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Evaluation of the Inhibitory Activity of Aqueous and Ethanolic Extracts of *Tilia cordata* Against Antibiotic-Resistant Escherichia coli Strains Isolated from Urinary Tract Infections

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تقييم النشاط التثبيطي للمستخلصات المائية والإيثانولية لنبات الزيزفون (*Tilia cordata*) ضد السلالات المقاومة للمضادات الحيوية من الإشريكية القولونية المعزولة من التهابات المسالك البولية

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Abstract:
The rapid emergence of multidrug-resistant Escherichia coli (MDR E. coli) in urinary tract infections (UTIs) has necessitated the search for alternative therapeutic agents derived from natural plants. This study aimed to evaluate the inhibitory activity of aqueous and ethanolic extracts of *Tilia cordata* (linden) against clinical isolates of antibiotic-resistant E. coli obtained from UTIs. A total of 30 clinical isolates were collected from urine samples of patients diagnosed with UTIs. Antibiotic susceptibility testing was performed using the disk diffusion method against commonly used antibiotics including ampicillin, gentamicin, ciprofloxacin, ceftriaxone, and imipenem. The minimum inhibitory concentrations (MICs) of T. cordata aqueous and ethanolic extracts were determined using the broth microdilution method, while the minimum bactericidal concentrations (MBCs) were assessed via subculturing on Mueller-Hinton agar. Phytochemical screening of both extracts was conducted using standard qualitative methods. Results revealed that 86.7% (26/30) of isolates were resistant to at least three antibiotic classes, classified as MDR. The ethanolic extract exhibited superior antibacterial activity compared to the aqueous extract, with MIC values ranging from 3.125 to 12.5 mg/mL, while the aqueous extract showed MICs between 6.25 and 25 mg/mL. The MBC/MIC ratio indicated a bactericidal effect for the ethanolic extract against 73% of tested isolates. Phytochemical analysis revealed the presence of tannins, flavonoids, saponins, and phenolic compounds in both extracts, with higher concentrations in the ethanolic extract. These findings suggest that *T. cordata* ethanolic extract possesses significant inhibitory activity against MDR E. coli isolates, supporting its potential as a natural alternative or adjunctive therapy for UTIs. Further in vivo studies and toxicity assessments are recommended.

Keywords: *Tilia cordata*, Escherichia coli, urinary tract infections, antibiotic resistance, aqueous extract, ethanolic extract, minimum inhibitory concentration, phytochemical screening.

الملخص

أدى التفشي السريع للإشريكية القولونية متعددة المقاومة للمضادات الحيوية في التهابات المسالك البولية إلى ضرورة البحث عن عوامل علاجية بديلة من النباتات الطبيعية. هدفت هذه الدراسة إلى تقييم النشاط التثبيطي للمستخلصات المائية والإيثانولية لنبات الزيزفون ضد العزلات السريرية للإشريكية القولونية المقاومة للمضادات الحيوية والمعزولة من التهابات المسالك البولية. تم جمع 30 عزلة سريرية من عينات بول مرضى تم تشخيص إصابتهم بالتهابات المسالك البولية. تم اختبار الحساسية للمضادات الحيوية بطريقة انتشار الأقراص ضد المضادات الحيوية شائعة الاستخدام بما في ذلك الأمبيسلين، الجنتاميسين، السيبروفلوكساسين، السيفترياكسون، والإيميبينيم. تم تحديد التركيزات المثبطة الدنيا للمستخلصات المائية والإيثانولية للزيزفون باستخدام طريقة التحليل الدقيق للبورون، بينما تم تقييم التركيزات القاتلة الدنيا عبر الزرع الفرعي على أكار مولر-هينتون. أظهرت النتائج أن 86.7% من العزلات كانت مقاومة لثلاثة فئات من المضادات الحيوية على الأقل، وتم تصنيفها كمتعددة المقاومة. أظهر المستخلص الإيثانولي نشاطاً مضاداً للبكتيريا متفوقاً مقارنة بالمستخلص المائي، حيث تراوحت قيم التركيزات المثبطة الدنيا بين 3.125 و12.5 ملغم/مل، بينما أظهر المستخلص المائي قيماً تراوحت بين 6.25 و25 ملغم/مل. أشارت نسبة التركيز القاتل الأدنى إلى التركيز المثبط الأدنى إلى تأثير مبيد للبكتيريا للمستخلص الإيثانولي ضد 73% من العزلات المختبرة. كشف التحليل النباتي الكيميائي عن وجود العفص، الفلافونويدات، الصابونينات، والمركبات الفينولية في كلا المستخلصين، مع تركيزات أعلى في المستخلص الإيثانولي. تقترح هذه النتائج أن المستخلص الإيثانولي للزيزفون يمتلك نشاطاً تثبيطياً مهماً ضد عزلات الإشريكية القولونية متعددة المقاومة، مما يدعم إمكاناته كبديل طبيعي أو علاج مساعد لالتهابات المسالك البولية. يوصى بإجراء المزيد من الدراسات الحيوية وتقييم السمية.

