

Article

Evaluation of the Inhibitory Activity of Tannin Extracts from the Leaves and Flowers of *Capparis spinosa* L. Against Multidrug-Resistant *Staphylococcus aureus*

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Citation: Salih, S. L. Evaluation of the Inhibitory Activity of Tannin Extracts from the Leaves and Flowers of *Capparis spinosa* L. Against Multidrug-Resistant *Staphylococcus aureus*. American Journal of Biology and Natural Sciences 2026, 3(9), 55-64.

Received: 18th Jul 2026

Revised: 05th Aug 2026

Accepted: 25th Aug 2026

Published: 12th Sept 2026


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Abstract: The results of the present study revealed the successful isolation and identification of *Staphylococcus aureus* bacteria from various clinical infections, obtaining 11 isolates out of 25 samples. The isolates exhibited very high resistance to aztreonam (100%), piperacillin (90.9%), and ceftriaxone (90.9%), while demonstrating complete susceptibility (100%) to both imipenem and meropenem. Furthermore, the results of the phenotypic detection of virulence factors showed the ability of some *Staph. aureus* isolates to produce beta-lactamase enzyme at a rate of 36.4% and hemolysin at 63.63%, in addition to the ability of all isolates to form a dense slime layer associated with biofilm formation. The floral tannin extracts of *Capparis spinosa* showed superior antibacterial inhibitory activity compared to leaf extracts, with the maximum inhibition zone diameter reaching 23 mm at 100% concentration. This result indicates that *C. spinosa* flowers may represent a promising natural source of compounds with antibacterial activity against *Staph. aureus*, particularly antibiotic-resistant isolates.

Keywords: *Staphylococcus aureus*, *Capparis Spinosa*, Tannin Extracts, Antibacterial Activity

Introduction

Medicinal plants are a significant part of the human diet [1]. Finding substitute medications with antibacterial qualities and less negative effects on human health has become crucial due to the growing resistance brought on by the usage of synthetic chemical antibiotics [2]. Tannins are widespread secondary metabolites in plants and are connected with many medicinal plants, so the tannin content and antimicrobial activity in select types of medicinal plants have been examined [3,4]. The genus *Capparis* L. is a member of the family *Capparaceae*, which is mainly found in arid and semi-arid regions and has about 45 genera and over 700 species [5]. *Capparis spinosa* L., the caper plant, is a perennial shrub that prefers hot, dry climates [6]. It is well-known for its edible flower buds, or capers. This plant can withstand temperatures as low as -8°C and is remarkably resilient to high temperatures and water scarcity [7]. The *Capparis spinosa* L. plant is utilised as a natural cure due to its various bioactive phytochemicals, such as tannins, phenols, flavonoids, triterpenoids,

steroids, saponins, and major and trace elements[8]. It possesses antimicrobial, cytotoxic, antidiabetic, anti-inflammatory, and antioxidant properties. [9]

Staphylococcus aureus is a highly virulent pathogen capable of causing diverse clinical infections in humans. It represents a primary etiological agent of infective endocarditis and bacteremia, in addition to inducing skin, soft tissue, osteoarticular, pleuropulmonary, and device-associated infections [10]. The bacterium *Staph. aureus* is characterized by the emergence of antibiotic-resistant strains, which represent a primary etiologic agent of nosocomial infections that record high prevalence rates globally[11]. Multiple studies have demonstrated that approximately 70% of clinical isolates in hospital settings exhibited resistance to antibiotics, particularly the β -lactams group, which until recently constituted the first-line defense in therapeutic protocols[12]. Therefore, the study aimed to isolate and identify *Staphylococcus aureus*, detect some of its phenotypic virulence factors, and evaluate the antibacterial activity of tannin extracts isolated from the flowers and leaves of the caper plant (*Capparis spinosa* L.) against some multidrug-resistant *Staph. aureus* isolates.

