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# **PUBLIKASI JURNAL**

Activity of Garlic (*Allium Sativum*) Methanol Extract as an Inhibitor of Biofilm Formation on Methicillin-Resistant Staphylococcus aureus (MRSA)

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# ABSTRACT FOR 4TH USIM INTERNATIONAL HEALTH E-CONFERENCE 2020 (IHEC 2020) IN CONJUNCTION WITH THE 3RD INTERNATIONAL CONFERENCE ON MEDICINE AND HEALTH SCIENCES (ICMHS)

Healthcare In A Pandemic Era: The New Norm

Held as Webinar from 16th – 17th December 2020

FACULTY OF MEDICINE AND HEALTH SCIENCES
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# Respiratory Symptoms and Risk of TSP Exposure at Public Transport Terminal

Reny Indrayani<sup>1\*</sup>, Dwi Martiana Wati<sup>2</sup>

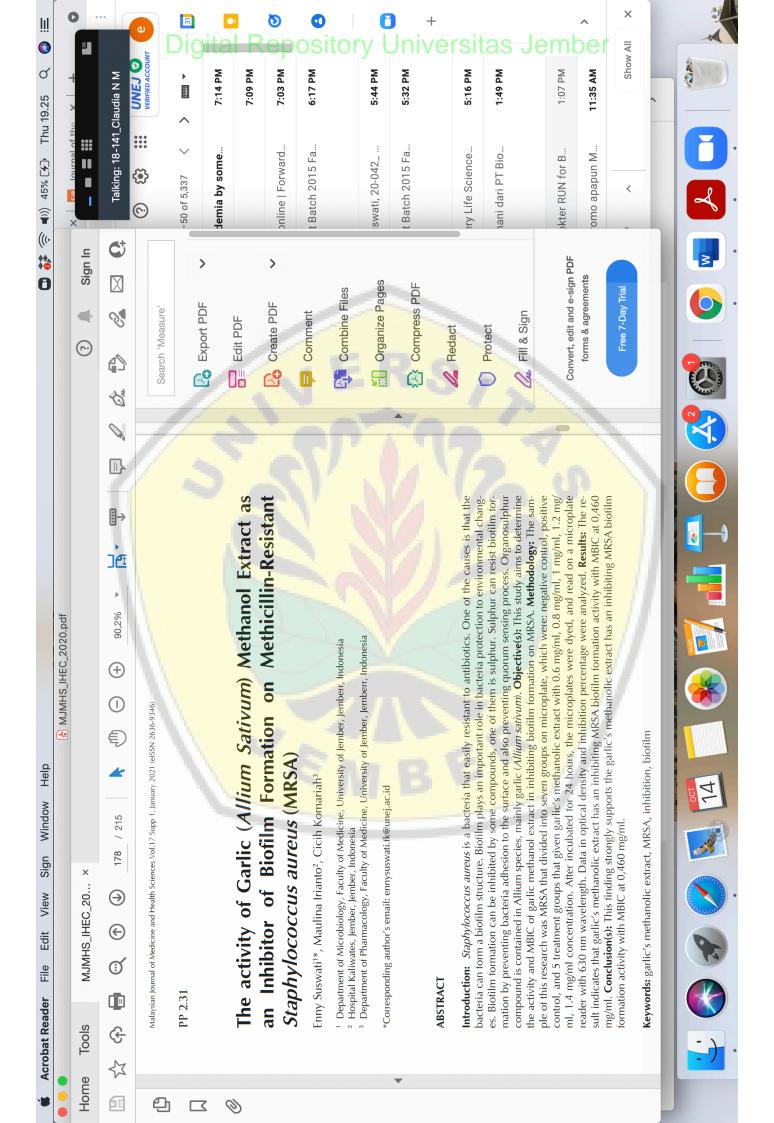
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### **ABSTRACT**

**Introduction:** Along with the progress of civilization, transportation plays an important role in advancing life. However, the growth of transportation has also brought an unfortunate impact on the community. One main part of the land transportation system is a public transport terminal which is common with total suspended particulate (TSP) as a risk agent for respiratory function. It may lead to an uncomfortable working environment, vision problems, poisoning, lung dysfunction and even cancer. **Objective:** This study aimed to describe the possibility of risk cancer due to TSP exposure among public transport terminal workers in Jember using ARKL assessment (which is based on EPA) which is consisted of cancer and non-cancer risk. **Methodology:** The study participants were 44 workers on terminal X. For assessing the respiratory symptom it used ARKL assessment based on EPA which is consisted of cancer and non-cancer risk. **Results:** According to the assessment, most workers experienced respiratory symptoms and could be able to have cancer risk (shown by ECR >1). However, they were safe from non-cancer risk. **Conclusion:** To conclude, TSP exposure seems to pose a potential risk of cancer. Therefore, it will be remarkably important to minimise the frequency of TSP exposure by evaluating working hours while ensuring the workers use a proper mask.

