Original Research Article

Effectiveness of Methanol Extract of *Moringa oleifera* Lam. Leaf as Antibacterial Drug to Bacterial Triggers of Urinary Tract Infections in Vitro

ABSTRACT

Background : Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus.* High recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections. This study aimed to measure the lowest concentration of *Moringa oleifera* Lam. Leaf that did not show any growth of the tested microorganisms.

Methods : The antibacterial activity test was adapted from the CLSI broth microdilution assay, with minor modifications, were used for the evaluation of the antibacterial activity of *M. oleifera* extracts against tested bacteria. Three concentrations higher than the MIC standard were cultured on the MHA. The lowest concentration on agar which was not found any growth of the bacterial colonies was determined as the MBC.

Results : The antibacterial activity test results showed that Moringa leaf extract could inhibit bacterial growth. Inhibition of bacterial growth based on extract concentration. For *E. coli* bacteria, the results showed the highest absorbance value at 100mg/ml concentration (2.86±0.02); even when diluted to 3,13 mg/ml (1.08±0.01), the moringa leaf extracts could inhibit the *E. coli* growth, although the value did not differ greatly from that of the negative control (0.13±0.06). The same as *E. coli,* the antibacterial activity test results showed that moringa leaf extract could inhibit others bacteria growth of UTIs.

Conclusions : Methanol extract of *Moringa oleifera* leaf can inhibit and bactericide the growth of urinary tract infections bacteria.

Keywords : Urinary tract infections, *Moringa oleifera* Lam., minimal inhibitory concentration, minimal bactericidal concentration

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INTRODUCTION

Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting 150 million people each year worldwide.[1] UTIs are one of the major causes of morbidity and

comorbidities in patients with underlying conditions, and it accounts for the majority of the reasons for hospital visit globally. Sound knowledge of factors associated with UTI may allow timely intervention that can easily bring the disease under control.[1,2] UTIs is known to cause short-term morbidity in terms of fever, dysuria, and lower abdominal pain (LAP) and may result in permanent scarring of the kidney. Urinary tract infections can be community acquired or nosocomial. Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices. Catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes.[3,4]

The majority of causative organisms of UTI are Gram negative bacteria in which *Escherichia coli* alone contribute to 80 percent of cases. *Proteus mirabilis, Klebsiella pneumonia*, and *Enterobacter aerogenes* are also involve in the pathogenesis of the disease. Gram-positive bacteria include *Staphylococcus saprophyticus* (10–15%), Enterococci, and Staphylococcus aureus (associated with calculi and catheterization). Microbiologically, UTI is defined as presence of at least 105 organisms/mL of urine in an asymptomatic patient or as more than 100 organisms/mL of urine in a symptomatic patient with accompanying pyuria (>5 WBCs/mL).[5] Conventional antibiotics are the first choice in an acute episode of UTI; therefore resistance of pathogenic bacteria to antibiotics is of high clinical concern.The concept of the control of drug resistance is rather widely held today. Several reports are available about the multidrug resistance of bacteria especially Staphylococcus, Pseudomonas, and Escherichia.[6,7]

Patients suffering from a symptomatic UTI are commonly treated with antibiotics; these treatments can result in long-term alteration of the normal micro-biota of the vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms. The availability of niches that are no longer filled by the altered microbiota can increase the risk of colonization with multidrug-resistant uropathogens. Importantly, the 'golden era' of antibiotics is waning, and the need for rationally designed and alternative treatments is therefore increasing.[8]

Moringa oleifera, an edible tree found worldwide in the dry tropics, is increasingly being used for nutritional supplementation. Leaves of *M. oleifera* have many phytochemical secondary metabolites of great pharmacological properties, such as alkaloids, flavonoids, saponins. All these metabolites were found to have antimicrobial properties. Many medicinal uses were also reported, various parts of this plant employed as anti-inflammatory, anti-hypertensive, antioxidant, hepato-protective, anti-diabetic and antimicrobial.[9,10]

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Studies on the antibacterial ability of *M. oleifera* have been carried out by several researchers, but there has been no study about antibacterial of *M. oleifera* extract against UTIs bacteria with microdilution method. Therefore, this study was aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of moringa leaf extract against UTIs bacteria.

