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Tube-Tests of *Eichhornia crassipes* and *Pistia stratiotes* Extracts as Bacterial Anti-Biofilm

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Abstract: Bacterial biofilms have strong adhesion to medical devices and are resistant to disinfectants, potentially triggering nosocomial infections. One promising approach involves the use of alternative phytochemical-based disinfectants. *Eichhornia crassipes* (*E. crassipes*) and *Pistia stratiotes* (*P. stratoites*) plants are known to possess antibacterial compounds; however, their anti-biofilm potential remains to be studied. This experimental study aimed to assess the antibiofilm potential of ethanol extracts of *E.crassipes* and *P.stratiotes* using a test tube method. The combination extract (EC+PS) was prepared at concentrations of 6.25% - 100% and tested in triplicate along with a control. Its ability was tested on three ATCC bacterial strains (*S. aureus, E. coli*, and *P.aeruginosa*) that form biofilms; confirmed by the growth of black colonies on Congo Red Agar (CRA). Qualitative observations determined the Minimum Biofilm Inhibitory Concentration (MBIC), revealing effective inhibition of 12.5% for *E. coli* and *S. aureus*, and 25% for *P. aeruginosa*. ANOVA analysis of biofilm intensity based on the Mean Gray Value (MGV) parameter showed significant variation between the EC+PS treatment and the control group ($p < 0.05$). Duncan's post hoc test showed the antibiofilm effect of EC+PS was comparable to chlorine: 75% for *S. aureus* and 100% for *E. coli* and *P. aeruginosa* ($p < 0.05$). In conclusion, the anti-biofilm activity of *E. crassipes* and *P. stratoites* shows promise as alternative disinfectants.
Keywords: Aquatic plants; biofilm-inhibitory activities; disinfectants; microbial biofilm.

INTRODUCTION

Nosocomial, or hospital-acquired infections, represent a significant challenge within the healthcare sector, exhibiting high global prevalence. Approximately 99% of bacterial populations exist in biofilm form, with only around 1% existing as free-floating (*planktonic*) cells (Jamal et al., 2018). Nearly 80% of clinical infections are thought to be associated with biofilm-forming bacteria (Zhao et al., 2023). Notably, *E. coli*, *P. aeruginosa,* and *S. aureus* are prominent biofilm producers. Biofilm development frequently occurs on medical device surfaces (Jamal et al, 2018; Mirani et al., 2018), leading to conditions including urinary tract infections, *endocarditis,* ventilator-associated pneumonia, *osteomyelitis, chronic* wound infections, and infections of prosthetic implants, including artificial joints and catheters (Shakibaie, 2018; Assefa & Amare, 2022). Biofilm formation contributes to treatment failure and increases healthcare costs. These complex microbial communities confer resistance against antibiotics, disinfectants, ultraviolet radiation, pH fluctuations, desiccation, heat,

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viruses, and host immune defenses. Consequently, biofilm-associated infections are particularly complex to manage (Zhao et al., 2023; Rather et al., 2021).

The disinfection of medical devices using disinfectants such as chlorine is of critical importance in preventing the transmission of nosocomial infections. Chlorine is particularly effective in this regard, as it can degrade the extracellular matrix of biofilms, which constitutes a major barrier to the effectiveness of antibiotic and antiseptic treatments. The implementation of routine cleaning protocols employing chlorine is therefore a vital measure to minimize the incidence of biofilm-related nosocomial infections (Tiwari et al., 2018; Waqas et al., 2023).

Deployment-related infections can be prevented through the application of disinfecting agents that inhibit microbial colonization or eliminate microorganisms (Artasensi et al, 2021). Biofilms exhibit a high tolerance to antimicrobial agents, necessitating the use of potent and adequately formulated disinfectants. *Chlorine or hypochlorite* remains among the most widely used disinfectants, offering broad-spectrum antimicrobial efficacy and the ability to suppress biofilm development (Tiwari et al., 2018). However, prolonged use of chlorine can promote microbial resistance and pose health risks, including dermatological irritation, ocular discomfort, and respiratory complications (Fabrizio et al., 2024). Furthermore, the efficacy of these disinfectants warrants careful evaluation, as exposure to pure microbial cultures can lead to biofilm bioaccumulation, affecting the viability, structural integrity, and biochemical properties of microbial cells (Fabrizio et al., 2024; Todorić et al., 2023).

