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Original Article

Antimicrobial effect of *Moringa oleifera* leaves extract against *Escherichia coli*

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ABSTRACT

At present, the use of medicinal plants has increased in the society as a different approach in seeking remedies to avoid adverse effects generated from synthetic chemicals. One of the popular medicinal plants in Indonesia, especially West Timor is *Moringa oleifera*. *Moringa* plants are known as the most valuable multipurpose tree as all parts of this plant can be processed into food, medicine, cosmetics, and even water purifiers (seeds). This study aims to investigate the antimicrobial potential of *M. oleifera* leaves against *Escherichia coli*. Various concentrations of 2%, 5%, 10%, 25%, 50%, 75%, and 100% of the ethanol extract of *M. oleifera* leaves were prepared and screened for antimicrobial activity using agar well diffusion assay against *E. coli.* Comparison with principle antibiotic, minimum inhibitory concentration (MIC) and qualitative phytochemical screening were undertaken to achieve optimal antimicrobial activity. The result of present study showed inhibition zone; 7.167 mm, 8 mm, 8.33 mm, 9 mm, 9 mm, 9.66 mm, and 11.33 mm, respectively, from the lowest concentration of the ethanol extracts. The MIC was determined at 2%. The qualitative phytochemical screening revealed the presence of flavonoid, tannins, saponins, and alkaloids. In conclusion, ethanol extracts of *M. oleifera* leaves exhibit potential antibacterial activity against *E. coli*.

Keywords: Antimicrobial, minimum inhibitory concentration, *Moringa oleifera*

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INTRODUCTION

Moringa oleifera is one of the plants that are widely used as medicinal purpose in Indonesia, especially in West Timor. *Moringa* plants is multipurpose due to the all parts of this plant that can be used as something useful, such as can be processed into food, medicine, cosmetics, and even water purifiers (seeds). This plant can also grow in various climates, although classified as tropical plants.[1]

The widely use of antibiotics in society to inhibit bacterial growth may lead to antibiotic resistance. Therefore, alternative active ingredients from medicinal plants are needed to be used as antibacterial agent.[2,3] The antibacterial activity can be tested and determined through several methods, including through the diffusion of liquid discs and dilution. *M. oleifera* leaves contain active ingredients such as flavonoids, alkaloids, phenolic compounds, and isothiocyanates, where these compounds are also found in other medicinal plants. The damage caused by the extract of *M. oleifera* to the membrane results in increased cell permeability and leakage, followed by the release of intracellular material.[4-6] Bacterial cell leaks can be caused by the destruction of the hydrophobic bonds of the components of bacterial cell membranes such as proteins, phospholipids, and components that are hydrophilically bound because they react with phenol, this results in increased cell membrane permeability and allows the entry of phytochemical compounds into bacterial cells, resulting in the release of cell substances such as proteins and nucleic acids which may lead to the death of bacteria. Thus, the identification of the bioactive components in medicinal plants, isolation, purification, and characterization of the active compounds by various analytical methods is essential.[7-9]

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MATERIALS AND METHODS

Sample Collection and Preparation

M. oleifera leaves were obtained from Liliba District, Kupang city, East Nusa Tenggara. The leaves harvested were rinsed with tap water, chopped, and air-dried until completely dried for 7 days. The dried leaves were stored until needed.

Extract Preparation

The dried *Moringa* leaves were blended and sifted into powder form. The sieve results were weighed and macerated with 70% ethanol 5 times the weight of the simplicia powder for 5 days. The ethanol extract was concentrated using the evaporator. The ethanol extract of *Moringa* leaves was stored in a sterile container at room temperature until needed.

Phytochemical Analysis

Phytochemical qualitative screening was carried out on the ethanol extract of *M. oleifera* leaves to detect the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and saponins.

Flavonoids

1 mg of the ethanol extract was taken and placed into a test tube, then added 1–2 drops of ethanol. Magnesium powder was also added along with 4–5 drops of concentrated HCl. A brownish, red, or orange color will form if there is flavonoids presence.

Saponins

1 mg of the ethanol extract was prepared mixed with water (1:1) and shaken vigorously for 1 min. If there is foam, then HCl 1 N is added to the mixture. The foam needs to last for 10 min with the height of 1–3 cm to confirm the presence of saponins.

Tannins

As much as 1 mg of the ethanol extract was inserted into a test tube, and then added 2–3 drops of 1% $FeCl_3$ solution. Color change was observed for dark blue or blackish green to depict the presence of tannins in the extract.

