Original Article

Multidrug Resistant- Escherichia Coli – Use of Plant Extracts as Alternative Treatment in Urinary Tract Infection

Use of Plant Extracts as Alternative Treatment in UTI

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ABSTRACT

Objective: To evaluate the in-vitro antibacterial activity of Moringa Oleifera (MO) and Murraya Koenigii (MK) leaf extracts against Multidrug resistant- Escherichia coli.

Study Design: In-vitro experimental study.

Place and Duration of Study: This study was conducted at the Ziauddin University from December 2022 to May 2023.

Methods: Extraction of MO and MK leaves was done by using rotary evaporator. Antibacterial activity of both the plants was evaluated by using Broth Dilution assay and Agar well diffusion assay.

Results: Concentrations from 7.812 mg/ml to 500mg/ml were made in 10% DMSO for both the extracts. No significant antibacterial activity was observed against MDR-E. coli at any tested concentration.

Conclusion: MO and MK leaf extracts were found to be inactive against MDR-E. coli isolated from urine samples of UTI.

Key Words: MIC, MDR-E. coli, UTI, Moringa Oleifera, Murraya Koenigii

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INTRODUCTION

Bacterial growth in a sterile urine culture from an affected person with signs and symptoms of frequent urination, burning sensation and dark colored urine is classified as a urinary tract infection (UTI). UTI is one of the most common bacterial diseases, according to data females are more likely to acquire UTI than adult males due to the fact that microorganism readily penetrate the bladder through the shorter woman urethra. It has been reported that Escherichia coli and Klebsiella Pneumoniae are the most prevalent causative organisms for causing resistant UTI throughout Pakistan[1].

Though the occurrence of multidrug resistant (MDR) E. coli and MDR K. pneumoniae strains has increased, however their antibacterial susceptibility pattern differs from country to country. Antibiotic resistance has been reported as global health concern that has rendered many antibacterial treatments ineffective. In Pakistan, due to the indiscriminate use of antibiotics resistance has been reported against beta lactam antibiotics particularly in treating UTIs[2]. The development of resistance by MDR E.Coli and MDR K.pneumonia has limited the use of broad spectrum antibiotics like Piperacillin-Tazobactam, Aminoglycosides and the last resort antibiotic Colistin. Keeping the above scenario, there is a pertinent need to explore alternative treatment options for these resistant bacteria that could be economical, possess optimal efficacy, good tolerability, and have fewer side effects[3].

Moringa Oleifera **(**MO) leaves possess anti-bacterial, antifungal, antipyretic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, anticancer, hepatoprotective, cardiac and circulatory stimulants activities[4], and Murraya Koenigii (MK) leaf called 'karri patta', has been reported to possess anti-diabetic, antioxidant, antimicrobial, anti-fungal, anti-inflammatory, anti-hypertensive, hepatoprotective, anti-hypercholesterolemia, anti-cancer, anti-diarrheal, analgesic, wound healing properties and it was found to be good for oral health as well with no known side effects[5]. Considering their antibacterial properties reported against various organisms this work was designed to check the antibacterial activity of leaves of MO and MK against MDR E.coli.

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METHODS

It was an in vitro pre-clinical experimental study conducted at Ziauddin University from December 2022 to May 2023. A total of 40 isolates per group were calculated for evaluation of antibacterial activity. The experimental samples of MDR-E. coli were collected from Microbiology Laboratory, Ziauddin Hospital. Plant Extraction was performed by using rotary evaporator. Minimum inhibitory concentration MIC was evaluated by broth dilution assay and Agar well diffusion methods. Samples of UTI showing growth of MDR-E. coli were included. Agar plates with growth of other organisms were excluded. The study got exemption from Ethical Review Committee of Ziauddin University.

Plant Collection and Authentication: Fresh MO and MK leaves were purchased from the commercial market of Karachi. The plants were washed and air dried in a shaded area for 2 weeks. Voucher number 96827 was allotted to MO specimen & 96826 was allotted to MK specimen from Herbarium, University of Karachi.

Preparation of MO Leaf and MK Leaf Extract: Air dried MO and MK leaves (500 g) were pulverized mechanically into fine particles. The powder residue (50g) was extracted by soaking in 500 mL of 80% ethanol into stoppered flask, kept for 48hrs with intermittent pulsating, the suspensions were filtered using clean filter paper (Whatman no.1), solvent was removed by evaporation using a rotary evaporator and stored in air tight bottles. Plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to make a stock solution[6].

