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MICROBIOLOGY

Antimicrobial, Antibiofilm, Antiquorum Sensing, and Antioxidant Activities of Some Medicinal Edible Thorny Plants

ÖZGÜR CEYLAN, AYSEL UĞUR, NURDAN SARAÇ, BÜŞRA E. ARSLAN & MEHTAP D. ŞAHIN

Abstract: The present study evaluated the antimicrobial, anti-quorum-sensing, antibiofilm, and antioxidant activities of *Silybum marianum*, *Scolymus hispanicus*, *Centaurea urvillei* subsp. *stepposa*, *Cirsium arvense* subsp. *vestitum* and *Onopordum illyricum*. Antimicrobial activity was tested by disc diffusion and microdilution methods, while quorum-sensing inhibition was assessed through violacein inhibition, swarming motility, and anti-quorum-sensing assays. Antioxidant potential was determined by DPPH and FTC methods. *S. hispanicus* and *O. illyricum* showed the highest inhibition zones (17 mm) against *Staphylococcus aureus* MU 47, and *O. illyricum* had the lowest MIC (5 mg/mL) against *Bacillus subtilis* ATCC 6633. The highest antibiofilm effect was recorded for *S. marianum* (41.83%) against *Escherichia coli* ATCC 25922. *S. marianum* showed 31.19% violacein inhibition, and *C. urvillei* subsp. *stepposa* inhibited swarming motility by 42.9%. *O. illyricum* extracts displayed the strongest antioxidant activity (IC_{50} = 5.76 mg/mL for DPPH; 67.79% inhibition by FTC). These findings suggest the studied plants hold promise as natural antimicrobial and antioxidant agents.

Key words: Antimicrobial, Antibiofilm, Anti-quorum Sensing, Antioxidant, Edible Thorny Plants.

INTRODUCTION

Antibiotics are a class of drugs known for their effective bactericidal and bacteriostatic properties. They are widely used not only in human medicine but also in treating poultry, livestock, fish, and other aquaculture species through infusion therapy. The extensive and continuous use of these antimicrobial drugs, combined with the large amounts of fecal waste from humans and animals, has increased the concentration of residual antibiotics in various environments, particularly water bodies. This accumulation of antibiotic residues has contributed significantly to the development of antibiotic resistance in bacteria. The emergence and spread of drug-resistant bacteria pose a major threat to public health, as they can render standard treatments ineffective, leading to longer illnesses, higher medical costs, and increased mortality. Addressing this problem may allow antibiotics to be administered more effectively and the resistance developed by bacteria to remain at more reasonable levels. The discovery of new natural agents, especially those with antimicrobial potential, may be of great benefit in overcoming this problem. Also, distinct medicinal plant treatments may be an option to help resolve multidrug-resistant bacterial infections (Ben et al. 2019, Zhou et al. 2022).

Plants are a promising option for discovering new antimicrobial agents due to their use in traditional medicine to treat bacterial diseases and their potential to provide an endless variety of

chemical compounds for research. These plants have great potential to develop effective solutions against existing drug resistance and to create safer and more effective treatment methods. Bioactive compounds of plant origin attract attention with their broad-spectrum antimicrobial properties and are evaluated in scientific studies. Various plant extracts are widely used, especially in pharmaceutical, food, chemical, and other industries because of their safety and fewer side effects (Anand et al. 2019, Odongo et al. 2023, Yahya et al. 2018). Among these, the Asteraceae family, comprising over 25,000 species worldwide, is particularly noteworthy (Rolnik & Olas 2021). Edible and medicinally important members such as *Silybum marianum*, *Scolymus hispanicus*, *Centaurea urvillei* subsp. *stepposa*, *Cirsium arvense* subsp. *vestitum*, and *Onopordum illyricum* are traditionally used in the Mugla region for various ailments (Wang et al. 2020, Kandil et al. 2020, Cekic et al. 2021, Balkrishna et al. 2024, Caddeo et al. 2023).

