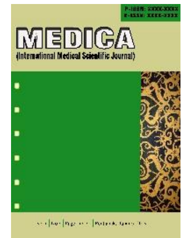
MEDICA

(International Medical Scientific Journal)
Vol.6, No.3, October 2024, pp.115 – 120
ISSN 2622-660X (Online), ISSN 2622-6596 (Print)
https://journal.ahmareduc.or.id/index.php/medica



The Antibacterial Effectiveness of Moringa Oleifera Leaves Extract Against the Growth of Escherichia coli Using the Diffusion Method

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Info Article

Article History:
Received:
14 August 2024
Accepted:
19 September 2024
Published:
30 October 2024

Keywords:
Moringa oleifera leaves
Escherichia coli
Diffusion Method

Abstrak

Moringa oleifera leaves a common herbal plant found in tropical regions and naturalized in Indonesia, are known to be rich in bioactive compounds such as flavonoids, tannins, terpenoids, alkaloids, and saponins. These compounds have potential medicinal properties, including antibacterial activity. This study aims to examine the antibacterial effectiveness of moringa leaf extract against the growth of Escherichia coli using the diffusion method. This descriptive-analytical research employed a purposive sampling technique with varying concentrations of moringa leaf extract: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Each concentration was tested in triplicate, resulting in a total of 30 samples. The antibacterial effectiveness test was conducted using the agar diffusion method with blank paper disks. The results showed that no inhibition zones were formed at concentrations of 10% and 20%. Inhibition zones began to appear at 30% concentration (7.3 mm), increasing with higher concentrations: 40% (9 mm), 50% (10.3 mm), 60% (11.3 mm), 70% (16.6 mm), 80% (23.3 mm), 90% (28.3 mm), and reaching 34.6 mm at 100% concentration. Based on the antibacterial activity category, 10% and 20% showed no activity, 30% and 40% were categorized as weak, 50% and 60% as moderate, 70% as strong, and 80%, 90%, and 100% as very strong. It can be concluded that an 80% concentration of moringa leaf extract is the most effective in inhibiting the growth of Escherichia coli.

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1. INTRODUCTION

Traditional medicinal plants are natural formulations traditionally used for treatment based on empirical knowledge. The rich diversity of medicinal plants supports the availability of ready-to-use traditional medicines. The use of medicinal plants to treat various diseases in communities has been preserved and passed down through generations. This knowledge has long been possessed and utilised by local communities, especially in the use of moringa leaves (Jumiarni & Komalasari, 2017).

Moringa leaves are an herbal plant commonly found in tropical regions and have been naturalised in Indonesia. The Indonesian community has long used moringa as both food and medicine. Apart from being used as a vegetable, moringa leaves are traditionally employed to treat purulent abdominal swelling by boiling them with mung beans and drinking the decoction every morning. Fresh moringa leaves are also used to treat ringworm. Other medicinal uses of moringa include acting as a diuretic for gonorrhea, a remedy for vomiting, and a treatment to alleviate hangovers (Ekawati, Singga, & Waangsir, 2022).

Despite being easily found in home gardens, the use of moringa plants has not been fully optimized. Moringa contains phytochemical compounds, and phytochemical screening results show that moringa leaves contain chemical components such as flavonoids, tannins, terpenoids, alkaloids, and saponins. These compounds have medicinal benefits such as skincare, anti-inflammatory, antihypertensive, antifungal, anticancer, and antibacterial properties. Furthermore, the high antioxidant activity in moringa leaves is attributed to active compounds like vitamin B6, vitamin B2, vitamin C, vitamin A, iron, and magnesium. Moringa also contains quercetin, a strong antioxidant known to help lower blood pressure (Santi, Yasa, & Nugroho, 2022). Due to these components, Moringa oleifera L. leaves contain compounds that can inhibit bacterial growth, including Escherichia coli.

Escherichia coli is a Gram-negative, rod-shaped bacterium. It can cause intestinal infections with symptoms such as diarrhea, abdominal pain, vomiting, and fever. These infections are generally treated with antibiotics such as sulfonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline, and aminoglycosides. The most commonly used antibiotic is ampicillin; however, ampicillin is ineffective against Pseudomonas, Klebsiella, and Enterococci. Based on existing data, E. coli has shown resistance to ampicillin, making it no longer viable for treatment. Therefore, safer alternative treatments are needed—one of which is utilizing beneficial plants like moringa leaves (Jaipah, Saraswati, & Hapsari, 2017).

