ORIGINAL ARTICLE

A Comparative Study About the Inhibitory Effect of *Allium Sativum* and *Anredera Cordifolia* (Ten.) *Steenis* on *Pseudomonas Aeruginosa* Bacterial Growth, Motility, and Biofilm Formation, in Vitro Study

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ABSTRACT

Introduction: *Allium sativum* (garlic) and *Anredera cordifolia* (Ten.) *Steenis* (Madeira vine) have shown antimicrobial activities against many pathogen species, although the results varied. We aimed to compare the anti-bacterial and anti-biofilm activity of *Allium sativum* and *Anredera cordifolia* against *Pseudomonas aeruginosa*, which can cause nosocomial infection by colonizing medical device surfaces and forming biofilm. **Methods:** The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of garlic extract against *P. aeruginosa* ATCC 9027. The effect of the extracts on biofilm formation was tested by using static microtiter plate biofilm assay. Allium and *Anredera cordifolia* leaf extracts with various concentrations were incubated in LB medium then stimulated with *P. aeruginosa* ATCC 9027 suspensions. Crystal violet staining was performed prior to optical density measurement on a microplate reader at 595 nm. The bacterial motility tests were performed on Nutrient Agar (NA) and BBL motility test medium. **Result:** *Allium sativum* and *Anredera cordifolia* leaf extracts inhibited the growth of *P. aeruginosa* with MIC values of 1250 and 2500 µg/ml, respectively. Both extracts inhibited bacterial adherence with doses as low as 62.5 µg/ml of *Allium sativum* and 125 µg/ml of *Anredera cordifolia*. Scanning electron microscopy (SEM) clarified the inhibition of both extracts on bacterial attachment. In a similar trend, both extracts inhibited bacterial swimming and swarming motilities. **Conclusion:** This study suggests that both extracts could inhibit bacterial biofilm formation and bacterial cell motility in the optimal concentration of 500 µg/ml.
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Keywords: *P. aeruginosa*, *Allium sativum*, *Anredera cordifolia*, Extract, Biofilm, Anti-bacterial

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, rod-shaped opportunistic pathogen that can survive in a wide range of environments (1). While normally inhabiting the soil and natural environment, these bacteria possess a high adaptability and intrinsic antibiotic resistance, causing them to survive at artificial settings, including the surfaces of various medical devices. *P. aeruginosa* is commonly found adhering to plastic surfaces of medical devices and forming biofilms, which are difficult to remove and treat (2) including dental unit waterlines . Severe *P. aeruginosa* infections are often nosocomial and mostly found in immunocompromised hosts, including patients recovering from surgery, patients with burns, diabetes mellitus, malignancies, and elderly patients (3, 4). It is estimated that *P. aeruginosa* has a prevalence of 7.1%–7.3% among all healthcare-associated infections. This bacterium is the most common Gram-negative organism identified in nosocomial pneumonia. Healthcare-associated pneumonia (HAP) and ventilator-associated pneumonia (VAP) are a significant source of this infection. The cases were up to 22% of all healthcare-acquired infections. In the USA in 2017 it had been reported that *P. aeruginosa* was estimated to have caused 32.600 infections and 2700 deaths among hospitalized patients. Then in 2019 to 2020, this case rate increased by 32% (5, 6, 7). A retrospective study in Thailand reported that 22% of *P. aeruginosa* infections in hospitalized patients were due to XDR strains, resulting in significantly higher mortality (8).

Usually, antibiotics are prescribed for the treatment of *P. aeruginosa* infections. Conventional antibiotic therapy for *P. aeruginosa* infection usually uses β-lactam, fluoroquinolone and aminoglycosides. However, the widespread use of antibiotics may lead to antibiotics resistance. *P. aeruginosa* clinical isolated from the Intensive Care Units (ICUs) demonstrating the highest incidence of resistance to these antibiotics (9). Previous study reported that in the Arabian Gulf countries from 2010–2021 antimicrobial resistance was highest to meropenem ranging from10.3–45.7% (10). With the growing emergence of degenerative diseases along with the increasing number of antimicrobial-resistant strains (11, 12), treatment options for *P. aeruginosa* infections are limited. Therefore, develop natural products as anti-bacterial agent and methods to preventing bacterial colonization is an urgent priority.

