

# Antimicrobial and antibiofilm activities of *Capparis ovata* Desf. methanolic extracts

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## ARTICLE INFO

### Article History

Received : 27.07.2024

Accepted : 18.09.2024

DOI: 10.33988/auvfd.1523402

### Keywords

Antibiofilm

Antimicrobial activity

*Capparis ovata*

Methanol extract

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**How to cite this article:** Bayezit M, Soyucok A, Yalçın H, Kart A (2025): Antimicrobial and Antibiofilm Activities of *Capparis ovata* Desf. Methanolic Extracts. Ankara Univ Vet Fak Derg, 72 (2), 155-163. DOI: 10.33988/auvfd.1523402.

## ABSTRACT

This study aimed to investigate the antibacterial, antifungal, and antibiofilm activities of methanol extract of *Capparis ovata* against a range of foodborne pathogenic bacteria as well as *Candida tropicalis*. The polyphenolic content of the extract was determined using high pressure liquid chromatography. Liquid microdilution, and microplate methods were used for the evaluation of antimicrobial and antibiofilm activities, respectively. The Minimum Inhibitory Concentration (MIC) values for *C. ovata* extract were determined as follow: 8 mg/mL against *Bacillus cereus*, 16 mg/mL against *Enterococcus faecalis*, and *C. tropicalis*, 32 mg/mL against *Staphylococcus aureus* strains, *S. epidermidis*, *Escherichia coli* 43895, *Listeria monocytogenes* strains, *L. innocua*, and 64 mg/mL against *E. coli* 35150 and *Salmonella* Typhimurium. The Minimum Bactericidal Concentration (MBC) values were 16 mg/mL (*B. cereus*), 64 mg/mL (*S. aureus* strains (except 13552), *E. faecalis*, *C. tropicalis*). For *E. coli* (43895 and 35150), *L. monocytogenes* strains, *L. innocua*, *S. Typhimurium*, *S. aureus* NCTC 13552, *S. epidermidis*, the MBC value was 128 mg/mL. The inhibition of biofilm formation by *C. ovata* extract was found at the following concentrations (in mg/mL): 8 for *S. aureus* strains (except 13552), *S. epidermidis*, *B. cereus*, and *C. tropicalis*, 16 for *L. innocua* and *E. faecalis*, 32 for *E. coli* 43895, *L. monocytogenes* strains, and *S. aureus* NCTC 13552, and 64 for *E. coli* 35150 and *S. Typhimurium*. Overall, these results indicate that *C. ovata* extract possesses potent antimicrobial and antibiofilm activities on the tested pathogenic microorganisms. Due to these properties, *C. ovata* extract can be considered as a promising plant-based antimicrobial agent for potential application in the food industry.

## Introduction

The treatment of infections that threaten public health has become quite complex due to the increasing number of bacteria developing resistance to antibiotics (55). Recently, antibiotic resistance has become a serious global health problem. The growing prevalence of infection caused by resistant microorganisms impacts human health by increasing mortality rates and hospitalizations, leading to higher economic costs (32). Yeasts can also colonize the human body and cause serious health problems ranging from simple infections to life-threatening conditions. The pathogenicity of *Candida* species is closely linked to their ability to form biofilms and adhere

to host cells (55). Today, the increased use of antifungal drugs has also led to the emergence of drug-resistant strains (34).

Microbial biofilms, particularly those formed by pathogenic bacteria (31, 41) consist of microorganisms embedded in an extracellular matrix composed of polysaccharides, proteins, nucleic acids and lipids. These biofilms adhere to both living and non-living surfaces, serving to protect the bacteria from environmental threats (16, 32, 55). It's well known that biofilms play a crucial role in the pathogenesis of various infectious diseases, posing significant challenges in treatment and severely limiting therapeutic. Biofilms are considered as a key

virulence factor, causing persistent, chronic and recurrent infections (32), and they also represent a major source of bacterial contamination in the food industry (17, 25). Of particular concern is the prevention and elimination of biofilms formed by *S. aureus* strain on surfaces (28), as these are highly resistant to antibiotics and can evade host immune response by preventing phagocytosis (54). The most common mechanism of resistance within microbial biofilms is the inability of antimicrobial agents to penetrate the biofilm barrier and reach the embedded microorganisms. In biofilm formation, resistance genes are more easily transferred from one microorganism to another (55).

