

Infusion or Decoction Extracts of *Helianthus annuus* Leaves: Potential Inhibitors for QS system and Biofilm Formation in *Pseudomonas aeruginosa*

Helianthus annuus Yapraklarının İnfüzyon veya Dekokasyon Özütleri: *Pseudomonas aeruginosa*'nın QS Sistemi ve Biyofilm Oluşumu Üzerine Potansiyel İnhibitörler

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Abstract

Pseudomonas aeruginosa is one of the drug-resistant opportunistic pathogens with the ability to form biofilm and to produce a number of virulence factors via Quorum Sensing (QS) regulation. Most researchers have focused on QS inhibition to overcome the drug resistance problem. QS inhibitor molecules are investigated from natural resources. In the present study, anti-QS activities of ethyl acetate extracts of decoction and infusion samples from *Helianthus annuus* leaves were tested on biosensor strains of *P. aeruginosa* (*lasB-gfp*, *rhlA-gfp* and *pqsA-gfp*), as well as anti-biofilm activities on PAO1 wild type. *H. annuus* leaf samples were firstly infused or decocted and then extracted with ethyl acetate. The efficacies of infusion or decoction extracts were examined at the concentrations of 240, 120, and 60 µg/ml in 96-well microplates and evaluated in Citation 3 multimode microplate reader (Biotek). The inhibition rates of decoction extracts were recorded as 70.61% for *las*, 44.09% for *rhl* and 83.77% for *pqs* system at 240 µg/ml. The biofilm inhibition percentages of the extracts were determined to be 50.82% (±1.36). Moreover, inhibition rates for infusion extracts were detected as 62.08% for *las*, 45.15% for *rhl* and 77.79% for *pqs*, and 53.88% (±3.94) for biofilm formation. In conclusion, the potential efficacies of the extracts of decocted or infused *H.annuus* leaves were demonstrated on QS system and biofilm formation of *P. aeruginosa*. However, there is a need for more detailed investigations and determination of the active substances that have QSI and anti-biofilm effect.

Keywords: *Helianthus annuus*, *las* system, *rhl* system, *pqs* system, quorum sensing inhibitor, anti-biofilm

Öz

Pseudomonas aeruginosa, Quorum Sensing (QS) regülasyonu yoluyla biyofilm oluşturma ve bir dizi virülans faktörü üretme kabiliyetine sahip ilaca dirençli fırsatçı patojenlerden biridir. Çoğu araştırmacı, ilaç direnci sorunlarının üstesinden gelmek için QS inhibisyonuna odaklanmıştır. QS inhibitör molekülleri doğal kaynaklardan araştırılmaktadır. Bu çalışmada, *Helianthus annuus* yapraklarından elde edilen dekoksasyon ve infüzyon örneklerinin etil asetat özütlерinin anti-QS aktiviteleri, *P. aeruginosa*'nın biyosensör suşları (*lasB-gfp*, *rhlA-gfp* ve *pqsA-gfp*) üzerinde ve ayrıca anti-biyofilm aktiviteleri PAO1 yabancıl tipinde test edilmiştir. *H. annuus* yaprak örnekleri önce infüze veya dekokte edildi ve daha sonra bunların etil asetat ile özütü çıkarıldı. İnfüzyon veya dekoksasyon özütlерinin etkisi 96 kuyucuklu mikroplakalarda 240, 120 ve 60 µg/ml konsantrasyonlarında incelendi ve Citation 3 multimod mikroplaka okuyucuda (Biotek) değerlendirildi. Dekoksasyon özütlерinin inhibisyon oranları, 240 µg/ml'de *las* için %70.61, *rhl* için %44.09 ve *pqs* sistemi için %83.77 olarak kaydedildi. Özütlерin biyofilm inhibisyon yüzdelerinin ise %50.82 (± 1.36) olduğu belirlenmiştir. Ayrıca infüzyon özütleri için inhibisyon oranları *las* için % 62.08, *rhl* için% 45.15 ve *pqs* için % 77.79 ve biyofilm oluşumu için %53.88 (± 3.94) olarak tespit edildi. Sonuç olarak, infüze veya dekokte edilmiş *H.annuus* yaprak özütlерinin potansiyel etkileri *P. aeruginosa*'nın QS sistemi ve biyofilm oluşumu üzerinde gösterilmiştir. Bununla birlikte, QSI ve anti-biyofilm etkiye sahip aktif maddelerin daha ayrıntılı araştırmalarına ve belirlenmesine ihtiyaç vardır.

Anahtar Kelimeler: *Helianthus annuus*, *las* sistemi, *rhl* sistemi, *pqs* sistemi, quorum sensing inhibitör, anti-biyofilm

I. INTRODUCTION

With the discovery of penicillin, significant progress has been achieved in the clinical practice and new antibiotic classes have been discovered in the period defined as the golden age of antibiotics (1, 2). These antibiotics have been effective in the treatment of many infections. Due to the cost-effectiveness and non-toxicity of these antibiotics produced from natural products and derivatives, they have been traditionally prescribed by physicians since ancient times (2). On the other hand, an increase in drug resistance of pathogenic bacteria because of excessive or incorrect use of conventional antibiotics has become a global health problem. Moreover, major

economic losses and high mortality and morbidity rates have been reported in patients due to the high prevalence of antibiotic-resistant strains. The Center for Disease Control and Prevention (CDC) reported that an average of 23,000 patients died in the United States due to lack of effective treatment options due to drug resistance, and approximately \$ 20 billion was spent per year for the treatment of antibiotic resistance-related infections (3). It has been also reported that more than 58,000 infants died in 2013 due to antibiotic resistance in India. (4). Drug resistance, unfortunately, causes difficulties in the treatment process of some hospital-acquired infections and immunosuppressive chronic diseases such as cystic fibrosis, and antibiotics remains insufficient. From this point of view, it seems impossible to prevent the increase of resistance to antibiotics. In clinical practice, most of the prescribed drugs are modifications of existing antibiotics and are only short-term solutions (5). Therefore, innovative alternative treatment approaches should be discovered to overcome antibiotic resistance problem.

