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Antibacterial Power of the Patikan Kebo Plant (*Euphorbia hirta*) Against Leaf Blight Bacteria (*Xanthomonas oryzae*)

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Abstract: Bacterial leaf blight (BLB) is a significant disease of rice plants. It is caused by the Gram-negative bacterium *Xanthomonas oryzae* and often leads to yield losses. Excessive use of synthetic chemicals to control BLB has raised concerns over resistance and chemical residues. Therefore, natural alternatives are needed, such as *Euphorbia hirta*, which is traditionally used as a medicinal plant. This study aimed to determine the inhibitory power of *E. hirta* extracts against *X. oryzae* and to identify the presence of flavonoids, tannins, and saponins. Plant extracts were prepared by ethanol maceration, hot-water infusion (~90 °C), and fresh squeezing. Antibacterial activity was evaluated by the well diffusion method on Mueller-Hinton Agar with concentrations of 25–100% (v/v), using chloramphenicol as positive control. Inhibition zones were measured after 24–120 h. The results showed that all preparations inhibited *X. oryzae*, with ethanol extract producing the strongest activity (10.65–15.94 mm), followed by infusion (3.09–6.31 mm) and fresh-juice (1.50–4.85 mm), compared to chloramphenicol (21.40 mm). Phytochemical tests confirmed flavonoids, tannins, and saponins in all extracts. In conclusion, *E. hirta* demonstrates antibacterial potential against , with ethanol extract being the most effective.

Keywords: *Euphorbia hirta*; Inhibitory power; *Xanthomonas oryzae*

Introduction

Rice crop failure is often caused by bacterial leaf blight (BLB). This disease is caused by the Gram-negative bacteria, *Xanthomonas oryzae*. BLB attacks cause rice crop losses of 21 to 36% in the rainy season and 18-28% in the dry season (Sariasih et al., 2020; Laraswati et al., 2021). The *X. oryzae* bacteria infect rice plants through wounds or stomata and damage the chlorophyll in the leaves. This reduces the plant's ability to photosynthesize. This pathogen can infect rice plants at all growth stages, from seedling stage to near-harvest. If the attack occurs early in growth, the plant will wilt and die, a symptom called kresek by Indonesian farmers. The wilt or kresek symptom usually occurs when the rice plant is infected by the pathogen through the roots or base of the stem. Previously, Yuliani et al. (2017), Shaheen et al. (2019), and Jiang et al. (2020) have reported that, if the attack occurs during flowering, the grain filling process will not be perfect, so that the grains are not fully filled or even empty.

Various efforts have been made to control BLB disease in rice, such as that implemented in NTB Province, namely planting a new superior variety (VUB) called Inpari 32 BLB. Another effort is the use of synthetic pesticides such as the brands Agrept 20 WP, Bactocyn 150 AL and Plantomycin 7SP (Sarwani & Muhrizal, 2014). However, these methods, especially the use of synthetic chemicals have not provided satisfactory results because the symptoms of rice blight have not decreased and instead the diversity of *X. oryzae* has increased, which may be caused by gene mutations, which produce more resistant mutants (Yanti et al., 2018; Chukwu et al., 2019; Hidayati et al., 2022). Thus, excessive use of synthetic antibacterial materials can be considered as wasteful action besides having a negative impact on the environment. Controlling a pathogen that triggers resistance to the pathogen itself makes

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subsequent control more difficult to carry out (Larasaty et al., 2020; Carsono et al., 2021). Therefore, several control alternatives such as the use of botanical pesticides have begun to become an urgent study to produce materials and control methods that are selective, environmentally friendly and, if necessary, feasible for farmers.

