

The phytochemical composition and antibacterial activity of the ethanol extract of the leaves *Capparis spinosa* L.

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ABSTRACT

Capparis spinosa L. (caper), a medicinal plant native to the Mediterranean region, has attracted considerable scientific interest due to its rich phytochemical composition and broad spectrum of biological activities. The present study was designed to comprehensively evaluate the *in vitro* antibacterial efficacy of ethanolic leaf extracts of *C. spinosa* against a panel of clinically significant bacterial pathogens, including *Micrococcus* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (UTI isolate), and *Staphylococcus aureus*. In parallel, the phytochemical profile of the extract was investigated, with quantitative assessment of total phenolic and flavonoid contents to elucidate their potential contribution to antimicrobial activity. Ethanolic extracts were prepared using the maceration extraction method, whereas antibacterial activity was determined by the agar well diffusion assay. Chemical characterization of the extracts was performed using high-performance liquid chromatography (HPLC). In addition, total phenolic content (TPC) and total flavonoid content (TFC) were quantified using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. The results revealed that the ethanolic leaf extract of *C. spinosa* is a rich source of bioactive phenolic constituents and possesses pronounced antibacterial activity against all tested bacterial strains. These findings underscore the therapeutic potential of *C. spinosa* as a promising natural antimicrobial agent and support its prospective application in the development of alternative strategies to combat bacterial infections and antimicrobial resistance.

Keywords: *Capparis spinosa*, ethanolic extract, antibacterial activity, total polyphenols, total flavonoids.

INTRODUCTION

Infectious diseases are a significant global health concern, accounting for 41% of the disability-adjusted life years (DALYS). The main cause of this problem is the widespread bacterial resistance to antibiotics, which poses a serious threat to global public health. To address this issue, attention is now shifted towards biologically active components isolated from plant species used as herbal medicine. These plant-based compounds have shown promising antimicrobial activity against various drug-resistant bacteria (Hemeg *et al.*, 2020).

Additionally, they offer several advantages over conventional antibiotics, such as lower toxicity and fewer

side effects. Researchers are now exploring the potential of these natural compounds to develop new and effective antimicrobial agents that can combat the growing threat of antibiotic resistance. This approach not only provides an alternative solution to the current crisis but also promotes the sustainable use of plant resources in healthcare (Gupta & Sharma, 2022).

One limitation associated with plant-derived antimicrobial agents is the variability in their efficacy, as they may not consistently achieve the therapeutic potency required to replace conventional antibiotics in the treatment of severe or systemic infections. Nevertheless, medicinal plants possess remarkable antimicrobial

potential owing to their ability to synthesize a wide range of secondary metabolites, including phenolics, flavonoids, alkaloids, and terpenoids, which act as natural defense mechanisms against pathogenic microorganisms such as bacteria, fungi, and viruses (Cowan, 1999; Allemailem, 2021). These bioactive compounds contribute significantly to the antimicrobial properties of medicinal plants and may provide promising alternatives for combating antimicrobial resistance (Silva and Fernandes Júnior, 2010).

Natural products are traditionally widely used for treatment because they are available and cheap compared to the synthetic drugs. Microorganisms include bacteria, fungi, certain algae and protozoa may be either harmless, disease producing or extremely harmful. Various bacteriological agents that are pathogenic to man and cause many infectious diseases include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus mirabilis*. Some of these organisms form normal flora of man but may become opportunistic pathogens when the host is immuno compromised or when they are coincidently transmitted to areas where they are not normally resident (Mbata, 2017).

Capparis spinosa (caper) is a Mediterranean medicinal plant whose extracts have gained considerable attention recently thanks to their rich content of bioactive phytochemicals and promising antibacterial (as well as antioxidant) properties. Recent phytochemical analyses reveal that caper extracts especially alcoholic (e.g. ethanolic or methanolic) extracts of leaves, buds or fruits contain substantial amounts of **phenolic compounds** (polyphenols), **flavonoids** (such as rutin, quercetin), **alkaloids**, and other secondary metabolites. (Nesrine *et al.*, 2024).

MATERIALS AND METHODS

Materials

Plant material

Capparis spinosa L. was collected in September 2024 from the Boudzira area located in Benghazi, northeast Libya. The plant was recognized and taxonomy in the Department of Botany, Faculty of Science, University of Benghazi, Libya.

Bacteria used

Micrococcus spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Clinical isolate obtained from the patients with urinary tract infection, Department of Microbiology, Al-Akeed Lab, Benghazi, Libya .

Media Used

Muller- Hinton agar and Nutrient ager. These media were taken from Sigma Company.

Chemicals

All the chemical in this study obtained from Biochemistry Laboratory in Benghazi University.

Reference Antibiotics Discs

Imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin (CN), ciprofloxacin (CIP), levofloxacin (LEV), azithromycin (AZM), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX) are standard antibiotics discs used as references in this study. They were bought from Bioanalyse Tibbi Malzemeler San.

Methods

Preparation of plant extract

The leaves of *Capparis spinosa* cleaned and dried at room temperature, after complete drying, the leaves grounded, in the next step, powdered leaves (250g) transferred the dark-colored flasks and mixed with 500 ml of ethanol and the mixture kept in the shaker for 48 hours. The mixture then was filtrated and the filtrate mixed again with ethanol and kept in the shaker for another 48 hours, and the mixture was filtrated again and this step repeated for third time. The ethanol collected after each time of filtration evaporated by rotatory at 40C°. The extracts stored at 4°C in dark air-tight containers .

