Original Article

Detection of In-Vitro Antibacterial Activity of Tinospora Cordifolia and Solanum Nigrum Leaf Extracts against Multidrug Resistant- Escherichia Coli

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ABSTRACT

Objective: It was to determine the in-vitro antibacterial activity of ethanolic leaf extracts of Tinospora cordifolia (TC) and Solanum nigrum (SN) against Multidrug resistant- Escherichia coli (MDR-E.coli).

Study Design: In-vitro experimental study.

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

Materials and Methods: TC and SN leaves were extracted using a rotary evaporator. Antibacterial sensitivity of bothextracts was determined by Agar well diffusion assay and the Broth Dilution assay.

Results: Total 8 concentrations from 3.90 to 500 mg/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: The leaf extracts of Solanum nigrum and Tinospora cordifolia were ineffective against MDR- E.coli of DFI.

Key Words: DFI, antibacterial activity, MIC, MDR- E.coli, Solanum nigrum leaf extract, Tinospora cordifolia leaf extract

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INTRODUCTION

DFI refers to an infection that occurs in the soft tissue or bone located beneath the malleoli in patients who are experiencing uncontrolled diabetes. DFIs encompass a diverse array of dermal infections from superficial infections to persistent osteomyelitis. These infections can result in grave complications, notably gangrene, potentially necessitating amputation of the lower extremities.[1]The microorganisms responsible for DFIs typically consist of gram-positive cocci in the initial acute phase, whereas the wound mainly contains a combination of gram-negative aerobes and anaerobes in the chronic stage [2]. Majority of these causative bacteria are aerobes, such as S. aureus, E. coli and anaerobes e.g., Clostridium perfringens. etc[3].

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It has been reported that among the various gram negative bacteria isolated from DFIs, MDR-E.coli are predominantly found. [4 5]

Antibiotic resistance is a major worldwide health concern that has rendered many antibacterial treatments ineffective. [6] Exudate from DFI increase the likelihood of colonization of damaged skin by various microbes.[7] Theworldwide medicinal market is dominated by herbal treatments due to large range of secondary metabolites. Crude extracts of many plants are being used therapeutically as these are inexpensive and safe too.

SN (black nightshade) is a Solanaceae plant. It is widely recognized for its role as an anti- inflammatory agent and also used in the management of several ailments like cancer[8,9]. Antibacterial property of leaf, stem and root extracts of SN has been validated by Devi G.B et.al as it was highlighted that SN possesses antibacterial activity against the isolated test organisms (E.coli, S.aureus) with ZOI ranging from 7 to 30 mm[10]. Additionally TC has been utilized for centuries to treat a wide range of diseases; furthermore its antibacterial properties have also been reported[11]. Hence considering the reported antibacterial properties of these herbs, the purpose of the current study was to evaluate antibacterial activity of TCLE and SNLE against MDR-E.coli isolated from DFIs.

MATERIALS AND 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 assessed for evaluation of antibacterial activity. The experimental samples of MDR-E.coli were collected from Microbiology Laboratory, Ziauddin Hospital. Rotary evaporator was used to achieve plant extraction. Minimum inhibitory concentration was determined by Agar well diffusion method and the broth dilution assay. Pus samples displaying MDR-E.coli growth were included. Agar plates that had grown additional organisms were disregarded. This research was granted exemption from Ethical Review Committee of Ziauddin University.

Plant Collection and Authentication: Fresh TC & SN leaves were purchased from the commercial market of Karachi. The dust was washed and then the leaves were air dried in a shaded area for 2 weeks. Voucher number 97677 and 97676 was allotted to TC & SN specimen respectively [12].

Preparation of Tcle and Snle: Air dried TC leaves and SN 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 and was kept for 48hrs with intermittent pulsating. After that, the suspensions were filtered using clean filter paper (Whatman no. 1) and then solvent was removed by evaporation using a rotary evaporator and stored in air tight bottles for further studies. Plant extracts were dissolved in 10% DMSO to produce stock solution.[13]

Isolation of Bacteria from Clinical Specimens: The isolates were identified using Gram staining, microbiological analysis using MacConkey culture medium, and biochemical tests. [14]

Identification of Mdr-E.Coli By Modified Kirby-Bauer Disk Diffusion Test: Isolates were identified as MDR on showing resistance to ≥ 1 antibiotic in ≥ 3 different antibiotic groups. For E.coli, Ampicillin (10 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Cefuroxime (30 µg) antibiotic discs were plated and the bacterial colonies were then cultured on MHA plates and incubated at a temperature of 37 ºC for 24 hours.[15,16]

Broth Dilution Assay: MIC values of TCLE and SNLE against the test organisms were determined using the broth dilution method (CLSI 2018). TCLE and SNLE 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 MH 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. After correct labeling, the bottles were subjected to incubation at 37°C for a full day before being checked for obvious turbidity. The MICs were computed as the lowest concentration that inhibited the test organisms from growing (turbidity). This experiment was performed in triplicates. The data was reported as the presence or absence of turbidity in the falcon tubes. [14]

Agar Well Diffusion Method: Antibacterial property of TCLE and SNLE against MDR-E.coli was determined by this method. The bacterial strains, when compared with the McFarland standard, were evenly distributed across MH agar plates. Round wells, measuring 7mm in diameter were created, with the help of a septic cork borer, in the plates that had been inoculated.

