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Preliminary Study on the Role of Medicinal Plant *Tinospora cordifolia* in Reducing Bacterial Load from Human Hand

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Abstract. Excessive and unregulated application of antimicrobial agents stands at the forefront as the major instigator for the development of bacterial strains exhibiting resistance to multiple drugs, commonly referred to as multi-drug resistance (MDR). In addition, synthetic drugs are more expensive for the treatment of diseases. Additionally, the cost of using synthetic medications to treat illnesses is much higher. Therefore, in order to control microbial infections, new infection-fighting techniques must be developed. The study was planned to evaluate the role of *Tinospora cordifolia* against various bacterial strains. In total of 500 samples were collected randomly from people working in university of Swabi and tested for presence of bacterial load. Three bacterial strains were isolated *i.e.*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*. The anti-bacterial profile of isolated strains was determined using *Ciprofloxacin*, *Tetracycline*, *Erythromycin*, *Ampicillin*, *Chloramphenicol* and *Amoxicillin*. The activity *Tinospora cordifolia* was evaluated against antibiotic resistant bacterial strains which showed inhibitory activity against various multi-drug resistance bacterial strains and these results were compared with already available antibiotics. The results of antibiotic profiling showed that the *Ciprofloxacin* and *Tetracycline* were most efficient against all strains with a largest zone of inhibition followed by *Chloramphenicol* while *Erythromycin*, *Ampicillin* and *Amoxicillin* show no zones of inhibition. Whereas the activity of the medicinal plant extract of *Tinospora cordifolia* showed inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.* It is concluded that the *Tinospora cordifolia* have antibacterial potential and uphold its replacement with of commonly used antibiotic drugs.

Keywords: antimicrobial, *Tinospora cordifolia*, medicinal plant, bacteria, pathogens

Introduction

Since ancient times, medicinal plants have been employed for health and disease treatment due to their minimal side effects. In developing countries more than 80% of the population relies on medicinal plants as source of medicine (Biswas *et al*., 2013). Medicinal plants serve as abundant reservoir of crucial compounds, showcasing a diverse array of biological capabilities. This renders them a valuable reservoir of various metabolites and an inexhaustible reservoir of antimicrobial agents (Oli *et al*., 2023). Extensive research has been conducted on the antibacterial properties of various plants to explore their antimicrobial potential and many of these have been utilized as alternatives in medicinal applications (Céline *et al*., 2009). There are now antimicrobials of plant origin that are helpful in treating infectious infections while also explaining many of the negative effects frequently connected to antimicrobials of synthetic origin (Joshi and Kaur, 2016). Based on the pharmaceutical research, many new promises of potential high yielding new antimicrobial agent have emerged.

The importance of *Tinospora cordifolia's* antimicrobial activity lies in its ability to combat infectious agents and contribute to the management of microbial related illnesses. Its diverse bioactive compounds, such as alkaloids, terpenoids and flavonoids which are believed to be responsible for its antimicrobial efficacy. Different plants can be used as natural medicine in form of crude extracts of plants. The plant is valued as immune modulator (Jagetia and Rao, 2006) anti-cancer (Johnson *et al*., 2011) antioxidant (Sivakumar *et al*., 2010), anthelmintic (drug used to treat infections of animals with parasitic worms) (Nagaprashanthi *et al*., 2012) antipsychotic and hypoglycaemic (Stanely *et al*., 2000).Various crude extracts of *T. cordifolia* were studied

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against enteric bacteria of respiratory tract pathogens and bacteraemia and used for skin infection. An aqueous extract of *T. cordifolia* condensed sneezing, allergic rhinitis, nasal obstruction and itching in an irregular clinical trial over eight weeks (Akhtar, 2010). The uniqueness of *Tinospora cordifolia* lies in its exceptional antimicrobial activity, setting it apart in the realm of medicinal plants. Extensive studies have revealed the distinctiveness of its bioactive compounds, showcasing potent antibacterial properties. The plant's antimicrobial efficacy extends across a spectrum of micro-organisms, making it a promising candidate for addressing diverse infectious agents. *Tinospora cordifolia's* novelty is not only attributed to its antimicrobial potency but also to its potential in serving as a source for developing novel therapeutic agents, presenting new avenues in the field of natural medicine (Oli *et al*., 2023)

The present study was designed to evaluate the antibacterial efficacy of *Tinospora cordifolia* against bacterial strain isolated from human hand. Gram negative and gram-positive bacterial strains were included in the study to evaluate antimicrobial activity of *Tinospora cordifolia* and compare its effect with standard drugs, in order to uphold its replacement with of commonly used antibiotic drugs.

