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Comparison of ethanolic and ethyl acetate fractions of Iraqi Medicago sativa for the treatment of urinary tract infection

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Abstract. *Urinary tract infections (UTIs), predominantly caused by uropathogenic Escherichia coli (UPEC), affect over 100 million people annually and are a leading cause of morbidity due to rising antibiotic resistance. Medicago sativa (alfalfa), a medicinal plant rich in phytochemicals, has shown antibacterial potential, yet its efficacy against UPEC in Iraq remains unexplored. This study investigates the antibacterial effects of M. sativa ethanolic and ethyl acetate fractions as potential alternatives to conventional antibiotics for UTI treatment.*

Methods. M. sativa was collected in Kirkuk, defatted with hexane, extracted with 85% ethanol, and fractionated into petroleum ether, chloroform, ethyl acetate, and ethanolic fractions. Phytochemical analyses, including Dragendorff's, Mayer's, and ppigh-performance liquid chromatography were performed. Urine samples from 85 UTI patients were cultured, yielding 30 UPEC isolates. Antibacterial activity was evaluated using the agar well diffusion method, with minimal inhibitory concentrations (MICs) determined via microplate serial dilution. Antibiotic susceptibility was tested using the Kirby-Bauer method against eight antibiotics. Data were analyzed using SPSS v26 (ANOVA, LSD).

Results. Most participants (56.7%) were under 40 years old, with females more affected. The ethanolic fraction demonstrated superior antibacterial activity, with a mean inhibition zone of 21.96 ± 1.9 mm at 75 mg/ml (p=0.001), compared to 17.32 ± 1.5 mm for the ethyl acetate fraction. High-performance liquid chromatography confirmed bioactive compounds, including gallic acid, quercetin, and myricetin. Meropenem exhibited 100% sensitivity, while cephalothin showed complete resistance.

Conclusions. M. sativa extracts, particularly the ethanolic fraction, exhibit significant antibacterial activity against UPEC, offering a promising alternative to antibiotics. Larger, multicenter studies are needed to validate these findings and explore clinical applications.

Keywords: *urinary tract infection, antibiotic, ethyl acetate fraction, M. sativa extract, ethanolic fraction.*

Conflict of interest. The authors declare no conflict of interest.

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Порівняння етанольної та етилацетатної фракцій іракської Medicago sativa для лікування інфекцій сечовивідних шляхів

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Резюме. *Інфекції сечової системи (ІСС), переважно спричинені уропатогенним Escherichia coli (UPEC), щороку вражають понад 100 мільйонів людей і є основною причиною захворюваності через зростання антибіотикорезистентності. Medicago sativa (люцерна), лікарська рослина, багата на фітохімічні сполуки, демонструє антибактеріальний потенціал, але її ефективність проти UPEC в Іраку досі не досліджена. Це дослідження спрямоване на вивчення антибактеріальних ефектів етанольної та етилацетатної фракцій M. Sativa, як потенційних альтернатив традиційним антибіотикам для лікування ІСС.*

Методи. M. sativa була зібрана в Кіркуку, знежирена гексаном, екстрагована 85% етанолом і фракціонована на фракції петролейного ефіру, хлороформу, етилацетату та етанолу. Проводили фітохімічний аналіз, включно з тестами Драгондорфа, Майєра та високоефективною рідинною хроматографією. Зразки сечі 85 пацієнтів з ІСС були культивовані, що дозволило визначити 30 ізолятів UPEC. Антибактеріальну активність оцінювали методом дифузії в агарі, а мінімальні інгібуючі концентрації (MIK) визначали шляхом серійного розведення в мікропланшетах. Чутливість до антибіотиків тестували методом Кірбі-Бауера проти восьми антибіотиків. Дані аналізували за допомогою SPSS v26 (ANOVA, LSD).

Результати. Більшість учасників (56,7%) були молодше 40 років та переважали жінки. Етанольна фракція показала вищу антибактеріальну активність із середньою зоною інгібування 21,96 ± 1,9 мм при 75 мг/мл (p=0,001) порівняно з 17,32 ± 1,5 мм для етилацетатної фракції. Високоефективна рідинна хроматографія підтвердила наявність біоактивних сполук, зокрема галовової кислоти, кверцетину та мірицетину. Меропенем мав 100% чутливість, тоді як цефалотин показав повну резистентність.

Висновки. Екстракти M. sativa, особливо етанольна фракція, демонструють значну антибактеріальну активність проти UPEC, пропонуючи перспективну альтернативу антибіотикам. Для підтвердження результатів і вивчення клінічного застосування необхідні більші багатоцентрові дослідження.

