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RESEARCH ARTICLE

Antimicrobial and anti-quorum sensing activities of giant fennel (*Ferula elaeochytris* Korovin) from the Hatay region

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Hatay bölgesinden çakşır otunun (*Ferula elaeochytris* Korovin) antimikrobiyal ve anti-quorum sensing aktiviteleri

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Öz

Amaç: Günümüzde giderek artan antibiyotik direnci, bilim dünyasını yeni antimikrobiyal moleküllerin keşfine veya alternatif mücadele yöntemlerinin geliştirilmesi üzerine odaklamıştır. Bu alternatif mücadele yöntemlerinden bir tanesinin bakterilerde quorum sensing (QS; çoğunluğu algılama) inhibisyonu olacağı öngörülmektedir. Çünkü QS sistemi bakterilerde virülans faktörlerinin sentezinde önemli bir rol oynar. Medikal öneme sahip birçok bitkinin umut verici antimikrobiyal ve anti-QS aktivitelere sahip olduğu bilinmektedir. Bu bitkilerden birisinin de Anadolu'da yıllardır yaygın olarak kullanılan çakşır otu (*Ferula elaeochytris* Korovin) olabileceğini düşünmekteyiz. Bu nedenle, bu çalışmada *F. elaeochytris* Korovin'in kök özütünün antimikrobiyal ve anti-QS aktivitelerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Özütün antimikrobiyal aktivitesi, çeşitli mikroorganizmalara karşı disk difüzyon yöntemi ile taranmıştır. Özütün etkili suş veya suşlara karşı minimum inhibitör konsantrasyonu (MİK) ve minimum bakterisidal konsantrasyonu (MBK) değerleri sıvı mikrodilüsyon yöntemi ile belirlenmiştir. Anti-QS aktivite, *Pseudomonas aeruginosa* ve *Chromobacterium violaceum* bioraportör suşlarında sırasıyla piyosiyanin ve viyolasin pigmentleri üretiminin inhibisyonu üzerine araştırılmıştır.

Bulgular: Özütün sadece Staphylococcus aureus suşuna karşı antimikrobiyal aktivitesi (zon çapı = 9.3±0.6 mm) tespit edilmiştir. MİK ve MBK değerleri sırasıyla 4.4±1.9 mg/mL ve >105 mg/mL olarak saptanmıştır. Viyolasin üretimi inhibisyonu belirlenmemiştir. Özütün MİK altı konsantrasyon değerleri (1.64 µg/mL ve 3.28 µg/mL) bakteri üremesini baskılamadan piyosiyanin üretimini sırasıyla %60 ve %82 oranında inhibe etmiştir.

Öneri: Bu çalışmanın sonuçları *F. elaeochytris* Korovin'in kök özütünün antistafilokokal ve anti-QS ajanların geliştirilmesi için iyi bir aday olabileceğini göstermektedir.

Anahtar kelimeler: Antimikrobiyal, ekstrakt, *Ferula elaeochytris* Korovin, piyosiyanin, quorum sensing

Abstract

Aim: Today, increasing antibiotic resistance has focused the science world on the discovery of new antimicrobial molecules or the development of alternative methods of struggle. One of the alternative methods is thought to be inhibition of bacterial quorum sensing (QS). Because the QS system performs a crucial part in the synthesis of virulence factors in bacteria. Numerous medicinal plants are known to have promising antimicrobial and anti-QS activities. One of these plants may be giant fennel (*Ferula elaeochytris* Korovin), which has been extensively used in Anatolia for years. Therefore, it was aimed to investigate the antimicrobial and anti-QS activities of the root extract of *F. elaeochytris* Korovin in this study. .

Materials and Methods: The antimicrobial activity of the extract was screened by disc diffusion assay against various microorganisms. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against sensitive strain(s) were determined using broth microdilution assay. Anti-QS activity investigated on inhibition of violacein and pyocyanin production in *Chromobacterium violaceum* and *Pseudomonas aeruginosa* bioreporter strains, respectively.