The application of phytochemicals as preventive agents against pathogenic microbial transmission has been widely investigated due to their low toxicity and greater environmental compatibility. Phytocompounds have demonstrated considerable potential as biofilm-inhibitory agents (Lu et al, 2019; Mishra et al., 2020). These compounds possess bioactive properties, demonstrating combined antimicrobial and biofilm-inhibitory activities (Nadaf et al., 2018). Secondary metabolites exert suppressive effects on single-species as well as polymicrobial biofilm communities (Hu et al., 2020). Classes of bioactive compounds with antibiofilm activity include flavonoids, alkaloids, tannins, saponins, and steroids (Lu et al, 2019; Mishra et al., 2020)

The application of these compounds as combination formulations for treating biofilm-associated infections is widely considered more efficacious and preferable than the use of single-compound preparations (Hu et al, 2020; Bonincontro et al., 2023). Common aquatic weeds include *E. crassipes,* widely known as water hyacinth, and *P. stratiotes,* known as water lettuce. Both species exhibit pharmacological properties, including diuretic, antidiabetic, antidermatophytic, antioxidant, antifungal, and antimicrobial activities. The phytochemical constituents of these aquatic plants include phenolics, flavonoids, tannins, and alkaloids. Additional antimicrobial compounds identified in these species comprise steroids, saponins, anthraquinones, and terpenoids (Tulika & Mala, 2015; Tyagi & Agarwal, 2017)

The development of natural products as novel therapeutic strategies for more effective biofilm inhibition is necessary to address pathogens resistant to synthetic drugs (Srinivasan et al., 2021). The effectiveness of an extract as an antibacterial and antibiofilm agent requires a solvent compatible with its phytochemicals. For example, testing the water extract of *E. crassipes* and *P. stratiotes* in a single preparation did not show an antibacterial effect, but in combination, it was able to reduce bacterial colonization (Budiarti et al., 2024a). Meanwhile, with ethanol extract in a single preparation, the resulting effect was still below the positive control. The effectiveness of inhibition appeared to be comparable to the positive control by administering the

combined extract (*E. crassipes* + *P. stratiotes*) on a range of every Gram bacteria (Budiarti et al., 2024a; Budiarti et al., 2024b). Ethanol, chloroform, methanol, and petroleum ether can be used as solvents for phytochemicals that have antibiofilm effects (Tumpa et al., 2023). Methanol extracts of the aquatic plants *Punica granatum* L. and *Rhus coriaria* L. are known as plant-based antimicrobial compounds effective in inhibiting biofilms of *S. aureus*, *P. aeruginosa,* and *E. coli* bacteria. Biofilms produced by these three pathogens contribute to bacterial protection against antimicrobials (Nostro et al., 2016). In vitro, biofilms formed by pathogenic bacteria can be detected using the Congo Red Agar (CRA) method; bacterial colonies with biofilms appear black (Normanita, 2020; Budiarti et al., 2024c). The phytochemical composition of aquatic plants is similar to that of *Eichhornia crassipes* and *Pistia stratiotes*; therefore, both represent promising candidates for antibiofilm applications.

Preliminary phytochemical examination of ethanol extracts of *Eichhornia crassipes* and *Pistia stratiotes (EC+PS)* revealed the presence of tannins, alkaloids, flavonoids, terpenoids, phenolics, saponins, and steroids. These compounds are known to possess antimicrobial and antibiofilm activity, with effectiveness significantly influenced by their phytochemical composition. In general, combinations of various secondary metabolites can produce synergistic therapeutic effects as antibiofilms (Mishra et al., 2020; Hu et al., 2020; Bonincontro et al., 2023), including against antibiotic-resistant microbes, without showing signs of acute toxicity (Ngemenya et al., 2019).

