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Antibiofilm Test of Ethyl Acetate Extracts of the Jarak Tintir (*Jatropha multifida* L.) Stem Against *Escherichia coli* Bacteria

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Abstract Biofilm is a population of bacterial cells that strongly attached to a surface and enclosed by a layer of Extracellular Polymeric Substances (EPS). Biofilms serve to protect bacteria from external environmental influences such as disinfectants, immune systems, and antibiotics. One of the bacteria species which is able to form biofilm is *Escherichia coli*. Jarak tintir (*Jatropha multifida* L.) is a medicinal plant used by the community to treat the infection. Ethyl acetate extract of *Jatropha multifida* L. stem contains flavonoids, alkaloids, and phenols. These compounds are suspected to be responsible as antibacterials agent. The aim of this study was to investigate the inhibition ability of ethyl acetate extract of *Jatropha multifida* L.stem against *Escherichia coli* biofilm. The biofilm inhibition test was carried out using the crystal violet microtiter plate assay method. Ethyl acetate extract of *Jatropha multifida* L.stem has the percentage inhibition against clinical isolate *Escherichia coli* biofilm (72.39%) and *Escherichia coli* ATCC 35218 biofilm (85.10%) at its MIC value or at a concentration of 250 µg/mL and 125 µg/mL, respectively. Ethyl acetate extract of *Jatropha multifida* L. stem has activity in inhibiting biofilm of *Escherichia coli.*

Keywords: Biofilm, *Escherichia coli*, *Jatropha multifida* L.

1. Introduction

Biofilms produced by bacteria can increase the number of antibiotic resistance [1]. Antibiotic failure to penetrate biofilms decreases the effectiveness of antibiotic therapy [2]. Antibiotics resistant bacteria cause the infection more severe and become difficult to cure [3]. *Escherichia coli* is a biofilm former bacteria that found in many wound infections. Efforts to overcome biofilms in an infectious disease are needed because the bacterial resistance problem is increasing.

During this several decades, infectious diseases are cured by using antibiotics. Antibiotics have a limited effect on bacteria in biofilm forms. This is because bacteria in biofilm forms are harder to be penetrated by antibiotics, so new treatment strategies are needed to prevent or treat biofilm-related infections. One of the new treatments to overcome biofilm-related infections is by utilizing chemical compounds found in plants. *Jatropha multifida* L. is a medicinal plant found in Indonesia that can overcome biofilms. Ethyl acetate extract of the *Jatropha multifida* L. stem has flavonoid, alkaloid and phenol compounds [4]. This extract has antibiofilm activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* bacteria with IC_{50} value 0,76 mg/mL and 0,3 mg/mL, respectively [5]. The aim of this study was to investigate the inhibition ability of ethyl acetate extract of *Jatropha multifida* L. stem against Gram-negative bacteria, *Escherichia coli* in biofilm forms.

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2. Material and Method

2.1. Ethyl acetate extract of Jarak Tintir (*Jatropha multifida* L.) stem

The extract was obtained from previous studies. *J. multifida* L. stem bark powder was purchased from CV. Merapi Pharma Company, Yogyakarta, Indonesia. The amount of 20 gram powder of this simplicia was extracted with 200 mL n-hexane and ethyl acetate gradually with by the soxhletation method. The ethyl acetate extract of *Jatropha multifida* L. was obtained in form of viscous extract.

2.2. Antibacterial Activity Test of Ethyl Acetate Extract of Jatropha multifida L. stem against Escherichia coli ATCC 35218 and Clinical Isolates

The antibacterial activity test was performed by microdillution method in 96-well microplate based on CLSI guideline [6]. The ethyl acetate extract of (*Jatropha multifida* L.) with various concentrations of 4 mg - 0.031 mg were dissolved using DMSO 10%. The various concentration of extract with an amount of 20 µl were dispensed into the well of a microplate containing 160 µl MHB and 20 µl *Escherichia coli* bacterial suspension 10^6 CFU. This experiment was used a media control which contained 200 µl MHB, a solvent control containing 160 µl MHB, 20 µl DMSO 10% and 20 µl bacterial suspension, and a bacterial control containing 180 µl MHB and 20 µl bacterial suspension in others well. This culture was then incubated at 37ºC for 24 hours. An aliquot of each sample was subcultured on the sterile MHA plates and incubated at 37ºC for 24 hours. The MIC for bacteria was determined as the lowest concentration of extract inhibiting the visual growth of the test cultures on the agar plate.