Isolation of Bacteria from Clinical Specimens: Isolates were identified by Gram stain, microbiological analysis on MacConkey medium, and biochemical testing.

Identification of MDR e. Coli by Modified Kirby-Bauer Disk Diffusion Test: Isolates were identified as multidrug resistant on showing resistance to ≥ 1 antibiotic in ≥ 3 different antibiotic groups using Ampicillin (10 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Cefuroxime (30 µg) and Gentamicin (10 µg) antibiotic discs. The bacterial colonies were also swabbed on Mueller Hinton agar plates and incubated at 37 ºC for 24 hours[7].

BROTH DILUTION ASSAY

MIC values of MOLE and MKLE against the test organisms were determined using the broth dilution method (CLSI 2018**).** MOLE and MKLE stock solutions (200 mg/ml) were reconstituted by mixing 2.5 g leaf extract with 12.5 ml sterile water. To obtain varied concentrations of the stock, the retrieved extract was serially diluted 2-fold in Mueller Hinton broth. From stock solutions of leaf extracts, 3 concentrations were prepared in 3 marked test tubes, resulting in 2.5 ml broth with extract concentrations of 500 mg/ml, 250 mg/ml and 125 mg/ml. For the procedure, the bottle was filled with 0.1 mL of each organism's inoculum. The MH broth served as negative control. The bottles were properly labeled and incubated at 37°C for 24 hours before being inspected for visible turbidity. The MICs were computed as the lowest concentration that inhibited the test organisms from growing[8]. Experiment was performed in triplicates. The data was reported as the presence or absence of turbidity in the falcon tubes.

Agar Well Diffusion Method: Agar well diffusion method was performed to investigate antibacterial efficacy of MOLE and MKLE against MDR E. coli. The fresh inoculums of MDR bacterial isolates compared with 0.5 McFarland standard were spread uniformly on the surface of MHA plates. Using sterile cork borer, wells of 7mm diameter were punched into the inoculated plates, 50µl of different concentrations of MOLE and MKLE (500, 250, 125, 62.5, 31.25, 15.625,7.81 mg/ml) were loaded to labeled wells with the help of micropipette. DMSO (10%) was used as negative control. The plates were left for diffusion of extract for 15 minutes and were incubated for 24 to 48 hours at 37°C. After 48 hours presence or absence of zone of inhibition was examined. The zones of inhibition of MOLE and MKLE were measured and compared using a diameter scale[9].

RESULTS

A) Results of the Broth Dilution Assay: Figure 1 and 2 represents the results of Broth Dilution Assay for MOLE and MKLE against MDR-E.coli the Experiment was performed in triplicates. A total of 3 concentrations (500 mg/ml, 250 mg/ml and 125 mg/ml) were evaluated for each plant extract. MOLE- The falcon tubes F1, F2 and F3 had a concentration of 500mg/ml , G1, G2 and G3 had a concentration of 250mg/ml and H1, H2 and H3 had a concentration of 125mg/ml for MOLE .MKLE- The Falcon tubes A1, A2 and A3 had a concentration of 500mg/ml, B1, B2 and B3 had concentrations of 250mg/ml whereas C1, C2 and C3 had a concentration of 125mg/ml for MKLE. All three tubes of both plant extracts F, G, and H for MOLE and A, B, C for MKLE were found to be turbid indicating negative results. Furthermore, negative control group didn't show activity.

Results of the Agar Well Diffusion Method: Figure No.3 indicates the results of Agar well diffusion assay. The MDR-E.coli strains were evaluated at 7 concentrations from 7.81, 15.62, 31.25, 62.5, 125, 250 and 500 mg/ml made for both the extracts in 7 labelled wells, while the 8th tube contain the DMSO as negative control. Both the extracts didn't inhibit the growth of MDR-E.coli at any tested concentration. Negative Control showed no antibacterial activity. Experiment was performed in triplicates.