In 2017, the World Health Organization (WHO) published a list of antibiotic priorities for critical, high, and moderate pathogens, highlighting the need for new antibiotics. According to this list, *Pseudomonas aeruginosa* is critical for the research and development of new antibiotics (5). According to CDC data, 13% of total *P. aeruginosa* infections and 400 deaths due to these infections are associated with multiple drug resistance (3). Therefore, it is believed that novel non-conventional antibiotic compounds can still be obtained from natural products. However, there are strict criteria for novel alternative compounds particularly such as effectiveness and safety criteria in drug discovery (drug size, ethical concerns, clinical trials, wide spectrum activity, and non-toxicity). In clinical practice, the number of natural-based antibiotics (approximately 28,000) versus their utilization rates (0.1%) is very low, indicating difficulties in drug discovery (2).

Recently, it has been proposed to combat against antibiotic resistance problem via antivirulence strategies. This strategy does not directly kill bacteria but prevents the host from bacterial infections and damages caused by bacteria. Researchers suggested that antivirulent drugs can potentially be utilized synergistically in combination with already established or newly discovered antimicrobials to prolong the life of these drugs. It is well known that many bacteria can secrete several virulence factors and form a biofilm resulting in worsening the course of the disease (6, 7). The bacterial communication mechanism, Quorum Sensing (QS), is responsible for the regulation of these characteristic features that negatively affect the pathogenesis of diseases. In this way, bacteria can combat against the host's immune system. Bacteria that reach a certain level of density secrete small diffusible chemical molecules [acyl homoserine lactones (AHLs), auto-inducing peptides (AIPs) and auto-inducers 2 (AI-

2)], which all of them are called autoinducers (AI). These signals are also perceived by other bacteria and cause them to behave in a coordinated manner (8-12). Bacteria in the biofilm form are resistant to antibiotics up to 10-1000 times compared to planktonic species (13).

Pseudomonas aeruginosa is a drug-resistant opportunistic pathogen and may cause both community- and hospital-acquired infections. This bacterium especially causes nosocomial infections such as cystic fibrosis, pneumonia, and also infections associated with the urinary tract, surgical site, bloodstream, and skin (14). It has been reported that *P. aeruginosa* has four hierarchically related QS systems: *las*, *rhl*, *pqs* and *iqs* (15, 16). Erickson *et al.* (2002) reported QS related AHL, virulence factors and *lasI-lasR* transcripts in the sputum specimens of patients with cystic fibrosis (17). It has been well established that the formation of biofilm structure and secretion of many virulence factors in *P. aeruginosa* affect adversely the course of diseases and these features are regulated by QS mechanism. Nevertheless, established biofilm formation cannot be ameliorated easily. Antivirulence approaches have been proposed in recent years and one of them is inhibition of QS by natural or synthetic molecules. Several QS inhibitors (QSI) obtained from various organisms in nature (plant, algae, animal, bacteria, etc.) are included in the literature (18).

Since ancient times, plants have been used for the treatment of several diseases in folk medicine. Traditional medicine and complementary/alternative medicine are considered as primary health care in countries where healthcare settings are limited. On the other hand, people in modern countries also prefer to use phytomedicine due to positive health effects. It has been reported that 75-90% of the world's population take advantage of plant and plant extracts as a primary health source. Various parts of plants have been used for treatment by direct consumption, boiling (decoction), adding to boiled water (infusion) or as a poultice (19, 20). Our knowledge of herbal medicine depends on the trial and error experiments that have been passed on from generation to generation and information about differences in the ways of preparation of herbal medicines (21-23). The widespread use of plant extracts in the treatment of diseases depends on the active compounds they contain. Various bioactive substances especially secondary metabolites from plants provide a continuous source of potential for new drug compounds (20, 24). About 25% of prescribed drugs are of plant origin directly or indirectly (21). Nowadays, bioactive substances from plants can be isolated and their probable side effects or toxicity and appropriate dosage for medication can be determined. For this reason, anti-QS potentials of plant species collected from different

localities are investigated as direct extracts or based on the compounds they contain (25).