Antibiotics used in the treatment of biofilm-related infections are not able to fully eliminate the biofilm, but they can reduce its presence. To effectively remove the biofilm layer, higher concentrations of chlorine-based antimicrobials are needed. For many reasons, "biofilm inhibition" is considered the main drug-targeted approach for treating a wide range of infections (32). Recently, the overuse of synthetic antimicrobials to prevent bacterial infections has posed serious threat to public health by contributing to the rise of drug resistance. In addition, various synthetic food additives commonly used in the food industry to preserve foods and extend their shelf life cause public health concerns. For this reason, there is a growing recommendation to use natural products as alternatives to conventional synthetic antimicrobials. Although new antibacterial and antifungal drugs can be developed, various new therapeutic agents specifically targeting biofilm formations, as these continue to cause significant health problems (55).

It is well established that microbial pathogens can develop resistance to many commonly used drugs. Along with such multidrug resistant pathogens, it can lead to treatment failures, increasing mortality rates, and higher healthcare costs. In addition, the problem of synthetic antimicrobials leaving residues in food is another problem. The problem of resistance and residue encourages researchers to develop natural antimicrobial products. In recent years, interest in the natural products of plants has been increasing. This situation forces researchers to develop drugs derived from natural origin. In recent years, there has been a growing interest in the plant-based natural products (30).

*Capparis* species, belonging to the *Capparis* genus within the *Capparaceae* family, are among the important perennial shrubs. There are approximately 39 genera and 650 species within the *Capparis* genus worldwide (22). These plants grow naturally in temperate (tropical/subtropical) regions across all continents, especially in Mediterranean countries (8, 34). The caper, widely used in Mediterranean cuisine (42), is generally

consumed as pickle due to its economic and nutritional benefits (34).

Different parts of the caper have been used for nutritional, cosmetic and medicinal purposes since ancient times. *C. spinosa*, *C. ovata* and *C. decidua* are among the most extensively studied species for their therapeutic and nutritional properties (22). *Capparis* species are used in traditional medicine to treat various illnesses, including rheumatic, stomach problems, headaches and toothaches, gout, joint inflammation, and anemia (33, 34). Additionally, *Capparis* species are commonly used as diuretics, appetite stimulant, vitamin C supplement in scurvy (6, 19), analgesic, and wound healer (5). Experimental studies conducted on various parts of caper species have demonstrated a wide range of biological and pharmacological properties, such as antitumoral, antioxidant, anti-inflammatory, antidiabetic, antihyperglycemic, antiatherosclerotic, antidepressant, antiallergic, antihyperlipidemic, antihypertensive, antifungal, antimicrobial, antiviral, and antihepatotoxic effects. These species are also used to enhance liver functions, treat stroke and hemophilia. It has been reported that they have a protective effect in preventing epileptic attacks and are effective in the treatment of skin and hair diseases (2, 5, 12, 20, 22, 33, 34, 40, 45).

In fact, several phytochemical studies have shown that many species from *Capparis* genus are rich in a wide range of nutritional and/or bioactive compounds. These include vitamins, lipids (such as fatty acids), terpenes, terpenoids, alkaloids (such as spermidine alkaloid), sugars, glucosinolates (such as glucocapparin), isothiocyanate glucosides, glycosides, phytosterols, and various beneficial compounds (saponins, tannins and sulfurs). They contain also some phenolic compounds with antioxidant properties (such as flavonoids, polyphenols and phenolic acids), such as tocopherols and carotenoids (such as lutein,  $\beta$ -carotene) (1, 3, 15, 21, 22, 29, 30, 34, 46, 49, 50, 52), many volatile compounds and essential oils (13). Capers are particularly rich in flavonoids such as kaempferol, quercetin, (13) and rutin (Vitamin P) (22). The most common flavonoids found in *Capparis* species are rutin (46) and quercetin (7). Studies have also revealed that capers are a rich source of many useful chemical compounds, such as campesterol, catechin, luteolin, coumarin, resveratrol, stigmaterol, caffeic acid, chlorogenic acid, ferulic acid, shergic acid, vanillic acid, aliphatic, and triterpenic alcohols (27, 47, 48, 49, 51, 52, 53). Moreover, the presence of glucocleomine distinguishes *Capparis* species from other fruits and vegetables (36). Toxicity studies have shown that the ethanolic extract of *Capparis sepiaria* leaves is not toxic at 300-5000 mg/kg in the experimental model (38). *Capparis spinosa* seed oils have been determined to have low toxicity and do not show signs of acute toxicity. Oral