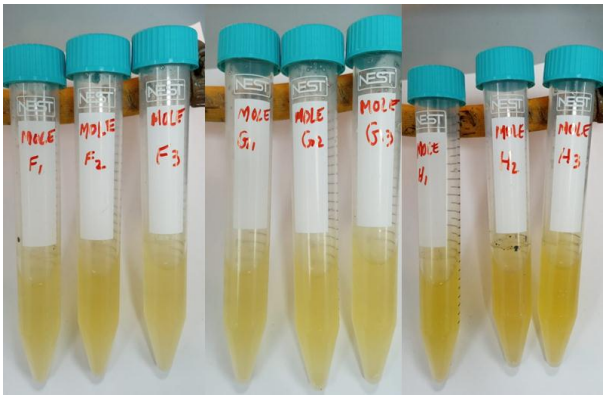

Figure No. 1: Turbidity in falcon tubes-broth broth dilution assay for mole

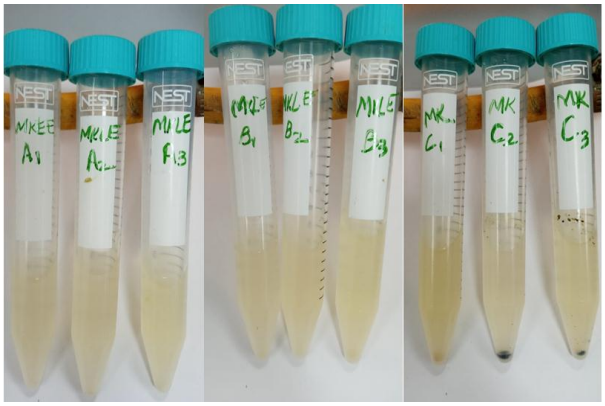

Figure No. 2: Turbidity in falcon tubes-broth dilution assay for mkle

Table No. 1: Zoi of Mole for Mdr E.Coli:

Sr. No.	Concentration of plant extract used (mg/ml)	ZOI (mm)	ZOI (mm)	ZOI (mm)
1.	500	N/D	N/D	N/D
2.	250	N/D	N/D	N/D
3.	125	N/D	N/D	N/D
4.	62.5	N/D	N/D	N/D
5.	31.25	N/D	N/D	N/D
6.	15.625	N/D	N/D	N/D
7.	7.8125	N/D	N/D	N/D
8.	Control	N/D	N/D	N/D

*N/D=No detection of anti-bacterial activity

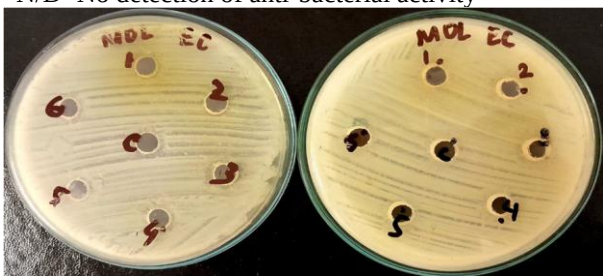

Figure No .3: Antibacterial activity (zois) of different concentrations of mole against mdr e.coli using agar well diffusion method

Table No.2: Zoi of Mkle for Mdr E.Coli:

Sr. No.	Concentration of plant extract used (mg/ml)	ZOI (mm)	ZOI (mm)	ZOI (mm)
1.	500	N/D	N/D	N/D
2.	250	N/D	N/D	N/D
3.	125	N/D	N/D	N/D
4.	62.5	N/D	N/D	N/D
5.	31.25	N/D	N/D	N/D
6.	15.625	N/D	N/D	N/D
7.	7.8125	N/D	N/D	N/D
8.	Control	N/D	N/D	N/D

*N/D=No detection of anti-bacterial activity

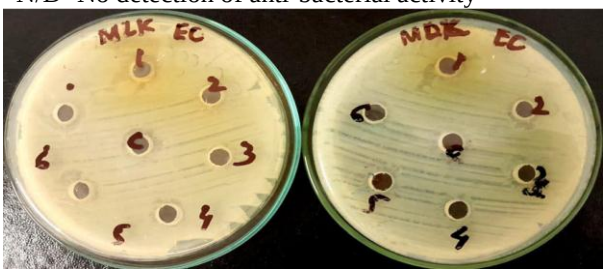

Figure No. 4: Antibacterial Activity of Different Concentrations of Mkle against Mdr e.Coli

DISCUSSION

Medical science has substantiated plants as the possible sources of drugs to inhibit and treat human infections. The World Health Organization has documented antimicrobial resistance as a global health security threat that needs action through government sectors and the general public entirely. Alarmingly, MDR pathogens are now considered a general warning signal due to their wide range of problems especially in developing countries like Pakistan[10]. In view of the above details, there is a need to explore new antimicrobial agents derived from medicinal plants to address the growing threat from these pathogenic microbes. In this study, it was tested against MDR E. coli on two edible plants, MO and MK.

