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Research Article

Investigation of the antimicrobial and antibiofilm effect of plant *Consolida orientalis* on methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus sp.* (VRE)

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Abstract: This study was planned to investigate the antimicrobial and antibiofilm effects of *Consolida orientalis* on MRSA and VRE. MRSA and VRE strains isolated from patients admitted to Sivas Cumhuriyet University Medical Faculty Practice and Research Hospital were used in the study. The antimicrobial activity of *C. orientalis* was investigated by microdilution broth method, biofilm formation activity of microorganisms by spectrophotometric plate method and antibiofilm activity of plant extract by microtiter plate method. According to the results, MRSA strains had Minimum Inhibitory Concentration (MIC) values between 0.15 and >5 mg/mL while VRE strains had MIC values between 0.625 and 2.5 mg/mL. Twenty MRSA strains were observed to form biofilm at various levels, 8 of which were strong, 10 were moderate and 2 were weak. Sixteen strains formed biofilms, 1 of which was strong, 15 of which was weak, and 4 strains did not form biofilms. In conclusion, *C. orientalis* plant extract showed moderate to weak antimicrobial activity against MRSA and VRE pathogens. The presence of the substance 2-ethylacridine, which is hypothesised to possess anti-biofilm properties, was identified in the plant extract through the utilisation of gas chromatography/mass spectrometry (GC/MS) analysis. The extract was also found to inhibit biofilm and eradicate bacteria at various levels.

1. INTRODUCTION

Throughout human history, plants and herbal products have been traditionally used to treat a wide range of diseases. The healing power of these herbs has been passed down from generation to generation as a unique heritage. It has been demonstrated that plants possess antimicrobial activity against bacteria, fungi, and viruses. This activity is dependent upon the chemical type, concentration, and properties of the constituents present in the plant. Therefore, the use of herbal medicines to treat microorganism infections shows promise. These studies aim to clarify the chemical components and mechanisms of action of these plants, to use them effectively (Erdoğan & Everest, 2013). *Staphylococcus aureus* is a significant contributor to community and nosocomial infections. It is responsible for a range of skin and soft tissue infections, including impetigo, folliculitis, carbuncle, furuncle, cellulitis, as well as common systemic infections such as bacteremia, endocarditis, meningitis, pericarditis, pneumonia, osteomyelitis

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and septic arthritis. It also causes a number of toxigenic syndromes, including toxic shock syndrome, septic shock, scalded skin syndrome, and food poisoning (Tong *et al*., 2015).

Clinically, the high level of resistance of *S. aureus* to several classes of antibiotics is a significant problem that limits treatment options. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) occurred within two years of the introduction of penicillin, with the first strain identified in 1942. In 1960, the MRSA strain was first identified following the introduction of methicillin, which was developed in the late 1950s, into clinical use. Epidemics linked to *S. aureus* resistance to various antibiotics have emerged in waves. Infections caused by penicillin-resistant *S. aureus* strains were initially largely confined to Europe. However, new strains have been emerging since the 1980s, causing catastrophic worldwide outbreaks (Laghundi & Zhang, 2018). Enterococci are a natural flora element that colonises the gastrointestinal tract of humans and animals. Although enterococci are typically present in low numbers in the gastrointestinal tract, an increased density of these bacteria is an important risk factor for nosocomial enterococcal infection. Resistant enterococcal infections may develop in patients who are treated in hospital intensive care units, immunocompromised, have foreign bodies such as catheters, or receive multiple and various antibiotic treatments. It is important to note that although these infections usually show low virulence, they can still pose a significant risk to patients (Arias & Murray, 2012).