Materials and Method

Isolation and identification of pathogenic bacteria

The study included the collection of 25 clinical samples by qualified medical staff from patients of both genders attending Tikrit Teaching Hospital. The sources of infection varied among the patients and included various clinical specimens. Subsequently, the samples were inoculated onto Mannitol Salt Agar using a sterile cotton swab. The plates were incubated at 37°C for 24 hours, after which a single colony was taken from each growth that appeared on the medium where the sample was cultured. The purification process was performed using the four-quadrant streak plate method on Nutrient Agar to isolate pure colonies. These pure colonies were then subcultured onto Nutrient Agar slants and stored at 4°C prior to subsequent testing [13]. Preliminary identification of the pathogenic bacteria began with microscopic identification after staining with Gram stain, relying on the morphological features of the colonies. Subsequently, the identity of the isolated bacteria was confirmed using standard biochemical tests. Finally, accurate and definitive identification was verified using the VITEK 2 Compact System at the hospital central laboratory[14].

Antibiotic sensitivity test

Antimicrobial susceptibility testing for the isolates was performed using the Kirby-Bauer method on Mueller-Hinton agar [15]. A homogeneous bacterial suspension was prepared by suspending 3–5 pure colonies in 5 mL of sterile saline, adjusting its turbidity to the 0.5 McFarland standard. The agar surface was inoculated with a cotton swab after expressing excess fluid, and allowed to dry for 3–5 minutes. Disks were applied using a dispenser, and the plates were incubated at 35–37 °C for 16–18 hours. Inhibition zone diameters were measured using a digital caliper, and isolates were categorized as sensitive, intermediate, or resistant according to CLSI criteria.

Phenotypic detection of virulence factors of *Staph. aureus* isolates

1. Hemolysin Production:

Hemolytic activity was detected using the culture method on plates. Each isolated sample was cultured on a blood agar plate and incubated at 37°C for 24 hours. Isolated samples that showed hemolytic areas surrounding the growth were considered positive [16].

2. β -lactamase production

The filter paper method was used for the β -lactamase production assay [17]. To make the penicillin solution, dissolve 0.2 g of starch in 100 mL of distilled water, then add 1 g of penicillin. One-by-five-centimeter strips of filter paper were cut, submerged in the penicillin solution, and left to dry for two hours. A 5 mm diameter section of the paper strip was covered with colonies that were applied in a circular motion. A loopful of the recently cultured test organism was then added to the strips, and they were incubated for 30 minutes at 37°C. Iodine solution was then used to saturate the filter paper, and any extra solution was eliminated. The decolorization of the inoculation site after 10 minutes showed a positive beta-lactamase test, while organisms that did not produce beta-lactamase kept their purple colour.

3. Biofilm Formation

The Tube Method (TM) was adopted as follows: a single fresh colony from a recent culture was transferred to tubes containing 3 mL of BHIB medium, incubated for 24 hours at 37 °C, then the medium was poured off and the tubes were washed with saline solution to remove non-adherent cells. Crystal violet stain 0.1% was added for 15-20 minutes, then the stain was removed and the tubes were washed to remove excess stain and the process was repeated three times. Staining of the tube was observed in the cultured area, particularly the appearance of the purple ring in the surface layer, and the results were recorded and evaluated based on the degree of staining of the inner walls of the tube[18].

Preparation of Tannin Extract

Tannin extracts previously prepared separately from the leaves and flowers of *Capparis spinosa* were used in the present study. The extracts were prepared according to the method described by [19]. Briefly, 0.5 g of powdered leaves and 0.5 g of powdered flowers were separately boiled in 50 mL of distilled water for 30 min. The extracts were then filtered and centrifuged at 2000 rpm for 20 min. The resulting supernatants were transferred separately into 100-mL volumetric flasks, followed by the addition of 20 mL of 4% lead acetate solution. The volume was adjusted to 100 mL with distilled water. The extracts were subsequently filtered and dried in an electric oven at 40°C until completely dry. The dried extracts were collected, placed in tightly sealed bottles, and stored in a refrigerator until further use [19].

Antibacterial assay of Tannin Extract.

