



**BioLink**  
**Jurnal Biologi Lingkungan, Industri, Kesehatan**

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***GREEN TEA ETHANOL EXTRACT EFFICACY AGAINST  
PSEUDOMONAS AERUGINOSA***

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Submitted : 01-06-2022; Reviewed :02-07-2022; Accepted : 08-08-2022

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***Abstract***

*Research about green tea ethanol extract efficacy against Pseudomonas aeruginosa has been done. The green tea leaves were sourced from tea distributor in Sidamanik District, North Sumatra, Indonesia. Green tea leaves extract was obtained by maceration technique in which green tea leaves were soaked with ethanol 96% for 24 hours. After that, the filtrate was concentrated to be thick extract with no liquid content again. After that, the concentrated extract was made by 5%, 10% and 15% as extract variations. The efficacy of green tea ethanol extract against Pseudomonas aeruginosa was signed by increasing the inhibitory diameter that using disc method, in which the distilled water was used as negative blank. The results obtained for blanks, 5%, 10% and 15% extracts were 0; 1.85; 2.9 and 4.45 mm. The conclusion of this study is that the concentration of the extract is increasing proportionally to its inhibitory power, the higher of extract concentration is higher of its inhibitory activity against Pseudomonas aeruginosa. The conclusion described the green tea ethanol extract was effective to be antibacterial agent against Pseudomonas aeruginosa.*

**Keywords:** *Pseudomonas aeruginosa; Green Tea; Maceration; Disc Method; Ethanol Extract*

**How to Cite:** *Fahmi, A., Syukur, S., Chaidir, Z., & Melia, S., (2022). Green Tea Ethanol Extract Efficacy Against Pseudomonas aeruginosa, BioLink: Jurnal Biologi Lingkungan, Industri dan Kesehatan, Vol. 9 (1): Hal. 26-32*

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## INTRODUCTION

*Pseudomonas aeruginosa* is a gram negative bacteria which known as obligate aerobic bacteria. It has encapsulated and polar flagella. The flagella has function as motility organelle which measuring about 0.5-1.0 m. These bacteria do not produce spores and cannot ferment carbohydrates. (Wu *et al.*, 2015)

In biochemical tests, these bacteria produced a positive impact on the indole, Methyl Red, and Voges-Proskauer assays. These bacteria are widely found in nature, for example in soil, water, plants, and animals. *P. aeruginosa* is an opportunistic pathogen. This bacteria is nosocomial pneumonia infection causing (Bassetti *et al.*, 2018).

When *P. aeruginosa* colonies grow on suitable media, they produce a bluish non-fluorescent pigment, pyocyanin. Some strains of *Pseudomonas* are also capable of producing a green fluorescent pigment, namely pioverdin (Sulviana *et al.*, 2017).

These bacteria are also often used to degrade pesticide substances. *P. aeruginosa* is a bacteria that is able to adapt to conditions of low oxygen and nutrients. It also grows well in a temperature range of 4-42°C. *P. aeruginosa* can live on medical equipment and other parts of the hospital, making it easy to infect patients

with decreased immunity (Jenny & Kingsbury, 2018).

Medicinal plants have been the main source of therapeutic agents for alleviation and curing diseases such as the tea plants (*Camellia sinensis*). The tea is main beverage which popular in many countries because the people believe with consuming tea will make more values for their health.

Based on the process, tea is classified into 4 types, namely white tea (non-fermented, withered, the main content is polyphenols and fluoride), green tea (non-fermented, withered and dried, the main content is Epigallocatechin-3-gallate (EGCG), tea oolong (partially fermented mainly dimethyl EGCG) and black tea (fermented entirely the main content is theaflavin) (Isemura, 2019).

In addition to these factors, there are several other factors that can affect the quality of tea include the age of the leaves, the type of picking and varieties and clones. In the processing, directly or indirectly, catechins in green tea influence with all properties of tea included the taste, color and flavor. Bitter taste from tea is strongly influenced by the catechins. It means that the higher catechin, will lead to higher the bitterness (Anjarsari, 2016).

There are the researches about the antimicrobial activities of various teas against *P. aeruginosa*, has learned about

inhibitory of green tea distilled water extract which taken from Tea Plantation, Bogor, Indonesia to the methicillin resistant *Staphylococcus aureus* and *P. aeruginosa* (Radji *et al.*, 2013).