Ключові слова: *інфекція сечової системи, антибіотик, етилацетатна фракція, екстракт M. sativa, етанольна фракція.*

Introduction. Urinary tract infections (UTIs) are among the most common bacterial infections, significantly contributing to morbidity and mortality, second only to respiratory tract infections (RTIs). UTIs can affect individuals of any age but are more prevalent in women, with over 50% of women and approximately 12% of men experiencing a UTI in their lifetime [1]. Uropathogenic Escherichia coli (UPEC) is the primary cause of UTIs [2]. Asymptomatic bacteriuria, defined as bacterial proliferation in urine without urinary symptoms, is commonly due to commensal colonization [3]. It occurs in 1–5% of healthy premenopausal women, 4–19% of healthy older adults, and 15–50% of institutionalized elderly individuals [4].

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Medicinal plants play a critical role in developing innovative medications. In many rural communities, natural remedies remain preferred due to the efficacy of plant-derived drugs and concerns about the adverse effects of synthetic medications. Plants are a vital source of commercially used pharmaceuticals, with many synthetic drugs originating from natural plant compounds [5]. Antibiotics such as ciprofloxacin, trimethoprim, and sulfamethoxazole are commonly prescribed for UTIs, but high recurrence rates and increasing antibiotic resistance pose significant challenges [6]. Identifying novel antimicrobial agents is a global priority [7]. Pharmacological and phytochemical research supports the traditional use of herbs, offering the potential for clinical research and the development of new medications [5].

Medicago sativa (alfalfa), a perennial legume from the Leguminosae family, exhibits antibacterial properties due to its phytochemical constituents [8]. Multidrug-resistant pathogens, including E. coli, are a growing global threat, necessitating the development of new antimicrobial drugs [9]. This study investigates

the antibacterial efficacy of M. sativa extracts against UPEC in urine samples from Iraqi patients, highlighting its potential as a superior alternative to conventional antibiotics.

Materials and methods. *Plant Material.* The entire *Medicago sativa* plant, belonging to the Fabaceae family, was collected near Kirkuk, Iraq. The Pharmacognosy Department at the College of Pharmacy, Ashur University, verified the plant's identity. The study was approved by the local ethics committee of the Pharmacology Department at Baghdad College of Medicine (approval code: 178, dated 20/10/2021). In November 2021, during full bloom, the plant was harvested, cleaned, air-dried at room temperature in the shade, coarsely crushed, and weighed.

Extraction and fractionation. A 350-gram sample of powdered plant material was defatted with hexane, dried, and extracted using 85% ethanol. The resulting extract was fractionated into four fractions—petroleum ether, chloroform, ethyl acetate, and ethanolic—and dried using anhydrous sodium sulfate [10].

Preliminary qualitative phytochemical analysis. Chemical analyses were conducted on crude and fractionated M. sativa extracts to identify active constituents, including alkaloids, flavonoids, terpenoids, and steroids, following established methods [11].

Chemical tests:

- *Dragendorff's test (alkaloids):* A solution of potassium bismuth iodide, containing basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI), produces an orange or reddish-brown precipitate upon contact with alkaloids, confirming their presence.
- *Mayer's test (alkaloids):* Two milliliters of alcoholic extract were mixed with 1–2 drops of Mayer's reagent (potassium mercury iodide in water). A white or creamy precipitate indicated alkaloids.
- *Lead acetate test (flavonoids):* One milliliter of 10% lead acetate solution was mixed with 2 ml of alcoholic extract. A yellowish-white precipitate confirmed flavonoids.
- *Salkowski test (terpenoids):* Two milliliters of organic extract or fractions were mixed with 2 ml of chloroform, evaporated to dryness, and treated with 2 ml of concentrated sulfuric acid. After heating for 2 minutes, a reddish-brown layer at the interface indicated terpenoids.
- *Liebermann-Burchard test (steroids):* Two milliliters of crude extract or fractions were mixed with 1 ml of chloroform, 2–3 ml of acetic anhydride, and 2 drops of concentrated sulfuric acid. A dark green color confirmed steroids.
- *H_2SO_4 test (steroids):* Two milliliters of extract or fractions treated with sulfuric and acetic acids developed a greenish color, indicating steroids.

High-performance liquid chromatography analysis of phenols and flavonoids in M. sativa extract. Phenols and flavonoids were analyzed using high-performance liquid chromatography (HPLC), not fast-liquid chromatography (FLC). A Nucleodur C18-DB column (50 x 4.6 mm, 3 µm particle size) was used under optimized conditions. The mobile phase consisted of a linear gradient of 0.05% trifluoroacetic acid (TFA) in deionized water (solvent A) and 0.05% TFA in methanol (solvent B).