MATERIALS DAN METHODS

Preparation and Extraction of Plant Material

The plant materials used for this study are the matured leaves of *Moringa oleifera* Lam. obtained from a naturist shop. The leaves were removed from the branches, washed properly with sterile distilled water and then air dried. Upon drying, the leaves were macerated in a sterile grinder into fine powdery form to produce the medicinal preparation. Extraction was performed as described elsewhere with minor modification. The powdered moringa leaves was percolated in 500 ml absolute methanol in one liter capacity conical flasks stopper and kept for two weeks with intermittent shaking. The percolates were filtered with Whatman no.1 filter paper. Then, it was concentrated at 40°C under reduced pressure using rotary evaporator which yields semi-solid residues. The semi-solid residues were left in Incubator at 40°C until totally dried (about two days). Dried extracts were reconstituted in 10% DMSO to make a concentration 200 mg/ml and kept in refrigerator at 4°C in dark well tight bottles until used in the antibacterial testing.[11]

Preparation of Tested Bacteria

Five referenced bacterial strains caused UTIs were used the collection of Department Microbiology, FK UKI, representing four Gram negative bacteria (*Escherichia coli* ATCC 25922, *Proteus mirabillis* ATCC 25933, *Klebsiella pneumonia* ATCC 27736, and *Pseudomonas aeruginosa* ATCC 27853) and one Gram positive bacteria (*Staphylococcus aureus* ATCC 25923). The bacterial cultures were inoculated into Muller Hinton Broth (MHB) media. Subsequently, it was incubated for 24 hours at 37 ^{o}C. Bacteria were inoculated into 0.9 ml NaCl solution as much as 3 ml. Then the turbidity was measured using a UV-Vis spectrophotometer at λ 600 nm.

Minimum Inhibitory Concentration (MIC):

The antibacterial activity test was adapted from the CLSI broth microdilution assay, with minor modifications. Brieflfly, the culture was diluted 1 in 50 with MHB to produce the MIC assay inoculum culture. Sterile broth was added (100 µL) to all wells of a 96-well plate (Nunc MicroWell NUN260860). The test extracts of moringa in each well were 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.13 mg/ml and 1.56 mg/ml (w/v)or control antibiotics (100 µL) were then added to the top row of each plate and 1 in 2 serial dilutions were prepared in each column. A growth control (without extract) and a sterile control (without inoculum) were included on each plate. The bacterial culture inoculum (100 µL) was then added to all wells except the sterile control wells and the plates were incubated at 37 °C for 24 h. After that, the absorbance of antibacterial activity was read at 600 nm in an automatic microplate ELISA reader (Bio-Rad Model 550, California). The antibiotic

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ciproloxacin was tested in parallel on each test occasion as a positive control for Gram negative bacteria, and antibiotic clindamycin for Gram positive bacteria.[12,13]

Determination of the Minimum Bactericidal Concentration (MBC)

MBC was determined for each set of wells from MIC determination, a loopful of broth was collected from those wells, which did not show any growth and inoculated on sterile Muller Hinton Agar (MHA). The methanolic extract dilutions were added into each well of a 96-well microtiter plate, and then the bacterial suspension was inoculated. The plate was incubated for 24 hours at 37°C. In order to determine the minimum bactericidal concentration (MBC), aliquots were pipetted out of each well and seeded onto the agar. The sample petri dishes were incubated for 24 hours at 37°C and determined growth of bacteria colony.

Statistical analysis

The data obtained from the measurement results are tabulated and analyzed by measuring the disc test's resistance zone's diameter. The calculation of the extract inhibition effectiveness is calculated based on the equation.