الكلمات المفتاحية: زيزفون، إشريكية قولونية، التهابات المسالك البولية، مقاومة المضادات الحيوية، مستخلص مائي، مستخلص إيثانولي، تركيز مثبط أدنى، فحص نباتي كيميائي..

1. Introduction

1.1 Research Problem

Urinary tract infections (UTIs) represent one of the most common bacterial infections worldwide, affecting millions of individuals annually across all age groups. Among the etiological agents, *Escherichia coli* accounts for approximately 70-80% of community-acquired UTIs and a significant proportion of hospital-acquired cases (Ben Hsin et al., 2025). The management of UTIs has become increasingly challenging due to the rapid emergence and dissemination of antibiotic-resistant *E. coli* strains, particularly those producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases. Multidrug-resistant (MDR) *E. coli* isolates have been reported globally, limiting therapeutic options and leading to increased morbidity, mortality, and healthcare costs. In Libya, similar trends have been documented, with alarming rates of resistance to commonly prescribed antibiotics such as ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolones (Ben Hsin et al., 2025; Salem, 2025).

The overuse and misuse of antibiotics in human medicine, agriculture, and livestock have accelerated the selection pressure favoring resistant bacterial clones. Consequently, the WHO has classified antibiotic resistance as one of the top ten global public health threats. This crisis has revived interest in exploring natural products, particularly medicinal plants, as alternative or adjunctive therapeutic agents. *Tilia cordata* (commonly known as linden or small-leaved lime) has been traditionally used in European and Middle Eastern folk medicine for its sedative, anti-inflammatory, diaphoretic, and antimicrobial properties. However, the scientific evidence

regarding its efficacy against clinically relevant MDR *E. coli* isolates from UTIs remains limited and requires rigorous investigation.

1.2 Objectives

The primary objectives of this study are:

1. To isolate and identify *E. coli* strains from urine samples of patients diagnosed with UTIs.
2. To determine the antibiotic resistance profiles of clinical *E. coli* isolates against commonly used antibiotics.
3. To prepare aqueous and ethanolic extracts of *Tilia cordata* leaves using standard extraction protocols.
4. To evaluate the in vitro inhibitory activity of both extracts against MDR *E. coli* isolates by determining the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).
5. To compare the antibacterial efficacy of aqueous versus ethanolic extracts.
6. To perform preliminary phytochemical screening of both extracts to identify major bioactive compounds potentially responsible for the observed activity.
7. To provide scientific evidence supporting the potential use of *T. cordata* as a natural alternative for managing UTIs caused by antibiotic-resistant *E. coli*.

1.3 Significance of the Study

This study holds significant scientific and clinical importance for several reasons. First, it addresses the urgent need for novel antimicrobial agents effective against MDR pathogens, particularly in developing countries where antibiotic resistance surveillance is limited and therapeutic options are constrained. Second, the study focuses on *T. cordata*, a plant with a long history of traditional use but insufficiently characterized for its antibacterial effects against uropathogenic *E. coli*. Third, by comparing aqueous and ethanolic extracts, the research provides insights into the most effective extraction solvent for maximizing bioactive compound yield. Fourth, the phytochemical screening offers preliminary data on the chemical constituents responsible for the antibacterial activity. Finally, the findings may contribute to the development of evidence-based phytotherapeutic protocols for UTIs and support local and regional efforts to combat antibiotic resistance through natural product research (Salem et al., 2025; Khalil et al., 2025; Salem & Lakwani, 2024).

2. Literature Review

2.1 Urinary Tract Infections: Epidemiology and Etiology

Urinary tract infections are among the most prevalent bacterial infections encountered in both community and hospital settings. The global annual incidence of UTIs is estimated at approximately 150 million cases, with women disproportionately affected due to anatomical, physiological, and behavioral factors (Ben Hsin et al., 2025). Recurrent UTIs affect up to 30% of women despite appropriate antibiotic therapy. The primary causative agent, uropathogenic *E. coli* (UPEC), possesses specific virulence factors including adhesins (type 1 and P fimbriae), toxins (hemolysin, cytotoxic necrotizing factor), iron acquisition systems, and capsules that facilitate colonization, invasion, and persistence within the urinary tract.

2.2 Antibiotic Resistance in Uropathogenic *E. coli*

The global spread of antibiotic-resistant *E. coli* has reached critical levels. Resistance to ampicillin, a former first-line agent, exceeds 50% in most regions. Fluoroquinolone resistance, driven by chromosomal mutations in gyrase and topoisomerase genes, has increased dramatically, with rates ranging from 20-60% worldwide. Third-generation cephalosporin resistance, mediated predominantly by CTX-M-type ESBLs, poses a major therapeutic challenge, while carbapenem resistance, though still relatively uncommon, is emerging as a threat through the dissemination of carbapenemase genes such as blaNDM, blaKPC, and blaOXA-48 (Salem, 2025; Salem & Salem, 2025).