Another research had compared the inhibitory activity between fresh green tea, commercial green tea and black tea from Ooti plantation, India. The research learnt about antimicrobial activity of these teas methanol extracts against pathogen bacterias including *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholera* (Archana & Abraham, 2011).

Then, the research had used green tea and black tea extract against *P. aeruginosa* using methanol as solvent to obtain the extract. The research evaluated the antibacterial activity of methanol extracts of green and black teas on *P. aeruginosa*. This research was carried out on burn wounds of 245 hospitalized patients in Kerman, Iran. From this research, green tea methanol extract had the higher antibacterial effect than black tea methanol extract (Taherpour *et al.*, 2016).

*P. aeruginosa* able to be isolated from patient who has eye infection illness from Al-Haytham Teaching Eye Hospital in Baghdad, Iraq. The researchers found out their ability as antibacterial agents. The research showed that the extract decreased

the bacterial viable count (Flayyih *et al.*, 2013)

Green tea has great benefits with few side effects. Therefore, more and more studies are focused on the effects of green tea on human health until now (Zhou *et al.*, 2016).

Here, We study about the efficacy of green tea ethanol extract (*Camelia sinensis*) from a distributor labeled Juma in Sidamanik District, North Sumatra against *P. aeruginosa* because this sample is not analyzed the antimicrobial activity of *P. aeruginosa* yet and to get information involve the activity of green tea.

## MATERIALS AND METHODS

The type of research used was experimental laboratory. Green tea was from commercial product labeled JUMA from Sidamanik district in North Sumatra. The extract of green tea was resulted with maceration and evaporation the ethanol as solvent until get thickly green tea ethanol extract. Green tea extract was made in to 5%, 10%, and 15% concentration with distilled water as solvent. then analyzed the antimicrobial activity in Microbiology Laboratory of Pharmacy Faculty, University of Sumatra Utara in August 2021.

### **Sample Preparation and Extraction**

The sample of green tea were dried leaves ready to be macerated. Amount 100g of simplicia powder was extracted with 96% ethanol by maceration method for 2 days in a macerator container with occasional stirring. Then, it was filtered using filter paper and then re-immersed on the filtered residue for 1 days with the same treatment as the previous stage. The macerate was evaporated in average temperature at 78°C to obtain a thick green tea ethanol extract (Fahmi, 2020).

### **Preparation of Bacterial Culture Stock**

*P. aeruginosa* colonies were taken using a round needle that had been sterilized and implanted on the surface of the nutrient media so that it was slanted by scratching and then incubated at 37°C for 24 hours (Fahmi, 2020).

### **Bacterial Inoculum Making**

*P. aeruginosa* isolates were grown as colonies and then taken from the culture stock using a sterilize ose needle first, after that suspended in a test tube containing 10

mL of nutrient broth media and incubated at 37°C for 24 hours which means fulfill to the standard (Mc. Farland) for the turbidity (Fahmi, 2020).

### **Inhibitory Efficacy**

0.1 mL of *P. aeruginosa* as inoculum was put in a petri dish, after that the nutrient agar (NA) that boiled with distilled water was poured into 15 mL petri dish with size 15 mL, then the petri dish was shaken on the table surface so that the media and bacterial suspension were homogeneous and allowed to be solid agar. Disc paper that has been given with the concentration of each test solution that placed on the surface of the media has solidified then, incubated at 37°C during 18 hours until 24 hours. Observed and measured the inhibition diameter which formed using a caliper (Fahmi, 2020).

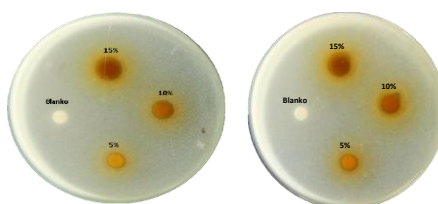
## **RESULTS AND DISCUSSION**

The inhibition diameter between green tea ethanol extract with various concentrations had been formed as table below with two repetitions.