Urine sample collection and culture. Urine samples were collected in sterile containers from 85 UTI patients at Medical City Hospitals, Iraq. After bacterial isolation, 30 samples were confirmed to contain *Escherichia coli.* The urine was inoculated onto agar plates and incubated aerobically at 37°C for 24 hours to isolate *E. coli.*

Antibiotic susceptibility testing. The susceptibility of bacterial isolates to six antibiotics – meropenem (10 µg), ceftriaxone (10 µg), levofloxacin (5 µg), ciprofloxacin (10 µg), nitrofurantoin (100 µg), and amikacin – was tested using the modified Kirby-Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates resistant to three or more antibiotics were classified as multidrug-resistant [12].

Evaluation of antibacterial activity. The ethanolic and ethyl acetate fractions of *M. sativa* were tested for their ability to inhibit Gram-negative bacteria, such as *E. coli*, using the agar well diffusion method [13, 14]. Pure *E. coli* colonies were grown on Mueller-Hinton Agar (MHA) at 37°C for 24 hours, suspended in sterile saline, and spread onto MHA plates. Three concentrations of plant extract were added to wells, with a 10 µg meropenem disk as a positive control and dimethyl sulfoxide as a negative control. Inhibition zones were measured after 24 hours at 37°C.

Minimal inhibitory concentration (MIC) determination. MICs were determined using a 96-well microplate. Each well received 100 µl of MHA, followed by 100 µl of plant extract in the first column. Serial dilutions were performed, discarding the final volume. A 50 µl bacterial suspension was added, and MIC was determined by the absence of turbidity [13].

Data analysis. Data were analyzed using IBM SPSS Statistics version 26. Descriptive statistics (means ± standard deviations, ranges, percentages, frequencies) were calculated for participant demographics, antibiotic susceptibility, inhibition zones, and HPLC retention times. One-way analysis of variance (ANOVA) with post-hoc least significant difference (LSD) tests was used to compare mean inhibition zones across Medicago sativa extract concentrations (25, 50, 75 mg/ml) and meropenem, with a significance level of $p=0.001$.

Results. *Bacterial development patterns in patients with UTI.* This study analyzed 85 urine samples from patients with UTI at hospitals in Baghdad, Iraq. Of these, 30 samples were confirmed to contain uropathogenic Escherichia coli (UPEC). The study participants, with a mean age of 38.0 ± 19.4 years, ranged from 20 to 72 years. The majority (56.7%) were under 40 years old, with 17 participants aged 20–39, 7 aged 40–59, and 6 aged 60–72. Females were more frequently affected than males.

Antibiotics susceptibility testing of UPEC isolates. Thirty UPEC isolates were tested against eight antibiotics: meropenem, amikacin, ceftriaxone, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim, and cephalothin. Meropenem exhibited the highest sensitivity (100%), followed by amikacin (83.3%) and nitrofurantoin (80.0%). Ceftriaxone, ciprofloxacin, levofloxacin, and trimethoprim showed moderate sensitivity, while cephalothin was completely resistant (100%) (Table 1). These findings underscore the importance of antibiotic susceptibility testing for effective UPEC treatment.

Table 1

Antibiotic Susceptibility of UPEC Isolates

Drug	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Meropenem	30 (100.0)	0 (0.0)	0 (0.0)
Amikacin	25 (83.3)	3 (10.0)	2 (6.7)
Ceftriaxone	10 (33.3)	1 (3.3)	19 (63.4)
Ciprofloxacin	14 (46.7)	0 (0.0)	16 (53.3)
Levofloxacin	14 (46.7)	0 (0.0)	16 (53.3)
Trimethoprim	16 (53.3)	0 (0.0)	14 (46.7)
Nitrofurantoin	24 (80.0)	3 (10.0)	3 (10.0)
Cephalothin	0 (0.0)	0 (0.0)	30 (100.0)

Phytochemical analysis of Medicago sativa extract. The crude extract of M. sativa contained alkaloids, flavonoids, terpenoids, and steroids, while the ethyl acetate fraction solely contained flavonoids, as indicated by the pre-eliminatory phytochemical study as shown in (Table 2).

