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The antibiofilm effects of some *Cistus* spp. against pathogenic microorganisms

Sevim Feyza Erdoğmuş[a,*], Cihat Bilecen[b], Özlem Erdal Altıntaş[c], Sevgi Ulukütük[d], Mustafa Kargıoğlu[e]

[a] *Afyonkarahisar Health Sciences University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Afyonkarahisar, Turkey*
[b] *Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Medical Biology and Genetics, Afyonkarahisar, Turkey*
[c] *Afyonkarahisar Health Sciences University, Şuhut Vocational School of Health Services, Department of Medical Services and Techniques, Afyonkarahisar, Turkey*
[d] *Afyon Kocatepe University, Şuhut Vocational School, Department of Food Processing, Afyonkarahisar, Turkey*
[e] *Afyon Kocatepe University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Turkey*

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ABSTRACT

Recently, the potential antibacterial or antibiofilm effects of some plant species belonging to the *Cistus* sp. have motivated investigation of their use as herbal remedies. In this study, antibiofilm activities of aqueous (dH_2O) leaf extracts of *Cistus laurifolius* L., *C. creticus* L. and *C. salviifolius* L. on some pathogenic microorganisms with biofilm forming ability were investigated. Biofilm forming ability of pathogen test microorganisms were evaluated by congo red agar method and microtiter plate method and all tested microorganisms were confirmed as biofilm producers. *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 11778, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 12600 were evaluated as strong biofilm producers. The highest concentration of examined extracts both showed biofilm inhibition and biofilm eradication against the tested pathogen microorganisms. In particular, studied plant extracts showed good antibiofilm effect, and biofilm eradication against *S. aureus* ATCC 6538 and *S. aureus* ATCC 12600. $MBIC_{50}$ values of *S. aureus* ATCC 6538 were found as 6.25 µg/ml of *C. laurifolius*, and 50 µg/ml of *C. creticus* extracts. Also, 50 µg/ml of *C. creticus* extract showed ≥ 90% inhibition of biofilm growth ($MBIC_{90}$ = 50 µg/ml). $MBEC_{50}$ values of *S. aureus* ATCC 12600 were determined as 6.25 µg/ml in all tested plant extracts and 50 µg/ml of *C. creticus* extract was required to induce ≥ 90% eradication ($MBEC_{90}$) of biofilm growth of *S. aureus* ATCC 12600. Our study revealed that aqueous leaf extracts of *C. laurifolius*, *C. creticus* and *C. salviifolius* could be potential candidates for drug discovery to treat pathogen test microorganisms capable to induce infectious diseases especially by their biofilm forming ability.

1. Introduction

Although the immune defence system of living organisms fighting against pathogenic microorganisms act as a first degree barrier, sometimes, microorganisms overcome this immune system barrier and cause various infections. The treatment of these infections is usually performed using antibiotics but the number of antibiotic-resistant microorganisms has significantly increased recently (Jasovský et al., 2016). Antibiotic resistance is a major problem to public health in which the biofilm forming abilities of some microorganisms contribute to this resistance and, therefore, treatment of infections become more difficult (Olsen, 2015; Richardson, 2017; Aslam et al., 2018; Yan and Bassler, 2019; Bowler et al., 2020). The biofilm is an extracellular matrix that surrounds microbial cells and is comprised of biological polymers such as exopolysaccharide (EPS), protein, and DNA (Leone et al., 2006; Flemming et al., 2016). Various environmental factors are effective in the formation of biofilms such as bacterial strain, surface structure, pH, nutrient amount, and temperature (Donlan and Costerton, 2002). Several researchers have revealed that several pathogen microorganisms show biofilm formation ability which is considered a protective mechanism (Fernandes et al., 2011; Darwish and Asfour, 2013; Silva et al., 2014,

* Corresponding author:
E-mail address: feyzakus@gmail.com (S.F. Erdoğmuş)
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Raza et al., 2013). Biofilm not only makes microorganisms resistant to adverse environmental conditions, but also protects them from phagocytes and complement systems (Rasmussen Givskov, 2006). Therefore, biofilm-forming microorganisms are considered as the main cause of persistent hospital infections, especially in immunocompromised individual (Roy et al., 2019). Studies have revealed that biofilm-producing bacteria can be 100-10000 times more antibiotic resistant to planktonic forms. (Monzón et al., 2002; Hall-Stoodley et al., 2004; Davies, 2003).

Medicinal and aromatic plants which have various pharmacological properties are important resources in the traditional folk medicine (Ghorbanpour et al., 2017; Ganaie, 2021; Fierascu et al., 2021; Ben Bakrim et al., 2022). Turkey has one of the largest floras in Europe and *Cistus* genus, which is one of the Mediterranean region's characteristic genera, is traditionally used in folk medicine (Catoni et al., 2012; Comandini et al., 2006; Cetin and Yanikoglu, 2006; Attaguile et al., 2000; Ustun and Baykal, 2016). Researchers showed that *Cistus* species are rich in bioactive compounds such as flavonoids, polyphenols, and terpenoids (Stepien et al., 2018; Barrajón-Catalán et al., 2011; Küpeli and Yesilada, 2007; Zalegh et al., 2021). These compounds are effective in using *Cistus* species as anti-inflammatory (Demetzos et al., 2001), antibacterial (Benali et al., 2020; Güvenç et al., 2005), antifungal (Barros et al., 2013), antiviral (Ehrhardt et al., 2007), analgesic (Sayah et al., 2017), and antitumoral (Dimas et al., 2000). Five *Cistus* species belonging to the Cistaceae family grow naturally in Turkey: *C. creticus* L., *C. laurifolius* L., *C. monspeliensis* L., *C. parviflorus* Lam., and *C. salviifolius* L. (Davis, 1988).

