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EVALUATION OF *THYMUS VULGARIS* AQUEOUS EXTRACT AS A NATURAL ANTIMICROBIAL AGENT AGAINST URINARY TRACT INFECTION PATHOGENS

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ABSTRACT

Introduction Urinary tract infections (UTIs) are often caused by bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) which can lead to major health problems. The purpose of this study was to to examine the antibacterial effects of extract of *Thymus vulgaris* aqueous against these uropathogens.

Material and methods The aqueous extract of *Thymus vulgaris* was prepared via hot water decoction (yield: 3.6 g from 30 g dried plant material 12%). Bacterial strains were isolated from 25 clinical urine samples and identified using selective culture media using the Gram staining and biochemical characterization. Antibacterial activity was assessed by using the agar well diffusion method with extract concentrations (50, 100 and 200 mg/mL) of *Thymus vulgaris* extract.

Results The results of this study showed dose dependent of inhibition zone with *S. aureus* showing greater susceptibility (ZOI: 10–27 mm) compared to *E. coli* (ZOI: 9–23 mm). Ciprofloxacin shown larger inhibition zone (28–30 mm).

Conclusions These findings demonstrate promising antibacterial potential of *T. vulgaris* aqueous extract as a natural alternative to combat antibiotic-resistant UTIs. These results support previous investigation about *T. vulgaris* as an alternative or complementary treatment for bacterial infections.

Keywords Ciprofloxacin, *E. coli*, *S. aureus*, *Thymus vulgaris*, urinary tract infections

EVALUAREA EXTRACTULUI APOS DE *THYMUS VULGARIS* CA AGENT ANTIMICROBIAN NATURAL ÎMPOTRIVA AGENȚILOR PATOGENI ALE INFECȚIILOR TRACTULUI URINAR

Introducere Infecțiile tractului urinar (ITU) sunt adesea cauzate de bacterii, precum *Staphylococcus aureus* (*S. aureus*) și *Escherichia coli* (*E. coli*), care pot cauza probleme majore de sănătate. Scopul acestui studiu a fost de a examina efectele antibacteriene ale unui extract apos de *Thymus vulgaris* împotriva acestor agenți patogeni.

Material și metode Extractul apos de *Thymus vulgaris* a fost preparat prin decoct în apă fierbinte, obținându-se 3,6 g din 30 g de material vegetal uscat (12%). Tulpinile bacteriene au fost izolate din 25 de probe de urină și identificate prin medii de cultură selective, folosind colorația Gram și caracterizarea biochimică. Activitatea antibacteriană a fost evaluată utilizând metoda de difuzie în agar cu godeuri la concentrații variabile (50, 100 și 200 mg/ml) de extract de *Thymus vulgaris*.

Rezultate Rezultatele acestui studiu au arătat o dependență de doză în funcție de zona de inhibiție, *S. aureus* prezentând o sensibilitate mai mare (ZI: 10–27 mm) comparativ cu *E. coli* (ZI: 9–23 mm). Ciprofloxacina a prezentat o zonă de inhibiție mai mare (28–30 mm).

Concluzii Extractul apos de *Thymus vulgaris* a demonstrat efecte antibacteriene distincte, evidențiind potențialul său ca alternativă naturală sau terapie complementară pentru combaterea infecțiilor urinare, susținând în același timp eforturile de a reduce rezistența la antibiotice. Aceste rezultate susțin investigațiile anterioare despre *Thymus vulgaris* ca tratament alternativ sau complementar contra infecțiilor bacteriene.

Cuvinte-cheie Ciprofloxacină, *E. coli*, *S. aureus*, *Thymus vulgaris*, infecții ale tractului urinar.

INTRODUCTION

Thymus vulgaris (commonly known as thyme) is a widely used medicinal herb with a long history in both traditional and modern therapeutics (1). Its pharmacological properties are largely attributed to bioactive constituents such as thymol and carvacrol, which exhibit strong antibacterial, antifungal, antiviral, and antioxidant activities (2, 3). Due to these properties, *T. vulgaris* has been traditionally applied in the treatment of respiratory infections, skin disorders, and urinary tract infections (UTIs), demonstrating effectiveness against pathogens including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (4).