Antioxidant activities assays

All of these experimental have been conducted in (Lab Biochemistry in Benghazi University)

PPH free radical scavenging activity (RSA)

The antioxidant activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH[•] method as modified by (Park *et al.*, 2006). The reaction mixture containing 2ml of the extract at different concentration"100,200,300,400,500µg/ml" and 2ml of DPPH[•] (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30minutes. When the DPPH[•] reacted with an antioxidant compound in the extract that can donate hydrogen, it was reduced and resulting decrease in absorbance at 517nm using UV-visible spectrophotometer, and the mean values was obtained from triplicate experiments. The percentage of the remaining DPPH[•] was plotted against the sample concentration. A lower value indicates greater antioxidant activity. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula: - %DPPH "RSA" = [Abs. of Control Abs. of Sample / Abs. of Control] x 100

Phytochemical screening of sample

The extract was analyzed for the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins,

steroids, terpenoids, carbohydrate and glycosides. The ethanol extract was screened for the presence of phytochemical classes by using the standard following methods (Table 1) (Kairupan *et al.*, 2019).

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean $\pm$ standard deviation (SD).

Statistical analyses were carried out using GraphPad Prism version 9.0 (GraphPad Software, USA). Differences among groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $P < 0.05$ was considered statistically significant.

Table(1): Protocol for phytochemical screening.

Component exit test	Protocol for test	Result for confirmation
Alkaloids	1.0ml extract + 3.0 drops of Dragendoff's reagent	Orange-red precipitate
Flavonoids	1.0ml extract + few drops of dil. NaOH	Intense yellow colour
Total phenol	1.0ml extract + 2.0ml water + few drops of 10% FeCl ₃	Blue-green colour
Tannins	100mg solvent free extract + 1ml 5% FeCl ₃	Bluish- black precipitate
Saponins	1.0ml extract + 20ml water + agitation for 15min	1cm layer of foam
Steroids	1.0ml extract + 10ml CHCl ₃ + 10ml conc. H ₂ SO ₄	Upper layer – red and lower layer – yellow-green
Terpenoids	5.0ml extract + 2.0ml CHCl ₃ + 3.0ml conc. H ₂ SO ₄	Reddish brown precipitate
Carbohydrate	2.0ml extract + 2 drops of Molish reagent (95% α -naphthol in ethanol) + 2.0ml conc. H ₂ SO ₄	Purple colour at junction
Glycosides	5.0ml extract + 2.0ml CHCl ₃ + 2.0ml CH ₃ COOH	Violet, blue to green colour

Total phenolic content (TPC)

TFC in the extract obtained from (*Capparis spinosa* leaves) was estimated using the colorimetric method based on the Folin-Ciocalteu reagent (Lee *et al.*, 2003 and Madhavi *et al.*, 1996) with some adjustments at an absorbance of 725 nm by a UV-visible spectrophotometer. Pyrogallol a natural phenolic compound used to calculate the standard curve equation and the results were expressed as mg pyrogallol equivalent /100 g of the dried plant material. The samples were carried out in triplicate.

Total flavonoids content (TFC)

The aluminum chloride colorimetric method was used for TFC determination (Mantle *et al.*, 2002 and McDonald and Prenzler 2001) the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. quercetin a natural flavonoid compound used to calculate the standard curve equation and the results were expressed as quercetin mg equivalent / 100 g of the dried plant material. The sample was measured in triplicates.

LC Chromatography / Mass Spectra MS

Thermo Scientific, Trace LC/MS Ultra & ISQ Single Quadrupole MS, DB-5 bonded-phase fused-silica capillary column was used for LC/MS analysis of the sample (ethanol extracted) in Forensic Evidence.

Preparation of Bacterial Suspension:

An overnight culture grown on nutrient agar slants was prepared for each of the bacterial strains under investigation, including both standard strains and clinical/environmental isolates obtained from the Department of Microbiology, Al-Akeed Laboratory, Benghazi, Libya. The studied bacteria comprised Gram-positive and Gram-negative organisms as follows: *Micrococcus spp.* and *Staphylococcus aureus* (Gram-positive), and *Pseudomonas aeruginosa*, *Acinetobacter spp.*, and *Klebsiella pneumoniae* (Gram-negative). The bacterial growth from each culture was aseptically washed with sterile 0.9% normal saline and adjusted to a turbidity equivalent to 1×10^8 CFU/mL, corresponding to the 0.5 McFarland standard. Nutrient agar was used

for routine cultivation and maintenance of bacterial strains, while **Mueller–Hinton agar (MHA)** was employed for antimicrobial susceptibility testing.

Antibacterial Activity Assays

Agar Well Diffusion

With the help of corn borer Wells or cups of 8mm responsible for the bitter taste. It has size were made with sterile borer into agar plates containing the bacterial inoculums. Three drills were made per plate in the pre inoculated MH-agar plates, Blood agar. The extract was added to these ditches (100µl) at all dilutions that were made in triplicate. (100µl) of ethanol (70%) were added as A negative control. Triplicate sample of each dilution was tested. After 24hrs the diameter of zone of inhibition were measured in mm and results were recorded with the help of scale were presented in the graph (**Junior and Zani, 2000**). The mean and standard deviation of the diameter of inhibition zones were calculated. Then plates were incubated at 37°C. After 24 hrs. diameter of clear zone produced surrounding the ditches were measured to the nearest mm with the help of scale and were presented in the graph (**Sana and Ifra, 2012**).

The Effects of the Commercially Used Antibiotics on Bacterial Growth

The effect of ethanol extract of leaves from *Capparis spinosa* used on the tested bacteria was compared with the sensitivity of the same bacteria to nine antibiotics Imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin (CN), ciprofloxacin (CIP), levofloxacin (LEV), azithromycin (AZM), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX). Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100 µl bacterial suspensions on plates (overnight cultures grown at 37°C on Muller Hinton agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate media ,the antibiotics discs were placed on the surface of the medium, (a distance of 2-4mm between each discs) and incubated at 37 °C for 24 hours .At the end of 24 hours, the diameters of the resulting zones of inhibition were measured and the average values were recorded.