Keywords: Respiratory symptoms, total suspended particulate (TSP) exposure, Public Transport Terminal workers







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# Activity of Garlic (*Allium sativum*) Methanol Extract as an Inhibitor of Biofilm Formation on Methicillin-Resistant *Staphylococcus aureus* (MRSA)



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# **Abstract**

**Introduction:** Staphylococcus aureus is a bacteria that easily resistant to antibiotics. One of the causes is that the bacteria can form a biofilm structure. Biofilm plays an important role in bacteria protection to environmental changes. Biofilm formation can be inhibited by some compounds, one of them is sulphur. Sulphur can resist biofilm formation by preventing bacteria adhesion to the surface and also preventing quorum sensing process. Organosulphur compound is contained in Allium species, mainly garlics (Allium sativum).

Objective(s)

This study aims to determine the activity and MBIC of garlic methanol extract in inhibiting biofilm formation on MRSA

**Methodology:** The sample of this research was MRSA that divided into seven groups on microplate, which were: negative control, positive control, and 5 treatment groups that given garlic's methanolic extract with 0,6 mg/ml, 0,8 mg/ml, 1 mg/ml, 1,2 mg/ml, 1,4 mg/ml concentration. After incubated for 24 hours, the microplates were dyed, and read on microplate reader with 630 nm wavelength. Data in optical density and inhibition percentage were analyzed **Results:** The result indicates that garlic's methanolic extract has an inhibiting MRSA biofilm formation activity with MBIC at 0,460 mg/ml.

**Conclusion(s):** This finding strongly supports the garlic's methanolic extract has an inhibiting MRSA biofilm formation activity with MBIC at 0,460 mg/ml. **Keywords**: garlic's methanolic extract, MRSA, inhibition, biofilm

# Introduction

MRSA in Indonesia mentioned that in 2001 only one (0,3%) MRSA was identified from 329 S.aureus that were isolated from 3995 patients. In 2011 in three educational hospitals, four percent of MRSA carriers were identified from cultures of nasal cavity, esophagus, and skin lesion cultures of 1502 patients. While in 2016, in Dr. Soetomo Hospital, Surabaya, the average of MRSA carriers were 8,1% of 643 patients. One of the reasons why S.aureus become susceptible to resistance is because it can form a biofilm structure. Biofilm is defined as bacterial multicellular aggregate which attached to a biotic or abiotic surfaces and it could produce Extracellular Polymeric Substance (EPS). The formation of biofilm in general involving two processes which are cell surface attachment and cell-cell interaction. Bacteria has an ability to interact between the cell that called quorum sensing. The formation of biofilm can be inhibited by compounds such as phenol, indole, triazole, sulfur, furanon, bromopyrrole, dan peptide. Sulfur can inhibit the formation of biofilm by preventing bacterial attachment dan quorum sensin]. One of the plants that contains a lot of sulfur is garlic. Allicin, one of sulfur compounds in garlic, is known to has an antibacterial effect against S.aureus.

The purpose of this research is to find out the activity of garlic's methanol extract as an inhibitor of MRSA biofilm formation.

# **Methods**

This is a quasi experimental research design with a post test only control group design., in Biology Labroratory of the Mathematics and Natural Sciences Faculty, Microbiology Labroratory, and Biomolecular Labroratory of Medicine Faculty, Jember University.

