

Antibiofilm Formation Activities Of Ethanol Extract Of *Curcuma Domestica* Val. Rhizome Against Multidrug-Resistant *Acinetobacter Baumannii*

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Abstract.

Multidrug resistance is still a major health problem worldwide. Biofilm formation was related to the resistance of bacteria mechanism. *Acinetobacter baumannii*, one of the bacteria that cause nosocomial infections, is given particular attention due to its high prevalence of resistance. The current study address observing the antibacterial activity and antibiofilm formation activity of *Curcuma domestica* rhizome extract against multidrug-resistant *Acinetobacter baumannii* (MDR *A.baumannii*). *Curcuma domestica* rhizome was macerated by ethanol 96%. Determination of Minimum Inhibitory Concentration (MIC) was carried out by the microdilution method. Antibiofilm formation analysis was determined by standard quantitative using crystal violet-stained assay. Ethanol extract of *C. domestica* possesses antibacterial activity against MDR *A. baumannii* with MIC value 250 µg/mL. Additionally, the ethanol extract of *C. domestica* could inhibit the biofilm formation of MDR *A.baumannii*, thus percentage of biofilm formed only 23.7% and significance different with control after analyzed using ANOVA post hoc test Tukey. According to these results, ethanol extract of *C. domestica* revealed antibacterial activity and had the potency in suppressing the biofilm formation of MDR *A.baumannii*, thus could be utilized in clinical application, especially in MDR *A.baumannii* infection.

Keywords: Antibiofilm, antibacterial, *Curcuma domestica*, multidrug-resistant and *Acinetobacter baumannii*.

I. INTRODUCTION

Curcuma domestica Val. is one of Indonesia's traditional plant species. This plant has various benefits, such as anti-inflammatory, anti-hyperlipidemic, antioxidant, antiseptic, and antibacterial properties [1]. *Curcuma* species was reported also have functions in immunomodulatory effects. These pharmacological activities were due to their main constituents, including curcuminoid (as the major bioactive compound in *Curcuma* species) [2]. *C. mangga* as also one of the *Curcuma* species, has immunomodulatory effect and could be developed as an immunotherapeutic agent [3]. This is also supported by a previous study by Momoh et al., (2022) which stated that essential oil, curcuminoids, curcumin, turmerol, valeric acid, and turmeric oil in *Curcuma longa* possess antibacterial activity against *S. aureus* and *E. coli* [4]. *Acinetobacter baumannii* is a bacterium that also contributes to a high number of infections in hospitals. This also followed by an increasing incidence of resistance to various antibiotics [5]. The high rate of resistance of this bacterium is due to its tendency to acquire a multidrug resistance (MDR) phenotype [6]. Mortality rate is reported to be up to 70% in cases of infection caused by MDR *A.baumannii* [7]. The resistance mechanisms of MDR *A. baumannii* are including produced β-lactamase enzymes, enzymes modified aminoglycosides, overexpression of efflux pumps, modified membrane permeability, and modifying drug target sites of action [8]. Modified of membrane permeability is due to decreasing the production of porins, which is porin as a channel for molecule to penetrate the outer membrane.

Therefore, modified in membrane permeability also has a significant role in resistance mechanism. In addition, LPS modifications can also reduce membrane integrity [8]. Biofilm is a population of bacteria, which are bound together or enclosed in a matrix produced by the bacteria themselves, formed and grows on a solid surface. Biofilms are important virulence factors that existed in bacteria, because they can lead to chronic persistent and recurrent infections, also causes high resistance to antibiotics and resistance to the

immune system of the host cells. Bacteria which protected in exopolysaccharide (EPS) biofilms are up to 1,000 times more resistant to antibiotics than in their planktonic cell form [9]. According to the World Health Organization (WHO), MDR *A. baumannii* is a critical priority pathogen and urgently needed a new type of antibiotics to overcome this problem. However, the discovery of new antibiotic compounds will take a long time and high cost. Now, several studies mostly interested in seeing the potential of natural compounds as antibacterial agent and analyze the mechanism of action. The potential of ethanol extract *C. domestica* against MDR *A. baumannii* still unknown. So, this study aims to determine the antibacterial activity and the antibiofilm formation activity in MDR *A. baumannii*.

II. MATERIAL AND METHODS

Chemical and Media

Brain Heart Infusion Broth (BHIB) as media to inoculate the bacteria. 96% ethanol, sodium chloride, crystal violet obtained from National Research and Innovation Agency. Phosphate Buffer Salin Tablet (Dulbecco A) was obtained from Oxoid BR0014G, dimethyl sulfoxide purchased from Sentra Teknosains (Indonesia).

