oleifera NA ILHA SEMAU, LESTE DE NUSA TENGGARA, CONTRA Escherichia coli

RESUMO

Objetivo: Este estudo foi realizado para investigar o constituinte fitoquímico do extrato de folhas de Moringa oleifera e determinar a inibição deste extrato contra a bactéria Escherichia coli.

Metodologia: Este foi um estudo experimental em laboratório. As folhas de Moringa foram coletadas na Ilha Semau, East Nusa tenggara, Indonésia. Testes quantitativos foram realizados para determinar os componentes bioativos do extrato de folhas de Moringa. O método de difusão em poço foi utilizado para testar a atividade inibitória do extrato de folhas de moringa contra E. coli. O tratamento foi dividido em 10 concentrações de extrato (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%).

Resultados: Este estudo mostrou a presença de flavonoïdes, fenóis, alcaloïdes, taninos e saponinas no extrato de folhas de M. oleifera. O extrato de folhas de M. oleifera possui diferentes propriedades inibitórias contra a bactéria E. coli. As concentrações de extrato de Moringa foram de 10%, 20% e não apresentaram atividade inibitória contra E. coli enquanto as demais concentrações foram capazes de inibir o crescimento de E. coli. O diâmetro médio da zona de inibição para cada concentração variou, ou seja, 1,91 mm (40%), 6,27 mm (50%), 8,53 mm (60%), 10,39 mm (70%), 12,80 mm (80%), 14,84 mm (90%) e 19,04 mm (100%). O mais eficaz para inibir o crescimento da bactéria E. coli foi 100%. Os resultados da análise estatística utilizando testes ANOVA repetidos obtiveram valor de p 0,001 (p<0,05) o que significa que o extrato de folhas de M. oleifera é capaz de inibir E. coli.

Contribuições: Concluímos que as folhas de M. oleifera têm potencial como antibacteriano, portanto são necessários mais estudos relacionados ao uso de M. oleifera na superação de diversos problemas de saúde ambiental.


1 INTRODUCTION

The M. oleifera is a plant that grow in the tropics and various regions in Indonesia. This plant is often used by certain communities as a vegetable and treatment of disease. The benefits obtained from this plant are not only in the leaves but in other parts including flowers, seeds, roots, bark. The plant is often call miracle tree. Indonesian people generally called it “kelor” but the people of East Nusa Tenggara generally name the plant “Marungga”.

M. oleifera leaves in various studies showed the presence of tannins, flavonoids, glycosides, terpenoids, phenols (Malhotra & Mandal, 2018). Tests for compound content
showed that various parts of the moringa plant contained tannins, saponins, phenols and flavonoids (Dodiya et al., 2015). Compound testing on *M. oleifera* leaves has been carried out using standard procedures to identify the content of bioactive compounds alkaloids, flavonoids, tannins and phenols (Taufik et al., 2021).

*E. coli* are bacteria indicator of sanitation. Not everyone has adequate basic sanitation facilities. People in Porto Seguro are dissatisfied with basic sanitation services and the majority do not know what basic sanitation services are (Faganello and Neto, 2021). The presence of *E. coli* in water indicates contamination of the water by human feces and warm-blooded animals. A study found the presence of *E. coli* bacteria in drinking water samples. Research in Guadalajara also showed that the total coliform bacteria in most of the water samples examined exceeded the allowable requirements (Rubino et al., 2019). Research using various methods of extraction and measurement of inhibition zones for various types of bacteria has shown its role for use in water treatment, especially in areas with difficulty in water supply (Bancesi et al., 2020).

*M. oleifera* which grows in East Nusa Tenggara has the advantage of being easy to cultivate and heat resistant. Several studies have been conducted on Moringa leaves from East Nusa Tenggara. Moringa leaves can reduce the expression of TRAIL R1 and Bcl-2 proteins in Wistar rats (Boy & Endang, 2013). However, there has been no research about the ability of *M. oleifera* leaves on Semau Island, East Nusa Tenggara as an antibacterial against *E. coli*. Therefore it is necessary to conduct a study on this matter.

This study aims to investigate the phytochemical constituent and determine the inhibitory activity of *M. oleifera* leaf extract against *E. coli* bacteria. The results of this research are expected to be useful in the development of further studies related to the use of *M. oleifera* in overcoming various environmental health problems.
2 THEORETICAL FRAMEWORK

3 METHODOLOGY

Study design. This study was a true experimental study using the agar well diffusion method with 3 repetitions. The test bacteria used were E. coli bacteria treated with Moringa leaf extract which was divided into 10 different concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%). This research was conducted in the microbiology laboratory of the faculty of science and technology, Widya Mandira Catholic University, Kupang in November 2022. Tests for the compounds in the extract were carried out in the integrated testing laboratory, Gadjah Mada University, Yogyakarta. The independent variable is the concentration of Moringa leaf extract while the dependent variable is the inhibition of the growth of E. coli bacteria.

