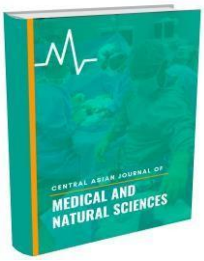

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Antibacterial Activity of Pyracantha Coccinea Ethanolic Fruit Extract Against Uropathogenic Escherichia Coli Isolated from Patients with Urinary Tract Infection in Kirkuk, Iraq

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Abstract: Introduction: Urinary tract infection (UTI) is one of the most common bacterial infections with high prevalence, especially in women. Uropathogenic *Escherichia coli* (UPEC) are the most frequent aetiological agents of uncomplicated and recurrent UTIs. Objective: The study was conducted to evaluate the phenotypic and antimicrobial susceptibility characteristics and antibacterial activity of *Pyracantha coccinea* (*P. coccinea*) ethanolic fruit extract against UPEC isolates from patients attending Kirkuk Governorate Maternity and Children's Hospital, Iraq. Methods: This cross-sectional laboratory-based study was carried out between October 2025 and March 2026 at Kirkuk Governorate Maternity and Children Hospital and the Department of Biology, College of Science, University of Kirkuk. Standard microbiological methods were used to culture midstream urine samples obtained from patients clinically suspected of UTI. Bacterial identification of *E. coli* isolates was performed using conventional biochemical tests and the automated VITEK2 Compact system from bioMérieux (France). Antimicrobial susceptibility testing was conducted utilizing VITEK2 and the Kirby-Bauer disk diffusion methods as per CLSI guidelines. The Kirby-Bauer method was used to test the antibacterial activity of ethanolic fruit extract of *P. coccinea* at concentrations of 25, 50, 75 and 100 μg/mL. Results: Positive cultures were detected on 110 (59.13%) patients. The most common isolate was *E. coli* 45 [76.27%]. Ampicillin, cefotaxime, ceftriaxone and tetracycline had very high resistance rates whereas carbapenems, nitrofurantoin fosfomycin and amikacin were more active. *P. coccinea* ethanolic extract has moderate antimicrobial activity against UPEC as denoted by the highest inhibition zone: 25 μg/mL (12.00 ± 0.58 mm). Although there were significant differences in extract concentrations (P = 0.008). Conclusion: A high rate of multidrug-resistant UPEC has been detected in UTI patients in Kirkuk. Based on the *in vitro* studies, *P. coccinea* ethanolic fruit extract had significant antibacterial activity and has the potential to be a natural source of antibacterials that can be further investigated through plant phytochemical and molecular characterization.

Keywords: Urinary Tract Infection (UTI), Uropathogenic *Escherichia Coli* (UPEC), Antimicrobial Resistance, Antibacterial Activity, *P. Coccinea*.

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1. Introduction

Urinary tract infection (UTI) is one of the most frequently observed bacterial infections around the world and a serious public health challenge. It is a burden due to its high prevalence, recurrence, healthcare cost and emergence of antimicrobial resistance. Around 50-60% of women have at least one UTI, and a significant proportion develop recurrent infections that reduce quality of life and increase the risk of complications [1].

The low prevalence of UTIs in adult men, on the other hand, can be explained by anatomical and physiological attributes (Klein & Hultgren, 2020), including short urethra, proximity to the anal site, hormonal factors and sexual activity favouring bacterial colonization and upward pass of infection.