RESULTS AND DISCUSSION

Percentage Yield of Extract

Table 2: The percentage yield of the ethanolic extract obtained from *C. spinosa* leaves was 55.76%, demonstrating the effectiveness of ethanol in extracting plant bioactive compounds

Sample	Weight of sample (gm)	Weight of extract (gm)	Yield %
leaves of <i>C.spinosa</i> ethanol extract	250g	139.4g	55.76%

The results shown in Table 2 demonstrate the extraction efficiency using ethanol. The ethanolic extract achieved a yield of 55.76%.

Qualitative and Quantitative Analysis :

Qualitative Phytochemical Analysis

The results of the phytochemical analysis (positive and negative) of the ethanol extract of *C. spinosa* leaves are shown in Table 3. The phytochemical analysis of the ethanolic extract of *C. spinosa* L. leaves revealed a wide range of secondary metabolites, including phenols, flavonoids, glycosides, carbohydrates, tannins, saponins, terpenoids, and steroids, while no alkaloids were detected. These results are partially consistent with those of **Awda et al. (2019)**, who reported the presence of polyphenols, flavonoids, and glycosides in *C. spinosa* L. leaf extracts. However, unlike our findings, their study indicated the absence of alkaloids, carbohydrates, tannins, saponins, terpenoids, and steroids in leaf extracts from Iraqi *C. spinosa* L. plants.

The results of the phytochemical analysis (positive and negative) of the extract are shown in Table 3. In contrast, **Abu-Rida and Salim (2023)** identified alkaloids, flavonoids, steroids, glycosides, carbohydrates, tannins, phenolic compounds, and saponins in methanolic extracts of *C. spinosa* L. fruit, while no terpenoids were observed. The differences between this study and that of **Abu-Rida and Salim (2023)** are likely due to variations in the plant parts analyzed (leaves versus fruit), extraction solvents and methodologies, as well as differences related to geographical origin.

Quantitative Analysis

Antioxidant Evaluation of Ethanol Extracted from *C. spinosa* Leaves

The antioxidant activities of this extracted from the *C. spinosa* leaves are evaluated by:

Total Phenolic Content (TPC)

Absorbance of standard compound (pyrogallol) at λ_{max} =760 nm in ethanol extracted from *C. spinosa* leaves. shown in table 4.

Table 3: Phytochemical Screening Tests for the Ethanol Extract Leaves of *C. spinosa*

Phytochemicals Compounds	Ethanol extract
Alkaloids	-
Flavonoids	+
Phenols	+
Tannins	+
Saponine	+
Steroids	+
Terpenoids	+
Carbohydrate	+
Glycosides	+
Fixed oils	-

Table 4: Presented the Contents of Total Phenols that Were Measured by Folin Ciocalteu Reagent (FCR) in Terms of Pyrogallol Equivalent and Total Flavonoid Content Expressed as (mg/g) Quercetin Equivalent.

Leaves of <i>C. spinosa</i>	Ethanol extract
Total phenolic content (mg pyrogallol/g sample)	22.1±0.92 mg/g
Total flavonoid content (µg quercetin /mg sample)	30.59± 2.86 µg/mg

Our study measured the total phenolic content of caper leaves (*Capparis spinosa* L.), which reached 22.1 ± 0.92 mg/g in the ethanolic extract. These results differ from those reported by **Yahya *et al.* (2020)**, who studied caper leaves collected from southern Tunisia using ultrasonic extraction and reported a lower total phenolic content of 9.72 mg/g. In contrast, **Tlili *et al.* (2017)** observed a slightly higher total phenolic content (23.37 mg/g) in methanolic extracts of Tunisian caper leaves obtained by infusion. Despite the geographical proximity of the sampling areas and previous studies, the marked variation in total phenolic content is attributed to several factors, including: (a) differences in environmental conditions affecting the biosynthesis of phenolic compounds in the plant; (b) variation in the genetic diversity of the studied *C. spinosa* strains; and (c) the effect of different extraction techniques and solvents used, such as ultrasonic extraction with ethanol in the study by **Yahya *et al.* (2020)** versus methanol extraction in the study by **Tellili *et al.* (2017)**.

Total Flavonoids Content (TFC)

The total flavonoid content for this extracts the *C. spinosa* leaves presented in Table 4 as well as absorbance of standard compound (quercetin) was shown in this table at different concentrations.

Our investigation revealed a total flavonoid content (TFC) of 30.59 µg/mg in the ethanolic extract. This value falls within the wide range reported by **Tlili *et al.* (2015)** for methanolic seed extracts of *C. spinosa* collected from different regions in Tunisia, which varied between 4.71 and 72.71 mg QE/g dry residue. However, direct comparison remains limited due to differences in the plant parts analyzed (leaves versus seeds), the solvents used for extraction (ethanol versus methanol), and the units of expression (µg quercetin equivalents per mg sample versus mg QE per g dry residue). Likewise, the TFC values reported by **Yahia *et al.* (2020)** for aqueous leaf extracts (ranging from 1.16 to 2.11 mg QE/g dry weight) and by **Rajhi *et al.* (2021)** for hydroethanolic leaf extracts and their fractions (13.546 to 107.29 mg QE/g) differ

from those obtained in the present study. These discrepancies may be attributed to variations in extraction techniques, geographical origin of the plant material, and possible genetic diversity among *C. spinosa* L. populations.

The DPPH[•] Radical Scavenging Activity

DPPH assay is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidant activity of *C. spinosa* leaves is expressed in terms of percentage of inhibition (%) and IC₅₀ values (µg/ml). Table 5 inhibition (%) of *C. spinosa* leaves and the well-known antioxidant ascorbic acid. The result of the IC₅₀ (µg/ml) value of the *C. spinosa* leaves is shown in Table (6), this result compared it to the value of for IC₅₀ (µg/ml) the ascorbic acid. IC₅₀ was calculated as amount of antioxidant present in the sample. The lower the IC₅₀ value the higher the antioxidant activity.