Plants can produce chemical compounds that protect themselves from pathogens. These compounds are generally secondary metabolites, including alkaloids, flavonoids, phenols, saponins, tannins, steroids, and triterpenes. Of these compounds, some are anti-insect and some are anti-fungal, anti-bacterial and anti-viral. These anti-pest compounds can be selectively extracted from plant tissue and then used as natural pesticides against certain pests. Using natural pesticides will be safer than using pesticides from synthetic chemical compounds. This is because natural pesticides are easier and quicker to decompose after application and their pesticide performance is selective (Hussein & El-Anssary, 2018; Suripto et al., 2023a).

Amban Several studies have reported that *E. hirta* plant extract can provide quite good antibacterial effects, such as against *Porphyromonas gingivalis* with an inhibition zone diameter of 35.5 mm (Kono et al., 2018), against *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* with inhibition zone diameters of 15.83, 18.20, 16.56, and 19.54 mm, respectively (Risdayanti et al., 2020).

Several studies have also tested the antibacterial power of *E. hirta* plants against pathogenic bacteria that attack plants. Kusampudi et al. (2023) have reported that, methanol extract of *E. hirta* plant with a concentration of 1280 mg/L is able to inhibit 90% of the growth of *Ralstonia solanacearum* and *X. axonopodis* pv *vesicatoria* bacteria. Ambang et al. (2023) have also proven that the *E. hirta* plant has antibacterial properties against *Xanthomonas campestris*, *R. solanacearum*, and *Pseudomonas celebensis*.

There has been no research on the antibacterial power of the *E. hirta* plant against *X. oryzae*. In addition, in the above studies, antibacterial substances were extracted from the *E. hirta* plant using organic solvents that are currently not recommended because they are toxic or carcinogenic, such as methanol. Therefore, research on the antibacterial power of *E. hirta* plant material, which is extracted through different techniques, namely extraction with ethanol, direct squeezing and boiling with water against *X. oryzae* needs to be carried out. Based on the background of the problem above, this study was conducted with the aim of determining the antibacterial power of ethanol extract, juice and infusion of *E. hirta* plants on the growth of *X. oryzae* bacteria.

Method

Providing Anti-Bacterial Ingredients from E. hirta Plant

Samples of mature and healthy patikan kebo plants were taken from wild plant community land in Kerandangan Hamlet, Senggigi Village, West Lombok Regency. Identification of patikan kebo plants was carried out by observing plant habitus variables for the *Euphorbia hirta* species as has been done by Gautam et al. (2023). The habitus of the *E. hirta* plant used in this study can be seen in Figure 1.

Figure 1. Habitus of the patikan kebo plant (*E. hirta*). a. roots; b. stems; c. leaves; d. flowers

Antibacterial materials from the *E. hirta* plant were made using three different extraction techniques, namely direct squeezing, boiling with water and extraction using ethanol, so that each produces material in the form of juice, infusion and ethanol extract.

All parts of the adult plant were cut into small pieces and then air-dried without being exposed to direct sunlight. Drying without sunlight is important to prevent damage to the bioactive content. UV rays from the sun can damage bioactive compounds of plant origin, which are generally the result of secondary metabolism (Suripto et al., 2023a; Rahmawati et al., 2023). Small pieces of dried plant material are then ground and the dry powder (simplisia) from the plant material is ready to be processed for making extracts using ethanol as the sole solvent and for making infusions using heated water.

The *E. hirta* plant simplicia was extracted with ethanol using a maceration technique, which was modified from Rathour et al. (2017), Yanti et al. (2018), Abubakar & Mainul (2020), Ravikumar et al. (2022), and Suripto et al. (2023a). The simplicia is soaked in Beckerglass containing ethanol in a ratio of 1:5 (100 grams of simplicia/500 mL of ethanol), stirred every 6 hours for 24 hours. The mixture was filtered using a funnel lined with filter paper to produce a filtrate (500 mL of ethanol extract solution) which was collected into an Erlenmeyer bottle. The ethanol solvent was

evaporated from the filtrate using a rotary vacuum evaporator and the resulting viscous extract was then dried to a viscous consistency using a cup in an evaporation chamber. This dry ethanol extract was dissolved in 500 mL of water to produce a stock solution of 100% ethanol extract.