UTIs are categorized by anatomic site and severity as either lower (cystitis, urethritis) or upper (Pyelonephritis) that can progress to urosepsis and renal damage without treatment. Presently, guidelines recommend diagnosis based on clinical signs validated by microbiological confirmation, especially urine culture and antimicrobial susceptibility testing [2]. Some of the common symptoms include dysuria, frequency, urgency, fever suprapubic or flank pain and hematuria. Among the causative agents, *Escherichia coli* - particularly uropathogenic *E. coli* (UPEC)- is the most common, responsible for 70-95% of uncomplicated cases [3], [4]

Multiple virulence factors allowing UPEC to adhere, invade, persist and evade immunity have been trained. Adhesins, fimbriae, toxins, iron-acquisition systems, capsules and flagella associated during biofilm development, also belong to those not-altered proteins. Novel virulence-related [5] and biofilm formation genes can be associated with recurrence, such as fimH, CNF1, csgA and Agn43 [6]. Flagella-mediated motility further enhances ascending infection. One consequence that has been a growing concern in the management of UTIs is the emergence of antimicrobial resistance among uropathogenic *E. coli* (UPEC) isolates, particularly during the past couple of decades very quickly.

The widespread use and misuse of antibiotics has accelerated the emergence and the spread of multidrug resistant (MDR) strains, including those producing extended-spectrum beta-lactamases (ESBLs), resulting in an increasing resistance to all classes of nowadays used antibiotics, such as ampicillin, cephalosporins, fluoroquinolones and trimethoprim-sulfamethoxazole) [7], [8]. This underscores the need for antimicrobial stewardship and culture-guided therapy. Data, especially regarding UPEC molecular epidemiology, virulence and resistance patterns in Iraq including Kirkuk province are relatively limited. While the incidences of MDR and ESBL are reported to be increasing, few studies combine phenotypic and genotypic conclusions [9]. It further complicates empirical treatment and infection control strategies with this gap.

The Maternity and Children's Hospital of Kirkuk Governorate is a large tertiary facility for women and pediatric populations at high risk for urinary tract infections (UTIs), thus making it an excellent candidate for epidemiological and microbiological evaluation. These studies can provide insights into local UPEC strains, their resistance profiles and pathogenic mechanisms, which would facilitate diagnosis, treatment and control of UTI.

Medicinally used plants are recently considered potential antimicrobial solutions. Pyracantha coccinea (Rosaceae) is reported to have bioactive compounds such as phenolic acids, flavonoids, tannins including chlorogenic acid and gallic acid, floppy trunk type quercetin derivatives which have high potential role in antimicrobial [10]. They may work by disrupting bacterial membranes, enzyme inhibition, nucleic acid synthesis interference or biofilm formation suppression [11].

Thus this study aimed at determination of the phenotypic characteristics, antimicrobial susceptibility patterns and antibacterial activity of *P. coccinea* ethanolic fruit extract against UPEC isolates from UTI patients in Kirkuk -Iraq which will be a step towards establishing an epidemiological data profile locally and exploring plant based antibacterial agents from domestic sources for use as alternative and/or supplementary approaches to treat diseases caused by multi-drug resistant (MDR) pathogens.

2. Materials and Methods

This cross-sectional observational laboratory-based study was performed from October 2025 to March 2026 to evaluate the phenotypic characteristics, antimicrobial susceptibility profile and antibacterial activity of *P. coccinea* ethanolic fruit extract against uropathogenic *E. coli* (UPEC) isolates recovered from UTI patients attending Kirkuk Governorate Maternity and Children's Hospital, Iraq. The hospital is one of the few key tertiary referral hospitals that offer women and children's healthcare for Kirkuk Governorate and serves both urban as well as rural populations which provides a representative sampling frame of community -and hospital-acquired UTIs. Traditional microbiological processing and urine culture was performed at the hospital microbiology laboratory, while advanced microbiological and molecular tests were performed in the molecular microbiology laboratory of the Department of Biology, College of Science, University of Kirkuk.

2.1. Patient Selection and Sample Collection

Inclusion/Exclusion Criteria Patients with clinical presentations consistent with UTI and patients presenting symptoms such as dysuria, urinary frequency, urgency, suprapubic pain or flank pain with fever associated with urinary symptoms were enrolled into the study. Under aseptic conditions, clean mid-stream urine specimens with a minimum volume of 10 mL were collected. In the case of catheterized patients, aseptic urine samples were collected via catheter sampling ports following approved disinfection protocols. Specimens were either transported to the microbiology laboratory within 1 hour from collection or stored at 4°C and processed within four hours according to standard microbiological guidelines.

