



Original Article

Antimicrobial activity of *Etingera elatior* leaf extract in *Staphylococcus aureus*-induced cystitis: an in vivo study

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ABSTRACT

Background: *Etingera elatior* has been reported to possess antimicrobial properties; however, its therapeutic effects in bacterial cystitis, particularly those caused by *Staphylococcus aureus* (*S. aureus*), remain inadequately explored in vivo.

Objective: This study aimed to evaluate the antimicrobial and anti-inflammatory effects of *Etingera elatior* leaf extract in an experimental model of *S. aureus* cystitis.

Methods: A true experimental posttest-only design was conducted using 28 male BALB/c mice induced with *S. aureus*. The animals were allocated into control and treatment groups receiving *Etingera elatior* leaf extract at concentrations of 40%, 50%, 60%, 70%, and 80%. Therapeutic outcomes were assessed based on urinary tract mucosal fluid secretion and bacterial colony counts. Data were analyzed using one-way ANOVA followed by post hoc testing.

Results: Treatment with *Etingera elatior* leaf extract significantly reduced cystitis severity compared with the infected control group ($p < 0.05$). The extract demonstrated both antimicrobial and anti-inflammatory effects, as indicated by reductions in bacterial load and mucosal secretion. Among the tested concentrations, the 40% extract showed the most consistent therapeutic effect, with outcomes comparable to ciprofloxacin.

Conclusions: *Etingera elatior* leaf extract exhibits potential as an alternative therapeutic agent for bacterial cystitis. Its antimicrobial and anti-inflammatory properties support its further development as a complementary treatment for urinary tract infections.

INTRODUCTION

Cystitis is a common manifestation of urinary tract infection (UTI) characterized by inflammation of the bladder caused by bacterial pathogens. UTIs represent a significant global health problem, with more than 150 million cases reported annually, and approximately 40–50% of adult women experiencing at least one episode during their lifetime. In Indonesia, UTIs remain prevalent, particularly among women and older adults, with an estimated incidence of around 6% in the adult population.¹

Although *Escherichia coli* is the most frequently reported causative agent, *Staphylococcus aureus* (*S. aureus*) has been identified as an important pathogen in certain cases of cystitis. Infection caused by *S. aureus* may lead to bladder inflammation and, if inadequately treated, can increase the risk of ascending infection and renal

complications. Moreover, the emergence of antibiotic resistance among *S. aureus* strains has become a growing concern, limiting the effectiveness of conventional antimicrobial therapy and highlighting the need for alternative treatment approaches.^{2,3}

Etingera elatior is a traditional medicinal plant widely used in Southeast Asia and is known to contain phenolic compounds with antioxidant and antimicrobial properties. Previous studies have reported that *E. elatior* leaf extract exhibits antimicrobial activity against several pathogenic bacteria, including *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*. These biological activities are attributed to its bioactive constituents, which inhibit bacterial growth.⁴

However, evidence regarding the therapeutic effects of *E. elatior* in cystitis, particularly that induced by *S. aureus*, remains limited. In vivo studies evaluating its efficacy using objective indicators of cystitis severity, such as urinary tract

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mucosal fluid secretion and bacterial colony counts, are still scarce. Furthermore, the association between the phenolic content of *E. elatior* extract and its potential to reduce inflammatory responses in cystitis remains poorly elucidated.⁵⁻⁷ Considering the increasing problem of antibiotic resistance and the potential of plant-based therapies, further investigation of *E. elatior* as an alternative treatment for cystitis is warranted. Therefore, this study aimed to evaluate the therapeutic effects of *E. elatior* leaf extract in a mouse model of *S. aureus* –induced cystitis.

METHOD

Study Design

This study employed a true experimental posttest-only design.⁸

Study Setting

The study was conducted at the Microbiology Laboratory, Tadris Biology Department, State Islamic Institute of Palangka Raya, Central Kalimantan, Indonesia, from November 30, 2024, to January 6, 2025.