This study aimed to determine the antibiofilm activities of aqueous (dH_2O) leaf extracts of *C. laurifolius*, *C. creticus* and *C. salviifolius*, used as herbal remedies in Turkish folk medicine, against some pathogenic test microorganisms. The present study is the first report on antibiofilm effects of *C. laurifolius*, *C. creticus* and *C. salviifolius* against pathogenic test microorganisms.

2. Materials and methods

2.1. Extraction of plant materials

C. laurifolius, was collected from Akdağ-Sandıklı/Afyonkarahisar region (Turkey) and *C. creticus*, *C. salviifolius* were collected from Ağva-Şile/İstanbul region (Turkey) in June-July 2021. These species were identified by Prof. Dr. Mustafa Kargioglu in Afyon Kocatepe University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, using "Flora of Turkey and the East Aegean Islands" (Davis, 1988). The herbarium name and registration numbers of the plants are AKU-10384 for *C. creticus*, AKU-10385 for *C. salviifolius* and AKU-10400 for *C. laurifolius*.

The fresh leaves of *C. laurifolius*, *C. creticus* and *C. salviifolius* were dried in a dryer at 45 °C, then fine powdered in a mill. The modified ultrasonic extraction method was used to prepare dH_2O extracts of *C. laurifolius* (Cl-dH_2O), *C. creticus* (Cc-dH_2O), *C. salviifolius* (Cs-dH_2O). Thirty (30) grams of powdered sample of each extract was prepared with 400 ml each of dH_2O ultrasonicated 1h, at room temperature (Latiff et al., 2021). The available aqueous extract was filtered and the extract was evaporated until dryness in a rotary evaporator (Heidolph) at 40 °C and followed by freeze-drying.

2.2. Pathogen test microorganisms

Listeria monocytogenes ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* NRRLB 4420, *Enterococcus faecalis* ATCC 51289, *Escherichia coli* ATCC 35218, *Bacillus subtilis* NRS 744, *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 12600, *P. aeruginosa* ATCC 11778 and *Candida albicans* ATCC 10231 were used as the test microorganisms. The test microorganisms obtained from the bacterial culture collection of Faculty of Pharmacy, Afyonkarahisar Health Sciences University (Afyonkarahisar, Turkey) were used in this study.

2.3. Determination of the biofilm formation of pathogen test microorganisms

Biofilm production of pathogen test microorganisms were evaluated by both congo red agar and microtiter plate methods. All experiments were repeated three times. For congo red agar method, pathogen test microorganisms inoculated to congo red agar medium (brain heart infusion broth 37 g/l, sucrose 50 g/l, congo red 0.8 g/l, and agar 10 g/l) at 37 °C for 24 h, then they were incubated at 25 °C for 48 h (Freeman et al., 1989; Saxena et al., 2014). After incubation periods, the colonies which are black, red, dry, rough, and transparent were evaluated as biofilm positive. Also, the colonies that pinkish red, flat, and central dark colonies were evaluated as biofilm negative (Jain and Agarwal, 2009; Szczuka and Kaznowski, 2014). Then 50 µl of broth culture distributed to a 96-well microtiter plate and re-incubated at 37 °C for 24 hours for microtiter plate method. After incubation period, medium was removed, the wells were washed 3 times by distilled water and 150 µl of crystal violet solution (0.5%, v/v) was transferred into the wells, incubated at 37 °C for 45 min. Then the wells were washed again and 150 µl of ethanol:acetic acid (95:5) was distributed to each well incubated in ambient temperature for 10 min to dissolve the dye. 100 µl of aliquots from each well were transferred to a new microtiter plate and the optical density (OD) of each well was measured at 570 nm using a microplate spectrophotometer (Thermo Scientific Multiskan Sky). The medium was used as a negative control and *P. aeruginosa* ATCC 11778 that is known to generate biofilm was used as the positive control (Kenar et al., 2020). According to the critical OD value (ODc) the biofilm production was scored as: non-biofilm producers [(-), OD ≤ ODc], weak biofilm producers [(+), ODc < OD ≤ 2 x ODc], moderate biofilm producers [(++), 2 x ODc < OD ≤ 4 x ODc], or strong biofilm producers [(+++), OD > 4 x ODc] (Gomes et al., 2019).