Helianthus annuus L. (Asteraceae) has an ethnobotany value of 3000 years and has been used as a traditional medicine in Asian and European countries. Different parts of *H. annuus* have been traditionally used for the treatment of several diseases. *H. annuus* seeds have been reported to be used in the treatment of heart disease, respiratory infections, cough, whooping cough and common colds (20). Also, a decoction of seeds in Iraq is used as an expectorant and diuretic (26). In India, its flowers and leaves in bronchiectasis, in Europe its seeds in pulmonary infections, in Russia its leaves in fever, in America its leaves in kidney diseases, in Mexico its roots have been used in the treatment of cuts and wounds (20). Tea prepared from leaves has anti-hemorrhagic, diuretic, expectorant, and antipyretic effects. On the other hand, tea prepared from flowers of *H. annuus* has been reported to be utilized in malaria and lung diseases. Pulverized leaves are known to be applied to the wound and snake-spider bites as a poultice (27). Tinctures of *Helianthus* flowers and leaves are used to treat malaria fever and bronchiectasis. It has also been reported that *H. annuus* roots are boiled and used as a hot bath in rheumatic pain (20, 28, 29). In Morocco, the roots and extracts of *H. annuus* have been reported to be used as hypoglycemic, gastrointestinal stimulant, diaphoretic, antihelmintic and emenagogue (30). Also, *H. annuus* was involved in the composition of some pomades in pharmacy. Its oil has been reported to be used as a constituent in skin-protective medicinal products in newborns (31) and dry skincare for its softening and noncomedogenic properties (32). Considering this ethnobotanical value of *H. annuus L.*, there are several studies evaluating biological activities such as anti-inflammatory, anti-malarial, anti-asthmatic, anti-diabetic, anti-hypertensive, anti-oxidant, anti-tumor, antioxidant, and antimicrobial (33). Many studies have been conducted on the isolation of chemical compounds responsible for biological activities belonging to *H. annuus L.* (20, 29). Most of these studies were carried out using aqueous extracts from leaves or extracts obtained from solvents. The number and variety of isolated compounds indicate the complexity of these extracts and the ability of *H. annuus L.* to produce secondary metabolites. The phytochemicals of *H. annuus* leaves, stems, and roots were reported as flavonoids, phenolic acids, terpenoids, steroids, saponins, and tannins (20, 33). On the other hand, according to our knowledge, there is no much study for QS activity of *H. annuus* leaves. *H. annuus* leaves are normally discarded after gathering sunflower oil from the plant and they are not used. In this context, *H. annuus* leaves have importance for being plant-based material with low cost and also they have potential biological activities. The utilization of leaves from this plant will provide also add value to the economy and also pharmaceutical industry.

The goal of this study to evaluate the QS inhibitory and anti-biofilm potentials of *H. annuus* leaves obtained from Thrace region. For this purpose, anti-QS and anti-biofilm activities of ethyl acetate extracts of the decoction and infusion samples from *H. annuus* leaves were examined on the biosensor strains of *P. aeruginosa* (*lasB-gfp*, *rhlA-gfp* and *pqsA-gfp*) and *PAO1* wild type strain, respectively.

II. MATERIAL AND METHODS

2.1. Sample Collection and Obtaining Ethyl Acetate Extracts from *H.annuus* leaves

The samples of *H.annuus* leaves were washed and dried on air. 10 gram of each sample was weighed and pulverized. Then, these samples were divided into two groups called the infusion and decoction group. The samples belonging to two groups were taken into sterile bottles. The ethyl acetate as a solvent was utilized. After the addition of the solvent, the bottles were placed in the dark for three days. The solvent evaporation was carried out in a rotary evaporator at 40 °C. The extracts of *H.annuus* leaves were weighed again to obtain the weight of crude extracts. These extracts were then dissolved in 100% DMSO with a stock concentration of 16 mg/ml and diluted with physiological saline for anti-QS and anti-biofilm tests.

2.2. Bacterial Strains

lasB-gfp, *rhlA-gfp* and *pqsA-gfp* biomonitor strains with *lasR*, *rhlR*, *pqsR*-regulated promoters, and green fluorescent protein (gfp) gene were developed by Hentzer *et al.* (34), Yang *et al.* (35) and Yang *et al.* (36). These strains were utilized in the experiments to evaluate QS inhibition. As growth medium, M9 minimal media supplemented with 2.5 mg/l thiamine, 0.5% (wt/vol) glucose, and 0.5% (wt/vol) casamino acids, was utilized.

2.3. QSI Screening

A modified method was carried out for the QSI screenings (37). The growth medium mentioned above was put into wells of 96-well black microplates (Nunc, Thermo Scientific). After two-fold serial dilutions of extracts were made, test concentrations of applied extracts were 240 µg/ml, 120 µg/ml and 60 µg/ml. Then, the overnight cultures of *lasB-gfp*, *rhlA-gfp* and *pqsA-gfp* biomonitor strains with an OD 450 nm of 0.1 were put into the test, positive and negative control wells. The blank wells were also included in the experiments. Cytation 3 multimode microplate reader (Biotek) was utilized for the monitorization of the bacterial growth and *gfp* expressions. Data were recorded for 16 hours by taking absorbance and fluorescence measurements every 30 minutes. The fluorescence expressions of biomonitor strains were measured at 485 nm excitation and 535 nm emission wavelengths.

2.4. Biofilm Experiments

P.aeruginosa PAO1 was incubated overnight at 37°C in the growth medium. Biofilm experiments were done in 96-well microplates. The ethyl acetate extracts of the decoctions and infusions from *H. annuus* leaves were tested at the dosages of 240, 120 and 60 µg/ml. The experiments included negative, positive control, and blind wells. The tests were performed in three replicates. The biofilm forms were stained with 0.1% crystal violet and measured at OD 590 nm in the microplate reader (Cytation 3-BioTek).

