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Comparison of the antibiofilm activity of plant-derived compounds furanone C30 and ellagic acid C11 with antibiotics against *Pseudomonas aeruginosa*

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ABSTRACT

OBJECTIVE: *Pseudomonas aeruginosa* is considered one of the leading pathogens responsible for community- and healthcare-associated infections. *P. aeruginosa* infections are difficult to treat due to antibiotic resistance, various virulence factors, and the capacity to build biofilms, which provide resistance to drugs and immune cells. Antibiotics used to treat biofilm-associated infections over an extended period resulted in the establishment of resistance strains. In this study, we comparatively investigated the efficacy of natural compounds (Furanone-C30 [F], Ellagic-acid C11 [EA], Tobramycin [TOB], Ciprofloxacin [CIP], and Meropenem [MEM]) and antibiotics in inhibiting and eradicating *P. aeruginosa* biofilm.

METHODS: The Minimum Biofilm Inhibition Concentrations (MBIC) and Minimum Biofilm Eradication Concentrations (MBEC) were determined using a micro broth dilution technique, and their effects on the biofilm were evaluated by crystal violet staining and cell viability tests (MTT).

RESULTS: F significantly suppressed *P. aeruginosa* biofilm formation in a dose-dependent way, with 100% inhibition at 512 and 256 g/mL and 92% inhibition at 128 µg/mL. F also eradicated 92.9% of the biofilm at 512 µg/mL and 90% at 256 µg/mL. EA provided 41.6% biofilm inhibition and 33.1% biofilm eradication at 512 µg/mL.

CONCLUSION: Our findings indicate that the natural compounds displayed a dose-dependent effect on *P. aeruginosa* biofilm, with F being found to be more effective than EA. In conclusion, this study suggests that furanone may hold promise as a natural alternative for the treatment of *P. aeruginosa* biofilm and highlights the need for further research to support this hypothesis.

Keywords: Biofilm inhibition and eradication; ciprofloxacin; ellagic acid C-11; furanone C-30; meropenem; Pseudomonas aeruginosa; tobramycin.

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Biofilms are complex microbial cell communities encased in an extracellular polymeric substance (EPS) matrix formed by some bacteria, which have improved antibiotic resistance and host immune response [1]. Microorganisms can exist in two distinct forms in nature: planktonic and biofilm. Planktonic cells are free-living cells, whereas biofilm is a group of organisms that attach to the surface. In regards to physiology and metabolism, microorganisms in a biofilm differ significantly from their planktonic counterparts [2].

These modifications include changes in motility, increased formation of extracellular polysaccharides in some cases, and greater antibiotic resistance [3]. The most frequent Gram-negative bacterium in nosocomial infections is *P. aeruginosa*, and its capacity to form biofilms is largely responsible for its high frequency in humans [4, 5]. The ability of *P. aeruginosa* to form biofilms on permanent medical devices such as prostheses and catheters and in the lungs of cystic fibrosis patients

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is thought to be due to persistent infections caused by the bacteria and the low efficacy of current treatments [6]. Biofilm formation inhibits the effect of antibiotics; therefore, antibiotic resistance spreads quite quickly among the bacterial population embedded in biofilms [5]. *P. aeruginosa* has been classified as a serious threat by the Centers for Disease Control (CDC) because of its ability to develop high levels of antibiotic resistance, especially when it forms biofilms [7]. In addition, *P. aeruginosa* is inherently resistant to several classes of antibiotics, so its therapeutic options are limited. It can also become a much more problematic pathogen due to acquired or mutational resistance [8]. Biofilm formation of *P. aeruginosa* is regulated by the quorum sensing (QS) system. QS is a bacterial communication system in which bacteria coordinate their behaviors according to bacterial cell density and regulate virulence factors [4, 9]. Today, pathogenic bacteria with multi-drug resistance are very common. Therefore, research on the determination of new treatment strategies that bacteria cannot develop resistance has started to gain momentum. Inhibition of the QS mechanism, which provides interbacterial communication, is one of the most important of these strategies [10].