Table 2

Phytochemical screening of crude extract and different fractions

Crude and fractions	Alkaloids	Flavonoids	Steroids	Terpenoids
Crude	+	+	+	+
F1	+	-	+	+
F2	+	-	+	+
F3	-	+	-	-

HPLC analysis. HPLC is a sensitive instrument for detecting low concentrations of phytochemicals in extracts. It allows for qualitative identifications by comparing retention periods with reference standards. HPLC analysis detects gallic acid, salicylic acid, caffeic acid, pyrogallol, quercetin, myricetin, naringin, and apigenin, as shown in Tables 3, 4, and Fig. 1 and 2.

Table 3

Standard HPLC results

Components	Retention Time	Area UV
Gallic Acid	2.33	32274
Pyrogallol	3.01	39079
Caffeic Acid	4.09	41169
Salicylic Acid	5.18	52672
Naringin	6.26	45870
Myrecetin	7.02	71387
Quercetin	7.92	47835
Apigenin	8.84	45761

Table 4

HPLC results of analyzed fractions with their retention

Fraction	Components	Retention Time	Area UV
Ethanol	Gallic Acid	2.363	24865
	Pyrogallol	3.04	25753
	Caffeic Acid	4.107	18919
	Salicylic Acid	5.183	20115
	Naringin	6.268	18261
	Myricetin	7.017	20899
	Quercetin	7.93	29338
	Apigenin	8.852	23199
Ethyl acetate	Gallic Acid	2.007	33379
	Pyrogallol	4.09	46455
	Caffeic Acid	5.163	25141
	Salicylic Acid	6.252	23256
	Naringin	6.992	16266
	Myricetin	7.902	43078
	Quercetin	8.828	25338

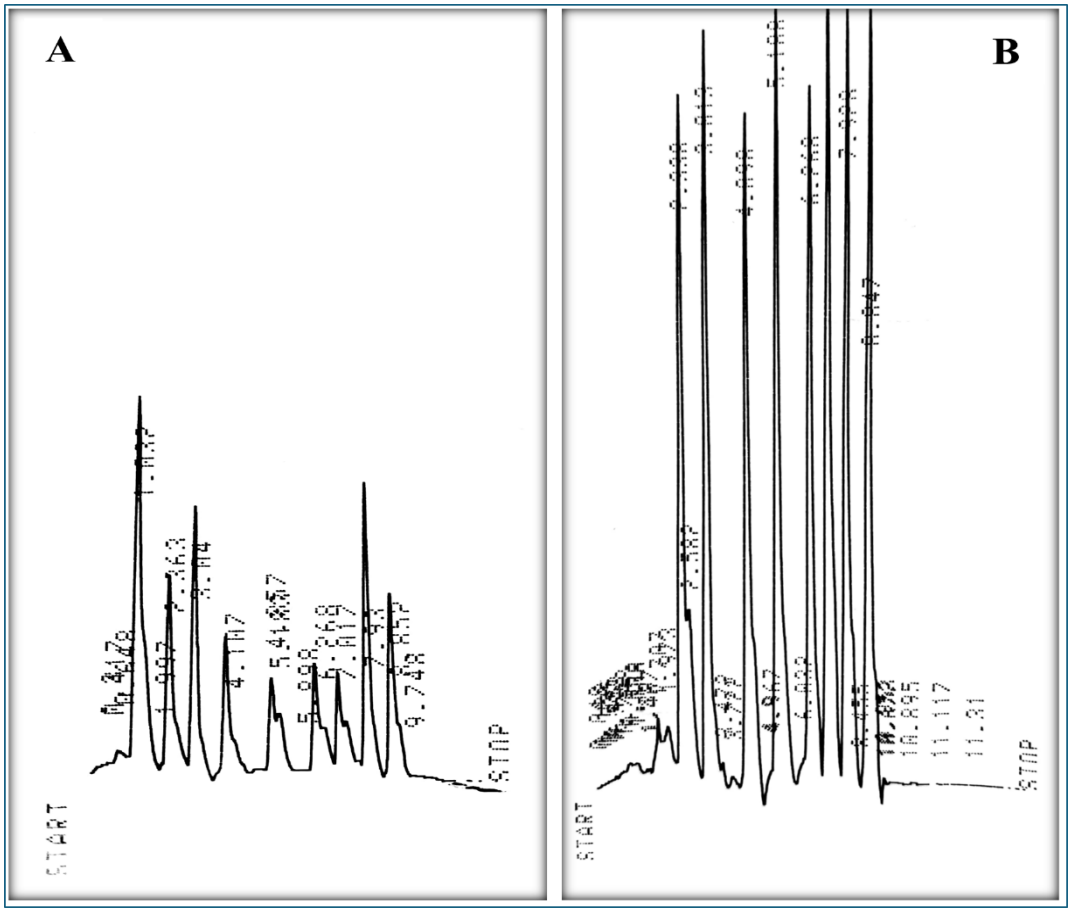

Fig. 1. HPLC chromatogram of the ethanolic fraction of Medicago sativa extract. (A) Sample; (B) Standards. Observed peaks and their retention times (min): Gallic Acid (2.363), Naringin (6.268), Myricetin (7.017), Quercetin (7.930), Apigenin (8.852).