The test tube method can be used to qualitatively assess antibiofilm activity by determining the Minimum Biofilm Inhibitory Concentration (MBIC) (Goeres et al., 2019), while biofilm intensity can be measured quantitatively using the Mean Gray Value (MGV) approach; higher MGV values indicate thinner biofilms (Artyushkova et al., 2016; Winarsih et al., 2023). This approach has been used, for example, with a combination of *Citrus hystrix* extracts, which showed variations in MGV values against *P. aeruginosa, E. coli,* and *S. aureus* compared to synthetic antibiofilms (Budiarti et al., 2024c). However, to date, scientific data on the antibiofilm activity of the combination of ethanol extracts of *E. crassipes* and *P. stratiotes* is still limited, especially against biofilm-producing clinical isolates using the combined MBIC and MGV approach, thus requiring further exploration. The aim of this study was to assess the antibiofilm potential of ethanol extracts from *Eichhornia crassipes* and *Pistia stratiotes* using the test tube method.

MATERIALS AND METHODS

This experimental posttest-only control group study was performed between October and December 2023 in the Pharmacology and Microbiology Laboratories, Faculty of Medicine (FK), Lambung Mangkurat University (ULM). The test plants, *Eichhornia crassipes* and *Pistia stratiotes*, were collected in August 2023 from Sungai Jingah Village and identified in the Mathematics and Natural Sciences Laboratory ULM, Banjarbaru.

Extracts of *E. crassipes* (EC) and *P. stratiotes* (PS) were prepared by maceration in ethanol (96%, Merck) and evaporated using a rotary evaporator (Buchi, Switzerland) to obtain concentrated extracts (purity ≥99.5%). The extracts were dissolved in Dimethyl Sulfoxide (DMSO, Merck). As a result, single extracts of *E. crassipes* (EC) and P*. stratiotes* (PS) were prepared at concentrations of 3.125%, 6.25%, 12.5%, 25%, 3.75%, and 50% (w/v). Preparation of the combination of E. crassipes + P. stratoites (EC + PS) with a ratio of 1:1, by mixing 2 mL of EC and 2 mL of PS into a sterile tube, so that the combination of EC + PS/tube tested was 100%,

75%, 50%, 25%, 12.5%, and 6.26%. (Lin et al., 2017; Budiarti et al., 2024b; Budiarti et al., 2024c). Each treatment was carried out in three replications.

Standard ATCC strains (*E. coli* 25922, *P. aeruginosa* 27333, *S. aureus* 25923). A bacterial suspension was prepared in Trypticase Soy Broth (TSB) (Merck) mixed with 10% glucose (Merck) (TSB+glu), then homogenized by vortexing and maintained at 37°C for a further 24 hours. A 100 µl sample of the incubated liquid culture was diluted with TSB + glu solution, and the turbidity level was compared with that of a 0.5 McFarland (1.5×10^8 CFU/mL).

Tested for biofilm production on Congo Red Agar/CRA (Merck): One loop of bacterial isolate was taken from TSB+glu medium, followed by streaking onto CRA plates and incubation at 37°C for 48-hours. Each culture was carried out in triplicate. Biofilm production was evaluated according to colony morphology, pigmentation, and the degree of slime formation, and classified as weak (black), moderate (almost black), or strong (very black) (Aniba et al., 2024).

Tube test (Qualitative test), observation of Minimum Biofilm Inhibitory Concentration (MBIC): Prepared EC+PS extracts at various concentrations and controls, test bacterial suspension (0.5 McFarland), also sterile tubes. A total of 2 ml of 100% EC+PS extract was put into tube-1 containing 2 ml of the test bacterial suspension. Tubes-2 to 6 each contained bacterial suspension, each containing EC+PS extract (75%, 50%, 25%, 12.5%, and 6.25%), and tube-7 only contained 2 ml of bacterial suspension without treatment/negative control. Tube-8 contained 2 ml of bacterial suspension combined with 2 ml of 0.0002% Chlorine (Merck), and tube-9 contained 2 ml of bacterial suspension combined with 2 ml of negative control (10% DMSO). All test tubes were maintained at 37°C for 24 hours (Budiarti et al, 2024c; Irfan et al., 2022).

