



Laboratory Journal of Infectious Diseases

Homepage: <https://jurnal.poltekkeskupang.ac.id/index.php/LJID>



Original Article

Inhibitory Effect of Moringa Leaf Extract on *Escherichia coli* Isolated from Urine of UTI Patients

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ARTICLE INFO

Article history:

Received 5 April 2025

Revised 26 April 2025

Accepted 20 May 2025

Keywords:

Moringa leaf extract

Escherichia coli

Urinary tract infection

Minimal inhibitory concentration

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Doi:

ABSTRACT

The moringa plant (*Moringa oleifera*) are a well-known medicinal plant in Indonesia, often referred to as the "Miracle Tree" due to their numerous health benefits. Urinary Tract Infection (UTI) is a clinical condition in which bacteria are present in the urinary tract, typically caused by microorganisms such as *Escherichia coli* (*E. coli*). Moringa leaves contain antibacterial phytochemicals such as flavonoids, saponins, alkaloids, terpenoids, steroids, catechol tannins, gallic tannins, and anthraquinones, which can inhibit the growth of *E. coli*. UTIs are commonly treated with antibiotics, but excessive use of antibiotic can have consequence, such as antibiotic resistance. This study aims to determine the inhibitory activity of moringa leaf extract against *E. coli* isolated from the urine of UTI patients. The bacteria were cultured using EMBA media, Gram staining, and biochemical tests. The extract was prepared through maceration, and the Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method. The MIC test used bacterial suspensions at McFarland 0,5 and 0,25 with extract concentrations of 0,25%, 0,5%, 1%, 5%, 10%, 20%, 30%, and 40%. Results were observed using the turbidimetry method or visual observation. The findings showed that moringa leaf extract inhibited *E. coli* growth at concentrations of 20%, 30%, and 40%, as the suspensions remained clear after incubation. Conclusion: Moringa leaf extract can inhibit *E. coli* growth isolated from UTI patient urine and may be recommended for UTI prevention and treatment. These results are expected to provide knowledge that moringa leaves can be processed as food ingredients that have the potential to be used in efforts to prevent UTIs.

Introduction

The Moringa Plant (*Moringa oleifera*) are plants in Indonesia that have long been recognized for their medicinal properties and various health benefits, which is why they are often dubbed the "Miracle Tree" (*Pohon Keajaiban*) (Purnamasari, 2020). The use of moringa leaves as antimicrobials is most commonly applied in testing their inhibitory activity against bacteria. Previous studies have shown that moringa leaf extract has antibacterial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) (Megawati, Prasetya, & Sanjiwani, 2023), *Staphylococcus epidermidis*, and *Propionibacterium acnes* isolated from acne (Riswana, Indriarini, & Etty, 2022), *Pseudomonas aeruginosa* (Yunita, Permatasari, & Lestari, 2020), as well as *Shigella dysenteriae* (Karo et al., 2021).

Citation:



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Urinary Tract Infection (UTI) is a clinical condition of the urinary tract characterized by the presence of bacteria in the urine, caused by microorganisms and bacteria that can potentially enter the urinary tract. Although the urinary tract is normally free from bacterial growth, bacteria typically ascending from the rectum can cause UTIs (Abbas et al., 2023). The most frequently identified bacterium in the urine of UTI patients is *E. coli*, with a prevalence of 60% (Megawati, Prasetya, & Sanjiwani, 2023). The primary treatment for UTI patients is antibiotic therapy. However, the use of antibiotics does not, but can also cause side effects such as nausea, diarrhea, stomach pain. Excessive and inappropriate use of antibiotics also risks serious consequences, such as antibiotic resistance. In addition, antibiotics have pharmacokinetic effects that can increase plasma concentrations or reduce the clearance of ceftriaxone and can affect the kidneys. (Hashary, Manggau, & Kasim, 2018).