Alkaloids

1 mg of the ethanol extract was placed into a test tube and added 0.5 ml of 2% HCl. The mixture was then divided equally into two tubes. In the first tube, 2–3 drops of Dragendorf reagent were placed and in the second tube 2–3 drops of Wagner reagent were dropped. A positive sample of alkaloids is when a white precipitate forms on the Dragendorf reagent and brown deposits also forms in the Wagner reagent.

Ethanol Free Test

A total of 5 ml of *M. oleifera* leaves ethanol extract were prepared and added concentrated H_2SO_4 and glacial acetic acid. Color change was observed after sulfuric acid and potassium dichromate were added as well. Odor change in the form of an ester aroma was also observed. If there is no ester aroma and no color change obtained, then the extract could be confirmed does not contain ethanol.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of the MIC of the ethanol extracts was carried out based on turbidity. Various concentrations of 2%, 5%, 10%, 25%, 50%, 75%, and 100% of the extract were prepared and placed in different test tubes along with 0.1 ml of the bacterial suspension using a micropipette as well as 0.9 ml of nutrient broth. The mixtures were incubated for 24 h in the incubator. Turbidity was observed on the test tubes; the lowest concentration is expressed as the MIC value.

Antimicrobial Susceptibility Test

Antimicrobial activity of the *M. oleifera* ethanol extracts was conducted using the Agar-well diffusion method with *Escherichia coli* as the test microorganism. Mueller-Hinton Agar media were prepared and the bacteria were swabbed on the solid agar media. Aliquots of 0.2 ml of each concentration of the ethanol extract (2%, 5%, 10%, 25%, 50%, 75%, and 100%) were carefully dropped on the paper discs and placed carefully on the media. The media were incubated at 37°C for 24 h in the incubator. The ability of the various ethanol extracts concentration to inhibit the growth of *E. coli* was measured and reported as the diameter of the inhibition zone (cm).

RESULTS

The results in Table 1 demonstrated that the ethanol extract of *M. oleifera* leaves contained saponins, alkaloids, flavonoids, and tannins compound.

The MIC was determined at 2% concentration of the ethanol extract of *M. oleifera* leaves [Table 2].

Based on Table 3, it can be seen that the inhibition zone was found at the concentration of 2% to the concentration of 100%.

Table 1: Phytochemical components of the *M. oleifera* leaves extract

Concentrations of ethanol extract of *M. oleifera* leaves							
Phytochemical Components (%)	**100**	**75**	**50**	**25**	**10**	**5**	**2**
Alkaloids	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+

M. oleifera: *Moringa oleifera*

Table 2: The MIC of the *M. oleifera* leaves extract on the test bacteria

	Concentrations of ethanol extract of *M. oleifera* leaves								
Test Bacteria (%)	**100**	**75**	**50**	**25**	**10**	**5**	**2**	**Control (-) Water**	**Control (+) Cotrimoxazole**
E. coli	–	–	–	–	–	–	–	+	–

E. coli: *Escherichia coli*; MIC: Minimum inhibitory concentration; *M. oleifera*: *Moringa oleifera*

Table 3: Diameter (mm) of the inhibition zone of the *M. oleifera* leaves ethanol extract and the controls on *E. coli*

	Concentrations of ethanol extract of *M. oleifera* leaves								
Repetition	**Control (+)**	**100%**	**75%**	**50%**	**25%**	**10%**	**5%**	**2%**	**Control(-)**
I	27	12	10	9	9	9	8	7.5	0
II	26	11	10	9	9	8	8	7	0
III	26	11	9	9	9	8	8	7	0
MEAN	26.33	11.33	9.66	9	9	8.33	8	7.167	0

E. coli: *Escherichia coli*; *M. oleifera*: *Moringa oleifera*

DISCUSSION

The MIC of the extracts concluded that antibacterial effect was detected at low concentrations; which is at 2%. This supports the theory that *M. oleifera* has the capacity to cause cellular destruction of *E. coli*.[10] Furthermore, the agar diffusion method was able to determine that the ethanolic extracts of *M. oleifera* had an antibacterial effect against *E. coli*. Olson and Fahey (2011) reported that the antibacterial effect of *M. oleifera* might be due to the chemical compound 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy)-benzylisothiocyanate which involves the inhibition of essential cellular membrane enzymes.[7] The phytochemical compounds present such as alkaloids, saponins, tannins, and flavonoids supported the antimicrobial activity depicted by the ethanolic extracts of *M. oleifera*.[3] Further study in this field needs to isolate the potential antimicrobial substances.

CONCLUSION

This study has demonstrated an antibacterial effect of *M. oleifera* leaves ethanol extract against *E. coli* at low concentration. More research is required to explore the cytotoxicity the active components as well as the adverse effects that may adhere.

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CONFLICT OF INTEREST

There is no conflict of interests during this study.

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