Plant-derived chemicals have long been utilized to cure microbial infections and are thought to be safe for human ingestion. These plant-derived chemicals are expected to aid in the development of molecules that inhibit the QS system, weakening bacterial pathogenicity and rendering pathogenic bacteria non-virulent without harming their survival [11]. Ellagic acid (EA) is a polyphenolic chemical present in many nuts, vegetables, and fruits, including pomegranates, walnuts, almonds, blackberries, raspberries, and strawberries [12]. *In vitro*, *in vivo*, and clinical research have shown its broad-spectrum physiological activities, which include antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, antiviral, immunomodulatory, and neuroprotective properties [12–14].

Another plant-derived compound, halogenated acyl-furanone, a class of secondary metabolites produced by the Australian sea red macroalgae *Delisea pulchra*, are promising compounds in the fight against bacterial infections of various species [15]. Furanone C-30 structurally contains the same lactone ring as the N-acyl-homoserine lactone (AHL) structure of *P. aeruginosa* and can impair communication based on the QS system by competing with AHL molecules to bind to the main QS (LasR) receptor in a concentration-dependent manner [16].

Highlight key points

- *P. aeruginosa* is the most common gram-negative bacteria causes nosocomial infections.
- Higher concentrations of natural compounds were more effective at inhibiting and eradicating biofilms.
- The natural compounds can use QS inhibitors against for P. aeruginosa biofilm.

Considering the rapid increase in antimicrobial resistance and the lack of new antibiotics used in the treatment of resistant microorganisms, new antimicrobial treatments are needed to fight resistant bacteria. Therefore, in our study, we focused on natural compounds to inhibit the biofilm growth of *P. aeruginosa* and eradicate mature biofilm. As a new treatment option, it was aimed to investigate the inhibition and eradication effects of natural compounds F and EA on *P. aeruginosa* biofilm in comparison with TOB, CIP, and MEM in an *in vitro* model.

MATERIALS AND METHODS

Bacterial Culture and Growth Conditions

Pseudomonas aeruginosa PAO1 (ATCC BAA-47) was used for *in vitro* analysis of biofilm in our study. The standard strain of *P. aeruginosa* (ATCC BAA-47) was diluted according to the manufacturer's recommendations. These samples were cultured on Tryptic soy agar (TSA) (Condalab, Spain) medium. Colonies of bacteria from the overnight cultures were adjusted to 0.5 McFarland standard in physiological saline. Then, a loopful of this suspension was inoculated into Tryptic soy broth (TSB) (Condalab, Spain) medium and incubated in a shaking incubator at 37 °C until $1.5x10^8$ cfu/mL (equivalent to 0.5 McFarland tube turbidity) was reached. After incubation, this bacterial suspension was diluted with TSB to $5x10^5$ CFU/mL.

Preparation of Antibiotics

To observe the inhibition and eradication effect of antibiotics on *P. aeruginosa* PAO1 biofilm, three antibiotics from different classes (aminoglycosides, fluoroquinolones and carbapenems) were included in the study. Tobramycin (Glentham, UK), Ciprofloxacin (Himedia, USA), and Meropenem (Glentham, UK) were obtained from commercial companies. Stock solutions of antibiotics were prepared considering the potency of the production batch number. The dilutions of the solutions were prepared with distilled water at desired concentrations.

Preparation of Furanone and Ellagic Acid

Two different natural compounds were used to observe the inhibition and eradication effect of natural compounds on the *P. aeruginosa* PAO1 biofilm. Furanone (Sigma Aldrich, Germany) was dissolved in absolute ethanol. Ellagic acid (Cayman Chemical, USA) was dissolved in dimethyl sulfoxide (DMSO, Neofroxx, Germany). Stock solutions of F and EA at desired concentrations were prepared.

Biofilm Formation Assay

Biofilm formation by *P. aeruginosa* was measured quantitatively in 96-well microplates using the crystal violet method [17]. 200 µL of *P. aeruginosa* culture was incubated at 37 °C for 18 hours under static conditions. To remove planktonic bacteria, the culture supernatant was removed and the wells were washed twice with phosphate-buffered saline (PBS, MP Biomedicals, USA). The microplates were dried by air. Each well received 200 µL of 99% methanol for 15 minutes before being dumped. The wells were dyed for 15 minutes with 200 µL of 1% crystal violet. Excess dye was rinsed away with water, and the microplate was allowed to dry. To destain crystal violet, 200 µL of 96% ethanol was added to wells and allowed for 30 minutes before measuring absorbance at 595 nm with a spectrophotometer (ThermoFisher Scientific, USA). Each plate included wells of TSB without *P. aeruginosa* served as negative control wells.