III. RESULTS

3.1. Findings on the QS and Biofilm Inhibition of Ethyl Acetate Extracts of Decoction from Leaves of *H.annuus*

The inhibition rates for the extracts are given in **Table 1**. The highest inhibition rates on *las*, *rhl* and *pqs* system were recorded at the highest concentration (240 µg/ml) by the treatment of ethyl acetate extracts of decoction samples from leaves of *H.annuus*. The inhibition percentages were 70.61 for *las*, 44.09 for *rhl* and 83.77 for *pqs*, respectively. The anti-biofilm effect of the same extracts was detected to be 50.82% (±1.36) at the 240 µg/ml. Dose response curves of *lasB-gfp*, *rhlA-gfp* and *pqsA-gfp* biomonitor strains of *P. aeruginosa* treated with ethyl acetate extracts of decoction from leaves of *H.annuus* are shown in **Figure 1-4**.

Table 1. The inhibition rates for all doses (240, 120 and 60 µg/ml) against biosensor strains of *P. aeruginosa* (*lasB-gfp*, *rhlA-gfp* and *pqsA-gfp*) on QS system and inhibition rates against PAO1 wild type on biofilm formation.

Strains		Concentrations	The inhibition rates % of ethyl acetate extracts of decoction from leaves of <i>H. annuus</i>
QS inhibition	<i>lasB-gfp</i>	240 µg/ml	70.61
		120 µg/ml	55.95
		60 µg/ml	41.50
	<i>rhlA-gfp</i>	240 µg/ml	44.09
		120 µg/ml	27.38
		60 µg/ml	13.85
<i>pqsA-gfp</i>	240 µg/ml	83.77	
	120 µg/ml	68.05	
	60 µg/ml	55.56	
Biofilm inhibition	PAO1 wild type	240 µg/ml	50.82 (±1.36)
		120 µg/ml	25.27 (±2.41)
		60 µg/ml	19.79 (±2.15)

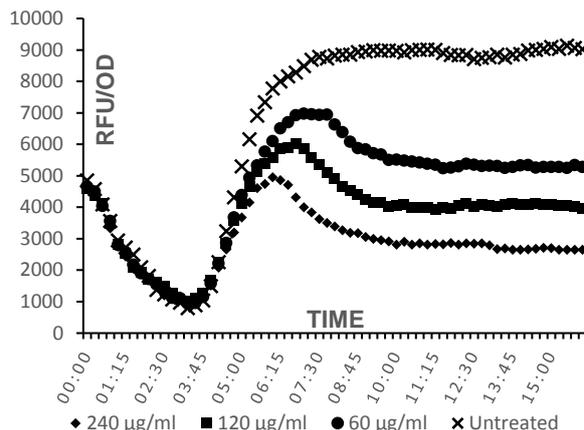


Figure 1. QS inhibition of *lasB-gfp* monitor strains treated extracts of decoction from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

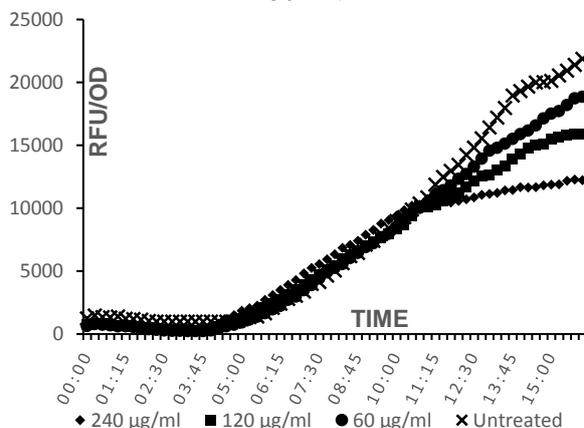


Figure 2. QS inhibition of *rhlA-gfp* monitor strains treated extracts of decoction from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

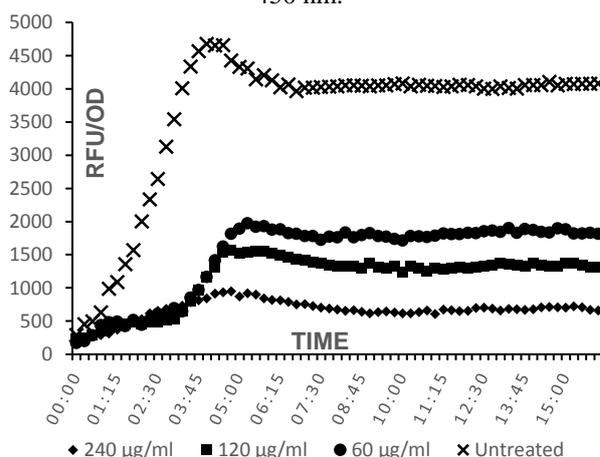


Figure 3. QS inhibition of *pqsA-gfp* monitor strains treated extracts of decoction from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

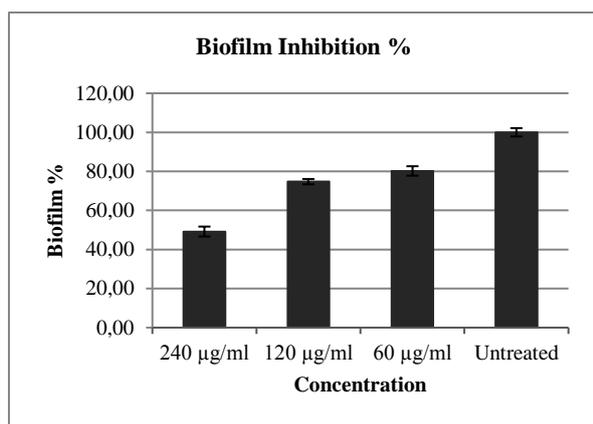


Figure 4. Anti-biofilm properties of ethyl acetate extracts of decoction from leaves of *H. annuus* at certain concentrations of 240, 120, 60 µg/ml against *PAOI* strain.