Results: The extract exhibited the antimicrobial activity against only Staphylococcus aureus (zone of inhibition (ZOI) = 9.3±0.6 mm). The MIC and the MBC values were determined as 4.4±1.9 mg/mL and >105 mg/mL, respectively. No inhibition of violacein production was detected. Pyocyanin production was reduced by 60% and 82% at sub-MIC concentrations (at 1.64 mg/mL and 3.28 mg/mL, respectively) as against the control ($p<0.05$) without suppressing bacterial growth.

Conclusion: This study shows that the root extract of *F. elaeochytris* Korovin may be a good candidate to develop antistaphylococcal and anti-QS agents.

Keywords: Antimicrobial, extract, *Ferula elaeochytris* Korovin, pyocyanin, quorum sensing

Introduction

The genus *Ferula* is found in the Apiaceae family and has about 170 species on earth. They grow naturally in the Mediterranean region east to central Asia. *Ferula* species have a long story of therapeutic use, and their biological properties demonstrated in various studies (Kose et al 2010, Ozek et al 2008, Khoury et al 2017). *Ferula* species are known to contain compounds such as sesquiterpen, sesquiterpene coumarins, sesquiterpene lactones and sulfur-containing compounds (Akaberi et al 2015). *Ferula elaeochytris* Korovin (giant fennel) is widely used among the *Ferula* species and has commercial preparations. In Anatolia, the leaves and roots of this plant have been consumed as a tea for aphrodisiac purposes (Altundag and Ozturk 2011, Güzel et al 2015, Sargin 2015).

Nowadays, increasing antibiotic resistance has led scientists to discover new antibacterial molecules and to find alternative treatment strategies to combat bacteria. Besides, the discovery of new antimicrobial molecules has declined in recent years. Quorum sensing (QS) is a system by which bacteria communicate with each other to regulate most of their pathogenic behaviors and synthesis of virulence factors via small signaling molecules. Anti-QS agents may be considered alternatives to antibiotics because of their capacity to interfere with the synthesis of virulence factors in bacteria (Jiang et al 2019).

Various signal molecules related to QS have been described in bacteria. Autoinducing peptides (AIP) used by Gram-positive bacteria, N-acyl homoserine lactone (AHL) used by Gram-negative bacteria, and autoinducer-2 (AI-2) signaling molecules used by both Gram-negative and Gram-positive bacteria have the most common of these. The QS system can also interfere in many ways. These are prevention of signal molecule production; destruction of signal molecules upon release; and prevention of signal molecule uptake into the cell (Lade et al 2014).

We need to come up with solutions to combat antibiotic resistance, which has become a major problem today. To the best of our knowledge, no investigations on antimicrobial and anti-QS activities, have been reported on the root extract of *F. elaeochytris* Korovin. Thus, we aimed to investigate the antimicrobial and anti-QS activities of the root extract of *F. elaeochytris* Korovin in the current study.

Material and Methods

Plant material and preparation of the extract

The roots of *F. elaeochytris* Korovin were collected in Hatay, Turkey, in September 2019. The root extract of *F. elaeochytris* Korovin was prepared described previously (Lin et al 1999). The collected root samples were properly cleaned and dried at room temperature conditions for 30 days. The dried samples were ground using a laboratory mill and passed through 1 mm mesh sieves to dark glass jars. Then, 100 g of the ground roots was extracted using 1 L from methanol (40% w/v) at 250 rev/min for 24 hours at room temperature. Then, the extract was percolated into a sterile container using Whatman No. 1 filter paper. The solvent was evaporated using a vacuum rotary evaporator at 60°C. The obtained extract was dissolved in sterile distilled water as 210 mg/mL final concentration and used to investigate antimicrobial and anti-QS activities.

Microorganism strains and growth conditions

The antibacterial activity of the extract was tested against two Gram-negative bacteria (*Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853*), two Gram-positive bacteria (*Enterococcus faecalis ATCC 29212* and *Staphylococcus aureus ATCC 25923*), and one fungus (*Candida albicans ATCC 10231*) as representative pathogens.

