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Evaluation of the Antibacterial Activity of Ethanolic Leaf Extract of Capparis spinosa Against a Clinical *Escherichia coli* Isolate

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تقييم النشاط المضاد للبكتيريا للمستخلص الإيثانولي لأوراق نبات القبار الشوكي (*Capparis spinosa*) ضد عزل سريري لبكتيريا الإشريكية القولونية (*Escherichia coli*)

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Abstract:

This study aimed to evaluate the inhibitory effect of ethanolic leaf extract of Capparis spinosa against a clinical Escherichia coli isolate using concentrations of 100, 300, 360, and 700 mg/ml. Preliminary chemical screening indicated the presence of a significant amount of saponins, alongside low levels of flavonoids and tannins. The antibacterial activity was assessed using the agar diffusion method. The results demonstrated that the ethanolic leaf extract of C. spinosa exhibited no antibacterial activity against the clinical E. coli isolate, as no inhibition zones were observed at any tested concentration. This lack of activity may be attributed to factors such as low concentrations of active compounds, limited permeability through the Gram-negative bacterial outer membrane, and potential degradation of heat-sensitive components during the extraction process. In contrast, the positive control antibiotics Cefotaxime (40 mm), Amikacin (16 mm), and Amoxicillin (28 mm) showed activity, while Penicillin showed no inhibition. The study concludes that the effectiveness of plant extracts is highly dependent on the solvent, extraction method, chemical composition, and the specific bacterial strain.

Keywords: *Capparis spinosa*, Antibacterial Activity, Ethanolic Extract, *E. coli*, Antibiotics.

الملخص:

هدفت هذه الدراسة إلى تقييم التأثير المثبط للمستخلص الإيثانولي لأوراق نبات القبار (*Capparis spinosa*) ضد عزل سريري لبكتيريا الإشريكية القولونية (*Escherichia coli*) باستخدام تركيزات 100 و300 و360 و700 ملجم/مل. أشار الفحص الكيميائي الأولي إلى وجود كمية كبيرة من الصابونين، بالإضافة إلى مستويات منخفضة من الفلافونويدات والتانينات. تم تقييم النشاط المضاد للبكتيريا باستخدام طريقة الانتشار في الآجار. أظهرت النتائج أن المستخلص الإيثانولي لأوراق نبات القبار لم يُظهر أي نشاط

مضاد للبكتيريا ضد عزل *E. coli* السريري، حيث لم تُلاحظ أي مناطق تثبيط في جميع التركيزات التي تم اختبارها. قد يُعزى غياب هذا النشاط إلى عوامل مثل انخفاض تركيز المركبات النشطة، وضعف نفاذيتها عبر الغشاء الخارجي للبكتيريا سالبة الجرام، واحتمالية تحلل المكونات الحساسة للحرارة أثناء عملية الاستخلاص. في المقابل، أظهرت المضادات الحيوية المستخدمة كضوابط إيجابية نشاطًا (سيفوتاكسيم 40 مم، أميكاسين 16 مم، أموكسيسيلين 28 مم)، بينما لم يُظهر البنسلين أي تثبيط. تخلص الدراسة إلى أن فعالية المستخلصات النباتية تعتمد بشكل كبير على نوع المذيب، طريقة الاستخلاص، التركيب الكيميائي، والسلالة البكتيرية المحددة.

الكلمات المفتاحية: *Capparis spinosa*، نشاط مضاد للبكتيريا، مستخلص إيثانولي، *E. coli*، مضادات حيوية.