In Libya, studies have documented high resistance rates among UTI isolates. Ben Hsin et al. (2025) reported that 72% of *E. coli* isolates from Libyan patients were resistant to ampicillin, 58% to trimethoprim-sulfamethoxazole, 45% to ciprofloxacin, and 38% to ceftriaxone. Notably, 31% of isolates exhibited MDR phenotypes, defined as non-susceptibility to at least one agent in three or more antibiotic classes. These findings underscore the urgent need for alternative strategies, including plant-derived antimicrobials.

2.3 Medicinal Plants as Sources of Antimicrobial Agents

Plants have served as primary sources of therapeutic agents for millennia, with approximately 25% of modern pharmaceuticals derived directly or indirectly from plant metabolites. The antimicrobial properties of plants are attributed to secondary metabolites such as phenolics, flavonoids, tannins, terpenoids, alkaloids, and saponins, which act through diverse mechanisms including membrane disruption, enzyme inhibition, DNA intercalation, and interference with quorum sensing. Unlike conventional antibiotics that typically target single bacterial pathways, plant extracts often exert multi-target effects, potentially reducing the likelihood of resistance development (Salem et al., 2026; Alshawish et al., 2025).

Recent research has documented promising antibacterial activities of various plant extracts against MDR *E. coli*. Khalil et al. (2025) demonstrated that alcoholic and aqueous extracts of *Plantago ovata* leaves inhibited the growth of antibiotic-resistant bacteria, with MICs ranging from 2.5 to 20 mg/mL. Similarly, Salem et al. (2025) reported that *Taraxacum officinale* extracts exhibited significant activity against *E. coli* and *Staphylococcus aureus*, with the ethanolic extract showing superior efficacy compared to the aqueous extract. Soof et al. (2025) identified bioactive isothiocyanates in *Sinapis alba* essential oil with rapid bactericidal kinetics against clinically relevant pathogens. Furthermore, Salem (2024) documented the antimicrobial activity of *Usnea barbata* extract against *E. coli* and *S. aureus*, suggesting that lichens also represent promising sources of antimicrobial compounds.

2.4 *Tilia cordata*: Botanical Description and Traditional Uses

Tilia cordata Mill., belonging to the family Malvaceae (formerly Tiliaceae), is a deciduous tree native to Europe and western Asia, commonly known as small-leaved lime, linden, or winter lime. The tree reaches heights of 20-30 meters and produces heart-shaped, serrated leaves and fragrant yellowish-white flowers. Traditional medicine systems across Europe have utilized linden flowers, leaves, and bracts for their sedative, spasmolytic, diaphoretic, anti-inflammatory, and diuretic properties. Linden tea is widely consumed for treating colds, coughs, fever, nervous tension, and hypertension. Topically, linden preparations have been applied for skin inflammation, burns, and wounds.

2.5 Phytochemistry of *Tilia cordata*

Phytochemical investigations have identified a diverse array of bioactive compounds in *T. cordata*. The main chemical constituents include:

1. **Flavonoids:** Quercetin, kaempferol, tiliroside (kaempferol-3-O-β-D-(6"-O-E-p-coumaroyl)glucopyranoside), astragalin, and hyperoside. These compounds exhibit potent antioxidant and antimicrobial activities.
2. **Phenolic acids:** Caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, and gallic acid, known for their anti-inflammatory and antibacterial properties.
3. **Tannins:** Condensed and hydrolyzable tannins contribute to astringent and antimicrobial effects.
4. **Mucilage polysaccharides:** Composed of arabinose, galactose, rhamnose, and galacturonic acid, responsible for demulcent and immunomodulatory activities.
5. **Volatile oils:** Including sesquiterpenes (farnesol, nerolidol), alkanes, and fatty acids.
6. **Other compounds:** Saponins, coumarins, and essential amino acids.

The composition varies significantly depending on plant part, harvest time, drying method, and extraction solvent. Ethanolic extraction typically yields higher concentrations of

flavonoids and phenolic compounds compared to aqueous extraction due to the differential solubility of these compounds.

2.6 Antimicrobial Activity of *Tilia* Species

Limited but growing evidence supports the antimicrobial potential of *Tilia* species. Previous studies have reported inhibitory effects of *Tilia* extracts against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*) and, to a lesser extent, Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). The activity is generally attributed to flavonoids and phenolic acids that disrupt bacterial cell membranes, inhibit efflux pumps, and interfere with nucleic acid synthesis. However, most studies have employed standard laboratory strains rather than clinical MDR isolates, and comparisons between aqueous and ethanolic extracts remain insufficient. Furthermore, no study to date has specifically evaluated the activity of *T. cordata* extracts against uropathogenic MDR *E. coli* isolates from Libyan patients.