Previous studies have shown that *T. vulgaris* is active against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains such as methicillin-resistant *S. aureus* (MRSA) (5). Its broad-spectrum antimicrobial potential has led to increasing interest in its application in medicine, food preservation, and personal care products, particularly as a natural alternative to synthetic antimicrobials (6).

In addition to its antimicrobial effects, *T. vulgaris* is valued for its expectorant, antispasmodic, anti-inflammatory, and antioxidant properties, which contribute to its use in managing respiratory ailments, inflammatory conditions, and oxidative stress-related disorders (7–9). As antimicrobial resistance (AMR) continues to pose a global public health challenge, there is a growing need to identify safe and effective plant-based alternatives that align with the One Health framework and help reduce reliance on conventional antibiotics.

Given this context, aqueous extracts of *T. vulgaris* may offer a promising natural therapeutic option for managing UTIs**.** We hypothesize that the aqueous extract of *T. vulgaris* exerts antibacterial effects against UTI-causing pathogens in a concentration-dependent manner. Therefore, this study aims to evaluate the antibacterial activity of aqueous *T. vulgaris* extract against *S. aureus* and *E. coli* isolated from human urinary tract infections. The agar well-diffusion method was employed to assess antimicrobial efficacy and compare the extract's activity with that of ciprofloxacin, a commonly used antibiotic.

MATERIALS AND METHODS

This study was conducted at Baquba Teaching Hospital in Baquba, Iraq, from May 2024 to January 2025. *Thymus vulgaris* was procured from a local market in Samawah. The plant material was washed three times with distilled water to remove surface contaminants and air-dried at room temperature. After drying, the plant material was ground into a fine powder using an electric blender and stored in a sealed container until extraction.

The aqueous extract of *T. vulgaris* was prepared using the decoction method. Briefly, 30 g of dried plant powder were mixed with 200 mL of distilled water in a beaker and heated until boiling. The mixture was then filtered through Whatman No. 1 filter paper to remove solid residues. The clear filtrate was collected in a sterile glass container and refrigerated at 4 °C for no longer than seven days prior to antimicrobial testing (10).

Urine samples were obtained from 25 patients clinically diagnosed with urinary tract infections (UTIs). Samples were collected in sterile containers and streaked onto MacConkey agar and eosin methylene blue (EMB) agar for *E. coli* isolation, and mannitol salt agar for *S. aureus* isolation. Blood agar was also used to support general bacterial recovery. Plates were incubated aerobically at 37 °C for 24 hours. Suspected colonies were selected based on

morphological characteristics and subjected to biochemical identification (11). Confirmed isolates were maintained on nutrient agar slants at 4 °C for subsequent analysis.

Antibacterial activity was evaluated using the agar well diffusion method. Mueller–Hinton agar plates were prepared and allowed to solidify. A standardized bacterial inoculum (100 µL) was evenly spread on each plate using a sterile cotton swab (12, 13). Wells of 6 mm diameter were created using a sterile cork borer. A volume of 100 µL of *T. vulgaris* extract at concentrations of 50 mg/mL (low), 100 mg/mL (medium), and 200 mg/mL (high) was dispensed into the wells. Sterile distilled water served as the negative control, while ciprofloxacin was used as the positive control. Plates were incubated at 37 °C for 24 hours, after which inhibition zones (mm) were measured. Larger inhibition zones indicated stronger antibacterial activity (14). The performance of the extract was compared with ciprofloxacin to assess relative efficacy.

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance using GraphPad Prism software (Version 8). Differences were considered statistically significant at $p \leq 0.05$ (15, 16).

RESULTS

The hot-water extraction of *Thymus vulgaris* yielded 3.6 g of extract from 30 g of dried plant material, corresponding to a 12% (w/w) yield. This value falls within the commonly reported range of 10–30% for aqueous herbal extractions, which varies depending on factors such as temperature, extraction duration, and plant composition.

A total of 25 urine samples from patients with urinary tract infections (UTIs) were cultured on selective and differential media to isolate *Escherichia coli* and *Staphylococcus aureus*. After 24 hours of incubation at 37 °C, bacterial growth was observed on MacConkey agar, eosin methylene blue (EMB) agar, mannitol salt agar (MSA), and blood agar. On EMB agar, *E. coli* produced characteristic green metallic sheen colonies due to vigorous lactose fermentation (fig. 1A). On MSA, *S. aureus* formed yellow colonies indicative of mannitol fermentation (fig. 1B). Blood agar cultures showed β-hemolytic colonies for *S. aureus*, whereas *E. coli* colonies were non-hemolytic or occasionally α-hemolytic (fig. 1C). Microscopic and biochemical analyses were performed to confirm species identity. Gram staining revealed *E. coli* as Gram-negative, rod-shaped bacteria occurring singly or in pairs, while *S. aureus* appeared as Gram-positive cocci arranged in clusters (17).