The Squeeze and infusion of *E. hirta* plants were made using techniques modified from Makalew et al. (2016) and Awad et al. (2021). To make the squeeze, 500 grams of fresh and clean *E. hirta* plants are squeezed using flannel paper to produce juice. This juice is the *E. hirta* plant squeeze stock with a concentration of 100%. The preparation of infusion of *E. hirta* plant is done by boiling 100 grams of the simplicia with 500 mL of water as a solvent heated to 90°C. The boiled water is filtered and the resulting filtrate (infusion) is not evaporated further, even if the volume decreases, add water until it reaches a volume of 500 mL and this is the stock infusion solution for a concentration level of 100%.

Each type of anti-bacterial material from the *E. hirta* plant, namely ethanol extract, infusion and squeezed water, was ready to be tested for its inhibitory power against *X. oryzae* bacteria with treatment concentrations of 0, 25, 50, 75, and 100% using water as diluent. However, before bioessaying, the anti-bacterial ingredients from *E. hirta* were examined for their bioactive content, especially compounds from the flavonoid, tannin and saponin groups using techniques adapted from Asha & Thirunavukkarasu (2015), Panche et al. (2016), Ningsih et al. (2017), Ghosh et al. (2019), Julianto (2019), Sariwating et al. (2022), Ravikumar et al. (2022), and Tripathi et al. (2022). The flavonoid test is carried out by taking 1 mL of the extract sample, then adding 0.1 grams of Mg powder and 5 drops of concentrated HCl. If the sample contains flavonoid compounds, a red or orange color will form. For the tannin test, 1 mL of the extract sample is added with 1 drop of 10% $FeCl_3$ reagent. The presence of tannin compounds is indicated by the formation of a dark blue or bluish green color. The presence of saponin compounds is tested by taking 1 mL of the extract sample, mixed with 5 mL of distilled water in a test tube, then shaken until foam forms. The solution is left for 2 minutes, then given 1 drop of 2 N HCl. The presence of saponin is indicated by the formation of a steady foam (the foam does not disappear even if HCl is added).

Preparation of Growth Media for X. oryzae Bacteria

X. oryzae bacteria were grown on Himedia Mueller Hinton Agar (MHA) media. The composition and preparation of the media was carried out according to the instructions on the label, adapted from Fioni et al. (2023) and Tidiane et al. (2024). 11.4 grams of Mueller Hinton Agar (MHA) powder was dissolved in 300 mL of distilled water using an Erlenmeyer. The media is stirred until homogeneous on a hot plate until it boils. The homogenized media was sterilized in an autoclave at a pressure of 1 atm, temperature 121℃ for 15 minutes. About 15-20 mL of sterile media is poured into a sterile petri dish and then left to stand until it solidifies.

Preparation of X. oryzae Bacterial Samples

The procurement of *X. oryzae* bacterial samples was carried out through the stages of isolate purity testing, rejuvenation, and bacterial suspension preparation. Testing the purity of bacterial isolates was carried out using Gram staining with positive criteria for *X. oryzae* which was indicated by the appearance of a red color and a uniform bacterial cell shape, namely all rod-shaped (bacilli) (Laraswati et al., 2021; Hidayati et al., 2022).

The pure isolate was rejuvenated using the streak plate method. One oasis of bacterial colonies was taken from the pure isolate stock, then streaked onto the surface of the MHA media, then incubated at 30^0C for 24 hours. The test *X. oryzae* bacterial suspension was made by taking 3 cycles of the rejuvenated culture, then suspending it in 5 mL of 0.9% NaCl solution and voxing until homogeneous. The results obtained were observed for turbidity and equated with a standard 0.5 McFarland solution (Hidayati et al., 2025).