3.2. Findings on the QS and Biofilm Inhibition of Ethyl Acetate Extracts of Infusion from Leaves of H.annuus

The inhibition rates for the extracts are given in **Table 2**. The ethyl acetate extracts of infusion samples from leaves of *H.annuus* potentially inhibited three QS system of *P. aeruginosa* at the concentration of 240 µg/ml. These inhibition rates were recorded as 62.08% for *las*, 45.15% for *rhl* and 77.79% for *pqs*, respectively. The inhibition ratio of the same extracts on the biofilm formation of *PAOI* strain was found to be 53.88% (±3.94) at the concentration of 240 µg/ml. Dose response curves are shown in **Figure 5-8**.

Table 2. The inhibition rates for all doses (240, 120 and 60 µg/ml) against biosensor strains of *P. aeruginosa* (*lasB-gfp*, *rhlA-gfp* and *pqsA-gfp*) on QS system and inhibition rates against *PAOI* wild type on biofilm formation.

Strains		Concentrations	The inhibition rates % of ethyl acetate extracts of infusion from leaves of <i>H.annuus</i>
QS inhibition	<i>lasB-gfp</i>	240 µg/ml	62.08
		120 µg/ml	48.20
		60 µg/ml	37.72
	<i>rhlA-gfp</i>	240 µg/ml	45.15
		120 µg/ml	30.15
		60 µg/ml	17.81
	<i>pqsA-gfp</i>	240 µg/ml	77.79
		120 µg/ml	68.45
		60 µg/ml	54.29
Biofilm inhibition	<i>PAOI</i> wild type	240 µg/ml	53.88 (±3.94)
		120 µg/ml	25.42 (±2.21)
		60 µg/ml	12.78 (±2.20)

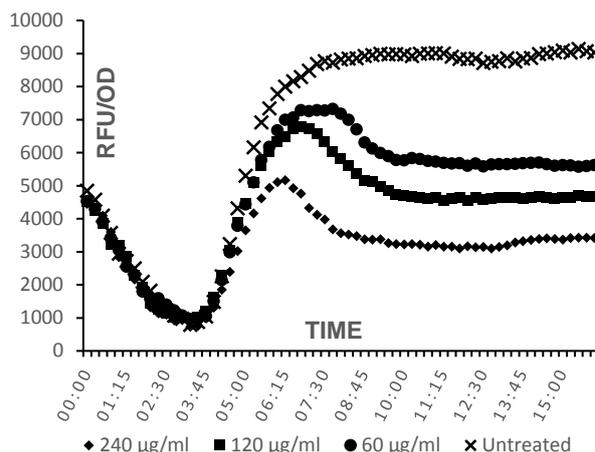


Figure 5. QS inhibition of *lasB-gfp* monitor strains treated extracts of infusion from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

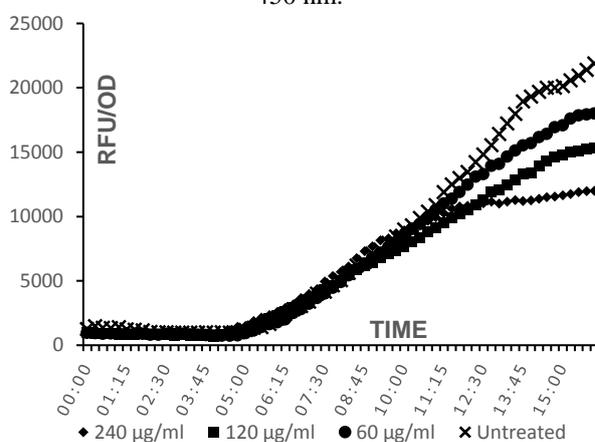


Figure 6. QS inhibition of *rhlA-gfp* monitor strains treated extracts of infusion from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

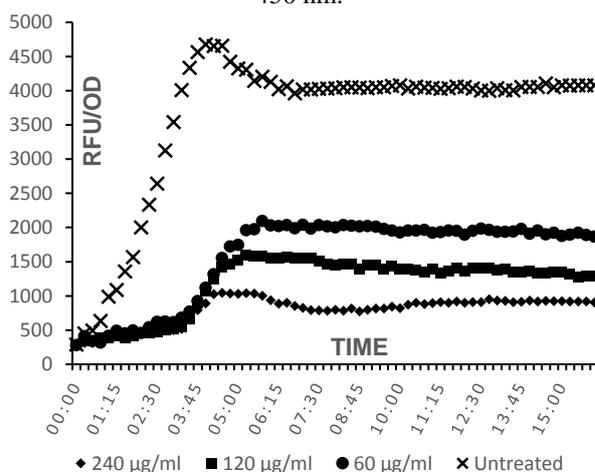


Figure 7. QS inhibition of *pqsA-gfp* monitor strains treated extracts of infusion from leaves of *H. annuus* at certain concentrations of 240, 120 and 60 µg/ml. Data are shown as relative fluorescence unit over OD 450 nm.