Herbal medicine used in traditional medicine practice or complementary and alternative medicine (CAM) is popular for 80% percent of the world in Asia, Latin America, and Africa and is reported to have minimal side effects (13). *Allium sativum* (garlic) and *Anredera cordifolia* (Ten.) Steenis (Madeira vine, or "binahong" in Indonesian) are commonly found in Indonesia and are recognized as medicinal herbs. Both plants showed therapeutic use in various diseases and have been recognized as a potent antimicrobial agent. A previous study reported that *Allium sativum* extract is an effective agent for controlling various bacterial strains including extended-spectrum beta-lactamases (ESBL) Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus cereu (14, 15). The water-soluble component of *Allium sativum*, allicin, has also been reported to possess antimicrobial activity against Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus mutans, Streptococcus sobrinus and Actinomyces oris (14). *Anredera cordifolia*, on the other hand, have been reported to lower blood pressure (16), blood glucose (17), and cholesterol (18) levels, while also improving the healing process in burn injury (19). The effects are hypothesized to result from flavonoid, saponin, and tannin contents in its leaves, which have antioxidant, anti-inflammatory, and antibacterial effects. In a previous study, flavonoids (20) and saponins (21) are reported to induce bacterial lysis by alteration of bacterial cell wall, while alkaloids may interfere with DNA transcription process and protein synthesis within the bacteria (21). In addition, we have previously observed that *Allium sativum* inhibited biofilm formation of *Streptococcus sanguinis* by preventing bacterial adherence and reducing bacterial motility (22). While the anti-bacterial potential of *Allium sativum* has been widely studied, scarce evidence is found on *Anredera cordifolia* extract, notably towards *P. aeruginosa*. In this study, we aim to compare the anti-bacterial properties of *Anredera cordifolia* leaves extract to *Allium sativum* inhibiting the growth of *P. aeruginosa*. We also aim to observe and compare both extracts' capacity in inhibiting bacterial adherence and motility.

MATERIALS AND METHODS

Sample preparation

The ethical approval of this research was received from the Komite Etik Penelitian Kesehatan (KEPK) Medical Faculty Universitas Kristen Duta Wacana Yogyakarta (no. 1181.A/C.16/FK/2022). *P. aeruginosa* ATCC 9027 was grown in Lactose Broth (LB; Oxoid., UK) medium for 24 h at 37°C. The concentration of bacterial stock suspension was prepared as 1.5×10^8 colony forming units (CFU)/ml. *Allium sativum* and *Anredera cordifolia* leaves were obtained and confirmed by a botanical expert at the herbal manufacturing company, CV Merapi Farma, Yogyakarta, Indonesia (ref. SK/SMPL/MFH/VII/2020 and S/10/MFH-01/02/2020), and were processed into an ethanolic extract using maceration technique at the Integrated Research and Testing Laboratory of Universitas Gadjah Mada Yogyakarta, Indonesia, as our previous study (22). Briefly, 350 grams of *Allium sativum* were washed and chopped into small pieces, then mixed with 1000 ml of 96% ethanol. The mixture was stored at 4^0C for 24 hours. The macerate was filtered three times and concentrated by a vacuum rotary evaporator at 40^0C to obtain an ethanolic extract of *Allium sativum*. The *Anredera cordifolia* leaf extract was prepared in the same way. One hundred grams of the leaves were powdered and dissolved in 96% ethanol solution. The mixture was filtered three times, then evaporated with a rotary evaporator for 90 minutes at 40oC. The extracts in paste form were stored in a refrigerator at 4^0C until used. Extracts would be diluted before every experiment was carried out by taking as needed to ensure consistency in experimental results. Extracts in paste form were dissolved with dimethyl sulfoxide 1% (DMSO, Merck, Germany) solution as a stock in a concentration of 10,000 µg/ml which was then further diluted for graded concentration assay. The stock solution of extract was filtered using a syringe filter 0.45 µm (Sartorius, Germany) prior to use.

Antibacterial susceptibility assay

The broth microdilution method was used to test the susceptibility of *P. aeruginosa* ATCC 9027 to *Allium sativum* and *Anredera cordifolia* extracts to determine minimum inhibitory concentration (MIC). To determine the initial concentration of the extract for the MIC test, the optimization was carried out based on modification of our previous study (22). Approximately 10 µl of 1.5×10^8 CFU/ml bacterial suspension was inoculated into 100 µl of LB broth containing either *Allium sativum* or *Anredera cordifolia* ethanolic extract at a varying concentration (10,000; 5000; 2500; 1250; 625; 312.5; and 156.25 µg/ml) in 96-well culture plate (Iwaki, Japan). Ciprofloxacin (Sigma-Aldrich, USA) was used as a standard antibacterial agent at a concentration of 5 µg/ml that can cause no turbidity condition. The experiments were performed by using 5 replicates. The

plates were incubated for 24 h at 37°C and observed for the absence of turbidity visually. The MICs of *Allium sativum* and *Anredera cordifolia* extracts were defined as the lowest concentration showing no turbidity (22, 23).