Materials and Methods

Sample collection. A total 500 samples were collected from students and employees working at University of Swabi, Khyber Pakhtunkhwa, Pakistan. The sampling procedure include collection of finger expressions on petri plates containing nutrient agar (Sigma Aldrich) media and transported to the microbiology laboratory, University of Swabi and were screened for bacterial load on human hands. The petri plates were labelled with name, gender and department. The samples were stored at 4 °C for further analysis.

Isolation of bacterial strains. Primary screening was carried out using nutrient agar (Sigma Aldrich) medium to observe the presence of bacterial strains. All plates were placed in incubator at 37C° for 24 to 48 h for bacterial growth. After incubation colonies were marked on the bases of colony morphology. A single colony was picked from primary culture and spread it again on agar to get isolated colonies and obtained pure bacterial culture strain were obtained. Morphological characters were determined by gram staining and motility was determined by hanging drops method Gurave *et al*. (2015). individual colony was picked and transfer to new nutrient agar media. Plates were incubated at room temperature for 24 h. Pure culture needs two different medias for separation gram-negative bacteria were isolated on Macconkey (Liofilchem) agar and gram-positive bacteria was isolated by mannitol salt agar (Oxoid) (MSA).

Identification of bacterial isolates. Bacteria was identified on the basis of colony morphology, gram staining and biochemical characteristics.

Gram staining. A standard procedure of gram stain reaction was carried for all isolates and examined under 40x and 100x lenses. A total of 3 bacterial strains were characterized as gram negative and gram positive.

Biochemical analysis. All biochemical tests were performed according to standard protocols (Benson *et al*., 2001). Citrate utilization test, indole and catalase tests were performed.

Antibacterial susceptibility pattern of selected strains. By using the whole set Kirby and Bauer method (disk diffusion method) antibiotic susceptibility pattern of all selected bacterial strains were determined according to CLSI guidelines (2013).

Antibacterial activity of Tinospora cordifolia. *Tinospora cordifolia* plant was cut into small pieces after being washed with sterilized distilled water followed by boiling and filtration. This solution was then kept for a week in orbital shaker. Antibacterial activity was carried out through well diffusion method using nutrient agar plate. Stock solution of extract was prepared by mixing 1mL of extract with 3 mL of ethanol. A bore of 6mm in diameter was prepared and were loaded with plant extract solution and were place on bacterial lawn containing agar surface. All the plates were incubated for 24 h at 35-37 °C. After incubation period all the plates were examined and result was recorded (Gaudreau and Gilbert, 1997).

Results and Discussion

Sample collection and gender wise distribution. A total of 500 sample were collected from the human hands and transported to the microbiology laboratory, University of Swabi. From male students 235 samples were taken while 265 samples from female students of different departments at University of Swabi were collected (Table 1). The number rate recorded was 47% for male students and 53% for female students.

Table 1. Gender and percentage wise distribution of hand's sample collected from students

Gender	Number	Percentage
Male	235	47%
Female	265	53%
Total	500	100%

Isolation and characterization of bacterial population from hands. A total of 500 samples were collected through finger expressions on petri plates from students of different departments at University of Swabi. After incubation period 490 plates showed positive growth results, from each plate approximately and 5 bacterial strains were isolated whereas 10 plates showed no growth. A total of 3 pathogenic bacterial strains were tested. Among these bacterial positive plates known number of bacterial isolates characterized through biochemical analysis were *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* and *Citrobacter*. Out of total 500 specimens 417 (83.4%) isolates were tested positive for *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* and *Citrobacter*, while 83 (16.6%) isolates cultured on different media showed no growth. The bacterial strains were sub cultured and pure cultures of all isolates were maintained for further analysis (Fig. 1)

Antibiotic susceptibility pattern determination. Antibiotic susceptibility pattern was determined using six classes of antibiotics *i.e. Ciprofloxacin*, *Tetracycline*, *Erythromycin*, *Ampicillin*, *Chloramphenicol* and *Amoxicillin* all of the strains isolated from hands showed resistance to *Penicillin*-G. *E. coli* was found susceptible to *Tetracycline* only followed by *S. aureus* susceptible to *Ciprofloxacin* and *Tetracycline* (Fig. 2). Whereas *P. aeruginosa* was found resistant to all antibiotics. (Table 2).

