



Antibacterial Activity of Leaf Extracts from the Desert Plant *Capparis spinosa*

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Abstract:

The spiny caper plant (*Capparis spinosa*) is a medicinal herb with potent antibiotic properties. This study aimed to evaluate the inhibitory effect of extracts from this plant on the growth of the four bacterial species studied. The efficacy of three leaf extracts (aqueous, methanolic, and ethanolic) from plants growing in the western desert of Anbar province, Iraq, the inhibitory activity of the three extracts was tested against the growth of four medically important pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). The study concluded that the methanol extract was the most effective in inhibiting the growth of the studied bacterial species. It had the maximum inhibitory influence of 24.4 mm on *B. subtilis*. The lesser influence of 17.3 mm was on *E. coli*. The ethanol extract was the most effective in inhibiting the studied bacterial species, reaching 18.6 mm for *B. subtilis*. While *Ps. aeruginosa* showed a lower effect, reaching 09.5 mm in the aqueous extract.

Key words: Caper, Extract, Leaves, Inhibition, Bacterial species, Desert plant.



Introduction

Herbs, plant extracts, and phytochemicals are widely used as food, nutritional supplements, and medicines [1, 2]. Because of their health-enhancing benefits and effects, and the numerous biological activities associated with their traditional uses, many of these herbs have been marketed. In some cases, the alleged vital activities are not always scientifically proven, so their information relies heavily on the history and folklore of the local communities [3, 4, 5].

The spiny caper plant (*Capparis spinosa* L.) belongs to the Capparaceae family [6, 7]. It grows in regions with low temperatures and humidity in western Central Asia. It has the advantage of being high in nutritional value and is traditionally used to treat many diseases. Moreover, its roots are valuable for being anti-diarrheal [8, 9, 10]. In traditional agriculture, different parts of this plant show bioactivity against viruses, as the bud extract prevents the replication of the Herpes simplex virus 2. It prevents infection stimulators like interferon- γ , interleukin-12, and tumour necrosis factor- α ; this is the influence of the hydroxycinnamic acid, which represents the antioxidants found in *C. spinosa* buds [11].

The fruits and roots of *C. spinosa* are considered potent antimicrobial agents

compared to extracts from many other medicinal herbs [12, 13, 14]. Studies exist on the traditional use of extracts from these plant species as popular natural antibiotics, or on their pharmacological efficacy, such as the protective effect of flower buds of some species, the effect of methanol extract on cartilage cells [8], the anti-inflammatory and anti-biofilm activity of *C. spinosa* fruits [15], and their anti-proliferative activity against cancer cells. The inhibitory effect of *C. spinosa* buds on HIV type 1 has also been confirmed [16].

Due to the continuous increase in bacterial resistance to commonly used antibiotics and the development of multiple strains of resistance, which has resulted in numerous therapeutic problems, it has become necessary to search for alternative antimicrobial substances. One of the most promising solutions is the use of herbal medicines, but they have to be based on scientific studies. Many studies have been published recently in this field, making it possible to assess the effects of aromatic and medicinal plants on human health [17].

The importance of the components has attracted the interest of many researchers worldwide. Since the discovery of inhibitory effects and their importance in the therapeutic field, the use of biologically active compounds has become an important

area of scientific research to address the problem of bacterial resistance to traditional chemical drugs and to reduce their potential side effects [18]. In addition, the use of antibiotics two to four times is sometimes necessary to treat many infections, such as skin, respiratory, urinary tract, and intra-abdominal infections. The germs responsible for the above-mentioned diseases are *S. aureus*, *E. coli*, and *Klebsiella*, and the study focused on the increased antibacterial activity of *C. spinosa*, which is known for its interesting therapeutic properties [19].

Using the minimum inhibitory concentration inhibition process, the inhibitory effect of acetone, ethanol, and methanol extracts of the plant species *C. deciduas* and *C. spinosa* on the growth of bacterial species, including *S. aureus*, *E. coli*, *B. subtilis*, and *P. multocida*, was evaluated. (MIC). The methanolic extracts of selected sections from both plants were found to substantially reduce the growth of the four bacterial species when all extracts were compared [4].