Determination of the Effect of Natural Compounds and Antibiotics on *P. aeruginosa* Biofilm

The inhibition and eradication potentials of F, EA, TOB, CIP, and MEM on *P. aeruginosa* biofilm were determined by the crystal violet microplate technique.

Determination of Minimum Biofilm Inhibitory Concentration (MBIC)

A micro broth dilution approach was used to assess the MBICs of F, EA, TOB, CIP, and MEM against *P. aeruginosa* biofilm. In the wells, ten different antibiotic concentrations were evaluated using two-fold serial dilutions. In wells, two-fold serial dilutions of natural substances were made to evaluate eight various concentrations. In a 96-well microplate, F and EA were diluted at concentrations ranging from 512–4 µg/mL, while TOB, CIP, and MEM were diluted at concentrations ranging from 64–0.125 µg/mL. Then, in each well, 100 µL of prepared bacterial suspension was introduced. A positive control was 200 µL of bacterial suspension in wells. Negative control wells were those with the TSB but no *P. aeruginosa*. The microplate was incubated at 37 °C for 18 hours. Inhibition of biofilm at the end of incubation time was assessed using the crystal violet staining technique as previously described. After staining, absorbance values were measured at 595 nm using a spectrophotometer. MBIC value was defined as the lowest concentration that significantly inhibited biofilm formation.

Determination of Minimum Biofilm Eradication Concentration (MBEC)

After 18 hours of incubation, *P. aeruginosa* biofilms formed in 96-well microplates were gently aspirated with the help of an automatic micropipette, then washed twice with 250 µL of PBS to eliminate planktonic bacteria before being air-dried. Two-fold serial dilutions of natural compounds and antibiotics were diluted as described in the MBIC section using another sterile microplate. MBECs of F, EA, TOB, CIP, and MEM against *P. aeruginosa* biofilm were determined with a micro broth dilution technique. In a 96-well microplate, F and EA were diluted with TSB at concentrations ranging from 512–4 µg/mL, TOB, CIP, and MEM were diluted at concentrations ranging from 64–0.125 µg/mL and added to wells. The microplate was incubated at 37 °C for 18 hours. Eradication of biofilm was determined using the crystal violet staining technique as previously described. Wells containing 200 µL of bacterial suspension in TSB without natural compounds or antibiotics were used as positive controls. Wells containing 200 µL of TSB were used as a negative control. After staining, absorbance values were measured at 595 nm using a spectrophotometer. MBEC values were defined as the lowest concentration where no growth was observed.

MTT Assay

The 3-(4.5 dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT, Boster Bio, USA) assay was used to measure the metabolic activity of live cells in biofilms. Microplates were made and incubated as previously described in the section on biofilm inhibition and eradication. To remove planktonic bacteria, the wells were gently aspirated and washed with PBS after incubation.

After drying the microplates, 100 µL of TSB and 10 µL of MTT solution were added to each well according to the MTT kit procedure. Microplates were incubated

at 37 °C for 4 hours in the dark. 100 µL of formazan solution (Boster Bio, USA) was then added into each well and incubated at 37 °C for 18 hours. Absorbance values were measured at 570 nm using a spectrophotometer. All experiments were performed three times.

Statistical Analysis

All experiments were performed in triplicate and repeated at least three times on different days. The data were analyzed using SPSS v.22 (Armonk, NY). The efficacy of the components was determined using the Wilcoxon test. The level of statistical significance was set at $p<0.05$.