2.7 Research Gap and Hypothesis

Based on the reviewed literature, the following research gaps were identified:

- Lack of studies evaluating *T. cordata* extracts against clinical MDR *E. coli* isolates from UTIs.
- Limited comparative data on aqueous versus ethanolic extraction efficacy.
- Absence of MIC and MBC data specific to Libyan uropathogenic isolates.
- Insufficient phytochemical correlation with antibacterial activity.

Hypothesis: The ethanolic extract of *Tilia cordata* exhibits significantly higher inhibitory activity against MDR *E. coli* isolates compared to the aqueous extract, with MIC values ≤10 mg/mL, and the activity correlates with higher concentrations of flavonoids and phenolic compounds.

3 .Methodology

3.1 Study Design and Setting

This was an experimental in vitro study conducted at the biology laboratory, Faculty of Education, Bani Waleed University, Bani Waleed, Libya, between January and May 2026. The study received ethical approval from the biology department review board (Ref No. BIO/2025-028).

3.2 Study Population and Sample Collection

Inclusion criteria: Adult patients (≥18 years) of both sexes presenting with clinical symptoms of UTI (dysuria, frequency, urgency, suprapubic pain, fever) and positive urine culture.

Exclusion criteria: Patients on antibiotic therapy within the preceding two weeks, patients with indwelling urinary catheters, pregnant women, and patients with known immunosuppression.

A total of 100 midstream clean-catch urine samples were collected from patients attending the outpatient clinics of Bani Waleed Hospital. Samples were transported to the laboratory within 2 hours of collection in sterile containers and processed immediately.

3.3 Isolation and Identification of *E. coli*

Each urine sample was inoculated onto Cysteine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar using a calibrated loop (1 µL) and incubated aerobically at 37°C for 24 hours. Significant bacteriuria was defined as $\geq 10^5$ colony-forming units (CFU)/mL. Presumptive *E. coli* colonies (lactose-fermenting, pink colonies on MacConkey agar) were subcultured for purity. Identification was confirmed using:

- Gram staining (Gram-negative rods)
- Biochemical tests: indole production (positive), methyl red (positive), Voges-Proskauer (negative), citrate utilization (negative), triple sugar iron agar (acid/acid with gas), urease (negative), and motility (positive)
- API 20E system (bioMérieux, France) for ambiguous isolates.

A total of 30 confirmed *E. coli* isolates were randomly selected for further analysis.

3.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) according to CLSI guidelines (CLSI M100, 2024). The following antibiotic disks (Oxoid, UK) were used: ampicillin (AMP, 10 µg), amoxicillin-clavulanate (AMC, 20/10 µg), piperacillin-tazobactam (TZP, 100/10 µg), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), aztreonam (ATM, 30 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), gentamicin (CN, 10 µg), amikacin (AK, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), nitrofurantoin (F, 300 µg), fosfomycin (FOS, 200 µg), and colistin (CT, 10 µg).

Plates were incubated at 37°C for 18 hours. Inhibition zone diameters were measured and interpreted according to CLSI breakpoints. *E. coli* ATCC 25922 was used as quality control. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. ESBL production was screened using cefotaxime (30 µg) and ceftazidime (30 µg) alone and in combination with clavulanic acid (10 µg), with a ≥5 mm increase in zone diameter indicating ESBL positivity.

3.5 Plant Material Collection and Authentication

Fresh leaves of *Tilia cordata* were collected from cultivated trees in the botanical garden of the Faculty of Agriculture, Bani Waleed, Libya, during April 2025 (flowering stage). The plant material was authenticated by a botanist, and a voucher specimen (TC-2025-009) was deposited in the university herbarium. Leaves were washed with distilled water, shade-dried at room temperature (25±2°C) for 10 days, and ground into fine powder using an electric grinder. The powder was stored in airtight amber glass containers at 4°C until extraction.

3.6 Preparation of Plant Extracts

3.6.1 Aqueous Extract

Fifty grams of powdered leaves were macerated in 500 mL of sterile distilled water in a conical flask. The mixture was shaken at 150 rpm on an orbital shaker at room temperature for 48 hours. The extract was filtered through sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrate was frozen at -80°C and lyophilized (freeze-dried) to obtain a dry powder. The yield was calculated as (weight of extract / weight of dry plant powder) × 100. The dried extract was stored at -20°C until use.

3.6.2 Ethanolic Extract

Fifty grams of powdered leaves were macerated in 500 mL of 70% ethanol (v/v) in a conical flask. The mixture was shaken at 150 rpm at room temperature for 72 hours. The extract was filtered as described above. The filtrate was concentrated using a rotary evaporator (Büchi, Switzerland) at 40°C under reduced pressure, followed by complete drying in a freeze-dryer. The yield was calculated, and the extract was stored at -20°C.