Numerous studies have contributed to the evaluation of the pharmacological importance and biological efficacy of the caper plant (*C. spinosa*) as a dry medicinal plant adapted to arid and semi-arid environments. Its capacity to withstand

drought, high temperatures, and poor soils makes the caper a valuable desert plant with promising applications in natural-product research. The plant's various organs—leaves, buds, fruits, roots, and aerial parts—are reported to contain many biologically active secondary metabolites, including alkaloids, phenolic acids, flavonoids, terpenoids, glucosinolates, and fatty-acid derivatives. These compounds are closely linked to antioxidant, anti-inflammatory, antimicrobial, and other pharmacological activities, which support the plant's traditional medicinal use and encourage further experimental evaluation of its biological potential. [11, 14].

Among the numerous biological activities evaluated for the caper plant (*C. spinosa*), its antimicrobial effect is of particular interest due to the increasing global resistance of bacteria to conventional antibiotics. Extracts rich in phenolic and flavonoid compounds may inhibit bacterial growth through multiple mechanisms, including disruption of the bacterial cell membrane, interference with microbial enzyme systems, suppression of biofilm formation, and modulation of oxidative stress. Recent studies on spiny caper extracts and preparations have demonstrated promising antibacterial activity against both Gram-positive and

Gram-negative bacteria, suggesting that this species may be a valuable source of natural antimicrobial agents [18, 19, 4].

Despite the increasing number of studies and research on the therapeutic and pharmaceutical properties of *C. spinosa*, the antibacterial activity of leaf extracts collected from desert environments remains under-researched, particularly in western Iraq. Given that environmental stress conditions can influence secondary metabolite accumulation in desert plants, evaluating locally collected *C. spinosa* leaves could provide valuable insights into their antibacterial potential. Therefore, the current study aimed to evaluate the inhibitory activity of aqueous, methanolic, and ethanolic leaf extracts of *C. spinosa* against the activity of selected pathogenic bacterial isolates. The importance of the current study lies in estimating the secondary metabolite components of a desert plant that thrives in the western Anbar desert, where high temperatures and aridity lead to stress in harsh desert environments, resulting in unique secondary metabolite profiles that distinguish these wild populations from those found in other geographical areas.

Methodology

Sample Collection and Extract Preparation

Plant specimens of the species *C. spinosa* were collected from the western Anbar Plateau in Iraq, specifically from Ramadi, Hit, and Al-Baghdadi. Several field visits were conducted between July 15 and August 15, 2023, during which specimens were collected at the flowering stage. The specimens were then examined, identified, and compared with Iraqi flora and with specimens preserved in the Iraqi National Herbarium. The botanical status of *Capparis spinosa* L. is shown below.

Family: Capparaceae

Genus: *Capparis*

Species: *Capparis spinosa*

The collected leaves were dried in paper bags by exposing them to sunlight for 72 hours. Then, they were dried in an electric oven at 65 ° for 24 hours. The next step was grinding the leaves using an electric blender. The dry powder samples of each part of the plant (20 g) were mixed separately with 100 mL of each of the three solvents (distilled water, ethanol, methanol) for 3 hours at room temperature in a water bath shaker. The residue was extracted with 100 mL of fresh solvent. The three extracts were reduced under reduced pressure at 45 °C using a rotary evaporator. After that, the concentrated crude extracts were stored in the refrigerator at 4 °C for use in the experiment [20] with some modifications.

Preparation for Bacterial Strains

The bacterial isolates, *S. aureus*: ATCC 25923, *E. coli*: ATCC 11775, *B. subtilis*: ATCC 21332, and *Pseudomonas aeruginosa*: ATCC 27853, were taken from the microbiology laboratory in the Department of Biology, University of Anbar. The bacteria were incubated for 24 hours at 37 °C in a solid agar medium. Then, they were transferred to a nutrient broth to obtain liquid cultures from the above isolates. The inhibitory activity of aqueous, methanolic, and ethanolic extracts of *C. spinosa* was evaluated individually against the studied bacterial species using the media etching method [21].