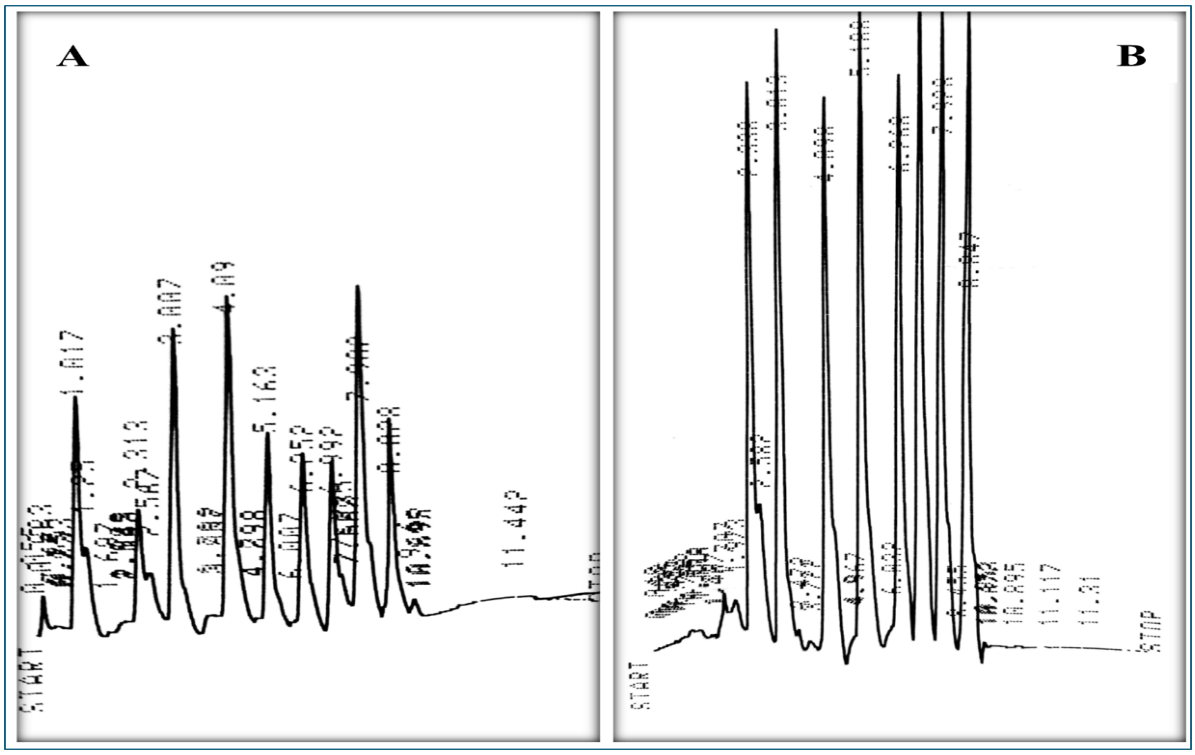

Fig. 2. HPLC chromatogram of ethyl acetate fraction: A.Sample, B. Standard. Peaks that were observed, Naringin= 6.992, Myricetin= 7.902, Quercetin= 8.828, and Gallic Acid, 2.007.

Antibacterial activity of different concentrations of M. sativa extracts against E. coli in comparison to meropenem. The sensitivity of uropathogenic Escherichia coli (UPEC) to Medicago sativa extracts, meropenem, and dimethyl sulfoxide (DMSO) was determined using the agar well diffusion method. Both ethanolic and ethyl acetate fractions exhibited growth inhibition zones, with higher concentrations of M. sativa extract demonstrating greater antibacterial activity (Fig. 3).