Therefore, an easily accessible and affordable alternative with potential as a natural antimicrobial source is needed—moringa leaves offer such a solution. Phytochemical screening studies have identified antibacterial compounds in moringa leaves such as flavonoids, saponins, alkaloids, terpenoids, and steroids (Fauziah et al., 2023), as well as tannins (Vinca et al., 2023). Previous studies using the MIC method with various concentrations of moringa leaf extract against *E. coli* demonstrated that the lowest antibacterial effect occurred at a 5% concentration, which was able to inhibit *E. coli* growth (Hutomo et al., 2023). In East Nusa Tenggara (NTT), moringa leaves are widely used for various purposes, such as anti-diabetic (reducing blood glucose levels) (Yasaroh et al., 2021), reducing uric acid levels (Putra, Azizah, & Clara, 2019), and as antioxidants (Susanty et al., 2019).

Based on this background, the present study was conducted to examine the inhibitory effect of moringa leaf extract on *E. coli*, specifically *E. coli* isolated from the urine of UTI patients. The research was carried out in the Bacteriology Laboratory of the DIII Medical Laboratory Technology Study Program at Poltekkes Kemenkes Kupang.

Methods

Time and Place of Research

This research was conducted at the Bacteriology Laboratory, DIII Medical Laboratory Technology Program, Poltekkes Kemenkes Kupang, during February to April 2025.

Preparation of Moringa Leaf Extract

The extraction method used for *Moringa oleifera* leaves was maceration. A total of 600 grams of dried moringa leaf powder (simplicia) was weighed and extracted using 6000 ml of 70% ethanol (OneLab Waterone™) at room temperature for 7 days with occasional stirring. After 7 days, the sample was filtered using filter paper to obtain a filtrate. The filtrate was then evaporated using a vacuum evaporator (Bioevopeak) and further concentrated on a water bath at temperatures below 60 °C until a thick extract with 100% concentration was obtained. Ethanol-free testing was performed by pipetting 2 ml of potassium dichromate into a porcelain dish, adding 6 drops of concentrated sulfuric acid, mixing, then adding 1 ml of moringa extract. A negative ethanol result was indicated by the solution remaining orange in color.

Sterilization of Equipment

All tools were sterilized before use by wrapping them in aluminum foil or brown paper and placing them in an oven (Memmert) at 100 °C for 15 minutes. Media in Erlenmeyer flasks covered with cotton were sterilized in an autoclave (Gemmy SA-232X) at 121 °C for 15 minutes.

Preparation of Eosin Methylene Blue Agar (EMBA)

A total of 9.375 g of EMBA powder (Merck) was weighed using an analytical balance (Sartorius), placed in an Erlenmeyer flask, and dissolved with 150 ml of distilled water until the volume reached 250 ml. The solution was heated and stirred until completely homogeneous. The flask was covered with cotton or aluminum foil and sterilized in an autoclave (Gemmy SA-232X) at 121 °C for 15 minutes at 1,5 atm pressure. The medium was poured into sterile petri dishes (15-20 ml each) and left at room temperature until solidified (Artanti et al., 2018).

Preparation of Mueller Hinton Agar (MHA)

A total of 8.5 g of MHA powder (Merck) was weighed using an analytical balance (Sartorius), placed in an Erlenmeyer flask, and dissolved with 225 ml of distilled water until reaching 250 ml. It was heated while stirring until fully dissolved. The Erlenmeyer flask was sealed with cotton or aluminum foil and sterilized in an autoclave (Gemmy SA-232X) at 121 °C for 15 minutes under 1,5 atm pressure. The medium was poured into 12 sterile petri dishes (15-20 ml each), allowed to solidify at room temperature, and was ready to use (Artanti et al., 2018).

Identification of *E. coli* in Urine Samples from UTI Patients and Microscopic Examination with Gram Staining and Biochemical Tests

E. coli isolation was done by inoculating urine samples onto EMBA media. The cultures were incubated in an incubator (Memmert) at 37 °C for 24 hours. The resulting green metallic colonies were observed. Gram staining was performed by fixing the bacteria over a flame using physiological saline. The staining steps included crystal violet for 1 minute, iodine for 1 minute, 70% ethanol for 30 seconds, and safranin for 1 minute. The bacteria were then examined under a microscope (Olympus CX23) at 100× magnification. Further, inoculation onto biochemical media such as SIM, MR, VP, Simmons Citrate, and TSIA was done. These media were incubated at 37 °C for 24 hours. SIM medium was added with Kovacs reagent, MR medium with methyl red reagent, and VP medium with 10% KOH and alpha-naphthol reagent (Widianingsih & de Jesus, 2018).