After MIC determination, the minimal bactericidal concentration (MBC) was determined by spreading 100 µl of 1.5×10^8 CFU/ml bacterial suspension from the wells in MIC value and up on Brain Hearth Infusion agar plate (BHI, Oxoid, UK). The plates were incubated for 24 h at 37°C. There is no bacterial colony growth at the love's concentration of an antimicrobial agent, it is termed the MBC endpoint (23).

Bacterial adherence assay

A static microtiter plate biofilm assay was performed to test inhibition by the extract on *P. aeruginosa* biofilm formation. The assay was performed in 96-well culture plate (Iwaki, Japan). Ten µl of either *Allium sativum* or *Anredera cordifolia* extract were added to 100 µl LB broth in 96-well culture plate and were incubated for 30 min at 37°C. Each culture was inoculated with 10 µl of 1.5×10^8 CFU/ml *P. aeruginosa* suspension and incubated at 37°C for 24 hours. After the incubation, the culture medium and the planktonic bacteria were removed from each well by washing with phosphate buffer saline solution (PBS, Sigma-Aldrich, Germany, pH 7.4) and fixed with 150 µl absolute methanol for 15 min. The biofilm adhering onto the wells was stained with 0.1% (wt/vol) crystal violet for 10 min at room temperature and rinsed with PBS twice. The stained biofilms were extracted from the wells with 200 µl of 96% ethanol and transferred to a fresh 96-well plate. The absorbance of the biofilms was measured at 595 nm using a microplate reader (Thermo Scientific, USA).

Scanning Electron Microscopy (SEM)

Observation with a scanning electron microscope (SEM) was done to provide a general overview of the bacterial adherence to the HA disks. Saliva-coated HA discs were placed in a 24-well culture plate (Iwaki, Japan) containing 500 µl BHI Broth (Difco Inc., USA). One hundred microliters of *Allium sativum* and *Anredera cordifolia* ethanolic extracts were added and incubated for 30 minutes at 37°C, followed by inoculation of 100 µl of 1.5×10^8 CFU/ml *P. aeruginosa* suspension and incubated at 37°C for 24 hours. The discs were rinsed with PBS, then fixed with 4% paraformaldehyde for 30 minutes. The discs were subsequently dehydrated in an ethanol series (50% for 10 min, 70% for 10 min, 95% for 10 min, and 100% for 20 min) and air dried overnight. Then the discs were coated with gold metal using auto fine coater (JEC-3000FC, JEOL, Japan), followed by bonding to carbon double-side tape for examination by SEM (JEOL JSM IT-200, Japan).

Observation of bacterial motility

The observation was conducted to provide a general overview of bacterial motility. The swarming motility of *P. aeruginosa* was tested using Nutrient Agar (NA; Oxoid, UK) medium, while for the swimming motility BBL Motility medium (Becton Dickinson, USA) was used. One hundred µl of either *Allium sativum* or *Anredera cordifolia* ethanolic extract was incubated in 1000 µl LB medium for 30 min at 37°C. One hundred µl of 1.5×10^8 CFU/ml *P. aeruginosa* suspension were stimulated to the cultures and incubated for 24 hours at 37°C. After incubation 1 µl of each culture was inoculated at the center of the agar surface, then further incubated for 24 hours at 37°C (25). The bacterial motility was observed after 24- and 48-hours incubation. Then the cultures were incubated for another 24 hours and were observed for its motility.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (La Jolla, USA). Optical density results were presented as mean values of the replicates along with each standard deviation in the data graphs, and One-way ANOVA analysis of variance followed by Newman–Keuls' posthoc test was performed for statistical analysis. Results with p-values < 0·05 were considered significant. Bacterial susceptibility and motility were determined through visual observation by a blind observer. Experiments were performed in triplicates unless stated otherwise.

