American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Study of the Effect of Terminalia Chebula Seed Extract on Inhibiting the Growth of Bacteria Isolated From Urinary Tract Infection

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Received: 2024, 15, Dec
Accepted: 2024, 21, Dec
Published: 2025, 09, Jan

Annotation: Herbal medications make up a significant portion of all accepted health systems worldwide. Additionally, medicinal plants are thought to be inexpensive and valuable sources of a variety of phytoconstituents that are often used in the creation of medications to treat a wide range of illnesses. 140 urine samples were collected between July and September 2024 from UTI-afflicted women who were sent to the laboratory after seeing a specialist and being admitted to Azadi Teaching Hospital in Kirkuk City. Following identification of the bacterial colonies on blood agar based on their culture properties, they were incubated at 37 0C for 24 hours. The data indicated that when cultivated in the optimal culture medium, 109 (77.9%) of the total samples demonstrated favorable results for bacterial growth. Thirty-one (22.1%) of the total samples had negative results for bacterial growth. With 51 (46.7%) isolates, E. coli had the highest incidence of urinary tract infections. S. aureus (20.2%), P. mirabilis (12.0%), P. aeruginosa (8.3%), and K.

pneumonia (16.7%) were next in line. E. coli, K. pneumonia, and P. aeruginosa all shown high resistance to ampicillin, making it the antibiotic most resistant to all bacterial taxa in the research. All of the bacteria under study, however, shown a significant susceptibility to tobramycin, amikacin, and ezetrenam. Additionally, the results demonstrated T. chebula's capacity to suppress bacteria based on concentration. The isolates of E. coli, P. mirabilis, K. pneumonia, P. aeruginosa, and S. aureus were inhibited by 50, 100, and 150 ul of T. chebula. E. coli was the most sensitive to the extract, with an average diameter of inhibition of 29.2±4.2, while S. aureus was the least sensitive, with an average diameter of inhibition of 19.5±3.5. The highest effective concentration for inhibiting bacterial growth was determined to be 150ul. T. chebula seed extract was found to exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria.

Keywords: T. chebula, UTI, E. coli, K. pneumonia, S. aureus.

Introduction

Researchers that screened a variety of medical plants found that Terminalia chebula, also known as Harad or Chebulic Myrobalan, is one of the most highly valued medicinal herbs [1-2]. Numerous phytoconstituents found in T. chebula have been shown to provide a wide range of therapeutic benefits. Since ancient times, the myrobalan fruit has been utilized as a home medicine for a variety of human ailments, demonstrating its many health benefits [3–4]. The combretaceae family includes Terminalia chebula, a modest tree used in traditional medicine. Common names for it include Chebulic myrobalan, Ink tree, and Black myrobalan. In addition to India, various nations in Asia and Africa also employ Terminalia chebula as a traditional medicine [5]. Furthermore, because of its many pharmacological properties linked to the physiologically active compounds it contains, the plant has a long history of being utilized extensively in homeopathic, ayurvedic, and unani medicine [6]. It has important active ingredients such tannins, flavonoids, and phenols. Gallic acid is abundant in it [7]. Rutin and quercetin are the two primary flavonoid chemicals identified from the fruits [8]. Furthermore, the pant has been widely utilized to cure a variety of illnesses, including cancer, heart

disease, paralysis, leprosy, ulcers, gout, arthritis, and more. Antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancer, antiulcer, antimutagenic, and wound-healing properties have also been thoroughly documented for the plant [9–10]. One of the most prevalent infectious diseases in the world, urinary tract infections (UTIs) can affect the kidneys, bladder, or urethra. They impact 150 million people annually and are associated with high medical expenses and substantial morbidity [11]. UTIs have a detrimental effect on patients' romantic and social interactions, which lowers their quality of life, even if the symptoms vary depending on where the infection occurs [12,13]. There are two types of UTIs: complicated (cUTIs) and uncomplicated (uUTIs) [14]. When there are no neurological or anatomical problems in the urinary tract, uUTIs usually affect healthy patients [15]. Complicated UTIs are those that are linked to urinary tract abnormalities that make the patient more vulnerable to infection, like catheterization or anatomical or functional abnormalities [15,16]. In order to determine the antibacterial activity of *Terminalia chebula* seed extract against certain harmful bacteria, this study was created.