Mechanisms such as biofilm formation and quorum sensing enhance bacterial survival and persistence, facilitating the development and maintenance of antimicrobial resistance and consequently diminishing the efficacy of current therapeutic strategies. To the best of our knowledge, although some studies have reported the antimicrobial and antioxidant activities of these species, their antibiofilm and quorum-sensing inhibitory activities, including the inhibition of violacein production and swarming motility, have not yet been investigated. Given the important role of these mechanisms in bacterial persistence and multidrug resistance, identifying natural compounds capable of targeting these processes is of considerable importance. Therefore, this study aims to investigate the in vitro antimicrobial, antioxidant, antibiofilm, and quorum-sensing inhibitory activities of ethanol and aqueous extracts of *Silybum marianum*, *Scolymus hispanicus*, *Centaurea urvillei* subsp. *stepposa*, *Cirsium arvense* subsp. *vestitum*, and *Onopordum illyricum*, which naturally grow in the Mugla region and are consumed as food, against clinically relevant microorganisms associated with treatment challenges.

MATERIALS AND METHODS

Plant Extracts

The five plants belonging to the Asteraceae family used in the study were collected by the first and fifth authors from the Mugla region in southwestern Türkiye during the spring-summer period, and were identified by the fifth author. These plants and herbarium numbers: *Silybum marianum* L. Gaertner (1216), *Cirsium arvense* (L.) Scop. subsp. *vestitum* (1717), *Centaurea urvillei* DC. subsp. *stepposa* WAGENITZ (1718), *Onopordum illyricum* L. (1719), *Scolymus hispanicus* L. (1720). These are kept in the Usak University/ Faculty of Education Laboratory Herbarium. The aerial parts of the plants were dried in air and turned into powder. Ethanol extracts were obtained using the Soxhlet apparatus, and aqueous extracts were lyophilized in the Freeze Dryer. All plant extracts were stored in sterile opaque vials in the refrigerator until use.

Microorganisms

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* NRLL B-4375, *Candida albicans* ATCC 10239, and multiresistant *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and several of *Staphylococcus*

strains, *Chromobacterium violaceum* CV12472, *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PA01 were used in the present study.

Assessing Antimicrobial Activity

The disc diffusion assay

Bacterial density was adjusted to 0.5 McFarland (Bauer et al. 1966, Collins et al. 1995, Murray et al. 1995). From here, 100 µL of each was spread onto plates containing Mueller-Hinton agar. Plant extracts were poured into blank discs in 20 µL volumes. After the discs dried, they were placed on plates. Petri dishes were incubated at 30 °C or 37 °C for 24 h according to the growth conditions of the microorganisms. The zone of inhibition was recorded after incubation. Discs embedded with solvents were assumed as the control. Disks containing penicillin G (10 U, Oxoid), chloramphenicol (30 µg, Oxoid), and nystatin (100 U, Oxoid) were used as standard antibiotics.

Minimum inhibitory concentrations (MIC)

MICs were determined by the microdilution method (CLSI 2006). The density of microorganisms in Mueller-Hinton Broth medium was 5×10^5 colony units (CFU) /mL. Extracts at different concentrations (20, 10, 5, 2.5, 1.25 mg/mL, ...) were put into the wells of a 96-well microtiter plate containing the test microorganisms. Wells without extracts were considered controls. Then, the microplates were incubated under appropriate conditions. Afterwards, reading was done on the reader. The MIC was taken as the minimum concentration at which no microbial growth was detected.

Determination of Antibiofilm Activity

Biofilm inhibition was evaluated as described by Merritt et al. (2011). The antibiofilm activities of plant extracts were tested in microtiter plates at MIC and MIC/2 values. Briefly, 1% of overnight cultures of test pathogens were added to 200 µL of TSB medium. The plates were incubated at 37°C for 48 hours in the presence and absence of extract. Wells containing only TSB were assumed to be controls. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining biofilms were stained with 0.1% crystal violet solution for 10 min at room temperature. The wells were washed again to remove the crystal violet dye. For Gram-negative bacteria and yeast, 200 µL of 95% ethanol was added to each well, whereas 200 µL of 33% glacial acetic acid was added to the wells containing Gram-positive bacteria. After waiting for 30 minutes, 125 µL of solution from each well was transferred to sterile tubes and the volume was adjusted to 1 mL with distilled water. Afterwards, % inhibition was calculated by measuring at 550 nm.