MBIC assessment: The turbidity level in the tube was observed based on the following categories: +0 (clear, no biofilm formation), +1 (slightly clear, if weak, slight biofilm formation), +2 (slightly turbid, if biofilm formation is present), and +4 (turbid, if biofilm formation is strong). The MBIC value is the lowest concentration, indicating biofilm formation in the +1 category (beginning to clear/slightly clear). MBIC was confirmed by taking one loop of suspension from the +1 tube, spreading it evenly on the CRA surface, and subsequently incubating under standard conditions (37°C) for a 48-h period. Blackish bacterial colonies that failed to grow or that grew the least on the petri dish were identified as MBIC (Budiarti et al., 2024c; Irfan et al., 2022).

Mean Gray Value (MGV) Measurement: After 24 hours of incubation at 37°C, the contents of the tube-test were removed and rinsed with phosphate-buffered Saline (PBS) (pH 7.3, Merck) and dried. Next, 0.5 ml of 0.1% Crystal Violet solution (Merck) was added to the tubes as a rinse, and excess stain was discarded. Each test tube was rinsed using sterile distilled water (deionized water) and dried. Each dried tube was photographed, and the MGV was analyzed using Adobe Photoshop Creative Suite 6 Extended (CS-6). The scale used ranged from 0-255. Photo film results with values close to 0 (zero) indicate high film color density, and values close to 255 indicate low film color density control (Winarsih 2023; Budiarti et al., 2024c; Irfan et al., 2022).

Data analysis: MBIC values were analyzed descriptively, MGV values by Shapiro-Wilk and Levene's tests, followed by ANOVA and Duncan's test ($p < 0.05$) (Nadaf et al., 2018). Ethical Applicability: The study complied with Biosafety Level 2 standards and was approved by the Health Research Ethics Committee, Faculty of Medicine, Lambung Mangkurat University (Ref: No. 165/KEPK-FK ULM/EC/VII/2023).

RESULTS AND DISCUSSION

Qualitative MBIC observations according to the outcomes of the +1 category tube test appeared to be clear and did not form biofilm colonies on CRA. The MBIC of the *Eichhornia crassipes* and *Pistia stratiotes* (EC+PS) extract, as shown in Table 1, was 12.5% for both *S. aureus* and *E. coli*, and 25% *for P. aeruginosa*. These results indicate that tubes with higher extract concentration suspensions appeared clearer and showed an increased antibiofilm effect.

Table 1. Tube-test Turbidity Level of *Eichhornia crassipes* and *Pistia stratiotes* (EC+PS) Extract and Controls in Bacterial Biofilm

Treatment	The Turbidity of Microbial Biofilm Suspensions in TSB+Glu		
	S. aureus	***E. coli***	***P. aeruginosa***
6.25% EC+PS	+++	+++	++++
12.5% EC+PS	+	+	++
25% EC+PS	0	0	+
50% EC+PS	0	0	0
75% EC+PS	0	0	0
100% EC+PS	0	0	0
Chlorine	0	0	0
DMSO	+++++	+++++	+++++

Description: 0: Clear, no biofilm formed; +1: Slightly clear, with weak inhibition of biofilm formation; +2: Slightly turbid, with moderate inhibition of biofilm formation; +3: Turbid, with strong biofilm formation; +4: Very turbid, very strong biofilm formation. MBIC was confirmed in a tube +1 (beginning clear) and without blackish bacterial growth/colonies at least on CRA.