In the present study, the ethanolic extract of *C. spinosa* leaves collected from Libya exhibited an IC₅₀ value of 145.7 µg/mL. This value differs markedly from the IC₅₀ of 43.03 µg/mL reported by **Tlili et al. (2017)** for methanolic leaf extracts of *C. spinosa* from Tunisia. Such discrepancies may reflect the influence of geographical factors on the biosynthesis of antioxidant compounds in *C. spinosa* L., a phenomenon that has also been reported in other plant species (**Chihi et al., 2021; Suleiman &**

Ateeg, 2020; Uddin et al., 2018). In particular, differences in environmental conditions-such as soil composition, climatic factors, and altitude-between Libya and Tunisia may result in distinct phytochemical profiles. The impact of environmental factors on plant secondary metabolite production is well documented (**Mostafa et al., 2018; Saleh et al., 2020**) and may account for the observed variation in antioxidant activity. Moreover, genetic variability among *C. spinosa* L. populations from different geographical regions may further contribute to these differences, as population-specific genetic traits have been shown to influence secondary metabolite production in various plant species.

In addition, our findings also diverge from those of **Yahia et al. (2020)**, who reported a substantially lower DPPH scavenging activity in aqueous leaf extracts of *C. spinosa* L. obtained via ultrasound-assisted extraction, with IC₅₀ values ranging from 33.98 to 40.20 mg/mL. Although both studies demonstrate the antioxidant potential of aqueous extracts, the pronounced differences in IC₅₀ values warrant further investigation. Variations in DPPH assay conditions, characteristics of the plant material, and specific extraction parameters may all contribute to these inconsistencies.

Collectively, these results highlight the importance of carefully considering geographical origin, extraction methodology, and plant part when comparing antioxidant activity across different studies.

Table 5: DPPH Radical Scavenging Activity of Vitamin C and *C.spinosa* Leaves % Inhibition.

Concentration of vitamin C " µg/ml "	Percent of inhibition (%)	concentration of <i>C. spinosa</i> Leaves ethanol extract " µg/ml "	Percent of inhibition (%)
1000	91.7%	1000	90.2%
500	88.2%	500	88.1%
250	85.6%	250	84.99%
125	77%	125	76.8%
62.5	53%	62.5	54.3%
31.25	39%	31.25	40.7%

Table 6: IC₅₀ Values of Ethanol Extract from *C. spinosa* Leaves and Ascorbic Acid of DPPH Scavenging Activity.

Sample	DPPH scavenging assay IC ₅₀ " µg/ml "
Ethanol extract from <i>C. spinosa</i> Leaves	145.7
Ascorbic acid	142.6

The LC/MS of the Ethanol Extract from Leaves of *C. spinosa*

Table (7) shows the results obtained from the LC/MS for the ethanol extract from leaves of *C. spinosa* where the results showed that the extract contains 15 compounds.

Table 7: The Chemical Constituent of the Ethanol Extract from Leaves of *C. spinosa* by LC/MS. Conc. (%) Based on Peak Area Integration.

No.	Name of the Compounds	Conc. (%)
1.	Phosphoenolpyruvate	2.0
2.	Citric acid	0.4
3.	6-phosphogluconate	2.4
4.	Phosphoarginine	0.4
5.	Glycyl-glutamine	3.8
6.	3-Nitro- tyrosine	2.3
7.	Fraxetin	18.8
8.	Chorismic acid	2.7
9.	S-succinylcysteine	3.3
10.	1-(1-Hydroxybutyl)-1,3,4,5,6,7-hexahydro-2-benzofuran-4,5,6,7-tetrol	4.3
11.	Porphyrilic acid	15
12.	Dehydrovariabilin	11.8
13.	15-Acetyldeoxynivalenol	20.9
14	3-Hydroxy-6-[[<i>(E)</i> -3-hydroxy-2,4-dimethylhept-4-enoyl]amino]-2,4-dimethyl-5-oxohexanoic acid	7.9
15	Fraxidinglucoside	4.4

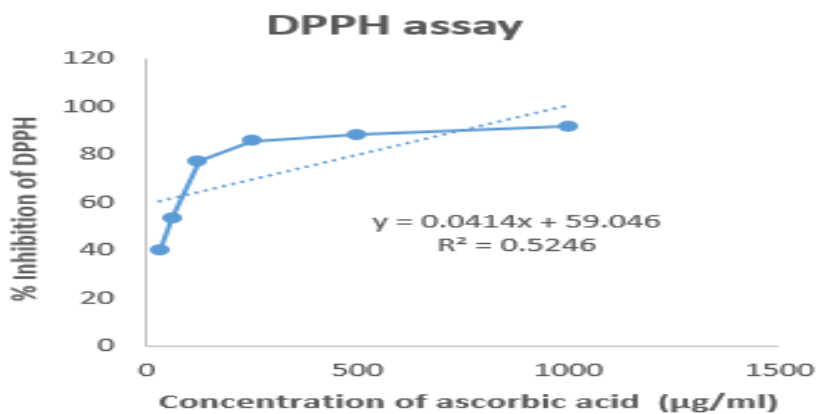


Figure 1: Percentage of Inhibition of DPPH Scavenging Activity for Ascorbic Acid.