The percentage of inhibition of the tested extracts was calculated using the following formula:

$$\text{Biofilm inhibition (\%)} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Violacein Inhibition Assay

The violacein inhibitory potential of test plants against *C. violaceum* CV12472 was performed as reported in our previous study (Tamfu et al. 2020). Firstly, the MIC values of the extracts were determined at concentrations of 25, 12.5, 6.25, 3.12 mg/mL, ... In sterile microtiter plates containing LB medium, a

total volume of 200 µL was prepared by adding 10 µL of the overnight culture of *C. violaceum* CV12472 (adjusted to OD_{600} = 0.4) and 20 µL of plant extracts. Plates were incubated at 30° C for 24 h at MIC and sub-MIC concentrations of test extracts. The inhibition of violet pigment produced by bacteria was examined. LB broth containing only *C. violaceum* CV12472 was used as a positive control. Absorbance was read at 585 nm.

The percentage of violacein inhibition was calculated by the following formula:

$$\text{Violacein inhibition } (\%) = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Quorum Sensing Inhibition (QSI) Assay

Quorum sensing inhibition was carried out, as in our previous study (Tamfu et al. 2020), using a slight modification of the method described by Koh & Tham (2011). MIC values were determined at concentrations of 100, 50, 25, 12.5 mg/mL, ... for aqueous extracts and in the range of 20, 10, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL for ethanol extracts. MIC and subMIC concentrations of plant extracts were tested against the *C. violaceum* CV026 strain. 5 mL of warm molten Soft Top Agar (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionized water) was seeded with 100 µL of an overnight *C. violaceum* CV026 culture, and 20 µL of 100 µg/mL C_6HSL was added as exogenous AHL source. The mixture was gently homogenized and immediately poured as an overlay onto the surface of a solidified LBA plate. After the overlay had solidified, wells with a diameter of 5 mm were punched into each plate. Each well was filled with 50 µL of filter-sterilized plant extract at MIC and sub-MIC concentrations. The plates were incubated in an upright position at 30° C for 3 days. After incubation, the diameters of the quorum sensing inhibition zones were measured in millimeters. Each experiment was performed in triplicate. The formation of a white or cream-colored halo around the well against the purple lawn of activated C. violaceum CV026 was considered indicative of quorum sensing inhibition.

Swarming Motility Inhibition Assay

Inhibition of swarming motility was performed as previously described by Packiavathy et al. (2012). Overnight cultures of *P. aeruginosa* PA01 strain were inoculated pointwise into the center of plates containing medium and plant extracts (100 mg/mL) containing 1% peptone, 0.5% NaCl, 0.5% agar, and 0.5% D-Glucose. The plate without extract was served as a control. Plates were incubated at an appropriate temperature in an upright position for 18 h. The reduction in swarming of bacterial cells in plates with extracts was recorded and used to deduce the percentage swarming migration inhibition compared to control plates.

Determination of Antioxidant Activity

DPPH radical scavenging assay

The antioxidant activity of the extracts was determined based on their ability to react with the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical (Yamasaki et al. 1994). 50 µL of the extract (1.25, 2.5, 5, and 10 mg/mL in ethanol/water (1:1, v/v)) was added to 5 mL DPPH solution (0.004%) in ethanol. After incubation at room temperature for 30 min, the absorbance of each solution was measured at

517 nm. Percentage of inhibition and the concentration of sample required for 50% scavenging of the DPPH free radical (IC_{50}) were determined. BHT and ascorbic acid were used as a positive control.

Ferric thiocyanate (FTC) method

A screw-cap vial containing a mixture of 4 mg of sample in 4mL of 99.5% ethanol, 4.1 mL of 2.51% linoleic acid in 99.5% ethanol, 8.0 mL of 0.02 M phosphate buffer (ph 7.0) and 3.9 mL of water (final concentration 0.02%, w/v) was placed in an oven at 40°C in the dark (Kikuzaki & Nakatani 1993). To 0.1 mL of this mixture in a test tube, 9.7 mL of 75% (v/v) ethanol, 0.1 mL 30% ammonium thiocyanate and finally, 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. Three minutes after the addition of ferrous chloride, the absorbance was measured at 500 nm. This step was repeated every 24 h until the control reached its maximum absorbance value. The results were expressed as percentage inhibition.