Extraction of M. oleifera leaf. The extract was made using the maceration method. Moringa leaves was obtained from plants that grow on Semau Island, East Nusa Tenggara, Indonesia. For extract preparation, M. oleifera leaves were dried in an oven at 60° C for 24 hours. The dried leaves were then crushed into powder and dissolved in ethanol solvent, stirred and allowed to stand for 24 hours. Moringa leaf powder as much as 100 g dissolved in 500 mL ethanol solvent. The macerated extract is filtered using a Buchner funnel, the vapor is dissolved with a rotary evaporator to produce a viscous
ethanol extract. Evaporation with a vacuum rotary evaporator at a temperature of 50 °C, 70 rpm to obtain extracts.

3.1 MEDIA PREPARATION

Nutrient Agar (NA) media was prepared by dissolving 4 grams of Nutrient Agar with 500 ml of distilled water in an Erlenmeyer and covered with aluminum foil, stirred with heat until boiling then sterilized by autoclaving at 121°C for 15 minutes and 1 atm pressure. After that the media was poured into a petri dish 10 mL each, allowed to cool to form a gel.

3.2 TEST OF ANTIBACTERIAL ACTIVITY

Beest layer media (12 mL) and a seat layer (10 mL) are made from nutrient agar media and then sterilized for 15 minutes at 121°C. Pour the beest layer media into the petri dish, after it cools and solidifies, pour the seat layer media that has been added with the inoculum solution. *E. coli* bacteria as much as 5 µl, let it cool and solidify. After it has solidified, then make wells as needed and drip the Moringa leaf extract solution into each well with various concentrations, namely 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. After each well was filled, it was incubated in an incubator at 32.5 °C for 24 hours. After that, it was removed from the incubator and the size of the inhibition zone was measured using a vernier caliper. This work was repeated 3 times.

3.3 DATA ANALYSIS

The data obtained was presented in table and figure. Statistical analysis was carried out using repeated ANOVA tests.

4 RESULTS AND DISCUSSION

4.1 PHYTOCHEMICALS CONSTITUENT IN *M. OLEIFERA* LEAF EXTRACT

Quantitative test of phytochemicals constituent in *M. oleifera* leaf extract showed the presence of flavonoids, phenols, alkaloids, tannins and saponins (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total % m(w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoids</td>
<td>11.17</td>
</tr>
<tr>
<td>Total phenol equivalent of gallic acid</td>
<td>7.56</td>
</tr>
<tr>
<td>Total alkaloid equivalents of quinine</td>
<td>0.10</td>
</tr>
<tr>
<td>Total tannin equivalent of tannic acid</td>
<td>12.76</td>
</tr>
</tbody>
</table>
The results of phytochemical testing on *M. oleifera* leaves found the presence of flavonoids, phenols, alkaloids, tannins and saponins (Table 1). Flavonoids are polyphenols that are widely found in plants and fruits (Xue et al., 2023). The results of the review have show that plant moringa contain potential phytochemicals, give benefit as antibacterial and beneficial to health people (Tiloke et al., 2018). Phytochemical screening showed the presence of alkaloids, flavonoids and tannins extract (Metsopkeng et al., 2020). Flavonoids and saponins insposible potentially in prevention disease, however need caution And need approach new For prove (Wang et al., 2023). The research was conducted in three Southern States (Rio Grande do Sul, Paraná, and Santa Catarina) to identify plants used in the treatment of bacterial diseases and compare them with scientific basis. Literature shows that the plant contains medicinal potential chemical components (Bones et al., 2022)

A study of inhibition mechanism showed that flavonoids can targeting the bacterial membrane and cause damage to membrane integrity and biosynthesis. Besides it's a flavonoid work by inhibiting cell wall synthesis, inducing hydrolysis and preventing bacteria from synthesizing biofilms. They also can be annoying with energy metabolism and interfere with the normal physiological activity of bacteria (Weng et al., 2023).

### 4.2 INHIBITION ACTIVITY OF *M. OLEIFERA* LEAF EXTRACT AGAINST *E. COLI*

The results of the inhibition of *M. oleifera* leaf extract at various concentrations against *E. coli* can be seen in Table 2.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Inhibition Zone (mm)</th>
<th>Average (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40%</td>
<td>3.70</td>
<td>1.023</td>
</tr>
<tr>
<td>50%</td>
<td>9.70</td>
<td>5.04</td>
</tr>
<tr>
<td>60%</td>
<td>9.73</td>
<td>10.71</td>
</tr>
<tr>
<td>70%</td>
<td>11.04</td>
<td>6.08</td>
</tr>
<tr>
<td>80%</td>
<td>10.34</td>
<td>12.05</td>
</tr>
<tr>
<td>90%</td>
<td>11.07</td>
<td>12.08</td>
</tr>
<tr>
<td>100%</td>
<td>19.71</td>
<td>22.06</td>
</tr>
</tbody>
</table>

Source: Prepared by the author (2023)
The results of statistical analysis using repeated ANOVA tests obtained p value 0.001 (p<0.05) which means that *M. oleifera* leaf extract is able to inhibit *E. coli* bacteria. The results of observations of extract activity at concentrations of 10%, 20% and 30% against *E. coli* bacteria are shown in Figure 1. The results of observations at concentrations of 40%, 50% and 60% are shown in Figure 2, concentrations of 70%, 80% and 80% in figure 3 and 100% concentration in figure 5.