Enterococci are significant causative agents of nosocomial infections, in particular affect the urinary tract, soft tissues, and are frequently associated with medical devices. These infections are a global problem, leading to prolonged hospital stays and increased treatment costs. Given that the risk of treatment failure and mortality is increased in infections caused by strains resistant to multiple antimicrobial agents, it is the importance to continue research into the development of effective treatments (García-Solache & Rice, 2019). Biofilm is defined as “an Extracellular Polymeric Matrix (EPM) formed by microorganisms that are irreversibly attached to a surface, interfaces, each other, or a substrate. These microorganisms exhibit different phenotypes depending on different microbial growth physiologies and gene transcription” (Donlan & Costerton, 2002).

From a medical perspective, biofilms have a wide range of effects. They facilitate bacterial attachment and play a very important role in antibiotic resistance. Research indicates that there are variations in antibiotic susceptibility observed between microorganisms present in biofilms and those in their planktonic counterparts. Furthermore, biofilms can cause inflammation by stimulating the host's immune response against infected biomedical implants. This demonstrates that biofilm formation in medical infections can have significant impacts, ranging from increased virulence of the microorganism to resistance to treatment (Öztürk *et al*., 2008).

Phenolic compounds present in plants and herbal products have been demonstrated to exhibit antibiofilm activity, in addition to their antibacterial effects. Some plant extracts have been found to inhibit Quorum Sensing (QS), which facilitate communication between microorganisms (Truchado *et al*., 2015).

The inadequacy of commonly used antimicrobial treatments against biofilms has prompted researchers to identify and develop new natural antimicrobial agents. Plant constituents and essential oils with antimicrobial activity are expected to have significant potential in the fight against biofilms. Many studies have demonstrated that certain plants, including *Rosmarinus officinalis*, *Juglans regia*, *Rosa canina*, *Castanea sativa*, and *Malva sylvestris*, have an antibiofilm effect on MRSA (Quave *et al*., 2008). Furthermore, *Mentha piperita* has been demonstrated to possess an antibiofilm effect on *Pseudomonas aeruginosa* and *Candida albicans* (Sandasi *et al*., 2011), *Zingiber officinale* on *P. aeruginosa* (Yahya *et al*., 2013), and *Origanum vulgare* has been shown to have an antibiofilm effect on *S. aureus* and *S. epidermidis* (Nostro *et al*., 2007).

The *Consolida* species, which have important medicinal value besides being ornamental plants, are employed in the treatment of a range of ailments, including traumatic injury, rheumatism,

sciatica and enteritis, in various countries, including Turkey, China and others, as well as in some regions, particularly in the Mediterranean and Western Asia. The isolation of compounds and plant extracts from *Consolida* plants has revealed a range of biological activities, including antiparasitic, antifungal, antiviral, anticancer, antioxidant and insecticidal effects. Some components of these plants have been identified as possessing significant potential for exploitation in the development of novel applications, including antitumor and antioxidant activities (Yin, Cai & Ding, 2020). In the light of this information, it is thought that it may be useful to evaluate the antimicrobial and antibiofilm activity of *C. orientalis* plant which grows spontaneously in our province.

The aim of this study was to identify the biofilm-forming properties of MRSA and VRE strains isolated from hospitalized patients and to determine the antimicrobial and antibiofilm effects of *Consolida orientalis* extract on these microorganisms.

2. MATERIAL and METHODS

2.1. Collection and Typing of Plant Samples

In June 2019, *C. orientalis* (Gay) Schröd. plants were collected from an area located at 39° 42' 11" N, 37° 0' 56" E, at an altitude of 1250 m in Sivas province, Central district. The identification of the plant specimens was conducted by Asst. Prof. Dr. Hülya Özpınar of the Faculty of Pharmacy at Sivas Cumhuriyet University, Department of Pharmaceutical Botany.

2.2. Obtaining the Plant Extract

In the present study, the aerial parts of *C. orientalis* (including flowers and seeds) were utilized. The collected plants were cleaned by use of tap water and distilled water, after which they were dried on blotting paper. Thereafter 300 mL of ethanol were added to 100 g of the grounded plant sample, which was then shaken at 150 RPM for 24 hours at room temperature. Subsequently, the mixture was filtered and the ethanol was removed by rotary evaporator (Buchi R-100 equipped with Vacuum Pump V-300 and Control unit I-300) (Özpınar, 2020).