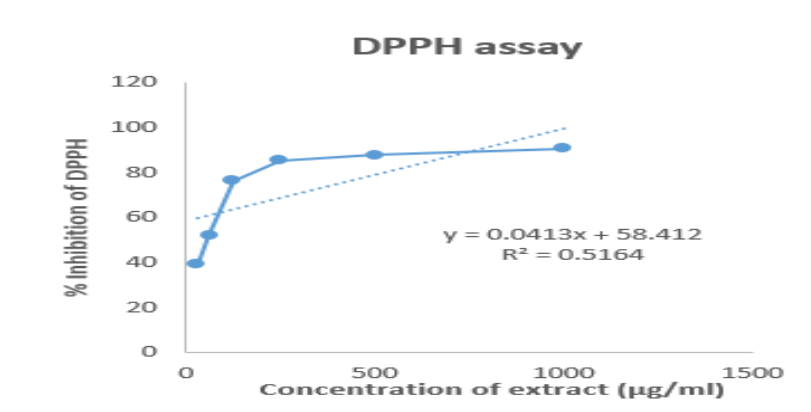


Figure 2: Percentage of Inhibition of DPPH Scavenging Activity for Ethanol Extracted from the *C. spinosa* Leaves

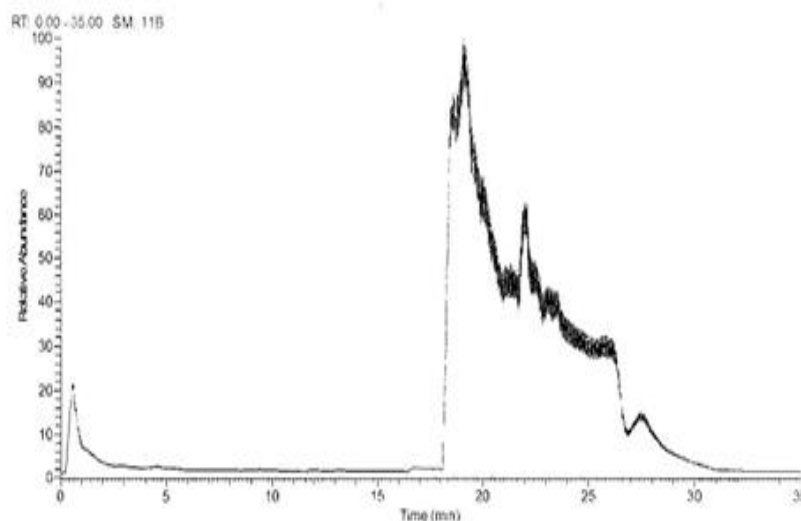


Figure3 : LC/MS of Ethanol Extract from Leaves of *C. spinosa*

Antibacterial Evaluation of Ethanolic Leaf Extract of *Capparis spinosa* L.

The antibacterial activity of the ethanolic extract of *Capparis spinosa* L. exhibited a clear concentration-dependent effect against the tested bacterial strains. At the highest concentration (100%), the extract demonstrated pronounced inhibitory activity against most isolates, with the largest zones of inhibition observed against *Acinetobacter* spp. (18 ± 4.20 mm), followed by *Pseudomonas aeruginosa* (12 ± 2.01 mm), *Klebsiella pneumoniae* (11 ± 2.77 mm), *Staphylococcus aureus* (10 ± 3.51 mm), and *Micrococcus* spp. (10 ± 2.11 mm). These findings indicate a broad-spectrum antibacterial potential of the extract when applied at elevated concentrations. In contrast, the extract at 70% concentration exhibited moderate to weak antibacterial activity, showing

measurable inhibition only against *Acinetobacter* spp. (14 ± 3.65 mm), *Pseudomonas aeruginosa* (7 ± 1.11 mm), and *Micrococcus* spp. (5 ± 1.84 mm), while *Staphylococcus aureus* and *Klebsiella pneumoniae* remained resistant. This variation in susceptibility highlights the influence of extract concentration on antibacterial efficacy and suggests that higher concentrations are required to overcome intrinsic resistance mechanisms, particularly in Gram-negative bacteria (Tili & Trabelsi, 2022).

The enhanced activity at higher concentrations may be attributed to the rich content of phenolic compounds and flavonoids, which are known to disrupt bacterial cell membranes and interfere with essential cellular processes. Phytochemical analyses of *C. spinosa* consistently document significant levels of these bioactive constituents, particularly flavonols such as quercetin and

kaempferol derivatives, which correlate with both antioxidant and antimicrobial functions (Al-Snafi, 2023). The reduced susceptibility observed among certain Gram-negative strains may be attributed to the structural complexity of their outer membrane, which limits the penetration of bioactive compounds. The antibacterial effects of the ethanolic extract of *C. spinosa* are likely associated with its rich content of phenolic compounds, flavonoids, and terpenoids, which are known to disrupt microbial cell membranes, interfere with enzyme activity, and induce oxidative stress in bacterial cells (Ben Mansour et al., 2021).

Furthermore, recent evidence suggests that ethanolic extracts of *C. spinosa* show stronger antibacterial effects than aqueous counterparts, consistent with the higher solubility of phenolic and flavonoid compounds in organic solvents, which enhances extract potency against Gram-positive and Gram-negative bacteria (Zhou et al., 2022).

The *Capparis* genus have exhibited notable anti-infective properties, including antibacterial, antifungal, antiviral, antiprotozoal, anthelmintic, and insecticidal activities. For instance, *C. decidua* has shown broad-spectrum antimicrobial activity through aqueous, chloroform, acetone, methanol, and ether extracts. Minimum inhibitory concentrations (MICs) as low as 0.028 µg/mL were observed against *Lactobacillus* spp., and isolated compounds outperformed standard antibiotics such as tetracycline and ciprofloxacin in growth inhibition assays (Upadhyay et al., 2010). Furthermore, the stem and flower extracts of *C. decidua* exhibited potent insecticidal and oviposition-deterrent activities against the pulse beetle *Bruchus chinensis*, a serious pest of stored food grains that causes significant damage to cowpea, gram, soybean, and other pulses (Upadhyay et al., 2006).

