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The Effect of Crude Jackfruit (*Artocarpus heterophyllus*) Leaf Extract on *Pseudomonas fluorescens* Bacteria In Vitro

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ABSTRACT

Cultivation activities often experience obstacles due to bacterial disease attacks, one of which is caused by *P. fluorescens* bacteria. Commercial treatment efforts include the use of antibiotics, but this can cause bacterial resistance. Therefore, an alternative treatment that is more environmentally friendly but can still be used as medicine is needed, namely the use of natural ingredients such as jackfruit (*A. heterophyllus*) leaves. This study aims to determine the effect of crude jackfruit (*A. heterophyllus*) leaf extract on *P. fluorescens* bacteria in vitro. This study used an experimental method with a Completely Randomized Design (CRD) consisting of 5 treatments, 2 controls, and 3 replications. The control treatment consisted of a positive control (Tetracycline 30 ppm), and a negative control (only using disc paper), as well as 5 different extract dose treatments, namely treatment A (60 ppm), treatment B (120 ppm), treatment C (180 ppm), treatment D (240 ppm) and treatment E (300 ppm). The results of the study showed that the use of crude jackfruit (*A. heterophyllus*) leaf extract affected *P. fluorescens* bacteria. The relationship between the use of crude jackfruit (*A. heterophyllus*) leaf extract with different doses and the resulting inhibition zone showed a quadratic regression pattern with the equation $y = -0.0001x^2 + 0.05x + 6.327$.

INTRODUCTION

Bacterial diseases are a serious threat to fish farming, as they can lead to decreased production, decreased water quality, and even total mortality (Linayati *et al*., 2024). One type of pathogenic bacteria frequently found infecting freshwater fish such as carp and tilapia is *Pseudomonas fluorescens* (Pękala-Safińska, 2018). This bacterium is an opportunistic pathogen and generally attacks fish experiencing stress due to poor environmental conditions, high density, or improper handling. *P. fluorescens* infection can cause various clinical symptoms such as skin lesions, bleeding, and decreased appetite. This bacterial infection can cause "red skin" disease and can even lead to death. This bacterium is considered a pathogen that causes hemorrhagic septicemia (Zhang *et al*., 2020). The pathogenicity of *Pseudomonas* sp. bacteria in fish causes the fish's swimming pattern to become skewed (Budianto *et al*., 2022). According to Maryani *et al*. (2018), other research found that *P. fluorescens* bacterial infections in fish can cause boils on the skin, fins, abdominal cavity, and internal organs.

Fish farmers use antibiotics as a treatment, but this can lead to resistance (Nurmala *et al*., 2015). Gram-negative bacteria are more likely to develop resistance, making treatment difficult. The use of natural ingredients as an alternative treatment is recommended because they reduce side effects and are environmentally friendly. One such alternative is jackfruit (*A. heterophyllus*) leaves. According to research by Darmawati *et al*. (2015), phytochemical tests on jackfruit leaves showed positive antibacterial compounds such as flavonoids, phenols, steroids, and tannins. Jackfruit leaves are known to have the highest antibacterial content compared to other parts of the plant. Alkaloids were 12.34 mg/100g, tannins 0.07 mg/100g, and flavonoids 3.91 mg/100g (Amadi *et al*., 2018).

Based on this problem, it is necessary to conduct research on the effect of crude jackfruit (*A. heterophyllus*) leaf extract on *P. fluorescens* bacteria in vitro, which can be used as an alternative treatment.

METHODS

The research was conducted at the Central Laboratory of Life Sciences (LSIH), Brawijaya University, Malang, from February to March 2024.

The equipment used in this study included an autoclave, Luminary Air Flow (LAF), refrigerator, hotplate, incubator, beaker glass, blender, blue tip, yellow tip, Bunsen burner, petri dish, funnel, Erlenmeyer flask, measuring cylinder, scissors, digital caliper, loop needle, micropipette, test tube rack, test tube, rotary vacuum evaporator, spatula, analytical balance, glass jar, vortex mixer, tweezers, sprayer, triangle. The materials used in this study included *P. fluorescens* bacteria, jackfruit leaf powder (*A. heterophyllus*), Tryptic Soy Broth (TSB), Pseudomonas Selective Agar (PSA), 10% DMSO, disc paper, 70% Ethanol, NaCl Pa, tissue, 70% Alcohol, filter paper, distilled water, aluminum foil, and cotton.

