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In vitro antimicrobial, antioxidant, antibiofilm, and quorum sensing inhibitory activities of *Scandix pecten-veneris* L., *Artedia squamata* L., *Oenanthe pimpinelloides* L.

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ABSTRACT

Plants have been widely used as a pharmaceutical to treat diseases in many cultures for centuries. In vitro biological activities of some plants consumed as food and used in traditional medicine in Southwestern Anatolia have been reported. The present study focused on the antimicrobial, antibiofilm, antioxidant, and quorum sensing (QS) inhibitory activities of aqueous and ethanolic extracts of aerial parts of *Scandix pecten-veneris* L., *Artedia squamata* L., *Oenanthe pimpinelloides* L. The antimicrobial activity was evaluated against fifteen bacteria and one yeast using disc diffusion and microdilution methods. The aqueous and ethanol extracts of *A. squamata* showed the highest activity against *Staphylococcus aureus* MU 47 with 20 and 23 mm zone diameters, respectively. The ethanol extract of *S. pecten-veneris* inhibited *Staphylococcus aureus* ATCC 25923 at a 5 mg/mL MIC value. The aqueous extract of *O. pimpinelloides* inhibited biofilm formation of *S. aureus* MU 40, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10239 at MIC value. All extracts displayed violacein inhibition against *Chromobacterium violaceum* CV12472 in the concentration range of 1.25 mg/mL to 25 mg/mL. The aqueous extract of *A. squamata* showed valuable anti-quorum sensing (anti-QS) activity with a pigment inhibition zone of 17 mm on *Chromobacterium violaceum* CV026. The aqueous extract of *S. pecten-veneris* inhibited the swarming motility of *Pseudomonas aeruginosa* PA01 by 47.6%. The aqueous and ethanol extracts of *A. squamata* indicated the best antioxidant activity. It was proved that these edible plant extracts could be used as natural alternative inhibitors to control microbial pathogenesis and diseases.

Key words: *Scandix pecten-veneris*; *Artedia squamata; Oenanthe pimpinelloides;* antimicrobial; antibiofilm; quorum sensing; antioxidant

Introduction

Microbial pathogens are life-threatening factors that cause the emergence of various diseases (Pandian et al., 2023). The high levels of such pathogenic microorganisms cause alarming situations for public health morbidity and mortality (Varela et al., 2021). In addition, the resistance of bacteria to the inhibitory and bactericidal effects of therapeutic agents is another concern. However, bacteria have begun to develop multiple resistant due to the unconscious and widespread use of antibiotics. This situation makes it more difficult to fight against bacteria that develop resistance. To extinguish the negative impact of this, the search for new therapeutic agents is becoming increasingly important. Antibiotic-resistant bacterial infections resulting from acquired resistance and/or biofilm formation require the development of new therapeutics. A 2017 World Health Organization (WHO) Global Antimicrobial Surveillance System report highlights that antibiotic resistance is a worldwide problem. The estimated cost of treating antibiotic resistance-related infections is ~$50,000 per person, with a societal cost of $20 billion (Dadgostar, 2019).

Existing antibiotics and drugs with antimicrobial activity are losing their effectiveness. This enables the emergence of new treatments based on natural antimicrobial compounds against multi-resistant microorganisms. Combinations of plant extracts containing different molecules, including polyphenols, are promising for antimicrobial activity. These combinations can be effective against many various bacterial molecular targets and sometimes potentially evade common