RESULTS

This study investigated the ability of M. oleifera leaf extracts to inhibit the growth of some bacterial triggers of UTIs. The tested bacteria used were *E. coli, P. mirabillis, P. aeruginosa, K. pneumoniae and S. aureus*. Bacterial cell density was quantified by determining the optical density of all cultures at 600nm. Analysis of optical density data obtained for all experimental repeats showed the following mean (± standard deviation) OD values for the positive growth control wells: *E. coli* 0.80 (± 0.06); *P. mirabilis* 0.68 (± 0.01), *P. aeruginosa* 0.51 (± 0.13), *K. pnemoniae* 0.81 (± 0.01), and *S. aureus* 0.49 (± 0.03).

Testing of the MIC moringa leaf extracts using the microdilution method, where the optical density of all microtitre plate wells was determined at 600nm using a spectrophotometer microplate reader. For *E. coli* bacteria, the results showed the highest absorbance value at 100mg/ml concentration (2.86±0.02); even when diluted to 3,13 mg/ml (1.08±0.01), the moringa leaf extracts could inhibit the *E. coli* growth, although the value did not differ greatly from that of the negative control (0.13±0.06)(Figure 1). The same as *E. coli,* the antibacterial activity test results showed that moringa leaf extract could inhibit others bacteria growth of UTIs are presented in figure; *P. mirabillis* (figure 2)*, P. aeruginosa* (figure 3)*, K. pneumoniae* (figure 4) *and S. aureus* (figure 5). The minimum inhibitory concentration was considered as the minimum concentration of the extract that inhibited bacterial growth.

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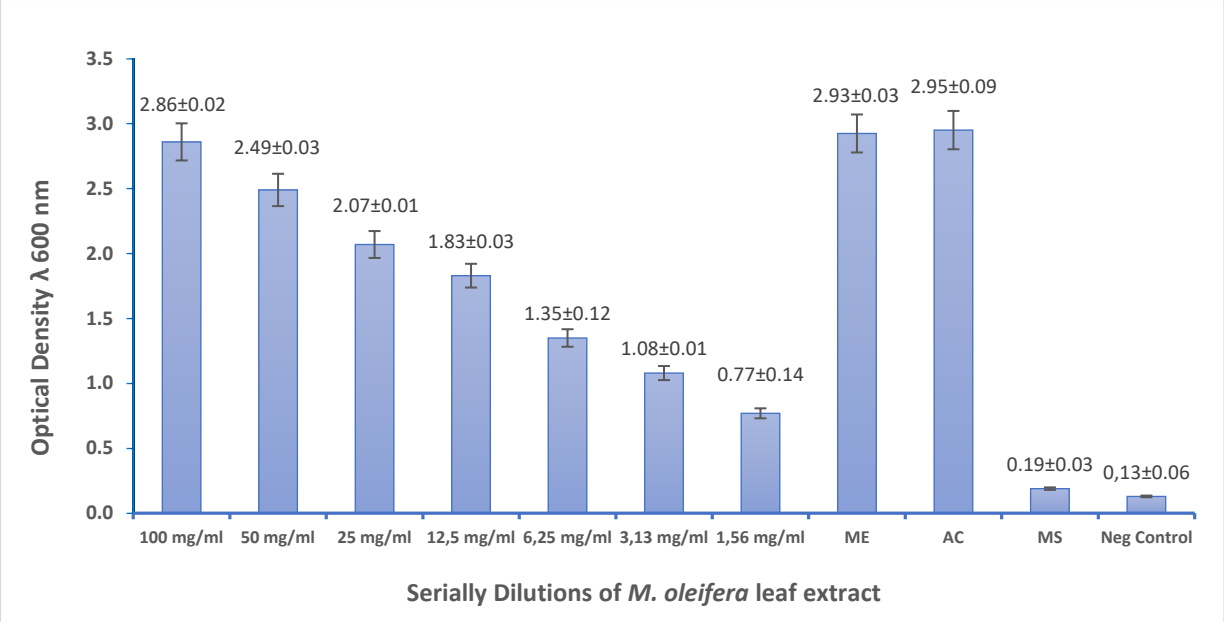

Figure 1. The measurements of optical density of moringa extract activity against *Escherichia coli*. ME (media + extract); AC (antibiotic control; ciprofloxacin); MS (media + solvent DMSO); Neg Control (Media MHB).