Jackfruit (*A. heterophyllus*) Leaf Extract Production

Jackfruit (*A. heterophyllus*) leaf extraction was carried out using the maceration method with 1 liter of 70% ethanol as solvent. Wet jackfruit leaves were first dried for 7 days and then blended into powder. A 100-gram sample of the powder was weighed, then placed in a glass jar and soaked in 70% ethanol for the first 6 hours with occasional stirring. Then, it was left for 18 hours and then covered with aluminum foil to prevent the ethanol from evaporating. The macerate was separated by filtration using filter paper. The filtration process was repeated at least twice using the same type and amount of solvent. The next step was to collect all the macerates and then evaporate them using a rotary evaporator until a thick extract was obtained (Kusumawati *et al*., 2017).

Sterilization of Tools and Materials

The sterilization process of tools and materials is that the tools to be sterilized are wrapped in newspaper (for test tubes and Erlenmeyer flasks, the top is given cotton). Aquades is poured sufficiently into the autoclave, then the tools that have been wrapped in newspaper are put into the autoclave and closed tightly diagonally. The switch is turned on, then the red siren button on the autoclave is turned to the red-light limit. Wait 15 minutes, after reaching a temperature of 121ºC, the alarm will sound, then turn off. Wait a few moments until the thermometer and manometer show the number 0. The power switch is turned off, and the autoclave lid is opened. Take the tools that have been sterilized and then stored. The sterilized materials are stored in the refrigerator.

PSA (Pseudomonas Selective Agar) Media

PSA media is used as an agar medium in conducting disc tests. The procedure is to weigh 7 grams of PSA media using a digital scale. It is placed in an Erlenmeyer flask and added with 280 mL of distilled water, and then homogenized. The Erlenmeyer flask is then heated using a hotplate and stirred until homogeneous using a spatula. The Erlenmeyer flask is then covered with cotton and wrapped in aluminum foil. The Erlenmeyer flask is then sterilized by autoclaving at a temperature of 121ºC for 15 minutes at a pressure of 1 atm. The sterile media is waited until lukewarm or around 300°C before being poured into sterile petri dishes.

TSB (Tryptic Soy Broth) Media

TSB media is a liquid medium used for bacterial culture. The process of making TSB media is by weighing 0.21 grams of media using a digital scale. The media is placed in an Erlenmeyer flask and then dissolved in 7 mL of distilled water. The Erlenmeyer flask containing the media and distilled water is then heated on a hotplate and stirred until homogeneous, then placed into a test tube. The test tube is then covered with cotton and wrapped in aluminum foil. The test tube is sterilized by autoclaving at a temperature of 121ºC for 15 minutes at a pressure of 1 atm. The sterile media is left until lukewarm before use.

Bacterial Rejuvenation

P. fluorescens bacteria obtained from the Faculty of Medicine, Brawijaya University, Malang. *P. fluorescens* were rejuvenated by preparing an agar slant and collecting the isolate using a sterile loop needle. The bacteria present on the loop needle were then streaked onto the agar slant using the streaking method. The agar slant, infused with bacteria, was then incubated at 32ºC for 24 hours.

Bacterial Culture

P. fluorescens bacterial culture was performed by sampling a rejuvenated bacterial culture on a slant agar using a sterile loop needle, making one streak. The bacteria on the loop needle were then dipped into TSB liquid medium and stored in an incubator at 32ºC for 24 hours.

In Vitro Test

The procedure for conducting in vitro tests using the disc method involves planting 100 µl of bacteria in a Petri dish containing PSA media and leveling it using a triangle fixed with a Bunsen burner. Sterile paper discs are soaked in the specified jackfruit leaf extract for 10-15 minutes, then drained and placed on the surface of the agar medium. The media, infused with bacteria and the discs, is incubated at 32°C for 18-24 hours. After incubation, the media is observed, and the clear zone formed around the discs is measured using a digital caliper.