The antibacterial activity of tannin extracts was evaluated against *Staph. aureus*. Briefly, 0.2 mL of fresh bacterial culture was added to 5 mL of sterile nutrient broth and incubated for 3–5 hours to reach a density corresponding to 10⁶ CFU/mL (0.5 McFarland standard). A 100 µL volume of the revived culture was spread onto Mueller-Hinton agar plates using a spreader. Wells of 6 mm diameter were punched into the agar [20], and 0.1 mL of various concentrations (100, 75 and 50) mg/ml of the were loaded extracts into the wells with a solvent control sample of deionized water. The plates were incubated overnight at 37°C and the zones of inhibition were subsequently measured in millimeters.

Results and Discussion

Isolation and identification

Among 25 clinical samples collected from patients suffering from various infections, a total of 11 isolates were identified as *Staph.aureus*. The remaining isolates belong to other Staphylococcus species ,both of which were isolated using mannitol salt agar, as it is a selective and differential medium specific for Staphylococcus bacteria. A total of 11 isolates were identified as *Staphylococcus aureus*, These isolates were characterized using various biochemical tests in addition to microscopic morphological criteria and the diagnosis was confirmed using the VITEK 2 Compact System, which were obtained from blood swabs, Urine, Diabetic patient swab and respiratory tract swab.

Sensitivity of bacterial isolates to antibiotics

The antibiotic susceptibility test results against 12 types of antibiotics, as shown in Table (1) and Figure (1) demonstrated that the eleven bacterial isolates exhibited the highest resistance to ATM, PRL, CRO and CTX at 100%, 90.9%, 90.9% and 72.7%, respectively, followed by N at 54.5%. Furthermore, the results showed that IPM and MEM antibiotics exhibited high efficacy against *Staph.aureus* at (100%), followed by CIP, OFX, and LEV at (90.9%).

The high resistance of bacteria to penicillin antibiotics may be attributed to the bacteria possessing beta-lactamase enzymes that hydrolyze the penicillin group, the genes for which are either chromosomal or plasmid in origin. In addition, these bacteria produce penicillin-binding proteins (PBPs) located in the cytoplasmic membrane associated with the cell wall. These proteins serve as the target for both penicillins and cephalosporins, as alterations in the target site of beta-lactam antibiotics lead to bacterial resistance against them[21].

Antibiotic resistance in bacteria arises from the misuse of antibiotics. Therefore, continuous monitoring and enhanced infection control programs are essential to reduce infection rates and

ensure adherence to appropriate guidelines for antibiotic use. A broader approach should include understanding resistance genes in both pathogenic and non-pathogenic bacteria, as well as the complete genome of resistant microorganisms [22].

Table 1. Results of antibiotic susceptibility testing for *Staph. aureus* isolates.

Antibiotic	Symbol	Concentration µg/disk	Resistant %	Sensitive %
Aztreonam	ATM	30mg	100	-
Imipenem	IPM	10mg	-	100
Lincomycin	L	10mg	18.2	81.8
Meropenem	MEM	10mg	-	100
Ofloxacin	OFX	10mg	9.1	90.9
Neomycin	N	10mg	54.5	45.5
Cefotaxime	CTX	30mg	72.7	27.3
Gentamycin	CN	10mg	18.2	81.8
Levofloxacin	LEV	5mg	9.1	90.9
Ceftriaxon	CRO	10mg	90.9	9.1
Pipracilin	PRL	30mg	90.9	9.1
Ciprofloxacin	CIP	5mg	9.1	90.9