and dermal exposures have been determined to have mean lethal doses of 7000 mg/kg and 3000 mg/kg, respectively (37). The results of these study indicate that this plant may have potential use in medical applications. Although there are studies investigating the polyphenolic content analysis and antimicrobial activities of various species of Capari, studies investigating the content analysis and antimicrobial activities of *Cappari ovata* are very limited. In this study, the *in vitro* antimicrobial and antibiofilm-forming activity of the methanol extract of *C. ovata* were investigated against some foodborn pathogenic microorganisms.

## Materials and Methods

**Plant Material and Extraction:** *Capparis ovata* (Marmara University, Faculty of Arts and Sciences, Herbarium no: 5091) was obtained from a local plant market (Aşçı Murat Kapari Ar-Ge Gıda Ürt. Mrk.) in Burdur Province of Türkiye in June-2023. *C. ovata* methanol extract was prepared according to the method described by Özkan *et al.* (35). According to this method, the plant samples were collected and cleaned. The aerial of the plant were dried in the shade. The dried samples were pulverized using a grinder (Arzum, Türkiye). 20 g of *C. ovata* powder was placed in a Soxhlet apparatus (Caliskan Cam Teknik) with cartridge, and extracted with 500 mL of methanol at 50 °C for 3 hours. The obtained extracts were filtered using Whatman no: 4 filter paper, and the solvent in the extract was evaporated at 40 °C using a rotary evaporator (IKA RV 10, Germany). The lyophilization was carried by using a freeze dryer (Labconco, Model: Freezone 6 plus). The obtained crude extract was then stored at -20 °C until use.

**Polyphenolic Compound Analysis:** Polyphenolic compound analysis of the extract was performed using Shimadzu Prominence Brand HPLC system (Japan) equipped with LC20 AT pump, CTO-10ASVp column oven, DAD detector (SPD-M20A), and a Zorbax C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of 3% formic acid (A) and methanol solvents (B), and the results were evaluated using the LC solution software. The content of 11 phenolic compounds, including gallic acid, vanillic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, ellagic acid, naringin, cinnamic acid, and quercetin were evaluated. For the analysis, 0.2 g of *C. ovata* extract was dissolved in 1 mL of mobile phase, filtered through a 0.45 µm membrane, and then injected into the HPLC system (9).

**Antimicrobial and Antibiofilm Activities of the Extract:** The antimicrobial activity was evaluated using the disc diffusion and microdilution methods, whereas the

antibiofilm-forming activity was assessed using the microplate method (43).

**Test Microorganisms:** Standard microorganisms were obtained from Burdur Mehmet Akif Ersoy University, Department of Food Hygiene and Technology. Various Gram-positive bacteria including *Staphylococcus aureus* ATCC 25923, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (Enterotoxin E) FRI 918, *S. aureus* NCTC 13552, *S. epidermidis* ATCC 12228, *Bacillus cereus* NRRL-B-3711, *Enterococcus faecalis* ATCC 29212, *Listeria innocua* ATCC 33090, *L. monocytogenes* RSKK 02028, and *L. monocytogenes* RSKK 472 have been used. The Gram negative bacteria used were *Escherichia coli* O157:H7 ATCC 35150, *E. coli* O157:H7 ATCC 43895, and *Salmonella* Typhimurium ATCC 14028. Additionally, the yeast fungus; *Candida tropicalis* ATCC 13803 has been included.