Data Analysis

The results obtained will be carried out ANOVA test (<0.05) to determine the effect of jackfruit (*A. heterophyllus*) leaves crude extract on *P. fluorescens* bacteria. To determine the relationship between treatment (jackfruit leaves crude extract) and test parameters (zone of inhibition) an orthogonal polynomial test was performed.

RESULTS

Bacterial Identification

Identification of bacteria was performed using the Gram staining method to observe the morphology of *P. fluorescens* bacterial cells and determine whether the bacteria are gram-positive or gram-negative. The results of this study indicate that *P. fluorescens* is a gram-negative bacterium. The Gram staining results (Figure 1) show a reddish-pink coloration of the bacteria because they cannot retain the purple color of crystal violet after being rinsed with alcohol.

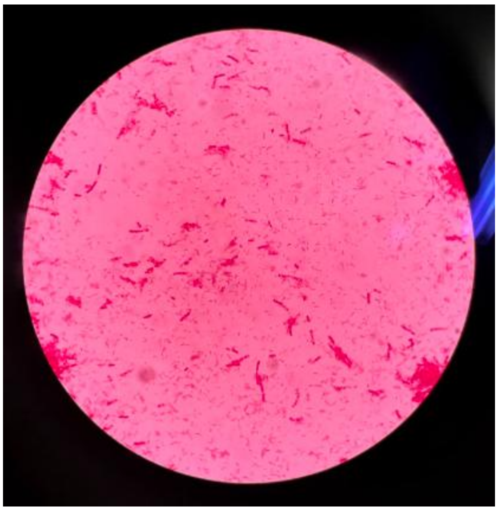

Figure 1. Gram Staining Results of *P. fluorescens* Bacteria (1,000x Magnification)

Disc Test

Observations during the 24-48-hour study on the effect of crude jackfruit (*A. heterophyllus*) leaf extract on *P. fluorescens* bacteria in vitro revealed inhibition zones formed in each treatment. The inhibition zone images are presented in Figure 2, and the inhibition zone measurement results are presented in Table 1.

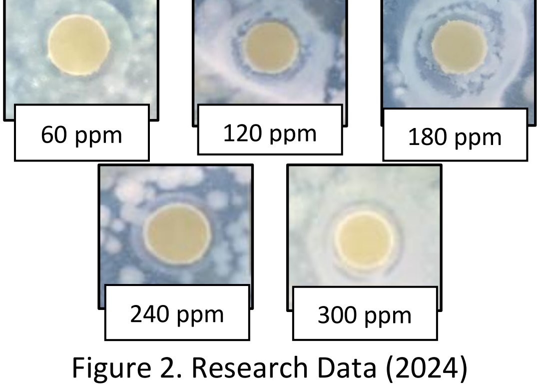

Figure 2. Research Data (2024)

Table 1. Average Inhibition Zone Results (Research Data, 2024)

Treatment	**Repeat**			**Total**	**Average**
	1	**2**	**3**		
A (60 ppm)	8.90	8.97	9.02	26.88	8.96 ± 0.06
B (120 ppm)	9.86	9.98	9.64	29.47	9.82 ± 0.17
C (180 ppm)	10.84	11.58	12.03	34.45	11.48 ± 0.60
D (240 ppm)	10.23	10.27	9.13	29.63	9.88 ± 0.65
E (300 ppm)	9.02	9.06	8.47	26.55	8.85 ± 0.33
				146.97	

The results of the study obtained an average value of clear zone measurements in treatment A (60 ppm) of 8.96 mm, B (120 ppm) of 9.82 mm, C (180 ppm) of 11.48 mm, D (240 ppm) of 9.88 mm, and E (300 ppm) of 8.85 mm. Based on Table 2, the classification of inhibition zone responses in treatments A (60 ppm), B (120 ppm), D (240 ppm) and E (300 ppm) is classified as a moderate inhibition response. Treatment C (180 ppm) is classified as a strong inhibition response. The following classification of inhibition zones according to Azis (2017) is presented in Table 2.