2.2. General Urine Examination

All collected specimens provided a routine general urine examination (GUE). Gross findings assessed urine color, turbidity and hematuria. Urine samples subjected to centrifugation at 1,500 × g for 5 min were examined microscopically (high-power microscopy) for leukocytes, erythrocytes, epithelial cells, casts, crystals, and bacteria. Pyuria: ≥5 cells/HPF at 7 X μmL. Among all the variables, chemical analysis was done using urine dipstick strips for detection of nitrite, leukocyte esterase (LE), glucose, ketones, protein, pH and specific gravity.

2.3. Bacterial Isolation and Identification

Bacteriological procedures included isolation and identification of bacterial pathogens as per standard guidelines. *E. coli* was isolated by inoculating urine specimens to MacConkey agar and blood agar with calibrated sterile loops followed by aerobic incubation at 37°C for 18-24 hours. Interpretation of significant bacteriuria was based on microbiological standards. Colony morphology, Gram staining and conventional biochemical tests (such as oxidase, catalase, indole, methyl red, Voges-Proskauer citrate utilization urease production kligler iron agar reactions motility) were used for identification of presumptive isolates of *E. coli*. Identification was also confirmed by the automated VITEK2 Compact system (bioMérieux, France).

2.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing performed by both the VITEK2 Compact system and Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) standard methods. Antimicrobial agents tested β-lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracycline, nitrofurantoin and trimethoprim–sulfamethoxazole. Quality control with *E. coli* ATCC 25922 reference strains were performed.

2.5. Preparation of *P. coccinea* Ethanolic Fruit Extract

The antibacterial activity of ethanolic fruit extract from *P. coccinea* was determined employing Kirby-Bauer disk diffusion assay. Mature fruits of *P. coccinea* were collected from different localities of Kirkuk Governorate and were taxonomically identified at the College of Science, University of Kirkuk. The fruits were shade-dried, powdered with a mechanical grinder and extracted with 96% ethanol by cold maceration for 48 hours in constant shaking. The extract was filtered then concentrated in a rotary evaporator under reduced pressure at 40°C, following preparation of stock solutions with Dimethyl Sulfoxide (DMSO), and the working concentrations were 25, 50, 75, and 100 μg/mL.

2.6. Evaluation of Antibacterial Activity

Antibacterial activity was measured as inhibition zone diameter against identified UPEC isolates after 24 hours at 37 °C. Ciprofloxacin and ceftriaxone discs served as the positive control, while DMSO-containing discs were used as negative controls.

2.7. Statistical Analysis

Data entered and coded were statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 26. Lesson The descriptive statistics were given in the number, ratio, mean, standard deviation. Chi-square test ($\chi 2$), Fisher's exact test, and one-way analysis of variance (ANOVA) were applied if appropriate. Statistical significance was defined as a P value of (< 0.05).

3. Results

The study showed that 110(59.13%) women with UTI were found to have positive bacterial culture and negative bacterial culture was seen in 76(40.86%) cases. In terms of isolated microorganism type, Gram-negative bacteria accounted for 7 species (63.64%) and Gram-positive bacteria for 4 species (36.36%). These results reveal that Gram-negative organisms were the major responsible pathogens of UTI s in the study women following.

Table 1. Results of Urine Culture Among the Study Group.