Anti-Bacterial Power Testing

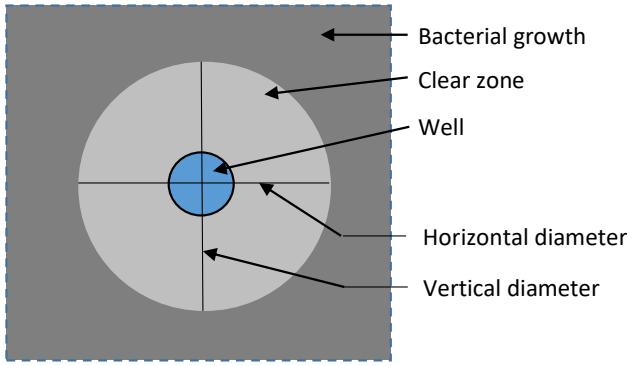

Figure 2. Scheme of observing the inhibition zone using the well method

Anti-bacterial activity testing was carried out using a well diffusion method adapted from Bassey & Okon (2021) and Hidayati et al. (2022, 2025). Take 100 µl of *X. oryzae* suspension using a micropipette and pour it into a petri dish containing MHA medium. The bacterial suspension was spread evenly on the surface of the media using a spreader, then dried for 15 minutes. Make wells with a diameter of 9 mm using a blue tip in the medium that has been inoculated with the test bacteria. Drop 100 µL of anti-bacterial material from the *E. hirta* plant at a predetermined concentration. As a comparison, 0.1% (v/v) antibiotic chloramphenicol was

also used as a positive control. Each of all treatments was carried out in 4 replications.

The growth medium for the test bacteria that had been treated was incubated at 300 °C for 5 days. The variable inhibitory power was observed by measuring the diameter of the clear zone formed during 5 x 24 hours of incubation. The observation scheme of the inhibition zone using the well diffusion method can be seen in Figure 2.

The quantity of inhibity power was determined based on the diameter of the inhibitory zone (D) and can be calculated using the following formula (Equation 1):

$$D = \frac{(V-W) + (H-W)}{2} \quad (1)$$

Description:
D = Diameter of inhibitory zone
H = Horizontal diameter
V = Vertical diameter
W = Diameter of well

Data Analysis

Data on the diameter of the inhibitory zone were analyzed descriptively to compare the inhibitory power between treatments of various *E Hirta* plant extracts according to variations in concentration and according to variations in incubation time.

In general, the work flow chart for the anti-bacterial power study can be seen in Figure 3.

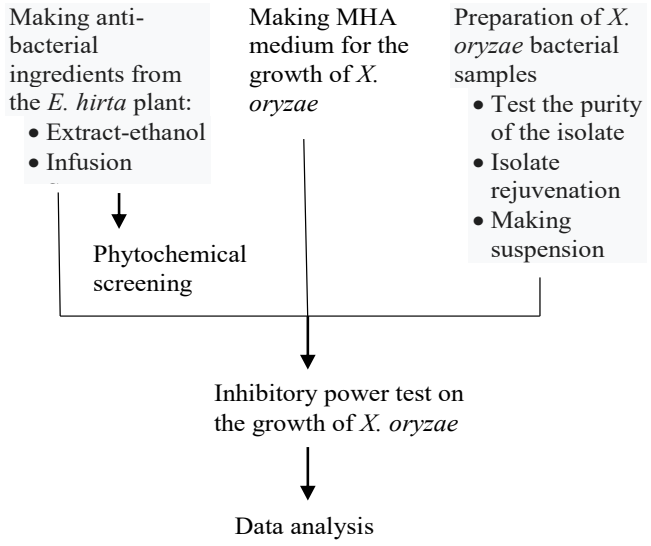

Figure 3. Work flow chart for testing the anti-bacterial power of *E. hirta* plants against *X. oryzae* bacteria

Results and Discussion

Inhibitory Power of E. hirta Plant Extracts on the Growth of X. oryzae Bacteria

The study showed that the ethanol extract had the highest inhibition against X. oryzae with with inhibitory zone diameter (IZD) of 9.78 to 15.94 mm, while the infusion and juice had smaller inhibition zones of of 2.12 to 6.31 mm and 0.98 to 4.85 mm (Table 1).