C. sepiaria was reported to have exhibited dose-dependent anthelmintic activity comparable to albendazole (Latha et al., 2009). *C. spinosa* has been extensively studied for

its anti-infective properties. Ex-tracts from various parts of the plant demonstrated antibacterial, antifungal, antiprotozoal, molluscicidal, larvicidal, and antiviral effects. Notably, flavonoids such as (Lauric acid) and (Linolenic acid) glycosides in methanol bud extracts were found to inhibit herpes simplex virus HSV- 2 replication and enhance immune responses in human peripheral blood mononuclear cells (Arena et al., 2008) Aqueous extracts suppressed dermatophytes (*Microsporum canis*, *Trichophyton violaceum*) at low concentrations (Ali et al., 1999), and chloroform extracts inhibited *Deinococcus radiophilus* growth (Boga et al., 2011).

Moreover, *C. spinosa* methanol and ethyl acetate extracts exhibited antimalarial activity against *Plasmodium falciparum* (IC₅₀ = 0.50 µg/mL) without cytotoxicity to human cells (Abdel-Sattar et al., 2010)), and seeds were shown to have anti- human immunodeficiency virus (HIV) and antifungal effects via protein constituents (Lam & Ng, 2009) Extracts have also demonstrated synergistic effects with antibiotics, enhancing their efficacy against both susceptible and resistant *E. coli* strains (Darwish & Aburjai, 2010) Additional molluscicidal effects targeting *Biomphalaria alexandrina* and interference with parasite *Leishmania* major development have been attributed to lectins and toxic proteins present in the plant (Mantawy et al., 2004).

Recent investigations have underscored the antimicrobial potential of *Capparis spinosa*, particularly against *Acinetobacter baumannii* and *Streptococcus mutans*. Leaf and flower extracts showed concentration-dependent antibacterial and anti-virulence effects, with *S. mutans* displaying greater sensitivity (MIC = 16 mg/mL) than *A. baumannii* (MIC = 32 mg/mL) (Mahdi, 2025). Ethanol extracts of *C. spinosa* (leaves, roots, fruits) collected in inhibited six *Helicobacter pylori* strains, with leaf extracts showing the strongest activity (inhibition zones: 12.0–30.7 mm at 100 mg/mL) (Al-Khafaji& Mohammed, D.Y, 2023).

Table 8: Evaluation of the antibacterial activity of the ethanolic leaf extract of *C. spinosa*. The assays were conducted in triplicate, and results are presented as mean inhibition zone diameters (mm) ± standard deviation following 24 h of incubation. R = resistant

Concentration Bacteria	Zone of inhibition (mm) ± standard deviation		
	Ethanol extract		
	100c	70c	Control
<i>Staphylococcus aureus</i>	10±3.51	R	R
<i>Micrococcus spp.</i>	10±2.11	5±1.84	R
<i>Klebsiella pneumonia</i>	11±2.77	R	R
<i>Acinetobacter spp.</i>	18±4.20	14±3.65	R
<i>Pseudomonas aeruginosa</i>	12±2.01	7±1.11	R

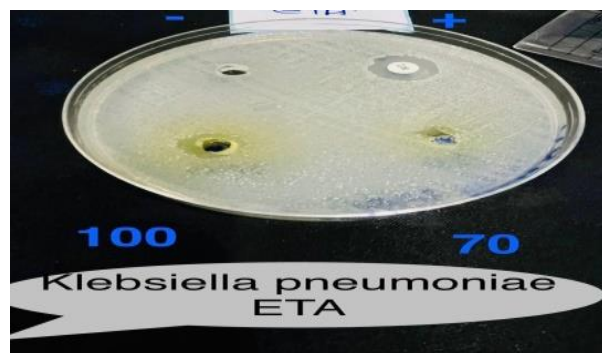


Figure (4): Antibacterial activity of Ethanol extract from leaves of *C. spinosa* against *Klebsiella pneumoniae* bacteria at 70% and 100% concentration
C=negative control (Ethanol)

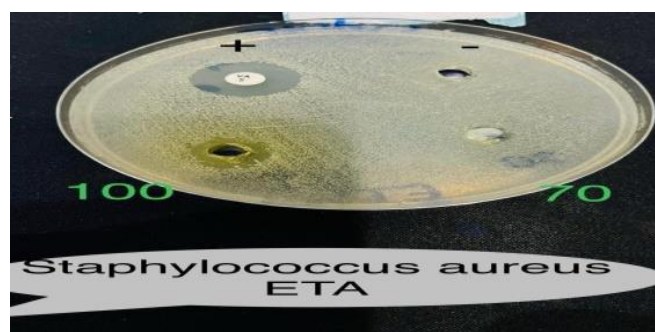


Figure (5): Antibacterial activity of Ethanol extract from leaves of *C. spinosa* against *Staphylococcus. aureus* bacteria at 70% and 100% concentration.
C=negative control (Ethano



Figure (6): Antibacterial activity of Ethanol extract from leaves of *C. spinosa* against *Acinetobacter spp* bacteria at 70% and 100% concentration.
C=negative control (Ethanol)



**Figure (7): Antibacterial activity of Ethanol extract from leaves of *C. spinosa* against *Pseudomonas aeruginosa* bacteria at 70% and 100% concentration
C=negative control (Ethanol)**

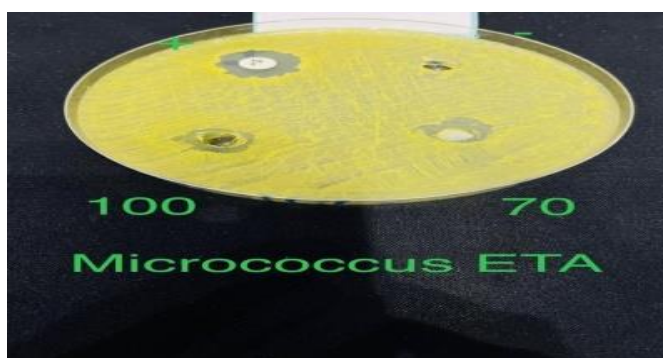


**Figure (8): Antibacterial activity of Ethanol extract from leaves of *C. spinosa* against *Micrococcus* bacteria at 70% and 100% concentration.
C=negative control (Ethanol)**

Antibiotic Susceptibility Patterns of Clinical and Environmental Bacterial Isolates.