**Minimum Inhibitory Concentration (MIC):** 200 µL of Mueller-Hinton II Broth containing *C. ovata* extracts at various concentrations (2.00, 4.00, 8.00, 16.00, 32.00, 64.00, 128.00 and 256.00 mg/mL) was placed into each well of a 96-well plate. Subsequently, 20 µL of a microorganism suspension was added to each wells. Wells containing only Mueller-Hinton II Broth were used as controls. The plates were then incubated for 24 hours at 37 °C. After incubation, the absorbance was measured at 600 nm using a spectrophotometer (Varioskan Lux, Thermo, Finland). The experiment was performed in triplicate.

**In Vitro Disc Diffusion Test:** Each bacterial strain was cultured on Tryptic Soy Agar (TSA) (Merck) for 18 hours at 37 °C. The turbidity of the bacterial cultures was then adjusted to 0.5 McFarland standard using a densitometer (Biosan, Latvia) in 0.9% NaCl. 100 µL of the adjusted bacterial suspension was spread uniformly onto the surface of Mueller-Hinton Agar petri dishes using a sterile spreader. 10 µL of *C. ovata* extracts prepared at concentrations of 25 mg/mL and 50 mg/mL were adsorbed onto 6 mm sterile discs (Whatman™ 2017–006). The discs containing the test substances were placed on the inoculated agar plates, which were then incubated at 37 °C for 48 hours. After incubation, the diameters of the inhibition zones (mean ± SD) were measured in millimeter (mm) (43).

**Minimum Bactericidal Concentration (MBC):** After incubation, 10 µL of the suspension from each well was inoculated onto fresh TSA plates. The TSA plates were then incubated at 37 °C for 24 h. The MBC was identified as the lowest concentration of *C. ovata* extract at which no bacterial growth was observed (43).

**In Vitro Antibiofilm Activity:** For the biofilm assay, pathogenic microorganisms were cultured in TSA at 37 °C for 16 h. The cultured bacteria were collected using a sterile swab and adjusted to 0.5 McFarland standard using a densitometer (Alla, France) in 10 mL of 0.9% NaCl. Then, 20 µL of this bacterial suspension was added to 200 µL Mueller Hinton Broth containing different doses of *C. ovata* extracts, and the plates were incubated at 37 °C for 24 h. Following incubation, the microplate was emptied and washed 3 times with 200 µL phosphate-buffered saline (PBS) to remove non-adherent bacteria. Adherent bacteria were fixed by adding 200 µL of methanol for 15 min. After discarding the methanol the plates were dried at 55 °C for 1 h. Bacteria adhered to the wells were stained with 200 µL of crystal violet for 5 min. Then, the stained microplates were washed with tap water to remove excess stain and dried again. The retained dye was solubilized 200 µL of 33% acetic acid and the absorbance was measured at 590 nm (45).

## Results

Phenolic compounds of the extract and wavelengths, retention times, and LOD values for the analytes were given Table 1. Standard and sample chromatograms are shown in Figures 1 and 2, respectively. The analysis showed that *C. ovata* methanolic extract was predominated by caffeic acid (15220.493 µg/g), rutin (8431.761 µg/g), ellagic acid (7487.660 µg/g), epicatechin (5710.737 µg/g), naringin (1835.020 µg/g).

The *in vitro* antimicrobial activity of *C. ovata* methanol extract against the tested microorganisms was determined using disc diffusion and microdilution methods, along with its *in vitro* antibiofilm effects (43). The MIC values for the tested bacterial strains and the yeast strain (*C. tropicalis* ATCC 13803) are presented in Figure 3. MIC, MBC and antibiofilm activity are summarized in Table 2.