Table 1. Diameter of the growth inhibition zone of *X. oryzae* in various *E. hirta* plant extract treatments (average in mm from 4 replications)

Various extracts	C* (%)	Incubation period (hours)				
		24	48	72	96	120
Ethanol extract	25	10.65	11.54	11.79	10.58	9.78
	50	12.01	12.79	12.64	13.09	12.8
	75	13.06	13.94	13.45	13.54	13.91
	100	15.94	14.34	13.75	13.81	14.37
Infusion	25	3.09	2.77	2.42	2.16	2.12
	50	4.46	4.05	3.45	3.28	3.17
	75	5.85	5.33	5.05	4.67	4.43
	100	6.31	6.18	5.56	5.35	5.10
Squeezed water	25	1.50	1.39	1.13	1.05	0.98
	50	2.84	2.60	2.58	2.56	2.62
	75	3.80	3.50	3.49	3.37	3.47
	100	4.85	4.32	4.42	4.39	4.45
Control (+)	0.1	21.40	21.25	19.40	19.05	17.28

*Application concentration

Table 2. Results of phytochemical screening for anti-bacterial substances from *E. hirta* plant

E. hirta plant material	Compound + reagen	Reaction		Result
		Standard	Sample	
Extract-ethanol	Flavonoids + Mg + Concentrated HCl	Red, orange to yellow	Red	+
	Tannin + $FeCl_3$ 10%	Greenish blue to black	Greenish blue	+
	Saponin Shaken + HCl 2N	The foam remains stable	The foam remains stable	+
Squeze	Flavonoids + Mg + Concentrated HCl	Red, orange to yellow	Orange	+
	Tannin + $FeCl_3$ 10%	Greenish blue to black	Blue	+
	Saponin Shaken + HCl 2N	The foam remains stable	The foam remains stable	+
Infusion	Flavonoids + Mg + Concentrated HCl	Red, orange to yellow	Yellow	+
	Tannin + $FeCl_3$ 10%	Greenish blue to black	Black	+
	Saponin Shaken + HCl 2N	The foam remains stable	The foam remains stable	+

As a comparison or positive control, the use of 0.1% antibiotic chloramphenicol showed strong inhibition of the growth of *X. oryzae* with an inhibition zone diameter of 17.28 to 21.4 mm. The higher inhibition of the ethanol extract may be attributed to the presence of flavonoids,

tannins, and saponins identified in this extract. These three groups of compounds are actually also found in the infusions and juices, as shown by the results of phytochemical examination, but the amounts may be much smaller (Table 2).

Ethanol has a different polarity than water, although it is still considered a polar solvent. Compounds such as flavonoids and tannins are often semipolar, making them easier to extract with ethanol than with water. These three compounds may be obtained in greater quantities if they are extracted with semi-polar solvents such as dichloromethane (DCM). A solvent that has the same polarity as the compound to be extracted will be more effective in extracting the compound (Suripto et al., 2023a; Pulukadang et al., 2024; Ojha, 2025).

These results can confirm that the antibacterial ability of *E. hirta* plant extracts is caused by the presence of chemical compounds such as flavonoids, tannins and saponins. These three groups of compounds from other plant species, namely the horsewhip plant (*Stachytarpheta jamaicensis*), have also been reported to have inhibitory power against *X. oryzae* (Hidayati et al., 2025). Several reports have stated that the *E. hirta* plant contains flavonoids such as quercetin, quercitrin, quercitol and their derivatives such as rhamnose, quercetin rhamnoside, chlorophenolic acid, routine, leucocyanidin, myricitrin, cyaniding 3,5-diglucoside, camphol, flavonol, inositol, tetraxerol, β-sitosterol, and kaempferol (Ghosh et al., 2019; Cabrera-Contreras et al., 2020), classes of tannin compounds such as dimeric hydrolysis, dehydro, ellagic tannins, and terchebin, monomeric hydrolyzed tannins, geranin and benzyl gallate (Tripathi et al., 2022). The bioactive content obtained from extraction, the results are highly dependent on various processing parameters such as solvent polarity, species and developmental stage of the plant, plant parts used (roots, stems, leaves, roots), geographical origin, harvest time, type of storage, and drying method if using simplicial (Ravikumar et al., 2022; Tidiane et al., 2024; Monon et al., 2024; Ouédraogo et al., 2025).