Table 2. Classification of Inhibition Zones (Azis, 2017)

Inhibition Zones (mm)	Resistance Response
<5	Weak
5-10	Moderate
10-19	Strong
>20	Very Strong

Table 3. Variance Analysis

Source of Diversity	**db**	**JK**	**KT**	**F.hit**	**F 5%**	**F 1%**
Treatment	4	13.33	3.33	18.18**	3.48	5.99
Random	10	1.83	0.18			
Total	14					

The analysis of variance calculation above shows that the administration of crude jackfruit extract (*A. heterophyllus*) leaf has a significantly different effect on the inhibition of *P. fluorescens* bacteria. This is indicated by the calculated F value being greater than the F table value of 5% and F table 1%. Therefore, it was continued with the Least Significant Difference (LSD) test. The results of the LSD test are presented in Table 4.

Table 4. BNT Test Results

Treatment	**Average**	**E**	**A**	**B**	**D**	**C**	**Notation**
		8.85	**8.96**	**9.82**	**9.88**	**11.48**	
E	**8.85**	-					a
A	**8.96**	0.11^{ns}	-				a
B	**9.82**	0.97^{*}	0.86^{*}	-			b
D	**9.88**	1.03^{*}	0.92^{*}	0.05^{ns}	-		bc
C	**11.48**	2.63^{**}	2.52^{**}	1.66^{**}	1.61^{**}		d

From the data above, it can be concluded that treatment C (180 ppm) is the most effective dose in inhibiting the growth of *P. fluorescens* bacteria. Next, orthogonal polynomial regression was performed to determine the treatment between the given test parameters. The graph of the results of the Orthogonal Polynomial calculation is presented in Figure 3.

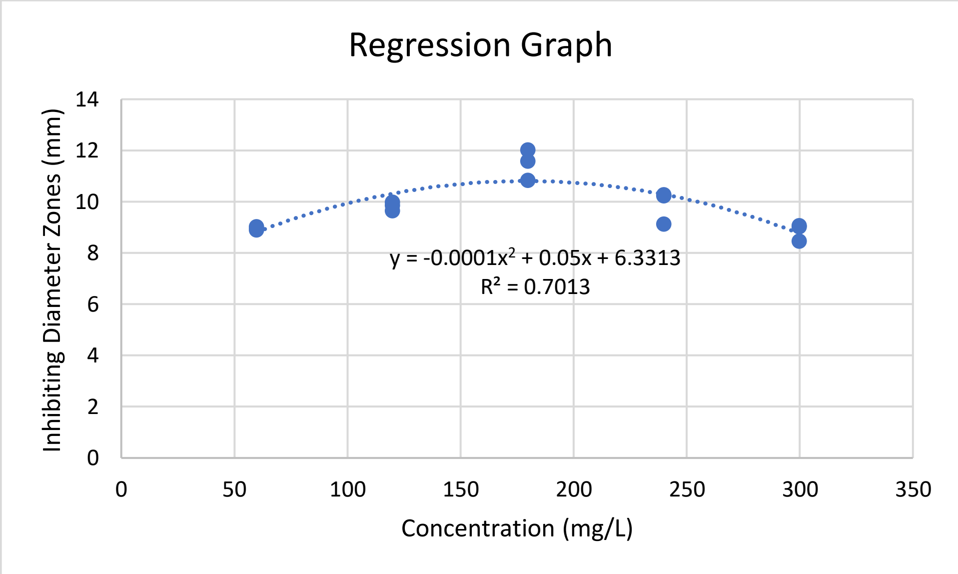

Figure 3. Antibacterial Activity of Jackfruit Leaves Crude Extract Against *P. fluorescens*

Based on the graph presented above, the results show that the relationship between the use of jackfruit (*A. heterophyllus*) leaf extract with different doses and the resulting inhibition zone shows a quadratic regression pattern with the equation $y = -0.0001x^2 + 0.05x + 6.327$ and coefficient $R^2 = 0.70$. The increase in the inhibition zone is not always proportional to the increase in the dose given.

DISCUSSION

Pseudomonas fluorescens is a Gram-negative environmental bacterium often studied as a key contributor to plant and soil health (Taylor *et al*. 2025). Pseudomonas bacteria are Gram-negative bacteria that appear pink after Gram staining because their cell walls have a thin peptidoglycan layer and an outer membrane containing lipopolysaccharide, making them unable to retain the crystal violet stain. Morphologically, Pseudomonas are bacilli (rod-shaped), motile with a single polar flagellum, and do not form spores. These bacteria are obligate aerobes, meaning they require oxygen for growth (Dash *et al*., 2022).

