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Anti-biofilm, Anti-quorum sensing, Anti-swarming, and Antimicrobial Activity of *Rubus fruticosus* L.

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ABSTRACT
Over the past decade, antibiotic resistance has increased at an unprecedented rate, posing a serious challenge to healthcare systems worldwide. Research indicates that this resistance issue, which is projected to cause significant loss of life by the 2050s, is particularly alarming. Consequently, alternative methods to effective antibiotics are being explored to combat resistance. *Rubus fruticosus* L., commonly known as blackberry, is a shrub plant famous for its fruit. This fruit holds significant medicinal, cosmetic, and nutritional value. In our study, methanol, ethyl acetate, ethanol, and hexane extracts of *Rubus fruticosus* fruit from the Rize region were screened for their antimicrobial and quorum-sensing activities. Antimicrobial activity was investigated using the agar diffusion method against various Gram-negative and Gram-positive bacteria, as well as two Candida species. Anti-quorum sensing and antibiofilm activities were evaluated using *Chromobacterium violaceum* ATCC 12472 and *Pseudomonas aeruginosa* PAO1. The results showed that the methanol extract of *Rubus fruticosus* exhibited antimicrobial activity, while the ethanol extract demonstrated antibiofilm and anti-swarming activities. These findings suggest that *Rubus fruticosus* has the potential to be used as a natural agent in combating antimicrobial resistance.

Microbiology

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Rubus fruticosus L. nin Anti-biyofilm, Anti-quorum sensing, Anti-swarming ve Antimikrobiyal Aktivitesi

ÖZET
Son on yılda dünya çapında benzeri görülmemiş bir oranda artan antibiyotik direnci sağlık sistemleri için ciddi bir zorluk oluşturmaktadır. Araştırmalar, bu direnç sorununun özellikle 2050'li yıllarda büyük can kayıplarına yol açacağını öngörmektedir. Dirençle mücadelede etkili antibiyotiklerin yerini alabilecek alternatif yöntemler araştırılmaktadır. *Rubus fruticosus* L., böğürtlen adıyla bilinen ve meyvesiyle ünlü bir çalı bitkisidir. Bu meyvenin tıbbi, kozmetik ve besin değeri oldukça yüksektir. Çalışmamızda, Rize iline ait *Rubus fruticosus* meyvesinin metanol, etil asetat, etanol ve hekzan ekstraktları antimikrobiyal ve antiquorum sensing aktivite açısından incelenmiştir. Çeşitli Gram-negatif ve Gram-pozitif bakterilere ve iki Candida cinsine ait türe karşı antimikrobiyal aktivite, agar difüzyon yöntemi ile araştırılmıştır. Anti-quorum sensing ve antibiofilm aktiviteleri ise *Chromobacterium violaceum* ATCC 12472 ve *Pseudomonas aeruginosa* PAO1 kullanılarak değerlendirilmiştir. Sonuçlar, *Rubus fruticosus*'un metanol ekstraktının antimikrobiyal aktiviteye sahip olduğunu, etanol ekstraktının ise antibiofilm ve antiswarming aktivitelerine sahip olduğunu göstermektedir. Bu bulgular, *Rubus fruticosus*'un antimikrobiyal dirençle mücadelede potansiyel bir doğal ajan olarak kullanılabileceğini düşündürmektedir.

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INTRODUCTION

Antimicrobial resistance (AMR) is a critical global health concern that has gained significant attention in recent years. The rise of multidrug-resistant bacterial strains, along with the limited development of new antibiotics, presents a complex challenge in combating infectious diseases (Terreni et al., 2021). Misuse and overuse of antimicrobials are recognized as key drivers in the development of drug-resistant pathogens (Zhou, 2023). This resistance poses a substantial threat to public health, emphasizing the need to explore innovative therapeutic approaches to address the issue (Kranjec et al., 2021).

Recent research has emphasized the importance of understanding the mechanisms of enhanced tolerance of biofilm-associated infections to antimicrobial therapy, given the escalation of drug resistance and the necessity for effective treatment strategies (Tsui et al., 2016). Moreover, exploring the incorporation of antimicrobial compounds beyond traditional antibiotics, particularly those with broader antimicrobial activity, is being considered to tackle emerging resistance (Bezuidenhout et al., 2015).