RESULTS

Antimicrobial activity

The disc diffusion assay

The disc diffusion method was used to determine the antimicrobial activities of plant extracts and given in Table I. Although the study showed that both aqueous and ethanol extracts of five plants exhibited no inhibition zone diameter against *E. coli* ATCC 25922, *P. aeruginosa* MU 187, *P. aeruginosa* MU 188, and *C. albicans* ATCC 10239, they were antimicrobial at different ranges of values against other test microorganisms. Among aqueous extracts of plants, *C. urvillei* subsp. *stepposa* performed the highest value with a zone diameter of 15 mm against *S. aureus* MU 40, for the ethanol extracts, *S. hispanicus* and *O. illyricum* exhibited the highest value with a zone diameter of 17 mm against *S. aureus* MU 47.

Minimum inhibitory concentration (MIC)

Another antimicrobial analysis of the extracts was performed by the microdilution method. In Table I, MIC values of plant extracts varied from 5 mg/mL to 20 mg/mL. The aqueous extracts (except *S. marianum*) inhibited *S. aureus* ATCC 25923, *S. aureus* MU 40, *S. aureus* MU 46, and *P. aeruginosa* MU 189 at 20 mg/mL concentration. The most sensitive microorganism to the 5 mg/mL concentration of *O. illyricum* ethanol extract, which is the most effective of alcohol extracts, was found to be *B. subtilis* ATCC 6633.

Antibiofilm Activity

The antibiofilm activity of the extracts was evaluated at MIC and MIC/2 concentrations against the test microorganisms and the results are presented in the Table II. The highest antibiofilm activity was observed with the aqueous extract of *S. marianum* against *E. coli* ATCC 25922, displaying an inhibition rate of 41.83% at MIC. Among the ethanol extracts, the highest biofilm inhibition was exhibited by the ethanol extract of *C. urvillei* subsp. *stepposa*, revealing a 26.27% effect against *P. aeruginosa* ATCC 27853.

Table I. The disc diffusion zone diameters (mm) and minimum inhibitory concentration values (mg/mL) of plant extracts.

Microorganisms	Extracts	*S. marianum*		*S. hispanicus*		*C. urvillei* subsp. *stepposa*		*C. arvense* subsp. *vestitum*		*O. illyricum*		P	C	N
		DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	DD	DD
B. subtilis ATCC 6633	Aqueous	-	-	-	-	-	-	-	-	-	-	NT	25	NT
	Ethanol	-	10	9	20	9	10	9	10	10	5			
M. luteus NRRL B-4375	Aqueous	-	-	-	-	-	-	-	-	-	-	NT	22	NT
	Ethanol	-	20	-	>20	-	10	-	20	8	20			
S. aureus ATCC 25923	Aqueous	7	-	8	20	-	-	-	-	7	-	31	NT	NT
	Ethanol	-	20	11	20	-	10	-	20	11	20			
S. aureus MU 38	Aqueous	-	-	-	-	-	-	9	-	-	-	28	NT	NT
	Ethanol	-	10	-	-	-	10	-	10	8	20			
S. aureus MU 40	Aqueous	-	-	11	-	15	20	7	-	7	-	30	NT	NT
	Ethanol	7	20	10	20	-	10	-	10	10	20			
S. aureus MU 46	Aqueous	-	-	11	-	14	20	-	-	-	-	31	NT	NT
	Ethanol	11	20	13	20	-	10	-	10	9	10			
S. aureus MU 47	Aqueous	9	-	7	-	10	-	11	-	11	-	29	NT	NT
	Ethanol	13	-	17	-	-	20	-	20	17	-			
S. epidermidis MU 30	Aqueous	-	-	-	-	-	-	9	-	8	-	NT	15	NT
	Ethanol	8	-	13	-	-	20	-	10	-	20			
E. coli ATCC 25922	Aqueous	-	-	-	-	-	-	-	-	-	-	19	NT	NT
	Ethanol	-	-	-	-	-	-	-	-	-	-			
P. aeruginosa ATCC 27853	Aqueous	8	-	8	-	8	-	8	-	-	-	NT	15	NT
	Ethanol	-	-	-	-	-	20	-	-	8	-			
P. aeruginosa MU 187	Aqueous	-	-	-	-	-	-	-	-	-	-	NT	15	NT
	Ethanol	-	-	-	-	-	20	-	-	-	-			
P. aeruginosa MU 188	Aqueous	-	-	-	-	-	-	-	-	-	-	NT	16	NT
	Ethanol	-	20	-	20	-	-	-	-	-	-			
P. aeruginosa MU 189	Aqueous	-	-	-	-	-	20	7	-	-	20	NT	15	NT
	Ethanol	-	20	-	20	-	20	-	-	-	20			
P. fluorescens MU 180	Aqueous	-	-	7	-	7	-	-	-	-	-	NT	18	NT
	Ethanol	-		-	-	-	-	-	-	-	-			
P. fluorescens MU 181	Aqueous	-		-	-	-	-	-	-	-	-	NT	18	NT
	Ethanol	-	20	-	-	-	10	-	-	8	20			
C. albicans ATCC 10239	Aqueous	-	-	-	-	-	-	-	-	-	-	NT	NT	19
	Ethanol	-	-	-	20	-	20	-	-	-	-			