2.4. Detection of the EPS production of pathogen test microorganisms

EPS production of pathogen test microorganisms was determined by Marshall and Rawson (1999) method. Broth cultures of pathogen test microorganisms were prepared in NB (Nutrient Broth) and adjusted to 0.5 McFarland turbidity (approximately 1 to 4 x 10^8 cfu/ml) were transferred into a fresh 5 ml NB medium and re-incubated for 20 hours at 37 °C. Then, volume of 1 ml from each culture was transferred into eppendorf tubes and incubated in a water bath at 100 °C for 10-15 minutes. After incubation period, 85% trichloroacetic acid (TCA, 0.17%) were added to the samples and were centrifuged at 14.000 rpm for 20 min. The supernatants were transferred to new eppendorf tubes and the same amount of ethanol was added to each eppendorf tube. Then the eppendorf tubes were centrifuged at 14.000 rpm for 20 min. EPS productions of pathogen test microorganisms were determined by phenol sulfuric acid method. Obtained pellets by centrifugation were dissolved in 100 µl of sterile distilled water and 50 µl of pure phenol was added. Then 500 µl of sulfuric acid was added and vortexed. After incubation for 20 min. at 37 °C, 50 µl of each sample were taken and their absorbance values were determined at 490 nm by a microplate spectrophotometer (Thermo Scientific Multiskan Sky). All

experiments were repeated three times. The results were evaluated according to the glucose standard curve.

2.5. Inhibition of biofilm formation

The biofilm inhibition effects of Cl-dH_2O, Cc-dH_2O, Cs-dH_2O extracts were determined by a 3-[4,5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium-bromide (MTT) colorimetric method of Kairo et al. (1999) with modification of Walencka et al. (2005) and Teanpaisan et al. (2014). Briefly, 100 µl of two-fold serial-diluted extract concentrations (50 to 6.25 µg/ml) were added in 96-well microtiter plates. The fluconazole (10 mg/ml) for *C. albicans* ATCC 10231 and penicillin G (30 mg/ml) for the remaining microorganisms were used as the positive controls, whereas the native medium was used as the negative control. An equal volume of each pathogen test microorganism (1×10^6 cfu/ml) was added and mixed, except in the well with medium alone, and incubated at 37 °C for 24 h. Then supernatants were discarded and washed three times with 150 µl of PBS, and 50 µl of MTT (0.3%) were added and incubated for 2 h at 37 °C. The MTT solution was removed from the wells and 150 µl of DMSO, 25 µl of 0.1 M glycine buffer (pH 10.2) were added to the wells to dissolve the formazan crystals and incubated for 15 min at ambient conditions. The optical density was measured with microplate spectrophotometer (Thermo Scientific Multiskan Sky) at the 570 nm wavelength. The minimal biofilm inhibitory concentration 50% ($MBIC_{50}$) and minimum biofilm inhibitor concentration 90% ($MBIC_{90}$) were calculated. All experiments were repeated three times.

The percentage (%) inhibition was calculated using the equation:

$$[1 - (A_{570} \text{ of the test}/A_{570} \text{ of untreated control})] \times 100$$

2.6. Eradication of biofilm formation

The eradication power of biofilm formation of Cl-dH_2O, Cc-dH_2O, Cs-dH_2O extracts were evaluated using minimum biofilm eradication concentration (MBEC) assay (Teanpaisan et al.,2014). 200 µl (10^6 cfu/ml) of each pathogen test microorganism was inoculated into the 96-well microtiter plate and incubated for 24 h at 37 °C. After the incubation period, the medium was removed and the wells were carefully washed three times with PBS in order to remove non-adherent cells. 200 µl of Cl-dH_2O, Cc-dH_2O, Cs-dH_2O extracts (serial two-fold dilutions from 50 to 6.25 µg/ml) were then added to the wells and incubated for 24 h at 37 °C. Then the adherent bacteria were washed three times PBS and the numbers of surviving microorganisms were determined by an MTT assay. The MBEC value was defined as the concentrations that showed 50% and 90% inhibition of biofilm formation on the medium. The fluconazole (10 mg/ml) for *C. albicans* ATCC 10231 and penicillin G (30 mg/ml) for other bacteria were used as positive controls and the native medium was used as a negative control. All experiments were repeated three times.

The percentage eradication was calculated using the equation of:

$$[1 - (A_{570} \text{ of the test}/A_{570} \text{ of nontreated control})] \times 100$$

3. Results and discussion

3.1. Determination of the biofilm formation of pathogen test microorganisms

In the present study, biofilm generating abilities of some pathogen test microorganisms were demonstrated. According to the congo red agar method, all pathogen test microorganisms in appearances of red, reddish, dry, rough, and transparent colonies were evaluated as biofilm producers. The biofilm forming abilities of pathogen test microorganisms were also evaluated using the microtiter plate method and the results were shown in Table 1. *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 11778, *P. aeruginosa* ATCC 11778, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 12600 were evaluated as strong biofilm producers. *L. monocytogenes* ATCC 19115, *K. pneumoniae* NRRLB 4420, *E. coli* ATCC 35218, and *C. albicans* ATCC 10231 were evaluated as moderate biofilm producers. On the other hand, *E. faecalis* ATCC 51289, *B. subtilis* NRS 744, and *E. coli* ATCC 25922 were evaluated as weak biofilm producers.