In response to the increasing antimicrobial resistance crisis, initiatives have been undertaken to promote alternative antimicrobial approaches like antimicrobial photodynamic therapy (aPDT), which has demonstrated potential as a light-based strategy to combat resistance (Cieplik et al., 2018). Additionally, strategies such as antimicrobial stewardship, surveillance, and leveraging artificial intelligence for the development of new antimicrobial medicines have been suggested to address the challenges posed by AMR (Ercan et al., 2023).

The global distribution of antimicrobial resistance in various settings, including in animals like swine, underscores the importance of monitoring antimicrobial use and resistance to protect public health (Hayer et al., 2022). The continuous evolution of research in antimicrobial properties, biofilm eradication, and the rational use of antibiotics reflects ongoing efforts to stay ahead of the increasing trend of antibiotic resistance (Permatananda et al., 2023). One of the critical mechanisms by which pathogenic microorganisms enhance their virulence is through quorum sensing. Quorum sensing is a bacterial communication system that regulates gene expression in response to population density via signaling molecules. This system controls the expression of virulence factors, secondary metabolite production, and stress adaptation mechanisms, allowing bacteria to become pathogenic (Merghni et al., 2022). Pathogens utilize quorum sensing to coordinate activities such as biofilm formation, violacein production, and swarming motility, which are crucial for their virulence. Inhibiting these quorum-sensing pathways is seen as a promising strategy to mitigate the impact of serious pathogenic bacteria and the infections they cause (Packiavathy et al., 2014).

Violacein is a natural bacterial pigment with significant biological activities. It has been extensively studied for its antimicrobial, antifungal, antiviral, anticancer, and antiparasitic properties (Kanade et al., 2022; Abedin, 2024). Recent research has focused on various aspects of violacein, including its biosynthetic pathway, production by different bacterial hosts, metabolic engineering for enhanced production, and its applications in synthetic biology, pharmaceuticals, and industry (Ahmed et al., 2021). Studies have shown that violacein induces cell death in tumor cells and inhibits tumor cell migration, suggesting potential therapeutic applications in cancer treatment (Mehta et al., 2015). Furthermore, the formulation of violacein in different carriers has been explored to improve its efficacy and overcome physiological barriers (Durán et al., 2007).

Historically, humans have relied on plants for both food and medicinal purposes. The World Health Organization (WHO, 2002) data shows that over 60% of the global population continues to use plant-based remedies due to the bioactivity of their phytochemicals (Acet, 2021). One such plant is *Rubus fruticosus* L., commonly known as blackberry, which belongs to the Rosaceae family. This plant is valued not only for its delicious taste and pleasant aroma but also for its nutritional and medicinal properties. Rubus is the largest genus within the Rosaceae family, encompassing around 700 species. The fruits of Rubus are consumed fresh and are also processed into various food products such as jams, wine, tea, ice cream, desserts, jellies, and pastries. In addition to their nutritional benefits, Rubus fruits have been used as natural antimicrobial agents. Studies have shown that *R. fruticosus* is effective against bronchitis and respiratory tract infections (Zia-Ul-Haq et al., 2014).

Rize province, located in northeastern Turkey on the eastern Black Sea coast, is a region rich in biodiversity and traditional knowledge of medicinal plants. Several studies highlight the significance of Rize province as a valuable source of medicinal plants. For instance, a study by Dalar (2018) emphasizes the importance of documenting traditional medicinal plants in Van province, Turkey, to preserve local medicinal knowledge threatened by

urbanization. This underlines the urgency of safeguarding the valuable traditional medicinal practices present in regions like Rize.

Furthermore, research by Yazıcı et al. (2023) focuses on the fruit quality parameters of mandarin genotypes from Rize province, indicating the diversity and richness of plant species in the region. Additionally, Abay et al. (2016) provide insights into the bryophyte checklist of Rize, highlighting the ecological importance of the province. Thus, *Rubus fruticosus* L., a species, was collected from Rize province based on its rich flora characteristics.