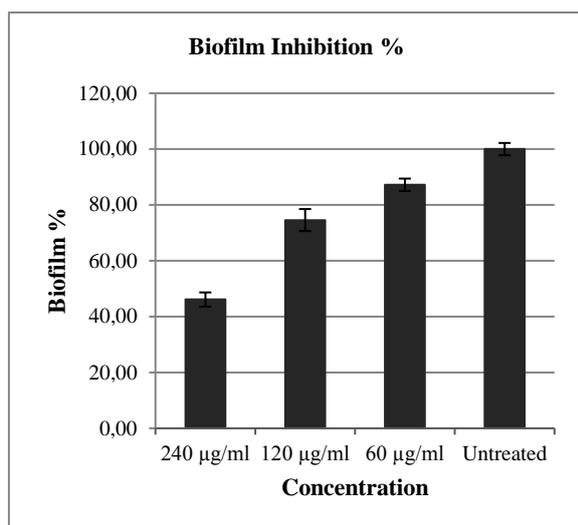


Figure 8. Anti-biofilm properties of ethyl acetate extracts of infusion from leaves of *H. annuus* at certain concentrations of 240, 120, 60 µg/ml against *PAOI* strain.

IV. DISCUSSION

To our knowledge, *H. annuus* leaves have not been evaluated before for anti-QS and anti-biofilm properties against *P. aeruginosa*. In the present study, the potential inhibitory efficacies of the ethyl acetate extracts of the decoction and infusion samples obtained from *H. annuus* leaves on *las*, *rhl* and *pqs* systems and biofilm formation of *P. aeruginosa* were demonstrated.

As known, the therapeutic effect of traditional antibiotics depends on the disruption of the growth cycle, cell wall and protein synthesis, DNA replication. These treatment approaches have been considerably effective over the years but then, overuse or misuse of these antibiotics led to emergence of resistant populations. Therefore, the need for alternative treatment strategies and new antibiotic components have come into prominence (6, 38-40). In many studies, QS inhibitors have been investigated from natural sources such as plants, animals, and algae and they are still currently under investigation. Also, studies on synthetic QS inhibitors are also available in the literature (41). In the view of many plants have various biological activities such as antibacterial, anti-tumor and anti-fungal, it is thought that plants may have also anti-QS effects. Therefore, there is increasing interest in plants for their probable potential for QS inhibition. At this point, inhibition of QS through ethnobotanically valuable plants with abundant bioactive potential can be an alternative strategy to the global antibiotic resistance problem. *H. annuus* is one of these plants that utilized for many years traditionally and there are several studies evaluating its biological potential such as anti-inflammatory, antioxidant, antitumor, antiasthmatic, antipyretic, antihypoglycemic, antifungal and antimicrobial activities from seed oil, shoots and tinctures (20).

In the literature, there are many studies about the antibacterial potential of different parts of this plant. The antibacterial properties of *H. annuus* were studied against various bacteria. However, there are no many studies about antibacterial properties of *H. annuus* against *P. aeruginosa*. The methanol extracts of *H. annuus* seeds were reported to be effective against some Gram-negative bacteria, and Gram-positive bacteria (29). In another study, *H. annuus* seed oil was found to be successful against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (42). On the other hand, the aqueous extracts of *H. annuus* were found to be ineffective against *E. coli*, *B. subtilis*, and *S. aureus* but showed high activity against *K. pneumonia* (28). The antibacterial potentials of *H. annuus* seem to be depending on several parameters such as tested bacterial species, used solvents and/or the parts of the plant. These studies reveal the fact that differences in the sensitivity of Gram-positive and Gram-negative bacteria might be dependent on the cell wall structure or expected/unexpected effect might be from different compounds obtained from various solvents.

In a recent study, QSI activity of sunflower oil has been reported. The researchers evaluated the QSI potential of sunflower oil depending on violacein production and they showed inhibitory effects of sunflower oil in a dose-dependent manner (43). In the literature, QS inhibition is generally assessed by the production of violacein pigment in the *C. violaceum* mutant strain *CV026*, which cannot synthesize AHL. On the other hand, fluorescence-based biosensor strains provide a direct evaluation of QS-related gene expression. In the present study, the inhibitory effects of tested extracts on QS system of *P. aeruginosa* were tested via biosensor strains (*lasB-gfp*, *rhlA-gfp* and *pqsA-gfp*).

Infusions and decoctions are used in modern phytotherapy where the active ingredients are water-soluble. The decoctions are made by boiling plant in water for some time to remove the soluble components. A mixture of 2 and 12 herbal compounds obtained by boiling in water is a conventional herbal dosage form commonly used. The decoctions are normally appropriate for rigid plant materials such as barks, roots and sometimes may also be prepared from plants with low water-soluble components. Since water is not evaluated amongst good solvents for most of the active ingredients in plants, a relatively short extraction time (typically 5 to 10 minutes) used in their preparations for decoctions and infusions (44). In this study, both decoction and infusion methods were tested because different active compounds from *H. annuus* leaves may have anti-QS and anti-biofilm effects. In addition, extraction with an additional ethyl acetate solvent was carried out to reveal more ingredients that are active.