Statistical Analysis:

Data were expressed as mean $\pm$ standard deviation (SD) from three independent replicates ($n = 3$). Two-way analysis of variance (ANOVA) was carried out separately for inhibition zone and minimum inhibitory concentration (MIC) results, to evaluate the effect of bacterial species, treatment, and the interaction

between them (bacterial species $\times$ treatment). When significant differences were found, Tukey's honestly significant difference (HSD) post-hoc test was used for multiple comparisons between treatment means. Differences were considered significant at $p < 0.05$. The statistical analyses were performed using SPSS software.

Results:

The plant extracts studied significantly affected the growth of the four bacterial species in the experiment. The methanol extract of *C. spinosa* effectively inhibited the growth of all selected bacteria. Its maximum inhibitory potential was greater against *B. subtilis*. The inhibition zone was 24.4 mm. Then *E. coli* came next, with an inhibition zone of 17.3 mm. The ethanol extract of *C. spinosa* had the greatest inhibitory effect on *B. subtilis*, reaching 18.6 mm; the aqueous extract of *C. spinosa* had the greatest inhibitory effect on *B. subtilis*, reaching 16.7 mm (Table 1).

Table 1. Antimicrobial activity of *C. spinosa* leaf extracts against bacterial species (mm).

Bacterial species	Ethanol	Methanol	Distilled water	Amoxicillin
<i>Staphylococcus aureus</i>	11.30 ± 0.20 ^c	14.70 ± 0.20 ^b	10.10 ± 0.10 ^c	32.50 ± 2.00 ^a
<i>Escherichia coli</i>	12.70 ± 0.20 ^c	17.30 ± 0.20 ^b	11.50 ± 0.20 ^c	33.20 ± 1.00 ^a
<i>Bacillus subtilis</i>	18.60 ± 0.30 ^c	24.40 ± 0.20 ^b	16.70 ± 0.20 ^d	33.03 ± 0.58 ^a
<i>Pseudomonas aeruginosa</i>	11.50 ± 0.20 ^c	14.30 ± 0.30 ^b	9.50 ± 0.20 ^d	30.90 ± 2.00 ^a

Values are expressed as mean ± SD (n = 3). Means with different superscript letters within the same row are significantly different according to Tukey's HSD test at p < 0.05. Means sharing the same superscript letter are not significantly different.

Lower MIC values indicate greater antibacterial activity, as a smaller concentration of the extract is required to inhibit bacterial growth. Accordingly, the methanolic extract exhibited the strongest antibacterial activity among the tested extracts, showing the lowest MIC values against all tested bacterial species. The lowest MIC value was recorded against *B. subtilis* (118.2 µg µL⁻¹), followed by *P. aeruginosa* (122.6 µg µL⁻¹).

The ethanol extract of *C. spinosa* also had a significant influence on *B. subtilis*, and its value was (155.3 µg. µl⁻¹). *S. aureus* came next with a value of (178.3 µg. µl⁻¹). The aqueous extract had a significant influence, and its value was (168.7, 192.3 µg. µl⁻¹) on both *B. subtilis* and *S. aureus*, respectively (Table 2).

Table 2. Minimum inhibitory concentration (MIC) of *C. spinosa* leaf extracts against the tested bacterial species (µg µL⁻¹).

Bacterial species	Ethanol	Methanol	Distilled water	Amoxicillin
<i>Staphylococcus aureus</i>	178.77 ± 2.04 ^c	141.13 ± 1.03 ^b	192.30 ± 2.30 ^d	23.40 ± 2.00 ^a
<i>Escherichia coli</i>	231.03 ± 2.00 ^c	175.17 ± 2.52 ^b	247.40 ± 3.40 ^d	14.30 ± 1.00 ^a
<i>Bacillus subtilis</i>	155.10 ± 3.00 ^c	118.07 ± 2.00 ^b	168.23 ± 2.04 ^d	11.50 ± 1.00 ^a
<i>Pseudomonas aeruginosa</i>	195.17 ± 3.01 ^c	122.60 ± 1.70 ^b	218.50 ± 1.00 ^d	28.60 ± 2.00 ^a

Values are expressed as mean $\pm$ SD (n = 3). Different superscript letters within the same row indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$. Lower MIC values indicate greater antibacterial activity.

Discussions:

Medicinal plants have been used for decades, but despite their use in herbal medicine and indirect use in pharmaceutical drugs, the emergence of antibiotic-resistant pathogenic bacteria, as well as the limitations of conventional methods for treating infectious diseases, has accelerated research. [23,24]. The antimicrobial activity of *C. spinosa* leaves was tested against four different bacteria in this study. Compared to synthetic drugs like amoxicillin, the extracts greatly slowed the growth of the four bacterial species.