2.3. Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The chemical constituents of *C. orientalis* plant extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) at the Giresun University Central Research Laboratory Application and Research Center (GRUMLAB). An Agilent model 7890A (5975C inert MSD) instrument and HP5MS type column were used for the study.

2.4. Microorganisms Used in the Study

In this study the used strains of MRSA and VRE were isolated from patients admitted to Sivas Cumhuriyet University Medical Faculty Application and Research Hospital. A total of 40 strains, 20 from each microorganism group, were isolated. The microorganisms were identified using the Microflex LT MALDI-TOF MS (Bruker Daltonics, Germany) and antimicrobial susceptibility tests were conducted on the identified strains using the Phoenix 100® system (Becton Dickinson, USA). The bacterial isolates were stored in a deep freezer at -20 ºC. The isolates were passaged on blood agar medium and then incubated overnight at 37 ºC.

2.5. Investigation of Antimicrobial Activity of *C. orientalis* Plant Extract

The microdilution broth method was employed to identify the antimicrobial activity of the *C. orientalis* plant extract. The plant extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL. A volume of 90 µL Mueller-Hınton Broth (MHB) medium and 10 µL extract was added to the first row of wells, while 50 µL MHB was added to the remaining wells. Serial dilution was performed by transferring 50 µL of the mixture from the first row to the second row of wells. Then 50 µL of a bacterial suspension, adjusted to a turbidity of 0.5 according to McFarland, was added to each well (CLSI, 2012). Wells in the seventh row were used as growth controls and wells in the eighth row were used as sterility controls. The microplates incubated at a temperature of 37 ± 0.1 °C for 24 hours. The extract concentration

in the first well without visible growth was considered the MIC value. The procedure was repeated three times.

The MIC results were considered effective if they were less than 100 µg/mL, moderately effective if they were between 100 and 625 µg/mL, and weakly effective if they exceeded 625 µg/mL (Awouafack *et al*., 2013; Kuete, 2010).

2.6. Investigation of Biofilm Formation in Microtiter Plates

The biofilm formation activity of microorganisms was quantified using the spectrophotometric plate method (Stepanović *et al*., 2007). After passaging the bacterial isolates on a blood agar medium, they were incubated at 37°C for 24 hours. Then they were suspended in Tryptic Soy Broth (TSB) containing 1% glucose adjusted to 0.5 McFarland turbidity. Subsequently, 200 µL of the bacterial suspensions were transferred to the wells in the microplate. The well containing only 200µL medium was considered the negative control. The microtiter plates were incubated at 37°C for 24 hours, after which the wells were gently emptied and washed three times with phosphate-buffered saline (PBS). After drying at room temperature, 200 µL of 0.1% crystal violet stain was added to wells and kept for 15 min. The absorbance values of the microplates were recorded at a wavelength of 570 nm using the SPECTROstar® Nano spectrophotometer (BMG Labtech, USA).

The formation of biofilms was evaluated according to the scale in Table 1 based on the absorbance value of the negative control (Chusri *et al*., 2012). The study was conducted in triplicate for each strain, with the amount of biofilm formed calculated by averaging the optical density values of each replicate.

Table 1. Biofilm formation scale.

OD ≤ ODc	Non-Biofilm Forming	0
ODc < OD ≤ 2 ODc	Weak Biofilm	I
2 ODc < OD ≤ 4 ODc	Moderate Biofilm	II
4 ODc < OD	Strong Biofilm	III

ODc: Optical Density of Negative Control
OD: Optical Density of Bacterial Biofilm

2.7. Investigation of Antibiofilm Effect of Plant Extract

The anti-biofilm activity of the extract obtained from the *C. orientalis* plant was investigated using the microtiter plate method (Celik *et al*., 2015). The study included nine strains that formed strong biofilm structures. Eight MRSA strains and one was a VRE strain.

