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Article

Antimicrobial Activity Test of Black Tea Ethanol Extract (*Camellia sinensis*) against *Pseudomonas aeruginosa*

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Abstract: A study about the antimicrobial activity test of black tea ethanol extract (*Camellia sinensis*) against *Pseudomonas aeruginosa* has been carried out. The black tea leaves are sourced from Sidamanik Plantation, North Sumatra. The dried black tea leaf extract was obtained by maceration technique in which black tea leaves were macerated for 24 hours. Then, the macerate was concentrated to obtain a thick extract and make sure that the ethanol had evaporated. After that, the concentrated extract was made with a variation of 5%, 10% and 15%. The antimicrobial activity test used was the disc method, where distilled water was used as a negative blank. The results obtained for average SD for blank with diameter 6 mm, 5%, 10% and 15% extracts were 6 ± 0,00; 7,75 ± 0,35; 9,05 ± 0,07 and 9,95 ± 0,07 mm with the inhibition diameter 0 mm; 1,75 ± 0,35; 2,05 ±0,07 and 3,95 ± 0,07 mm. The conclusion of this study is that the concentration of the extract is straightly proportional to its inhibitory power, namely the higher the concentration of black tea ethanol extract, the higher its inhibitory activity against *Pseudomonas aeruginosa*, so it can be used as an antibacterial agent for *Pseudomonas aeruginosa*.

Keywords: *Pseudomonas aeruginosa*; Sidamanik black tea; maceration; disc method; ethanol extract.

1. Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an obligatory aerobic bacterium, an opportunistic pathogen, encapsulated, gram-negative which is motile because it has polar flagella, size about 0.5-1.0 m (Boyd, 1995; Horneck et al., 2010; Madigan et al., 2008; Tortora et al., 2018). *P. aeruginosa* colonies do not produce spores and disable carbohydrates. These bacteria are widely found in nature, such as in soil, water, plants, and. This bacterium is the main cause of nosocomial pneumonia infection in animals (Horneck et al., 2010). When *P. aeruginosa* colonies can live on suitable media, they produce pyocyanin pigment. Some strains of *Pseudomonas* are also capable of producing pioverdin pigment (World Health Organization,

2004). These bacteria are also often used to degrade pesticide substances. *P. aeruginosa* is a bacterium that can adapt to conditions of low oxygen and nutrients. This bacterium can also grow in a temperature range of 4-42 °C (Stover et al., 2000).

Patients will be easily infected because *P. aeruginosa* can live on medical equipment and other parts of the hospital (Engel & Balachandran, 2009). Medicinal plants have been the main source of therapeutic agents for the alleviation and cure of diseases such as tea plant (*Camellia sinensis).* Tea is famous for leaves which have been consumed for centuries as a beverage and is valued for its medicinal properties. In the case of black tea, the leaves and buds are fermented or oxidized after they have been dried. Much research has been successful in finding their activities such as antimicrobial activity (Archana & Abraham, 2011). Thus, we noticed that the antimicrobial activity test of black tea ethanol extract (*Camellia sinensis*) from Sidamanik Plantation North Sumatera against *Pseudomonas aeruginosa.*

2. Literature Review

2.1. Black Tea

Tea (*Camellia sinensis*) is cultivated worldwide. There are two main varieties of the tea plant: *Camellia sinensis var. sinensis* which is characterized by its small plants such as leaves and shrubs, comes from China and grows in several countries in Asia with a moderate cold climate, and *Camellia sinensis var. assamica* which is a large leaf tree found in the southwest region of China and India and exported to several other countries with semi-tropical climates. Due to its unique flavor and taste, sinensis tea dominates the production of green tea, while *assamica* tea is mainly used in the production of black tea due to its high content of catechins and tannins. Tea is the most widely consumed flavored functional drink in the world. Tea generally consists of green tea, oolong tea, white tea, and black tea (Li et al., 2013).

Black tea contains relatively high levels of polyphenols with the main phenolics being flavan-3-ols (epi) catechins, (epi) gallocatechins and their gallate esters, flavonols (mono-, di-, and tri-glycoside conjugates of myricetin, quercetin and kaempferol) flavones and quinic acid esters of gallic, coumaric and caffeic acids. Black tea has a reduced content of flavan-3-ol monomers and higher levels of theaflavin polymerized derivatives, which account for about 10-30% of the converted catechins, and thearubigines (de Mejia et al., 2009; Rouanet et al., 2010).