RESULTS

Antibacterial susceptibility assay

We observed a growth inhibition comparable to ciprofloxacin (used as positive control) on *P. aeruginosa* treated with 1250 µg/ml of *Allium sativum* ethanolic extract, while *Anredera cordifolia* extract inhibited bacterial growth at the concentration of 2500 µg/ml (Figure 1). Among the five replicates, we consistently observed no turbidity of the LB media with the same dose of extracts, suggesting no bacterial growth. No bacterial growth was observed in BHI agar plates at a concentration of 5000 µg/ml and up for both extracts. Therefore, the MBC value for both extracts was at a concentration of 5000 µg/ml.

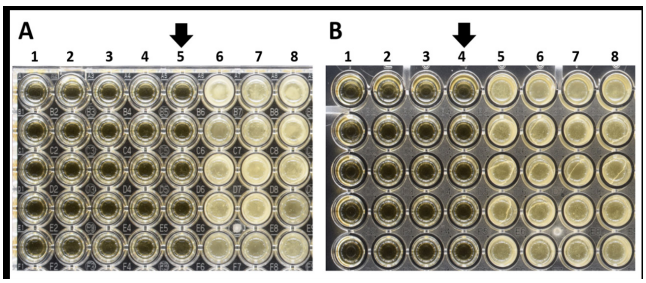

Figure 1: Bacterial susceptibility assay of P. aeruginosa treated with multiple concentrations of A) *Allium sativum* extract; and B) *Anredera cordifolia* extract. *P. aeruginosa* was treated with ciprofloxacin (5 µg/ml, column 1 in both figures) as positive control, columns 2-8 used treatment with 10,000; 5000; 2500; 1250; 625; 312.5; and 156.25 µg/ml, respectively, of extracts. Arrows showed minimum inhibitory concentration (MIC) values of 1250 and 2500 µg/ml of Allium sativum and Anredera cordifolia extract, respectively.

Inhibition of bacterial adherence and biofilm formation

As we previously found that a dose of 1250 µg/ml of *Allium sativum* and 2500 µg/ml of *Anredera cordifolia* extract elicits anti-bacterial activity to *P. aeruginosa*, we adapted a range of extract concentrations lower than 1250 and 2500 µg/ml for *Allium sativum* and *Anredera cordifolia* extract, respectively, for further analysis to avoid result bias due to bacterial inactivity.

The inhibition of bacterial adherence was characterized by a decreased OD value compared to the untreated (control) group. We observed that the OD value decreased proportionally with the increasing concentration of both *Allium sativum* and *Anredera cordifolia* extract, although *Allium sativum* obviously showed a more potent inhibition of bacterial adherence. Inhibition of bacterial adherence was seen at incubation with as low as 62.5 µg/ml of *Allium sativum* extract, and 125 µg/ml of *Anredera cordifolia* leaves extract (Figure 2). When treated with *Allium sativum*, we observed a dose-dependent decrease of the OD along with the increasing dose of extract. The reduction was significant between tested concentrations, although the optimum dose was seen in 500 µg/ml *Allium sativum* extract, as there was no statistically significant OD reduction between 500 and 1000 µg/ml (marked as ns in pink bracket). On the other hand, inhibition of bacterial adherence appeared similar among the *Anredera cordifolia* concentrations tested, with statictically significant OD reduction seen in between 250 to 500 µg/ml (marked with brown brackets, ns: not significant, ***: p value < 0.001). Therefore, we proposed that the optimal extract dose for inhibition of bacterial adherence to be 500 µg/ml in both extracts. The presented data were values from three replicates experiments.

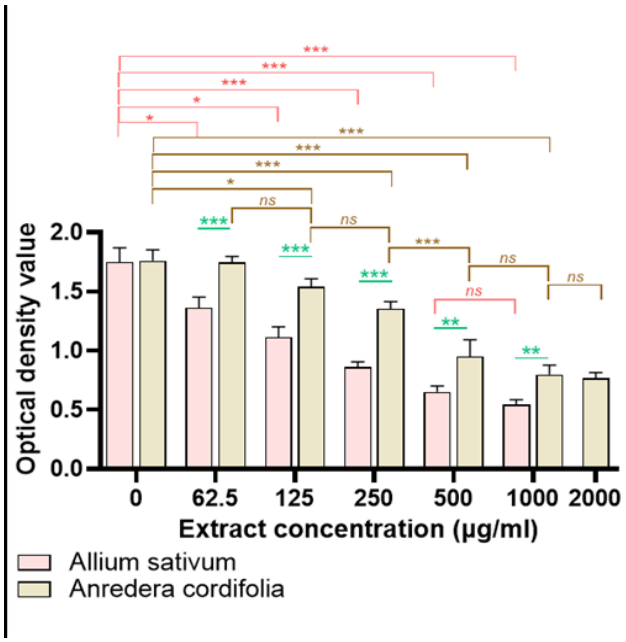

Figure 2: Optical density values of *P. aeruginosa* adherence at various concentrations (ns: not significant; *: p<0.05; **p<0.01; ***p<0.001)