RESULTS

Determination of Minimum Biofilm Inhibitory Concentration (MBIC)

When the absorbance values of the effects of F, EA, TOB, CIP and MEM on biofilm formation in 96-well microplates were evaluated compared to the negative and positive controls, concentration-dependent biofilm inhibition was detected. Concentration-dependent biofilm suppression was seen when the absorbance values of the impacts of F, EA, TOB, CIP, and MEM on biofilm development in 96-well microplates were compared with the negative and positive controls. According to negative and positive control; TOB and MEM at 16 µg/mL, and CIP at 8 µg/mL inhibited the biofilm 100%. F; 100% at concentrations of 512–256 µg/mL; 92% at 128 µg/mL inhibited biofilm. EA; inhibited 41.6% biofilm at a concentration of 512 µg/mL. F compound also showed a biofilm inhibition effect of 35% at 16 µg/mL concentration. It was observed that the inhibitory effect on the biofilm was maintained in a dose-dependent manner (Fig. 1A). The lowest concentration at which biofilm formation was inhibited was called MBIC (Table 1).

Determination of Minimum Biofilm Eradication Concentration (MBEC)

Biofilms were allowed to be formed for 18 hours on microplates. After incubation, the eradication effects on biofilm were investigated by adding different concentrations of natural compounds and antibiotics. The effect of antibiotics and natural compounds on mature *P. aeruginosa* biofilm was determined by measuring absorbance values at 595 nm of the bacterial culture. When the absorbance values measured in the wells were evaluated, TOB and CIP showed an eradication

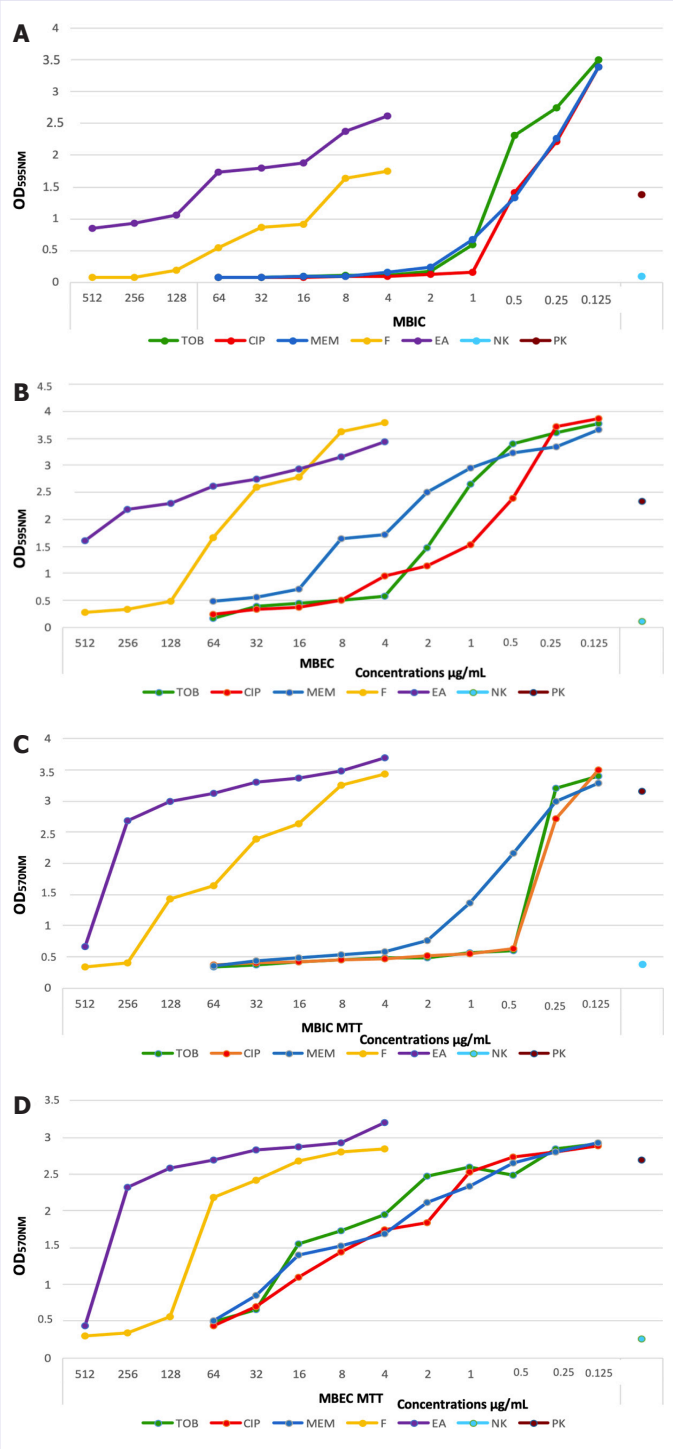

Figure 1. **(A)** Determination of minimum biofilm inhibitory concentration. **(B)** Determination of minimum biofilm eradication concentration. **(C)** Determination of cell viability of the *P. aeruginosa* in biofilm inhibition. **(D)** Determination of cell viability of the *P. aeruginosa* in biofilm eradication.