The selection of solvents is a critical factor in plant extraction processes, impacting both the extraction yield and the quality of the compounds obtained. Various studies emphasize the importance of choosing suitable solvents based on their polarity and efficiency in extracting specific bioactive compounds from plants. For example, ethanol and water are commonly utilized solvents due to their low toxicity, high extraction yield, and the ability to adjust solvent polarity by varying their ratios (Singh et al., 2022). Moreover, the use of aqueous mixtures of organic solvents such as ethanol, methanol, acetone, and others with water has been demonstrated to enhance the antioxidant efficiency of plant products compared to using water alone (Venkatesan et al., 2019).

To further explore the antimicrobial properties of *R. fruticosus*, researchers investigated the activity of different solvent extracts of the plant, including methanol, ethyl acetate, ethanol, and hexane, using the agar well diffusion method. This study focused on the effects of these extracts on quorum sensing in *Chromobacterium violaceum* strains and *Pseudomonas aeruginosa* PAO1. The quorum-sensing system in these bacteria plays a vital role in their pathogenicity, and disrupting this system could provide a novel approach to combating bacterial infections.

The ongoing research into plant-based antimicrobials is promising, particularly as antimicrobial resistance continues to rise. By understanding and harnessing the bioactive compounds in plants like *Rubus fruticosus*, scientists aim to develop new treatments that can work alongside existing drugs or as standalone therapies. The dual approach of targeting bacterial virulence factors and utilizing natural plant extracts offers a comprehensive strategy to address the growing challenge of antimicrobial resistance.

MATERIALS and METHODS

Collection of fruits and preparation of extracts

Methanol, ethyl acetate, ethanol, and hexane extracts of the *R. fruticosus* fruits (Figure 1) belong to Rize/İkizdere were prepared using the maceration method (Solanki & Nagori, 2012). 10-20 g of the fruits were powdered, and 10 times the relevant solvents were added to it. It was stirred at room temperature with a magnetic stirrer for 48 hours. Then, the extracts were filtered through filter paper, and the solvent was evaporated at 40 °C in the evaporator, and they were prepared in DMSO (Dimethyl sulfoxide) at concentrations of 50-200 mg mL.

Figure 1. *Rubus fructicosus* L.
Şekil 1. Rubus fructicosus L.

Antimicrobial Activity

Antimicrobial activity was tested using the agar well diffusion method. The plates were incubated at 18 h for bacteria, 48 h for *Candida* strains, and examined for any growth inhibition zones (Woods et al., 2003; Denev et al., 2014; Gür, 2016). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 *Pseudomonas aeroginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Bacillus cereus A*TCC, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Yersinia pseudotuberculosis* ATCC, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumonia* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 10231, *Mycobacterium smegmatis* ATCC 607, *Chromobacterium violaceum* ATCC 12472 strains were used for the experiments.

Minimal Inhibitory Concentration (MIC) (µg/mL) was determined by the broth microdilution method (Murray et al., 2009). Tested derivatives were diluted with 100 µL of Mueller-Hinton broth-II in half increments starting from the first well of the microtiter plate. Brain heart infusion Broth (BHBI) was used for *M. smegmatis* (Woods et al., 2003). RPMI 1640 with 0.2 % glucose was used for *Candida* species (EUCAST, 2022). To determine the Minimum Bactericidal Concentration (MBC), samples from the MIC well and the previous three wells were inoculated into their respective media. The lowest concentration without growth in the media was determined as MBC (Saliha et al., 2020).

Antiquorum Sensing Activity

To detect the inhibition of violacein pigment, sub-MIC values of *R. fruticosus* against *C. violaceum* (ATCC 12472) were determined according to the strain. *C. violaceum* was cultured in 5 mL of Luria-Bertani (LB) medium in a shaking incubator for 24 hours. The prepared cultures were then poured onto LB agar plates and allowed to dry. Wells were created in the dried agar plates, and 50 microliters of the extracts at sub-MIC concentrations were added to each well. Wells that exhibited bacterial growth, but no pigment formation, were considered positive for violacein inhibition (Mclean et al., 2004; Koh & Tham, 2009, Üreyen Esertaş et al., 2022).