The anti-QS activity of the extract was tested against *Chromobacterium violaceum ATCC 12472* and *P. aeruginosa* PAO1. *Chromobacterium violaceum ATCC 12472* generates a blue-purple pigment called violacein, using long-chain AHL molecules (C10-C16) as QS signal molecules and is widely used bioreporter strain in screening studies for anti-QS agents (Morohoshi et al 2008). *Pseudomonas aeruginosa* PAO1 produces a bluish pigment called pyocyanin, using short-chain AHL molecules (C4-AHL) and is used as a bioreporter strain like *C. violaceum* (Kalia et al 2015).

Pseudomonas aeruginosa PAO1 is an opportunistic pathogen and widely used as a reference strain in laboratories. It was acquired from the Department of Medical Microbiology, Faculty of Medicine, Kocaeli University. The other strains were ATCC (American Type Culture Collection) reference strains and were get from the culture collection of the Department of Medical Microbiology, Faculty of Medicine, Karadeniz Technical University.

Unless and otherwise stated, *P. aeruginosa* PAO1 and *C. violaceum* and strains were cultured in peptone water and Luria Bertani (LB; NZYTech, Lisbon, Portugal) media. The others were cultured on Mueller hinton agar (MHA; Merck, Darmstadt, Germany) and potato dextrose agar (PDA; Pronadisa, Spain). All culture processes were conducted aerobically at 37°C.

Disc diffusion assay

The antimicrobial activity of the extract was screened according to EUCAST disc diffusion methodology (EUCAST 2020). Shortly, overnight cultures of each strain grown on agar me-

dia were adjusted to 0.5 McFarland turbidity standard (1.0 – 2.0 × 10^8 CFU/mL for bacteria and 1.0 – 2.0 × 10^6 CFU/mL for *Candida albicans*) with colony suspension method. Then, a sterile cotton swab was submerged to the suspension and seeded by swabbing in three directions on MHA for bacteria and PDA for *C. albicans*. Blank discs (6 mm diameter) were set on the surface of the seeded agar media and impregnated with 10 µL extract (210 mg/mL). Imipenem (10 µg/disc; Bioanalyse®, Turkey) and erythromycin (15 µg/disc, only for *S. aureus*; Bioanalyse®, Turkey) were used as positive controls according to EUCAST. No antifungal agent was used as a positive control. Distilled water was used as negative control.

The cultures were incubated at 18 hours for bacteria and 48 hours for *C. albicans*. After the incubation, zone of inhibition (ZOI) surrounding the discs were measured using a ruler (mm).

Broth microdilution assay

The minimum inhibitory concentration (MIC) was determined using the broth microdilution assay as described by Wiegand et al (2008) with some modifications. Briefly, the assay was performed in 96-well microtiter plate containing 100 µL/well of Mueller hinton broth (MHB; Merck, Darmstadt, Germany). One hundred microliters from the stock concentration of the extract (210 mg/mL) was transferred to the first well and two-fold diluted to obtain concentrations ranging from 105 mg/mL to 0.20 mg/mL. Erythromycin (Molekula, UK) solution as a control antibiotic was used, and its tested concentration ranged from 64 µg/mL to 0.125 µg/mL. The cells suspension adjusted to 0.5 McFarland density as described above was added to each well in a volume of 5 µL except sterility control wells. The plate was sealed with a sterile lid and incubated for 18 hours. The lowest concentration of the extract which there was no visible growth of the microorganisms was determined as the MIC value.

The minimum bactericidal concentration (MBC) value was found after the MIC determination. For this, viable cell counts were performed on MHA with subculturing method from concentrations of MIC and above. The first concentration counted as below 100 colonies on the agar medium was interpreted as the MBC value.

Violacein inhibition assay

Violacein inhibitory activity of the extract was investigated against *C. violaceum* ATCC 12472 using the soft agar method described as McClean et al (1997) with some modifications. Briefly, five mililiters of molten soft LB agar (0.5% w/v) was transferred with 50 µL of *C. violaceum* overnight culture in LB broth. The soft agar-culture mixture was gently vortexed and directly poured over the surface of prewarmed LB agar plates. When the soft agar had solidified, wells of 6 mm in diameter were punched in the agar with a sterile cork borer and 50 µL of the extract (210 mg/mL) was pipetted into the agar wells. Vanillin (500 µg/mL; Merck, Darmstadt, Germany) and distilled water were used as positive and negative controls, respectively. The culture was incubated for 18 hours, then analysed for violacein production. Quorum sensing inhibition was determined by a white, opaque, but viable halo surrounding the wells.