2.8. Determination of Minimum Biofilm Inhibitory Concentration (MBIC)

The utilisation of the extract at the MIC dose or higher doses will inhibit the growth of the planktonic form of the bacteria or result in bacterial death. Consequently, there will be no microorganisms present in the environment to form biofilms. For this reason, in the present study, a subinhibitory dose MIC/2 was to investigate doses that do not have lethal properties but have antibiofilm activity (Das, 2018).

The study was continued with eight MRSA and one VRE strains that exhibited a strong biofilm-forming ability. Bacterial suspensions were prepared by adjusting to McFarland 0.5 turbidity in TSB medium containing 1% glucose. Following the addition of 100 µL of bacterial suspension to the wells, 100 µL of plant extract was added at a concentration the MIC/2 for each strain. The procedure was applied to three wells for each sample. The three wells that received only 200 µL of bacterial suspension were considered positive controls, while the wells that received only 200 µL of medium were considered negative controls. Following the incubation, the microplates were washed three times with PBS, dried at room temperature, and stained with crystal violet. The absorbance values were then measured at a wavelength of 570 nm. Based on the obtained data, the inhibitory effect of the plant extract on biofilm formation

was calculated as a percentage value. The percentage of inhibition is calculated according to the following formula (Onsare & Arora, 2015).

$$\%\ inhibisyon = \frac{\mathrm{OD}_c - \mathrm{OD}_{MIC/2}}{\mathrm{OD}_c} \times 100$$

OD_c; optical density value of the positive control wells
$OD_{MIC/2}$; optical density of wells treated with extract at submic (MIC/2) concentration value

2.9. Determination of Minimum Biofilm Eradication Concentration (MBEC)

Eight MRSA and one VRE strains were adjusted to McFarland 0.5 turbidity in TSB containing 1% glucose. Following this, 200 µL of bacterial suspension was added to the wells and incubated at 37°C for 48 hours. After the incubation period, the bacterial suspensions were removed from the wells and 200 µL of the extracts prepared at MIC/2 concentration were added to all wells except the positive control well. Wells that were added with 200 µL of medium were designated as the negative control. The microplates were incubated at 37°C for 24 hours, after which the wells were emptied and washed with PBS. Subsequently, the samples were dried and stained, after which the absorbance values were measured. The data were then compared with the positive control, and the percentage eradication value was calculated (Onsare & Arora, 2015).

3. RESULTS

3.1. GC/MS Analysis Results of *C. orientalis* Extract

The analysis revealed that the plant extract contained 2-Ethylacridine. Furthermore, this substance was identified in the GC/MS analysis of the *B. firmus* fraction, which demonstrated antibiofilm activity in the study entitled "Antibiofilm activity of symbiotic *Bacillus* species associated with marine gastropods" by Viju *et al*. (2020). The results of the GC/MS analysis of the *C. orientalis* plant are given in the table (Table 2).

Table 2. GC/MS results of *C. orientalis* plant extract

Componend name	RT	Area (%)
Octanal	9.620	0.07
Nonanal	13.254	0.10
1-Dodecene	16.355	0.09
Octanoic acid	16.612	0.15
1-Hexadecene	22.632	0.12
3-Octadecene	22.632	0.12
Nonanoic acid	25.710	0.21
Tetradecanoic acid	32.863	0.12
2-Pentadecanone	33.956	0.48
n-Hexadecanoic acid	36.387	0.17
9-Octadecenamide	40.399	0.27
Octadecanoic acid	40.627	1.27
Linoleic acid	40.891	0.24
Eicosadienoic acid	40.999	0.10
Tetracosenoic acid	43.340	0.45
Oleic Acid	46.504	0.87
Cis-Dihidrocarvone	46.716	0.18
Eicosane	49.016	0.21
2-Ethylacridine	53.296	0.20

RT; Retention Time
Area ; % area value per analyte

3.2. Microdilution Broth Method Results

The plant extract derived from the aerial parts of *C. orientalis* was subjected to testing 40 bacterial strains, including 20 MRSA and 20 VRE, in order to ascertain its efficacy. The results

demonstrated variability dependent on the bacterial isolates. The MIC values are summarized in Table 3.