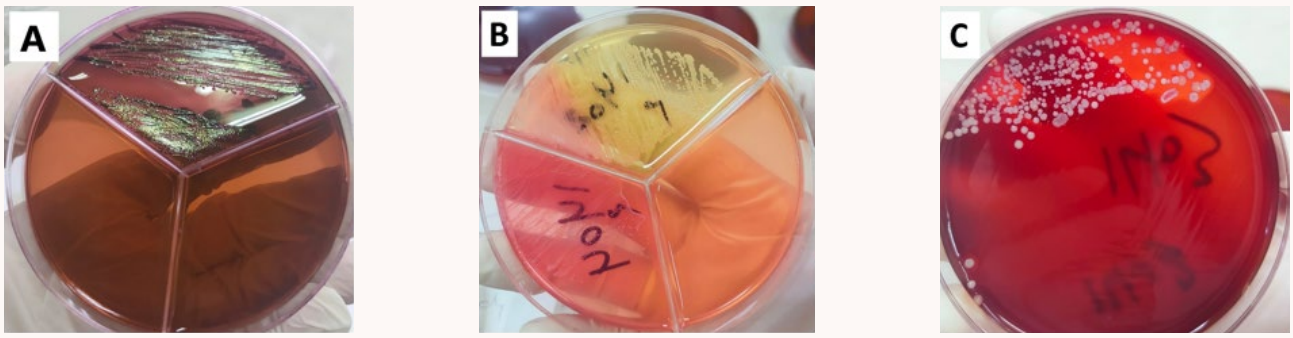

Figure 1. Identification of *Escherichia coli* and Staphylococcus aureus:
(A) *E. coli* identified on EMB agar, (B) *S. aureus* mannitol salt agar,
(C) *E. coli* and *S. aureus* colonies on blood agar.

The antibacterial activity of *Thymus vulgaris* extract was assessed against *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method as illustrated in Figure 2.

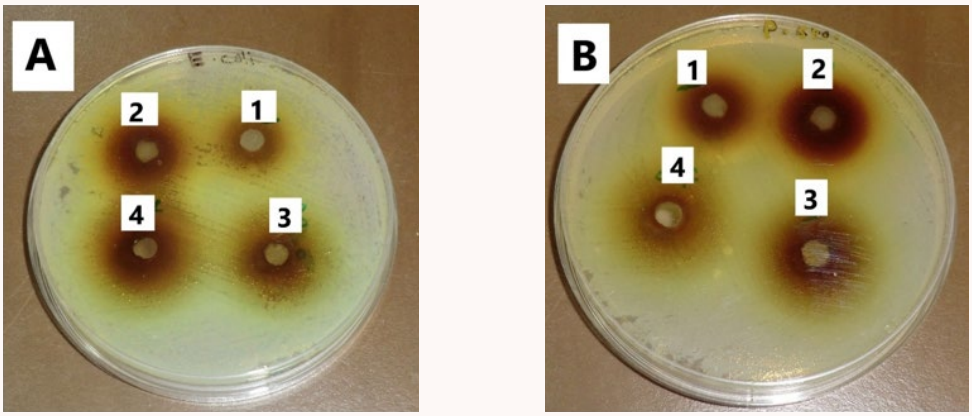

Figure 2. Comparison of inhibition zones of *Thymus vulgaris* extract:
(A) E. coli, (B) S. aureus. (1) Low concentration (50 mg/mL), (2) Medium concentration (100 mg/mL), (3) High concentration (200 mg/mL), (4) Ciprofloxacin (5 µg/disc).

The results, expressed as mean ± standard deviation (SD) of the inhibition zone diameter (ZOI) in millimeters, are summarized in Table 1. The results showed the zone of inhibition (ZOI) widths varied but the extract had inhibitory effects on both bacterial species. The antibacterial activity was assessed by measuring the diameter in mm of inhibition zones formed around the wells containing the extract. The results are summarized in Table 1 and Figure 3.