Pyocyanin inhibition assay

Pyocyanin inhibitory activity of the extract was investigated against *P. aeruginosa* PAO1 using pyocyanin extraction method described as Essar et al (1990) with slight modifications. Prior to this experiment, the MIC value of the extract against *P. aeruginosa* PAO1 was determined. The sub-MIC concentrations of the extract were used as final concentrations in this assay to avoid any antibacterial effect.

Overnight culture of *P. aeruginosa* PA01 was adjusted to $OD600_{nm}$ 0.1 and transferred to sterile two culture tubes, in volumes of 4922 µL and 4961 µL. The extract was added to one of the tubes in a volume of 78 µL (for the final concentration of 3.28 mg/mL) and to the other in the volume of 39 µL (for the final concentration of 1.64 mg/mL). Thus, the final volume was completed to 5 mL. Besides, one tube was also used as a negative control (sterile distilled water in the same volume). After the tubes were wrapped in aluminum foil, they were incubated for 24 hours at 150 rev/min shaker. Then, 1.5 mL culture from each tube was centrifuged at 10 000 rev/min for 5 min to obtain cell-free supernatant. Chloroform (0.9 mL; Merck, Darmstadt, Germany) was added to the supernatant and mixed vigorously. The chloroform layer was then taken to a new sterile microcentrifuge tube and re-extracted with 0.3 mL of 0.2 N hydrochloric acid (HCl). After centrifuge process, 0.2 mL volume of the top layer (HCl) was transferred to 96-well microplate. The absorbance was determined at 520 nm against 0.2 N HCl using a UV-visible spectrophotometer (BioTek Epoch, Vermont, USA).

Although the extract was studied at sub-MIC concentrations, whether the cell growth was suppressed was confirmed by viable cell count from cultures after 24 hours. The cultures were serially diluted to factors of 10^{-1}–10^{-7} in serum physiological solution, and 50 µL from 10^{-7} dilutions were spread on MHA plates. The plates were incubated for 24 hours, and colony counts were compared with the control.

Statistical analysis

All the experiments were carried out at least three times and results were presented as mean ± standard deviation (SD) values. The data were analysed for normality using the model Wilks-Shapiro test (Shapiro and Wilks, 1965). Paramet-

ric data was analysed with independent samples t-test using IBM-SPSS statistics version 23.0 (IBM Inc., Armonk, NY, USA), and $p<0.05$ was recognized as statistically significant.

Results

The antimicrobial activity of the root extract of *F. elaeochytris* Korovin was tested against various microorganisms by the disc diffusion assay and results are shown in Table 1. The extract possessed the antimicrobial activity against *S. aureus* (ZOI = 9.3±0.6 mm; Figure 1). The extract had no antimicrobial activity against other tested microorganisms. After the antimicrobial activity was determined by the disc diffusion method, the MIC value was detected by the broth microdilution assay. The MIC and MBC values of the extract against *S. aureus* were found to be 4.4±1.9 mg/mL and >105 mg/mL, respectively (Table 1).

Anti-QS activity of the extract was investigated against *P. aeruginosa* PAO1 and *C. violaceum* ATCC 12472 bioreporter strains, which produced pyocyanin and violacein pigments interacting with the QS mechanism, respectively. Pyocyanin production was reduced by 60% and 82% at concentrations of 1.64 mg/mL and 3.28 mg/mL compared with the control ($p<0.05$) without interfering with bacterial growth (Figure 2). No inhibition of violacein pigmentation was observed.