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antibiotic resistance mechanisms. The existence of polyphenols and plant extracts that can disrupt the bacterial plasma membrane, inhibit efflux pumps, inhibit biofilm formation, and inhibit the effect of antimicrobial resistance-related proteins such as PBP2a have been demonstrated and contributed to the discovery of new compounds and plant extracts (Álvarez-Martínez et al., 2020; 2021). The traditional medical system based on the usage of medicinal plants still plays a significant role in the healthcare system. In recent years, medicinal herbs have gained wider acceptance due to the perception that these herbs, which are natural products, have fewer side effects and increased effectiveness than their synthetic counterparts (Abushouk et al., 2017a, 2017b). Currently, approximately 80% of the world's population relies on traditional medicines as the main form of primary healthcare. The increasing use of natural products in traditional medicine is due to the bactericidal, virucidal, fungicidal, spasmolytic, sedative, analgesic, and local anesthetic activities that the plants have. Many of these plants and plant extracts show medicinal activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral activities. Moreover, these medicinal plants can play a vital role in drug synthesis and development. Also, medicinal plants have a crucial role in biological applications such as cancer treatment, cardiovascular disease treatment, neurological disease treatment, and skin regeneration (Batiha et al., 2018; Beshbishy et al., 2019, Parham et al., 2020). Many plant species have been reported to have pharmacological activities attributable to their phytoconstituents, such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes (Batiha et al., 2020; Arslan, 2023).

The genus *Artedia* is represented only by the species *A. squamata*. Besides, *A. squamata* is known in the region as "Katırtırnağı". Infusion of *A. squamata* leaves are used internally as an antihypertensive and seeds are used internally for indigestion in folk medicine (Yıldız et al., 2021). *O. pimpinelloides*, grows naturally in Türkiye and is known as "Orman gazyağı" in the region of Muğla. Diverse research has been conducted on the antioxidant, antimicrobial, and anti-inflammatory properties of *O. pimpinelloides* extract. Moreover, literature studies show that there are studies on determining the nutritional content of *O. pimpinelloides* and its traditional use in human nutrition (Dal et al., 2023). *Scandix pecten-veneris*, commonly known as "Deli kişkiş", is used against infectious diseases in traditional medicine. The plant is native to Eurasia and is present in Europe, extending as far as England in the west and Denmark in the North, is also found in some parts of Türkiye (Wahab et al., 2018).

There are limited studies in the literature examining the antimicrobial and antioxidant activities, and there is no research about biofilm, swarming, swimming inhibition activity, violacein production inhibition, and quorum-sensing inhibitory effects of S*candix pecten-veneris*, *A. squamata*, *O. pimpinelloides*. Hereby, this study aims to investigate in vitro antimicrobial, antioxidant, antibiofilm activities, and quorum-sensing inhibitory effects of ethanol and aqueous extracts of *S. pecten-veneris*, *A. squamata*, *O. pimpinelloides*, which grow naturally in the Muğla region and are consumed as vegetables, in microorganisms that are clinically important and cause problems in treatment.

Materials and Methods

Preparation of plant extracts

Scandix pecten-veneris L., *Artedia squamata* L., and *Oenanthe pimpinelloides* L., belonging to the *Apiaceae* family, were collected from natural populations around Muğla in southwestern Türkiye. Ethanol extract was obtained from the aerial parts of air-dried and powdered plants using the Soxhlet apparatus. Aqueous extracts were prepared through the infusion method and lyophilized. The lyophilized plant extract was stored in small sterile opaque bottles until used under refrigeration.

Microbial strains

The microbial strains were three Gram-positive bacteria *(Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRLL B-4375, and *Bacillus subtilis* ATCC 6633), the two Gram-negative bacteria *(Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), one yeast *(Candida albicans* ATCC 10239), and multiresistant strains of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* and various species of *Staphylococcu*s. The strains coded MU (the multiresistant bacteria) were obtained from the Muğla University Culture Collection. The turbidity of the microorganism medium adjusts the 0.5 McFarland standard dilutions. The strains of *C. violaceum* CV12472, CVO26, and *Pseudomonas aeruginosa* PAO1 were obtained from the Spanish Type Culture Collection (CECT) (Valencia, Spain).

Determination of antimicrobial activity with the disc diffusion method

The antimicrobial activity was assayed with the disc diffusion method (Bauer et al., 1966; Collins et al., 1995; Murray et al., 1995) using bacterial cell suspensions whose concentration was equilibrated to a 0.5 McFarland standard. One hundred microliters of each bacterial suspension were spread on a Mueller-Hinton agar plate. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each extract dissolved in the solvent used for extraction at 25 mg/mL. The discs were allowed to dry and then placed on the inoculated agar. The plates were incubated at the appropriate temperature and time for microorganisms. Discs impregnated with ethyl alcohol and distilled water were used as controls. After

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incubation time, the zone of inhibition was measured. The experiment was performed in triplicate.