3.7 Phytochemical Screening

Qualitative phytochemical analysis was performed on both extracts using standard protocols to detect the presence of:

- **Alkaloids:** Mayer's, Wagner's, and Dragendorff's reagents
- **Flavonoids:** Ferric chloride test, Shinoda's test (magnesium and hydrochloric acid)
- **Tannins:** Ferric chloride and gelatin tests
- **Saponins:** Frothing test
- **Phenolics:** Ferric chloride test
- **Terpenoids:** Salkowski's test (chloroform and sulfuric acid)
- **Glycosides:** Keller-Kiliani test (for cardiac glycosides)
- **Carbohydrates:** Molisch's test
- **Proteins and amino acids:** Ninhydrin and biuret tests

The intensity of reactions was recorded as: (-) absent, (+) low, (++) moderate, (+++) high.

3.8 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth microdilution method according to CLSI guidelines. Stock solutions of each extract were prepared in 1% dimethyl sulfoxide (DMSO) at a concentration of 50 mg/mL, followed by two-fold serial dilutions in Mueller-Hinton broth (MHB) to obtain final concentrations ranging from 25 to 0.195 mg/mL. Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and further diluted 1:100 in MHB to achieve a final inoculum of approximately 5×10^5 CFU/mL. In a 96-well microtiter plate, 100 µL of each extract dilution was mixed with 100 µL of bacterial suspension. Positive controls (bacteria + MHB without extract) and negative controls (MHB + extract without bacteria) were included. Plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of extract that inhibited visible bacterial growth, as determined by optical density measurement at 600 nm using a microplate reader (Biotek, USA). Each isolate was tested in triplicate. *E. coli* ATCC 25922 was included as a reference strain.

3.9 Determination of Minimum Bactericidal Concentration (MBC)

Following MIC determination, 10 µL aliquots from wells showing no visible growth were subcultured onto MHA plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of extract that killed ≥99.9% of the initial inoculum, i.e., no bacterial growth on agar. The MBC/MIC ratio was calculated to determine whether the extract was bactericidal (MBC/MIC ≤4) or bacteriostatic (MBC/MIC >4).

3.10 Data Analysis

Data were analyzed using SPSS version 26.0 (IBM, USA). Descriptive statistics were used to summarize resistance frequencies and MIC/MBC values. The MIC50 and MIC90 (concentrations inhibiting 50% and 90% of isolates, respectively) were calculated. Differences in antibacterial activity between aqueous and ethanolic extracts were analyzed using the Wilcoxon signed-rank test. A p-value <0.05 was considered statistically significant. Results were presented as mean ± standard deviation (SD) and range.

4. Results

4.1 Bacterial Isolates and Identification

Out of 100 urine samples, 62 (62%) showed significant bacteriuria (≥10^5 CFU/mL). Among these, *E. coli* was identified in 41 samples (66.1% of positive cultures), representing the predominant uropathogen. Other isolates included *Klebsiella pneumoniae* (17.7%), *Proteus mirabilis* (8.1%), *Enterococcus faecalis* (4.8%), and *Pseudomonas aeruginosa* (3.2%). A total of 30 *E. coli* isolates were randomly selected for further characterization.

4.2 Antibiotic Resistance Profiles

The antibiotic susceptibility patterns of the 30 *E. coli* isolates are presented in Table 1 and Figure 1. Alarmingly high resistance rates were observed: ampicillin (90%), trimethoprim-sulfamethoxazole (76.7%), ciprofloxacin (66.7%), ceftriaxone (63.3%), gentamicin (46.7%), and nitrofurantoin (23.3%). Notably, 26 isolates (86.7%) exhibited MDR phenotypes, with 12 isolates (40%) resistant to four classes, 8 isolates (26.7%) resistant to five classes, and 6 isolates (20%) resistant to six or more classes. ESBL production was confirmed in 18 isolates (60%). All isolates remained susceptible to imipenem, meropenem, and colistin. Fosfomycin showed high activity with 93.3% susceptibility.

Table 1: Antibiotic Susceptibility of 30 E. coli Isolates

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin (AMP)	3 (10.0)	0 (0)	27 (90.0)
Amoxicillin-clavulanate (AMC)	10 (33.3)	4 (13.3)	16 (53.4)

Piperacillin-tazobactam (TZP)	22 (73.3)	3 (10.0)	5 (16.7)
Cefoxitin (FOX)	24 (80.0)	2 (6.7)	4 (13.3)
Ceftriaxone (CRO)	9 (30.0)	2 (6.7)	19 (63.3)
Ceftazidime (CAZ)	11 (36.7)	2 (6.7)	17 (56.6)
Cefepime (FEP)	15 (50.0)	3 (10.0)	12 (40.0)
Aztreonam (ATM)	14 (46.7)	2 (6.7)	14 (46.6)
Imipenem (IPM)	30 (100)	0 (0)	0 (0)
Meropenem (MEM)	30 (100)	0 (0)	0 (0)
Gentamicin (CN)	14 (46.7)	2 (6.7)	14 (46.6)
Amikacin (AK)	25 (83.3)	3 (10.0)	2 (6.7)
Ciprofloxacin (CIP)	8 (26.7)	2 (6.7)	20 (66.6)
Levofloxacin (LEV)	12 (40.0)	2 (6.7)	16 (53.3)
Trimethoprim-sulfamethoxazole (SXT)	5 (16.7)	2 (6.7)	23 (76.6)
Nitrofurantoin (F)	21 (70.0)	2 (6.7)	7 (23.3)
Fosfomycin (FOS)	28 (93.3)	1 (3.3)	1 (3.4)
Colistin (CT)	30 (100)	0 (0)	0 (0)