50µl of different concentrations of TCLE and SNLE (500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90 mg/ml) were loaded to labeled wells with the help of micropipette. 10% DMSO was used as negative control. Agar plates were then incubated for 24 to 48 hours at 37°C. After 48 hours, ZOIs of TCLE and SNLE were measured using a diameter scale. [17]

RESULTS

(A) Results of the Broth Dilution Assay: The results of broth dilution assay of TCLE and SNLE against MDR-E.coli isolates can be seen in Fig. 1 and 2.Three concentrations (500 mg/ml, 250 mg/ml and 125 mg/ml) were evaluated for each extract. Tubes labelled as K1, K2 and K3 had a concentration of 500mg/ml of TCLE, L1, L2 and L3 had a concentration of 250mg/ml whereas M1, M2 and M3 had 125mg/ml of the same extract. Tubes labelled as P1, P2 and P3 had a concentration of 500mg/ml, Q1, Q2 and Q3 had a concentration of 250mg/ml, whereas R1, R2 and R3 had 125mg/ml of the same extract. Turbidity was seen in all three tubes K, L, M for TCLE and P, Q, R for SNLE, indicating negative results. Negative control didn't show activity.

(A) Results of the Agar Well Diffusion Method: Fig.3 & 4 indicates the findings of Agar well diffusion assay. The MDR-E.coli strains were evaluated at 8 different concentrations from 3.90 to 500 mg/ml made for both the extracts using 8 labeled wells while the 9th well had the negative control, DMSO. 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.

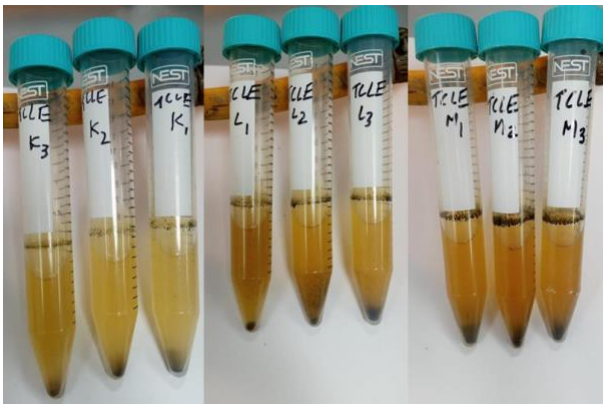

Figure No. 1: Turbidity in Falcon Tubes- Broth Dilution Assay For Tcle

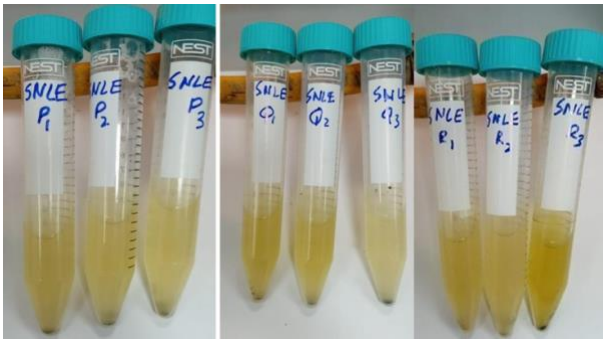

Figure No. 2: Turbidity in Falcon Tubes- Broth Dilution Assay For Snle

Table No.1: ZOI of TCLE for MDR-e-Coli

Sr. #	Concentration of plant extract used(mg/ml)	ZOI (mm)	ZOI (mm)	ZOI (mm)
1.	500	No activity	No activity	No activity
2.	250	No activity	No activity	No activity
3.	125	No activity	No activity	No activity
4.	62.5	No activity	No activity	No activity
5.	31.25	No activity	No activity	No activity
6.	15.625	No activity	No activity	No activity
7.	7.8125	No activity	No activity	No activity
8.	3.90	No activity	No activity	No activity
9.	Control	No activity	No activity	No activity