DD: Disc diffusion, MIC: Minimum inhibitory concentration, P: Penicillin-G, C: Chloramphenicol, N: Nystatin, -: No inhibition, NT: Not tested.

Table II. Antibiofilm activity of plant extracts.

Microorganisms	Extracts	*S. marianum*		*S. hispanicus*		*C. urvillei* subsp. *stepposa*		*C. arvense* subsp. *vestitum*		*O. illyricum*	
		% Inhibition									
		MIC	MIC/2	MIC	MIC/2	MIC	MIC/2	MIC	MIC/2	MIC	MIC/2
B. subtilis ATCC 6633	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	21.90	-	-	-	20.00	-	-	-	23.90	7.46
M. luteus NRRL B-4375	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
S. aureus ATCC 25923	Aqueous	-	-	9.04	4.73	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
S. aureus MU 38	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	24.08	-	7.59	-	-	23.03	18.06	1.30
S. aureus MU 40	Aqueous	-	-	-	-	26.13	12.76	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
S. aureus MU 46	Aqueous	-	-	-	-	8.44	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
S. aureus MU 47	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
S. epidermidis MU 30	Aqueous	-	-	-	-	29.33	7.10	7.07	-	-	-
	Ethanol	12.16	-	10.81	-	-	-	-	-	19.41	11.65
E. coli ATCC 25922	Aqueous	41.83	19.52	22.50	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
P. aeruginosa ATCC 27853	Aqueous	38.07	8.50	-	-	13.17	-	33.81	24.90	12.00	-
	Ethanol	-	-	-	-	26.27	13.82	5.99	-	-	-
P. aeruginosa MU 187	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	21.12	9.48	-	-	-	-
P. aeruginosa MU 188	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
P. aeruginosa MU 189	Aqueous	-	-	-	-	31.28	10.09	-	-	14.36	-
	Ethanol	11.72	-	18.79	-	-	-	-	-	6.89	-
P. fluorescens MU 180	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-
P. fluorescens MU 181	Aqueous	-	-	-	-	24.22	16.06	12.70	-	15.20	8.19
	Ethanol	-	-	-	-	-	-	-	-	12.36	-
C. albicans ATCC 10239	Aqueous	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	16.95	-	2.73	-	-	-	19.67	10.93

-: No inhibition.

Violacein Inhibition

The capacity of extracts to inhibit the violacein pigment produced by *C. violaceum* CV12472 was measured at MIC and MIC/2 values. Before calculating violacein inhibition, the MIC values of *C. violaceum* CV12472 were detected (Table III). MIC values of all plant extracts against CV12472 strain ranged between 2.5 and >25 mg/mL. Plant extracts displayed different percentages of inhibition at different concentrations. The ethanol extract of *S. marianum* exhibited the highest violacein production inhibition with a value of 31.19% at MIC.