Materials

The study used 28 male BALB/c mice aged 3–4 weeks with an average body weight of approximately 28 g. A total of 2500 g of *E. elatior* leaf powder was prepared and processed into extract concentrations of 40%, 50%, 60%, 70%, and 80%. Chemicals and reagents included 70% ethanol, 96% ethanol, chloroform, gallic acid standard solution, Folin–Ciocalteu reagent, sodium carbonate solution (Na_2CO_3), ciprofloxacin (250 mg), sterile distilled water, and aluminum foil. The instruments used included a laminar airflow cabinet, an autoclave, glassware, a magnetic stirrer, a Bunsen burner, micropipettes, an incubator, a digital balance, a UV–Vis spectrophotometer (760 nm), a gastric sonde, syringes, Petri dishes, test tubes, a colony counter, and a centrifuge.

Plant Extraction

Fresh *E. elatior* leaves were washed under running water, cut into small pieces, air-dried at room temperature, and pulverized into powder (2500 g). The powder was extracted using the maceration method with 96% ethanol for 24 hours.

Phytochemical Analysis

Qualitative Analysis

Qualitative screening of secondary metabolites was performed using *E. elatior* extract (1 mg/mL) to detect flavonoids, alkaloids, saponins, and tannins.

Quantitative Analysis

Antioxidant activity was assessed using the DPPH method at 517 nm. Total flavonoid content was measured using the AlCl_3 method (415 nm), and total polyphenols were determined using the Folin–Ciocalteu method (765 nm), expressed as gallic acid equivalents (mg GAE/g extract).

Experimental Procedure

Cystitis was induced by intraperitoneal injection of *S. aureus* culture (1 mL), diluted in 0.1% peptone solution (pH 4.5), for 7 consecutive days. On day 8, infection was confirmed based on clinical indicators (external genital appearance and urine color) and bacterial colony counts ($>10^3$ CFU/mL). Mice were then treated orally with *Etingera elatior* leaf extract via gastric sonde three times daily for 7 days. The treatment groups received doses equivalent to 200, 400, 600, 800, and 1000 mg/kg body weight. On day 8 post-treatment, all animals were euthanized. Urinary tract mucosal fluid secretion was measured, and total *S. aureus* colonies were quantified.

Clinical Scoring

Clinical assessment was performed based on two visual indicators: external genital appearance and urine color, each evaluated using a four-level scoring system. External genital appearance was graded as follows: 1 (normal), 2 (slightly reddish), 3 (reddish), and 4 (intensely red). Urine color was scored as: 1 (clear yellow/normal), 2 (pale yellow or slightly cloudy), 3 (dark yellow), and 4 (yellowish-brown). These parameters were used as supportive indicators of cystitis severity.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by post hoc testing. A p-value < 0.05 was considered statistically significant.⁹

Ethical Consideration

This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Palangka Raya (No. 185/UN24.9/LL/2024).

RESULTS

Phytochemical characteristics and antioxidant activity

Qualitative phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, terpenoids, and saponins in *E. elatior* leaf extract. DPPH analysis demonstrated a concentration-dependent increase in antioxidant activity, with inhibition observed from 40% up to 80%. Linear regression analysis showed a strong positive correlation between extract concentration and antioxidant activity ($R^2 = 0.923$) (Figure 1).

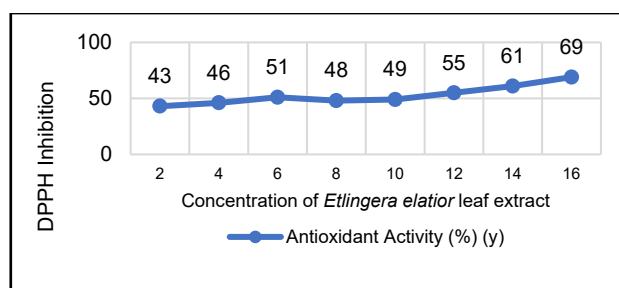


Figure 1. Antioxidant activity of *E. elatior* leaf extract determined by DPPH assay

Bioactive Compound Content

A positive linear relationship was observed between extract concentration and antioxidant activity. Total flavonoid and polyphenol contents increased with higher extract concentrations (Figure 2).