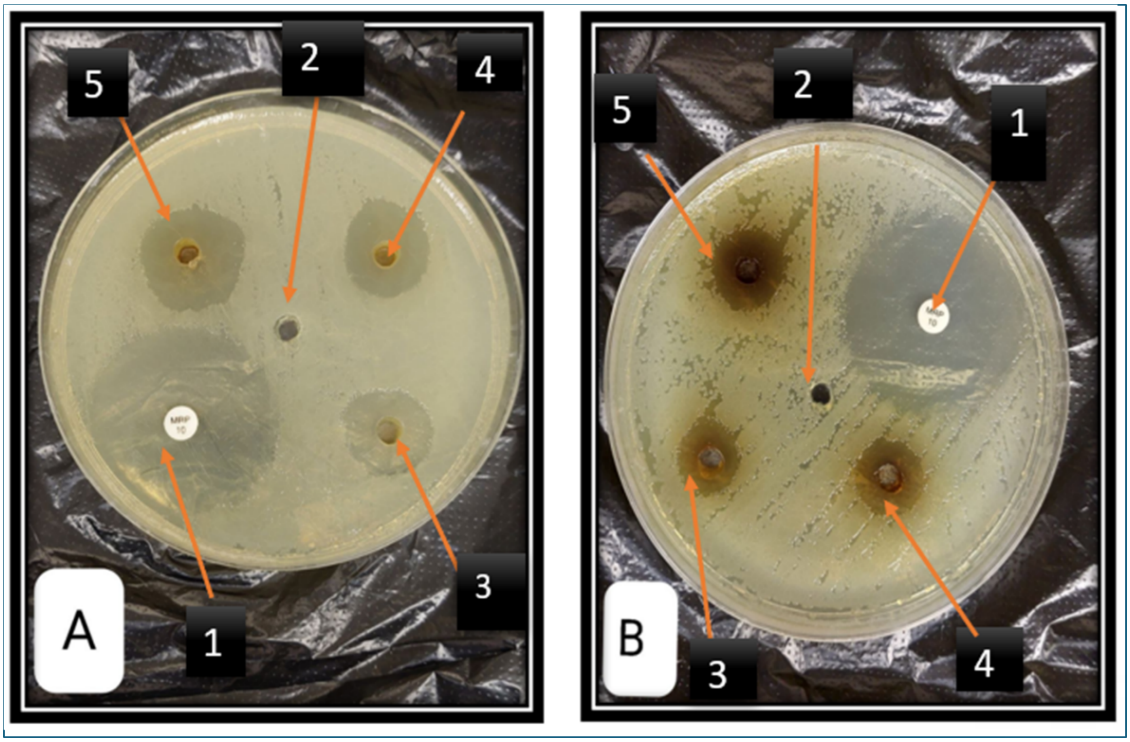

Fig. 3. Sensitivity of *Escherichia coli* to varying concentrations of *Medicago sativa* extracts. (A) Ethanolic fraction; (B) Ethyl acetate fraction. Treatments: (1) DMSO (negative control); (2) Meropenem (10 µg, positive control); (3) 25 mg/ml; (4) 50 mg/ml; (5) 75 mg/ml.

Meropenem produced a significantly larger mean inhibition zone compared to both the ethanolic and ethyl acetate fractions (p=0.001), as shown in Table 5 and Figure 4.

Table 5

Comparison of mean inhibition zones for *Medicago sativa* fractions and meropenem

Fraction/control, concentration (mg/ml)	Mean inhibition zone (mm, Mean ± SD)	P-value
Ethanol fraction	32.85 ± 2.7	0.001
Meropenem		
Ethyl acetate fraction	32.96 ± 2.6	0.001
Meropenem		