Antiswarming activity

P. aeruginosa strain was prepared for the experiment. Then, 50 microliters of *R. fruticosus* extracts (100 µg/mL, 50 µg mL) were added to LB soft agar medium. Prepared soft agars were poured onto LB plates and left to dry. A sample of *P. aeruginosa*, whose overnight culture was prepared with the help of a sterile toothpick, was dipped in the middle of the dried Petri dishes. After incubation, the result was interpreted according to the growth pattern of the bacteria in the petri dish (Rashid & Kornberg, 2000; Uğurlu 2013, Esertaş & Cora, 2024).

Antibiofilm activity

For the preparation of the experiments, a liquid culture of *Pseudomonas aeruginosa* was first established. The culture was grown overnight in LB broth to reach the appropriate density for the experiments. Once the culture was ready, the experimental setup began by adding specific volumes of the extract, bacterial culture, and medium to the wells of a microtiter plate. In each well designated for the experimental conditions, 40 µL of the extract was pipetted first. This was followed by the addition of 35 µL of the Pseudomonas aeruginosa PAO1 culture. Finally, 125 µL of LB broth was added to each well to achieve a final volume of 200 µL per well. This combination ensured that each well contained the precise ratios needed for the experiment. For the control wells, the setup was slightly different. No extract was added to these wells to serve as a baseline for comparison. However, the same volumes of bacterial culture (35 µL) and LB broth (125 µL) were added to maintain consistency in the total volume and conditions across all wells. Once all the wells were prepared, the microtiter plates were incubated under optimal conditions, usually at 37°C for a duration that allows for sufficient biofilm formation, typically 24 hours. After the incubation period, the medium was carefully removed from each well. The wells were then washed with sterile water to remove any non-adherent cells. To stain the biofilms, 200 µL of 0.3% crystal violet solution was added to each well. The plates were left to stand for 15 minutes, allowing the crystal violet to bind to the biofilm matrix. Following the staining step, the excess crystal violet was removed by washing the wells multiple times with sterile water. This step is crucial to ensure that only the biofilm-bound crystal violet remains in the wells. Next, 200 µL of 95% ethanol was added to each well to solubilize the bound crystal violet, and the plates were left for another 15 minutes to ensure complete dissolution. Finally, the optical density of the ethanol-crystal violet solution was measured at 570 nm using a spectrophotometer (Truchado et al., 2009; Esertaş et al., 2024).

Statistical Analysis

Statistical analysis was conducted using the SPSS 15.0 software. Mean ± standard deviation was employed to depict continuous variables conforming to a normal distribution. In cases where a normal distribution was not

evident, the median value was utilized. Qualitative variables were represented as percentages. Parametric variables underwent statistical assessment through the utilization of Student's T-test, applied to compare two distinct groups. A significance threshold of 0.05 was adopted to determine statistical significance.

RESULTS and DISCUSSION

Indigenous communities have historically utilized medicinal flora to address a variety of bacterial and fungal infections, primarily due to their affordability, accessibility, and the traditional belief in the antimicrobial properties attributed to these plants. Researchers worldwide are actively involved in scientifically validating the pharmacological activities that have been traditionally claimed for these plants. Moreover, the extensive use of antibiotics to treat microbial infections has led to increasing resistance among bacteria and fungi to the conventional antibiotics available commercially. (Abdova et al., 2018). Consequently, it is imperative to explore and identify novel natural resources, such as plants, for their potential to treat microbial infections. Our findings demonstrate that *Rubus fruticosus* different extracts exhibit considerable antimicrobial activity against common pathogens. In particular, the methanolic extract of *R. fruticosus* produced inhibition zones, showing notable efficacy against *C. albicans*, *A. haemoliyticus*, *S. aureus,* and *C. violaceum*. The antibacterial activity observed may be attributed to the presence of tannins (Cavanagh et al., 2003) (Table 1).