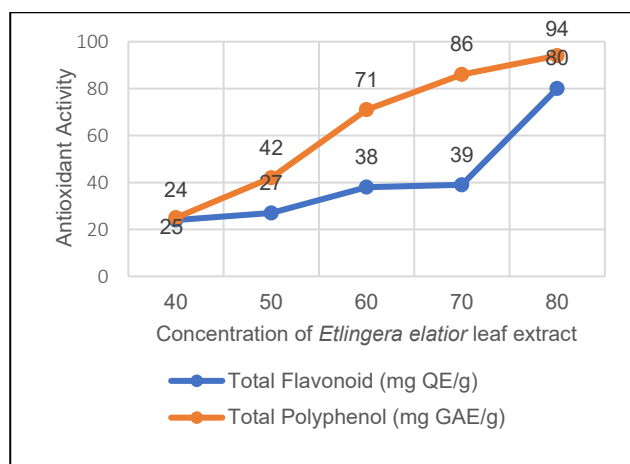


Figure 2. Total flavonoid and polyphenol contents of *E. elatior* leaf extract

Effect on urinary tract mucosal fluid secretion

Urinary tract mucosal fluid secretion was used as an indicator of inflammation. One-way ANOVA showed a significant difference among groups ($F = 2.711, p = 0.041$) (Table 1). Post hoc analysis demonstrated that extract concentrations of 40% and 70% resulted in lower secretion levels than the negative control and showed effects comparable to those of ciprofloxacin (Table 2).

Table 1. One-way ANOVA results for urinary tract mucosal secretion and *S. aureus* colony counts

Source	Sum of squares	Mean Square	F	p-value
Urinary Tract	1330.714	221.786	2.711	.041
Mucosal Secretion	1718.000	81.810		
Total	12236.714	2039.452	6.538	.001
Colonies of <i>S. aureus</i>	6550.250	311.917		

Table 2. Effect of *E. elatior* leaf extract on urinary tract mucosal fluid secretion

Group	Concentration (%)	Mean UTM Secretion (µL)	Notation
P2	Ciprofloxacin	4.7500	a
P3	40%	11.2500	ab
P6	70%	12.7500	ab
P1	Aquadest	14.2500	ab
P4	50%	22.5000	b
P5	60%	24.0000	b
P7	80%	24.0000	b

Exp: Values are presented as mean. Different superscript letters (a,b) indicate statistically significant differences among groups based on Duncan's multiple range test ($p < 0.05$).

Effect on Bacterial Colony Counts

The antimicrobial effect was evaluated based on total *S. aureus* colonies. One-way ANOVA revealed a significant difference among groups ($F = 6.538, p = 0.001$) (Table 1). Post hoc analysis indicated that extract concentrations of 40%, 50%, and 60% produced colony counts comparable to ciprofloxacin. The 40% concentration showed the lowest mean colony count, indicating the strongest antimicrobial effect (Table 3).

Table 3. Antimicrobial effect of *E. elatior* leaf extract on *S. aureus* colony counts in the urinary tract mucosa

Group	Concentration (%)	Total <i>S. aureus</i> Colonies (UTM)	Notation
P3	40%	21.7500a	a
P4	50%	27.5000a	a
P2	Ciprofloxacin	28.7500a	a
P5	60%	38.0000a	a
P1	Aquadest	65.0000b	b
P7	80%	67.0000b	b
P6	70%	76.7500b	b

Exp: UTM = (Urinary Tract Mucosal); Values are presented as mean. Different superscript letters (a,b) indicate statistically significant differences among groups based on Duncan's multiple range test ($p < 0.05$).