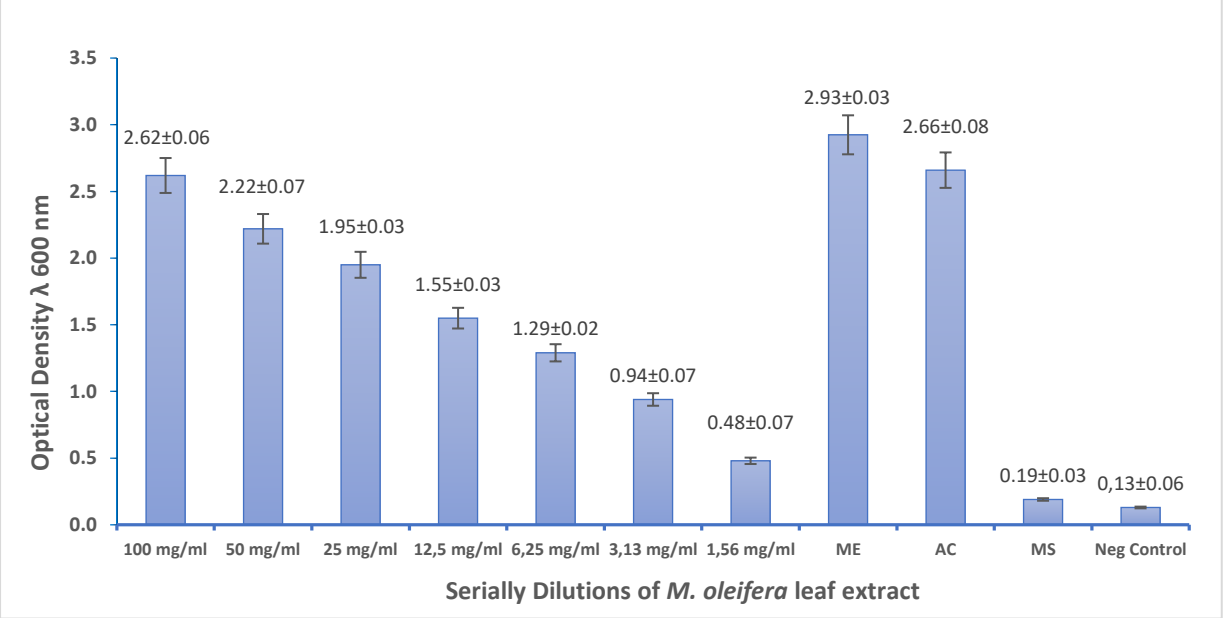

Figure 2. The measurements of optical density of moringa extract activity against *Proteus mirabilis*. ME (media + extract); AC (antibiotic control; ciprofloxacin); MS (media + solvent DMSO); Neg Control (Media MHB).