MBIC: Minimal biofilm inhibitor concentration; TOB: Tobramisin; CIP: Ciprofloxacin; MEM: Meropenem (64–0.125 µg/mL); F: Furanone; EA: Ellagic acid (512–4 µg/mL); PK: Positive control; NK: Negative control; OD: Optical density.

Table 1. MBIC percentages of antibiotics and natural compounds

µg/mL	TOB	CIP	MEM	F	EA
512				100	41.6
256				100	34.6
128				92	25.7
64	100	100	100	64	–
32	100	100	100	39	–
16	100	100	100	35	–
8	98	100	99	–	–
4	97	99	95	–	–
2	93	97	88		
1	61	95	55		
0.5	–	–	4.6		
0.25	–	–	–		
0.125	–	–	–		

MBIC: Minimal biofilm-inhibiting concentration; TOB: Tobramycin; CIP: Ciprofloxacin; MEM: Meropenem (64–0.125 µg/mL); F: Furanone; EA:– Ellagic acid (512–4 µg/mL); –: Ineffective.

Table 2. MBEC percentages of antibiotics and natural compounds

µg/mL	TOB	CIP	MEM	F	EA
512				92.9	33.1
256				90	6.8
128				83	1.4
64	98	94.2	83.7	30.3	–
32	88	90	77.9	–	–
16	85.4	88	73.7	–	–
8	82.7	82	30.9	–	–
4	79.6	62	27.7	–	–
2	38.6	54	–		
1	–	36.4	–		
0.5	–	–	–		
0.25	–	–	–		
0.125	–	–	–		

MBIC: Minimal biofilm-inhibiting concentration; TOB: Tobramycin; CIP: Ciprofloxacin; MEM: Meropenem (64–0.125 µg/mL); F: Furanone; EA:– Ellagic acid (512–4 µg/mL); –: Ineffective.

effect of 98.94%, respectively, on the biofilm at 64 µg/mL concentration, while MEM provided an eradication of 83% on the biofilm at 64 µg/mL concentration. F showed an eradication effect on biofilm with a rate of 92.9% at a concentration of 512 µg/mL. At the same time, F provided an eradication effect of 90; 83; 30.3 at 256; 128; 64 µg/mL, respectively. EA eradicated the biofilm by 33% at a concentration of 512 µg/mL (Fig. 1B). Our results showed that higher concentrations are needed to destroy preformed biofilms (Table 2).

Determination of Cell Viability of the *P. aeruginosa* in Biofilm Inhibition Microplate (MTT Assay)

Bacterial viability within biofilms was determined by the MTT method. When the absorbance measurements were evaluated according to the controls, TOB; at 32 µg/mL, CIP and MEM; at 64 µg/mL, and F at 512 µg/mL concentrations provided 100% inhibition of living cells in the biofilm. At 512 µg/mL, EA inhibited the number of viable cells by 90% (Fig. 1C).

Determination of Cell Viability of the *P. aeruginosa* in Biofilm Eradication Microplate (MTT Assay)

It was observed that F showed the closest activity to eradication in terms of viable cell number when evaluated at a concentration of 512 µg/mL.

At a concentration of 512 µg/mL, F eradicated 98.7% of viable cells in the biofilm. EA eradicated 93% of viable cells in the biofilm at a concentration of 512 µg/mL. At 64 µg/mL concentration, TOB, CIP, and MEM exhibited the activity closest to eradication in the number of viable cells. At 64 µg/mL concentration, TOB, CIP, and MEM eradicated viable cells in the biofilm at a rate of 90, 92, and 90%, respectively (Fig. 1D).