Determination of minimal inhibitory concentrations (MIC)

The MIC was evaluated by a broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006). The test medium was Mueller Hinton Broth and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (200µL) were inoculated into the wells of 96-well microtitre plates in the presence of extract with different final concentrations (1.25, 2.5, 5, 10, 20, mg/mL). The MIC was defined as the lowest concentration of extract at which no visible microbial growth was observed. The inoculated microplates were incubated at 37 °C for 24 h before being read.

Effect of extracts on bacterial biofilm formation

The effect of different concentrations of extract (ranging from 1 to 0.125 MIC) on biofilm-forming ability was tested on polystyrene flat-bottomed microtitre plates as described by Merritt et al., (2005). Briefly, 1% of overnight cultures (OD adjusted to 0.4 at 600 nm) of test pathogens were added into 200 µL of fresh TSB medium and cultivated in the presence/absence of extract without agitation for 48 h at 37ºC. The wells containing only TSB served as control. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 min at room temperature. Wells were washed once again to remove the crystal violet solution. Gram-negative bacteria/*C.albicans* and Gram-positive bacteria wells poured 200 µL of 95% ethanol and 33% glacial acetic acid, respectively. After shaking and pipetting of wells, 125 µL of the solution from each well were transferred to a sterile tube and the volume was adjusted to 1 mL with distilled water. Finally, optical density (OD) of each well was measured at 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). The percentage of inhibition of the tested extracts was calculated using the following formula:

$$\text{Biofilm inhibition (\%)} = \frac{\text{OD 550 control} - \text{OD550 sample}}{\text{OD 550 control}} \times 100$$

Violacein Inhibition Assay

C. violaceum ATCC 12472 was used in qualitative violacein inhibition screening (Tamfu et al., 2020). 10 µL of overnight culture of *C. violaceum* ATCC 12472 (OD adjusted to 0.4 at 600 nm) was added into wells of sterile microtiter plates containing 200 µL of LB broth and incubated in the presence and absence of sub-mıcs of test extracts at 30°C for 24 h and observed for inhibition in violacein pigment production. LB broth containing *C. violaceum* ATCC 12472 was used as a positive control. The absorbance was read at 585 nm. The percentage of violacein inhibition was calculated with the following formula:

$$\text{Violacein inhibition (\%)} = \frac{\text{OD 585 control} - \text{OD585 sample}}{\text{OD 585 control}} \times 100$$

Bioassay for Quorum sensing inhibition (QSI) activity using CV026

Quorum sensing inhibition was evaluated as described elsewhere (Koh & Tham, 2011) with slight modifications. 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 CV026 culture, and 20 µL of 100 µg/mL C_6HSL was added as an exogenous AHL source. This was gently mixed and poured immediately over the surface of a solidified LBA plate as an overlay. Wells of 5mm in diameter were made on each plate after the overlay had solidified. Each well was filled with 50 µL of sub-MIC concentrations of filter-sterilized herb extract.

The limit of activity detection was also determined by applying serial dilutions of the extracts (1:1 to 1:16, using LB broth as a diluent). Endpoints were estimated as the lowest dilution of extract giving discernible inhibition of violacein synthesis. Each experiment was repeated and the assay plates were incubated at 30°C for 3 days.

Anti-swarming in PA01

Inhibition of swarming motility assay was done as described previously (Packiavathy et al., 2012). Briefly, overnight cultures of *P. aeruginosa* PA01 strain were point-inoculated at the center of swarming plates consisting of 1% peptone, 0.5% nacl, 0.5% agar, and 0.5% of filter-sterilized D-glucose with various concentrations of plant extracts (100 mg/mL), and the plate without the extract was maintained as a control. Plates were incubated at an appropriate temperature in an upright position for 18 h. The swarming migration was recorded by measuring the swarm zones of the bacterial cells.