According to Putri *et al*. (2016), the diameter of the inhibition zone formed is not always proportional to the increase in the dose concentration administered. This is because at higher doses, the intermolecular bonds in the antibacterial compounds in the extract become stronger, resulting in larger molecules of the active compounds contained in the extract. This results in these molecules losing their ability to penetrate the pores of the agar medium used, thereby reducing the effectiveness of the active compounds in destroying bacterial cell membranes. Marfuati & Weni (2023) explain that the inhibitory effect can be attributed to the activity of flavonoid compounds present in jackfruit leaf extract (*Artocarpus heterophyllus*). Flavonoids are known to inhibit bacterial growth mechanisms such as nucleic acid synthesis inhibition and disruption of cell membrane function. The specific mechanisms include intercalation or hydrogen bonding between flavonoid rings and nucleic acid bases, disruption of cell membrane integrity, and inhibition of energy production and bacterial motility.

Based on phytochemical tests, jackfruit (*A. heterophyllus*) leaves contain active compounds, including flavonoids, saponins, tannins, and alkaloids. Auliah *et al*. (2019) stated that phytochemical tests on jackfruit (*A. heterophyllus*) leaves indicate that they contain flavonoids, saponins, and tannins, which can be used as antibacterials, antidiarrheals, antipyretics, antipyretics for fever, boils, skin diseases, and analgesics. Another opinion from Hudaya *et al*. (2024) say that Jackfruit leaf extract is known to contain various secondary metabolites such as flavonoids, saponins, tannins, alkaloids, phenols, and terpenoids. Jackfruit leaf can used as natural antioxidants, antimicrobial and anti-inflammatory properties, antiseptic, analgesic, antimicrobial, and antidiabetic activities, antioxidant and anticancer activities and anti-inflammatory effects and helps strengthen the immune system.

Armanda *et al*. (2017) stated that flavonoids can be used as antifungal and antibacterial agents. Flavonoids can also be used as antibacterial compounds due to their ability to interact with bacterial DNA by disrupting the hydrogen bonds in the double-stranded DNA. Flavonoids come into contact with DNA in the cell nucleus, and due to the difference in polarity between the lipids that make up DNA and the alcohol groups in the flavonoids, a reaction occurs, damaging the lipid structure of the DNA, leading to bacterial cell lysis and death. Other research, according to Azzahra & Trimulyono (2024), suggests that flavonoids can enhance the immune system by stimulating phagocytic cells as a cellular immune response. The hydroxyl groups in flavonoids interact with proteins in the bacterial cell membrane through hydrogen bonds, causing the proteins to lose their function.

Another study, according to Ernawati & Sari (2015), states that the antibacterial mechanism of saponins is by inhibiting bacterial cell wall permeability by forming a complex compound in the form of hydrogen bonds with the cell membrane. This can lead to bacterial cell death. Febriza & Kartika (2025) states that saponins can also lower cell surface tension, inhibit enzyme formation, and disrupt ion balance within cells. As a result, bacterial cells undergo lysis (rupture) and ultimately die.

Pendit *et al*. (2016) states that the antibacterial mechanism of tannins is by causing cell wall shrinkage, thereby disrupting bacterial cell permeability. Antibacterial activity is influenced by the polarity of the compounds extracted by each solvent, as well as their ability to disperse in the different media used in the antibacterial activity testing.

A supporting parameter in this study was the use of an incubator temperature of 32°C, which is suitable for the growth of P. fluorescens bacteria. This is in accordance with Trivedi *et al*. (2015) who stated that *P. fluorescens* bacteria are gram-negative bacteria that can grow optimally at temperatures below 32°C.

CONCLUSION

Based on the research results obtained, it can be concluded that the use of crude jackfruit (*A. heterophyllus*) leaf extract has an effect on *P. fluorescens* bacteria. The relationship between the use of crude jackfruit (*A. heterophyllus*) leaf extract with different doses and the resulting inhibition zone shows a quadratic regression pattern with the equation $y = -0.0001x^2 + 0.05x + 6.327$. The best dose was obtained in treatment C at 180 mg/L with an average inhibitory power of 11.48 mm.

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