Amongst tested concentrations, the maximum inhibitory effect was recorded at 240 µg/ml in our study. The more prominent potential inhibitory effects

were recorded against the *las* (70.61%) and *pqs* (83.77%) systems than *rhl* (44.09%) system for the decoction extracts. Similarly to the extracts of the decoction samples, the most inhibitory effects were detected for the *pqs* system (77.79%) by the extracts of infusion samples from the leaves of *H.annuus*. For these extracts, the inhibition rates in *las* and *rhl* system were detected to be 62.08% and 45.15%, respectively. Approximately 9% difference in inhibition percentages on *las* system were observed between decoction (70.61%) and infusion (62.08%) extracts. Also, a 6% difference was observed for *pqs* system between decoction (83.77%) and infusion (77.79%) extracts. From these results, our decoction and infusion extracts were both more effective on *pqs* system and also on *las* system but slightly effective on *rhl* system. The inhibitory effect of decoction and infusion extracts from leaves of *H.annuus* on *rhl* system was found to be similar (44.09% and 45.15%, respectively). As in other test groups, most inhibition by the extracts against *rhl* system was obtained at the concentration of 240 µg/ml. Taken together, we may suggest that decoction extracts were more effective on *las* and *pqs* systems. Biofilm formation was also inhibited by the tested extracts and inhibition ratios were similar between the extracts (50.82% and 53.88%).

On the other side, *H.annuus* is mainly used as the source of sunflower oil and its leaves are not normally used for any purpose and they are discarded. In this sense, the re-evaluation of leaves of this plant that will be disposed may provide highly possible add value to the country's economy. *H. annuus* leaves may also ensure beneficial effects in terms of health without any cost. Therefore, there are many positive aspects of the use of *H. annuus* leaves in healthcare because the leaves are plant-based material and have cost-effectiveness as well as their potential therapeutic aspects and evaluation of the material to be discarded.

Given the ethnobotanical value and biological activities of *H. annuus*, and the fact that it is a plant-based and low-cost material, the ethyl acetate extracts of decoctions and infusions from *H. annuus* leaves may alternatively be utilized for the inhibition of QS system and biofilm formation of *P. aeruginosa*.

V. CONCLUSIONS

In the present study, we demonstrated the potential inhibition of the ethyl acetate extracts of the decoction and infusion samples from the leaves of *H.annuus* on *P. aeruginosa las, rhl* and *pqs* QS system and biofilm formation. The fact that *H. annuus* has anti-QS and anti-biofilm activities indicates that it may have potential raw material in drug discovery. Considering the abundance of our country in terms of sunflowers, *H.annuus* extracts and/or its constituents can be easily integrated into the pharmaceutical industry. However, there is a need for more detailed investigations and determination of the active substances that have QSI effect.

REFERENCES

- [1] Butler, M.S., Blaskovich, M.A. and Cooper, M.A. (2013). Antibiotics in the clinical pipeline in 2013. *J. Antibiot. Res.*, 66(10), 571-591.
- [2] Wright, G.D. (2017). Opportunities for natural products in 21 st century antibiotic discovery. *Nat. Prod. Rep.*, 34(7), 694-701.
- [3] Centres for Disease Control and Prevention (US). (2013). Antibiotic resistance threats in the United States.
- [4] Pandhare, A. (2015). Bacterial resistance in India: Studying plasma antibiotic levels. *IJCCM*, 19(10), 574.
- [5] World Health Organization. (2017). Antibacterial agents in clinical development. World Health Organization: Geneva, Switzerland.
- [6] Rasko, D.A. and Sperandio, V. (2010). Anti-virulence strategies to combat bacteria-mediated disease. *Nat. Rev. Drug Discov.*, 9(2), 117-128.
- [7] Dickey, S.W., Cheung, G.Y. and Otto, M. (2017). Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. *Nat. Rev. Drug Discov.*, 16(7), 457.
- [8] Miller, M.B. and Bassler, B.L. (2001). Quorum sensing in bacteria. *Annu. Rev. Microbiol.*, 55(1), 165-199.
- [9] Smith, R.S. and Iglewski, B.H. (2003). *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. *J. Clin. Investig.*, 112(10), 1460-1465.
- [10] Williams, P. and Cámara, M. (2009). Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. *Curr. Opin. Microbiol.*, 12(2), 182-191.
- [11] Antunes, L.C.M., Ferreira, R.B., Buckner, M.M. and Finlay, B.B. (2010). Quorum sensing in bacterial virulence. *Microbiology*, 156(8), 2271-2282.
- [12] Rutherford, S.T. and Bassler, B.L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb. Perspect. Med.*, 2(11), a012427.
- [13] Olson, M.E., Ceri, H., Morck, D.W., Buret, A.G. and Read, R.R. (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Can. J. Vet. Res.*, 66(2): 86.
- [14] Driscoll, J.A., Brody, S.L. and Kollef, M.H. (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs*, 67(3), 351-368.
- [15] Lee, J., Wu, J., Deng, Y., Wang, J., Wang, C., Wang, J., Chang, C., Dong, Y., Williams, P. and Zhang, L.H. (2013). A cell-cell communication signal integrates quorum sensing and stress response. *Nat. Chem. Biol.*, 9(5), 339.
- [16] Lee, J. and Zhang, L. (2015). The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein & Cell*, 6(1), 26-41.
- [17] Erickson, D.L., Endersby, R., Kirkham, A., Stuber, K., Vollman, D.D., Rabin, H.R., Mitchell, I. and Storey, D.G. (2002). *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic

- fibrosis. *Infect. Immun.*, 70(4), 1783-1790.
- [18] Kalia, V.C. (2015). Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New Delhi, India.
- [19] Yesilyurt, E.B., Simsek, I., Tuncel, T., Akaydin, G. and Yesilada, E. (2017). Marmara Bölgesi'nin bazı yerleşim merkezlerinde halk ilacı olarak kullanılan bitkiler. *Marmara Pharm. J.*, 21: 132-148.
- [20] Bashir, T., Zahara, K., Haider, S. And Tabassum, S. (2015). Chemistry, pharmacology and ethnomedicinal uses of *Helianthus annuus* (sunflower): a review. *PAB.*, 4(2), 226.
- [21] Fowler, M.W. (2006). Plants, medicines and man. *J. Sci. Food Agric.*, 86(12), 1797-1804.
- [22] Karunamoorthi, K., Jegajeevanram, K., Vijayalakshmi, J. and Mengistie, E. (2013). Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. *J. Evid. Based Complementary Altern. Med.*, 18(1), 67-74.
- [23] Kocabas, A. (2017). Ease of phytochemical extraction and analysis from plants? *Ant. J. Bot.*, 1(2):6-31.
- [24] Jarić, S., Popović, Z., Mačukanović-Jocić, M., Djurdjević, L., Mijatović, M., Karadžić, B., Mitrović, M. and Pavlović, P. (2007). An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). *J. Ethnopharmacol.*, 111(1), 160-175.
- [25] Cepni, E. and Gurel, F. (2011). Bitkilerden elde edilen anti quorum sensing bileşikleri ve yeni ilaç geliştirmedeki potansiyelleri. *Turk. J. Microbiol.*, 41 (4):131-138.
- [26] Al-douri, N.A. (2000). A survey of medicinal plants and their traditional uses in Iraq. *Pharm. Biol.*, 38(1), 74-79.
- [27] Gaur, R.K., Petrov, N.M., Patil, B.L. and Stoyanova, M.I. (Eds.). (2016). *Plant Viruses: Evolution and Management*. Springer.
- [28] Sharma, D., Lavania, A.A. and Sharma, A. (2009). *In vitro* comparative screening of antibacterial and antifungal activities of some common plants and weeds extracts. *Asian J. Exp. Sci.*, 23(1), 169-172.
- [29] Subashini, R. and Rakshitha, S.U. (2012). Phytochemical screening, antimicrobial activity and *in vitro* antioxidant investigation of methanolic extract of seeds from *Helianthus annuus L.* *Chem. Sci. Rev. Lett.*, 1(1), 30-34.
- [30] Bnouham, M., Mekhfi, H., Legssyer, A. and Ziyat, A. (2002). Ethnopharmacology forum medicinal plants used in the treatment of diabetes in Morocco. *Int J Diabetes & Metabolism*, 10, 33-50.
- [31] Visscher, M.O. (2009). Update on the use of topical agents in neonates. *Newborn and Infant Nursing Reviews*, 9(1), 31-47.
- [32] Stoia, M. and Oancea, S. (2015). Selected evidence-based health benefits of topically applied sunflower oil. *App. Sci. Rep.*, 10, 45-49.
- [33] Guo, S., Ge, Y. and Jom, K.N. (2017). A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus L.*). *Chem. Cent. J.*, 11(1), 95.
- [34] Hentzer, M., Riedel, K., Rasmussen, T.B., Heydorn, A., Andersen, J.B., Parsek, M.R., Rice, S.A., Eberl, L., Molin, S., Høiby, M., Kjelleberg, S. and Givskov, M. (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology*, 148(1), 87-102.
- [35] Yang, L., Barken, K.B., Skindersoe, M.E., Christensen, A.B., Givskov, M. and Tolker-Nielsen, T. (2007). Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology*, 153(5), 1318-1328.
- [36] Yang, L., Rybtke, M. T., Jakobsen, T.H., Hentzer, M., Bjarnsholt, T., Givskov, M. and Tolker-Nielsen, T. (2009). Computer-aided identification of recognized drugs as *Pseudomonas aeruginosa* quorum-sensing inhibitors. *Antimicrob. Agents Chemother.*, 53(6), 2432-2443.
- [37] Bjarnsholt, T., Jensen, P. Ø., Rasmussen, T. B., Christophersen, L., Calum, H., Hentzer, M., Hougen, H.P., Rygaard, J., Moser, C., Eberl, L., Høiby, N. and Givskov, M. (2005). Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. *Microbiology*, 151(12), 3873-3880.
- [38] Walsh, C. (2003). Antibiotics: actions, origins, resistance. ASM. Press, Washington DC, USA.
- [39] Werner, G., Strommenger, B. and Witte W. (2008). Acquired vancomycin resistance in clinically relevant pathogens. *Future Microbiol.*, 3(5), 547-562.
- [40] Ventola, C.L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharm. Ther.*, 40(4), 277.
- [41] LaSarre, B., and Federle, M.J. (2013). Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol. Mol. Biol. Rev.*, 77(1), 73-111.
- [42] Alibe, I.M. and Inuwa, B. (2012). Physicochemical and anti-microbial properties of sunflower (*Helianthus annuus L.*) seed oil. *Int. J. Sci. Technol.*, 2(4), 151-154.
- [43] Pérez-López, M., Garcia-Contreras, R., Soto-Hernández, M., Rodríguez-Zavala, J.S., Martínez-Vázquez, M., Prado-Galbarro, F.J. and Castillo-Juárez, I. (2018). Antiquorum sensing activity of seed oils from oleaginous plants and protective effect during challenge with *Chromobacterium violaceum*. *J. Med. Food*, 21(4), 356-363.
- [44] Bone, K. and Mills, S. (2012). Principles and practice of phytotherapy modern herbal medicine: Dosage and dosage forms in herbal medicine. Churchill Livingstone Elsevier. 2nd ed. 121-133.