Discussion

The emergence of antibiotic-resistant pathogens makes the cure and control of infectious diseases difficult (Nellums et al 2018). The commercial antibiotics may also be insufficient to combat these resistant pathogens (Towse et al 2017). For this reason, it is very important to find compounds that will support antibiotics or strengthen their effects. We believe that traditional medicinal plants have very significant potential in this regard. In our study, the antimicrobial activity of the root extract of *F. elaeochytris* Korovin was screened against several reference strains representing medically important pathogens by the disc diffusion method.

The antimicrobial activity of the extract was observed only

Table 1. Antimicrobial activity of the root extract of *F. elaeochytris*.*

	ZOI (mm)	MIC (mg/mL)	MBC (mg/mL)
S. aureus ATCC 25923	9.3±0.6	4.4±1.9	>105
E. faecalis ATCC 29212	0	-	-
E. coli ATCC 25922	0	-	-
P. aeruginosa ATCC 27853	0	-	-
C. albicans ATCC 10231	0	-	-

* Data are presented as the mean ± SD. -, not tested.

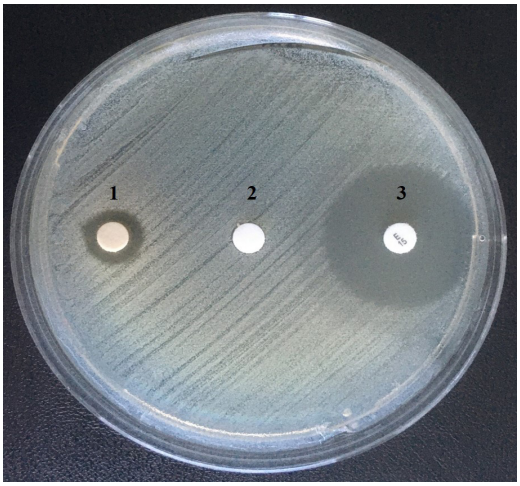

Figure 1. Antimicrobial activitiy of the root extract of *F. elaeochytris* against *S. aureus* ATCC 25923, 1; the root extract of *F. elaeochytris* Korovin, 2; negative control (distilled water), 3; positive control (erythromycin).

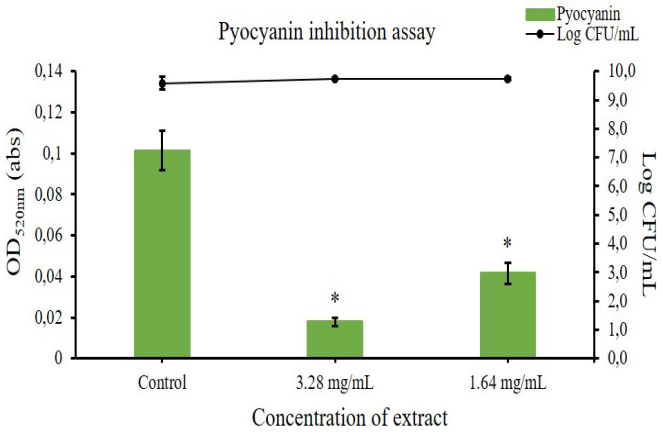

Figure 2. Effects of different concentrations of the root extract of *F. elaeochytris* on the production of pyocyanin and bacterial growth on *P. aeruginosa* PAO1. Data are presented as the mean ± SD. * $p<0.05$.

against *S. aureus*. However, the antimicrobial activity of the extract against *S. aureus* was rather weak compared to erythromycin (ZOI = 28.3±0.6 mm) used as a positive control (Figure 1). The MIC value of the extract against *S. aureus* was found to be 4.4±1.9 mg/mL while the MBC value as >105 mg/mL. This figure suggests that the root extract of *F. elaeochytris* has a bacteriostatic effect. Meanwhile, the MIC value of erythromycin against *S. aureus* was 0.25 µg/mL.