We further examined the architecture of bacterial attachment to the surface of hydroxyapatite disc using SEM. Figure 3A shows a representative photomicrograph of the untreated group. In this control group, more *P. aeruginosa* colonization is seen on the surface of the hydroxyapatite disc, compared when it's treated with 1000 µg/ml *Allium sativum* extract (Figure 3B), and 1000 µg/ml *Anredera cordifolia* extract (Figure 3C). Bacterial cells were covering most of the surface area of the untreated hydroxyapatite disc. Photomicrographs also showed that the number of bacterial colonies attached to the surface of the discs in the treatment group was less than those in the control group. Neither group treated with extracts formed an extracellular matrix as seen in the control group. The results of this study confirmed that the presence of *Allium sativum* extract and *Anredera cordifolia* extract inhibited bacterial attachment.

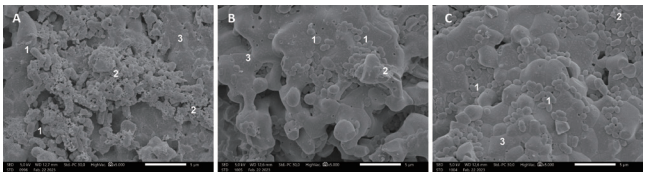

Figure 3: Scanning electron microscopy (SEM) of *P. aeruginosa* adherence: A) without treatment (control); B) following treatment with 1000 µg/ml of *Allium sativum extract*; and C) following treatment with 1000 µg/ml of *Anredera cordifolia* extract

The number of *P. aeruginosa* colonies (1) were higher in the untreated disk (A) compared to that of the 1000 µg/ml extract treated disk (B and C). The extracellular matrix (2) of the P. aeruginosa biofilm also detected in a higher intensity on the HA crystal (3) of the untreated disk (magnification 5,000X)

Inhibition of bacterial motility

As presented in Figure 4 and Figure 5, we observed that the bacteria migrated further in untreated control, compared to that of the 500 µg/ml extract treated cultures, after incubation for 24 and 48 hours. Similarly, in the case of bacterial swarming motility (Figure 4) and swimming motility (Figure 5), the spreading of colony in both *Allium sativum* and *Anredera cordifolia* extracts-treated cultures were less compared to the control group. Swarming motility is a movement of bacteria over the surface with the help of its flagella, while swimming motility is defined as the movement in liquid or low-viscosity conditions. In this study, *P. aeruginosa* swarming motility in the nutrient agar medium surface formed macroscopic colony pattern (Figure 4), while in swimming motility showed diffused colonies in the BBL semi solid medium (Figure 5). This phenomenon was seen in bacteria cultured on different media, although the typical colony spreading patterns of *P. aeruginosa* motility in the NA medium were different from those in the BBL medium. Therefore, we found that *Allium sativum* and *Anredera cordifolia* extract inhibited *P. aeruginosa* swarming and swimming motility.

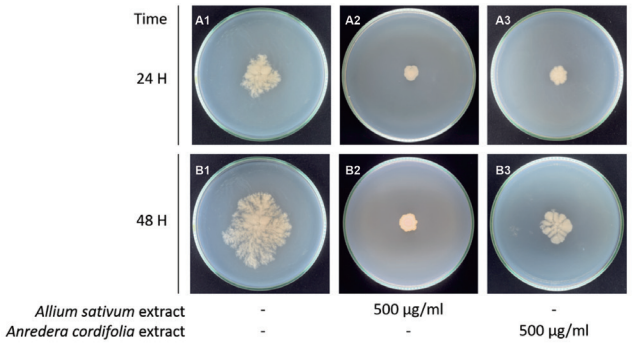

Figure 4: Observation of swarming motility of P. aeruginosa following treatment with either *Allium sativum* (A2 and B2) or *Anredera cordifolia* (A3 and B3) extract.
Bacterial swarming motility is observed after 24 hours (A2 and A3, upper lane) and 48 hours (B2 and B3, lower lane) compared with no treatment (A1 and B1).