2.2. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a ubiquitous environmental bacterium that causes opportunistic infections in humans. Many metabolic pathways and regulatory genes make these bacteria highly adaptive to a wide range of growth conditions. Its nutritional versatility, large number of virulence factors and high antibiotic resistance make this bacterium very difficult to eradicate from infected individuals, especially lung infections in cystic fibrosis patients. Key features of bacteria related to their virulence genes and their regulation, antibiotic resistance and future trends of anti-*Pseudomonas* approaches are discussed (Wu et al., 2015). P. aeruginosa is intrinsically resistant to many structurally unrelated antimicrobial agents due to the low permeability of the outer membrane (Papadimitriou-Olivgeris et al., 2022; Strateva & Yordanov, 2009).

2.3. Black tea against *P. aeroginosa*

Many studies have been conducted regarding the ability of black tea to attack *P. aeruginosa*. Before, there was research on the relationship between black tea against *Pseudomonas aeruginosa.* Taherpour et al. (2016) researched about the efficacy of methanolic extract of green and black teas against extended-spectrum beta-Lactamase-producing *Pseudomonas aeruginosa* and Flayyih et al. (2013) about antimicrobial effects of black tea (*Camellia sinensis*) on *Pseudomonas aeruginosa* isolated from eye infection. Ratnasooriya et al. (2016) examined the antibacterial properties of three types of Sri Lankan black tea which have the most potential to be used in inhibiting the growth of several pathogenic bacteria including *Pseudomonas aureginosa*.

Apart from black tea, the properties of green tea are also beneficial in inhibiting the growth of the *Pseudomonas aeruginosa* bacteria (Bazzaz et al., 2016). The research about both of teas also had been done. Muhammad et al. (2015) said that *P. aeruginosa* was most susceptible to all different teas (black and green tea). A recent study on determining the safety and efficacy of black tea extract in the treatment of *P. aureginosa* and *Stapylococcus aureus* bacterial conjunctivitis in a rabbit model and comparing it with gatifloxacin drops. The results showed that black tea extract had an antimicrobial effect similar to that of gatifloxacin in the rabbit model of conjunctivitis (Abdullatif et al., 2023).

Commercial black tea methanol, ethyl acetate and acetone extracts were prepared by soxhlet extraction. Antimicrobial activity was analyzed for gram positive bacteria *Bacillus cereus ATCC13061, Staphylococcus aureus ATCC6538p, Staphylococcus saprophyticus KCTC3345, Listeria monocytogenes ATCC7644* and bacteria *Proteus vulgaris KCTC2512, Pseudomonas aeruginosa KCTC2004, Pseudomonas putida ATCC49128* and *Serratia marcescens KCTC42171*. From the research, it was found that black tea methanol extract 100 mg/ml had good antimicrobial activity against the eight bacteria, but in ampicillin, *P. aeruginosa* and *Serratia marcescens KCTC42171* were not detected (Patil et al., 2016).

Research has also been conducted to determine antimicrobial activity by diffusion inhibition test of black and green tea extracts (cold and hot) against the antibiotic resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* after injuring the skin of rats due to burning. It was found that hot aqueous green and black tea extracts were more effective against both types of bacteria than cold aqueous based on zone of inhibition assessment. Green tea extract can be used for wound healing and burnt skin of rats and black tea extract showed a reduction in the number of inflammatory cells and regulates collagen bands. We conclude that green tea has greater activity as indicated by a higher anti-inflammatory effect compared to black tea (Sorchee, 2020).

Pseudomonas aeruginosa is a bacterial pathogen that causes eye infections in people who wear contact lenses. Researchers conducted a disc diffusion test using green tea and black tea concentrations of 100 mg/ml each to study its inhibitory effect on contact lenses containing *P. aeruginosa* bacteria. The results showed that the inhibition of green tea was better than black tea against *P. aeruginosa*, but both have the potential to be used as a cleaning solution for contact lenses (Bigaud & Yeung-Cheung, 2017).

3. Materials and Methods

3.1. Equipment

The equipment that used are autoclave, stir bar, hot plate, incubator (memmert), caliper, laminar air flow cabinet, micro pipette, tweezers, ose neddle, vortex and other glassware (beaker glass, petri dish, reaction tube, Erlenmeyer glass etc).