4.3 Extraction Yields

The extraction yields are presented in Table 2. The aqueous extract produced a higher yield (15.4%) compared to the ethanolic extract (11.2%), reflecting the higher solubility of polar compounds including polysaccharides in water.

Table 2: Extraction Yields of Tilia cordata Leaves

Extract	Initial powder (g)	Final extract (g)	Yield (%)
Aqueous	50	7.70	15.4
Ethanolic (70%)	50	5.60	11.2

4.4 Phytochemical Screening Results

Qualitative phytochemical analysis revealed the presence of several bioactive compounds in both extracts, with notable differences in intensity (Table 3; Figure 2). The ethanolic extract showed higher concentrations of flavonoids, phenolics, and terpenoids, while the aqueous extract contained more saponins and carbohydrates. Tannins were present in moderate to high amounts in both extracts.

Table 3: Phytochemical Composition of Tilia cordata Extracts

Phytochemical	Aqueous Extract	Ethanolic Extract
Alkaloids	-	+
Flavonoids	++	+++
Tannins	+++	++
Saponins	+++	+
Phenolics	++	+++
Terpenoids	-	++
Glycosides	+	+
Carbohydrates	+++	++
Proteins/Amino acids	+	+

Intensity: (-) absent, (+) low, (++) moderate, (+++) high

4.5 Minimum Inhibitory Concentration (MIC) Results

The MIC values of both extracts against the 30 clinical *E. coli* isolates are summarized in Table 4 and Figure 3. The ethanolic extract exhibited significantly lower MICs ($p < 0.001$) compared to the aqueous extract, indicating superior antibacterial activity. The MIC50 and MIC90 values for the ethanolic extract were 6.25 mg/mL and 12.5 mg/mL, respectively, compared to 12.5 mg/mL and 25 mg/mL for the aqueous extract.

Table 4: MIC Distribution of *T. cordata* Extracts Against *E. coli* Isolates

MIC (mg/mL)	Aqueous Extract (n isolates)	Ethanolic Extract (n isolates)
1.56	0	2
3.125	2	8
6.25	8	12
12.5	12	6
25	6	2
>25	2	0

4.6 Minimum Bactericidal Concentration (MBC) Results

The MBC values and MBC/MIC ratios are presented in Tables 5 and 6. The ethanolic extract demonstrated bactericidal activity (MBC/MIC ≤4) against 22 isolates (73.3%), whereas the aqueous extract showed bactericidal activity against only 12 isolates (40%). The remaining isolates were inhibited at concentrations that were not lethal (bacteriostatic effect).

Table 5: MBC Values of *T. cordata* Extracts

MBC (mg/mL)	Aqueous Extract (n)	Ethanolic Extract (n)
3.125	0	1
6.25	1	5
12.5	5	10
25	6	6
50	8	6
>50	10	2

Table 6: MBC/MIC Ratio Classification

MBC/MIC Ratio	Interpretation	Aqueous Extract n (%)	Ethanolic Extract n (%)
≤4	Bactericidal	12 (40)	22 (73.3)
>4	Bacteriostatic	18 (60)	8 (26.7)

4.7 Correlation Between Antibiotic Resistance and Extract Susceptibility

Interestingly, no significant correlation was observed between the level of antibiotic resistance (number of antibiotic classes to which isolates were resistant) and the MIC values of either extract (Spearman's rho = 0.12, $p = 0.51$ for ethanolic extract). This suggests that the mechanisms conferring resistance to conventional antibiotics do not confer cross-resistance to *T. cordata* phytochemicals.

5. Discussion

5.1 Interpretation of Antibiotic Resistance Patterns

The high prevalence of MDR *E. coli* (86.7%) observed in this study is alarming but consistent with recent reports from Libya and neighboring countries. Ben Hsin et al. (2025) reported that 72% of *E. coli* isolates from Libyan UTI patients were resistant to ampicillin, and 45% to ciprofloxacin, closely matching our findings. The 60% ESBL positivity rate exceeds regional

averages (approximately 40-50%) and likely reflects extensive cephalosporin use in clinical settings. The universal susceptibility to carbapenems (imipenem, meropenem) and colistin is reassuring but must be monitored vigilantly, as carbapenem-resistant *E. coli* has been reported in other Middle Eastern countries.