Plant Materials

Curcuma domestica rhizome was collected in North Sumatera, Indonesia. Determination of this plant sample was performed by Herbarium Medanense (MEDA), Faculty of Mathematics and Natural Science (FMIPA) Universitas Sumatera Utara (Voucher No. 192/MEDA/2022).

Bacterial Strains

The Multidrug-resistant *Acinetobacter baumannii* were collected from and has been identified by Marine Educational and Research Organization (MERO) Foundation, which located in Bali, Indonesia.

Extraction Process

Fresh rhizome of *C. domestica* were washed, sliced, put in the oven at a maintained temperature 45-50°C, then grounded to obtain a fine powder of the sample. As much as 472 g of sample powder was then extracted by maceration method with ethanol 96% (1:10 w/v). Extract was concentrated by rotary evaporator at 50°C – 60°C.

Determination of Minimum Inhibitory Concentration

The antimicrobial activity was analyzed in vitro by two-fold broth dilution [10]. The lowest concentration of a substance needed to stop bacterial growth totally is known as the minimum inhibitory concentration (MIC, measured in µg/mL). Dimethyl sulfoxide (DMSO) was used to dissolve the extract, and the final concentration of DMSO is not greater than 1%. The MIC of tetracycline also investigated as comparative data on extract. The final concentration of this solution was arranged from 500 µg/mL until 3.9 µg/mL which diluted by BHIB. The bacterium suspensions consisted of 1×10^6 CFU/mL adjusted to 0.5 Mc. Farland and diluted with normal saline. MIC value were obtained after the incubation process at 37°C for 24 h. DMSO 0.5% v/v was used as a negative control in this analysis.

Antibiofilm Formation Assay

Antibiofilm formation activity was analyzed using a slightly modified version of the crystal violet assay [11]. The activity of extracts on the biofilm formation of MDR *A. baumannii* was tested in a 96-wells microplate. Briefly, 100 µL of fresh inoculated MDR *A. baumannii* in BHIB (with the final concentration of 1×10^6 CFU/mL) was aliquoted into each well in presence of MIC concentration of extract and tetracycline. DMSO 0.5% (v/v) conducted as a negative control for this assay. The plate were incubated at 37°C for 24 h to allowed the process of biofilm formation. The plates were twice rinsed with PBS pH 7.3 after the bacterium suspension was removed, then the microplate were dried in room temperature. As much as 200 µL of crystal violet 0.3% was added to stained the biofilm formed, and then incubated at 37°C for 30 min. Rinsed the microplate again with PBS pH 7.3, and then added 200 µL of 96% ethanol to dissolve the stained biofilms. The percentage of biofilm formed was determined by measuring the absorbance at 560 nm using microplate reader and then the absorbance value of sample divided with absorbance of control (DMSO 0.5% v/v) and then multiplied by 100% .

Statistical Analysis

Result data were presented as Mean \pm standard deviation. The datas were analysis with ANOVA and continued by post hoc test Tukey that performed using SPSS 26 version. The value of *p* under 0.05 was indicates the significant different between groups and signed with (*) on the figure result.

III. RESULT AND DISCUSSION

Yield of Extraction Process

Extraction of simplicia from *C. domestica* rhizome was carried out by maceration method using ethanol 96% as solvent. Solvents with polar properties are expected to attract polar antibacterial compounds such as curcumin, flavonoids, and other polyphenolic compounds which are known to have antibacterial activity. The extract was concentrated using a rotary evaporator to obtain a viscous extract. The yield percentage of the extract reached 29.81% after being calculated by comparing the weight of the extract and the weight of the simplicia.

Minimum Inhibitory Concentration (MIC)

Table 1 shows that ethanol extract of *C. domestica* has a potential activity against MDR *A. baumannii* with MIC value 250 $\mu\text{g/mL}$. This result was supported with previous study which reported that *Staphylococcus aureus* growth can be inhibited by turmeric oil in concentration of 1 to 5000 ppm [12]. Study by Nerkar (2020), stated that turmeric extract and also the essential oil of *C. longa* can inhibit the growth of bacteria, fungi, and also parasites [13].