Materials & methods

Specimen Collection

140 urine samples were collected from UTI-afflicted women who were hospitalized to Azadi Teaching Hospital in Kirkuk City between July and September 2024 after consulting a physician and being transferred to the lab.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics

Following identification of the bacterial colonies on blood agar based on their culture properties, they were incubated at 37 0C for 24 hours.

Direct examination

By using a microscope to examine the morphological characteristics of germ cells—specifically, their interactions with the gram stain, which indicates the nature of the interaction as well as the shape and arrangement of the bacterial cells—bacterial colonies were found.

Biochemical reaction

Numerous biochemical tests, such as the methyl red, citrate, urease, voges-proskauer, catalase, oxidase, and indole assays, were carried out to detect and diagnose microorganisms.

Antibiotic susceptibility test (AST)

In accordance with the standards established by the Clinical Laboratory Standards Institute (CLSI, 2020), the AST for each isolate was performed using the Kirby-Bauer disc diffusion method with Muller Hinton (MH) agar [14]. The antibiotic discs (Bioanalyse (USA)) used in this study were Ampicillin, Gentamicin, Tetracyclic, Azetrenam, Ceftazidime, Levofloxacin, Amikacin, and Tobramycin. Multidrug-resistant (MDR) organisms were defined as those that showed resistance to several antimicrobial agent types, classes, or subclasses.

Collection of plant samples

The local market is where the plants were purchased. They were cleaned with distilled water to get rid of dust and impurities. After that, they were allowed to dry at 4 °C. The plant's seeds were then ground in an electric mill and stored in plastic containers until the lab required them.

Preparation of aqueous extract

For infusion, 20g of the plant sample and 400m of distilled water were combined at a weight/volume ratio of 20:1 to create the aqueous extract of the plant utilized in the study. The raw aqueous extract of each plant was obtained by filtering the mixture through multiple layers of gauze after it had been

in a shaker water bath at 40°C for 24 hours. The mixture was then dried in an electric oven set at 40°C and kept in airtight plastic containers until it was needed [18–19].

The test of antimicrobial activity for extracts

The diffusion approach was used to assess the antibacterial activity on agar [20]. A bacterial strain culture was made with 1×106 cells per milliliter using the McFarland opacity standard. Using a technique known as the spreading method, 0.1 ml of each bacterial isolate (1×106 cells per ml) was added to the Mueller Hinton agar plates. Ten milliliters of extract were applied to each well as a control. Thirty minutes before being put in the incubator, petri plates were held. For a whole day, each petri dish was incubated at 37°C. Inhibitory zone measurements were performed.

Statistical analysis

Version 18 of the SPSS software (Statistical Package for Social Science) was used to code and enter the data onto a computer for statistical analysis. The Chi-square test was used to look at correlations between variables after each data point was sorted based on its frequency. A p-value of less than 0.05 was regarded as significant [21–22].

Results and discussion

The current investigation includes 140 urine samples from UTI patients (table 1). The data indicated that when cultivated in the optimal culture medium, 109 (77.9%) of the total samples demonstrated favorable results for bacterial growth. Thirty-one (22.1%) of the total samples had negative results for bacterial growth.

Table (1): Distributed of study samples according to UTI

	No. (%) **+ve culture**	No. (%) **-ve culture**	Total No.(%)	P value
UTI Patients	109(77.9%)	31(22.1%)	140(100.0%)	0.032

The bacterial isolates' morphology, diameter, and forms were assessed using MacConkey agar and blood agar. Additionally, microscopic and biochemical tests, including the particular tests for each category, were used to validate the results of the biochemical identification, table (2).