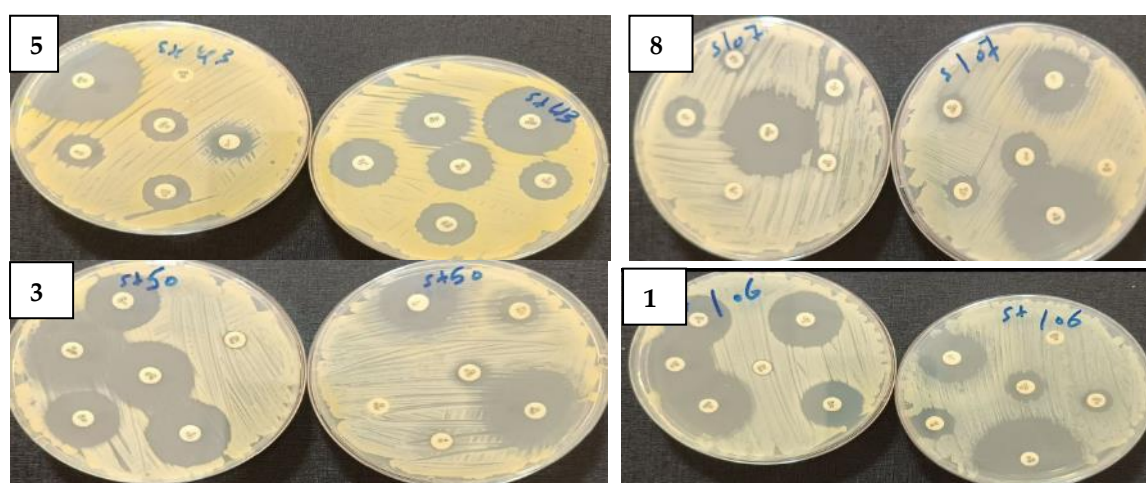


Figure 1. Sensitivity of *Staph.aureus* isolates to some antibiotics

Phenotypic Detection of Virulence Factors in *Staph.aureus*.

The ability of *Staph. aureus* bacterial isolates to produce virulence factors β -lactamase and β -hemolysin enzyme and biofilm formation, which increases their ability to cause various infections in humans, has been revealed as shown in table (2). The β -lactamase test was carried out using the filter paper method as shown in Figure (2). the results showed that *Staph.aureus* isolates were able to produce the beta-lactamase enzyme at a rate of 36.4%. These results were differed from those obtained by[23,24] which showed that *Staph. aureus* bacteria were able to produce this enzyme at a rate of 100%.

β -lactam antibiotics are a major factor in the treatment of bacterial infections worldwide and account for approximately 63.6% of antibiotic use. Resistance to this important class of antibiotics has increased globally in recent years, One of the important mechanisms of resistance to these antibiotics is the production of β -lactamase enzymes, which are capable of rendering β -lactam antibiotics inactive by covalently binding to their carbonyl group and hydrolyzing the β -lactam ring, thereby allowing bacterial resistance to these antibiotics[25]. Some β -lactamase enzymes are encoded by chromosome-borne genes, which can spontaneously translocate in certain species to conjugative plasmids, thereby increasing their ability to disseminate among other species [26] .

Furthermore, the results of the current study showed that 63.63% of *Staph. aureus* isolates were hemolysin producers, as shown in figure(3). This result differs from the study of [27,28] where the *Staph.aureus* bacterial isolates were 100% producers of this factor. Hemolysin is considered an important virulence factor for bacteria, as it breaks down red blood cells, causing anemia and weakening the immune system, thus providing large amounts of iron that benefit the bacteria in their metabolic activities. [29] The hemolysin enzyme also plays an important role in exacerbating bacterial tissue infections and the formation of a subsequent bacterial invasion, and its effect is manifested in the destruction of urinary epithelial cells [30].

The results also showed that all *Staphy. aureus* isolates were able to produce a dense mucus layer on the membrane surface, as shown in Figure (4). The results were consistent with [31]what the researcher stated, as the isolates produced biofilms at a rate of 90.9%, while they differed from [32]what he stated in that the isolates produced this factor at a rate of 45% .Bacterial growth within a biofilm community leads to increased antibiotic resistance through multiple mechanisms, including plasmid exchange and enhanced antibiotic resistance Furthermore, localized changes in microbial environments weaken antibiotic efficacy [33].