Variables	No. (%)
Positive bacterial culture	110 (59.13%)
Negative bacterial culture	76 (40.86%)
Gram-positive bacteria	4 (36.36%)
Gram-negative bacteria	7 (63.64%)
Total	186 (100%)

The number of patients with positive urine culture was more in the age group 19-40 years, having a share of 34(37.78%), followed by the age group with 41-60 years having a share of 14(15.56%). Out of the 90 cases females represented 68(75.56%) while, boys were only 22(24.44%). The highest number was seen in Gynecology/Antenatal Outpatient Clinics 26(28.89%), then Pediatric Outpatient Clinics 22(24.44%). In terms of risk factors, recent antibiotic consumption was the most prevalent factor detected, which was seen in 30(33.33%) patients followed by recurrent UTI history in 25(27.78%) and pregnancy in 24(26.67%). Dysuria was the commonest clinical presentation affecting 66(73.33%) of patients, urinary frequency/urgency was reported in 61(67.78%) individuals respectively. Fever ≥38 °C was found in 31(34.44%) patients and hematuria in 15(16.67%) patients respectively.

Table 2. Demographic and clinical characteristics of patients with positive urine culture.

Variable	Category	n (%)
Age (years)	< 5	10 (11.11%)
	5-12	12 (13.33%)

	13-18	11 (12.22%)
	19-40	34 (37.78%)
	41-60	14 (15.56%)
	> 60	9 (10.00%)
Sex	Female	68 (75.56%)
	Male	22 (24.44%)
Ward	Outpatient (paediatric)	22 (24.44%)
	Outpatient (gynaecology / antenatal)	26 (28.89%)
	Inpatient (paediatric)	16 (17.78%)
	Inpatient (obstetric / gynaecology)	14 (15.56%)
	Emergency	12 (13.33%)
Risk factors	Pregnancy	24 (26.67%)
	Diabetes mellitus	13 (14.44%)
	Indwelling urinary catheter	11 (12.22%)
	Recurrent UTI history	25 (27.78%)
	Recent antibiotic use	30 (33.33%)
Clinical features	Dysuria	66 (73.33%)
	Frequency/urgency	61 (67.78%)
	Suprapubic / flank pain	38 (42.22%)
	Fever ≥ 38 °C	31 (34.44%)
	Haematuria	15 (16.67%)

E. coli was the most common bacterial isolate obtained from positive urine cultures, with a total of 45(76.27%) isolates, as demonstrated in the study. The second most common isolate was *Klebsiella pneumoniae* 6(10.17%) followed by Proteus mirabilis 3(5.08%). Regarding the recovery of microorganisms and their total number, data are also summarized in Table 2. *Staphylococcus saprophyticus was isolated in 2(3.39%) cases; Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis* and *Acinetobacter baumannii* were each isolated in 1(1.69%) case. These observations corroborate the predominance of Gram-negative bacteria, mainly *E. coli*, as the most common uropathogen among women with UTI.

Table 3. Distribution of bacterial isolates among positive urine cultures.

Bacterial Isolates	No. (%)
Escherichia coli	45 (76.27%)
Klebsiella pneumoniae	6 (10.17%)
Proteus mirabilis	3 (5.08%)
Pseudomonas aeruginosa	1 (1.69%)
Staphylococcus saprophyticus	2 (3.39%)
Staphylococcus aureus	1 (1.69%)
Enterococcus faecalis	1 (1.69%)
Acinetobacter baumannii	1 (1.69%)
Total eligible culture-positive	**59 (100%)**

Out of the 45 (100%) *E. coli* isolates, all were oxidase negative and formed typical metallic green sheen due to acid production in EMB agar. All 100 isolates were also indole-positive, methyl red-positive, Voges-Proskauer-negative, citrate-negative, urease-negative and H_2S-negative. All isolates show acid/acid (A/A) reaction on KIA media without H_2S production and positive motility. Results confirmed the typical biochemical pattern of UPEC isolates in specimens examined.

Table 4. Biochemical and phenotypic characteristics of *E. coli* isolates (n = 45).