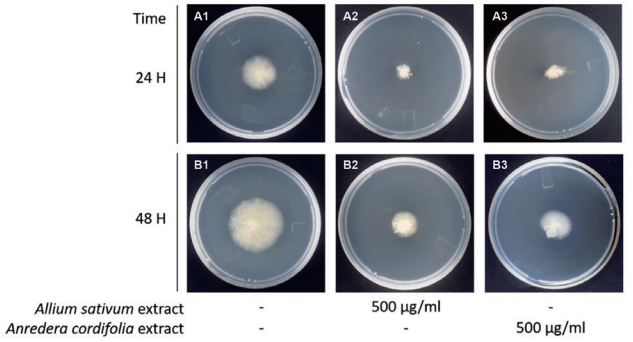

Figure 5: Observation of swimming motility of *P. aeruginosa* following treatment with either *Allium sativum* (A2 and B2) or *Anredera cordifolia* (A3 and B3) extract.
Bacterial swarming motility is observed after 24 hours (A2 and A3, upper lane) and 48 hours (B2 and B3, lower lane) compared with no treatment (A1 and B1).

DISCUSSION

In this study, we observed and compared the antimicrobial properties of garlic ethanolic extract with madeira vine leaves extract. Both herbal extracts preparations showed capacity in inhibiting bacterial growth, preventing bacterial adherence, and lowering bacterial motility, although we observed that garlic showed a significantly higher potency. The concentration of *Allium sativum* extract needed to inhibit bacterial growth in this study was higher than it was in our previous study using the same extract to a Gram-positive bacterium (22). Gram-negative bacteria are generally more sensitive to *Allium sativum*, but in this study, the opposite was observed. We estimated that the exact concentration of phytochemical content within the *Allium sativum* extract used in this study may contribute to such inconsistencies, as specific phytochemicals like ajoene, a sulfur-containing compound derived from *Allium sativum*, were previously found to inhibit the growth of the Gram-positive bacteria more effectively than the Gram-negative bacteria (26). In addition, different extraction procedures of the natural plant affected the phytochemical concentration in the extract. For instance, extraction with water or ethanol provides an allicin-rich product, with the latter showed higher yield (27), while oil-maceration produces an extract with high proportion of ajoene and thiosulfinates (28).

The modes of anti-microbial activity are correlated with the major component present *Allium sativum* ethanolic extract showed a concentration-dependent anti-bacterial effect against H. pylori (29), M. tuberculosis and S. aureus, including its drug-resistant strains (30-32). Water extract of *Allium sativum* reduced the mortality rate of Caenorhabditis elegans exposed to *P. aeruginosa* infections (33). From other studies *Allium sativum* extract also modulates pro-inflammatory cytokine and improves the clearing of pulmonary pseudomonas infection in mice (34). The antibacterial activity of garlic is widely attributed to allicin. Several peptides, including flavonoids, alkaloids and saponin also have minor contributions (35). On the other hand, *Anredera cordifolia* leaves contain flavonoid, saponin, and tannin (20, 21). The mechanism of antibacterial action of flavonoids is already unknown. However, a recent study suggested that the inhibition of nucleic acid synthesis was the antibacterial character of these compounds (36), while saponins works by reducing surface tension, thereby increasing cell permeability or leakage and causing intracellular compounds to come out (37). Tannin acts as an antimicrobial agent by its ability to pass through bacterial cell walls and reach the internal membrane to disrupt cell metabolism. This compound also inhibits the bacteria attachment to the surface. A lack of bacteria adhesion to the surface results in bacteria cell death (38, 39). The mechanisms of these active substances could the *P. aeruginosa* cell death.

In this study, garlic showed significantly higher potency than the madeira vine leaves extract. It is estimated that the presence of allicin in garlic, which is not found in madeira vine leaves, was the reason why garlic is more potent than madeira leaves.

One important anti-microbial mechanism of *Allium sativum* is the inhibition of biofilm formation. Many bacteria including *P. aeruginosa* are known to form biofilm to facilitate attachment and survival on an inapt environment through a chemical communication system known as quorum sensing (QS) signaling, leading to its pathogenesis and antibiotic resistance (39).

