

## Antibiofilm formation activities of ethanol extracts from *Curcuma domestica* rhizome against bacteria resistant *Pseudomonas aeruginosa*

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### Abstract

The Biofilm *P.aeruginosa* communities are characterized by close juxtaposition of bacterial cells and the presence of an exopolysaccharide matrix that surrounds the bacterial population. Many reports have postulated that the exopolysaccharide matrix provides an effective barrier that restricts penetration of chemically reactive biocides, cationic antibiotics and antimicrobial peptides. This study aims to antibiofilm formation activities of ethanol extract from *Curcuma domestica* against *Pseudomonas aeruginosa* resistant bacteria. These antibiofilm formation was determined using a modified-method of microdilution assay. Ethanol extract of *C. domestica* strong activity against *P.aeruginosa* with Anti-biofilm activity was observed through biofilm inhibition and degradation activities, determined by Cristal Violet assay. Data were analyzed using ANOVA test and Tukey HSD post hoc test. The significant inhibition of *P.aeruginosa* the biofilm degradation was obtained at a concentration of 31.2 µg/ml until 250 µg/ml. The ethanol extract of *C. domestica* have potency as alternative antibiofilm formation against selected bacteria resistant.

**Keywords:** Antibiofilm Formation; *Curcuma domesticae*; *Pseudomomas aeruginosa*; Optical density

### 1. Introduction

*Pseudomonas aeruginosa* is a gram-negative bacterium that continues to be a major cause of opportunistic nosocomial infections, causing around 9–10% of hospital infections. It is also the dominant cause of chronic lung infections contributing to the death of patients with cystic fibrosis. A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics a modest change in susceptibility can thwart their effectiveness (Hancock, 1998). Generally, the major mechanisms of *P. aeruginosa* used to counter antibiotic attacks can be classified into intrinsic, acquired and adaptive resistance. The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell and the production of antibiotic-inactivating enzymes. The acquired resistance of *P. aeruginosa* can be achieved by either the horizontal transfer of resistance genes or mutational changes (Breidenstein, et al. 2011). The adaptive resistance of *P. aeruginosa* involves the formation of biofilm in the lungs of infected patients where the biofilm serves as a diffusion barrier to limit antibiotic access to the bacterial cells (Drenkard, E., 2003).

*Curcuma longa L.* is commonly called turmeric and is a member of the ginger family. Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family (Gupta, et al. 2013). The yellow color of turmeric is due to the presence of three main curcuminoids in the rhizome namely: curcumin, demethoxycurcumin, and bis-demethoxycurcumin. Dry turmeric contains: 5.1% oils, 6.3% proteins, 69.43% carbohydrates, 3.5% minerals, and other elements (Islam, et al. 2002). The bioactive chemical constituents in turmeric have been investigated. Approximately 235 compounds, primarily terpenoids and phenolics, have been identified from

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various species of turmeric, including 22 diarylheptanoids and diarylpentanoids, 8 phenylpropenes as well as other phenolics, 109 sesquiterpenes, 68 monoterpenes, 5 diterpenes, 4 sterols, 3 triterpenoids, 2 alkaloids, and 14 other compounds (Li, et al. 2011). Throughout the Orient, turmeric is traditionally used for both prevention and therapy of diseases. Modern in-vitro studies reveal that turmeric is a potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer agent (Parasuraman, et al. 2014).

Biofilm communities are characterized by close juxtaposition of bacterial cells and the presence of an exopolysaccharide matrix that surrounds the bacterial population. Many reports have postulated that the exopolysaccharide matrix provides an effective barrier that restricts penetration of chemically reactive biocides, cationic antibiotics and antimicrobial peptides (Shiegeta, et al. 1997). Although numerous studies have analyzed the role played by diffusion limitation in biofilm resistance, many of the results reported on the subject are contradictory. The general consensus, however, is that *P. aeruginosa* biofilms delay penetration and delivery of aminoglycosides, but penetration of fluoroquinolones such as ciprofloxacin and ofloxacin occurs without delay (Vranny, et al. 1997). The report study during the early stages of biofilm development, changes in gene expression induced by surface attachment lead to the emergence of a biofilm-specific phenotype that potentially increases biofilm resistance. Later on, the production of the exopolysaccharide matrix contributes to increasing cell survival by delaying antimicrobial penetration. As biofilms mature, the increase in cell density creates gradients of nutrient and oxygen availability leading to a reduction in metabolic activity and growth rate.

Furthermore, the increase in cell density also leads to the activation of quorum-sensing systems. On the other hand, nutrient starvation and oxygen limitation induce the general stress response and up-regulation of efflux pumps. Finally, environmental conditions present in the biofilm also induce or select for phenotypic/persister variants resistant to high concentrations of antimicrobials. Additional studies are still necessary to further elucidate precisely how each of these different mechanisms contributes to the overall resistance displayed by bacterial biofilms (Drenkard, E., 2003). The purpose of this study was to look into the effect of *C. domestica* extract ethanol on biofilm formation were also studied

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## 2. Methods

### 2.1. Bacterial Strain

Clinical isolate of *Pseudomonas aeruginosa* was obtained from The MERO Foundation in Bali.

### 2.2. Chemical and Media

Natrium klorida (NaCl<sub>2</sub>), ethanol 96 %, crystal violet phosphate buffer saline (PBS) tablets were obtained from Sigma-Aldrich, UK. Brain Heart Infusion Broth (BHIB) were purchased from sigma-Aldrich (St Louis, MO, USA).

### 2.3. Preparation of Samples

*Curcuma domesticae* was soaked in ethanol (for 5 days). The ethanol extract was obtained by removing solvent under reduce pressure using rotary evaporator.