Table 1. Biofilm formation of pathogen test microorganisms

Pathogen test microorganisms	Mean OD ± SD	Biofilm formation
L. monocytogenes ATCC 19115	0.74 ± 0.10	++
S. aureus ATCC 25923	1.35 ± 0.15	+++
K. pneumoniae NRRLB 4420	1.03 ± 0.25	++
P. aeruginosa ATCC 11778	1.32 ± 0.18	+++
E. faecalis ATCC 51289	0.34 ± 0.09	+
E. coli ATCC 35218	0.96 ± 0.16	++
B. subtilis NRS 744	0.36 ± 0.03	+
S. aureus ATCC 6538	1.41 ± 0.09	+++
E. coli ATCC 25922	0.41 ± 0.01	+
P. aeruginosa ATCC 27853	1.25 ± 0.11	+++
S. aureus ATCC 12600	1.39 ± 0.17	+++
C. albicans ATCC 10231	0.87 ± 0.04	++

OD: optical density, SD: Standard deviation, +: Weak biofilm producer, ++: Moderate biofilm producer, +++: Strong biofilm producer

Table 2. EPS production by pathogen test microorganisms

Pathogen test microorganisms	EPS (mg/l) ± SD
L. monocytogenes ATCC 19115	6.23 ± 0.45
S. aureus ATCC 25923	6.98 ± 0.23
K. pneumoniae NRRLB 4420	2.27 ± 0.35
P. aeruginosa ATCC 11778	9.15 ± 0.87
E. faecalis ATCC 51289	5.45 ± 0.56
E. coli ATCC 35218	2.20 ± 0.25
B. subtilis NRS 744	1.77 ± 0.78
S. aureus ATCC 6538	7.68 ± 1.12
E. coli ATCC 25922	3.69 ± 0.56
P. aeruginosa ATCC 27853	9.78 ± 0.67
S. aureus ATCC 12600	7.75 ± 0.89
C. albicans ATCC 10231	4.35 ± 0.47

The biofilm formation ability of microorganisms both complicates the treatment of infections and causes serious economic costs in the health and food sectors (Van Houdt and Michiels, 2010; Sabir et al., 2017). Researchers have shown that many microorganisms show biofilm forming ability (Soares et al., 2016; Jamal et al., 2019). In support of our results, Croes et al. (2009) investigated the *in vitro* biofilm formation of clinical *S. aureus* isolates of distinct clonal lineages. This study revealed that *S. aureus* isolates had strong biofilm production ability under specific conditions. The antimicrobial properties and biofilm production of *P. aeruginosa* and *Staphylococcus* spp. strains were also demonstrated by Heidari et al. (2018).

3.2. Detection of the EPS production of pathogen test microorganisms

A glucose standard curve was prepared by using a glucose solution of 10–100 mg/ml to calculate the EPS amounts generated by pathogen test microorganism (Figure 1). EPS production by pathogen test microorganisms are shown in Table 2. The highest EPS production was found in *P. aeruginosa* ATCC 27853 (9.78 ± 0.67 mg/l) and followed by *P. aeruginosa* ATCC 11778 (9.15 ± 0.87 mg/l). The lowest EPS production was determined in *B. subtilis* NRS 744 (1.77 ± 0.78). Wu et al. (2020), evaluated the biofilm formation by some pathogen microorganisms and reported that some of these pathogens, with biofilm forming ability, showed resistance against various antibiotics used. Researchers revealed that the produced EPS were responsible for the cohesion of microorganisms, adhesion of biofilms to surfaces and also acted as adhesives between cells (Costa et al., 2018).

3.3. Inhibition and eradication of biofilm formation

The concentrations of Cl-dH_2O, Cs-dH_2O and Cc-dH_2O extracts required to inhibit ≥ 50% biofilm formation of pathogen test microorganisms are shown in Figures 2, 3 and 4. $MBIC_{50}$ values of Cl-dH_2O and Cs-dH_2O extracts on *S. aureus* ATCC 6538 were found as 6.25 and 50 µg/ml, respectively. Also, 50 µg/ml of Cc-dH_2O extract was required to inhibit ≥ 90% of biofilm growth ($MBIC_{90}$ = 50 µg/ml). $MBIC_{50}$ values of *E. coli* ATCC 25922 were found as 12.5 µg/ml for both Cl-dH_2O and Cs-dH_2O, and 25 µg/ml for Cc-dH_2O extracts. The volume of Cl-dH_2O and Cc-dH_2O extracts required to inhibit ≥ 50% of *S. aureus* ATCC 12600 biofilm formation ($MBIC_{50}$) was found as 50 µg/ml. On the other hand, $MBIC_{50}$ of the Cl-dH_2O extract on *C. albicans* ATCC 10231 was determined as 50 µg/ml. Biofilm inhibition of pathogen test microorganisms by positive controls are shown in Figure 5.