In line with our findings, *Allium sativum* was reported to inhibit *Streptococcus sanguinis* biofilm formation in a concentration-dependent manner (22). This is similar with the inhibition pattern of allicin to the biofilm formation of Proteus mirabilis (40). Water and toluene extract as well as isolated ajoene and allicin have been identified as a potent QS inhibitor by suppressing the expression of *P. aeruginosa* QS-controlled virulence genes (34, 42, 43). Diallyl-disulfide from *Allium sativum* oil also

inhibits in vitro biofilm formation and bacterial motility (43). In addition, *Allium sativum* acts synergistically with antibiotic by increasing drug penetration into the biofilm (33), increase serum sensitivity and phagocytic uptake, while reducing bacterial load (44).

We also showed that upon exposure to *Allium sativum* and *Anredera cordifolia* leaves extracts, the cell walls of *P. aeruginosa* changed in morphology. The bacterial cells attached to the hydroxyapatite disk appeared to vary in size and shape. Some bacterial cells attached solitarily and did not cluster, in contrast to the normal bacterial colonies in the control group. This phenomenon also occurred in *P. aeruginosa* bacterial cells that were exposed to 5-aminolevulinic acid photodynamic therapy (ALA-PDT), in which the bacterial cells changed in shape and size, cracked, while also accompanied by a significant decrease in population density (45). The SEM observation in our study showed altered biofilm architecture in cells exposed to the extracts. The process of forming a *P. aeruginosa* biofilm mass was hampered by *Allium sativum* and *Anredera cordifolia* extracts, so that a biofilm mass did not form as it was in the control group. This ultimately hinders the formation and development of biofilms.

P. aeruginosa, like other motile bacteria, will move to the surface to attach, colonize, and form a biofilm. The process of attachment and colonization is accompanied by the formation of extracellular polymeric substances (EPS) which its functions are uniting and protecting bacterial cells from environmental disturbances, as well as providing an attachment medium for the next colony (47). In addition to EPS formation, the process of biofilm development is also strongly influenced by bacterial motility. Swarming motility greatly determines the structure of *P. aeruginosa* biofilms (48), while twitching motility plays important role in facilitating the expansion of established biofilms (49).

Recent study reported that these substances may inhibit the nucleic acid synthesis of bacteria and induce bacterial lysis by alteration of bacterial cell wall (25, 51). Tannin, another component present in *Anredera cordifolia* leaves, could pass through the bacterial cell wall and interfere with cell metabolism and eventually lead to bacterial cell destruction. The activity of tannin in the Gram-negative bacteria is slower than it is in the Gram-positive bacteria as a result of the bilayered membrane in the Gram-negative bacteria (51, 52). In contrary to the abundant resources on *Allium sativum*, less is known on the effect of *Anredera cordifolia*, although its use as a medicinal herb has been widely applied within the community. The leaves of *Anredera cordifolia* are rich in flavonoids, aporins, tannins, alkaloids, and polyphenols (52). The motility of *P. aeruginosa* also occurred due to stimulation by inorganic material. Rutin coated zinc oxide nanoparticles were synthesized. The ZnO-Rutin NPs significantly reduced the swimming and twitching motility of *P. aeruginosa* (53). Phenolic compounds from polyphenols found in *Anredera cordifolia* leaves may act as antioxidant that made the antibacterial capability of this extract stronger (52, 54). In this study we were not performing an antioxidant assay. We suggest exploring the phenolic content of the leaves to determine their antioxidant effect for further study.

This study is limited by the use of crude ethanolic extracts of *Allium sativum* and *Anredera cordifolia* leaves, and the exact phytochemicals that may possess the observed effects could not be elucidated from this current study. In addition, the toxic dose of *Allium sativum* and *Anredera cordifolia* leaves ethanolic extract to human cells and tissues, and whether it may alter cellular functions are unknown, and should be one of the perspectives of further studies.

CONCLUSION

Altogether, we reported a study of the antimicrobial properties of *Allium sativum* and *Anredera cordifolia* leaves ethanolic extract against *P. aeruginosa*. At the lower concentrations of MIC, these ethanolic extracts inhibit bacterial adherence and motility, along with preventing biofilm formation. At a higher concentration of MIC, they directly inhibit bacterial growth. These extracts inhibit *P. aeruginosa* biofilm formation through bacterial motility mechanisms. In further study, we need to explore the toxic dose of *Allium sativum* and *Anredera cordifolia* leaves ethanolic extract to human cells and tissues, and whether it may alter cellular functions.

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