Antibacterial activities of Tinospora cordifolia. The zone of inhibition of *Tinospora cordifolia* was study which vary from 4mm to 18mm (Table 3). *Tinospora cordifolia* showed maximum zone of inhibition against *S. aureus* followed by *P. aeruginosa* and *E. coli* (Fig. 3).

The antimicrobial activity of *Tinospora cordifolia* holds significant importance due to its potential therapeutic applications. *Tinospora cordifolia*, also known as giloy or guduchi, is renowned for its broad-spectrum antimicrobial properties. It has demonstrated effectiveness against various micro-organisms, including

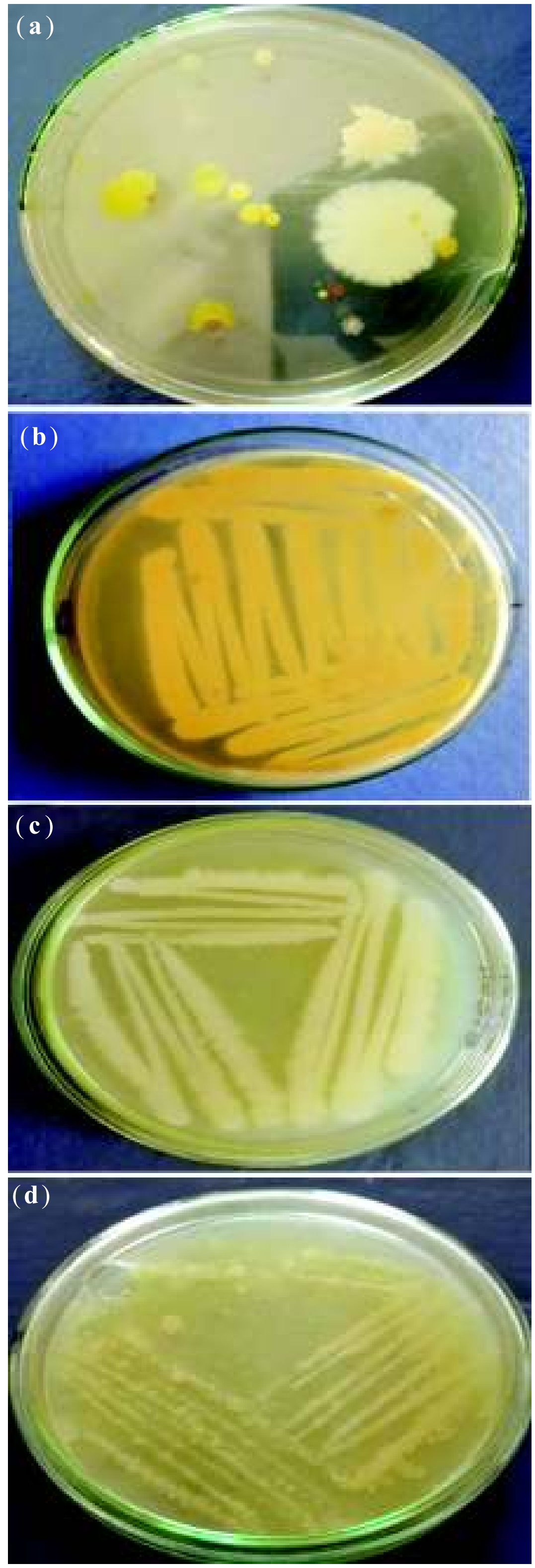

Fig. 1. Isolation and purification of bacterial strains. (**a**) Mix culture of bacterial isolates (**b**) *S. aureus* (**c**) *E. coli* (**d**) *P. aeruginosa*.