3.2. Ingredients

The materials that used are nutrient agar media (Oxoid), nutrient broth (Oxoid), blank disc, aquadest, *P. aeruginosa* bacteria, black tea ethanol extract

3.3. Procedures

3.3.1. Preparation of Bacterial Culture Stock

P. aeruginosa colony was taken using a round needle that had been sterilized by incandescence and then implanted on the surface of the nutrient media so that it was slanted by scratching and then incubated at 37 ^{0}C for 24 hours (Ditjen Produksi dan Distribusi Kefarmasian, 2020).

3.3.2. Bacterial Inoculum Making

P. aeruginosa colonies were taken from the culture stock using a sterile ose needle, then suspended in a test tube containing 10 mL of nutrient broth media and incubated at 37 ^{0}C for 24 hours until the turbidity was equivalent to the standard Mc. Farland (Ditjen Produksi dan Distribusi Kefarmasian, 2020).

3.3.3. Antibacterial Activity Test

A total of 0.1 mL of *P. aeruginosa* inoculum was put in a sterile petri dish, then the Nutrient Agar medium was poured into 15 mL petri dish, then the petri dish was shaken on the table surface so that the media and bacterial suspension were homogeneous and allowed to solidify. Disc paper that has been given with the concentration of each test solution, then placed on the surface of the media that has solidified. Incubated at 37 ^{0}C for 18 to 24 hours. Observed and measured using a caliper diameter of the inhibition zone formed around the paper disc (Ditjen Produksi dan Distribusi Kefarmasian, 2020).

3.4. Data Collection

The inhibit zone diameter between green tea and black tea ethanol extract had been formed and listed in Table 1 below.

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

C	Repetition	Inhibition Diameter *P.aeruginosa*
Control/Blank	P1	6
	P2	6
Average ± SD		6 ± 0,00
15%	P1	9,9
	P2	10,0
Average ± SD		9,95 ± 0,07
10%	P1	9,1
	P2	9,0
Average ± SD		9,05 ± 0,07
5%	P1	8,0
	P2	7,5
Average ± SD		7,75 ± 0,35

Table 1 displays the increasing inhibition activity with increasing the concentration. SD diameter of blank, 5%, 10% and 15% concentration variations were 6 ± 0,00; 7,75 ± 0,35; 9,05 ± 0,07 and 9,95 ± 0,07 mm with the inhibition diameter 0 mm; 1,75;3,05 and 3,95 mm. ID is Inhibition Diameter (Total Diameter – Blank Diameter)

4. Results and Discussion

4.1. Numerical Results

The numerical result of this study in detail can be seen in figure 1 below:

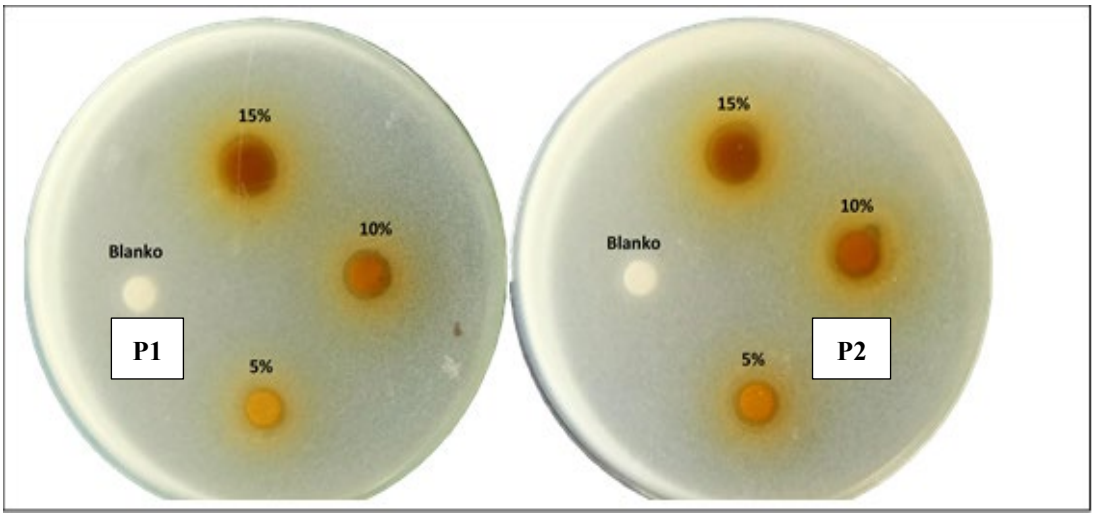

Figure 1. Black tea ethanol extract against *P. aeruginosa* (two repetitions)

Figure 1 shows that samples P1 and P2 are repetitions of the antibacterial activity of black tea ethanol extract samples in inhibiting the growth of *P. aeruginosa* where the two samples did not have a significant difference.