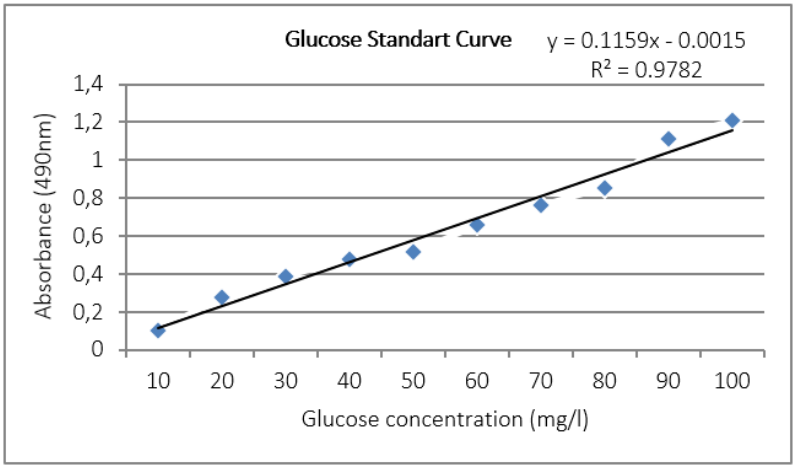

Figure 1. Glucose standart curve

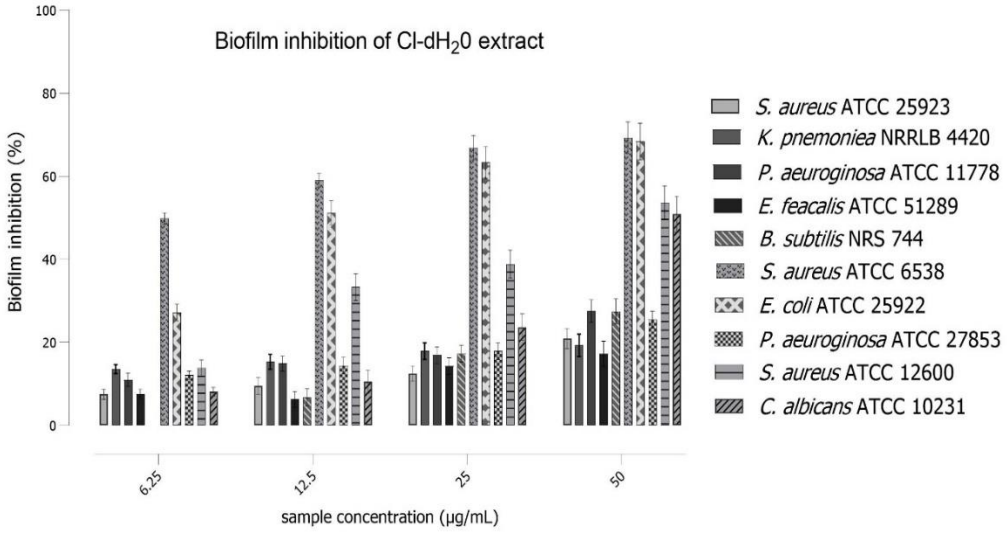

Figure 2. Biofilm inhibition of pathogen test microorganism by Cl-dH_2O extract

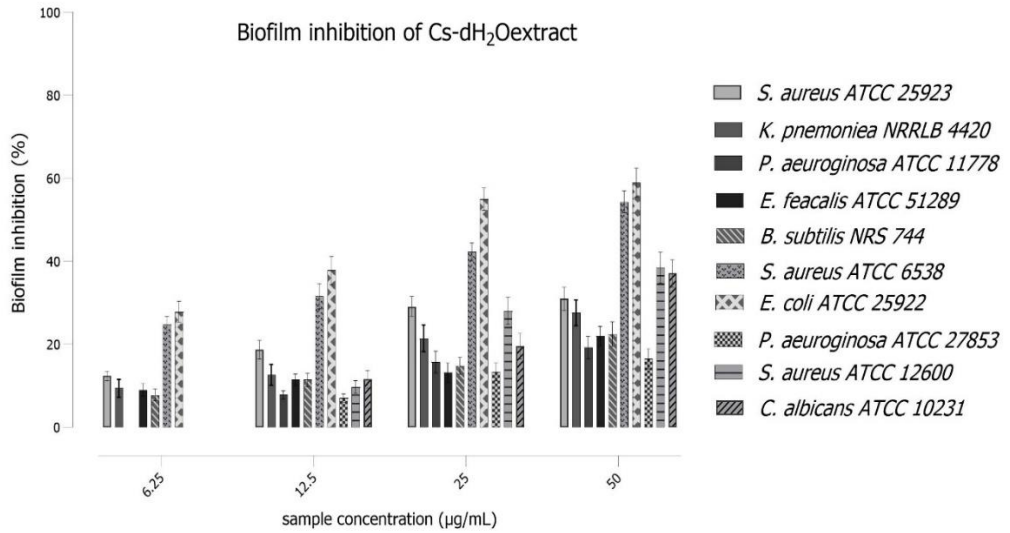

Figure 3. Biofilm inhibition of pathogen test microorganisms by Cs-dH$_2$O extract