Determination of DPPH radical scavenging activity

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

The ferric thiocyanate (FTC) method

A screw-cap vial containing a mixture of 4 mg of sample in 4 mL of 99.5% ethanol, 4.1 mL of 2.51% linoleic acid in 99.5% ethanol, 8.0 mL of 0.02M 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 of 30% ammonium thiocyanate, and finally, 0.1 mL of 2×10^{-2}M ferrous chloride in 3.5% hydrochloric acid were 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 hours until the control reached its maximum absorbance value.

Results

The antimicrobial activity of *S. pecten-veneris*, *A. squamata* and *O. pimpinelloides* extracts were determined by the disc diffusion method and a broth microdilution method. The results of the antimicrobial activity of the extracts are given in Table 1. The aqueous and ethanol extracts of the three plants showed various antimicrobial activities in the tested microorganisms. According to the results obtained by the disc diffusion method, aqueous extracts of *S. secten-veneris*, *A. squamata,* and *O. pimpinelloides* revealed inhibition against test microorganisms with 7-8 mm, 8-20 mm, and 8-11 mm zone diameters, respectively. For three plant ethanol extracts, the inhibition zones varied between 9-10 mm, 7-23 mm, and 7-11 mm. The aqueous and ethanol extracts of *A. squamata* exhibited the most effective antimicrobial activity against *S.aureus* MU 47 with a zone diameter of 20 and 23 mm, respectively.

The percentage inhibition of biofilm formation against the test microorganisms is given in Table 2. The aqueous extract of *S. pecten-veneris* reduced the biofilm formation of *P. aeruginosa* ATCC 27853 by 18.14% at MIC and 4.27% at MIC/2. The aqueous extract of *A. squamata* exhibited the highest antibiofilm activity with 19.4% inhibition against *S.aureus* MU 46 at MIC, while *O. pimpinelloides* reduced *P.aeruginosa* ATCC 27853 biofilm production by 26.80% at MIC and 2.55 % at MIC/2.

Table 1. *Antimicrobial activity of S. pecten-veneris, A. squamata, and O. pimpinelloides extracts*

Microorganism	Extract	*S. pecten-veneris*		*A. squamata*		*O. pimpineloides*	
		DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)
B. subtilis ATCC 6633	Aqueous	-	>20	10	20	-	>20
	Ethanol	9	20	12	10	11	10
M.luteus NRRL B-4375	Aqueous	-	>20	-	>20	-	>20
	Ethanol	-	20	7	10	8	10
S. aureus ATCC 25923	Aqueous	-	>20	11	>20	-	>20
	Ethanol	9	5	8	20	8	10
S. aureus MU 38	Aqueous	-	>20	-	>20	-	>20
	Ethanol	9	20	-	10	8	10
S. aureus MU 40	Aqueous	-	>20	-	>20	11	20
	Ethanol	10	>20	-	>20	8	20
S. aureus MU 46	Aqueous	-	>20	11	20	-	>20
	Ethanol	-	10	8	20	-	10
S. aureus MU 47	Aqueous	-	>20	20	20	-	>20
	Ethanol	-	>20	23	>20	11	>20
S. epidermidis MU 30	Aqueous	-	>20	-	>20	-	>20
	Ethanol	-	>20	11	>20	8	20
E. coli ATCC 25922	Aqueous	-	>20	7	>20	-	>20
	Ethanol	-	>20	-	>20	8	>20
P. aeruginosa ATCC 27853	Aqueous	8	20	8	>20	8	20
	Ethanol	-	>20	7	>20	9	>20
P. aeruginosa MU 187	Aqueous	-	>20	8	>20	-	>20
	Ethanol	-	>20	-	>20	-	>20
P. aeruginosa MU 188	Aqueous	-	>20	-	>20	-	>20
	Ethanol	-	10	-	>20	-	>20
P. aeruginosa MU 189	Aqueous	-	>20	-	20	-	>20
	Ethanol	-	10	-	20	-	10
P. fluorescens MU 180	Aqueous	7	>20	-	>20	-	>20
	Ethanol	-	>20	-	>20	-	>20
P. fluorescens MU 181	Aqueous	-	>20	-	>20	-	>20
	Ethanol	-	20	-	20	7	10
C. albicans ATCC 10239	Aqueous	-	20	-	20	-	20
	Ethanol	-	>20	-	>20	-	>20