2.3. Antibiofilm Test of Ethyl Acetate Extracts of Jarak Tintir (Jatropha multifida L.) Stem Against Escherichia coli ATCC 35218 and Clinical Isolates

The antibiofilm test was carried out using the crystal violet microtiter plate assay method [7]. A microplate polysterene flat bottom 96 wells were used in this method. The amount of 20 µl ethyl acetate extracts of *Jatropha multifida* L. with concentration ½ MIC, ¼ MIC and ⅛ MIC were dispensed into the well of a microplate which contains 160 µl TSB+1% glucose and 20 µl bacterial suspension with 10^6 CFU/ml. Many controls were used in this experiment such as: a media control which contains of 200 µl TSB+1% glucose, a bacterial control which contains of 180 µl TSB+1% glucose and 20 µl bacterial suspension, and a solvent control which contains of 160 µl TSB+1% glucose, 20 µl bacterial suspension, and 20 µl DMSO 10%. This microplate culture was then incubated at 37ºC for 48 hours. After incubation, all of the solutions in each well was discarded and microplate 96 wells were washed three times with 300 µl of 0.9% NaCl. The plates were vigorously shaken in order to remove all non-adherent bacteria. The biofilm which formed and attach into the wells were stained with 150 µl of 0,1% crystal violet. After 15 minutes, the microplate was washed with aquadest 200 µl. The ethanol 96% was added into the microplate, then the Optical Density (OD)of biofilm was determined spectrophotometrically by using microplate reader at 570 nm. The percentage of biofilm inhibition was calculated using the equation below:

$$\text{Percentage of inhibition} = \frac{((\text{OD bacterial control} - \text{OD media control}) - (\text{OD sample} - \text{OD media control}))}{(\text{OD bacterial control} - \text{OD media control})} x\ 100\% \quad (1)$$

3. Result and Discussion

3.1. Antibacterial Activity Test

Antibacterial activity of ethyl acetate extracts of *Jatropha multifida* L. stems was higher in inhibiting *Escherichia coli* ATCC 35218. It was due to the MIC and MBC was smaller than clinical isolates (**Table 1**). The compound in the ethyl acetate extract of *Jatropha multifida* L. stem had functioned as an antibacterial. MIC value is used to determine the amount of the extract which must be added to the bacterial culture in the antibiofilm activity test.

Table 1. MIC and MBC value of ethyl acetate extracts of *Jatropha multifida* L. stems against *Escherichia coli*

Bacteria	Ethyl Acetate Extract	
	MBC (µg/mL)	MIC (µg/mL)
Escherichia coli ATCC 35218	500	250
Escherichia coli clinical isolate	1000	500

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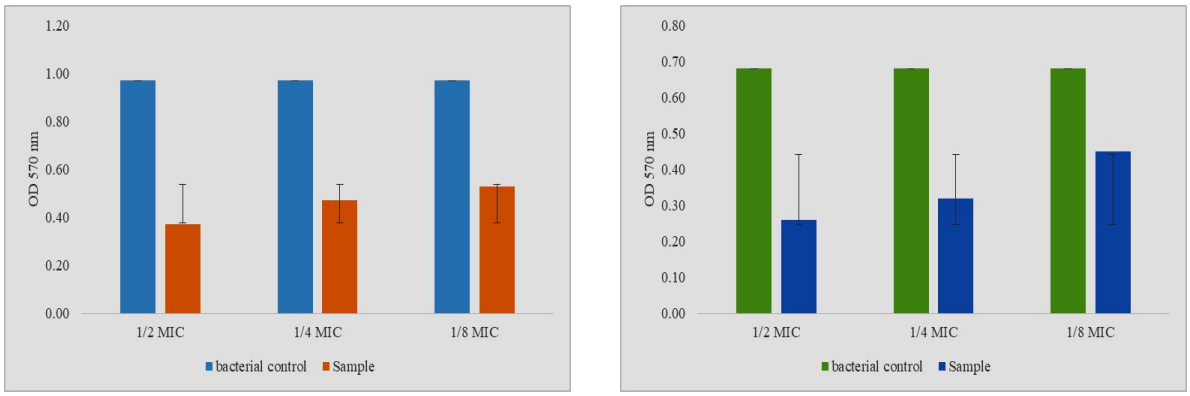
3.2. Antibiofilm Activity Test