The antibiotic susceptibility profiles of the tested bacterial isolates revealed distinct resistance and sensitivity patterns that are largely consistent with their taxonomic classification and known resistance mechanisms. *Acinetobacter spp.* demonstrated complete resistance to all tested antibiotics, including carbapenems (IPM, MEM), aminoglycosides (AK, CN), fluoroquinolones (CIP, LEV), macrolides (AZM), and β -lactams (AMC, CTX), reflecting its well-documented multidrug-resistant (MDR) phenotype commonly associated with clinical isolates and attributed to efflux pumps, carbapenemase production, and reduced membrane permeability (Antunes *et al.*, 2021).

Similarly, *Pseudomonas aeruginosa* exhibited resistance to most tested antibiotics, with sensitivity observed only to azithromycin, which aligns with its intrinsic resistance mediated by low outer membrane permeability and multiple efflux systems (Pang *et al.*, 2021). In contrast, *Klebsiella pneumoniae* showed susceptibility to carbapenems, aminoglycosides, and fluoroquinolones, while displaying resistance to β -lactam antibiotics (AMC, CTX), a pattern frequently associated with extended-

spectrum β -lactamase (ESBL) production (Wyres, & Holt, 2021).

Among Gram-positive bacteria, *Staphylococcus aureus* showed broad susceptibility to most tested antibiotics, except for resistance to azithromycin and cefotaxime, whereas *Micrococcus spp.* remained highly susceptible to all antibiotics, reflecting their limited resistance mechanisms and lower clinical pressure (Tong *et al.*, 2020 and Kandi *et al.*, 2021).

The antibiotic susceptibility profiles revealed pronounced multidrug resistance among several tested bacterial isolates, particularly Gram-negative pathogens. *Acinetobacter spp.* exhibited resistance to most tested antibiotics, including ceftazidime (CAZ), ceftriaxone (CRO), ceftiofur (FOX), trimethoprim-sulfamethoxazole (SXT), vancomycin (VA), oxacillin (OX), and piperacillin-tazobactam (TPZ), while retaining susceptibility to doxycycline (DO) and chloramphenicol (CT). This resistance pattern is characteristic of clinical *Acinetobacter* isolates and is commonly associated with efflux pump overexpression, reduced membrane permeability, and β -lactamase production (Wong *et al.*, 2022).

Similarly, *Pseudomonas aeruginosa* demonstrated complete resistance to all tested antibiotics, consistent with its intrinsic and acquired resistance mechanisms, including low outer membrane permeability and multiple multidrug efflux systems, particularly in environmental isolates (Tacconelli et al., 2022). In contrast, *Klebsiella pneumoniae* and *Escherichia coli* showed selective susceptibility to doxycycline, chloramphenicol, and piperacillin–tazobactam, while exhibiting resistance to several cephalosporins, suggesting the possible presence of extended-spectrum β -lactamase (ESBL)-producing strains (Bush& Bradford ,2020).

Among Gram-positive bacteria, *Staphylococcus aureus* displayed susceptibility to doxycycline, ceftriaxone, cefoxitin, vancomycin, and oxacillin, while resistance to ceftazidime and trimethoprim–sulfamethoxazole was observed, indicating variable resistance phenotypes. *Micrococcus* spp. remained largely susceptible to most antibiotics, reflecting their limited exposure to selective antimicrobial pressure and lower resistance potential (Pitout& DeVinney 2017). Overall, these findings highlight the escalating challenge of multidrug-resistant Gram-negative pathogens and underscore the need for alternative antimicrobial strategies.

Table (9): Antibiotic activity of different type of bacteria. R=resistance. S=Sensitive

Bacteria	SOURCE	PATHOGEN	Antibiotic								
			IP M	M E M	A K	C N	C I P	L E V	A Z M	A M C	C T X
<i>Acientobacter</i>	Clinical	Gram Negative Cocci	R	R	R	R	R	R	R	R	R
<i>Klebsiella pneumonia</i>	Clinical	Gram Negative Bacilli	S	S	S	S	S	S	R	R	R
<i>Pseudomonas aeruginosa</i>	Environm ental	Gram Negative Bacilli	R	R	R	R	R	R	S	R	R
<i>Escherichia coli</i>	Clinical	Gram Negative Bacilli	S	S	S	S	S	S	R	R	R
<i>Staphylococcus Aureu</i>	Clinical	Gram positive Cocci	S	S	S	S	S	S	R	S	R
<i>Micrococcus</i>	Clinical	Gram positive Cocci	S	S	S	S	S	S	S	S	S

Imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin (CN), ciprofloxacin (CIP), levofloxacin (LEV), azithromycin (AZM), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX).

Synergistic Antibacterial Activity of Ethanolic Leaf Extract of *Capparis spinosa* at Different Concentrations (100% and 50%,).