Introduction

Since ancient times, humans have relied on medicinal plants for healing, utilizing their innate intuition and cognitive abilities to explore the therapeutic properties offered by nature. According to reports from the World Health Organization, approximately 80% of the global population continues to depend on traditional medicine to meet their primary healthcare needs, due to its availability and relative effectiveness (WHO, 2020). The excessive use of antibiotics across various medical fields has contributed to the emergence of numerous resistant bacterial strains and the widespread prevalence of infectious diseases (Centers for Disease Control and Prevention, 2023). This increase in antimicrobial resistance is driven by several factors, including the over-prescription of antibiotics by healthcare practitioners and their misuse through self-medication (Laxminarayan et al., 2017; Hsin., et al 2025; Salem and Salem, 2025). Consequently, researchers have increasingly focused on identifying safe and effective alternatives, particularly those derived from medicinal plants. Numerous studies have evaluated plant extracts to assess their antimicrobial properties and identify novel bioactive compounds (Patel et al., 2021). Plant-based extracts have demonstrated a strong ability to inhibit the growth of pathogenic bacteria due to their complex mixture of bioactive constituents, which reduces the likelihood of microbial resistance developing against them. This contrasts with conventional antibiotics, which typically rely on a single active ingredient. Well-known medicinal plants with potent antimicrobial activity include wild thyme, garlic, turmeric, and ginger (Annaz et al., 2022). Among these plants, *Capparis spinosa*—commonly known as the caper plant or “Flinders rose”—is one of the most widely used species in traditional medicine. It belongs to the Capparidaceae family and is a perennial, thorny shrub that grows abundantly in arid and semi-arid regions across Southwest and East Africa, the Pacific Islands, and Southeast Asia (Saadaoui & Rzigui, 2023).

Figure (1) *C. spinosa* in the Khnifriyat area, Bani Walid, Libya.

The caper plant has long been valued for its medicinal benefits. Historically, various plant parts, including the roots, bark, leaves, and buds, were used to manage gastrointestinal disorders, spleen diseases, ear pain, skin conditions, joint inflammation, fever, cough, and asthma (Al-Quraishi & Al-Zoubi, 2020). More recently, experimental and clinical studies have highlighted the pharmacological potential of *C. spinosa*, demonstrating a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer effects (Annaz et al., 2022). Additionally, these studies have shown that the plant exhibits antimicrobial activity against several pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis* (Salem, 2024). These properties are attributed to its rich composition of biologically active compounds—most notably flavonoids such as quercetin, rutin, and kaempferol which contribute to the inhibition of bacterial growth (Annaz et al., 2022).

Previous Studies

Previous research has demonstrated the strong antibacterial potential of *Capparis spinosa*. For instance, (Ibrahim & Salih, 2020) reported that the ethanolic extract of caper leaves exhibited remarkable antimicrobial activity, producing inhibition zones of 16 mm against *Bacillus cereus* and 13.75 mm against the fungi *Candida albicans* and *Saccharomyces cerevisiae*. A high inhibition percentage (98.47%) was also recorded against *Renalam spp.*, while the aqueous extract showed no measurable activity. (Jian et al., 2021), which examined the chemical composition and traditional uses of the plant, revealed that *C. spinosa* demonstrates antibacterial activity across all its parts—including roots, stems, and fruits. This activity was attributed to the presence of phenolic and flavonoid compounds capable of directly affecting bacterial cells.

In another study conducted in Iraq, (Al-Ekdi et al., 2012) tested aqueous and ethanolic extracts of caper plants at different concentrations (200, 400, and 500 mg/mL) against various pathogenic microbes. The results indicated that Gram-positive bacteria were generally resistant to the extracts, whereas Gram-negative bacteria showed variable responses depending on the bacterial species and plant part used. Higher extract concentrations were associated with increased antibacterial activity. (Abdulridha & Saliem, 2023) investigated the effect of caper fruit extract in comparison with the antibiotic ciprofloxacin against resistant *E. coli* strains. The results demonstrated that combining the ethanolic extract with half the therapeutic dose of ciprofloxacin enhanced its antibacterial activity (synergistic effect), making previously resistant bacteria more susceptible and restoring the effectiveness of the antibiotic. (Jasim & Mohammed, 2023) evaluated the antibacterial activity of crude caper extracts against *Helicobacter pylori* isolates obtained from patients in Baghdad. The extracts produced strong inhibitory effects, with inhibition zones ranging from 12.0 mm to 30.7 mm, indicating potent antibacterial activity. (Ismiou, 2025) assessed the therapeutic properties of *C. spinosa* extract and reported significant protective effects against liver and kidney damage induced by cisplatin, in addition to demonstrating notable antibacterial properties. A more recent study (Annaz et al., 2022) revealed that the plant is rich in potent bioactive compounds, including flavonoids, alkaloids, and glucosinolates. The findings further indicated that extracts from the leaves and roots exhibited strong activity against various bacterial and fungal strains. Finally, (Adwan, 2023) reported that the ethanolic extract of caper fruits showed clear antibacterial activity against *Staphylococcus* species compared to aqueous extracts, which were less effective. The minimum inhibitory concentration (MIC) values for the ethanolic extract ranged between 12.5 and 25 mg/mL, suggesting good antibacterial potency.