The antibacterial performance of caper leaf extracts (*Capparis spinosa*) in this study is consistent with the findings of [25], who studied caper plants in Iraq and confirmed their significant inhibitory activity against several pathogenic bacteria. Their study also found high concentrations of polyphenolic compounds in organic solvent extracts, which were found to destabilize bacterial cell membranes.

The results also show that *Bacillus* bacteria were effectively inhibited by the methanol extract, although other bacterial species showed some tolerance. The differences in bacterial responses to

solvents may be attributed to differences in cell membrane permeability between Gram-negative and Gram-positive bacteria [26].

C. spinosa leaves are also high in antioxidants [27]. In contrast to synthetic drugs such as amoxicillin and ciprofloxacin, *C. spinosa* extracts exhibited strong antimicrobial potential, as this study demonstrated. The antimicrobial agents present in extracts prepared from fermented *C. spinosa* leaves are effective against drug-resistant bacterial strains [26, 27]. All of the tested bacteria were inhibited by the leaf extracts of *C. spinosa*. This ability to suppress bacterial growth may be attributed to plant compounds, such as phenols and flavonoids, present in the extracts. The elevated levels of phenolics and flavonoids found in different parts of the *Capparis* plant may therefore explain the antibacterial capacity of its extracts [28]. In a related study, [29] examined the antimicrobial phenolic acid activity.

Reversed-phase HPLC with UV detection has been used to elucidate the structure of phenolic acids, and GC-MS to characterise them. Plant extracts with higher levels of phenolic compounds and flavonoids—both of which are abundant in

Capparis extracts—were found to exhibit antibacterial activity [28, 30, 30]. The flavonoids present in the extracts were found to suppress the growth of *E. coli* and *S. aureus* [32, 33]. Consistent with these findings, extracts rich in phenolic acids and flavonoids exhibited stronger antimicrobial activity [34].

Conclusion: The methanolic leaf extract of *Capparis spinosa* showed the strongest antibacterial activity against the tested bacterial species, with the highest inhibition zone recorded against *Bacillus subtilis*. These findings suggest that *C. spinosa* leaves, especially methanolic extracts, may represent a promising natural source of antibacterial bioactive compounds.

Conflict of Interest: The authors declare that there is no conflict of interest.

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النشاط المضاد للبكتيريا لمستخلصات أوراق نبات الكبر

كُنْبَات صحراوي *Capparis spinosa*

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ملخص

(نباتاً عشبياً فعالاً في مجال المضادات الحيوية. هدفت هذه الدراسة إلى *Capparis spinosa* يُعدّ نبات الكبر)
 تحديد التأثير المثبط لمستخلصات هذا النبات على نمو أنواع معينة من البكتيريا. تم اختبار فعالية ثلاث مستخلصات من
 أوراق النبات (مائي، ميثانولي وإيثانولي) من نباتات تنمو في الصحراء الغربية لمحافظة الأنبار، وذلك لتحديد نشاطها
 ضد *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* المثبط ضد أربعة أنواع من البكتيريا الممرضة ذات الأهمية الطبية)
 خلصت الدراسة إلى أن المستخلص الميثانولي *Pseudomonas aeruginosa*, and *Staphylococcus aureus* .
B. subtilis هو الأكثر فعالية في تثبيط نمو أنواع البكتيريا المدروسة، حيث حقق أعلى تأثير مثبط بلغ 24.4 ملم على
 . أما المستخلص الإيثانولي، فكان الأكثر فعالية في تثبيط نمو أنواع *E. coli* وكان تأثيره أقل، حيث بلغ 17.3 ملم على
 تأثيراً *Ps. aeruginosa*. بينما أظهرت بكتيريا *B. subtilis* المدروسة، حيث بلغ تأثيره المثبط 18.6 ملم على
 أقل، حيث وصلت إلى 9.5 ملم في المستخلص المائي.

الكلمات المفتاحية: الكبر ، مستخلص الأوراق ، التثبيط، انواع البكتيريا، نبات صحراوي