DD: Disc Diffusion, MIC: Minimum Inhibition Concentration, -: No inhibition

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Table 2. *Antibiofilm activity of S. pecten-veneris, A. squamata, and O. pimpinelloides extracts*

Microorganism	Extract	*Scandix pecten-veneris* % inhibition				*Artedia squamata* % inhibition				*Oenanthe pimpinelloides* % inhibition			
		MIC	MIC/2	MIC/4	MIC/8	MIC	MIC/2	MIC/4	MIC/8	MIC	MIC/2	MIC/4	MIC/8
B. subtilis	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 6633	Ethanol	-	-	-	-	24.21	4.16	-	-	22.14	-	-	-
M.luteus	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
NRRL B-4375	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 25923	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
MU 38	Ethanol	14.39	-	-	-	8.11	-	-	-	11.25	-	-	-
S. aureus	Aqueous	-	-	-	-	-	-	-	-	23.27	3.98	-	-
MU 40	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	Aqueous	-	-	-	-	19.4	-	-	-	-	-	-	-
MU 46	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	Aqueous	-	-	-	-		-	-	-	-	-	-	-
MU 47	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
S. epidermidis	Aqueous	-	-	-	-		-	-	-	-	-	-	-
MU 30	Ethanol	-	-	-	-	-	-	-	-	10.36	-	-	-
E. coli	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 25922	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	Aqueous	18.14	4.27	-	-	-	-	-	-	26.80	2.55	-	-
ATCC 27853	Ethanol	-	-	-	-	-	-	-	-	13.82	-	-	-
P. aeruginosa	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
MU 187	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
MU 188	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	Aqueous	-	-	-	-	10.07	-	-	-	-	-	-	-
MU 189	Ethanol	14.48	6.2	-	-	-	-	-	-	-	-	-	-
P. fluorescens	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
MU 180	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
P. fluorescens	Aqueous	-	-	-	-	8.87	-	-	-	-	-	-	-
MU 181	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	Aqueous	-	-	-	-	-	-	-	-	26.14	6.53	-	-
ATCC 10239	Ethanol	21.85	-	-	-	-	-	-	-	-	-	-	-

-: No inhibition

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Table 3. *Violacein inhibition percentage and quorum-sensing inhibition zones of S. pecten-veneris, A. squamata, and O. pimpinelloides extracts*

Plant extract		Violacein activity (CV 12472)			Anti-quorum sensing activity (CV 026)		
		MIC (mg/mL)	Conc. (mg/mL)	Violacein inhibition (%)	MIC (mg/mL)	Antimicrobial activity (mm)	Anti- QS Activity (mm)
S. pecten-veneris	Aqueous	10	5	14.73	100	-	-
			2.5	8.76			
			1.25	3.32			
	Ethanol	> 25	25	17.94	0.7	-	12 (0.5 mg/mL)
A. squamata	Aqueous	10	5	5.38	100	11 (100 mg/mL) 7 (50 mg/mL)	17 (25 mg/mL)
	Ethanol	25	12.5	8.97	2	-	13 (1.0 mg/mL) 10 (0.5 mg/mL)
O. Pimpinelloides	Aqueous	5	2.5	7.86	100	-	-
	Ethanol	25	12.5	15.43	1	12 (1.0 mg/mL) 11 (0.5 mg/mL)	-

-: No inhibition

The highest antibiofilm inhibition activities of ethanol extracts were found to be 21.85% against *C. albicans* ATCC 10239 of *S. pecten-veneris* at MIC and 24.21 and 22.14% against *B.subtilis* ATCC 6633 of *A.squamata* and *O. pimpinelloides* at MIC, respectively.