Research Aim

The aim of this study is to evaluate the antibacterial activity of the ethanolic leaf extract of *Capparis spinosa* against a *clinical isolate of Escherichia coli*, using different extract concentrations and standard antimicrobial testing methods.

Materials and Methods

1. Plant Collection and Preparation of the Ethanolic Extract

Leaves of *Capparis spinosa* were collected from the Khnifriyat area in Bani Walid, Libya, during December 2024. The leaves were thoroughly washed, shade-dried, and ground into a fine powder. A total of 50 g of the powdered material was soaked in 250 mL of 95% ethanol to obtain a homogeneous suspension. The extract was concentrated using a rotary evaporator under reduced pressure to remove the solvent, then dried and stored at –20 °C until use, following the procedures described by (Shareef, 1998; El-Stal et al., 2005).

2. Preparation of Extract Solutions

One gram of the dried extract was dissolved in Dimethyl Sulfoxide (DMSO) to prepare solutions at concentrations of 100, 300, 360, and 700 mg/mL. All solutions were subjected to pasteurization at 62C^{o} for 15 minutes to ensure sterility and minimize thermal degradation (Deeb, 2019).

3. Bacterial Strain and Culture Conditions

A clinical isolate of *Escherichia coli* (E. coli) was obtained from a diagnostic laboratory. The bacterial identity was biologically confirmed using standard diagnostic tests.

4. Antibacterial Activity Assay (Disc Diffusion Method) and Controls

Petri dishes were prepared by pouring 20–25 mL of Muller-Hinton Agar (MHA) or Nutrient Agar into each plate and allowing it to solidify. The plates were then inoculated with a standardized bacterial suspension (0.5 McFarland turbidity). Sterile paper discs (6 mm diameter) were saturated with 20 µL of each extract concentration. Four standard antibiotic discs were used as positive controls:

- Cefotaxime (CTX)
- Amikacin (AK)
- Amoxicillin (AML)
- Penicillin (P)

The plates were incubated at 37C^{o} for 24 hours. The diameter of the inhibition zone around each disc was measured in millimeters (mm). Three replicates were performed for each treatment.

5. Preliminary Phytochemical Screening of Active Compounds

Qualitative analysis was conducted to identify the presence of key secondary metabolites:

- **Alkaloids**: Detected using Mayer's or Dragendorff's reagent.
- **Flavonoids**: Detected by adding $AlCl_3$ and observing the formation of a pale-yellow color.
- **Tannins**: Detected using 5% $FeCl_3$, solution, indicated by a blue-green coloration.
- **Saponins**: Confirmed by the formation of dense, stable foam after shaking the extract in water.

All tests were conducted at least twice to ensure accuracy.

Results and Discussion

1. Preliminary Phytochemical Screening of Bioactive Compounds

To identify the main bioactive constituents in the extract, preliminary phytochemical screening was conducted Table (1). The qualitative analysis of the ethanolic leaf extract of *C. spinosa* indicated the presence of several secondary metabolites. The screening revealed a strongly

positive result for Saponins, confirmed by the formation of dense and stable foam (Foam Test). Conversely, Flavonoids and Tannins were detected at low levels, showing only a pale-yellow color with $AlCl_3$ and a light blue/green color with $FeCl_3$, respectively. Alkaloids were found to be absent (Negative) as no precipitate was observed with Mayer's or Dragendorff's reagents.

Table (1) Active compounds, results, and additional observations.

Active Compound	Result	Additional Observations
Alkaloids	Negative	No precipitate observed
Flavonoids	Weakly positive	Pale yellow color
Tannins	Weakly positive	Light blue/green color
Saponins	Strongly positive	Formation of dense and stable foam

The absence of antibacterial activity observed in this study can be attributed, in part, to the chemical composition of the extract. Specifically, the low concentration of known active compounds like flavonoids and tannins may not be sufficient to exert a strong inhibitory effect. Additionally, the limited permeability of these compounds through the outer membrane of Gram-negative bacteria (such as *E. coli*) presents a significant challenge. Furthermore, the heat- or evaporation-sensitivity of certain bioactive constituents may have led to their degradation or loss during the concentration and preparation stages (Deeb, 2019; Salem, & Lakwani, 2024).