Table 1. Zone of inhibition (ZOI) for *Thymus vulgaris* extract (mm) against *E. coli* and *S. aureus*, p value ≤ 0.05.

Thymus vulgaris Extract (mg/mL)	N	*E. coli* mean ± SD of ZOI in mm	P value	*S. aureus* mean ± SD of ZOI in mm	*p value*
50 mg/mL (Low Concentration)	9	8.844 ± 1.51 ^{c***}	< 0.0001	10.37 ± 1.86 ^{c***}	< 0.0001
100 mg/mL (Medium Concentration)	9	13.94 ± 4.65 ^{c***}	< 0.0001	15.08 ± 4.61 ^{c***}	< 0.0001
200 mg/mL (High Concentration)	9	22.81 ± 3.80 ^{b**}	= 0.0015	26.98 ± 4.67 ^{a*}	= 0.0500
Ciprofloxacin (5 µg/disc)	9	28.32 ± 2.09	-	30.44 ± 5.53	-

*ZOI: Zone of inhibition

(a) Low Significant *, (b) Significant **, (c) High Significant *

For *E. coli*, the inhibition zones increased in a concentration-dependent manner. The lowest extract concentration (50 mg/mL) produced a zone of inhibition (ZOI) of 8.84 ± 1.51 mm ($p < 0.0001$), while the medium concentration (100 mg/mL) yielded a significantly larger ZOI of 13.94 ± 4.65 mm ($p < 0.0001$). The highest concentration (200 mg/mL) resulted in a ZOI of 22.81 ± 3.80 mm ($p = 0.0015$). However, all extract concentrations produced smaller inhibition zones than the positive control, ciprofloxacin (28.32 ± 2.09 mm).

A similar trend was observed for *S. aureus*. The low concentration (50 mg/mL) produced a ZOI of 10.37 ± 1.86 mm ($p < 0.0001$), while the medium concentra-

tion (100 mg/mL) generated a ZOI of 15.08 ± 4.61 mm ($p < 0.0001$). The highest concentration (200 mg/mL) exhibited the strongest antibacterial effect, with a ZOI of 26.98 ± 4.67 mm ($p < 0.05$), closely approaching the activity of ciprofloxacin (30.44 ± 5.53 mm).