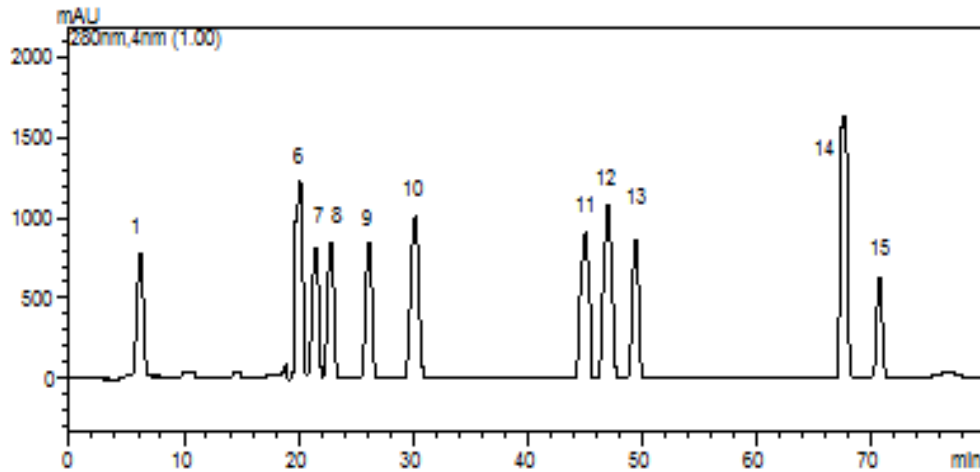
**Table 1.** Concentration of major phenolic compounds in the extract, and LOD, wavelength and retention time values for analysis.

Compounds	Concentration (µg/g)	LOD (ppm)	Wavelength (nm)	Retention time (min)
Gallic acid	91.130	0.01	280	6.8
Vanillic acid	485.940	0.11	320	19.2
Caffeic acid	15220.493	0.01	280	22.7
Epicatechin	5710.737	0.43	260	21.3
p-Coumaric acid	72.964	0.01	320	26.1
Ferulic acid	189.094	0.01	320	30.1
Rutin	8431.761	0.57	360	45.6
Ellagic acid	7487.660	0.57	240	47.7
Naringin	1835.020	0.40	280	49.7
Cinnamic acid	910.190	0.01	280	71.1
Quercetin	366.202	0.57	360	70.4

**Note:** This table focuses on major phenolic compounds, showcasing their concentrations, limits of detection (LOD), wavelength values, and retention times for a comprehensive overview. Standard and sample chromatograms are illustrated in Figures 1 and 2, respectively.

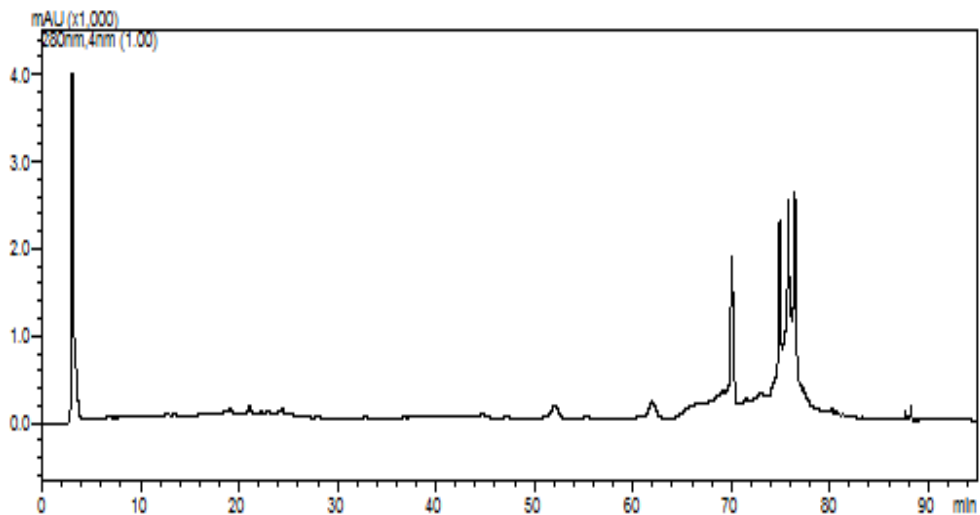
**Table 2.** Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC), and antibiofilm values of *C. ovata* methanol extract.