Preparation of Bacterial Suspension

A test tube was filled with 10 ml of sterile 0,9% NaCl solution. Bacteria were taken using a sterile inoculating loop and suspended into the saline solution. The bacterial suspension was adjusted to the turbidity standard of McFarland 0,5 and 0,25.

Preparation of Positive and Negative Controls

E. coli bacterial suspension was streaked on MHA media using a sterile cotton swab and allowed to stand for 15 minutes at room temperature. Then, ciprofloxacin antibiotic (Oxoid) and disks pre-soaked in distilled water for 30 minutes were placed on the MHA using sterile forceps and incubated at 37 °C for 24 hours (Boisala, 2024).

Testing the Inhibitory Effectiveness of Moringa Leaf Extract on *E. Coli*

E. coli suspensions with McFarland 0,25 and 0,5 standards were incubated with sterile broth for 4-8 hours. Moringa leaf extract was prepared at concentrations of 0,25%, 0,5%, 1%, 5%, 10%, 20%, 30%, and 40%, diluted with sterile distilled water, and filtered until clear. Sixteen sterile test tubes were prepared. Into each, 1 ml of extract and 1 ml of bacterial broth (aged 4-8 hours) were added. Tubes were incubated for 18-48 hours in an incubator (Memmert) at 37 °C. After incubation, bacterial growth was assessed based on turbidity (Subekti, Molek, & Sim, 2018).

Results

The inhibitory activity of moringa leaf extract against *E. coli* was tested using the broth dilution method to determine the MIC. The results were assessed based on the level of turbidity, which served as an indicator of bacterial growth. Table 1. shows that among the eight tested concentrations of moringa leaf extract-namely 0,25%, 0,5%, 1%, 5%, and 10% none were able to inhibit the growth of *E. coli* in suspensions prepared at McFarland standards 0,5 and 0,25, as indicated by the turbidity observed. However, the remaining three concentrations 20%, 30%, and 40% successfully inhibited the growth of *E. coli*, as evidenced by the clear appearance of the suspensions, resembling that of the pure moringa extract control. Based on these findings, the MIC of moringa leaf extract that effectively inhibited *E. coli* growth was determined to be 20%.

Table 1. Results of the inhibitory activity test of moringa leaf extract against *E. coli* using the MIC method

NO	Concentration %	Bacterial Suspension with McFarland Standard	
		0,5	0,25
1	Control	(-)	(-)
2	40%	(-)	(-)
3	30%	(-)	(-)
4	20%	(-)	(-)
5	10%	(+)	(+)
6	5%	(+)	(+)
7	1%	(+)	(+)
8	0,5%	(+)	(+)
9	0,25%	(+)	(+)

Notes:

(-): no bacterial growth (clear)

(+): bacterial growth present (turbid)

Based on the inhibitory test conducted, the antibiotic ciprofloxacin was used as a positive control, and sterile distilled water (aquadest) soaked into a sterile disk was used as a negative control, utilizing the disk diffusion method. In this method, if *E.coli* is sensitive to the antibiotic, a clear zone will form around the antibiotic disk placed on the inoculated media. The clear zone measured for the positive control was 32,30 mm. This indicates that *E. coli* isolated from the urine of UTI patients is susceptible to ciprofloxacin (Clinical and

Laboratory Standards Institute, 2020). In contrast, the negative control showed no clear zone, indicating that sterile distilled water does not inhibit the growth of *E. coli*.