Table (2): biochemical diagnosis of bacteria isolated from from UTIs

Isolates Testes	E. coli	P. mirabilis	P. aeruginosa	K. pneumoniae	S. aureus
Gram stain	-	-	-	-	+
Urease test	-	+	-	+	+
Oxidase test	-	-	+	-	-
Catalase Test	+	+	+	+	+
MR test	+	+	-	+	-
VP test	-	-	-	+	+
Indol test	+	-	-	-	-
Citrate test	-	-	+	+	-

Escherichia colonies have been seen on the MacConkey Agar medium, which is solid, medium-sized, convex, dry, regular, negative for the oxides test, positive for the catalase test, negative for the urea test, positive for the mandolin and red instance test, negative for the Voges-Proskauer test, and incapable of consuming citrate. The fermentation of lactose sugar is what gives it its pink hue. Proteus mirabilis bacteria are detected using the swarming movement phenomena on blood agar medium as a preliminary diagnostic method. They can also be recognized by their pale colonies on MacConkey agar medium, which are brought on by the accumulation of metabolic components that elevate the medium's pH, the non-fermentation of lactose sugar, and the intake of peptone, a source of nitrogen. The ripple movement phenomenon is compatible with this phenomenon [20]. Due to

its huge circular colonies, unevenly formed pink scales, mucus-containing capsules, and transparent and brilliant blood vessel colonies that were incapable of breaking down blood, Klebsiella pneumoniae was diagnosed using the MacConkey agar medium [23]. According to Table 3, E. coli caused the most urinary tract infections, with 51 isolates (46.7%), followed by S. aureus (20.2%), P. mirabilis (12.0%), P. aeruginosa (8.3%), and K. pneumonia (16.7%).

Table (3): isolate percentages of bacteria isolates

Bacteria	No.	%
E.coli	51	46.8
P. mirabilis	12	11.0
P. aeruginosa	8	7.3
K. pneumonia	16	14.7
S. aureus	22	20.2
Total	109	100

With a percentage of 46.8%, the results in Table (3) showed a statistically significant increase ($P < 0.05$) in the number of E. Coli isolates. These findings support those of other research [24], which found that E. coli was the most prevalent pathogen linked to UTIs, accounting for 44.7% of all cases. Additionally, they noted that the current study's findings are in line with the 8% and 16% isolation rates of P. aeruginosa and K. pneumonia from female patients with UTIs, respectively. E. Coli was found to be 68%, Klebsiella pneumonia to be 15%, Pseudomonas aeruginosa to be 10%, and Proteus mirabilis to be 6% in another study conducted in Iraq by [25]. According to a study conducted in Iraq by [26], out of 143 urine samples, E. coli was the most common cause of UTIs in patients (28.6%), followed by Klebsiella spp. (21.7%) and Staphylococcus spp. (16.7%).

Since E. coli, K. pneumonia, and P. aeruginosa all shown great resistance to Ampicillin, Table (4) demonstrated that Ampicillin is the medication most resistant to all bacterial kinds in the study. On the other hand, every bacterium under investigation shown a high sensitivity to tobramycin, amikacin, and azetrenam.

Table (4): show the effect of antibiotics on bacteria

Treatment / Bacteria	AMP (%)		CN (%)		TET (%)		CEZ (%)		LEV (%)		AZT (%)		AK (%)		Tob (%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E.coli	4.8	95.2	88.1	11.9	28.6	71.4	21.7	78.3	21.6	78.4	84.1	15.9	98.3	1.7	100	0.0
P. mirabilis	0.0	100	39.2	60.8	32.3	67.3	26.1	73.9	20.5	79.5	92.2	7.8	89.5	10.5	100	0.0
P. aeruginosa	6.3	93.7	25.6	74.4	29.6	70.4	51.4	48.6	28.3	71.7	88.6	11.4	90.3	9.7	100	0.0
K. pneumonia	9.8	91.2	52.7	47.3	42.4	57.6	31.8	68.2	18.5	81.5	80.4	19.6	84.3	15.7	100	0.0
S. aureus	12.1	87.9	61.5	38.5	38.5	61.5	19.5	80.5	36.2	63.8	73.1	26.9	88.8	11.2	100	0.0