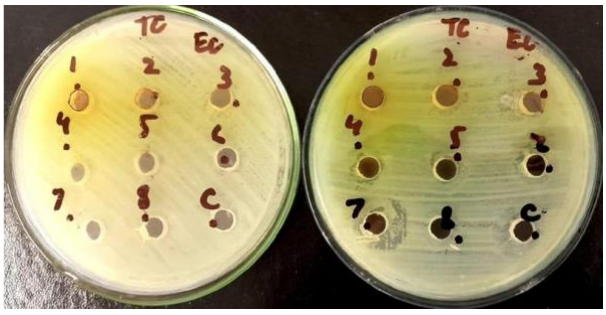

Figure No. 3: Antibacterial activity of different concentrations of Tcle against mdr-e.coli using agar well diffusion method

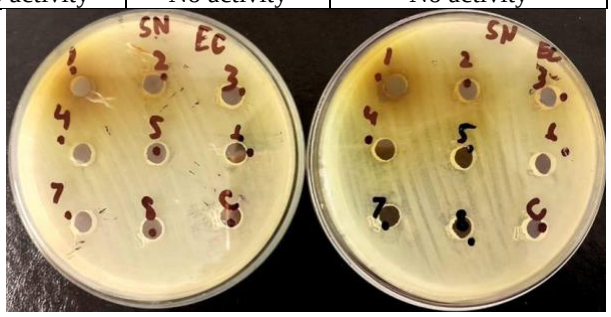

Figure No. 4: Antibacterial Activity of Different Concentrations of Snle Against Mdr E.Coli Using Agar Well Diffusion Method

Table No. 2: ZOI of SNLE For MDR-E.coli

S.No.	Concentration of plant extract used (mg/ml)	ZOI (mm)	ZOI (mm)	ZOI (mm)
1.	500	No activity	No activity	No activity
2.	250	No activity	No activity	No activity
3.	125	No activity	No activity	No activity
4.	62.5	No activity	No activity	No activity
5.	31.25	No activity	No activity	No activity
6.	15.625	No activity	No activity	No activity
7.	7.8125	No activity	No activity	No activity
8.	3.90	No activity	No activity	No activity
9.	Control	No activity	No activity	No activity

DISCUSSION

MDR bacteria have imposed socioeconomic calamity on a worldwide scale. Therefore, it is crucial to develop new antibacterial agents against these resistant pathogens.

A study conducted in Saudi Arabia in order to determine the antibacterial properties of Eucalyptus grandis extracts. The leaf extracts of the plant were screened against MDR-E.coli, MDR- P. aeruginosa and MRSA, using the agar well diffusion method. Except for the extract prepared from water, all the other extracts exhibited antimicrobial properties against the tested bacteria[18]. In a separate investigation, it was determined that the extracts derived from Andrographis paniculata possessed antibacterial properties against MDR-E. coli strains isolated from urine samples[19].

In contrast to some of the in-vitro studies mentioned above, in the present study TCLE & SNLE were ineffective against MDR- E. coli.

The bacterial isolates may have exhibited resistance as a result of the development of biofilms. The bacteria are protected by biofilms, which give them more resistance power and enable them to withstand harsh conditions as well as antibiotics, potentially developing bacteria that are MDR, extensively drug resistant, and fully drug resistant. The rate at which a wound heals may be dramatically impacted by biofilms. In light of this, even though the study did not investigate the involvement of biofilms in multi-drug resistance but they may be regarded as contributory factors.[20].

There exist numerous efflux mechanisms within the bacterium E. coli that are linked to the phenomenon of drug resistance. These mechanisms facilitate the expulsion of drugs and various other substances from bacterial cells [21] [22]. It is probable that these efflux pumps are liable for the decreased effectiveness of TCLE & SNLE used in the current study as the MDR-E. coli strains might have such resistance mechanisms.

Such resistant flora might have degraded the chemistry of the active metabolites present in the selected extracts as the chemical breakdown of antibacterial agents is an additional mechanism used by bacteria to fight against the antimicrobial agents**.** This might have caused the ineffectiveness of the selected herbs against the strains of MDR-E.coli in our study. Additionally, the ability of extracts to inhibit bacterial growth is also influenced by the combined effects of the chemical components.

The utilization of medicinal plants for research and therapeutic purpose can have an impact on the process of developing novel antimicrobials because of various inadequate agricultural methods, substandard standardization during the process of preparation, and unfavorable storage conditions.

Hence, the evaluation of the distinct literature information concerning the antimicrobial efficacy of botanical extracts might present challenges due to the fluctuation in the constitution of said extracts, which is contingent upon the regional climate and environmental circumstances.

Geographic regions experience significant variation in terms of rainfall and humidity, which may affect the active chemical constituents when these herbs are grown in various regions globally.[23].

CONCLUSION

TCLE & SNLE were found to be inactive against MDR-E.coli of DFI.

Author's Contribution:

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

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

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