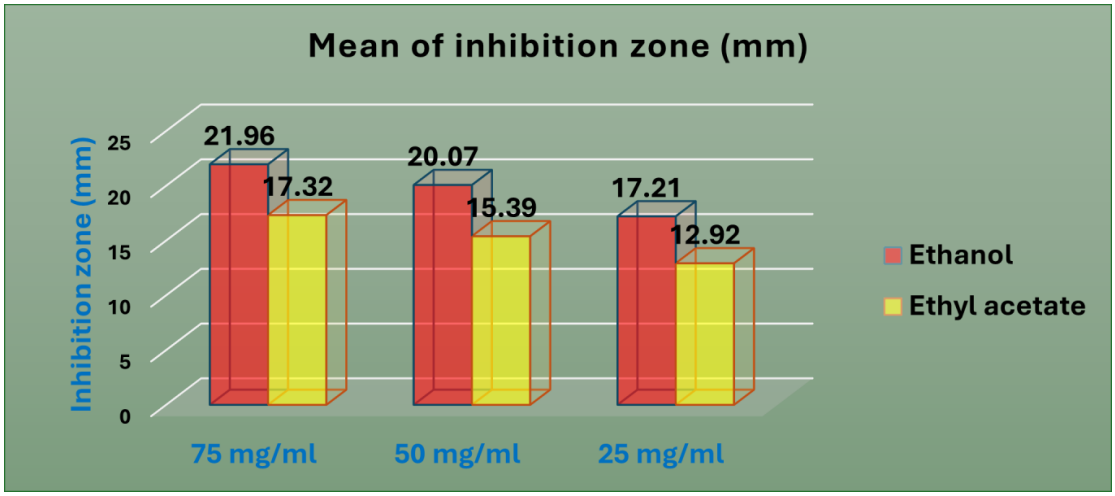

Fig. 4. Comparison of mean inhibition zones across varying concentrations of ethanolic and ethyl acetate fractions of *Medicago sativa* extracts.

Discussion. UTIs, affecting over 100 million people annually, are increasingly prevalent due to the rise of multidrug-resistant pathogens, leading to treatment challenges and higher mortality rates [15]. The overuse of antibiotics has driven the emergence of drug-resistant bacteria, necessitating novel antimicrobial agents [5]. Previous studies have identified diverse chemical constituents in Medicago sativa, with bioactive compounds contributing to its beneficial properties [16].

Phytochemical analyses revealed alkaloids, flavonoids, terpenoids, and steroids in the crude M. sativa extract, with only flavonoids detected in the ethyl acetate fraction, consistent with prior findings [16]. The ethanolic fraction also contained flavonoids, aligning with Grechana et al. [17]. High-performance liquid chromatography (HPLC) confirmed the presence of phenolic compounds, including gallic acid, caffeic acid, pyrogallol, salicylic acid, naringin, myricetin, quercetin, and apigenin, supporting previous research [18].

Flavonoids, a class of plant phenolic compounds, exhibit antibacterial properties by increasing bacterial membrane permeability, leading to leakage of intracellular contents and cell death. They also inhibit nucleic acid synthesis by interfering with DNA and RNA transcription, disrupt bacterial energy pathways by reducing ATP production, and prevent quorum sensing and biofilm formation, thereby reducing pathogenicity [19–22].

Quercetin exerts antibacterial effects by binding to DNA, causing strand cleavage, inhibiting DNA gyrase, and disrupting DNA replication. It also downregulates virulence genes, inhibits single-stranded DNA-binding proteins, and prevents biofilm formation by interfering with quorum sensing pathways, reducing bacterial adhesion and colonization [11, 23, 24]. Gallic acid inhibits bacterial growth by disrupting metabolic pathways and compromising membrane integrity, leading to intracellular leakage and cell death. It also prevents biofilm formation, reducing bacterial colonization in the urinary tract [25, 26].

Myricetin inhibits nucleic acid synthesis, cell envelope synthesis, and bacterial toxin production [27]. Apigenin disrupts bacterial membranes and inhibits cellular processes, including nucleic acid synthesis, cell envelope synthesis, biofilm formation, quorum sensing, and Ala-Ala synthetase. Naringin acts as a quorum-sensing inhibitor [27].

This study confirmed that females are more susceptible to UPEC infections due to their shorter and wider urethra, facilitating bacterial access to the bladder. Consistent with our findings, the highest UTI prevalence was observed in individuals aged ≤50 years (49.2%) and 50–69 years (57.3%) [28]. Women (77.3%) were more prone to UTIs than men (22.6%) [29].

Antibiotic susceptibility testing aligned with prior studies, showing high sensitivity to amikacin (83.3%) and nitrofurantoin (91.7%) for UTI treatment [30]. Previous research reported UPEC isolates were most susceptible to ciprofloxacin (86.5%), ofloxacin (75%), and nitrofurantoin (84.6%), supporting our results [31]. Gharavi et al. noted high sensitivity to nitrofurantoin (92.8%), imipenem (99.2%), amikacin (97.9%), and meropenem (97.2%) [32].

Medicago sativa (alfalfa) extract was found to inhibit *E. coli papC* and *rcsA* gene expression, reducing biofilm formation, consistent with our findings [33]. Meropenem, a broad-spectrum antibiotic, exerts its activity by covalently inhibiting penicillin-binding proteins (PBPs), which are peptidoglycan transpeptidases essential for bacterial cell wall integrity [34].

Prior studies reported resistance to cephalosporins and fluoroquinolones, with the highest susceptibility to tigecycline (100%), colistin (96.2%), amikacin (90.5%), fosfomycin (86.7%), and nitrofurantoin (84.9%), aligning with our results [29]. *E. coli* isolates exhibited complete resistance to cephalothin (100%), consistent with a reported resistance rate of 85.8% [35].