2. Antibacterial Activity Against the Clinical *E. coli* Isolate

The antibacterial activity of the ethanolic leaf extract of *Capparis spinosa* was evaluated against the clinical *E. coli* isolate using four different concentrations (100, 300, 360, and 700 mg/mL) via the disc diffusion method Figure (2). The results consistently indicated no antibacterial activity at any tested concentration, as no inhibition zones were observed around the extract discs (Figure 2). This lack of inhibition confirms the isolate's resistance to the extract under the present experimental conditions.

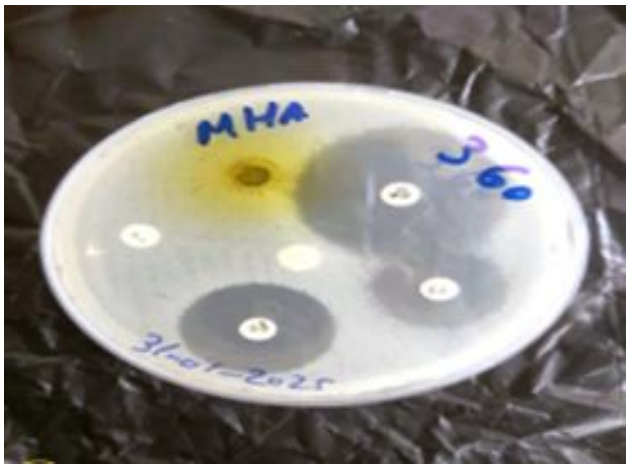

Figure (2) Effect of the alcoholic extract of *Copparis spp* leaves at 360 mg/ml on *E. coli*.

In contrast, the standard antibiotics used as positive controls exhibited clear and measurable inhibition zones, confirming the viability of the bacterial strain and the reliability of the experimental method. The observed inhibition zone diameters were 40 mm for Cefotaxime

(CTX), 16 mm for Amikacin (AK), and 28 mm for Amoxicillin (AML). The antibiotic Penicillin (P) showed no inhibition, indicating that the clinical *E. coli* isolate is resistant to penicillin, which is a common finding for Gram-negative bacteria due to inherent resistance mechanisms (Bauer et al., 1966).

Table (2) Samples, concentrations, and inhibition zone diameters.

Sample	Concentration (mg/mL)	Inhibition Zone (mm)
Ethanolic extract	100	No inhibition
Ethanolic extract	300	No inhibition
Ethanolic extract	360	No inhibition
Ethanolic extract	700	No inhibition
Cefotaxime (CTX)	30	40
Amikacin (AK)	30	16
Amoxicillin (AML)	10	28
Penicillin (P)	1	No inhibition

This finding, which shows the absence of activity against *E. coli*, is consistent with general literature reporting that the efficacy of *C. spinosa* extracts is often limited against Gram-negative bacteria, while higher activity is commonly reported against Gram-positive bacteria (Annaz et al., 2022; Deeb, 2019). However, a related study reported that the volatile oils extracted from caper leaves exhibited clear antibacterial activity against *E. coli* (El Aty et al., 2025). This difference is critical, as it suggests that the most active compounds may be volatile or heat-sensitive, and thus may not have been efficiently extracted or preserved using the 95% ethanol solvent and the thermal processes employed in this study. This highlights the crucial role that the type of solvent and the concentration of active constituents play in determining antibacterial activity, with methanolic extracts often showing higher activity compared to aqueous extracts (Watery, 2023; Salem., et al 2025).

Conclusion

Based on the results of this study:

1. The ethanolic leaf extract of *Capparis spinosa* exhibited no antibacterial activity against the clinical *E. coli* isolate at concentrations of 100, 300, 360, and 700 mg/mL.
2. The successful activity of the positive control antibiotics (Cefotaxime, Amikacin, and Amoxicillin) confirms the reliability of the experimental procedure and the validity of the results.
3. The absence of antibacterial activity is attributed to a combination of factors, including: the low levels of active compounds (Flavonoids and Tannins) identified in the extract, the limited permeability of these compounds through the outer membrane of the Gram-negative *E. coli* strain, and the potential degradation or loss of heat-sensitive components during the extraction and preparation processes.
4. The study highlights that the effectiveness of plant extracts is strongly influenced by the solvent type, the extraction method, the resultant chemical composition, and the biological characteristics of the bacterial strain tested.

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