Quorum Sensing Inhibition

Firstly, the sub-MIC values of the *C. violaceum* CV026 strain were selected for anti-quorum sensing. Then, the diameters of the cream or semi-transparent zones were measured in millimetres using the agar well diffusion method. The quorum sensing inhibition of the extracts is presented in Table IV. Among the test plants, both extracts of *O. illyricum* demonstrated inhibition of quorum sensing. The aqueous extract revealed a zone diameter of 13 and 12 mm at 25 mg/mL and 12.5 mg/mL concentration, respectively. The ethanol extract had a zone inhibition of 11 mm at 0.4 mg/mL. Moreover, all extracts of *S. hispanicus* and aqueous extract of *C. urvillei* subsp. *stepposa*, *C. arvense* subsp. *vestitum* and *O. illyricum* showed antimicrobial activity against the CV026 strain.

Swarming Motility Inhibition

Swarming motility is a key virulence factor in flagellated bacteria like *P. aeruginosa* PA01. This ability allows bacteria to migrate across surfaces, which is crucial for establishing long-term contamination. The inhibition of swarming was assessed using plant extracts on the *P. aeruginosa* PA01 strain. For swarming inhibition of strain PA01 applied 100 mg/mL concentration of all extracts. Swarming

Table III. Inhibition of violacein production in *C. violaceum* CV12472 by extracts.

Plants	Extracts	Violacein inhibition (%)		
		CV12472 MIC (mg/mL)	MIC	MIC/2
S. marianum	Aqueous	10	10.2	9.40
	Ethanol	25	31.19	25.58
S. hispanicus	Aqueous	2.5	10.50	-
	Ethanol	12.5	15.76	-
C. urvillei subsp. *stepposa*	Aqueous	>20	-	-
	Ethanol	>25	-	-
C. arvense subsp. *vestitum*	Aqueous	5	9.4	5.43
	Ethanol	25	10.32	-
O. illyricum	Aqueous	20	7.1	-
	Ethanol	25	-	-

-: No inhibition.

inhibition percentages are provided in Table V. The alcohol extract of *C. urvillei* subsp. *stepposa* was the highest extract that inhibited the swarming motility of PA01 by 42.9%. Moreover, when the aqueous extracts were compared, the most noteworthy effect was seen in this plant.

Antioxidant Activity

DPPH radical scavenging activity

In the study, one of the methods used for antioxidant activity is the DPPH free radical scavenging assay. Table VI shows that alcohol extracts of five plants had a better effect on DPPH free radical than aqueous extracts (except *C. arvense* subsp. *vestitum*). In particular, the ethanol extract of *O. illyricum* exhibited better radical scavenging activity with an IC_{50} value of 5.76±0.16 mg/mL. However, the antioxidative capacity of all extracts was weaker than the standard substances. BHT and α-tocopherol had low IC_{50} values of 0.487 mg/mL and 1.75 mg/mL, respectively.

Ferric thiocyanate (FTC) method

Another assay used for antioxidant activity is the FTC method, in which the amount of peroxide in the initial stages of lipid peroxidation is measured. FTC assay results indicated that the aqueous extracts had antioxidant potential in inhibiting lipid peroxidation chain reactions (Table VI). Especially, *O. illyricum* aqueous extract exhibited the highest inhibition with 67.79%. It can also be said that the ethanol extract of this plant has better inhibition ability than other ethanol extracts.

Table IV. Quorum sensing inhibition zones in *C. violaceum* CV026 by extracts.

Plants	Extracts	CV026 MIC (mg/mL)	Antimicrobial		Anti-QS	
			Concentration (mg/mL)	Inhibition zone (mm)	Concentration (mg/mL)	Inhibition zone (mm)
S. marianum	Aqueous	100	-	-	-	-
	Ethanol	20	-	-	-	-
S. hispanicus	Aqueous	>100	100	11	-	-
	Ethanol	2	1	11	-	-
C. urvillei subsp. *stepposa*	Aqueous	>100	100	9	-	-
	Ethanol	0.8	-	-	-	-
C. arvense subsp. *vestitum*	Aqueous	100	100	14	-	-
	Ethanol	2	-	-	-	-
O. illyricum	Aqueous	100	100	13	25	13
			50	11	12.5	12
	Ethanol	0.6	-	-	0.4	11

-: No inhibition.