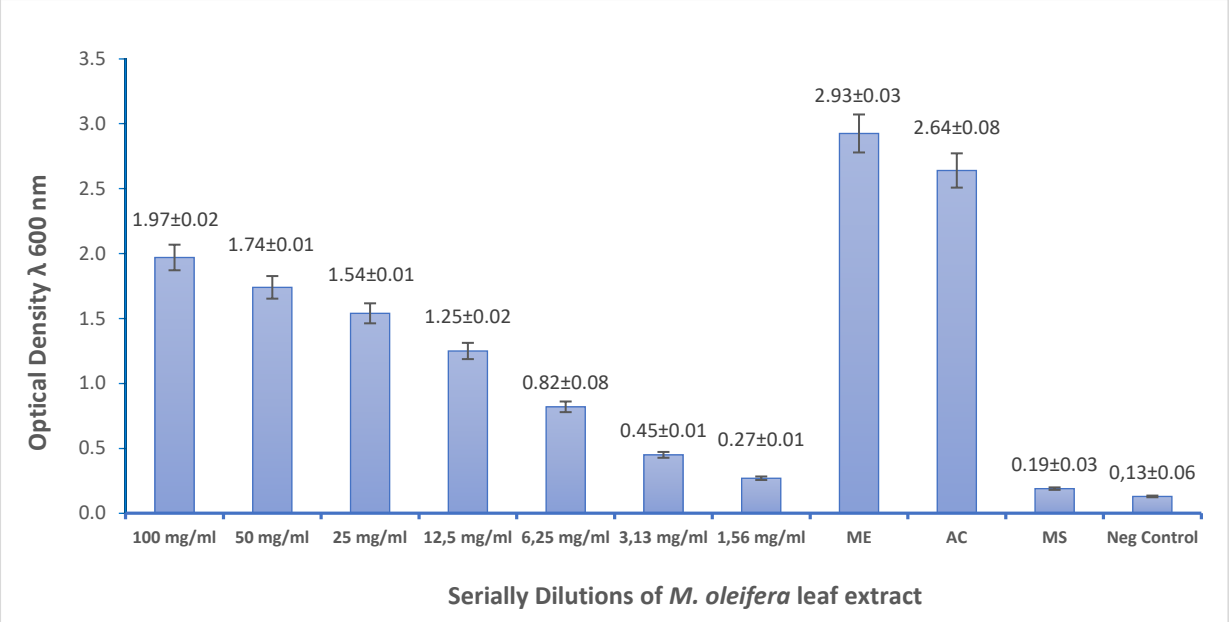

Figure 3. The measurements of optical density of moringa extract activity against *Pseudomonas aeruginosa*. ME (media + extract); AC (antibiotic control; ciprofloxacin); MS (media + solvent DMSO); Neg Control (Media MHB).

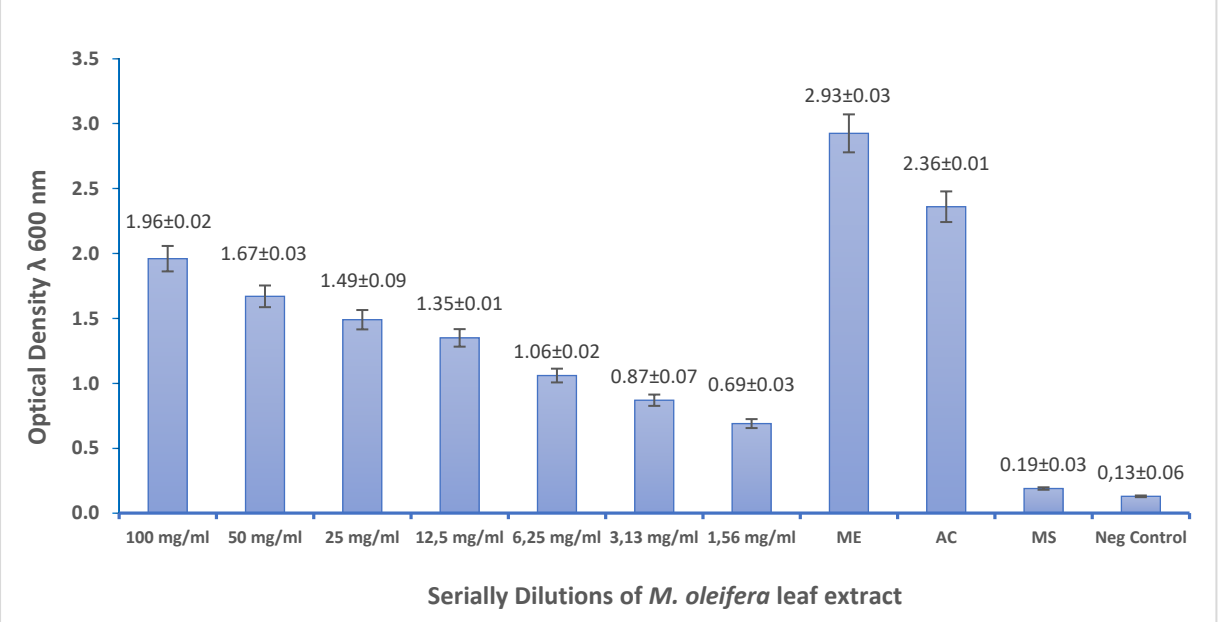

Figure 4. The measurements of optical density of moringa extract activity against *Klebsiella pneumoniae*. ME (media + extract); AC (antibiotic control; ciprofloxacin); MS (media + solvent DMSO); Neg Control (Media MHB).