Figure 1 is the comparison of biofilm in a bacterial culture which added with the extract and a biofilm which form in bacterial suspension without any addition of extract. There was a significant decreased of biomass biofilm formation in the sample which added with the extract.These phenomena occurred in all variations concentration of the extracts from 1/2 MIC value until 1/8 MIC value. This result indicates the inhibition biofilm formation activity of the extract.



(a) (b)

Fig.1 Effect of ethyl acetate extract of *Jatropha multifida* L. stems on biomass biofilm of *Escherichia coli* clinical isolate (a) and *Escherichia coli* ATCC 35218 (b)

Table 2 shows the percentage inhibition of *Escherichia coli* biofilm by the extract. This extract inhibits more than 50% of biofilm formation in both types of *Eschericia coli* at its MIC value and below its MIC value. At the MIC value or at the concentration of 250 µg / mL, the ethyl acetate extract exhibits the highest percentage inhibition (72.39%) meanwhile the concentration 62.5 µg / mL of the extract, showed the lowest percentage inhibition (52.61%) of *Escherichia coli* clinical isolates. In standard isolate of *Escherichia coli* ATCC 35218, ethyl acetate extract of *Jatropha multifida* L. stems has the greatest biofilm inhibition (85.10%) at the concentration of 125 µg / mL and showed the lowest percentage inhibition (47.30%) at the addition of 31.25 µg/mL of the extract. This indicates that biofilm inhibition is influenced by the amount of the extract which added in bacterial culture.

Table 2. Percentage Inhibition of *Escherichia coli* Biofilm

	E. coli Clinical Isolates			*E.coli* ATCC 35218		
Concentration of extract (µg/mL)	250	125	62,5	125	62.5	31.25
Inhibition of biofilm formation (%)	72.39	60.92	52.61	85.10	73.98	47.30

Based on the percentage of inhibition, ethyl acetate extract of *Jatropha multifida* L. stem had better activity in inhibiting a standard isolate of *Escherichia coli* ATCC 35218 biofilm than a clinical isolate. This result can occur because *Escherichia coli* clinical isolate was used may has multi-drug resistant characteristic, which is resistant to several antibiotics, while the *Escherichia coli* ATCC 35218 is an *Escherichia coli* of a bacterial standard which has characteristics of resistance to antibiotics β-lactam group [8]. It can be concluded that *Escherichia coli* clinical isolates were more resistant than *Escherichia coli* ATCC 35218 so the greater concentrations of ethyl acetate extract of *Jatropha multifida* L. stem were needed to inhibit the bacterial biofilm. Several compounds in plants are known to inhibit biofilm formation by suppressing the expression of genes that encode proteins and enzymes involved in biofilm formation [9].The greater concentrations of ethyl acetate extract of *Jatropha multifida* L. stem were needed to suppressing the expression of genes that encode proteins and enzymes involved in *Escherichia coli* clinical isolate biofilm formation.

Escherichia coli clinical isolates more resistant because they have a greater chance of interacting with other bacteria. The *Escherichia coli* in the body interact with other bacterial species which are multi-drug resistant bacteria through conjugation mechanism. Conjugation is the process of transferring resistance genes from other bacteria to *Escherichia coli* bacteria, consequently, *Escherichia coli* may have the same characteristics as multi-drug resistant bacteria because it is able to express resistance genes [10-12].

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4. Conclusion

Ethyl acetate extracts of *Jatropha multifida* L. stem have inhibition activity against *Escherichia coli* biofilms. This extract had the highest percentage inhibition of *Escherichia coli* clinical isolate biofilm (72.39%) at concentration 250 µg / mL and of *Escherichia coli* ATCC 35218 biofilm (85.10%) at concentration 125 µg / mL. Based on the percentage of biofilm inhibition, the activity of ethyl acetate extract of *Jatropha multifida* L. stem was better in inhibiting *Escherichia coli* ATCC 35218 bacterial biofilm.

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