Biochemical Test	Positive n (%)	Negative n (%)	Typical Reaction of *E. coli*
Oxidase test	0 (0%)	45 (100%)	Negative
EMB agar	45 (100%)	0 (0%)	Metallic green sheen
Indole test	45 (100%)	0 (0%)	Positive
Methyl red (M.R) test	45 (100%)	0 (0%)	Positive
Voges-Proskauer (V.P) test	0 (0%)	45 (100%)	Negative
Simmons citrate test	0 (0%)	45 (100%)	Negative
Kligler Iron Agar (KIA)	45 (100%)	0 (0%)	A/A reaction
H_2S production	0 (0%)	45 (100%)	Negative
Urease test	0 (0%)	45 (100%)	Negative
Motility test	45 (100%)	0 (0%)	Positive

Ampicillin was found to confer the highest resistance rate among UPEC isolates, with a detection frequency of 39 (86.67%) isolates, followed by cefotaxime in 31(68.89%), cephalexin and ceftriaxone each in 30(66.67%), and cefuroxime and tetracycline each in 29(64.44%) isolates [19]. Ciprofloxacin, levofloxacin and trimethoprim–sulfamethoxazole all showed moderate rates of resistance. In contrast, the two carbapenems were highly effective antimicrobials with imipenem and meropenem susceptibility rates of 43(95.56%) and 42(93.33%), respectively. Amikacin was similarly susceptible (40/45 [88.89%], 95% C.I. = 78.00, 94.18); as were fosfomycin and nitrofurantoin (39/45 [86.67%] (95% C.I. = 74,87 to96,68) and37/4582.22. Conclusions These results demonstrate a high level of multidrug resistance in UPEC isolates.

Table 5. Kirby-Bauer Disc Diffusion-Antimicrobial Susceptibility Profile of Confirmed UPEC Isolates.

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	4 (08.89%)	2 (04.44%)	39 (86.67%)
Amoxicillin-clavulanate	14 (31.11%)	4 (08.89%)	27 (60.00%)
Cephalexin	10 (22.22%)	5 (11.11%)	30 (66.67%)
Cefuroxime	12 (26.67%)	4 (08.89%)	29 (64.44%)
Cefotaxime	11 (24.44%)	3 (06.67%)	31 (68.89%)
Ceftriaxone	12 (26.67%)	3 (06.67%)	30 (66.67%)
Ceftazidime	13 (28.89%)	4 (08.89%)	28 (62.22%)
Cefepime	18 (40.00%)	5 (11.11%)	22 (48.89%)
Imipenem	43 (95.56%)	1 (02.22%)	1 (02.22%)
Meropenem	42 (93.33%)	2 (04.44%)	1 (02.22%)
Gentamicin	24 (53.33%)	3 (06.67%)	18 (40.00%)

Amikacin	40 (88.89%)	2 (04.44%)	3 (06.67%)
Ciprofloxacin	18 (40.00%)	3 (06.67%)	24 (53.33%)
Levofloxacin	20 (44.44%)	3 (06.67%)	22 (48.89%)
Trimethoprim–sulfamethoxazole	15 (33.33%)	2 (04.44%)	28 (62.22%)
Nitrofurantoin	37 (82.22%)	3 (06.67%)	5 (11.11%)
Fosfomycin	39 (86.67%)	3 (06.67%)	3 (06.67%)
Tetracycline	12 (26.67%)	4 (08.89%)	29 (64.44%)

The ethanolic extract of *P. coccinea* fruits exhibited antibacterial activity against UPEC isolates in vitro. Among the concentrations, 25 μg/mL exhibited highest antibacterial activity with a mean inhibition zone of 12.00 ± 0.58 mm, moderate antibacterial activity. Contrary to the non-volatile fraction, the bacteriostatic activity decreased with increasing concentrations of extracts as inhibition zones were identified at 50 μg/mL (9.00 ± 0.33 mm) and 75 μg/mL (7.00 ± 0.58 mm). The inhibition zone was expected to increase in a concentration dependent manner, with 8.00 ± 0.33 mm at 100 μg/mL as an example. Inhibition zones of ciprofloxacin and ceftriaxone were significantly higher (54.00 ± 1.00 mm and 50.00 ± 0.58 mm, respectively). One-way analysis of variance (ANOVA) showed significant differences among different concentrations of the extracts ($F = 9.74$, $P = 0.008$).