Clinical Observations

Clinical observations showed improvements in urine color and external genital appearance following treatment. These findings were consistent with reductions in bacterial load and mucosal secretion. However, these observations were considered supportive and not included as primary quantitative outcomes.

Integrated Evaluation

Overall, *E. elatior* leaf extract demonstrated both antimicrobial and anti-inflammatory effects. Among the tested concentrations, the 40% extract showed the most consistent therapeutic effect, with efficacy comparable to ciprofloxacin.

DISCUSSION

This study demonstrates that *E. elatior* leaf extract exerts significant antimicrobial and anti-inflammatory effects in an in vivo model of *S. aureus*-induced cystitis. Notably, the 40% extract concentration showed the most consistent therapeutic effect, as evidenced by the lowest bacterial colony counts and improvement in inflammatory indicators. These findings suggest that the extract is effective even at moderate concentrations and may not exhibit a linear dose-response relationship.

The observed antimicrobial effects may be mediated through multiple biological mechanisms. Plant-derived secondary metabolites, including flavonoids, tannins, alkaloids, and saponins, are known to disrupt bacterial cell membranes, alter permeability, and interfere with intracellular processes such as DNA replication and protein

synthesis.^{10,11,12} Additionally, these compounds may induce oxidative stress in microbial cells through the generation of reactive oxygen species, contributing to bacterial cell death and inhibition of biofilm formation.¹³ These mechanisms likely explain the reduction in *S. aureus* colony counts observed in this study.

In parallel, the improvement in inflammatory indicators suggests that *E. elatior* also possesses anti-inflammatory and antioxidant properties. During cystitis, activation of the immune response triggers the release of proinflammatory cytokines, leading to tissue damage and edema.¹⁴ Flavonoids have been reported to suppress inflammatory mediators such as IL-6 and TNF- α , while plant-derived compounds may also inhibit prostaglandin synthesis and oxidative stress pathways.^{15,16,17} Furthermore, terpenoids contribute to antioxidant activity by reducing oxidative damage and vascular permeability.^{18,19} These combined effects may underlie the observed improvements in external genital appearance and urine characteristics.

The present findings are consistent with previous studies demonstrating the antimicrobial and anti-inflammatory properties of *E. elatior*.²⁰ However, most earlier studies were limited to in vitro models or did not specifically evaluate *S. aureus*-induced cystitis in vivo. This study extends existing evidence by providing in vivo data that integrate microbiological outcomes with inflammatory indicators, supporting the therapeutic potential of *E. elatior* in urinary tract infections.²¹⁻²²

From a clinical perspective, these findings suggest that *E. elatior* leaf extract may serve as a potential complementary therapy for urinary tract infections, particularly in the context of increasing antibiotic resistance.²³⁻²⁵ However, translation to clinical practice requires further investigation, including clinical trials and safety evaluation.

Several limitations should be acknowledged. First, the inflammatory indicators used were observational and may be subject to bias. Second, this study did not include quantitative biomarkers, histopathological analysis, or standardized extract characterization, limiting mechanistic interpretation. Third, the absence of toxicity evaluation and pharmacokinetic data restricts assessment of safety and optimal dosing. Finally, as this study was conducted in an animal model, direct extrapolation to human clinical settings should be interpreted with caution.

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that *E. elatior* leaf extract has significant therapeutic potential in an experimental model of *S. aureus*-induced cystitis. The treatment effectively reduced inflammatory indicators and bacterial load, with the 40% concentration showing the most consistent and optimal response. These effects are likely associated with bioactive secondary metabolites possessing antimicrobial, anti-inflammatory, and antioxidant properties. Further studies are recommended to validate these findings using

objective biomarkers, histopathological evaluation, standardized extract characterization, and toxicity assessment. In addition, clinical studies are required to determine the efficacy and safety of *E. elatior* as a complementary therapy for urinary tract infections.

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