The antibiofilm effect of the extract in suppressing P. aeruginosa biofilm development requires a higher concentration than *S. aureus* and *E. coli*. The chlorine-treated tube appeared clearest, while the DMSO-treated tube appeared most turbid, indicating that DMSO has no antibiofilm activity. The antibiofilm efficacy of EC+PS was evaluated by assessing the intensity of biofilm formation in test tubes using the Mean Gray Value (MGV) method (Figure 1).

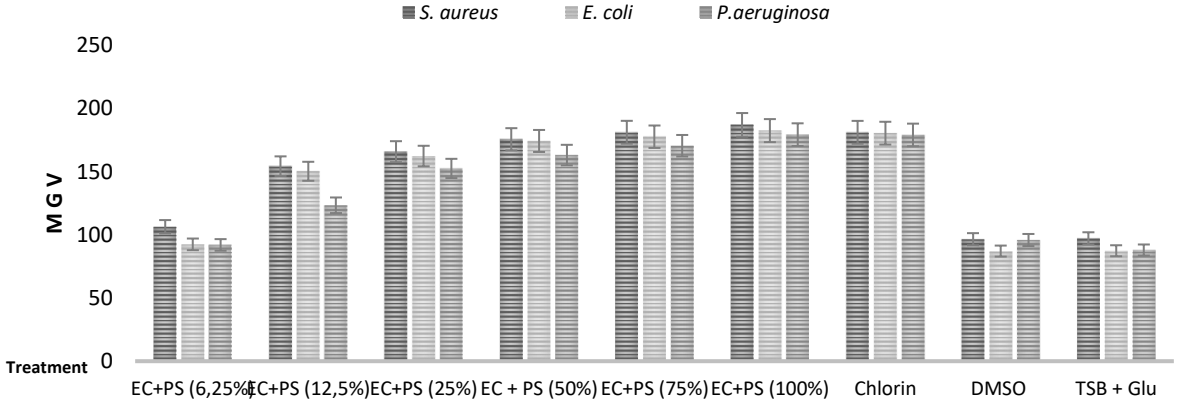

Figure 1.The Average Mean Gray Value (MGV) of Bacterial Biofilms in Extract of *Eichhornia crassipes* and *Pistia stratiotes* (EC+PS) and Controls

Figure 1 shows how increasing EC+PS extract concentration can increase the MGV value, indicating an increased antibiofilm effect with thinner film formation on the tube wall. The lowest MGV value was seen in the negative control, while the highest MGV was seen in chlorine and 100% EC+PS. The average MGV of the extract on *S. aureus* and *E. coli* was relatively similar, while the MGV of *P. aeruginosa* was lower than the other two bacteria.

Table 2. Comparison of Mean Gray Value (MGV) Averages of Bacterial Biofilms in *Eichhornia crassipes* and *Pistia stratiotes* (EC+PS) Treatments and Controls

Treatment	Mean Gray Value and Standard deviation		
	S. aureus	*E. coli*	*P. aeruginosa*
6.25% EC+PS	106.29±1.97780^{f}	92.39±0.30889^{g}	91.94±0.11150^{g}
12.5% EC+PS	154.27±0.44613^{e}	150.18±0.61857^{f}	123.3±0.57597^{e}
25% EC+PS	165.69±0.35796^{d}	162.25±0.48773^{e}	152.42±0.43705^{d}
50% EC+PS	175.5±0.66343c	174.16±0.17010^{d}	162.97±0.65767^{c}
75% EC+PS	181.02±0.07965^{b}	177.52±0.25929^{c}	170.37±0.32585^{b}
100% EC+PS	186.89±0.92465^{a}	182.34±0.29339^{a}	179.16±0.92467^{a}
Chlorine	180.98±0.25861^{b}	180.33±0.50518^{b}	178.92±1.04711^{a}
DMSO	96.44±0.54249^{g}	87.07±0.63893^{h}	95.81±0.24007^{f}
TSB + Glucose	97.1±040437^{g}	87.31±0.46592^{h}	87.91±0.19029^{h}

Note: The same letter notation within the same column denotes no significant difference, whereas different letter notations indicate significant differences ($p > 0.05$).