A study conducted by Dima et al., (2016) showed that moringa leaf extract (Moringa oleifera L.) at concentrations of 5%, 10%, 20%, 40%, and 80% had antibacterial activity ranging from moderate to strong. The largest inhibition zone was observed at 80% concentration (21.50 mm for Staphylococcus aureus and 24.00 mm for Escherichia coli), while the smallest was observed at 5% (11 mm for Staphylococcus aureus and 12 mm for Escherichia coli). The aim of this study is to evaluate the antibacterial effectiveness of moringa leaf (Moringa oleifera L.) extract against the growth of Escherichia coli using the agar diffusion method.

2. METHOD

This study employed a descriptive-analytic design, aiming to explain and describe the characteristics of each research variable. The study focused on presenting the percentage distribution of each concentration variation and categorized the antibacterial activity based on the diameter of the inhibition zones. Descriptive data such as the minimum, maximum, and average values, as well as the category and percentage of inhibition zone diameters across different concentrations, were calculated based on the results obtained by the researcher.

Data collection was carried out after measuring the width of the inhibition zones (in millimetres), specifically the distance between the outermost edge of the paper disk soaked with moringa extract and the Escherichia coli colonies. The test was conducted using concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, applied on the surface of Mueller Hinton Agar (MHA) medium. A ruler was used to measure the diameter of the clear zones.

The collected data were then analysed using descriptive statistics. The inhibition zones were categorised based on their diameter into four levels of antibacterial activity: weak, moderate, strong, and very strong. Additionally, the most effective concentration of Moringa oleifera leaf extract was determined based on the largest average inhibition zone (Notoatmodjo, 2018).

3. RESULTS AND DISCUSSION

This study used a total of 1,500 grams of moringa leaves. In each replication, 500 grams of leaves were pressed, yielding 75 mL of moringa leaf extract. The extract was prepared at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Each treatment was repeated three times, resulting in a total of 30 samples. An analysis was then conducted to determine the level of antibacterial effectiveness of the moringa leaf extract against the growth of *Escherichia coli* using the diffusion method.

Results of Moringa Plant Determination Test. The moringa leaves used in the study underwent a plant determination test to confirm that the sample belonged to the species *Moringa oleifera* Lam. and the family Moringaceae. The test confirmed that the leaves used in this study were indeed of the species *Moringa oleifera* Lam., family Moringaceae.

Results of Phytochemical Compound Identification in Moringa Leaf Extract. A qualitative phytochemical screening was conducted to identify active metabolite compounds in the moringa leaf extract (*Moringa oleifera* L.), which have the potential to act as antibacterial agents. The results of the phytochemical compound identification are shown in the following table:

Table 1. Phytochemical Screening Results of Moringa Leaf Extract

No	Test Parameter	Reagent	Test Result	Note
1	Saponin	HCl 2N	Persistent foam	+
2	Tannin	$FeCl_3$	Dark green coloration	+
3	Alkaloid	Dragendorff's reagent	Yellow precipitate	+
4	Flavonoid	HCl + Mg powder	Orange coloration	+
5	Terpenoid	Liebermann–Burchard	Brown coloration	–

1. Results of the Antibacterial Activity Test Against *Escherichia coli*

The following are the results of the antibacterial inhibition test of *Moringa oleifera* L. extract against *Escherichia coli*:

Table 2. Inhibition Zone Diameters of Moringa Leaf Extract Against *Escherichia coli* (Diffusion Method)

Replication	Control	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
R1	0	0	0	7	8	10	11	22	25	30	35
R2	0	0	0	8	9	11	12	13	22	26	34
R3	0	0	0	7	10	10	11	15	23	29	35
Min	0	0	0	7	8	10	11	13	22	26	34
Max	0	0	0	8	10	11	12	22	25	30	35

Average	0	0	0	7.3	9	10.3	11.3	16.6	23.3	28.3	34.6
Category		NA	NA	W	W	M	M	S	VS	VS	VS

Notes: NA = No Activity, W = Weak, M = Moderate, S = Strong, VS = Very Strong

Table 2 shows that inhibition zones began to appear after treatment with moringa leaf extract. Each concentration was tested in triplicate, and average inhibition zones were calculated. The results show that:

- 10% and 20% concentrations showed No Activity (NA)
- 30% and 40% were categorized as Weak (W)
- 50% and 60% were Moderate (M)
- 70% was Strong (S)
- 80%, 90%, and 100% were categorized as Very Strong (VS)

These findings can be interpreted into five levels: No Activity**,** Weak**,** Moderate**,** Strong**,** and Very Strong**.**

Table 3. Percentage of Inhibition Zone Categories of Moringa Leaf Extract Against *Escherichia coli*

Inhibition Category	No Activity (NA)	Weak (W)	Moderate (M)	Strong (S)	Very Strong (VS)
Percentage (%)	20%	20%	20%	10%	30%

This table indicates that:

- 20% of samples showed no antibacterial activity,
- 20% showed weak activity,
- 20% showed moderate activity,
- 10% showed strong activity,
- 30% showed very strong antibacterial activity against *Escherichia coli*.