	MIC	Concentrations (mg/mL)	
		MBC	Antibiofilm
<i>B. cereus</i> NRRL-B-3711	8	16	8
<i>S. aureus</i> ATCC 25923	32	64	8
<i>S. aureus</i> (MRSA) ATCC 43300	32	64	8
<i>S. aureus</i> (enterotoksin E) FRI 918	32	64	8
<i>E. faecalis</i> ATCC 29212	16	64	16
<i>C. tropicalis</i> ATCC 13803	16	64	8
<i>E. coli</i> O157:H7 ATCC 43895	32	128	32
<i>E. coli</i> O157:H7 ATCC 35150	64	128	64
<i>L. monocytogenes</i> RSKK 02028	32	128	32
<i>L. monocytogenes</i> RSKK 472	32	128	32
<i>L. innocua</i> ATCC 33090	32	128	16
<i>S. Typhimurium</i> ATCC 14028	64	128	64
<i>S. aureus</i> NCTC 13552	32	128	32
<i>S. epidermidis</i> ATCC 12228	32	128	8

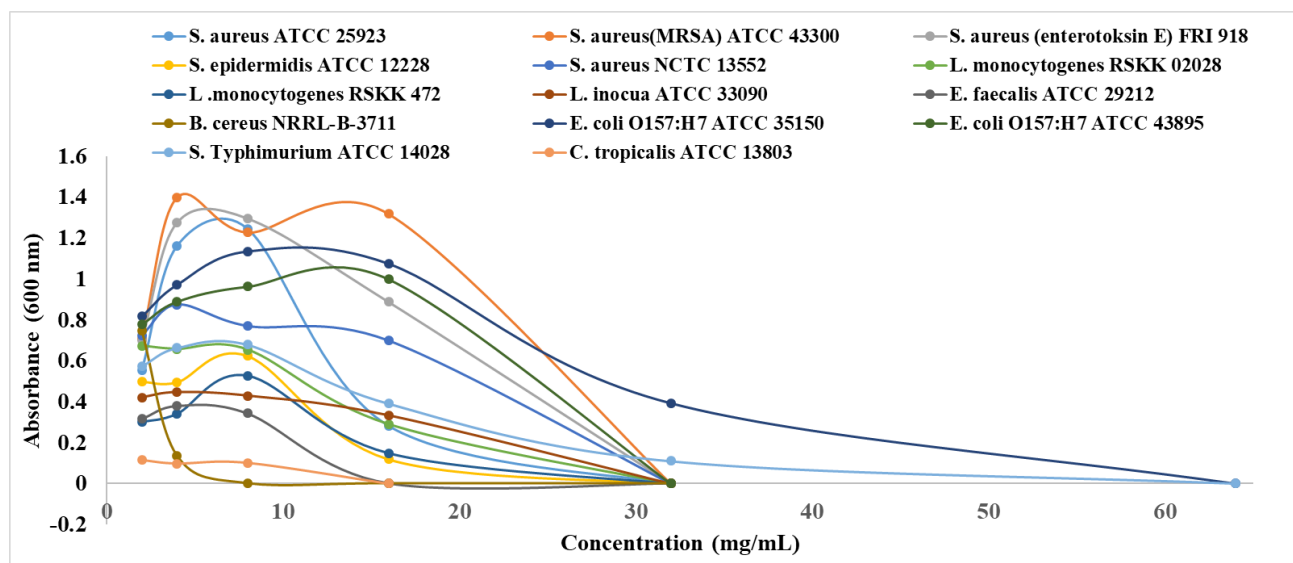


**Figure 1.** Standard Chromatogram.

Gallic acid (1), Vanillic acid (6), Caffeic acid (8), Epicatechin (7), p-Coumaric acid (9), Ferulic acid (10), Rutin (11), Ellagic acid (12), Naringin (13), Cinnamic acid (14), and Quercetin (15).



**Figure 2.** Sample Chromatogram of *C. ovata* extract.



**Figure 3.** MIC values of all tested bacteria and *C. tropicalis*.



**Table 3.** The mean  $\pm$  SD diameter of inhibition zones (mm) of the extract of *C. ovata* against the bacteria.

Bacteria	Concentration of <i>C. ovata</i> extract	
	25 mg/mL	50 mg/mL
<i>S. aureus</i> ATCC 25923	6.6 $\pm$ 0.14	7.15 $\pm$ 0.07
<i>B. cereus</i> NRRL-B-3711	7.8 $\pm$ 0.14	12.65 $\pm$ 0.21
<i>C. tropicalis</i> ATCC 13803	-	7.45 $\pm$ 0.21