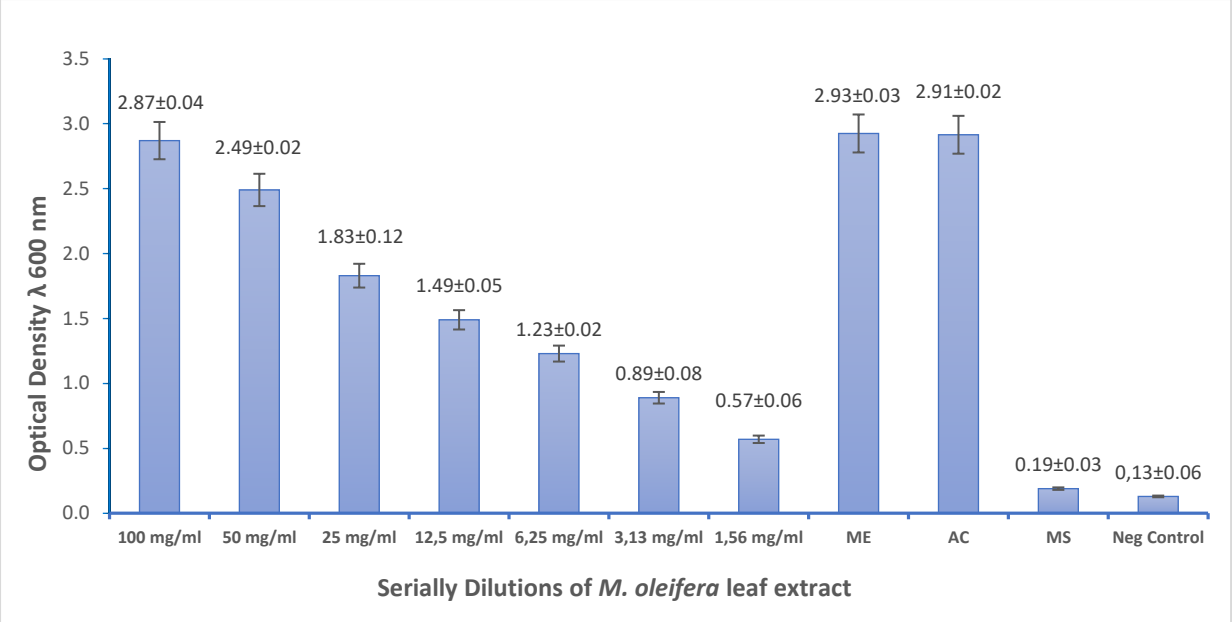

Figure 5. The measurements of optical density of moringa extract activity against *Staphylococcus aureus*. ME (media + extract); AC (antibiotic control; clindamycin); MS (media + solvent DMSO); Neg Control (Media MHB).

The MBC is defined as the minimum concentration required to inhibit any bacterial growth. The test results showed that the methanol extract of moringa had bactericidal activity against the tested bacteria is presented in table 1.

Table 1. The results of MBC of moringa extract activity against tested bacteria.

Conc. (mg/ml)	**Tested Bacteria**				
	E.coli	*P.mirabilis*	*P. aeruginosa*	*K. pneumoniae*	*S.aureus*
100 mg/ml	-	-	-	-	-
50 mg/ml	-	-	MBC	MBC	-
25 mg/ml	-	MBC	-	-	MBC
12.5 mg/ml	MBC	-	-	-	-
6.25 mg/ml	-	-	-	-	-
3.13 mg/ml	-	-	-	-	-
1.56 mg/ml	-	-	-	-	-

The percentage of antibacterial activity of moringa extract against tested bacteria is presented in table 2. At concentration of 100 mg/ml methanol extract of moringa can inhibit the growth of *E.coli* bacteria by 96.95%; at the same concentration, moringa extract inhibit *P. mirabilis* growth up to 98.49%; *P. aeruginosa* (74.42%); *K. pneumoniae* (83.05%); and *S. aureus* (98.63%).