Table 2. Observation results of inhibitory activity of positive and negative controls on *E. coli* using the disk diffusion method

No	Control	Diameter	Description
1	Positive Control (Ciprofloxacin)	32,30 mm	Susceptible
2	Negative control (Sterile distilled water)	-	-

Discussion

Based on previous studies, moringa leaf extract has been proven to inhibit the growth of *E. coli*, with a minimum effective concentration of 20%. These results align with the MIC test in this study, where the lowest concentration that inhibited *E. coli* growth was also found to be 20%. The difference in this study lies in the method used to determine the inhibitory activity: this study used the broth dilution method, while previous studies used the disk diffusion method (Sudarwati & Sumarni, 2016). Another earlier study showed that the lowest concentration of moringa leaf extract capable of inhibiting *E. coli* growth was 5000 µg/ml, equivalent to 5%. This discrepancy may be due to the use of the broth microdilution method in the previous study (Hutomo et al., 2023).

The ability of moringa leaf extract to inhibit the growth of *E. coli*, particularly the strains isolated from the urine of UTI patients, either through dilution or diffusion methods, is supported by the antibacterial compounds found in moringa leaves themselves. Previous phytochemical screening studies have shown that moringa leaves contain antibacterial compounds such as flavonoids, terpenoids, saponins, alkaloids, steroids (Faudziah et al., 2023), and tannins (Vinca et al., 2023).

The MIC method used in this study has the advantage of being relatively quick, as it does not require solid media dilutions, making it highly suitable as an initial screening method to assess the antibacterial potential of moringa leaf extract (Hutomo et al., 2023). However, using visual observation of turbidity in test tubes can be less effective, especially at high concentrations, due to the risk of misinterpretation.

Antibiotic resistance remains a common issue, particularly in UTI patients. This can result from inappropriate antibiotic use, high-dose usage without proper regulation, combining multiple antibiotics, or bacterial mutations. This study provides scientific insights to support the development of new herbal antibiotics using moringa leaves. Furthermore, this research raises public awareness about the potential of moringa leaves as a functional food that could help prevent UTIs.

Previous studies have shown that moringa leaf decoctions offer numerous health benefits. For example, they have been proven to reduce blood glucose levels in diabetic patients (Waruwu et al., 2022), lower blood pressure in elderly patients with hypertension (Antika, 2021), and decrease uric acid levels in the elderly (Laksono, 2025). Based on the MIC results in this study, moringa leaves show potential as an antibacterial agent against *E. coli*, a primary cause of UTIs. Therefore, moringa leaves could be used as an alternative for UTI prevention, such as in the form of decoctions. It is recommended to prepare them by boiling 40 grams of fresh moringa leaves (about 4 stalks) in 100 ml of water.

Following this study, it is recommended that future researchers: Conduct further testing using the Minimum Bactericidal Concentration (MBC) method. Perform MIC tests using moringa leaf extract concentrations between 10-20%. Apply the disk diffusion method using moringa leaf extract on *E. coli* isolated from UTI patient urine. Test the inhibitory effect of moringa leaf extract against other bacteria found in the urine of UTI patients, aside from *E. coli*.

Conclusions

Based on the research conducted, it can be concluded that moringa leaf extract has inhibitory activity against *E. coli* isolated from the urine of UTI patients, as tested using the MIC method, with effective concentrations at 20%, 30%, and 40%. The lowest concentration that successfully inhibited *E. coli* growth was 20%.

Author contributions

DATS, WO and NTK contributed to the study's concept and design. YN assisted with experimental studies and data acquisition. WO managed the literature search, data analysis, and statistical analysis. All authors participated in manuscript preparation, with DATS and NTK responsible for editing and review. All authors have read and agreed to the published version of the manuscript.

Aknowledgements

The authors would like to express their gratitude to the Director of Poltekkes Kemenkes Kupang, the Head of the DIII Medical Laboratory Technology Study Program at Poltekkes Kemenkes Kupang, the person in charge of the Bacteriology Laboratory of the DIII TLM Study Program at Poltekkes Kemenkes Kupang, and St. Carolus Borromeus Hospital Kupang.

Funding

There is no specific funding for this study.

Ethical approval statement

The study received ethical approval from the Ethics Committee of Poltekkes Kemenkes Kupang with certificate number No.LB.02.03/1/0034/2025.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

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