Figure 2. Moringa leaf inhibition zona against E. coli at 10%, 20%, 30% concentrations

Source: Prepared by the author (2023)

Figure 3. Moringa leaf inhibition zona against E. coli at 40%, 50%, 60% concentrations

Source: Prepared by the author (2023)

Figure 4. Moringa leaf inhibition zona against E. coli at 70%, 80%, 90% concentrations

Source: Prepared by the author (2023)
Moringa may be a source antimicrobial natural potential (Özcan, 2020). This may be due to the presence of compounds that are antibacterial. Treatment using M. oleifera leaf extract in this study showed different results. At low concentrations, it does not show antibacterial activity against *E. coli*. Treatment at a concentration of 40% began to show inhibitory activity against bacteria (Figure 3). As the concentration of the extract increases, the inhibitory activity also increases. It is proved that at a concentration of 100% it shows the highest inhibitory activity (Figure 5).

A study was done by integrating *M. oleifera* with filtration BioSand. The result showed that efficiency modification bucket filter and moringa, almost the same result with conventional BioSand filters, i.e range 81 - 98% (Kisakye, 2023). Other studies showed different results. *M. oleifera* plants have the ability to inhibit the growth of microorganisms but their activity is low to moderate. The study also combined *M. oleifera* with conventional antibiotics and proved more effective against some of the bacteria tested. Several antibiotics with low inhibitory activity returned to substantial activity when tested in combination with these extracts (Ilanko et al., 2019). The ethanol extract of *M. oleifera* leaves inhibit *E. coli* like inhibition by common antibiotics. The results of the proteomic test showed that the ability of *M. oleifera* to lyse *E. coli* was through a mechanism of changing the expression of proteins that play a role in the biological processes of bacteria (Smith et al., 2020).

A systematic review has been done for analyze extract antibacterial test results leaf and seed Moringa. Extract showed minimal antibacterial activity against gram negative bacteria. Whole seed extract showed more activity good compared to with extract dry. Methanol extract showed activity somewhat antibacterial more good compared to aqueous extract (van den Berg & Kuipers, 2022). Besides characteristic antibacterial, water extract of Moringa leaves on a number of concentration also showed
antifungal and yeast activity (Al_husnan & Alkahtani, 2016).

Various studies results plant including Moringa stenopetala showed significant antibacterial activity to resistant Staphylococcus aureus against methicillin. Organic solvents with high and medium polarity very good in extracting antimicrobial compared with nonpolar solvents. Moringa extract is known bacteriostatic. The anti-biofilm test showed that the extract the enough to inhibit growth resistant S. aureus to methicillin in a preformed biofilm matrix. The results of the analysis showed that there were twelve compounds withantimicrobial activity in these plants (Manilal et al., 2020).

A study has examined the abiotic factors of Moringa extract against bacteria (S. aureus). Continuity life bacteria rated using the colony forming unit (CFU) method. Increasing extract concentration significantly inhibited the number of colonies bacteria, concentration and cell count are inversely related. Incubation temperature and extract concentration significantly affect the number of bacteria. So that said that the use of Moringa extract can be alternative water treatment (Metsopkeng et al., 2020). Study also has done for test effectiveness methanol extract of M. oleifera leaves various concentration to lactobacillus bacteria (Owusu-Ansah et al., 2023).

The limitation of this study was not examining proteomic and genomic mechanisms of Moringa leaves inhibiting E. coli. Therefore, further research needsto be done. The results of this research have become the basis for developing further studies on the potency of moringa.

5 CONCLUSIONS

M. oleifera leaf extract has different inhibitory properties against E. coli bacteria. Concentrations of 40%, 50%, 60%, 70%, 80%, 90% and 100% were able to inhibit the growth of E. coli bacteria, and the most effective for inhibiting the growth of E. coli bacteria was 100%. Thus, the leaf extract has the potential as an antibacterial to provide solutions to public health and environmental health problems. Therefore further research on the potential utilization of moringa is needed.

ETHICAL CLEARANCE

This research has received ethical approval from the health ethics committee ofthe health polytechnic of the ministry of health Kupang (No.LB.02.03/1/0064/2022).
FUNDING

This work was supported by the health polytechnic of the ministry of health Kupang in 2022.
REFERENCES


Masriadi, Rahmawati Azis, eha Sumantri, Anwar Mallongi. Effectiveness of non pharmacological therapy through surveillance approach to blood pressure degradation


Xue, J. C., Yuan, S., Meng, H., Hou, X. T., Li, J., Zhang, H. M., Chen, L. L.,