**Table 1. Inhibition Activities Green Tea Ethanol Extract to *P. aeruginosa***

Initials	Concentration	Repetition	Diameter (mm)
Blank (Aquadest)	0%	I	6
		II	6
		Average ± SD	6 ± 0,00
Average ± ID	0		
Extract in aquadest	5%	I	7,7
		II	8,0
		Average ± SD	7,85 ± 0,21
Average ± ID	1,85		
Extract in aquadest	10%	I	8,9
		II	8,9
		Average ± SD	8,90 ± 0,00
Average ± ID	2.9		
Extract in aquadest	15%	I	10,3
		II	10,6
		Average ± SD	10,45 ± 0,21
Average ± ID	4.45		

From table 1, we concern that the increasing inhibition activity was directly increasing the concentration. SD diameter of blank, 5%, 10% and 15% concentration variations were  $6 \pm 0,00$ ;  $7,85 \pm 0,21$ ;  $8,90 \pm 0,00$  and  $10,45 \pm 0,21$  mm with the inhibition diameter 0 mm; 1,85; 2,9 and 4,45 mm. ID is Inhibition Diameter (Total Diameter – Blank Diameter). For detail, let us see the figures below:



**Figure 1. Inhibition zone of green tea ethanol extract against *P. aeruginosa***

For more detail, it could be seen as the diagram below where x as Inhibitory inhibition activity of green tea ethanol extract against *P. aeruginosa* on the concentrations. Diameter (mm) versus y as various concentrations.

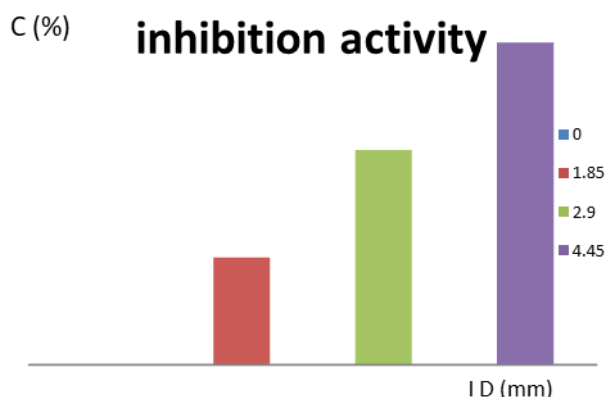


Figure 2. Diagram of x as Inhibitory Diameter (ID) versus y as concentration variations of green tea ethanol extract

The antibacterial activities can be described according to (Morales *et al.*, 2003), the activity of the antimicrobial inhibition zone was grouped into four categories, namely weak activity (<5 mm), moderate (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm). The antimicrobial inhibitory activity was expressed based on the clear zone produced around the paper disc. The diameter of the zone of inhibition of bacterial growth was measured in mm (Hombach *et al.*, 2016).

The formation of the inhibition zone was due to the antibacterial content in green tea ethanol extract and increasing as the extract concentration too. Green tea mechanism can prevent the attachment of pathogenic bacteria on the cell membrane of host. Also, green tea can inhibit the bacteria adhesion on it. The green tea also able to act as a potential anti-adhesive agent (Lee *et al.*, 2009).

Epigallocatechin gallate (EGCG), which is catechin derivative contained in green tea has also been reported to interact with the outer membrane bacterial and may prevent the adhesion to mammalian epithelial cells (Sharma *et al.*, 2012).

Green tea may affect the activity of dihydrofolate reductase, an enzyme that is needed by pathogenic bacteria to synthesize purine and pyrimidine as well as increase the thickness of the epidermis. *Pseudomonas aeruginosa* is binded by EGCG contained in green tea and the binding located in the bacterial cell membrane (Jeon *et al.*, 2014; Xiong *et al.*, 2017)

## CONCLUSION

Green tea ethanol extract against pathogen bacteria of *P. aeruginosa* has been carried out. The results obtained for blanks, 5%, 10% and 15% green tea ethanol extracts variation using distilled water as solvent were  $6 \pm 0,00$ ;  $7,85 \pm 0,21$ ;  $8,90 \pm$

0,00 and 10,45  $\pm$  0,21 mm with the inhibition diameter 0; 1.85; 2.9 and 4.45 mm with concentration variations that used. The conclusion of this study is that higher extract concentration make the inhibitory activity is higher too and they are increasing proportionally against *P. aeruginosa*.

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