### 2.4. Anti-biofilm formation assay

The activity of *C. domestica* extract ethanol on biofilm formation was performed in a 96-well microplate with slight modifications (Meah et al., 2020). In brief, 100 mL of the bacterial suspension were mixed into the wells containing the mixture of BHI supplemented with essential oil at various concentrations (1/4 MIC, 1 /2 MIC, MIC and 2 MIC) according to the MIC obtained (data not show) against the selected clinical isolates, and then were incubated at 37 °C for 24 h. The untreated bacterial suspension was used as a negative control. Afterward, the bacteria suspension

was discarded, and the plates were washed twice with PBS After air-drying, the wells were stained with 200 mL (0.1% crystal violet), incubated at 37 °C for 30 min. Then the plates were washed again with PBS and the stained biofilms were solubilized with 200 mL of DMSO. Absorbance was recorded at 560 nm and the percentage of biofilm formation was then calculated.

### 2.5. Statistical analysis

All tests were conducted in triplicate and the results were reported as mean ± SD. Data were statistically analyzed using oneway ANOVA followed by Tukey's HSD post hoc test, and p<0.05 was considered to be dignificantly different.

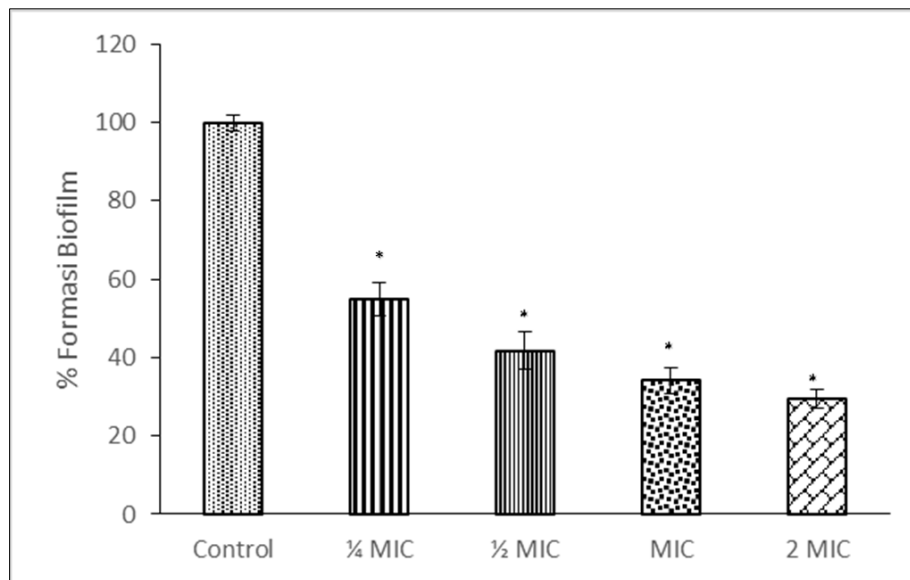
### 3. Results and discussion

#### 3.1. Extraction sample

Extraction of *C. domestica* simplicia was carried out by maceration method using 96% ethanol solvent. After the maceration process is complete, liquid ethanol extract is obtained, then the liquid ethanol extract is separated from the solvent using a rotary evaporator at 60°C to obtain a thick ethanol extract. The yield percent yield according to the Indonesian Herbal Pharmacopoeia (FHI) of the five ethanol extracts of *C. domestica* is not less than 7.2%, namely 56.6 so that the results of this extraction meet the requirements according to the Indonesian Herbal Pharmacopoeia (FHI).

#### 3.2. Anti-biofilm formation

Based on the broth microdilution method, extract ethanol *C. domestica* exhibited activity against clinical isolate of *P. aeruginosa* with MIC value of 125 µg/mL more than effective as antibacterial. The research results showed that there was an inhibition of biofilm formation in the treatment given *C. domestica* ethanol extract and a negative control comparison of concentrations of 2 MIC (250 µg/mL), MIC (125 µg/mL), ½ MIC (62.5 µg/mL) to ¼ MIC (32.1 µg/mL) as shown in Figure 1 for measurement results at OD 560 nm which represents the amount of biofilm formed and decrease in the rate of biofilm formation in the test sample shown in Figure 1.



**Figures 1** Effect of *Curcuma domesticae* extract at difference concentrations on tested clinical isolate biofilm formation. The mean ± SD for triplicate. \*: sample exhibits significant differences compared to control ( $p < 0.05$ )

The research results on the sample showed a greater decrease than the control, namely at the contrast of 2 MIC (125 µg/ml) up to ¼ MIC (31.2 µg/ml). The ethanol extract of *C. domestica* was able to inhibit biofilm growth by showing a decrease in the absorbance of crystal violet in *C. domestica*. The higher the concentration of the test sample used, the greater the inhibition of biofilm formation in bacteria resistant to *P. aeruginosa* (Ahmed et al, 2020). Biofilm formation is a virulence factor in *P. aeruginosa* resistant bacteria. Previous researchers reported that essential oils from the Zingiberaceae family were effective in inhibiting biofilm formation. Turmeric essential oil has antibiofilm activity against *Streptococcus mutans* (Lee et al, 2011). Then red ginger essential oil (*Zingiber officinale*) significantly inhibited *Candida albicans* biofilm formation by reducing the Optical Density (OD) of crystal violet (Rinanda et al, 2018).

### 4. Conclusion

Extract Ethanol *C. domestica* strong antibacterial activity against clinical isolates of the Gram-negative and The ethanol extract of *C. domestica* was able to inhibit biofilm formation to bacteria resistant *P. aeruginosa* with Nevertheless, further studies are still needed to evaluate its mechanism of action to overcome the resistance problem.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The author declares no conflict of interest in conducting this study.

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