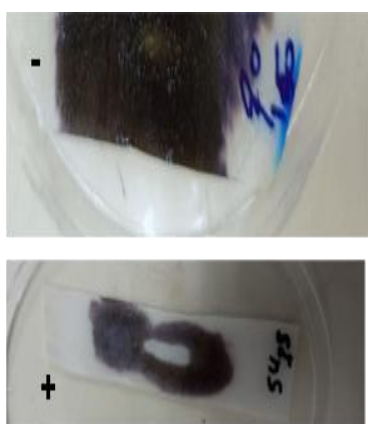


Figure 2. The ability of *Staph.aureus* to produce β - lactamase enzymes.

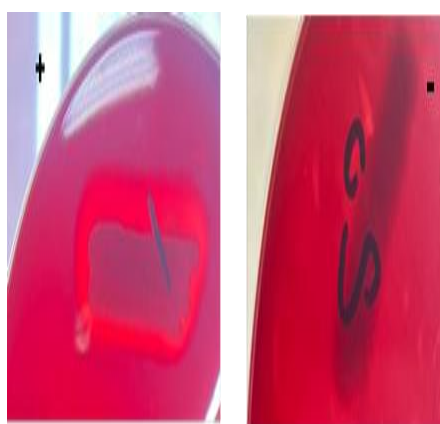


Figure 3. The ability of *Staph.aureus* to produce β -hemolysin enzyme.



Figure 4. The ability of *Staph.aureus* to biofilm produce.

Table 2. Phenotypic detection of virulence factors among *Staph. aureus* clinical isolates

Isolate No.	β -lactamase	β -hemolysin	Biofilm
1	—	—	+
2	—	—	+
3	+	—	+
4	+	—	+
5	+	—	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	—	+
10	—	—	+
11	—	+	+

The inhibitory activity of tannin extract

Bacterial resistance to drugs is increasing as a result of their intensive use in clinical medicine. Drug-resistant bacterial strains continue to be a problem for pharmacologists, despite the fact that scientists are undoubtedly always searching for and creating new medications to treat microbial diseases. Investigating natural items with antibacterial activity in their extracts is crucial. Although medicinal plants have been employed in traditional medicine and indirectly in pharmaceutical medications since ancient times, their antibacterial potential as a source of pharmaceuticals has not

been thoroughly investigated. Research on the effectiveness of plant extracts or phytochemical substances generated from plants is moving more quickly due to the resistance of harmful bacteria to medications and the custom of utilising medicinal plants to treat infectious disorders[34,35] .

The inhibitory activity of tannin extract prepared from the flowers and leaves of *Capparis spinosa* was evaluated against eight isolates of *Staph. aureus*. The flower extract showed the highest antibacterial activity, with the largest inhibition zone reaching 23 mm at a concentration of 100%, whereas the smallest inhibition zone was 13 mm at a concentration of 50% as shown in Table (3) and Figure (5). In contrast, the leaf extract showed lower inhibitory activity against the tested isolates, with the largest inhibition zone reaching 11 mm at a concentration of 100% and the smallest measuring 7 mm at a concentration of 50%, as shown in Table (4) and Figure (6). The results observed in the present study regarding the inhibitory efficacy of *C. spinosa* tannin extracts agree with what has been reported in the scientific literature; as a previous study indicated the broad inhibitory capacity of the aerial parts of *C. spinosa* against both Gram-positive and Gram-negative bacteria[36]. In addition[37] shown that *C. spinosa* and *C. decidua* have abundant antioxidant chemicals in their fruits and flowers. Where their extracts showed antimicrobial efficiency compared to some manufactured antibiotics such as amoxicillin and ciprofloxacin[37,38]. The study [38] also revealed the effectiveness of many extracts made from *C. spinosa* in inhibiting diverse bacterial species. Specifically, with an inhibitory zone of 23.7 mm, the methanolic extract of *C. spinosa* flowers demonstrated significant antibacterial activity against *Staph. aureus*. These markers corroborate the findings of the study by [39], which stated that *C. spinosa* flower extracts were effective in inhibiting both Gram-positive and Gram-negative bacteria.