Medicago sativa extracts demonstrated significant antibacterial activity against *E. coli* at varying concentrations, with larger inhibition zones at higher concentrations (p=0.001) [36]. The ethanolic fraction showed the highest mean inhibition zones at 75 mg/ml (21.96 ± 1.9 mm), 50 mg/ml (20.07 ± 1.8 mm), and 25 mg/ml (17.21 ± 2.2 mm), with significant differences (p=0.001). The ethyl acetate fraction exhibited lower inhibition zones at 75 mg/ml (17.32 ± 1.5 mm), 50 mg/ml (15.39 ± 1.3 mm), and 25 mg/ml (12.92 ± 1.6 mm) (p=0.001). The ethanolic fraction's superior activity is likely due to its potent extraction solvent, consistent with Khan et al. [37].

The ethanolic extract of *M. sativa* also showed antibacterial activity against Gram-negative bacteria, including *Pseudomonas aeruginosa* and *E. coli*, at various concentrations, supporting its efficacy in UTI treatment [38, 39]. Additionally, *M. sativa* extract inhibited *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Lactococcus lactis*, and *Bacillus licheniformis*, confirming its broad antibacterial properties [40].

In vitro tests demonstrated that *M. sativa* extracts exhibited significant antibacterial activity compared to meropenem (ethanolic fraction: 32.85 ± 2.7 mm; ethyl acetate fraction: 32.96 ± 2.6 mm; p=0.001). At 25 mg/ml, the ethanolic extract completely inhibited *E. coli* growth in broth media, with no growth observed on Mueller-Hinton agar. The ethyl acetate extract at 30 mg/ml exhibited bacteriostatic and bactericidal effects, as evidenced by no growth on Mueller-Hinton agar.

A recent study in India identified *E. coli, Enterococcus faecalis, Staphylococcus aureus*, and *Klebsiella pneumoniae* as primary UTI pathogens, with *E. coli* being the most common. *Tamarindus indica* and *Clitoria ternatea* outperformed ceftriaxone and piperacillin, reinforcing the potential of plant-based treatments [41]. Another study highlighted the efficacy of *Punica granatum, Aronia melanocarpa*, and *Cornus mas* for UTI prevention or combined antibiotic therapy, attributed to their tannins, steroids, terpenes, coumarins, flavonoids, and polyphenols [42].

The study has several limitations. First, the small sample size (85 urine samples) limits the generalizability of the findings to broader populations. Second, the study was conducted at a single center in Baghdad, Iraq, which may introduce regional bias and fail to account for geographic variations in UPEC strains or patient demographics. Third, the lack of in vivo testing restricts conclusions about the clinical efficacy and safety of *Medicago sativa* extracts for UTI treatment. Fourth, the study did not assess the potential synergistic effects between *M. sativa* extracts and conventional antibiotics, which could enhance therapeutic outcomes. Fifth, variability in plant material (e.g., differences in growth conditions or harvest timing) may affect the consistency of phytochemical profiles and antibacterial activity. Finally, the absence of long-term follow-up data limits understanding of the extracts' impact on UTI recurrence or resistance development. Larger, multicenter studies with in vivo models and diverse populations are needed to address these limitations and validate the findings.

Conclusions. This study demonstrates that Medicago sativa extracts, particularly the ethanolic fraction, exhibit significant antibacterial activity against UPEC, offering a promising alternative to conventional antibiotics for UTI treatment. The ethanolic fraction's superior efficacy, with inhibition zones of 21.96 ± 1.9 mm at 75 mg/ml (p=0.001), is attributed to bioactive compounds such as gallic acid, quercetin, and myricetin, which disrupt bacterial membranes, inhibit nucleic acid synthesis, and prevent biofilm formation. High resistance to cephalothin (100%) and moderate sensitivity to ciprofloxacin and levofloxacin underscore the need for novel treatments. Our results support the potential of M. sativa as a natural antimicrobial agent. Future research should focus on larger, multicenter trials to validate these findings and explore clinical applications, including synergistic effects with existing antibiotics.

Conflict of interest statement. The authors declare no conflict of interest.

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Author's contributions.

Ahmed Ibrahim Al-Yousif: ideation, approach, formal analysis, research, materials, data gathering, and composing the first draft.

Alaa Ghaith Ahmed: research, materials, data collection, and creation of the first draft.

Sheelan Amir Al-Darwish: writing, editing, data curation, formal analysis, research, resources, and technique.

Farah Fawzi: research, materials, and composing the first draft.

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