To the best of our knowledge, there is no study in the literature on the antimicrobial activity of the root extract of *F. elaeochytris* Korovin. Therefore, our study will be the first to evaluate the antimicrobial activity of the root extract of *F. elaeochytris*. In only one study conducted by Khoury et al (2017), the antimicrobial activity of the essential oils of *F. elaeochytris* fruits was tested against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *C. albicans* ATCC 10231, *Cryptococcus neoformans SNB-CN1, Candida parapsilosis ATCC 22019, Aspergillus fumigatus SNB-AF1, Trichophyton tonsurans SNB-TT1, Trichophyton soudanense SNB-TS1, Trichophyton mentagrophytes SNB-TM1, Trichophyton rubrum SNB-TR1, and Trichophyton violaceum SNB-TV1.* They detected antimicrobial activity against all tested microorganisms except *E. coli ATCC 25922, C. parapsilosis ATCC 22019, and A. fumigatus SNB-AF1.*

Ghasemi et al (2005) found that the essential oil from the fruits of *Ferula* gummosa possessed high antibacterial and antifungal activities against Gram-positive (*Staphylococcus epidermidis PTCC 1114, S. aureus PTCC 1112, and Bacillus subtilis PTCC 1023*) and Gram-negative (*E. coli* PTCC 1338, *Salmonella Typhi PTCC 1609, and Pseudomonas aeruginosa PTCC 1074*) bacteria and fungi (*C. albicans ATCC 14053* and *Candida kefyr ATCC 38296*).

Iranshahi et al (2008) reported that the polysulphide-rich fruit oil of Ferula latisecta showed antibacterial activity against Gram-positive (*Bacillus cereus ATCC 10876* and *S. aureus* ATCC 6538) but not Gram-negative bacteria (*P. aeruginosa ATCC 9027* and *E. coli ATCC 10536*). Also, it had a comparatively potential inhibitory activity against *C. albicans* ATCC 10231.

Asili et al (2009) stated that the essential oil of the fruits of Ferula badrakema exhibited moderate antibacterial activity against *S. aureus ATCC 6538* and *B. cereus ATCC 10876* as Gram-positive bacteria and *C. albicans ATCC 10231* as fungus. However, Gram-negative bacteria such as *E. coli ATCC 10536* and *P. aeruginosa ATCC 9027* determined not to be susceptible to inhibitory effects of this essential oil.

In our study, we found that the root extract of *F. elaeochytris* Korovin showed antimicrobial activity against only *S. aureus* as a Gram-positive, albeit weak. However, we believe that this extract may have a potential candidate for developing new antistaphylococcal agents.

Many bacterial species regulate the synthesis of virulence factors such as swarming, biofilm formation, toxin, exopolysaccharide, and pigment productions using the QS signal molecules (Eberl et al 1996, McClean et al 1997, Ohtani et al 2002, Marketon et al 2003, Rice et al 2005). Therefore, QS inhibition in bacteria may be an alternative treatment strategy to combat infectious diseases, and research on QS inhibitors is also gaining importance nowadays. To date, many agents that have been shown to be potential QS inhibitors have been gained to the literature. However, the fact that many of them, such as halogenated furanones, have toxic properties restrict their use in practice (Hentzer and Givskov, 2003). Therefore, it is important to introduce new reliable QS inhibitors to the literature. In the current study, we investigated the QS inhibitory activity of the root extract of *F. elaeochytris* Korovin. Considering our results, it was determined in qualitative screening on violacein inhibition that the extract could not inhibit the production of violacein *C. violaceum*. Pyocyanin production was inhibited by 60% and 82% at two sub-MIC concentrations compared with the control ($p<0.05$) without interfering with bacterial growth. Testing the extract at sub-MIC concentrations and the absence of a decrease in viable cells indicated that the suppression of pyocyanin production in *P. aeruginosa* was due to the QS inhibitory activity of the extract.

In particular, *Ferula asafoetida* extracts including oleo-gum-resin and essential oil were researched for anti-quorum sensing activity in before reports. These extracts of *F. asafoetida* were effective in different levels on tested bioreporter strains. Sepahi et al (2015) and Khambhala et al (2016) reported that the essential oil of *F. asafoetida* inhibited violacein and pyocyanin production in *C. violaceum* and *P. aeruginosa*, respectively. Also, Sepahi et al (2015) stated that elastase, pyoverdine, and biofilm formation was decreased on *P. aeruginosa* by the essential oil of *F. asafoetida*. Jomehpour et al (2016) investigated the effect of *F. asafoetida*'s oleo-gum resin on the expression of pathogenesis-related *tst* and *hdl* genes regulated by QS in MRSA and MSSA (methicillin-sensitive *S. aureus*) strains. They determined that the decrease of the *hld* gene expression on MRSA.