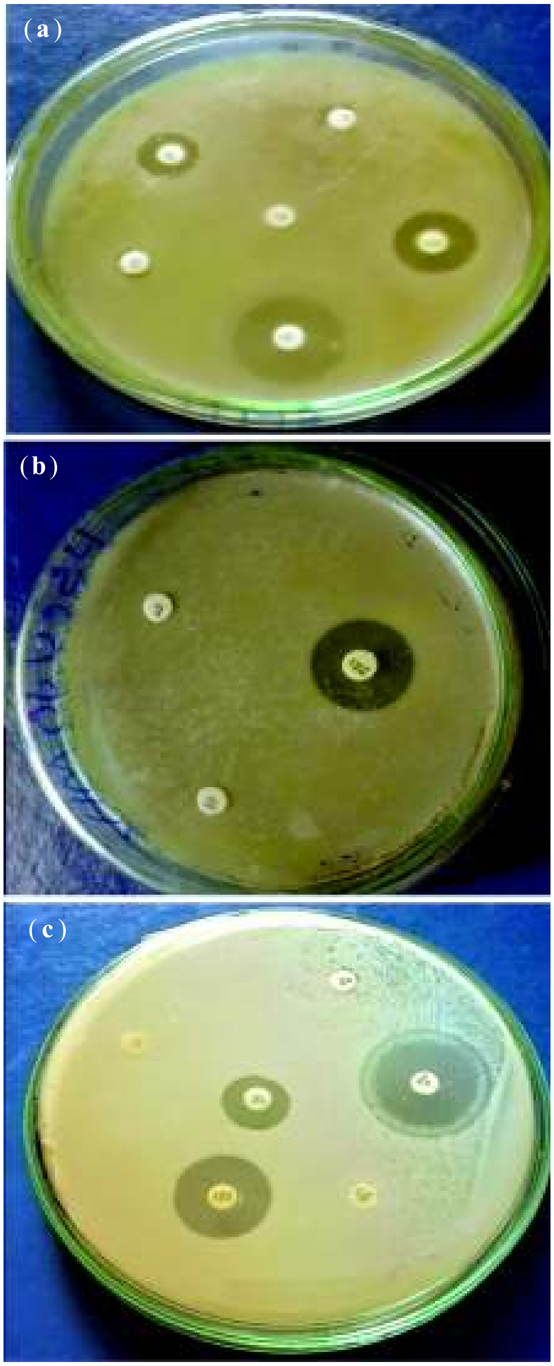

Fig. 2. Antibiotic susceptibility patterns of bacterial strains (**a**) *S. aureus* (**b**) *E. coli* (**c**) *P. aeruginosa*.

bacteria, viruses and fungi. Plants and their extracts have served as valuable components in pharmaceuticals, offering alternative medicines and natural therapies. Plant extracts, in particular, have shown promise as