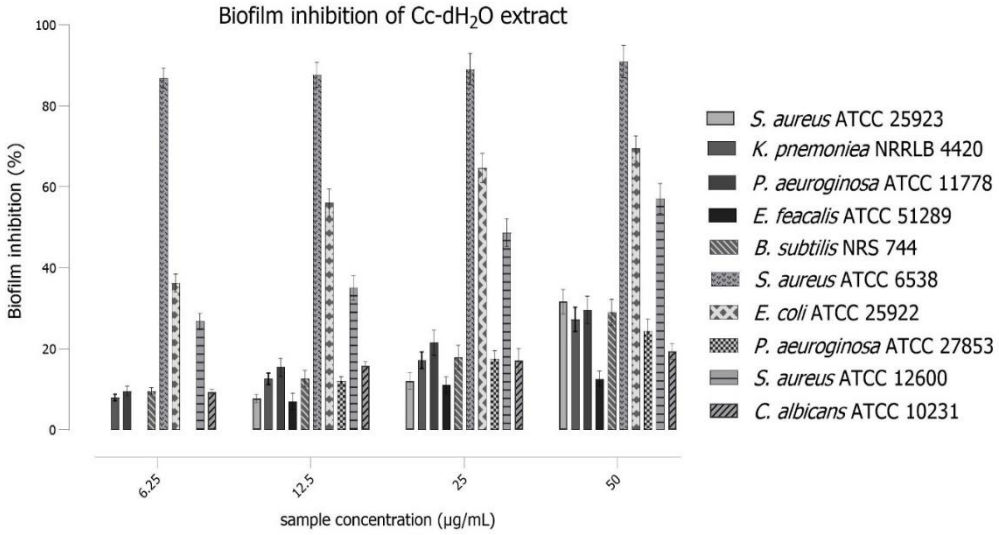

Figure 4. Biofilm inhibition of pathogen test microorganisms by Cc-dH$_2$O extract

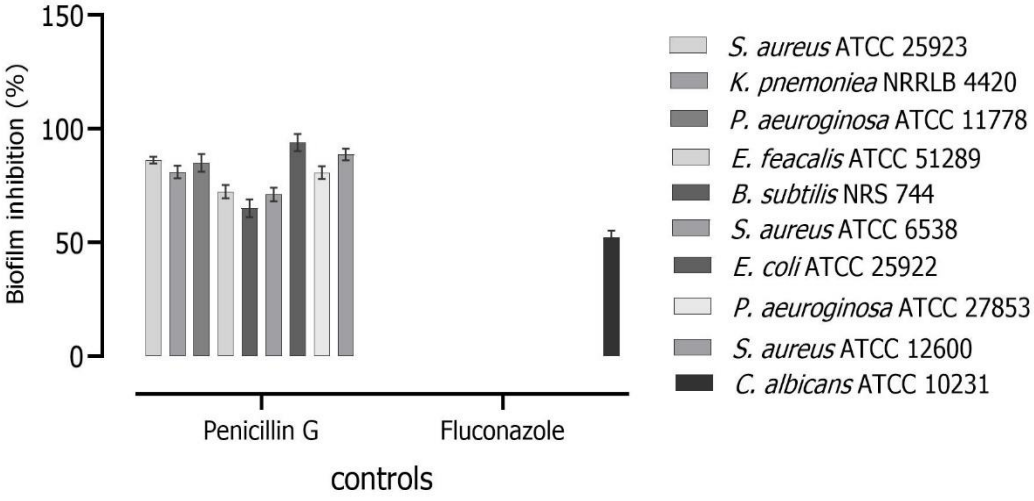

Figure 5. Biofilm inhibition of pathogen test microorganisms by positive controls

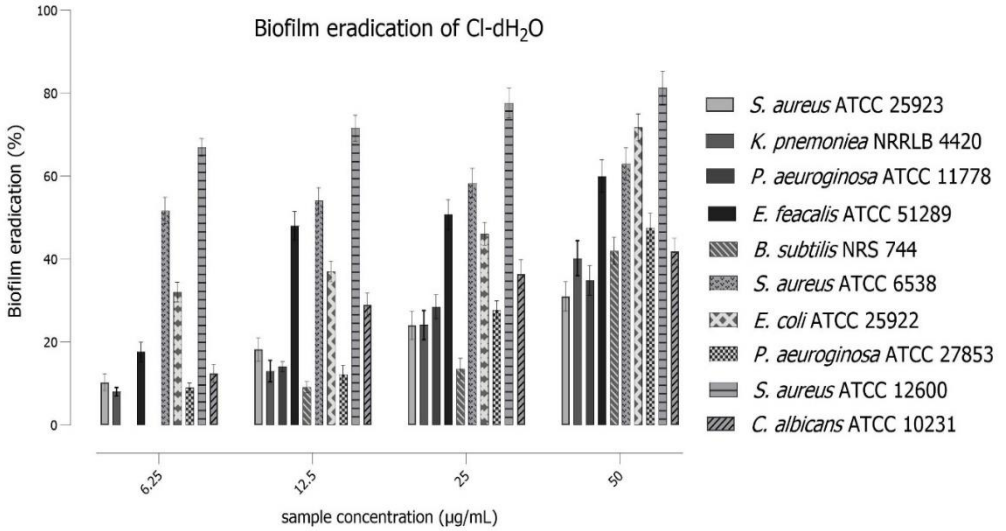

Figure 6. Biofilm eradication of pathogen test microorganisms by Cl-dH_2O extract