Violacein production in *C. violaceum* ATCC 12472 is regulated by long-chain (C10-C16) AHL molecules while pyocyanin production in *P. aeruginosa* PAO1 is regulated by short-chain AHL (C4-AHL) molecules. In our study, the fact that the extract was not able to inhibit the production of violacein pigment and inhibited the production of pyocyanin pigment suggested that compounds of the extract affected short-chain AHL molecules. Apart from pyocyanin, *P. aeruginos*a strains also regulate the expression of genes responsible for elastase, siderophore, and rhamnolipid synthesis with C4-AHL signal molecules. Therefore, if it is assumed that these virulence factors will also be inhibited, the root extract of *F. elaeochytris* Korovin may have significant potential in controlling

the pathogenesis of *P. aeruginosa*. However, uncontrolled and overuse of the consumption of this plant may cause liver toxicity. For this reason, it should be kept in mind that this plant may cause liver toxicity when excessively used in various forms such as tea.

Limitations of the study: It is necessary to identify the compounds responsible for the antibacterial activity by performing a phytochemical analysis of the extract. We also recommend combining bioactive compounds with commercial antibiotics and testing against strains that exhibit a specific resistance phenotype such as MRSA (methicillin-resistant *Staphylococcus aureus*) in future work.

Conclusion

To the best of our knowledge, this research is the first study to investigate the antimicrobial and anti-QS activities of the root extract of *F. elaeochytris* Korovin. The results showed that the root extract of *F. elaeochytris* Korovin has the potential to develop an antistaphylococcal agent. Furthermore, the root extract of *F. elaeochytris* Korovin showed that it is a good candidate for the development of anti-QS agent. All the data obtained in this study are preliminary results. The determination of the chemical contents of the extract and the effectiveness of bioactive molecules in combination with reference antibiotics should be evaluated.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study. or no moral support.

References

Akaberi M, Iranshahy M, Iranshahi M, 2015. Review of the traditional uses, phytochemistry, pharmacology and toxicology of giant fennel (*Ferula communis* L. subsp. *communis*). Iran J Basic Med Sci, 18(11), 1050-1062.

Altundag E, Ozturk M, 2011. Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Procedia – Social and Behavioral Sciences, 19, 756-777.

Asili J, Sahebkar A, Bazzaz FBS, Sharifi S, et al., 2009. Identification of essential oil components of *Ferula badrakema* fruits by GC-MS and 13C-NMR methods and evaluation of its antimicrobial activity. J Essent Oil-Bear Plants, 12(1), 7-15.

Eberl L, Winson MK, Sternberg C, Stewart GS, et al., 1996. Involvement of N-acyl-L-hormoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. Mol Microbiol, 20(1), 127–136.

Essar DW, Eberly L, Hadero A, Crawford IP, 1990. Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. J Bacteriol, 172(2), 884-900.

Ghasemi Y, Faridi P, Iraj M, Mohagheghzadeh A, 2005. *Ferula gummosa* fruits: An aromatic antimicrobial agent. Chem Nat Compd, 41(3), 311-314.

Güzel Y, Güzelşemme M, Miski M, 2015. Ethnobotany of medicinal plants used in Antakya: A multicultural district in Hatay Province of Turkey. J Ethnopharmacol, 174, 118-152.

Hentzer M, Givskov M, 2003. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest, 112(9), 1300-1307.

Iranshahi M, Hassanzadeh M, Bazzaz F, Sedigheh B, et al., 2008. High content of polysulphides in the volatile oil of *Ferula latisecta* Rech. F. et Aell. fruits and antimicrobial activity of the oil. J Essent Oil-Bear, 20(2), 183-185.

Jiang Q, Chen J, Yang C, Yin Y, et al., 2019. Quorum sensing: A prospective therapeutic target for bacterial diseases. BioMed Res Intern, 2019, 2015978.