Prior to violaceum inhibition, the MIC values of the extracts were determined against the *C. violaceum* CV12472 strain and are presented in Table 3. MIC values of all three plant extracts against CV12472 strain ranged from 5 to >25 mg/mL. The aqueous extracts were more active than ethanol extracts on *C. violaceum* CV12472, and the highest activity belonged to the aqueous extract of *O. pimpinelloides* (MIC = 5 mg/mL). Inhibition of violacein production was evaluated at sub-MIC as shown in Table 3. Among the plant extracts, both aqueous and ethanol extracts of *S. pecten-veneris* exhibited the most effective inhibitory activities against violacein production of *C. violaceum* CV12472. The ethanol extract of *S. pecten-veneris* delivered the highest violacein inhibition percentage of 17.94% at 25 mg/mL concentration. Afterwards, the aqueous extract inhibited violacein formation by 14.73% (5mg/mL), 8.76% (2.5mg/mL), and 3.32% (1.25mg/mL).

The MIC values of the extract over *C. violaceum* CV026 were found before specifying the quorum-sensing inhibition zones. The MIC values were 100 mg/mL for all aqueous extracs. MIC values for alcohol extracts of *S. pecten-veneris*, *A. squamata,* and *O. pimpinelloides* were 0.7, 2, and 1 mg/mL, respectively (Table 3). Both quorum sensing inhibition and antimicrobial activity zone diameters were measured in millimeters and are given in Table 3. The ethanol extract of *S. pecten-veneris* and all extracts of *A. squamata* showed inhibition of quorum sensing, and the highest value was the aqueous extract of *A. squamata* with a zone diameter of 17 mm at a concentration of 25 mg/mL. Interestingly, the aqueous extract of *A. squamata* labelled as QSI in the CV026 test also appeared to display antimicrobial activity. In addition, neither extract of *O. pimpinelloides* showed any inhibition of quorum sensing. However, it was determined that the alcohol extract of this plant showed the highest antimicrobial activity with a zone diameter of 12 mm (1 mg/mL concentration).

Swarming motility inhibition determinations of plant extracts were carried out using *P. aeruginosa* PA01 strain. Among the plant extracts, the aqueous extract of *S. pecten-veneris* exhibited the highest swarming inhibition activity of 47.6%. This is followed by the aqueous extract of *O. pimpinelloides* (33.3%) (Table 4).

Table 4. *Swarming motility inhibition on P. aeruginosa PA01 by S. pecten-veneris, A. squamata, and O. pimpinelloides extracts*

Plant extract		Swarming inhibition (%)
		100 mg/mL
S. pecten-veneris	Aqueous	47.6
	Ethanol	-
A. squamata	Aqueous	9.5
	Ethanol	14.3
O. pimpinelloides	Aqueous	33.3
	Ethanol	19

-: No inhibition

Antioxidant activities of aqueous and ethanol extracts of plants were evaluated using FTC and DPPH free radical scavenging methods. According to the DPPH assay results, both aqueous and ethanol extracts of *A. squamata* exhibited better radical scavenging activity than the others, with IC_{50} values of 23.11 ±0.28 mg/mL and 16.71± 1.45 mg/mL, respectively. Nevertheless, all extracts had weaker antioxidative capacity than the positive control. BHT and α-tocopherol had too low IC_{50} values (0.487 mg/mL and 1.75 mg/mL) in the DPPH assay (Table 5).

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Table 5. *Results of extracts on the in vitro free radical (DPPH) scavenging assay and Ferric thiocyanate assay (FTC)*

Plant extracts		DPPH	FTC
		IC_{50} (mg/mL)	İnhibition %
S. pecten-veneris	Aqueous	98.5 ±1.28	62.36±2.14
	Ethanol	57.46 ±0.66	20.00±0.26
A. squamata	Aqueous	23.11 ±0.28	65.54±1.79
	Ethanol	16.71±1.45	12.45±0.92
O. pimpinelloides	Aqueous	73.85 ±0.92	53.27± 2.54
	Ethanol	93.59±0.36	29.18±1.75
BHT		0.487±0.014	63.36±2.52
α-tocopherol		1.75±0.04	39.96±1.65
Ascorbic acid		NT	77.67±2.81

NT: Not tested

As shown in Table 5, the FTC method measures the amount of peroxide in the initial stages of lipid peroxidation. A low absorbance value in the FTC method indicates a high level of antioxidant activity. The absorbance was measured on the final day (4th day) in the FTC assay. The results obtained from the FTC experiment revealed that the aqueous extracts had antioxidative potential for chain-breaking inhibition of lipid peroxidation and free radical scavenging. In particular, it was determined that *A. squamata* aqueous extract showed the highest inhibition with 65.54%.