Since primordial times, plant derived medications have been consumed for their valuable antimicrobial activity. The utility of plant extract and its mixture with standard drugs compensate the resistance mechanism against various microbes[11].

In contrast to the results of our study on MDR E. coli numerous studies have shown that medicinal plants are able to produce a variety of bioactive compounds that potentially inhibit the growth of this human pathogenic bacterium. In a study conducted in Bangladesh using the broth dilution assay, green tea extract showed potent inhibitory effects against gram-negative MDR pathogens[12].

Similarly, in a study conducted in India, 11 plants were tested for antibacterial activity against MDR E. coli, among 11 plants, only Punica granatum showed potential antibacterial activity

Similar to our study, a study conducted at the University of the Netherlands found that MO has moderate to no antibacterial activity in vitro [13]. In accordance to our findings a study conducted in Nigeria investigated the antibacterial effect of amygdalin vernonia leaf extracts on MDR E. coli bacterial isolates. They reported that MDR E. coli has resistance to amygdalin Vernonia extract at all concentrations. The resistance was supposed to be due to the presence of drug-inactivating phytochemical enzymes in E. coli[14].

However, according to Hala et al, in 2020, the antibacterial activity of MO was remarkable, suggesting that the bioactive compounds of this extract are not substrates of bacterial efflux pumps against MDR bacteria, which does not support our conclusions[15]. Likewise, one more study showed positive antibacterial activity of Murraya koenigii against E. coli, which is not in accordance to our results[16].

Various mechanisms of resistance (alteration in penicillin-binding proteins, drug modifications, mutant drug targets, increased expression of the efflux pump, and altered membrane permeability) have been reported in multidrug-resistant bacteria. This makes it increasingly difficult to kill the growing bacterial population by herbal extracts due to the presence of well-evolved resistance genes[17].

In addition, most UTIs and other resistant infections are associated with development of biofilm. Therefore there is an urgent need to search for potential biofilm inhibitors with a specific or multifocal mechanism of action[18]. Besides the resistance mechanisms mentioned above, in particular the efflux pumps, which could be responsible for the ineffectiveness of the two extracts against MDR-ECs, a second factor could be the formation of a strong biofilm by resistant bacteria. Also, the chemistry of the active metabolites contained in plant extracts can be destroyed by bacteria, further weakening the antibacterial effect[19].

Hence, a large number of ethno medical studies would be required to compare the antibacterial activity of plant extracts, since local factors such as environmental quality, climate change, a nd other environmental factors contribute to the genetic and physical changes in plants in different parts of the world. Therefore, a plant that is effective against an organism in one part of the world may not be effective in another part of the world, as also demonstrated in our study[20].

In order to counter such a resistant organism, it is highly recommended to combine these plant extracts with other plants and antibiotics to develop a new ecological medicine.

One of the study conducted in Nigeria claims that MOLE possess a versatile antibacterial activity against non-resistant E.Coli, having MIC of 25mg/ml and ZOIs 7.25[21]. Similarly for non-resistant E. coli ZOIs by MK leaf extract varied from 1.9mm at the range of concentration 100-300mg/ml.[22]. The evolution in structure and function of MDR organism has made the point that in past the herbal extracts which were effective against non-resistant E. coli are not effective against MDR E. Coli.

CONCLUSION

MO and MK leaf extracts were found to be inactive against MDR-E. coli isolated from urine samples of UTI.

Author's Contribution:

Concept & Design of Study:	Humaira Arif
Drafting:	Akhtar Ali, Muhammad Owais Ismail
Data Analysis:	Faisal Iqbal Afridi, Tanveer Abbas, Sehrish Mahmood
Revisiting Critically:	Humaira Arif, Akhtar Ali
Final Approval of version:	Humaira Arif

Conflict of Interest: The study has no conflict of interest to declare by any author.

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