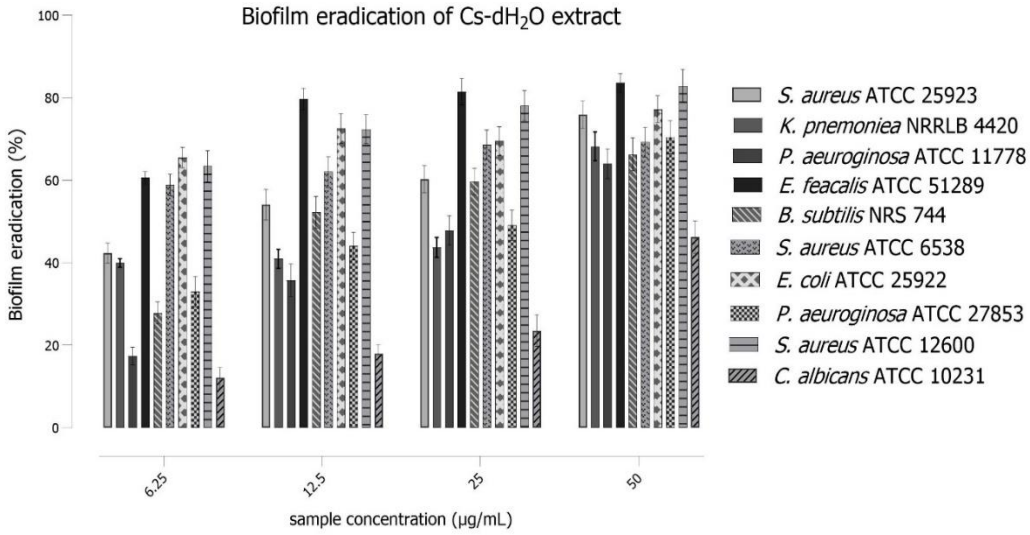

Figure 7. Biofilm eradication of pathogen test microorganisms by Cs-dH_2O extract

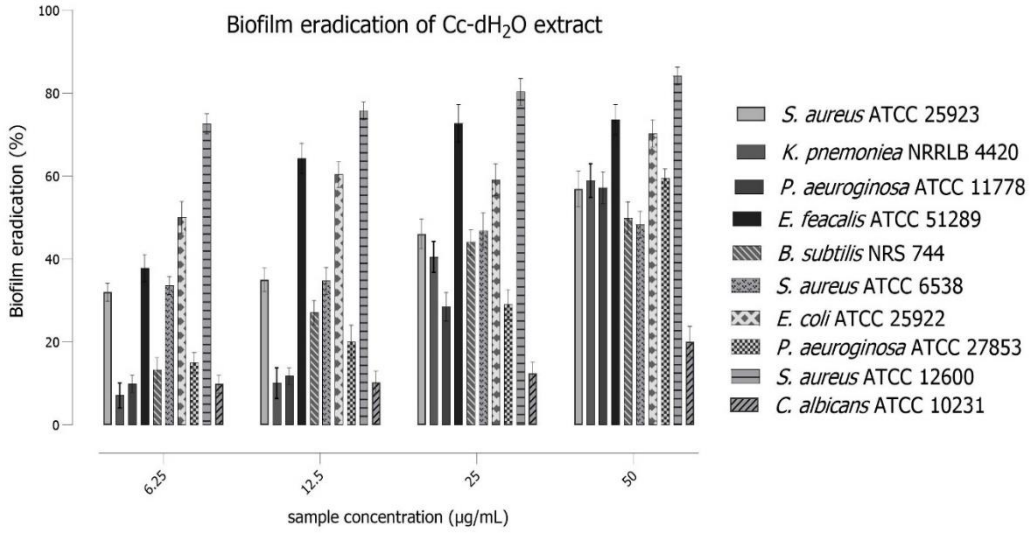

Figure 8. Biofilm eradication of pathogen test microorganisms by Cc-dH_2O extract

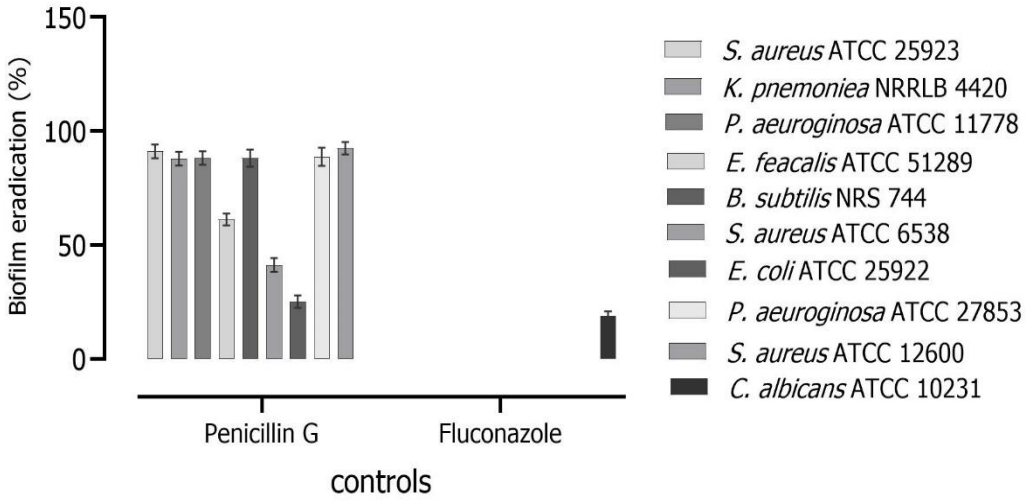

Figure 9. Biofilm eradication of pathogen test microorganisms by positive controls