Table 1…Antimicrobial activity of Rubus fructicosus
Çizelge. 1. Rubus fructicosus'un antimikrobiyal aktivitesi

Microorganisms	Solvents			Controls
	MeOH	EtOAc	EtOH	
S. aureus	14.33 ±4.72	11.0 ±2.64	12.0 ±0	30.33 ±0.47
B. subtilis	16.0 ±1.73	0	0	22.33 ±0.47
E. faecalis	0	10.33 ±1.52	9.0 ±1.73	15.0 ±0
E. coli	0	12.0	8.33 ±2.30	25.0 ±0
P. aeruginosa	0	12.0	0	13.0 ±0.47
A. haemoliyticus	14.0 ±1.73	12.33 ±2.08	16.33 ±1.52	23.0 ±0
K. pneumoniae	0	0	0	22.0 ±0
E. aerogenes	0	0	0	19.0 ±0.47
S. thymurium	12.0 ±4.58	0	0	17.0 ±0
C. violaceum	15.0 ±0	8.0 ±3.46	9.0 ±0	30.33 ±0.47
C. albicans	16.33 ±2.51	16.33 ±2.08	13.0 ±1.0	28.0 ±0
C. parapsilosis	0	0	0	25.0 ±0
M. smegmatis	0	0	0	24.33 ±0.47

Controls; Gram positive; Ampicilin, Gram negative; Gentamicin, *Candida*; Amphotericin B, *Mycobacterium*; Ciprofloxacin

The table was created by taking the average of the zone diameters obtained by repeating the agar well method 3 times. It was seen that the antimicrobial activity results of the extracts were similar, giving similar zone diameters. There is no significant difference between groups ($p<0.05$).

MIC and MBC Results of Extracts

In this study, the MIC and MBK concentrations of bacteria with a zone diameter of 15 mm and above were determined, and when the results were compared within themselves, it was observed (Table 2) that the activity in *S. aureus* was better than the others.

Table.2…MIC and MBC (µg mL) results of Rubus fructicosus
Çizelge 2. Rubus fructicosus'un MİK ve MBK (µg mL) sonuçları

			S. aureus	*B. subtilis*	*C. violaceum*	*C. albicans*
R. fruticosus	MeOH	MIC	250	500	500	500
		MBC	500	1000	1000	1000

Weli et al. (2020), in their study, stated that hexane, chloroform, and ethyl acetate extracts of *R. fruticosus* leaves showed antimicrobial activity, but no activity was detected in *S. aureus.* In study shows that ethyl acetate extracts are effective on *S. aureus*. Abachi et al. (2013) in their study, evaluated the activity of the ethanolic extract of *R. fruticosus* against nalidixic acid-resistant *Helicobacter pylori* and determined that it had a strong effect with an MIC value of 400-450 µg/mL. Researchers have determined a similar range of MICs in this study.

Violacein Inhibition Results

The different extracts were effective at inhibiting violacein production at all tested concentrations, as shown in Table 3. There are limited articles about violacein inhibition analysis of *R. fruticosus*. According to Oliveira et. al. 2016, it has been found that violacein inhibition by the phenolic extracts of *R. fruticosus*, especially at 118.60 mg GAE/L, inhibited 88.6%, a value higher than the positive control for this experiment (furanone), which inhibited 68.6%.

Table 3. Anti-violacein activity results of Rubus fructicosus
Çizelge 3. Rubus fructicosus'un antiviyolasin aktivite

	Solvent	*C.violaceum* 12472
R. fruticosus	MeOH	+
	EtOAc	-
	EtOH	+
	Hxn	-

Anti-Swarming Results

Antiswarming activity is crucial in inhibiting the collective movement of bacterial populations, with implications in agriculture, medicine, and waste management. Studies have investigated the use of natural compounds from fruit peel extracts and essential oils like Origanum vulgare to deter swarming behavior in bacteria such as *Salmonella enterica* and *Pseudomonas aeruginosa* (Mahadwar et al., 2015; Merghni et al., 2022). This antiswarming activity is vital for controlling the spread of pathogenic bacteria and biofilms, contributing to the development of novel antibacterial strategies.

Pseudomonas aeruginosa is a versatile bacterium known for its ability to exhibit various group behaviors, including swarming motility. Swarming motility is a coordinated movement of bacterial populations across surfaces facilitated by flagella. Studies have shown that the presence of sub-populations of P. aeruginosa deficient in flagellar activity can repress swarming in the entire population (Bru, 2023). Additionally, phage infection of P. aeruginosa can abolish swarming motility in infected subpopulations and induce the release of the Pseudomonas quinolone signaling molecule PQS, which repels uninfected subpopulations from approaching the infected area (Bru et al., 2019).