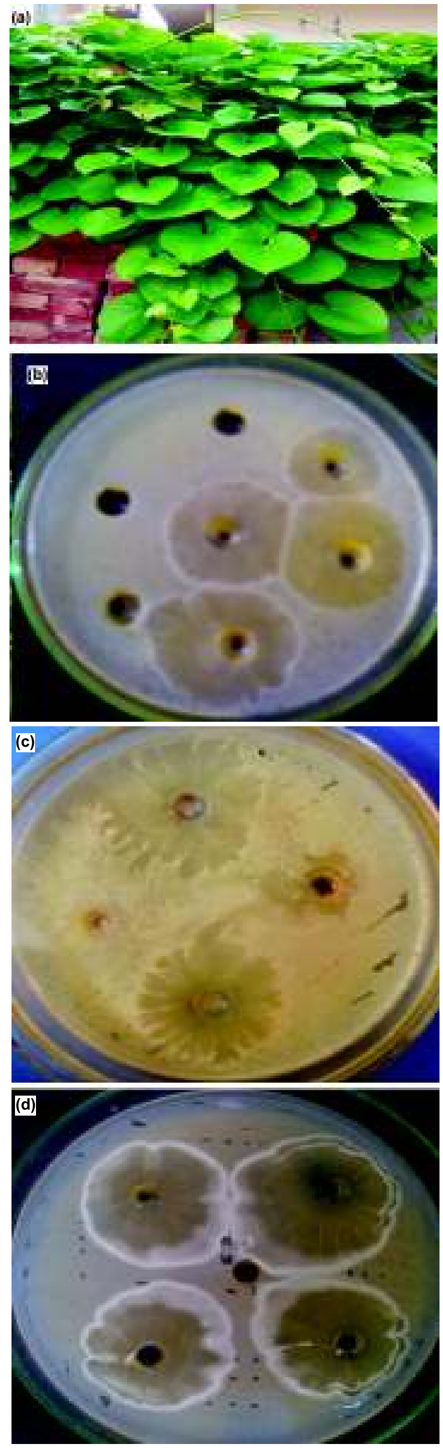

Fig. 3. Antibacterial activity of *Tinospora cordifolia* plant extract against pathogenic bacterial strains. (**a**) *Tinospora cordifolia* Plant; (**b**) Antibacterial activity against *S. aureus*, (**c**) Antibacterial activity against *E. coli*, (**d**) Antibacterial activity against *Pseudomonas aeruginosa*.

Table 2. Antibiotic Susceptibility pattern of bacterial strains isolated from hand (CLSI guideline 2013)

Antibiotics	Conc (µg)	*S. Aureus* zone (mm)	*E. coli* zone (mm)	*P. aeruginosa* zone (mm)
Ciprofloxacin	5	23.3 ± 0.6 (S)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)
Chloramphenicol	30	8.6 ± 0.4 (R)	0.0 ± 0.0 (R)	15.6 ± 0.2 (S)
Tetracycline	30	14.0 ± 0.5 (R)	23.0± 0.9 (S)	12.6 ± 0.6 (R)
Ampicillin	10	0.0 ± 0.0 (R)	na	0.0 ± 0.0 (R)
Amoxicillin	10	0.0 ± 0.0 (R)	na	0.0 ± 0.0 (R)
Erythromycin	15	0.7 ± 0.01 (R)	na	0.0 ± 0.0 (R)

Table 3. Antibacterial activity of *Tinospora cordifolia* against bacterial isolates

Medicinal plant	Conc. of stem extract	*S. aureus*	*P. aeruginosa*	*E. coli*	*Klebsiella*	*Citrobacter*
Tinospora cordifolia	1.5 ± 0.01 mL	18 ± 0.7 mm	16 ± 0.01 mm	12 ± 0.03 mm	4 ± 0.01 mm	8.7 ± 0.6 mm

reservoirs of novel antimicrobial compounds, especially effective against bacterial pathogens (Silva *et al*., 2016). The escalating issue of microbial resistance to existing antibiotics has led to treatment failures, heightened morbidity and mortality and a surge in healthcare costs (Friedman *et al*., 2016; Maragakis *et al*., 2008). Human hands generally harbour micro-organisms equally as part of standard micro flora and micro-organisms contacted from the environment. Microflora such as *Staphylococcus aureus* inhabitant be able to transfer from one individual to other, other microbes that are not inhabitant of the hands are come in contact from surfaces such as doorknobs or handles, toilet handles and taps in restrooms. In this research 500 samples were collected from students according to random statistical formula among which 417 isolates showed positive result for bacterial growth while rest of samples showed negative result. This recent research showed high percentage for female that is 53% and percentage for male students were 47%. The results of our study are in contour with (Noah, 2008), compared to males, females have extensively larger range of microorganisms on their hands. Their study showed that pH might play an important part in larger variety of bacteria on hands. Females skin is usually less acidic so bacteria easily grow on female skin, while males' skin have higher pH so bacteria on male hands are less because micro-organisms cannot easily grow in environment which is more acidic.

In this research, a substantial quantity of bacteria was isolated from the hands of students, with *Staphylococcus aureus* being the predominant species, followed by *E. coli*, *Pseudomonas*, *Klebsiella* and *Citrobacter*. Our findings align with these outcomes (Hedderwick *et al*., 2000), They isolated and identified four genera of bacteria from the hands, including *Staphylococcus aureus* spp. and *Pseudomonas* spp. Among the identified organisms, *Staphylococcus aureus* accounted for 41.7%, followed by *E. coli* at 74%. Another study demonstrated the presence of *Staphylococcus* spp. and *Escherichia coli* in all swab samples collected from the hands of students in both government and private schools. The results consistently indicate that *Staphylococcus* and *Escherichia coli* are part of the indigenous human skin flora. These human resident florae are latent pathogens of human beings. Comparative study of bacteria associated with the hands of nurses and homemakers (Aiello and Larson, 2003) resulted that *S. aureus* was present on hands of four nurses and 32 homemakers. Among 204 homemakers, five prevalent types of bacteria were identified on their hands including *Pseudomonas*, *Staphylococcus warneri*, *Klebsiella* and *Enterobacter*. Another study about hands flora was conducted which showed occurrence of hands flora and the effect of local plant *Tinospora cordifolia* effect against the hands bacteria included *Escherichia*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterobacter* (Narayanan and Sakthivel, 2011). These findings support the use of *T. cordifolia* as a hepatoprotective, anti-inflammatory and antioxidant agent (Saha and Ghosh, 2012; Upadhyay *et al*., 2010).