Table V. Swarming motility inhibition on *P. aeruginosa* PA01 by extracts.

Plants	Extracts	Swarming Inhibition (%)
		100 mg/mL
S. marianum	Aqueous	9.5
	Ethanol	14.3
S. hispanicus	Aqueous	9.5
	Ethanol	14.3
C. urvillei subsp. *stepposa*	Aqueous	19
	Ethanol	42.9
C. arvense subsp. *vestitum*	Aqueous	-
	Ethanol	28.6
O. illyricum	Aqueous	9.5
	Ethanol	9.5

-: No inhibition.

Table VI. Antioxidant activities of plant extracts.

Plants	Extracts	DPPH	FTC
		IC_{50} (mg/mL)	Inhibition %
S. marianum	Aqueous	29.19±0.43	67.12±2.74
	Ethanol	25.68±1.02	-
S. hispanicus	Aqueous	18.85±0.47	65.05±1.44
	Ethanol	16.47±0.31	8.75±0.57
C. urvillei subsp. *stepposa*	Aqueous	87.12±2.55	63.84±2.78
	Ethanol	22.73±1.14	-
C. arvense subsp. *vestitum*	Aqueous	28.55±0.58	61.22±1.92
	Ethanol	35.86±1.22	20.64±0.71
O. illyricum	Aqueous	47.16±2.06	67.79±2.98
	Ethanol	5.76±0.16	22.18±0.12
Standards	BHT	0.018±0.81	63.36±2.52
	α-tocopherol	0.037±0.26	39.96±1.65
	Ascorbic acid	NT	77.67±2.81

DPPH: 1,1-diphenyl-2-picrylhydrazyl. FTC: Ferric thiocyanate. -: No inhibition.

DISCUSSION

There has been a significant increase in the focus on natural resources, particularly plants frequently used in traditional medicine, to develop alternatives to the resistance mechanisms developed by bacteria. Based on this, the antimicrobial activity of thorny and edible plants in Mugla was investigated. It was determined that the ethanol extract of *S. hispanicus* and *O. illyricum* showed the highest activity. Aboukhalaf et al. (2023) stated that *S. hispanicus* did not show activity against *S. aureus*, but the inhibition zone diameter against *E. coli* was 9 mm, and, in other studies, the highest activity was detected against *S. aureus* with 19 mm (Aboukhalaf et al. 2023), in a different work, Petropoulos et al. (2019) reported that the highest inhibition was detected against *E. coli* and *B. cereus*, and in another study, it was reported that it showed different levels of activity against different microorganisms (Marmouzi et al. 2017). Considering the available information, although the data obtained are mostly similar, this difference may be related to the collection of the plant from different regions and at different times. In addition, the MIC values of these plants subject to the research were determined and it was revealed that *O. illyricum* ethanol extract was effective at the lowest concentration. Considering this context, making a direct comparison is not possible because there was no study on the antimicrobial potential of *O. illyricum*. However, there are studies on various activities of *O. illyricum* such as anti-HIV-1 (Sanna et al. 2018), anti-inflammatory (Formisano et al. 2016), cell viability, anti-rosacea, and antioxidant activity (Caddeo et al. 2023).

Antibiofilm activity is another parameter subject to this research, and in this context, *C. arvense* subsp. *vestitum* stands out as the plant that inhibits biofilm formation at the highest rate. No research has been found on the antibiofilm activity of *C. arvense* subsp. *vestitum*, but different researchers have tested different biological and chemical activities to reveal the potential of the *Cirsium* genus. Shin et al. (2022a) determined in their study that a different species of *Cirsium* showed high antimicrobial, antioxidant, and cytotoxic activity. Another study revealed that it could be an important source of antimicrobial activity (Tang et al. 2024). Moreover, it has been determined that it can be used in the treatment of diabetes (Shin et al. 2022b) and in reducing the environmental damage caused by *Microcystis aeruginosa*, which poses a major environmental problem (Liu et al. 2022).