The swarming activity of microorganisms is one of the steps of the quorum sensing system and is an important virulence factor regulated by signal communication. *R. fruticosus* EtOH extract was found to have high swarming properties. It was seen that this effect is dose-dependent, and the swarming activity disappears as the dose decreases (Table 4, Figure 2).

Several studies have reported that lower MICs of essential oils from various plants, such as tea tree, eucalyptus, and clove, inhibit swarming activity in *P. aeruginosa* PAO1 in a concentration-dependent manner (Noumi et al., 2018). Merghni et al. (2022) revealed that their results showed a differential reduction in the swarming activity of PAO1 by the tested agents, similar to this study.

Table 4. Swarming suppression results with Rubus fructicosus EtOH extract (100/50 µg/ml) in P.aeruginosa PAO1 strain
Çizelge 4. Rubus fructicosus EtOH (100/50 µg/ml) özütünün P. aeruginosa PAO1 suşunda swarming baskılama aktivitesi

	Zone Diameter (mm)
PAO1 control	29
PAO1+100 µg/mL EtOH extract	7
PAO1+50 µg/mL EtOH extract	19
PAO1 + 3% DMSO (solvent)	27

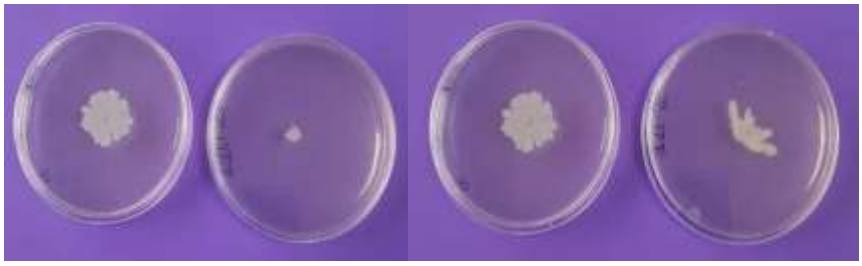

Figure 2. Swarming suppression results of R. fruticosus EtOH at 100 and 50 µg/mL
Şekil 2. R. fruticosus EtOH 100 ve 50 µg/mL'de swarming baskılama sonuçları

Biofilm Inhibition Results

Biofilm formation in Quorum sensing steps can provide protection against the immune system defense of the host and plays an important role in gaining resistance against various antimicrobials. As a result of biofilm experiments, it is seen that *R. fruticosus* EtOH extract inhibited biofilm formation intensively at 100 µg/mL. It is observed that biofilm suppression activity is lost at low doses, depending on the dose.

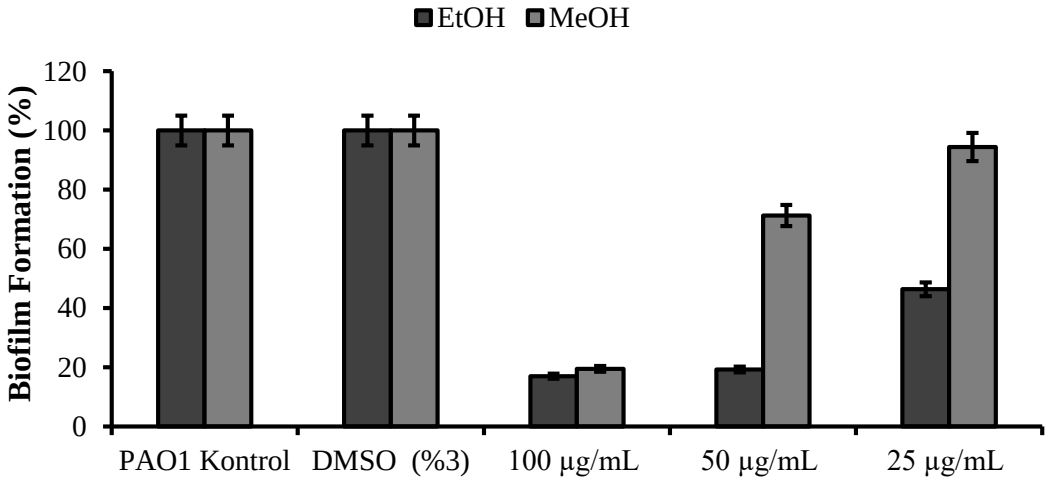

Figure 3. R. fruticosus antibiofilm activity
Şekil 3. R. fruticosus antibiyofilm aktivitesi