AMP= Ampicillin, CN=Gentamicin, TET= Tetracyclic, AZT= Azetrenam, CEZ= Ceftazidime, LEV= Levofloxacin, AK=Amikacin, Tob=Tobramycin

The Kirby Bauer disk diffusion method was used to test microorganisms for antibiotic susceptibility on Mueller-Hinton agar. Every bacterial isolate that was detected was subjected to the antimicrobial drugs listed in table (4). The results demonstrated that E. coli exhibited a high degree of ampicillin resistance. This aligned with research by [27–28], who found that 90%, 95%, and 92% of E. coli isolates from UTIs were ampicilin-resistant. Antimicrobial resistance to ampicillin, levofloxacin, and tetracyclic was also rising in other gram-negative bacteria, including P. mirabilis, P. aeruginosa, and K. pneumonia. A comparable outcome was documented in [29] and [30]. Gram-positive and gram-negative bacteria that were isolated for our study were both resistant to various antibiotic classes. S. aureus showed 100% resistance to ampicillin among the gram-positive isolates. This result is consistent with earlier research conducted in Libya [32] and Iraq [31]. In addition to other resistance mechanisms and unchecked antibiotic use, S. aureus's high level of resistance may result

in the production of penicillinase enzymes and other penicillin-binding proteins that aid in the organism's conversion to beta-lactam antibiotic resistance [33]. The current study's higher sensitivity of Gram-positive isolates for tobramycin, gentamicin, amikacin, and ezetrenam is consistent with that of [33].

Table (5) showed the ability of *T. chebula* for bacteria inhibition according to concentration. 50, 100, 150ul of *T. chebula* was caused in inhibitory effect on E. coli, *P. mirabilis*, *K. pneumonia*, *P. aeruginosa*, S. aureus isolates. It was found that the most efficient concentration in inhibiting bacterial growth was 150ul, and *E. coli* was the most sensitive to the extract, as the average diameter of inhibition was 29.2±4.2, while the least sensitive was S. aureus, as the average diameter of inhibition was 19.5±3.5.

Table (5): show the inhibition (mm) effect of aqueous extract of *T. chebula* at different concentration

Treatment / Bacteria type	Inhibition zone diameters (mm)		
	50ul	100ul	150ul
E.coli	1.16± 0.3	8.6± 2.7	29.2± 4.2
K. pneumonia	2.5±0.9	7.2±2.1	22.1±5.7
P. mirabilis	2.1±1.1	11.3± 2.4	20.8± 2.8
P. aeruginosa	1.46± 0.3	10.7± 2.03	25.2± 4.2
S. aureus	1.05±0.6	6.4±1.5	19.5±3.5

In the current study, the aqueous extract of T. chebula inhibited the growth of both Gram-positive and Gram-negative bacteria. The strength of phenolic compounds, particularly terpenes and tannins, which have been demonstrated to impact substrate deprivation, may be responsible for the possible inhibitory actions of T. chebula [34]. Due to the presence of numerous beneficial components, including phenols, flavonoids, alkaloids, tannins, volatile oils, and others, the T. chebula seed extract demonstrated great efficacy. The findings were in line with those of [35], who found that the alcoholic and aqueous extracts of T. chebula seeds have antioxidant and antibacterial properties, making them resistant to many medicines.

Conclusions

Based on the current findings, it is concluded that *T. chebula* seed extract showed antibacterial activity against Gram-negative and Gram-positive bacteria, and these extracts can play a role in the therapy of infection diseases.

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