The ANOVA and Duncan's analysis results (Table 2) indicated that the antibiofilm effect of the extract at the same concentration was significantly different in the three bacteria. The biofilm inhibition effect on *S. aureus* was greater than on *E. coli* and *P.aeruginosa* biofilms. The antibiofilm activity of chlorine was below the effect of 75% extract on *S. aureus* and 100% on *E. coli*, while the effect of 100% extract on *P.aeruginosa* was equivalent to the effect of chlorine.

Biofilms consist of a matrix composed of extracellular polymeric substances (EPS), primarily consisting of *exopolysaccharides*, proteins, and nucleic acids. This matrix surrounds microbial cells and facilitates intercellular communication via biochemical signaling and genetic exchange, and serves multiple functions, including nutrient distribution and enzyme localization. Extracellular DNA (eDNA) constitutes a crucial structural element in many microbial biofilms; hence, enzymatic degradation of eDNA can disrupt biofilm structural stability, compromising biofilm architecture and promoting the release of microbial cells from surfaces (Gebreyohannes et al., 2019). The findings of this study indicate that EC+PS extract treatment resulted in biofilm thinning, which is consistent with the susceptibility of eDNA and EPS to antibiofilm agents.

Biofilm formation poses a major challenge for the control of healthcare-associated infections, especially within hospitals, nursing homes, and other long-term care settings, largely because of the innate resistance of biofilms to antimicrobial therapies (Srinivasan et al., 2021). Opportunistic pathogens frequently implicated in nosocomial infections possess biofilm-forming capabilities (Mirani et al., 20189). Biofilms enriched with glycocalyx layers contribute to bacterial persistence, resisting host immune defenses and conventional antimicrobial or disinfection strategies (Kekeç et al., 2016). Furthermore, biofilms commonly develop on medical device

surfaces, facilitating microbial colonization and enhancing infection risk through dissemination of both individual and clustered cells (Srinivasan et al., 2021). This is consistent with research findings showing that all three clinical isolates, *S. aureus*, *E. coli*, and P*. aeruginosa,* were capable of forming thick biofilms in negative controls, as indicated by low MGV values.

Chlorine, particularly commonly used as sodium hypochlorite, is effective in preventing infection transmission due to its broad-spectrum antimicrobial properties, effectively eliminating vegetative cells, spores, and biofilms (Kekeç et al., 2016). Its antibiofilm activity increases with concentration and operates by disrupting protein structures in the biofilm matrix, inhibiting DNA synthesis, and inducing oxidative stress. Mechanistically, chlorine reacts with amino acids and undergoes chlorination, which interferes with the attachment and stability of biofilms (Günther et al., 2017; Clayton et al., 2021). This consistency is seen in studies, where chlorine showed the highest or near-highest MGV values across all bacteria used as positive antibiofilm controls.

Antibiofilm activity was demonstrated in this study for both chlorine and the ethanol extracts from *E. crassipes* and *P. stratiotes* against *P. aeruginosa, S. aureus,* and *E. coli.* The combined use of these plant extracts may confer a synergistic effect, as evidenced by prior research where mixtures of *P. granatum L*. and *R. coriaria L*. extracts exhibited antibiofilm effects against multiple pathogens *(Haroon & Daboor, 2019)*. Previous findings also support the efficacy of *E. crassipes* and *P. stratiotes* in inhibiting *E. coli* colonization, exhibiting effects comparable to chlorine (Budiarti et al., 2024a). The results of this study corroborate these findings, where *E. crassipes* and *P. stratoites* extracts resulted in an increase in MGV with increasing concentration, indicating concentration-dependent antibiofilm effectiveness.