In recent years, the increase in multi-antibiotic-resistant and biofilm-forming bacteria has led to the ineffectiveness of conventional antibiotic therapy, and this problem has become a global threat (Cepas et al., 2019). Quorum sensing is a successful cell-cell communication network that manages numerous mechanisms, such as antibiotic production, biofilm formation, and virulence factor production, especially in pathogenic microorganisms. Current research focuses on the identification of new antimicrobials of natural origin and their effects on virulence factors in order to control the spread of dangerous pathogens.

The search for new antimicrobial agents has focused on understanding the protective mechanisms created by plants that can live with high bacterial density for infections. In this direction, herbal products such as plant extracts and herbal polyphenolic compounds present a new strategy as non-toxic inhibitors in the inhibition of the QS system. In a study to show the effects of plant extracts and similar ones used as traditional medicine on the quorum sensing mechanism (Chu et al., 2013). *Fructus gardeniae* and *Ancdrographis paniculata* plants showed anti-QS activity using *C. violacaum* 12472 and *P. aeruginosa* PAO1. The results of the study on the antibiofilm activity of *Rubus ulmifolius* extracts show that plant-derived sugars exhibit different effects on microbial biofilm formation. Phenolic compounds are known to have growth inhibitory activity as well as antibiofilm activity. This suggests that interactions between sugar and biological targets are important (Fontaine et al., 2017).

Vattem et al. reported that raspberry (*Rubus idaeus*), blueberry (*Vaccinium angustifolium*), and grape (*Vitis* sp.) plants inhibited violacin production due to AHL mechanism by 60%, 42%, and 20% (Vattem et al., 2007). Literature data includes *R. rosaefolius* and *R. idaeus* species in quorum sensing studies with *Rubus* species. In this respect, in study is the first quorum sensing screening with *R. fruticosus.*

CONCLUSION

The results of the study demonstrate that *R. fruticosus* extracts possess significant antibacterial, quorum-sensing, antibiofilm, and anti-swarming properties. These findings suggest that *R. fruticosus* could be effectively evaluated as a potent antimicrobial agent for the treatment of bacterial infections. The antibacterial properties of the extracts indicate their potential to inhibit the growth of various bacterial strains, thereby reducing the prevalence and severity of bacterial infections. The quorum-sensing activity highlights the ability of *R. fruticosus* extracts to disrupt bacterial communication systems, which play a crucial role in the regulation of virulence factors and biofilm formation. This disruption can prevent bacteria from coordinating their pathogenic activities, making them less effective in establishing infections.

Furthermore, the antibiofilm activity of the extracts suggests that *R. fruticosus* can inhibit the formation of biofilms, which are protective matrices that bacteria produce to shield themselves from environmental stresses and antibiotic treatments. By preventing biofilm formation, *R. fruticosus* extracts can enhance the susceptibility of bacteria to antimicrobial agents, thereby improving treatment outcomes. Additionally, the antiswarming activity indicates the potential of the extracts to inhibit bacterial motility, further limiting the spread and colonization of pathogenic bacteria.

In future studies, it is planned to isolate and purify the active compounds present in *R. fruticosus* extracts by thoroughly investigating their chemical properties. This purification process will enable researchers to identify the specific bioactive molecules responsible for the observed antimicrobial effects. By understanding the chemical nature of these compounds, it will be possible to optimize their efficacy and develop targeted antimicrobial therapies. Additionally, further research will involve assessing the safety and toxicity profiles of the purified compounds to ensure their suitability for clinical applications. Overall, the promising results of this study pave the way for the development of novel antimicrobial agents derived from *R. fruticosus*, offering a natural and effective solution to combat bacterial infections and address the growing challenge of antibiotic resistance.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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