Jomehpour N, Eslami G, Khalili M, 2016. The effect of *Ferula assa-foetida L* and *Carum copticum* hydroalcoholic extract on the expression levels of *Staphylococcus aureus* genes involved in quorum sensing. Jundishapur J Microbiol, 9(10), e33879.

Kalia M, Yadav VK, Singh PK, Sharma D, et al., 2015. Effect of cinnamon oil on quorum sensing-controlled virulence factors and biofilm formation in *Pseudomonas aeruginosa*. PLoS One, 10(8), e0135495.

Khambhala P, Verma S, Seshadri S, Kothari V, 2016. Inhibition of bacterial quorum-sensing by *Ferula asafoetida* essential oil. Adv Genet Eng, 5, 2.

Khoury M, Beyrouthy ME, Eparvier V, Ouaini N, et al., 2017. Chemical diversity and antimicrobial activity of the essential oils of four Apiaceae species growing wild in Lebanon. J Essent Oil-Bear, 30(1), 25-31.

Kose E, Aktas O, Deniz I, Sarikurkcu C, 2010. Chemical composition, antimicrobial and antioxidant activity of essential oil of endemic *Ferula lycia* Boiss. J Med Plant Res, 4(17), 1698-1703.

Lade H, Paul D, Kweon JH, 2014. Quorum quenching mediated approaches for control of membrane biofouling. Int J Biol Sci, 10(5), 550-565.

Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, et al., 1999. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol, 68(1-3), 267-274.

Marketon MM, Glenn SA, Eberhard A, González JE, 2003. Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti.* J Bacteriol, 185(1), 325-331.

McClean K, Winson M, Fish L, Taylor A, et al., 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology, 143(Pt.12), 3703-3711.

Morohoshi T, Kato M, Fukamachi K, Kato N, et al., 2008. N-acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. FEMS Microbiol Lett, 279(1), 124-130.

Nellums LB, Thompson H, Holmes A, Castro-Sánchez E, et al., 2018. Antimicrobial resistance among migrants in Europe: a systematic review and meta-analysis. Lancet Infect Dis, 18(7), 796-811.

Ohtani K, Hayashi H, Shimizu T, 2002. The luxS gene is involved in cell-cell signalling for toxin production in *Clostridium perfringens*. Mol Microbiol, 44(1), 171–179.

Ozek G, Ozek T, Iscan G, Baser KHC, et al., 2008. Composition and Antimicrobial Activity of the Oils of *Ferula szowitsiana* DC. from Turkey. J Essent Oil-Bear, 20(2), 186-190.

Rice SA, Koh KS, Queck SY, Labbate M, et al., 2005. Biofilm formation and sloughing in *Serratia marcescens* are controlled by quorum sensing and nutrient cues. J Bacteriol, 187(10), 3477–3485.

Sargin SA, 2015. Ethnobotanical survey of medicinal plants in Bozyazı district of Mersin, Turkey. J Ethnopharmacol, 173, 105-126.

Sepahi E, Tarighi S, Ahmadi FS., Bagheri A, 2015. Inhibition of quorum sensing in *Pseudomonas aeruginosa* by two herbal essential oils from *Apiaceae* family. J Microbiol, 53(2), 176–180.

Shapiro SS, Wilk MB, 1965. An analysis of variance test for normality (complete samples). Biometrika, 52, 591-611

The European Committee on Antimicrobial Susceptibility Testing, 2020. Disk diffusion method, version 8.0. http://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/

Towse A, Hoyle CK, Goodall J, Hirsch M, et al., 2017. Time for a change in how new antibiotics are reimbursed: Development of an insurance framework for funding new antibiotics based on a policy of risk mitigation. Health Policy, 121(10), 1025-1030.

Wiegand I, Hilpert K, Hancock RE, 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc, 3(2), 163-175.

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Ethical Approval

The data and information presented in this article were obtained within the framework of academic and ethical rules. Ethical declaration that the evaluation results were in accordance with scientific and ethic rules, was received from the authors.

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