The agar diffusion assay revealed a synergistic interaction between the ethanolic extract of *Capparis spinosa* leaves and levofloxacin against *Micrococcus* spp. and *Staphylococcus aureus* (Figures 9 and 11). This effect was indicated by enlarged and partially overlapping inhibition zones between the antibiotic disc and extract wells at concentrations of 100 and 50 mg/mL, despite a constant separation distance of approximately 1 cm. The observed deformation and mutual extension of inhibition zones toward each other suggest an enhanced antimicrobial response beyond simple additivity. At 100 mg/mL, the inhibitory activity was more pronounced, with a clear extension toward the levofloxacin disc, implying that phytochemical constituents of *C. spinosa* may increase bacterial

susceptibility to the antibiotic. This interaction is likely mediated through multiple mechanisms, including increased membrane permeability, disruption of cell wall integrity, and inhibition of efflux pump activity, thereby promoting higher intracellular accumulation of the drug (Hemaiswarya et al., 2008). The persistence of this effect at 50 mg/mL further indicates a concentration-dependent potentiation. These results are consistent with previous reports highlighting plant-derived secondary metabolites, particularly flavonoids and alkaloids, as modulators of antibiotic resistance that can enhance the activity of conventional antimicrobial agents (Cushnie et al., 2014; Farhadi et al., 2019). In addition, *Capparis spinosa* has been widely recognized for its antimicrobial potential due to its rich phytochemical composition (Bouyahya et al., 2020). The pronounced effect against Gram-positive bacteria such as *Micrococcus* spp. may be related to the

simpler peptidoglycan architecture, which facilitates interaction with both phytochemicals and antibiotics (Silva *et al.*, 2016).

In contrast, no synergistic interaction was observed between the ethanolic extract and levofloxacin against *Acinetobacter* spp. and *Pseudomonas aeruginosa* (Figures 12 and 13). The inhibition zones around the antibiotic disc and extract wells (100% and 50%) remained clearly separated, with no evidence of merging, distortion, or zone enhancement at an inter-distance of approximately 1 cm. This pattern indicates independent

rather than cooperative antimicrobial activity. While true synergy in diffusion assays is typically reflected by zone bridging or expansion, such features were absent under the tested conditions. Although *C. spinosa* possesses documented antimicrobial constituents, its lack of potentiating effect in this case may be explained by intrinsic resistance mechanisms in Gram-negative bacteria, including reduced outer membrane permeability and active efflux systems that limit intracellular access of both antibiotics and phytochemicals (Nikaido, 2003).

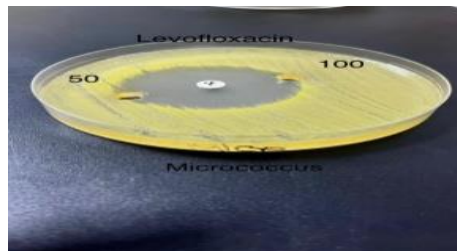


Figure (9): The Synergistic Inhibition of Ethanol Extracted at 100%, 50% from Leaves of *C. spinosa* against *Micrococcus*

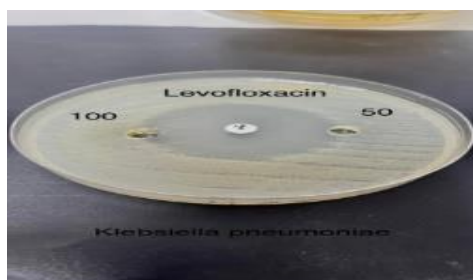


Figure (10): The Synergistic Inhibition of Ethanol Extracted at 100%, 50% from leaves of *C. spinosa* Against *Klebsiella pneumoniae*

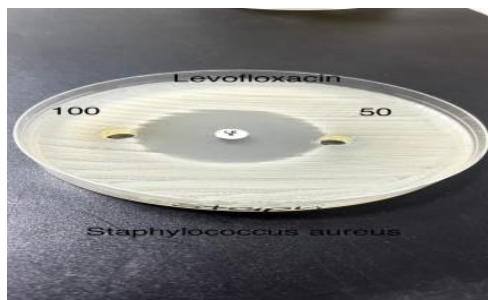


Figure (11): The Synergistic Inhibition of Ethanol Extracted at 100%, 50% from Leaves of *C. spinosa* against *Staphylococcus aureus*



Figure 12: No Synergistic Effect of the Ethanolic Leaf Extract of *C. spinosa* at 100% and 50% Concentrations Against *Acinetobacter* spp

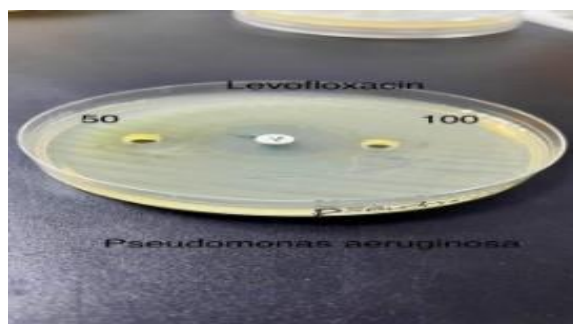


Figure (13) : No Synergistic Effect of the Ethanolic Leaf Extract of *C. spinosa* at 100% and 50% Concentrations Against *Pseudomonas aeruginosa*

CONCLUSION

In conclusion, the ethanolic leaf extract of *Capparis spinosa* exhibits potent, concentration-dependent antibacterial activity and significant synergistic effects with antimicrobial agents against both Gram-positive and Gram-negative bacteria. These findings support its potential use as a natural antimicrobial adjuvant and highlight its relevance in addressing antibiotic resistance.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

The study was conducted in accordance with institutional ethical guidelines. Clinical bacterial isolates were obtained from the Department of Microbiology, Al-Akeed Laboratory, Benghazi, Libya, following institutional regulations.

Informed Consent

Patient informed consent was waived because anonymized bacterial isolates were used and no patient-identifiable information was collected.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

TPC – Total Phenolic Content

TFC – Total Flavonoid Content

DPPH – 2,2-Diphenyl-1-picrylhydrazyl

LC/MS – Liquid Chromatography–Mass Spectrometry

MHA – Mueller–Hinton Agar

RSA – Radical Scavenging Activity

MIC – Minimum Inhibitory Concentration

MDR – Multidrug Resistant

CFU – Colony Forming Unit

HPLC – High-Performance Liquid Chromatography

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