Table 6. Antibacterial Activity of Ethanolic Fruit Extract of *P. coccinea* and Antibiotic Disc Against Isolated UPEC.

Tested Material	Concentration / Disc Potency	Mean Inhibition Zone (mm)	Activity Interpretation
P. coccinea ethanolic extract	25 μg/mL	12.00 ± 0.58	Moderate activity
P. coccinea ethanolic extract	50 μg/mL	9.00 ± 0.33	Low–moderate activity
P. coccinea ethanolic extract	75 μg/mL	7.00 ± 0.58	Low activity
P. coccinea ethanolic extract	100 μg/mL	8.00 ± 0.33	Low–moderate activity
Ciprofloxacin (positive control)	5 μg/disc	54.00 ± 1.00	High activity
Ceftriaxone (positive control)	30 μg/disc	50.00 ± 0.58	High activity
Ethanol (negative control)	----	0.00 ± 0.00	No activity

Figure 1. Wells diffusion method - zones of inhibition of UPEC with ethanolic fruit extract and antibiotic controls from *P. coccinea*

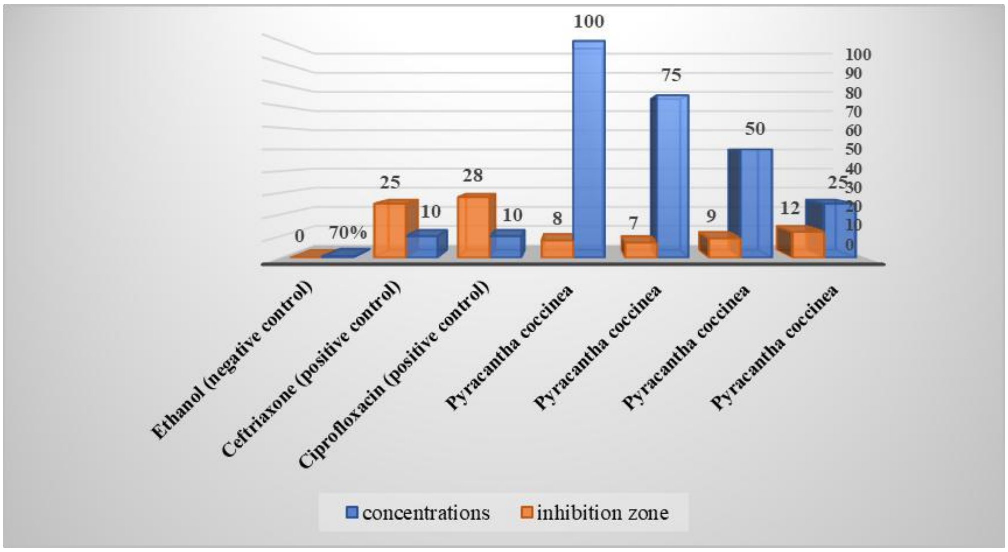

Figure 2. MIC and zone of inhibition for *P. coccinea*, Ceftriaxone and Ciprofloxacin.

4. Discussion

We have seen that in the present study, among those with UTI positivity on urine culture, a total of 110 (59.13%) women showed positive bacterial culture and Gram-negative bacteria were also more dominant isolated microbiologic agents. These results conform with previous studies where it was also reported that the Gram-negative enteric bacteria are still the leading causes of UTIs worldwide, particularly among women and in hospitalized cases. The variables : Foxman [12]; Flores-Mireles et al. [13] stated UTIs are one of the most widely spread bacterial infections worldwide, mainly being produced by members from *Enterobacteriaceae* family [14], [15]. The high number of females (68 (75.56%)) observed in our study is consistent with other studies observing females were more prone for UTIs due to anatomical and physiological differences. According to Klein and Hultgren [16], the colonization by bacteria as well as ascending infection is easier in women than in men due to the shorter female urethra, and due to the proximity of the anal region. Czajkowski et al. reported similar results. [17] and Yang et al. in 2022, showing that women of reproductive age have the greatest risk group for UTIs.