DISCUSSION

Today, the main reason *P. aeruginosa* infections cannot be treated is the resistance developed by bacteria against antibiotics. Recent research indicates that biofilms play a key role in bacteria's capacity to resist antibiotics. For this reason, antibiotics alone in the treatment of biofilm-associated infections are not sufficient. To tackle these diseases, new antimicrobial medicines must be created, and it is advised that the factors that lead to biofilm growth inhibition, biofilm disruption, or biofilm eradication be investigated to improve the efficacy of novel treatment options. In this regard, it is emphasized that QS inhibitors can be alternative antibiofilm agents. It is also thought that the combined treatment of QS inhibitors with antibiotics will increase the effectiveness of antibiotics. Therefore, in our study, we evaluated the antibiofilm activity of natural compounds F and EA against *P. aeruginosa* PAO1 biofilm.

It is known that biofilm formation is a serious problem in chronic infections and causes increased resistance to traditional antibiotics [18, 19]. There is a need to identify new effective agents with both antibacterial and antibiofilm properties against the increasing resistance of bacteria to conventional antibiotics [20]. For this reason, scientists are conducting research on antibiofilm agents and QS inhibitors as alternatives to traditional antimicrobial agents to prevent antibiotic resistance in bacteria [21]. Because the QS system is a cell-to-cell communication mechanism that is critical in the regulation of virulence factors and biofilm formation, therapeutic techniques based on its disruption are being examined [22]. Plant extracts have recently been identified as a rich reservoir of chemicals with biological activities such as antibacterial and antibiofilm characteristics [23]. Natural compounds are also recognized as a vital resource for the detection of many new therapeutic compounds [5]. Furanone is produced by the marine red macroalgae *Delisea pulchra*, and this natural compound is thought to contain a natural chemical defense mechanism that can control biofilm formation by targeting the receptors of the QS system [9]. The chemical structure of furanone is similar to acyl homoserine lactones (AHL), which are signal molecules of the QS system. Because of this similarity, furanones in *P. aeruginosa* compete with AHL binding by binding the LasR protein, the regulatory protein that responds to AHLs [24]. As a result of a study by Manefield et al. [25] it was observed that furanone, which is very similar to homoserine lactone, which is the signal molecule of the AI-I system, interferes with the QS mechanism of bacteria. In another study with furanone, it was shown that halogenated furanone compound can interact with AHL-mediated QS in *P. aeruginosa* and reduce the production of important virulence factors, and it has been reported to inhibit biofilm formation caused by *P. aeruginosa* [26]. Zhang et al. [9] reported that the combination of furanone C-30 and colistin not only inhibit bacterial biofilm formation but also has a better destructive effect on preformed mature biofilms. In our study, it was observed that F inhibited biofilm 100% at concentrations of 512 and 256 μg/mL. In our MBIC MTT assay, it was determined that viable cells were 100% inhibited at a concentration of F 512 μg/mL, and a 99.8% decrease in the number of viable cells at a concentration of F 256 μg/mL. In our eradication assay, it was observed that F exhibited the closest activity to eradication at a concentration of 512 μg/mL. It was determined that 93% of mature biofilm was eradicated at the same concentration. In our MBEC MTT assay, it was determined that the F compound showed the closest activity to eradication in the number of viable cells at a concentration of 512 μg/mL. At this concentration, a 98.7% reduction in the number of viable cells was detected. Our results were found to be consistent with previous studies, which found that F showed a decrease in biofilm formation, and it was determined that *P. aeruginosa* inhibited biofilm formation in a dose-dependent manner, as in the current studies.

EA is a naturally occurring polyphenolic compound with potent antioxidant and anticancer properties that is found in high amounts in the leaves, fruits, and seeds of various plants [27]. It has been reported that EA derivatives from the fruit of Terminalia chebula Retz. as well as aqueous extracts from Callistemon viminalis, Bucida buceras, and Conocarpus erectus inhibit the QS system of *P. aeruginosa* [28]. In another study, the effect of EA on *P. aeruginosa* virulence factors in *P. aeruginosa* biofilm was investigated. It has been reported that EA at a concentration of 0.5 mg/mL causes only a 10–15% reduction in the production of *P. aeruginosa* virulence factors [11]. As a result of the study by Huber et al. [29] it was observed that EA showed anti-QS activity on *Pseudomonas putida* biofilm formation, inhibiting swarming motility and biofilm formation. Since there are few studies on this subject in the literature, adequate comparisons could not be made. Our study is the first study on this subject in Turkey. In our study, EA showed an inhibitory effect on biofilm in the range of 512–256 μg/mL. It was determined that EA had a 41.6% inhibitory effect on biofilm at a concentration of 512 μg/mL. In MBIC MTT assay, a 90% reduction in the number of viable cells was detected at EA 512 μg/mL concentration. In our eradication assay, it was observed that EA eradicated the mature biofilm by 33% at a concentration of 512 μg/mL. In our MBEC MTT assay, EA again showed a 93% reduction in the number of viable cells at a concentration of 512 μg/mL.