In this investigation assessed the antibiotic susceptibility patterns of *Staphylococcus*, *Escherichia coli*, *Pseudomonas*, *Klebsiella* and *Citrobacter*. The antibiotics tested included *Amoxicillin*, *Ciprofloxacin*, *Erythromycin*, *Tetracycline*, *Ampicillin* and *Chloramphenicol*. *Tetracycline* and *Chloramphenicol* demonstrated higher effectiveness, whereas *Amoxicillin* and *Erythromycin* exhibited lower efficacy. According to Motayo *et al*. (2013) *Erythromycin*, *Ampicillin* and *Tetracycline* were evaluated for their susceptibility against *S. aureus*, *E. coli* and *Pseudomonas*, demonstrating a moderate susceptibility with percentages of 34%, 30.8% and 33.4%, respectively. G1-4A, a constituent from *T. cordifolia* which induces TLR-2 signaling that is important to inhibit the intracellular pathogen Gupta *et al*. (2016). The current appearance of resistance by microbes to different antibiotics recommended substitute treatment, to use the plants for treatment of diseases (Rachana *et al*., 2022). Medicinal plants are a source of natural compounds used by traditional communities for curative purposes in the manufacturing of medicines. These drugs included tubocuraine, colchicines, quinine and nicotine etc (Briskin, 2000). In most developing countries the primary means of obtaining medicines are medicinal plants (Bordes *et al*., 2013).

The findings of this study revealed the antibacterial impact of the medicinal plant *Tinospora*, *cordifolia* against *S. aureus*, *E. coli*, *Pseudomonas*, *Citrobacter* and *Klebsiella*, which were isolated from the hands of students. This assessment was conducted using the well diffusion method and the applied extract produced distinct inhibition zones with varying measurements. Another study conducted showed that methanolic extract of *T. cordifolia* has antimicrobial activities (Jeyachandran *et al*., 2003). This metabolic extract has been used against *Staphylococcus aureus*, *Pseudomonas aerogenosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typii*, *Enterobacter* and *Proteus vulgaris* (Nath *et al*., 2023). Various bacteria such as *Pseudomonas aerogenosa*, *E.coli*, *S.aureus* and *Klebsiella* were isolated and checked for *Tinospora cordifolia* extract activity. The result showed antibacterial effect extenuating the presence of isolated bacteria or inhibiting the growth of these bacteria (Tambekar *et al*., 2009) The result of study conducted by Thatte *et al*. (1992) showed the antibacterial properties of methanolic stem extract of *Tinospora cordifolia* against bacteria such as *Staphylococcus. aureus*, *Escherichia coli*, *Staphylococcus albus*, *Pseudomonas*.

Conclusion

In the current investigation, 417 out of 500 samples collected from students exhibited positive results for bacterial growth. Among these isolates, 53% were obtained from females, while 47% were from males. The predominant isolates included *Staphylococcus aureus*, followed by *E. coli, Pseudomonas*, *Klebsiella*, and *Citrobacter*. In terms of antibiotic effectiveness, *Tetracycline* and *Chloramphenicol* demonstrated higher efficacy, while *Amoxicillin* and *Erythromycin* showed less effectiveness against the isolated bacterial strains. Additionally, the antibacterial properties of the stem extract of *Tinospora cordifolia* against *Staphylococcus aureus*, *E. coli*, *Pseudomonas*, *Citrobacter* and *Klebsiella* were assessed in terms of various zones of inhibition.

Conflict of Interest. The authors declare that they have no conflict of interest.

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