The eradication of the biofilm formation of pathogen test microorganisms by Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O at various concentrations is demonstrated in Figures 6, 7 and 8. The concentrations of Cs-dH$_2$O and Cc-dH$_2$O extracts to eradicate ≥ 50% biofilm formation (MBEC$_{50}$) of *S. aureus* ATCC 25923 were found as 12.5 and 50 µg/ml, respectively. MBEC$_{50}$ values of Cs-dH$_2$O and Cc-dH$_2$O extracts on *K. pneumoniae* NRRLB 4420 strain were determined as 50 µg/ml. MBEC$_{50}$ values of Cs-dH$_2$O and Cc-dH$_2$O extracts on *P. aeruginosa* ATCC 11778 were found as 25 µg/ml and 50 µg/ml, respectively. The amounts of Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O extracts required to inhibit preformed biofilm formation by ≥ 50% (MBEC$_{50}$) in *E. faecalis* ATCC 51289 strain were determined as 25.0, 12.5 and 6.25 µg/ml, respectively. MBEC$_{50}$ values of Cc-dH$_2$O and Cs-dH$_2$O on *B. subtilis* NRS 744 were found as 50 µg/ml and 12.5 µg/ml, respectively. MBEC$_{50}$ of Cl-dH$_2$O and Cs-dH$_2$O on *S. aureus* ATCC 6538 were both determined as 6.25 µg/ml, whereas it was found 50 µg/ml for the Cc-dH$_2$O extract. MBEC$_{50}$ values of Cs-dH$_2$O and Cc-dH$_2$O extracts on *E. coli* ATCC 25922 were both found as 6.25 µg/ml. MBEC$_{50}$ values of Cl-dH$_2$O and Cc-dH$_2$O on *P. aeruginosa* ATCC 27853 were both determined as 50 µg/ml, whereas it was calculated as 25 µg/ml for Cs-dH$_2$O extract. Moreover, MBEC$_{50}$ values of all Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O extracts on *S. aureus* ATCC 12600 were determined as 6.25 µg/ml. Also, 50 µg/ml of Cc-dH$_2$O extract was sufficient to inhibit ≥ 90% of the preformed biofilm formation (MBEC$_{90}$) of *S. aureus* ATCC 12600. Finally, the MBEC$_{50}$ of Cs-dH$_2$O extract on *C. albicans* ATCC 10231 strain was found as 50 µg/ml. Biofilm eradication levels of pathogen test microorganisms by positive controls are shown in Figure 9.

In this study, the antibiofilm effects of Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O leaf extracts were evaluated against some pathogenic test microorganisms. The results of the present study clearly demonstrated that the percentages of biofilm inhibition and biofilm eradication of pathogen test microorganisms increased depending on the increasing concentrations of Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O leaf extracts. The highest concentration of Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O extracts were effective as MBIC$_{50}$ and MBEC$_{50}$ of positive controls applied to pathogen test microorganisms. Although numerous studies have been conducted to evaluate biological effects of *Cistus* sp., there is limited research on antibiofilm activity of *Cistus* sp. against pathogen microorganisms (Zalegh et al., 2021). Hannig et al. (2008) revealed that *Cistus*-tea may be used to reduce the initial bacterial adhesion. Lekbach et al. (2018) showed that *C. ladanifer* extract inhibited the growth of *P. aeruginosa* planktonic cells and the biofilm formation. In another study, Álvarez-Martínez et al. (2021) revealed that *C. salviifolius* extract exhibited higher antimicrobial activity against *S. aureus* isolates. Previous studies have demonstrated a relationship between polyphenols of plant extracts and their antimicrobial activities (Zalegh et al., 2021). In a previous study, it was suggested that the antibiofilm activities of Cl-dH$_2$O, Cc-dH$_2$O and Cs-dH$_2$O leaf extracts against pathogen test microorganisms could be associated with bioactive substances present in these extracts.

4. Conclusions

The results of this study revealed that the plant species that examined in this study showed varying degrees of antibiofilm activities against *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 25923, *K. pneumoniae* NRRLB 4420, *E. faecalis* ATCC 51289, *E. coli* ATCC 35218, *B. subtilis* NRS 744, *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 12600, *P. aeruginosa* ATCC 11778, and *C. albicans* ATCC 10231. The percentage of biofilm eradication values of these extracts were more effective than the biofilm inhibition values against the pathogen test microorganisms. Among the examined extracts, Cs-dH$_2$O showed greater antibiofilm effect against tested pathogen microorganisms. Therefore, Cs-dH$_2$O leaf extracts could be a potential candidate for drug discovery, particularly in the treatment of immune deficient patients. Also, these plant extracts may be potential candidates for further investigation to isolate antimicrobial compounds and to determine the mechanism of activity.

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None.

Conflict of interest

The authors declare no confict of interest in conducting and reporting this study.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

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CRediT authorship contribution statement

Sevim Feyza Erdoğmuş: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Writing, Review and Editing
Cihat Bilecen: Resources, Conceptualization, Visualization, Formal analysis, Investigation, Methodology
Özlem Erdal Altıntaş: Resources, Formal analysis, Methodology, Investigation, Review and Editing
Sevgi Ulukütük: Investigation, Review and Editing
Mustafa Kargıoğlu: Review and Editing

ORCID Numbers of the Authors

S.F. Erdoğmuş: 0000-0002-4319-7558
C. Bilecen: 0000-0002-0499-7317
Ö. Erdal Altıntaş: 0000-0003-4680-1738
S. Ulukütük: 0000-0002-5760-8493
M. Kargıoğlu: 0000-0003-0386-9716

Supplementary File

None.

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Reviewed by:

Safiye Elif KORCAN: Usak University, Usak, TURKEY
Arzu UNAL: Iğdır University, Iğdır, TURKEY