4.2. Graphical Results

For inhibition activity of black tea ethanol extract against *P. aeruginosa* so attached can be seen in Figure 2 below.

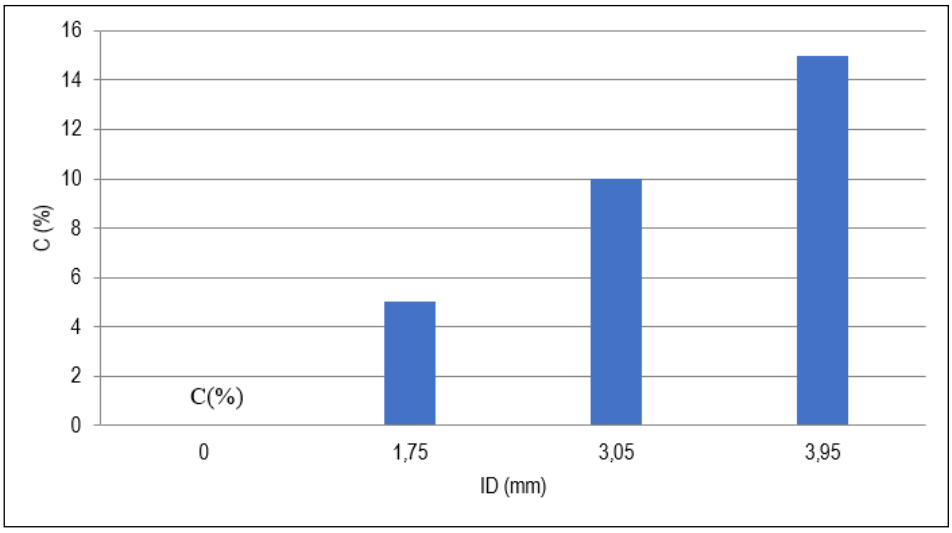

Figure 2. Inhibition activity of black tea ethanol extract against *P. aeruginosa*

Figure 2 captures the increasing inhibition activity with increasing the black tea ethanol extract concentration. The inhibition diameter as X and black tea ethanol extract concentration as Y. The inhibition diameter 0 mm; 1,75;3,05 and 3,95 mm which are taken from "total diameter – blank diameter".

4.3 Proposed Improvements

Testing of antibacterial activity according Davis & Stout (1971) that antibacterial activity is classified as weak when the inhibition zone is less than 5 mm, classified as moderate with an inhibition zone of 5 -10 mm, classified as strong if the inhibition zone is 11-20 mm, and classified as very strong if the inhibition zone is 20-30mm. The formation of the inhibition zone was due to the antibacterial content contained in the ethanol extract of black tea leaves and go straightly to the thicker concentration. This happens because of the phenolic content in black tea. Black tea is produced by fermentation process with the oxidation reaction of the phenolase enzyme contained in the tea leaves that changed sensory of that tea (Xu & Chen, 2002).

4.4 Validation

The percentage of catechin in black tea is 5 % and the oxidized phenolic compounds is 25 % (Song & Seong, 2007). Tea is reported to contain about 4000 bioactive compounds of which one third is contributed to polyphenols. Polyphenols are bonded benzene rings with multiple hydroxyl groups (Flayyih et al., 2013). Anti-microbial activities of tea extracts are very selective. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on the bacterial species so that they may be either growth inhibitory or stimulatory (Tiwari et al., 2005). The highest antimicrobial activity of tea is due to presence of catechins and polyphenoles which damages bacterial cell membrane (Mbata et al., 2008). Black tea contains large amounts of main polyphenols which are theaflavins and thearubigins (Li et al., 2013).

5. Conclusions

The inhibitory activity test comparison of ethanol extract of black tea leaves (*Camellia sinensis*) against *P. aeruginosa* has been done. The results obtained for blanks, 5%, 10% and 15% black tea ethanol extracts variation with aquadest as solvent were 6 ± 0,00; 7,75 ± 0,35; 9,05 ± 0,07 and 9,95 ± 0,07 mm with the inhibition diameter 0 mm; 1,75; 3,05 and 3,95 mm. The conclusion of this study is that the concentration of the extract is straightly proportional to its inhibitory power, namely the higher the concentration of black tea ethanol extract, the higher its inhibitory activity against *Pseudomonas aeruginosa*, so it can be used as an antibacterial agent for *Pseudomonas aeruginosa*.

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