The high resistance to trimethoprim-sulfamethoxazole (76.7%) renders this agent unsuitable for empirical UTI treatment in our region. Similarly, the 66.7% ciprofloxacin resistance rate contraindicates fluoroquinolone use without susceptibility testing. These findings underscore the urgent need for updated local antibiograms and antimicrobial stewardship programs. Salem (2025) emphasized that antibiotic resistance in Libya is driven by over-the-counter antibiotic availability, incomplete treatment courses, and inadequate infection control practices.

5.2 Comparative Efficacy of Extracts

The superior antibacterial activity of the ethanolic extract compared to the aqueous extract is consistent with previous studies on other medicinal plants. Khalil et al. (2025) reported that alcoholic extracts of *Plantago ovata* exhibited lower MICs (2.5-10 mg/mL) against resistant bacteria compared to aqueous extracts (10-20 mg/mL). Similarly, Salem et al. (2025) found that ethanolic *Taraxacum officinale* extract was more effective against *E. coli* than the aqueous counterpart. This differential activity is explained by the higher extraction efficiency of ethanol for hydrophobic and moderately polar bioactive compounds, particularly flavonoids and phenolic acids, which are known to possess strong antimicrobial properties.

The MIC50 of the ethanolic extract (6.25 mg/mL) is within the range considered pharmacologically relevant. According to standard criteria, plant extracts with MIC values ≤10 mg/mL are considered promising antibacterial agents, while those with MICs >10 mg/mL may still be useful as adjunctive therapies or for topical applications. The ethanolic extract met this criterion for 73% of isolates (MIC ≤10 mg/mL), whereas only 33% of isolates were inhibited by the aqueous extract at this concentration.

5.3 Bactericidal Versus Bacteriostatic Activity

The bactericidal effect of the ethanolic extract against 73.3% of isolates (MBC/MIC ≤4) is clinically significant, as bactericidal agents are generally preferred for treating UTIs, particularly in immunocompromised patients, those with anatomical abnormalities, or severe infections. The aqueous extract, by contrast, was predominantly bacteriostatic (60%). This distinction may be attributed to the higher concentrations of membrane-active compounds such as flavonoids and terpenoids in the ethanolic extract, which can cause irreversible bacterial cell damage (Salem & Salem, 2025).

The bactericidal mechanism of *T. cordata* phytochemicals likely involves multiple targets. Flavonoids such as quercetin and kaempferol have been shown to disrupt bacterial outer membranes, inhibit DNA gyrase, and interfere with efflux pumps. Tannins bind to bacterial adhesins, inhibit cell wall synthesis, and chelate iron essential for bacterial growth. The synergistic interactions among these compounds likely contribute to the enhanced bactericidal activity observed (Soof et al., 2025).

5.4 Correlation with Phytochemical Composition

The phytochemical analysis provides plausible explanations for the observed antibacterial differences. The ethanolic extract contained higher levels of flavonoids, phenolics, and terpenoids compared to the aqueous extract. These compound classes are well-documented for their antimicrobial properties. Flavonoids, in particular, exert antibacterial effects through multiple mechanisms including inhibition of nucleic acid synthesis, cytoplasmic membrane damage, and attenuation of bacterial virulence factors (Salem et al., 2026; Alshawish et al., 2025).

Tiliroside, a characteristic flavonoid of *Tilia* species, has been reported to possess significant antibacterial activity against Gram-negative bacteria by disrupting lipopolysaccharide layer integrity. Similarly, chlorogenic acid and caffeic acid present in the ethanolic extract exhibit

synergistic antibacterial effects when combined with other phenolics. The aqueous extract, while rich in saponins and carbohydrates, lacked sufficient concentrations of these potent antimicrobial compounds, explaining its weaker and primarily bacteriostatic action.

The lack of correlation between antibiotic resistance level and extract susceptibility is particularly noteworthy. This suggests that the mechanisms of bacterial resistance to beta-lactams, fluoroquinolones, and aminoglycosides (e.g., ESBL production, efflux pumps, target site mutations) do not confer resistance to the complex mixture of phytochemicals in *T. cordata* extracts. This finding supports the potential use of plant extracts against MDR strains that no longer respond to conventional antibiotics (Kadak & Salem, 2020; Salem & Lakwani, 2024).

5.5 Comparison with Previous Studies

Our MIC values for *T. cordata* are comparable to or better than those reported for other medicinal plants against MDR *E. coli*. Salem (2024) reported MICs of 2-8 mg/mL for *Usnea barbata* extract against *E. coli*, while Soof et al. (2025) documented rapid bactericidal activity of *Sinapis alba* essential oil with MICs of 1.56-6.25 mg/mL. The MIC50 of 6.25 mg/mL for the ethanolic extract in our study places *T. cordata* among moderately active plant extracts. However, direct comparisons are complicated by differences in extraction methods, plant parts used, bacterial strains, and susceptibility testing protocols.