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Table 2. The percentage of moringa extract activity against tested bacteria.

Conc. (mg/ml)	% Inhibition				
	E.coli	*P.mirabillis*	*P. aeruginosa*	*K.pneumoniae*	*S.aureus*
100 mg/ml	96.95%	98.49%	74.62%	83.05%	98.63%
50 mg/ml	84.42%	83.46%	65.91%	70.76%	85.57%
25 mg/ml	70.17%	73.31%	58.33%	63.14%	62.89%
12.5 mg/ml	62.03%	58.27%	47.35%	57.20%	51.20%
6.25 mg/ml	45.76%	48.49%	31.06%	44.92%	42.27%
3.13 mg/ml	36.66%	35.34%	17.05%	36.86%	30.58%
1.56 mg/ml	26.10%	18.05%	10.23%	29.24%	19.59%

DISCUSSION

The use of medicinal plants in the treatment and prevention of various diseases including UTIs is a very ancient practice. Due to easy availability, fewer reported side effects, cost-effectiveness, tolerance towards the patients with UTIs, and lack of bacterial resistance, herbal remedies are regaining more and more popularity and reliability worldwide. Given the growth of resistance to antibiotics, more and more researchers are assessing the antibacterial properties of various plants and their constituents.[14] Most of these researches are carried out *in vitro* to determine the effectiveness of the antibacterial activity of specific plant extracts against uropathogenics (Ups), to isolate the active phytocompounds, to green-synthesize nanoparticles and test their antibacterial properties on UPs, to study the synergy between the plant extracts and conventional antibiotics, and to determine the minimum inhibitory concentration (MIC) and bactericidal inhibitory concentration (MBC).[15] MIC of an antimicrobial extract was determined using broth serial dilution technique as was done in this study, antimicrobial substance was diluted several times using tube test contained of nutrient compound and then reacted with the pathogenic bacteria. The tube test then incubated, the growth of pathogenic bacteria was detected using spectrophotometer 600 nm. Concentration on the tube test which showed the bacterial growth increases dramatically expressed as MIC.

This study investigated the ability of M. oleifera leaf extracts to inhibit the growth of some bacterial triggers of UTIs. The antibacterial activity test results showed that Moringa leaf extract could inhibit bacterial growth. Inhibition of bacterial growth based on extract concentration. The results showed that increasing the concentration of the methanol extracts increased the absorbance of inhibition. The medicinal plant *Moringa oleifera* exhibits good antibacterial activity against*, S. aureus, P. mirabilis, E. coli, K. pneumoniae, and P. aeruginosa* in this study.

The effectiveness of the inhibition of Moringa leaf extract against UTIs bacteria is caused by the content of bioactive compounds in Moringa leaf extract, which can damage the protein synthesis system, damage to the cell wall, which causes lysis resulting in cell wall damage that can interfere with the mechanism of bacterial cell wall synthesis. Inhibition of bacterial

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growth from alkaloid antimicrobial substances by inhibiting enzymes that play a role in the DNA replication process. Inhibition of DNA replication will cause bacteria to not be able to divide so that it inhibits bacterial growth. Meanwhile, the alkaloids contained in the extract can interfere with the formation of cross-bridges of peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not fully formed and causes certain cell death.[7,16]

Research of Antibacterial Activities of *Moringa oleifera* leaf extract by Fadia *et al*, showed that the extract is active against bacterial isolates, whereas the inhibitory effect of the isolate is dose depending, where higher activity was clear by dose 200 mg/L. Also, the sensitivity of the bacterial isolate to the extract differs whereas *Klebsiella* is more sensitive to the extract with average zone 3.73 mm while *E. coli* less sensitive by average zone of inhibition 3.47 mm at a maximum concentration 200mg/L in comparison with a control.[17]

Conclusions

UTIs are very common in most countries of the world and very current in women and the elderly. The treatment of these pathologies which uses conventional antibiotics is increasingly hard given the growing resistance to antibiotics. Medicinal plants have various advantages because they are safe, economical, and easy to use and their main advantage is that bacteria have not yet developed resistance against them.