CIP's ability to suppress biofilm formation has also been examined in other clinical strains. In their investigation with *P. aeruginosa* PAO1 and four clinical *P. aeruginosa* strains, Gupta et al. [30] evaluated the biofilm inhibition capacity of CIP. According to the findings of the investigation, CIP at a dosage of 0.06 g/mL significantly reduced biofilm development in all strains.

In a study on the eradication of the biofilm formed by *P. aeruginosa* strains, it was observed that the MBEC value of CIP against *P. aeruginosa* strains was 16 times higher than the minimum inhibitory concentration (MIC) value

reported for the planktonic form [31]. The data obtained in our study support the findings of the current studies. In another study with *P. aeruginosa* strains isolated from various clinical specimens, the effect of TOB and CIP on the planktonic and biofilm forms of *P. aeruginosa* was investigated. They reported MIC value ranges of 0.25–2 µg/mL for CIP and 0.25–16 µg/mL for TOB in their planktonic forms. On biofilm forms, they reported ranges of MBEC values of 80–1280 µg/mL for CIP and 160–2560 µg/mL for TOB [32]. In a study on *P. aeruginosa* biofilm from samples isolated from patients with cystic fibrosis, the effects of TOB, CIP, and MEM on biofilm inhibition were evaluated and biofilm inhibition concentrations were determined as 1; 4; 2 mg/L for CIP, TOB and MEM, respectively [33]. In studies with antibiotics, it has been observed that antibiotics cannot completely eradicate bacterial biofilms, even if they trigger cell death in the biofilm [34]. In our study, the antibiotics we used to inhibit early *P. aeruginosa* biofilm were sufficient at lower concentrations compared to the mature biofilm structure, and it was found that they were compatible with previous studies in terms of biofilm inhibition and eradication effect potentials. In our study, TOB, CIP, and MEM provided 100% inhibition of biofilm in concentration ranges of 16–64 µg/mL. On the other hand, F inhibited biofilm 100% at 512–256 µg/mL concentration and 92% at 128 µg/mL, while EA provided 41.6% and 34.6% biofilm inhibition at 512 and 256 µg/mL, respectively. TOB, CIP, and MEM provided 98, 95, 83% biofilm eradication at 64 µg/mL concentration, respectively. F provided eradication of 92, 90, 83%, respectively, on biofilm at 512, 256,128 µg/mL concentrations, and 33%, 6.8%, respectively, at EA at 512, 256 µg/mL. The higher the concentrations of the natural compounds, the more effective the biofilm inhibition and eradication were dose-dependently. In our study, compound F was found to be more effective than EA in the treatment of biofilm.

It is possible to use natural compounds in higher doses than antibiotics. Antibiotic resistance and toxicities are the main factors limiting the use of antibiotics. Toxicity and resistance have been observed since the discovery of antibiotics used to treat a variety of bacterial diseases [35]. Furthermore, antibiotic residues can affect the human microbiota and produce health issues such as allergic reactions, chronic toxic effects after long-term exposure, and decreased digestive system functions [36]. In this study, it was determined that natural compounds F and EA gave effective results in biofilm inhibition and eradication dose-dependently manner.

Conclusion

Natural compounds have advantages such as low toxicity and safety for the human body, which is one of the reasons why they are preferred in biofilm studies. Therefore, natural compounds have the advantage of using higher concentrations than antibiotics. To protect against the toxic effects of antibiotics, especially in resistant bacteria that require high doses of antibiotics, natural compounds are considered a promising new natural alternative therapy to fight against biofilm.

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