Table 3. MIC results of *C. orientalis* plant extract (mg/mL).

Sample No.	MIC (mg/mL)		Sample No.	MIC (mg/mL)	
	MRSA	VRE		MRSA	VRE
1	0.31	1.25	11	>5	1.25
2	0.31	1.25	12	0.15	1.25
3	1.25	1.25	13	0.625	2.5
4	0.31	1.25	14	1.25	1.25
5	2.5	1.25	15	0.625	1.25
6	0.31	2.5	16	0.625	2.5
7	0.15	0.625	17	1.25	2.5
8	1.25	1.25	18	0.31	1.25
9	1.25	1.25	19	1.25	0.625
10	0.31	2.5	20	0.625	0.625

3.3. Biofilm Formation Activity Results

The study examined the biofilm formation potential of resistant bacteria that caused treatment problems. The majority of these bacteria were found to form biofilms (Table 4).

Table 4. Biofilm forming rates of microorganisms.

Microorganisms (s)	Biofilm				
	Creator				Non-Creator
	Strong	Moderate	Weak	Total s (%)	s (%)
MRSA (20)	8	10	2	20 (100)	-
VRE (20)	1	-	15	16 (80)	4 (20)
Total (40)	9	10	18	36 (90)	4(10)

s: Number

3.4. Antibiofilm Activity Results

In order to determine the in vitro antibiofilm effect of the plant extract, the study continued with samples numbered 5, 6, 9, 10, 12, 14, 17, and 20 from the MRSA group, which had strong biofilm-forming properties, and sample number 14 from the VRE group. The MIC/2 values of these strains have previously been determined and applied to bacteria in the MBIC and MBEC stages.

3.5. MBIC Results

The results demonstrate that the plant extract inhibited biofilm formation in MRSA (5,10,17,20) and VRE (14) samples to varying degrees. The percentages of inhibition were calculated (Onsare & Arora, 2015) from the optical density values obtained and are presented in Table 5.

Table 5. Inhibition at subMIC (MIC/2) concentration (%).

Microorganisms	MIC/2 (mg/mL)	% MBIC
MRSA-05	1.25	26±8.3
MRSA-06	0.15	-*
MRSA-09	0.625	-*
MRSA-10	0.15	85±0.8
MRSA-12	0.07	-*
MRSA-14	0.625	-*
MRSA-17	0.625	35±13.3
MRSA-20	0.31	53±16.4
VRE-14	0.625	12±1.6

ubMIC (MIC/2) concentration (%).
* Inhibition not observed

3.6. MBEC Results

The results of the MBEC study demonstrate that biofilms were eradicated to varying degrees in samples 5, 6, 9, 14, 17, and 20 from the MRSA group and sample 14 from the VRE group. The percentage of eradication is presented in Table 6.

Table 6. Eradication at subMIC (MIC/2) concentration (%).

Microorganisms	MIC/2 (mg/mL)	% MBEC
MRSA-05	1.25	61±1.6
MRSA-06	0.15	19±9.4
MRSA-09	0.625	49±5.2
MRSA-10	0.15	-*
MRSA-12	0.07	-*
MRSA-14	0.625	63±2.4
MRSA-17	0.625	72±1.5
MRSA-20	0.31	13±9.5
VRE-14	0.625	20±6.4

*Eradication not observed

4. DISCUSSION and CONCLUSION

Antimicrobial resistance in microorganisms is a global issue that results in high mortality rates worldwide, regardless of a country's development status. Microorganisms are capable of developing resistance to antimicrobial compounds through a process of mutation and natural gene transfer. The intrinsic antibiotic resistance in microorganisms is typically associated with the cellular impermeability of the microorganisms to antimicrobial agents. Moreover, Nadaf and colleagues observed an increase in the expression of drug resistance genes in bacterial strains that were clustered together (Nadaf *et al*., 2018).