In summary, at presentase the data shows that *M. oleifera* Lam. can potentially used for antibacterial of UTIs. Accuracy and sensitivity should be explored in larger study using in vivo method.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis. 2001; 183 (Suppl 1):S1–S4. [PubMed: 11171002].
2. Odoki M, Alieora AA, Tibyange J, Maniga JN, Wampande E, Kato CD, *et al*. Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. International Journal of Microbiology. 2019; Article ID 4246780, 8 pages.
3. Motse DFK, Ngaba GP, Foko LPK, Ebongue CO, Adiogo DD. Etiologic profile and sensitivity pattern of germs responsible for urinary tract infection among under-five children in Douala, Cameroon: a Hospital-Based Study. Avicenna J Clin Microbiol Infect, 2019a; 6(2):49–56.
4. D. Prakash and R. S. Saxena, "Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in Urban Community of Meerut City, India," *ISRN Microbiology*, vol. 2013, Article ID 749629, 13 pages, 2013.

5. K. Parveen, A. Momen, A. A. Begum, and M. Begum, “Prevalence of urinary tract infection during pregnancy,” *Journal of Dhaka National Medical College & Hospital*, vol. 17, no. 2, pp. 8–12, 2011.
6. D. Debasmita, M. C. Sahu, S. Rath, B. P. Paty, N. K. Debata, and R. N. Padhy, “Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria,” *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 2, supplement, pp. S846–S854, 2012.
7. Maurya SK, Singh AK. Clinical Study: Clinical Efficacy of *Moringa oleifera* Lam. Stems Bark in Urinary Tract Infections. International Scholarly Research Notices. 2014, Article ID 906843, 7 pages.
8. Subashchandranose S, et al. Host-specific induction of *Escherichia coli* fitness genes during human urinary tract infection. Proc Natl Acad Sci USA. 2014; 111:18327–18332. [PubMed: 25489107].
9. Mgbeahuruike A.C, Edeh G, Eze CS, Parker J, Ekere SO, Kanu OO, *et al*. Comparative evaluation of the antimicrobial profile of *Moringa* leaf and seed oil extracts against resistant strains of wound pathogens in orthopedic hospitals. *Afr. J. Microbiol. Res*. 2017; 11(39): 1484-1494.
10. Dzotam JK, Touani FK, Kuete V. Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosoma mafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria. *BMC Complement. Altern. Med*. 2016; 16(9): 1-8.
11. Ilanko P, McDonnell PA, Vuuren Sv, Cock ID. Interactive antibacterial profifile of Moringa oleifera Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflflammatory diseases. South African Journal of Botany. 2019; 124: 420-435.
12. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 11th Edition. CLSI document M07-A11. Clinical and Laboratory Standards Institute, Wayne, PA, USA. 2018.
13. Eloff J. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1988; 64: 711–713.
14. Shaheen G, Akram M, Jabeen F, Ali Shah SM, Munir N, Daniyal M, Zainab R. Therapeutic potential of medicinal plants for the management of urinary tract infection: a systematic review. Clin Exp Pharmacol Physiol. 2019; 46(7):613–24.
15. Arsene MMJ, Viktorovna PI, Davares AKL, Esther N, Urinary tract infections: Virulence factors, resistance to antibiotics, and management of uropathogenic bacteria with medicinal plants—A review. Journal of Applied Pharmaceutical Science. 2021; 11(07): 001-012.
16. Ernawati and Sari, K., 2015. Kandungan senyawa kimia dan aktivitas antibakteri ekstrak kulit buah alpukat (*Persea americana* P.Mill) terhadap bakteri *Vibrio alginolyticus*. *Jurnal Kajian Veteriner*, *3*(2), pp.203-211.
17. Taufik TM, Rashed A, Oshkondali STM, Alacrouk SA,Sleman K. Antibacterial Activities of Moringa oleifera Leaf Extract on Some Human Pathogenic Bacteria. Saudi J Med Pharm Sci. 2021; 7(8): 426-431.

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