A total of 20% of the samples, specifically those at 10% and 20% concentrations, did not produce any inhibition zones, indicating no antibacterial activity. Another 20% of the samples at 30% and 40% concentrations showed weak inhibition zones, suggesting low antibacterial activity. Similarly, 20% of the samples at 50% and 60% concentrations demonstrated moderate effectiveness, indicating a partial ability to inhibit bacterial growth. Only 10% of the samples, represented by the 70% concentration, exhibited strong antibacterial activity. The highest proportion, 30% of the samples, came from the 80%, 90%, and 100% concentrations, which showed very strong antibacterial effects. These results highlight a clear correlation between increasing concentrations of moringa leaf extract and its effectiveness in inhibiting the growth of Escherichia coli.

DISCUSSION

Research results indicate that the juice extract of Moringa leaves (Moringa oleifera) has the ability to inhibit the growth of Escherichia coli bacteria. This is evident from the clear zones formed around blank paper discs that had been soaked in various concentrations of moringa leaf extract for 15 minutes. The moringa leaf extract has proven to be very effective in inhibiting E. coli, as higher concentrations result in larger inhibition zones. From the research findings, the inhibitory effect was categorized as follows: no inhibition at 10% and 20%, weak inhibition at 30% and 40%, moderate inhibition at 50% and 60%, strong inhibition at 70%, and very strong inhibition at concentrations of 80%, 90%, and 100%. The 80% concentration was found to be the most effective in inhibiting E. coli, as the inhibition zone formed was categorized as very strong. Similar findings were reported by Dima et al. (2016), which also demonstrated that moringa leaves can inhibit E. coli with a very strong category. The presence of inhibition zones at each concentration of moringa leaf extract is attributed to the presence of active compounds such as tannins, alkaloids, saponins, and flavonoids, which act as the primary antibacterial agents.

According to Sovia et al. (2022), flavonoids are secondary metabolites from the polyphenol group that are water-soluble and naturally found in plants, with bioactive properties such as antiviral, antibacterial, and anti-inflammatory effects. The antibacterial mechanism of flavonoids involves three actions: inhibiting cell membrane functions, inhibiting nucleic acid synthesis, and inhibiting energy metabolism. The interaction between flavonoids and bacterial DNA can lead to permeability damage in bacterial cell walls, microsomes, and lysosomes. Tannins also serve as antibacterial agents by inhibiting reverse transcriptase and DNA topoisomerase enzymes, which prevents bacterial cell formation. Tannins are believed to shrink the bacterial cell wall or membrane, thereby disrupting cell permeability (Putra et al., 2016).

Alkaloids are structurally diverse natural compounds with various biological activities. These basic nitrogen-containing heterocyclic compounds are thought to interfere with the peptidoglycan components of bacterial cells, preventing the proper formation of the bacterial cell wall and leading to cell death (Meigaria, Mudianta, & Martiningsih, 2016). Alkaloids have long been sourced from plants and used in medicines, with well-known examples including morphine, quinine, strychnine, and cocaine (Rivai, 2020). Saponins exhibit a range of biological activities and beneficial pharmacological effects, including antibacterial action. They disrupt microbial cell membrane permeability, causing membrane damage and leakage of important intracellular components such as proteins, nucleic acids, nucleotides, and others. Saponins are also known for their anticholesterolemic, anti-inflammatory, antiparasitic, antibacterial, and antiviral properties (Sharma & Paliwal, 2012).

4. CONCLUSION

Based on the research results, it was found that the average inhibition zones of moringa leaf juice (Moringa oleifera L.) at concentrations of 10% and 20% showed no inhibition zone, 30% had an average of 7.3 mm, 40% had 9 mm, 50% had 10.3 mm, 60% had 11.3 mm, 70% had 16.6 mm, 80% had 23.3 mm, 90% had 28.3 mm, and 100% had 34.5 mm. The antibacterial activity of moringa leaf juice (Moringa oleifera L.) in inhibiting Escherichia coli at concentrations of 10% and 20% showed no inhibition zone, 30% and 40% were categorized as weak, 50% and 60% as moderate, 70% as strong, and 80%, 90%, and 100% as very strong. Therefore, the most effective concentration in inhibiting Escherichia coli using the diffusion method is 80%.

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