The antibiofilm activity to initiate biofilm formation inhibition (MBIC) in *P. aeruginosa* requires a higher concentration than in *S. aureus* and *E. coli*. This difference is influenced by *P. aeruginosa*'s resistance to antimicrobial agents; this bacterium is more stable in aqueous environments and more tolerant to disinfectants (Zhang et al., 2015). Differences in antibiofilm activity were also reported for *C. hystrix* extract (Budiarti et al., 2024c) and the cefadroxil–A. cordifolia combination (Mulyani & Natassya, 2025). Thus, antibiofilm activity is influenced by the type of extract and isolate tested. The results of this study also showed a similar pattern, with *P. aeruginosa* having a lower MGV value at initial concentrations than the other two bacteria, confirming its relative resistance.

The mechanisms of antibiofilm action include inhibition of initial cell adhesion, suppression of exopolysaccharide synthesis, disruption of established biofilms, and bactericidal effects on adherent cells. The vulnerability of the bacterial membrane plays a key role in the effectiveness of the extract: the membrane of gram-positive bacteria is more polar, making it more permeable to polar antimicrobial agents (Harika et al., 2020). The hydrophobicity of the biofilm-forming phenotype also influences adhesion dynamics; For example, *P. aeruginosa* is more hydrophobic than *S. aureus* and *E. coli* (Mirani et al., 2018). This helps explain the study results where the extract was more effective against S*. aureus*, reflected in higher MGV values at low concentrations. Antibiofilm activity is also influenced by biofilm structure: *P. aeruginosa* forms dense, organized microcolonies, while *E. coli* forms smaller, more fragmented biofilms (Harika et al., 2020). This structural difference is evident in the variation in MGV values between bacteria in this study.

The observed antibiofilm activity was related to the bioactive compounds in the *E. crassipes* and *P. stratiotes* extracts. Phytochemical analysis identified alkaloids, tannins, phenolics, flavonoids, terpenoids, saponins, and anthraquinones, consistent

with previous reports (Guzzo et al., 2020). The antibacterial mechanisms of these compounds include cell wall disruption, enzyme inhibition, and membrane damage (Goeres et al., 2019; Günther et al., 2017). Antibiofilm mechanisms include disruption of virulence factors and biofilm formation pathways. The presence of these compounds may explain the consistent increase in MGV values in high-concentration extracts in this study. Flavonoids inhibit the adhesion genes icaA and icaD, alkaloids disrupt quorum sensing, tannins are anti-adhesive, saponins inhibit EPS formation, and terpenoids inhibit initial surface attachment formation (Jain & Parihar, 2018; Lahiri et al., 2019; Hamzah et al., 2019; Laskoski et al., 2020; Tatli Cankaya & Somuncuoglu, 2021). This combination of mechanisms likely contributes to the antibiofilm effects of the *E. crassipes* and *P. stratiotes* extract observed in this study.

The findings of this study support previous reports on the biological activities of *E. crassipes* and *P. stratiotes,* including antibiofilm activity. However, limitations of the study lie in its in vitro design, which may not be representative of in vivo conditions. The MGV method is susceptible to technical variations such as lighting and tube position. Nevertheless, the clear concentration-response pattern in MGV values strengthens the validity of the observed antibiofilm trends.

CONCLUSION

This research found that the administration of *Eichhornia crassipes* and *Pistia stratiotes* extracts inhibited biofilm formation in three bacterial isolates, based on tube tests*. Eichhornia crassipes* and *Pistia stratiotes* extracts began to inhibit biofilm formation with MBICs of 12.5% for *S. aureus* and *E. coli,* and 25% for *P. aeruginosa*. The extracts' antibiofilm efficacy compared to chlorine differed significantly for the three bacteria ($p<0.05$). The activity of 75% extract against *S. aureus* biofilms, and 100% extracts against *E. coli* biofilms, was greater than chlorine, while the effect of 100% extract on *P. aeruginosa* biofilm was equivalent to chlorine. In conclusion, *Eichhornia crassipes* and *Pistia stratiotes* extracts have potential as alternative disinfectants with antibiofilm effects. Their effectiveness in disinfection can be further tested in accordance with applicable health standards.

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CONFLICT OF INTEREST

The research team and the conduct of this research have no conflict of interest.

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