Among the test plants, *O. illyricum* demonstrated anti-quorum sensing activity. Although no similar studies have been found, Holasová et al. (2022) found that *S. marianum* flavonolignans showed anti-quorum-sensing activity. This is the first study for the plants included in this study in terms of violacein production inhibition and swarming motility activity, which is another indicator of Quorum sensing. Therefore, previous studies are not suitable for comparison since they only cover the different biological activities or chemical contents of these plants.

In this study, antioxidant activity was tested in two different ways, *O. illyricum* ethanol extract showed the highest DPPH scavenging rate, and *O. illyricum* aqueous extract showed the highest FTC inhibition. Caddeo et al. (2023) determined the DPPH scavenging capacity of *O. illyricum* ethanol extract as 673 µg TE/g. Another study determined DPPH radical scavenging as 118.17 µmol TE/100 g (Ayaz et al. 2017). Although the most of studies have been conducted in the form of trolox equivalence, when we look at this situation in general, it is seen that these plants have high importance in terms of antioxidants.

CONCLUSIONS

This study showed that extracts from *S. marianum*, *S. hispanicus*, *C. urvillei* subsp. *stepposa*, *C. arvense* subsp. *vestitum* and *O. illyricum*, traditionally used and consumed as edible plants, possess significant antimicrobial, anti-protection sensing, antibiofilm and antioxidant activities. The edible nature of these plants supports their potential safety profile and may facilitate their inclusion in functional food products as natural preservatives or antioxidants. In addition, their bioactivities point to promising applications in wound care formulations and antimicrobial surface treatments, especially in preventing biofilm-associated infections and microbial resistance. As a next step, it is essential to isolate and characterise the active phytochemical compounds responsible for these effects. Subsequent cytotoxicity evaluations on various cell lines and in vivo evaluations in appropriate animal models will be critical to determine safety and efficacy. This study is expected to contribute to developing new therapeutic and preventive products based on plant-derived and safe compounds for use in food, pharmaceutical, and healthcare fields.

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ÖZGÜR CEYLAN[1]
https://orcid.org/0000-0002-1865-1093

AYSEL UĞUR[2]
https://orcid.org/0000-0002-5188-1106

NURDAN SARAÇ[3]
https://orcid.org/0000-0001-7676-542X

BÜŞRA E. ARSLAN[4]
https://orcid.org/0000-0002-4858-4397

MEHTAP D. ŞAHIN[5]
https://orcid.org/0000-0002-6081-6699

[1]Mugla Sitki Kocman University, Ula Ali Kocman Vocational School, Food Quality Control and Analysis Program, 48640 Ula, Mugla, Turkey

[2]Gazi University, Faculty of Dentistry, Department of Basic Sciences, Section of Medical Microbiology, 06490 Emek, Ankara, Turkey

[3]Mugla Sitki Kocman University, Faculty of Science, Department of Biology, 48000 Kotekli, Mugla, Turkey

[4]Mugla Sitki Kocman University, Faculty of Science, Department of Chemistry, 48000 Kotekli, Mugla, Turkey

[5]Usak University, Education Faculty, Science Education Department, 64200 Centre, Usak, Turkey

Correspondence to: **Özgür Ceylan**
E-mail: ozgurceylan@mu.edu.tr

Author contributions

Özgür Ceylan: Design of study, Data Analysis, Final Approval and Accountability, Technical or Material Support, Supervision; Aysel Uğur: Design of study, Critical Revision of Manuscript, Final Approval and Accountability, Supervision; Nurdan Saraç: Data Acquisition, Critical Revision of Manuscript, Final Approval and Accountability, Supervision; Büşra Eroğlu Arslan: Data Analysis, Final Approval and Accountability, Technical or Material Support; Mehtap Dönmez Şahin: Data Acquisition, Critical Revision of Manuscript, Final Approval and Accountability, Technical or Material Support.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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