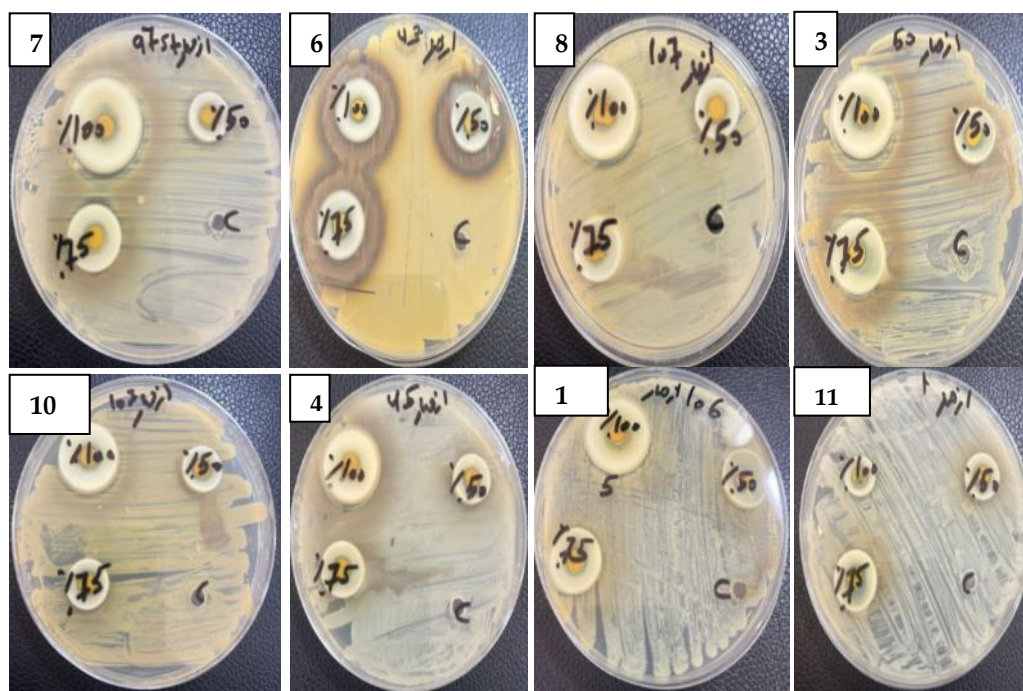
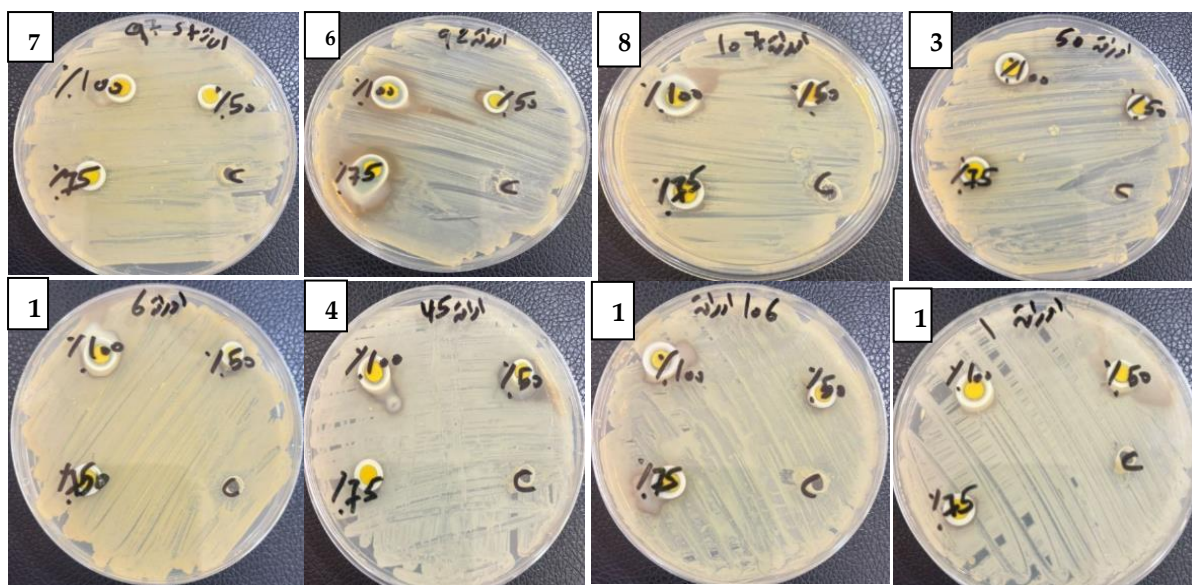
The antimicrobial mechanism of action of tannins involves multiple mechanisms: inhibition of cell wall synthesis, iron chelation, and interference with fatty acid synthesis. Tannins also inhibit quorum sensing, disruption of bacterial membranes, which disrupts virulence factors such as motility, biofilm formation, enzyme secretion, and toxin production [40,41]. Recent advances, such as tannin-loaded nanoparticles and hydrogels, have contributed to enhancing their antibacterial effectiveness and their ability to inhibit virulence [42]. Furthermore, a previous study reported researcher that tannin content and DPPH radical scavenging capacity are positively and significantly correlated [43]. Previously published data have indicated the antimicrobial activity of various extracts prepared from the caper plant (*Capparis spinosa*) against a wide spectrum of pathogenic bacteria [44,45]. Phenols and flavonoids, two active plant components, may be responsible for plant extracts' ability to inhibit bacterial growth. Thus, the high concentration of phenolic and flavonoid chemicals present in various regions of caper species may be the cause of the antibacterial action of caper plant extracts [46,47]. Researchers [48] looked into the connection between phenolic acids and plant extracts' antibacterial properties. They employed GC-MS to characterise the phenolic acids and high-performance liquid chromatography-reverse phase chromatography with UV detection to analyse their structure. They discovered that plant extracts with a greater concentration of flavonoid and phenolic chemicals have strong antibacterial properties. Antioxidants, phenolic substances, flavonoids, rutin, tocopherols, carotenoids, and vitamin C are also abundant in caper plant extracts[47,49,50].