The research used the *Microtiter Plate Biofilm Assay* method. The sample is MRSA ATCC® BAA-1026™\*, divided into seven groups on microplate which consist of negative control, positive control, and 5 treatments group (P1-P5). The negative control group contains 100 L/well of MRSA and 100 L/well of aquades. While the positive control contains 100 □L/well of MRSA and 80 mg/ml N-Acetylcystein (NAC) as much as 100□L/well. Treatment groups, P1-P5, contains 100 □L/well MRSA and garlic's methanol extract. With a concentration 0.6 mg/mL, 0.8 mg/mL, 1 mg / mL, 1.2 mg / mL, and 1.4 mg / mL for each, as much as 100 □L / well. The Microplate is incubated in incubator on a 37°C temperature for 24 hours. And then microplate is washed three times by using 100 \( \text{IL/well of} \) sterile PBS, fixed it with 100 \( \preceq \text{L/well of methanol for five minutes, color it } \) using 100 \( \text{L/well of 1\% crystal violet, wait for twenty minutes. For the rest of the coloration is washed off using 100 \( \preceq \text{L/well of sterile } \) aquadest. After that, add 100 L/well of 33% glacial acetic acid. Last, do the microplate reading using a microplate reader at 630nm wavelength.

This research has received an approval from the ethics commission of Medicine Faculty, Jember University. The results are presented in the form of mean ± standard deviation (SD) *optical density* (OD) of biofilm. For the data analysis is using One Way Anova analysis and logarithmic regression.

Data in the form of biofilm inhibition percentage obtained from the formula:

%inhibition =  $(OD_{negative control} - OD_{sample})$ x100%

OD<sub>negative contro</sub>

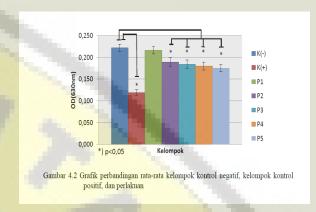


Fig 1. OD from spectofotometry

The logarithmic regression test was examined. Then, from the regression equation's result, enter the minimum inhibition percentage, so that *Minimum Biofilm Inhibitory Concentrations* (MBIC) from the garlic's methanol extract as the inhibitor of biofilm formation, on a concentration of 0,460 mg/mL.

# **Results and Discussion**

The results are shown using optical density (OD) form. Recording to the normality test of biofilm OD, the results got p>0,05 for all groups and the test of homogeneity got p=0,948. For the *One Way Anova* test, the value was p=0,000, so there was a significant difference in at least two treatment groups The LSD test showed if there was a <0,05 significant difference between the negative control groups, also there was a significant difference between the negative control and the P2,P3,P4,P5 treatment groups (Picture 1).

The results, which in biofilm OD form, got a significant outcome between the positive control and negative control groups. This shows that by giving aquades can not inhibit the formation of MRSA biofilm. Meanwhile by giving 80 mg/ml of NAC, the formation of MRSA biofilm can be inhibited because NAC can reduce the formation of bacterial EPS so the maturation process is delayed. It shown by the reduced of the biofilm's thickness on an examination using a *Confocal Lase Scanning Microscope* (CLSM). The biofilm OD results in negative control groups is significant to the P2, P3, P4, and P5 groups. It means that garlic's methanol extract has an activity as an inhibitor of biofilm formation on MRSA. This is because the garlic's methanol extract has sulfur content which prevents bacterial the attachment and *quorum sensing* process [10]. The previous research showed that garlic's methanol extract has an antibacterial effect which presented with the formation of inhibition zone on agar media.

Quantitatively with the RT-PCR analysis, *allicin* in garlic can suppress the expression of *icaA* in *Staphylococcus*. But the exact mechanism by which garlic suppresses biofilm-related genes in Staphylococcus is unclear yet. Moreover, organosulfur compouds can act as *quorum sensing* inhibitors by preventing the enzymatic activity of the LuxS cleavage precursor. And also a competitive inhibitor of Al-2 binding receptors in *methyl cycle. Quorum sensing* which involves LuxS and Al-2 has an influence on the regulation of bacterial biofilm formation. So when Al-2 and LuxS are inhibited by the *quorum sensing* then the formation of biofilm will be disrupted.

# Conclusion and Suggestion

Based on the research results, it can be concluded that the garlic's methanol extract (*Allium sativum*) can inhibit the formation of biofilm on MRSA. With the MBIC is 0,460 mg/mL. Further research is needed regarding to the garlic's methanol extract in inhibiting the formation of biofilm and it is also necessary to examine biofilms with other qualitative methods, for example with *Congo Red Agar* (CRA) or CLSM.