The MIC values for *C. ovata* extract were determined as follows: 8 mg/mL for *B. cereus* NRRL-B-3711, 16 mg/mL for *E. faecalis* ATCC 29212, and *C. tropicalis* ATCC 13803, 32 mg/mL for *S. aureus* ATCC 25923, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (enterotoxin E) FRI 918, *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* RSKK (02028, and 472), and *L. innocua* ATCC 33090, *S. aureus* NCTC 13552, *S. epidermidis* ATCC 12228, and 64 mg/mL for *E. coli* O157:H7 ATCC 35150, and *S. Typhimurium* ATCC 14028 strains.

The MBC values were found to be 16 mg/mL for *B. cereus* NRRL-B-3711, 64 mg/mL for *S. aureus* ATCC 25923, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (Enterotoxin E) FRI 918, *E. faecalis* ATCC 29212, and *C. tropicalis* ATCC 13803, 128 mg/mL for *E. coli* O157:H7 ATCC 43895, *E. coli* O157:H7 ATCC 35150, *L. monocytogenes* RSKK (02028, and 472), *L. innocua* ATCC 33090, *S. Typhimurium* ATCC 14028, *S. aureus* NCTC 13552, and *S. epidermidis* ATCC 12228 strains.

The methanol extract of *C. ovata* inhibited biofilm formation effectively. At a concentration of 8 mg/mL, it prevented biofilms in *S. aureus* ATCC 25923, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (enterotoxin E) FRI 918, *B. cereus* NRRL-B-3711, *C. tropicalis* ATCC 13803, and *S. epidermidis* ATCC 12228. At 16 mg/mL, it was effective against *L. innocua* ATCC 33090 and *E. faecalis* ATCC 29212. A higher concentration of 32 mg/mL was needed for *S. aureus* NCTC 13552, *E. coli* O157 ATCC 43895, and both strains of *L. monocytogenes* RSKK 02028 and RSKK 472. The highest concentration tested, 64 mg/mL, was required to inhibit biofilm formation in *E. coli* O157 ATCC 35150 and *S. Typhimurium* ATCC 14028. According to our findings, biofilm formation persisted in other tested bacteria under our experimental conditions. The extract was most effective against *B. cereus* NRRL-B-3711, *S. aureus* ATCC 25923, *S. aureus* (MRSA) ATCC 43300, *S. aureus* (enterotoxin E) FRI 918, *C. tropicalis* ATCC 13803, and *S. epidermidis* ATCC 12228 strains at the lowest concentration of 8 mg/mL. However, it exhibited the least inhibitory effect on *E. coli* O157 ATCC 35150 and *S. Typhimurium* ATCC 14028 at the highest concentration of 64 mg/mL.

The disc diffusion mean values for *C. ovata* methanol extract were determined as follows: *S. aureus* ATCC 25923 for 25 mg/mL; 6.6  $\pm$  0.14 mm, for 50

mg/mL; 7.15  $\pm$  0.07 mm, *B. cereus* NRRL-B-3711 for 25 mg/mL; 7.8  $\pm$  0.14 mm, for 50 mg/mL; 12.65  $\pm$  0.21 mm, *C. tropicalis* ATCC 13803 only for 50 mg/mL; 7.45  $\pm$  0.21 mm (Table 3).

## Discussion and Conclusion

In the current study, the methanol extract of *C. ovata* was assessed for its antimicrobial and antibiofilm activities against a panel of foodborne pathogenic microorganisms. The results showed the methanolic extract of *C. ovata* demonstrated effective activities against some pathogenic bacteria, as well as against the yeast fungus *C. tropicalis*. As with various parts and extracts of the genus *Capparis* (22, 39, 49), the species *C. ovata* species is recognized for its antibacterial effects (10) and contain phenolic acids with documented antibacterial properties (39, 49). It has been suggested that the antimicrobial and antifungal potential of this medicinal plants may be due to phenolic compounds, quaternary ammonium and glycosinolates in the plant (22). Additionally, terpenes, flavonoids, tannins, alkaloids and reducing sugars have been identified as having antimicrobial activities and could contribute to their antibacterial effects (30). It has been reported that capers exhibit antimicrobial and disinfectant effects due to their long-chain alkyl groups (26). However, it is important to consider various factors that affect the chemical composition and biological activities of these plants (30). The leaves and buds of the Caper plant are exceptionally rich in phenolic compounds (26). In the current study, high amounts of caffeic acid, rutin, ellagic acid, epicatechin, and naringin were found in the extract of *C. ovata*. Studies indicate that caffeic acid, rutin, ellagic acid, and naringin have high antimicrobial activity (11, 18, 23, 44). The antimicrobial activity in the current study can be attributed to these high amounts of compounds in the extract.