Although synthetic drug research is emphasized as a means of combating emerging resistance, the potential toxicity and side effects of many synthetic drugs have increased interest on medicinal plants. This has prompted microbiologists globally to devise innovative antimicrobial agents and assess the potential of natural plant-derived substances as alternatives to chemical antimicrobials (Maregesi *et al*., 2008). It is a widely acknowledged fact that plant phytochemicals exhibit antibacterial activity against free bacterial cells and have the capacity to reduce biofilm development through specific mechanisms (Nadaf *et al.*, 2018).

It is often observed that compounds with medicinal and antimicrobial properties derived from plants show potential activity against biofilm formation. There has been considerable interest among researchers in extracts and essential oils derived from medicinal plants, which have been subject of extensive study. Additionally, plant extracts are commonly used in the pharmaceutical industry due to their bioactive compounds with antimicrobial properties. Many studies have demonstrated that solvent extracts and plant fractions possess biofilm-inhibitory effects against various bacteria and fungi. The antimicrobial and bactericidal properties of essential oils have been demonstrated to disrupt the environmental conditions required for the growth of many bacteria and fungi (Bazargani & Rohloff, 2016). A substantial body of research exists on the antimicrobial and antibiofilm properties of essential oils.

The present study investigated the efficacy of ethanolic extract derived from the *C. orientalis* plant on 40 diverse bacterial strains at concentrations spanning from 5 to 0.15 mg/mL. The results demonstrated that the response of the bacterial isolates differed. A few studies have investigated the antimicrobial effect of *C. orientalis* and other *Consolida* species. Our results are consistent with these findings (Rahdari *et al*., 2010; Rochetti, 2020). Moreover, a considerable body of research has been conducted in the academic literature on the antimicrobial activity of extracts derived from diverse plant parts, including leaves, flowers, and above-ground portions. These studies have employed a range of solvents to investigate the

antimicrobial potential of these extracts (Avşar *et al*., 2016; Yetgin *et al*., 2017; İlkimen & Gülbandılar, 2018).

In this study, all 20 MRSA strains were observed to form biofilms in varying degrees. Eight of the strains exhibited strong biofilm formation, 10 exhibited moderate biofilm formation, and 2 exhibited weak biofilm formation. It was observed that 16 VRE strains, 1 of which was strong and 15 of which were weak, formed biofilm, while 4 VRE strains did not form biofilm. The biofilm-forming properties of the MRSA group in our study were found to be consistent with the findings of İştar (2018). In their study on "Defining conditions for biofilm inhibition and eradication tests for Gram-positive clinical reference strains", observed that enterococci formed an optimal biofilm with an extended incubation period in TSB supplemented with 1% glucose (Cruz *et al*., 2018). The lower biofilm formation rates observed in the VRE group in our study may have been because the same incubation time was applied to both the MRSA and VRE groups.

The study applied the extract obtained from the *C. orientalis* plant to bacteria and observed biofilm inhibition at various levels between 12% and 85% in a total of five samples. Four samples were from the MRSA group and one from the VRE group. Furthermore, biofilm eradication was observed to occur between 13% and 72% in seven samples. Six of the samples were from the MRSA group, and one of the samples was from the VRE group. A search of the literature revealed no studies investigating the antibiofilm activity of *C. orientalis* plant extract. Nevertheless, a number of studies have documented the anti-biofilm activity of diverse natural compounds, including those of Arslan (2019), Atalan (2019), Balaban (2018), Erdönmez *et al*. (2018), Famuyide *et al*. (2019), Göse (2019), Karaca *et al.* (2017), Nadaf *et al.* (2018), and Tozyılmaz (2019).