A previous study on *Tilia platyphyllos* (a related species) reported MICs of 20-40 mg/mL against standard *E. coli* ATCC 25922, which is higher than our findings. This discrepancy may be attributed to differences in extraction solvent (pure ethanol vs. 70% ethanol) and plant part (flowers vs. leaves). Our use of leaves, which are richer in tannins and flavonoids compared to flowers, may explain the superior activity observed.

5.6 Limitations of the Study

Several limitations should be acknowledged. First, this was an in vitro study; the results may not directly translate to in vivo efficacy due to factors such as poor bioavailability, metabolism, protein binding, and tissue distribution of phytochemicals. Second, we did not perform quantitative analysis (HPLC or GC-MS) to precisely identify and quantify individual bioactive compounds. Third, toxicity studies were not conducted, limiting conclusions about safety for human use. Fourth, the sample size (30 isolates) may not fully represent the diversity of *E. coli* strains circulating in the Libyan population. Fifth, we did not investigate the molecular mechanisms of action or potential synergy between *T. cordata* extracts and conventional antibiotics. Finally, we did not assess the activity of the extracts against biofilm-forming *E. coli*, which is highly relevant to recurrent UTIs.

5.7 Implications for Clinical Practice and Future Research

Despite these limitations, our findings have important implications. The ethanolic extract of *T. cordata* shows promise as a natural alternative for managing UTIs caused by MDR *E. coli*, particularly in resource-limited settings where new antibiotics are unavailable or unaffordable. The extract could be developed as a phytotherapeutic agent either alone or in combination with low-dose antibiotics to reduce resistance emergence (Salem et al., 2025).

Future research should focus on: (1) in vivo efficacy studies using animal models of UTI, (2) acute and sub-chronic toxicity assessments, (3) identification and isolation of active compounds using bioassay-guided fractionation, (4) evaluation of synergistic effects with conventional antibiotics, (5) formulation development (e.g., capsules, solutions, or topical preparations), (6) clinical trials in human subjects with UTIs, and (7) investigation of mechanisms of action including effects on efflux pumps and biofilm formation.

6. Conclusion and Recommendations

6.1 Conclusion

This study evaluated the inhibitory activity of aqueous and ethanolic extracts of *Tilia cordata* leaves against 30 clinical isolates of MDR *E. coli* from UTIs. The key findings are:

1. An alarmingly high prevalence of MDR (86.7%) and ESBL-producing (60%) *E. coli* among UTI patients in Bani Waleed, Libya, with complete susceptibility only to carbapenems and colistin.
2. The ethanolic extract of *T. cordata* exhibited superior antibacterial activity compared to the aqueous extract, with MIC50 values of 6.25 mg/mL and 12.5 mg/mL, respectively ($p < 0.001$).
3. The ethanolic extract demonstrated bactericidal activity (MBC/MIC ≤4) against 73.3% of isolates, whereas the aqueous extract was predominantly bacteriostatic.
4. Phytochemical analysis revealed higher concentrations of flavonoids, phenolics, and terpenoids in the ethanolic extract, explaining its enhanced activity.
5. No cross-resistance was observed between conventional antibiotics and *T. cordata* extracts, suggesting independent mechanisms of action.

The hypothesis that the ethanolic extract exhibits significantly higher inhibitory activity compared to the aqueous extract, with MIC values ≤10 mg/mL, is supported by the data.

6.2 Recommendations

Based on the findings, the following recommendations are proposed:

For clinical practice:

1. Local antibiograms should be updated regularly to guide empirical antibiotic selection for UTIs, given the high resistance rates observed.
2. Carbapenems should be reserved for confirmed MDR infections to preserve their efficacy.
3. *Tilia cordata* extracts may be considered as adjunctive therapy for recurrent or MDR UTIs under medical supervision, pending further safety data.

For healthcare policy:

1. Antimicrobial stewardship programs must be implemented in Libyan hospitals to combat resistance emergence.
2. Over-the-counter antibiotic sales should be restricted by enforcement of prescription-only regulations.
3. Investment in natural product research should be prioritized as a cost-effective strategy to discover new antimicrobial agents.

For future research:

1. Conduct in vivo studies to evaluate efficacy and safety of *T. cordata* extracts in animal models of UTI.
2. Perform toxicity assessments (acute, sub-chronic) to establish safety margins.
3. Isolate and characterize the individual bioactive compounds using chromatographic and spectroscopic techniques.
4. Investigate synergistic effects with conventional antibiotics to potentially lower therapeutic doses and reduce resistance.
5. Develop and validate standardized extraction protocols for consistent phytochemical composition.
6. Conduct randomized controlled clinical trials comparing *T. cordata* extract with standard antibiotic therapy for uncomplicated UTIs.
7. Explore the activity against biofilm-forming uropathogenic *E. coli* using biofilm inhibition and eradication assays.
8. Investigate mechanisms of action at molecular level, including effects on efflux pumps, quorum sensing, and outer membrane proteins.

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