Table 3. Inhibition zone diameters(mm) of *C. spinosa* floral tannin extract against *Staph. aureus* isolates

Isolate No.	100 mg/ml	75 mg/ml	50 mg/ml
1	22 mm	17 mm	16mm
3	23 mm	20 mm	19mm
4	22 mm	15 mm	13mm
6	21 mm	17 mm	13mm
7	23mm	16mm	12mm
8	21mm	13mm	12mm
10	19mm	19mm	13mm
11	16mm	13mm	12mm

Table 4. Inhibition zone diameters(mm) of *C. spinosa* leaves tannin extract against *Staph. aureus* isolates

Isolate No.	100 mg/ml	75 mg/ml	50 mg/ml
1	10 mm	9mm	8mm
3	10 mm	8 mm	8mm
4	9 mm	8 mm	8mm
6	11 mm	12 mm	8mm
7	9mm	7mm	7mm
8	10mm	7mm	7mm
10	9mm	8mm	7mm
11	9mm	8mm	8mm

**Figure 5.** Inhibitory activity of *C. spinosa* floral tannin extract against eight isolate of *Staph. aureus***Figure 6.** Inhibitory activity of *C. spinosa* leaves tannin extract against eight isolate of *Staph. aureus*

Conclusion

The study demonstrated elevated resistance levels among *Staph. aureus* isolates to several antibiotics, alongside their possession of multiple virulence factors. Furthermore, tannin extracts from the flowers and leaves of *C. spinosa* exhibited inhibitory activity against the studied isolates, with a clear superiority of the floral extract over the leaf extract, as the maximum inhibition zone diameter reached 23 mm at a concentration of 100%. These results indicate that *C. spinosa* represents a promising natural source of antibacterial compounds. Future investigations into these bio-active compounds may contribute to the development of alternatives or adjuvants to conventional therapy, as well as their potential exploitation in reducing overreliance on antibiotics and targeting specific bacterial virulence factors, thereby potentially mitigating the increasing risks associated with antimicrobial resistance.

Acknowledgment

I sincerely thank Dr. Rehab Salman for her contribution to the laboratory work.

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