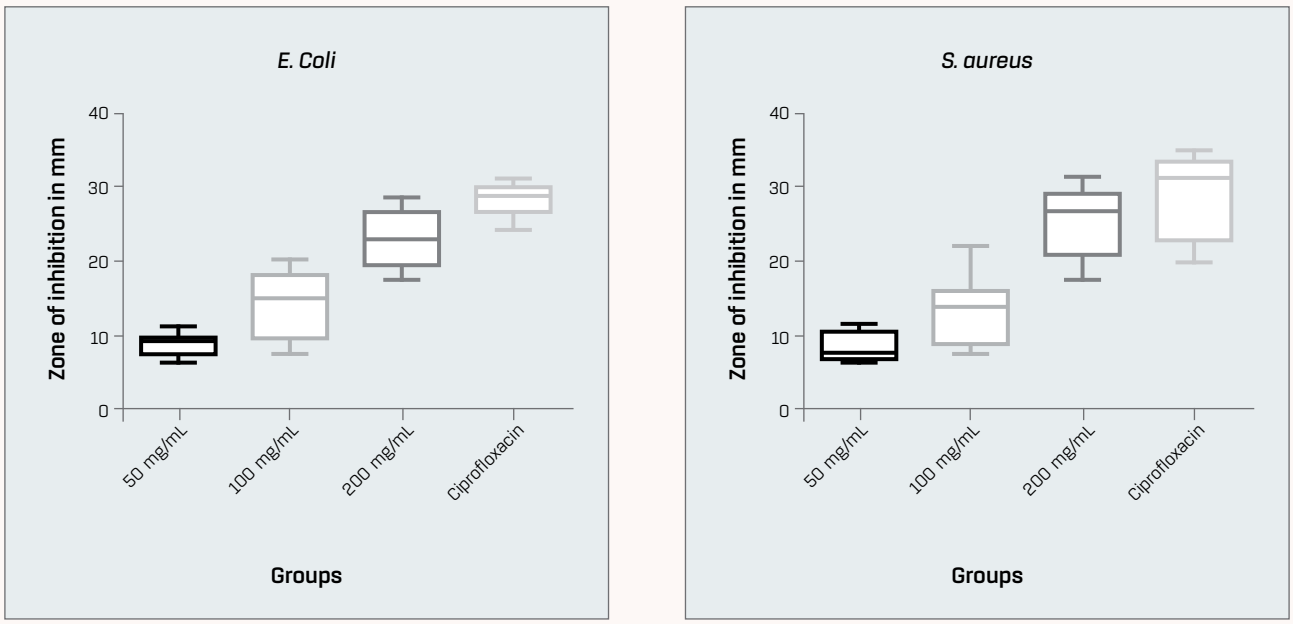

Figure 3. Statistical analysis of inhibition zones of *Thymus vulgaris* extract against *E. coli* and *S. aureus*, p value ≤ 0.05.

DISCUSSION

Thyme contains numerous bioactive compounds with demonstrated biological activity against a wide range of infectious agents. The findings of this study indicate that hot-water extraction was effective in isolating hydrophilic phytochemicals such as polyphenols, flavonoids, and tannins, all of which are known for their antimicrobial properties (18). However, because water has limited ability to dissolve hydrophobic compounds, aqueous extraction generally yields a lower quantity of extract compared to organic solvents such as ethanol or methanol (19). Despite this, the extract obtained in the present study provided a sufficient concentration of bioactive constituents to exert measurable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

The virulence of *E. coli* in UTIs is primarily attributed to its fimbriae-mediated adhesion, biofilm formation, and multiple antibiotic resistance mechanisms, all of which contribute to its high prevalence in clinical urine samples (20). In contrast, *S. aureus*–associated UTIs are often linked to immunocompromised conditions, catheter use, or hospital-acquired infections (HAIs).

As shown in Figure 2, inhibition-zone diameters increased proportionally with extract concentration, confirming a clear dose-dependent antibacterial effect. Moreover, *S. aureus* exhibited larger inhibition zones than *E. coli*, suggesting that Gram-positive bacteria are more susceptible to *Thymus vulgaris* extract than Gram-negative bacteria (21). This difference is likely related to structural distinctions in bacterial cell envelopes; the outer membrane of Gram-negative bacteria, such as *E. coli*, acts as a barrier that restricts the penetration of many antimicrobial compounds (22).

The antimicrobial activity of *T. vulgaris* can be attributed to its rich phytochemical profile, particularly thymol and carvacrol, which possess strong antibacterial properties. These compounds disrupt bacterial cell membranes, alter membrane permeability, and interfere with essential metabolic processes, ultimately leading to leakage of intracellular components and cell death (23).

Although the inhibition zones produced by the extract were smaller than those produced by ciprofloxacin, the results still demonstrate that *T. vulgaris* has meaningful antibacterial activity and may serve as a promising natural antimicrobial agent. Its bioactive constituents – including thymol, carvacrol, flavonoids, and phenolic acids – are known to damage bacterial membranes, inhibit enzymatic activity, and disrupt overall metabolic function (24).

A limitation of this study is the relatively small sample size (25 bacterial isolates), which may reduce the statistical power and generalizability of the findings. Larger studies with expanded sample sets are recommended to further validate and strengthen these observations.

CONCLUSIONS

1. *Thymus vulgaris* extract exhibits significant antibacterial activity against *E. coli* and *S. aureus* in a concentration-dependent manner.
2. The strong antimicrobial effects of the extract are primarily attributed to its diverse phytochemical composition, including thymol, carvacrol, and flavonoids, which disrupt bacterial cell membranes and interfere with essential cellular functions.
3. Although the extract produced inhibition zones smaller than those of ciprofloxacin, it demonstrated particularly potent activity against *S. aureus*, highlighting its potential as a natural antimicrobial agent and its promise for the management of urinary tract infections.

CONFLICT OF INTEREST

Author declare there is no conflict of interest

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ETHICAL APPROVAL

All experimental procedures involving animals were reviewed and approved by “the Ethics Committee of the College of Science, University of Diyala” (Approval No.: 2024 AEBT 144, Date: 8/2/2024). All procedures were done in accordance with the principles of research integrity, biosafety, and environmental protection.

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