In an *in vitro* study conducted by Muhaidat *et al.* (30), the antibacterial activity of *C. ovata* extract and essential oils extracted using various organic solvents (butanol, aqueous methanol, hexane) and methods was evaluated. The study found that the highest antibacterial activity among all tested fractions was against the Gram-positive *Streptococcus faecalis* (ATCC 29212). Conversely, the lowest activity was observed against the Gram-negative *S. Typhimurium* (ATCC 13311) when

using the petroleum ether fraction. In the current study, the highest antibacterial activity was found against *B. cereus* NRRL-B-3711 with MIC value of 8 mg/mL. In contrast, the lowest activity was noticed against *E. coli* O157:H7 ATCC 35150 and *S. Typhimurium* ATCC 14028 with MIC value of 64 mg/mL. The MIC values from the current study were higher than results of aqueous methanol extraction of *C. ovata* by Muhaidat *et al.* (30). The region where the plant was collected and the extraction method may have changed the content of the extract and thus caused differences in antibacterial activity.

Studies have demonstrated that various *Capparis* species possess antifungal activity against various fungal pathogens such as *Valsa valii* and *Rhizoctonia solani* (4, 24, 34). The methanolic extract of *C. ovata* was found to exhibit strong antifungal activity against *C. albicans* (ATCC 90028), *Candida parapsilosis* (ATCC22019), *Candida krusei* (ATCC 6258), *Malassezia pachydermatis* yeast strains and filamentous fungal strains *Microsporum canis*, *Microsporum gypseum*, *Microsporum nanum* and *Trichophyton mentagrophytes* (MIC values: 100-200 mg/mL) (34). In the current study, methanolic extract of *C. ovata* showed antifungal activity against *C. tropicalis* ATCC 13803 (Table 1). In our present research, the *C. ovata* extract effectively inhibited biofilm formation in all bacterial strains and the *C. tropicalis* ATCC 13803 strain tested, as detailed in Table 1. This study confirmed the antifungal activity of the methanolic extract of *C. ovate* against *Candida* species.

To date, there are no studies specifically addressing the antibiofilm activity of the *C. ovata* species. Reports on other *Capparis* species demonstrating such activity are also scarce. Notably, El-Subeyhi *et al.* (14) discovered that potassium oxide nanoparticles (K<sub>2</sub>O NPs), employed as reducing and capping agents in *C. spinosa* flower extract, exhibited significant antibiofilm activity. In the current study, *C. ovari* methanolic extracts exhibited anti-biofilm activities against all tested bacteria.

In conclusion, this study demonstrate that the methanolic extract of *C. ovata* holds potential as a natural antimicrobial agent for therapeutic formulations aimed at controlling the tested microorganisms and treating associated diseases. The bacteria investigated in this study are predominantly associated with food-derived products, suggesting that *C. ovata* could serve as a plant-based alternative to chemical preservatives for food preservation and safety. Furthermore, it may offer a viable option for biocontrol strategies and maintaining biofilm-free systems. However, additional studies are necessary to further confirm the role of this plant in herbal medicine and to explore its potential for discovering new natural bioactive compounds.

## Financial Support

This research received no grant from any funding agency/sector.

## Ethical Statement

This study does not present any ethical concerns.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Author Contributions

MB, AS, HY and AK conceived and planned the experiments. MB, AK and AS contributed to extraction. MB and AK conducted the extract content analyses. HY and AS conducted antimicrobial activity studies. MB made the first draft of the manuscript. MB, AS, HY and AK contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis and manuscript.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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