In agreement with He et al. [18], those 19-40 years had the most positive urine cultures [19] and Medina and Castillo-Pino [20], who attributed the higher prevalence of UTI's to sex, pregnancy or by hormones. *E. coli* represented in this study the majority isolate (76.27%) and that corresponds with previous global as well as regional reports documented UPEC which recognized as the main uropathogen [21][22][23]. Multiple virulence determinants facilitating colonization and persistence contribute to its predominance, including adhesins, fimbriae, toxins, iron-acquisition systems and motility or biofilm formation [24], [25].

The typical identification of *E. coli* was further confirmed by biochemical characteristics, such as the oxidase-negative and indole-positive results, metallic green sheen on EMB agar and motility which indicated that classical means might be efficient [26], [27]. Antimicrobial susceptibility testing indicated the high rate of resistance to popular antibiotics, especially ampicillin, cefotaxime, ceftriaxone and tetracycline indicating reports of multidrug resistant [28], [29] Burkholderia aggressive response to treatment in Iraq. The reason for this resistance is probably from the widespread use of antibiotics and spread of β-lactamase-producing strains. Increased resistance to third generation cephalosporins indicates dissemination of ESBL-producing Enterobacterales [30].

In line with Ormeño et al (2022) moderate to high levels of resistance to fluoroquinolones were also seen. This trend relates to misuse of antimicrobials and plasmid-mediated resistance [7], [9] (2022), Hooper Jacoby (2015). In contrast, the efficacy of carbapenems -imipenem and meropenem- remained very high (>93% susceptibility), consistent with that reported by Karlowsky et al. (2017). Our results support previous reports that nitrofurantoin, fosfomycin and amikacin have potent activity against resistant UPEC isolates (Nguyen et al., 2024; Başkan et al., 2026).

Antibacterial activity of ethanolic fruit extract of *P. coccinea* was moderate ($p < 0.05$) at 25 µg/mL which is also parallel to the results obtained by Karacaer (2023) and Keser et al.[51]. (2014). The responsible compound of this activity could be phenolic compounds, flavonoids, tannins and other polyphenols that disrupt the bacterial membrane and inhibit enzymatic processes (Daglia, 2012; Cushnie & Lamb, 2011; Borges et al., 2013). As previously indicated by Jafarzadeh et al., the large difference in inhibition zones ($P = 0.008$) suggests concentration-dependent action. (2020) and Jalali et al. (2026).

The findings of the present study confirm the predominance of multidrug-resistant UPEC isolates among women with UTIs and highlight the potential antibacterial activity of *P. coccinea* ethanolic fruit extract as a possible complementary therapeutic agent against resistant urinary pathogens.

5. Conclusion

The study confirmed the high frequency of culture-proven UTIs in Kirkuk and revealed UPEC as a main pathogen predominantly between women of childbearing age. Marked multidrug resistance to ampicillin, third-generation cephalosporins and tetracycline, and trimethoprim sulfamethoxazole was seen while carbapenems amikacin, fosfomycin, and nitrofurantoin showed limited effectiveness support their use as preferred therapies. The ethanolic fruit extract of Pyracantha coccinea exhibited moderate, concentration-dependent antibacterial activity against UPEC and warrants further investigation by way of phytochemical analysis, minimum inhibitory concentration determination, and *in vivo* testing. Conclusions These data support the need for culture-directed therapy, antimicrobial stewardship and future research on potential phytotherapeutic agents to combat multidrug-resistant uropathogens.

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