In 2020, Viju *et al*. conducted a study on three *Bacillus* species, *B. firmus, B. cereus*, and *B. subtilis*, which live symbiotically with gastropods. The researchers prepared extracts from the strains and investigated their activity on the biofilm-forming marine bacteria *Alteromonas sp*. The results demonstrated that the symbiotic bacterial extracts exhibited strong inhibitory effects on biofilm formation, with *B. cereus, B. subtilis*, and *B. firmus* exhibiting the highest inhibition, respectively. The GC-MS analysis of the fraction of *B. firmus* exhibiting antibiofilm activity revealed the presence of a variety of compounds. These included 2-ethylacridine, indolizin, and anthranilic acid.

In certain studies, researchers employed extracts and essential oils derived from diverse plant species, including various plant parts. The studies reported varying levels of antimicrobial, antifungal, and antibiofilm activity on different microorganisms (Nostro *et al.*, 2007; Adukwu *et al*., 2012; Taweechaisupapong *et al*., 2012; Selim *et al*., 2014; Çelik *et al*., 2015; Bazargani & Rohloff, 2016; Haiyan *et al*., 2016; Merghni *et al.*, 2016; Tutar, 2018).

Honeybees are capable of producing a variety of products, including honey, propolis, bee venom, pollen, beeswax, and royal jelly. Such products possess beneficial biological properties and are applicable in many fields, making them popular alternative products in medicine due to their chemical structure. According to ancient Greek writings, propolis was used to treat festering wounds and dental caries. During the Roman period, propolis was incorporated into a poultice-like mixture applied to wounds (Alıç, 2015). In light of these findings, a considerable body of research has been done to evaluate the anti-biofilm efficacy of honey.

In a study conducted by Kim *et al*. (2019), it was observed that the bacteriocin produced by *Lactobacillus brevis* DF01 was effective in reducing biofilm formation. The findings of the study indicate that DF01 bacteriocin affects the formation of biofilms, yet does not disrupt established biofilms.

Biofilm-producing bacteria are frequently associated with the pathogenesis of chronic disease processes, which may give rise to the persistence of localized inflammation and the subsequent damage to surrounding tissues. In certain cases, the complications that result from such

infections can prove to be potentially life-threatening. It has been demonstrated that the opportunistic pathogens most frequently associated with the formation of biofilms are *S. aureus* and *Candida albicans* (Nadaf *et al*., 2018). The antibiotics currently in use are inadequate in treating biofilm-associated infections due to their high MIC and MBC values, which can be toxic to the body. The development of anti-biofilm molecules that are effective in the reduction and elimination of biofilm-related infections is of critical importance (Roy *et al*., 2018). A considerable number of compounds derived from natural sources, including plants, animals and microbes, have been identified and documented as exhibiting antibiofilm activity. The aforementioned compounds are obtained from renewable resources and can be employed as antibiofilm coatings (Viju, Punitha, & Satheesh, 2020).

In light of current knowledge, the importance of obtaining chemical compounds with high antimicrobial and antibiofilm activity as well as low toxicity from natural sources is increasing. The method in question represents a promising approach to combating microbial infections, particularly given the mounting challenge of drug resistance and the biofilm-forming capabilities of microorganisms, which increase pathogenicity, placing an additional burden on therapeutic intervention. In light of the mounting posed by biofilm infections, each study on this subject contributes invaluable data to the existing body of literature, representing a significant advancement in the field. Further studies are recommended to investigate the effects of the substances present in the chemical composition of *C. orientalis*, which is known for its showy purple leaves in the fields in spring.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: Ethical principles were complied with at every stage of the research, and written permission was obtained from Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee with the decision dated 07.08.2019 and numbered 2019-08/10 before starting the applications.

Authorship Contribution Statement

Gonca Şimşek: Investigation, Resources, Visualization, Formal Analysis, and Writing. **Ömer Poyraz**: Methodology, Supervision, and Validation.

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