Discussion

The process of biofilm formation is a dynamic process that involves a series of sequential steps (Floyd et al., 2017). This begins when bacteria approach the surface. The majority of bacteria can switch between two forms; these are planktonic single cells and sessile biofilms. Planktonic cells and biofilms differ remarkably in their gene expression and morphological and physiological aspects. Biofilm-producing sessile cells display increased surface adhesins, innate tolerance to antibiotics, and increased resistance to environmental stress (Nadar et al., 2022). Thus, biofilms are quite dangerous and are responsible for the virulence of microbial infections and the evolution of resistance to traditional antimicrobials. Therefore, the antibiofilm activity of plants is important in the treatment of infections caused by resistant bacteria originating from biofilms. Although biological properties such as antimicrobial and antioxidant of *S. pecten-veneris*, *A. squamata,* and *O. pimpinelloides* have been investigated, their ability to prevent antibiofilm and anti-QS activity has not yet been reported.

C. violaceum CV12472 produces a purple pigment called violacein, which indicates the proper and normal functioning of its growth and acts as a signaling molecule. Violacein production is mediated by the quorum sensing (QS) process in *C. violaceum* CV12472. Inhibition of violacein at sub-MIC concentrations is crucial because it indicates the capacity of the compounds or extracts to inhibit signal molecule production in bacteria (Tamfu et al., 2022). The mutant *C. violaceum* CV026 strain does not produce violacein and this is supplied with external acylhomoserine lactone (AHL). This bacteria is usable to detect impairment of quorum sensing by determining quorum sensing inhibition zones. The quorum sensing inhibition zones were designated at sub-MIC concentrations. In test plates with purple lawn colour produced by activated *C. violaceum* CV026 bacteria, the formation of a cream or yellowish halo around the well was an indicator of QS inhibition, and a transparent or cloudy halo was an indicator of antimicrobial activity. The reduced pathogenicity of a microorganism can be achieved through the disruption of QS communication, which is responsible for pigment production (Asfour, 2018). This activity against bacterial virulence mechanisms necessary for disease development may render pathogenic bacteria nonvirulent by interfering with the QS communication system. QS has recently become an attractive target for the development of new anti-infective agents as alternatives to the use of antibiotics (Maksimovic et al., 2023). Study results show that almost all of the extracts of the aerial parts of the three plants have violacein production inhibition and anti-QS activity. This is the first report of the anti-QS activity of the tested extracts against CV12472 and CV026. The anti-QS results obtained are important considering that these plants are widely used locally as edible vegetables.

Quorum sensing-mediated swarming motility is known to play an important role in Gram-negative bacteria (Tamfu et al., 2020). This swarming behaviour, which is one of the bacterial virulence factors in biofilm formation, ensures adhesion to the surface in the early stages of biofilm formation and supports the development of biofilms (Packiavathy et al., 2014; Merghni et al., 2018). The aqueous and ethanol extracts of the plants used in this study reduced the swarming motility of target pathogens.

According to literature reviews, this study is the first on the antibiofilm activity, violacein inhibition, antiquorum sensing activity, and inhibition of swarming motility of aqueous and ethanol extracts of aerial parts of these plants. Therefore, since there are no studies on these biological activities, it may be advantageous to increase studies on this subject. Data on antioxidant, antimicrobial, and antibiofilm effects confirm the medicinal value of this plant and suggest new therapeutic uses for its metabolites or derivatives. Moreover, based on the popular use and medicinal potential of these plants in food, they can be of great importance to the pharmaceutical industries if used appropriately. Therefore, it is necessary to reveal the compounds of these plants and their potential biological activities. In addition, it is essential to conduct

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comprehensive studies on this subject to fill the gap in the literature.

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