Table 1. Antibacterial activity of the ethanol extract *C. domestica* and tetracycline against Multidrug-resistant *Acinetobacter baumannii*

Samples	MIC ($\mu\text{g/mL}$)
Ethanol extract of <i>C. domestica</i>	250
Tetracycline	31.25

Note : MIC = Minimum Inhibitory Concentration

Antibiofilm Formation of Ethanol Extract of *C. domestica*

One of the virulence factors in bacteria that can defense of antibiotic to penetrate into bacteria cell is formation of biofilm. Biofilm are communities of bacteria, that formed on living and nonliving solid surfaces, also grow within a self-replicating matrix [14]. Several results of the study reported that biofilm development is related to the failure of antibiotic therapy [15]. The antibiofilm activity of *C. domestica* extract consider as a concentration-dependent reduction using the standard method in analyze the inhibition biofilm formation. The ethanol extract of *C. domestica* had better activity in inhibited the biofilm formation compared with tetracycline. The biofilm formed in treated sample with ethanol extract of *C. domestica* (250 $\mu\text{g/mL}$) only 23.7%, while percentage of biofilm formation in tetracycline (31.25 $\mu\text{g/mL}$) treated sample still at value of 35.5% (**Figure 1**). Quorum sensing system is one of factor in production of biofilm in bacteria [16]. According to a previous study, curcumin as a major bioactive compound in *C. domestica*, significantly reduced the expression of quorum sensing regulating genes [17].

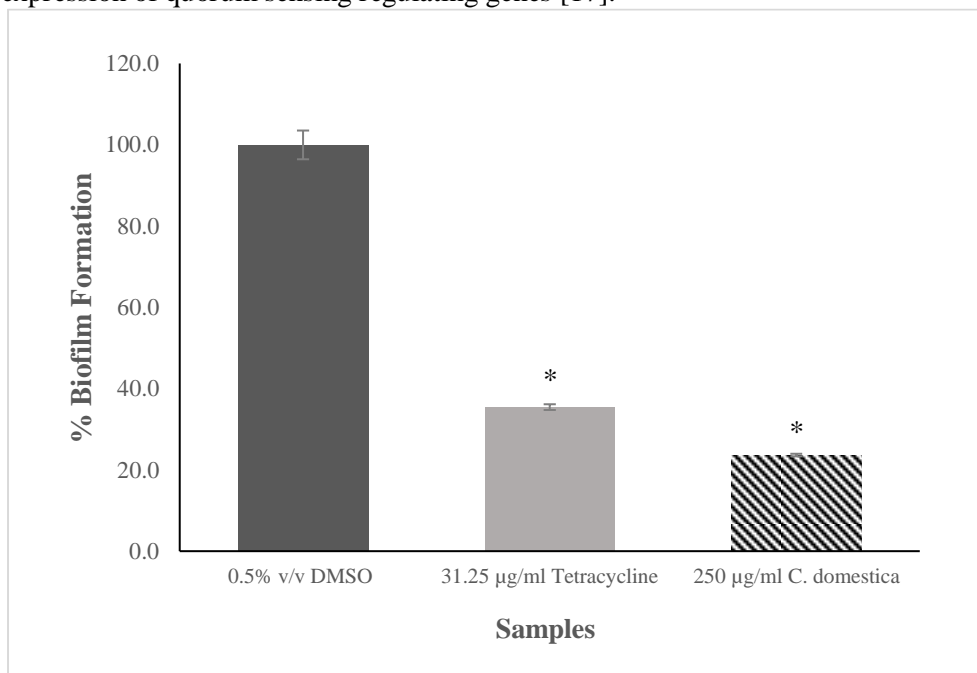


Fig 1. Effect of ethanol extract of *Curcuma domestica* compare with tetracycline on biofilm formation of multidrug-resistant *A. baumannii*. (*) sample present significant differences compared to negative control ($p < 0.05$)

IV. CONCLUSION

The ethanol extract of *C. domestica* has a potent antibacterial effect against MDR *A. baumannii*. Furthermore, the ethanol extract of *C. domestica* could inhibit the biofilm formation in MDR *A. baumannii*. It was suggested that an alternative approach to dealing with the problem of drug resistance would be to use natural compounds. Meanwhile, further studies is required to fully understand the toxicity and the molecular level of their activity.

V. ACKNOWLEDGMENTS

The authors would like to thanked the Universitas Sumatera Utara that has been supported this study by research funding of TALENTA with contract number 11119/UN5.1.R/PPM/2022 and the National Research and Innovation Agency for all research facilities to accomplished this study.

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