These three groups of compounds have previously been shown to possess strong antibacterial properties (Hemdan et al., 2019; Puspitasari et al., 2022; Putri et al., 2023; Ojha, 2025). The ethanol extract is likely more effective in dissolving these compounds than water, which is used in infusions and direct juices. This strengthens the research results which state that in the well method, the osmolality of the extract solution resulting from extraction with organic solvents is higher, more thorough and more homogeneous so that it is stronger in inhibiting bacterial growth (Retnaningsih et al., 2019; Sariwating et al., 2022).

Extraction of the *E. hirta* plant using ethanol as the sole solvent seems to be able to attract higher anti-bacterial bioactive content against *X. oryzae* compared to extraction using water, such as infusion and squeezed water. Maceration method with ethanol was able to extract more active antibacterial compounds compared to squeezing and infusion methods. Plant bioactive compounds are generally the result of secondary metabolism, some of which are reserve compounds stored in vacuoles and some of which are part of the membrane structure in cells. These secondary metabolite compounds can be withdrawn from the cell if the vacuole or tonoplasmic membrane and membrane structures in the cell are destroyed and this is not enough just to grind the extracted plant tissue. Membrane lipid components do not decompose or dissolve completely when extraction only uses water and is not accompanied by the use of organic solvents. Thus, there are still few secondary metabolite compounds that can be pulled out of the cells if extracted only with water as in the infusion and squeezing methods (Noviyanty et al., 2019; Suripto et al., 2023a; Qoriasmadillah et al., 2024).

The squeezing process only extracts compounds that are easily soluble in water, which are limited in number compared to compounds that dissolve in organic solvents such as ethanol. The infusion method produces a larger zone of inhibition than squeezed, but still lower than ethanol extract. The use of hot water in the infusion method allows more active compounds, especially those that are soluble in water, to be extracted. However, active compounds that dissolve in water tend to be less than compounds that dissolve in ethanol. In addition, the heating process in infusion can cause some active compounds to experience degradation, thereby reducing their antibacterial activity compared to extraction using ethanol (Risfianty & Indrawati, 2020; Erinle et al., 2021; Miftahurrohma & Wahyuni, 2022; Yulinar & Suharti, 2022; Rivani et al., 2024).

The results of the anti-bacterial test from *E. hirta* plants as a whole showed that the higher the treatment concentration, the larger the diameter of the inhibition zone produced. The higher the concentration of the extract, the more active compounds contained in the solution, so the antibacterial effect is stronger (Simanungkalit et al., 2020; Dixit & Tiwari, 2022; Naseri et al., 2025). The results of this study also show that in general, including positive control treatment, that was use of the antibiotic chloramphenicol), the maximum diameter of the inhibition zone occurred after 24 hours of incubation and after that the inhibition zone became smaller as the time increased up to 120 hours of incubation (Figure 4).

The decrease in the diameter of the clear zone or inhibition zone as the incubation time increases is caused by the growth of test bacteria around the clear

zone and even spreading towards the inside of the clear zone. The antibacterial substance from the test plant did not appear to cause the death of the test bacteria, but only inhibited their growth, and when the inhibitory power of the antibacterial substance from the plant was lost due to biodegradation, the bacteria began to grow again rapidly. This fact shows that the anti-bacterial ingredients from *E. hirta* plant, the same as the antibiotic chloramphenicol, appear to be bacteriostatic against the *X. oryzae*. This strengthens the results of previous studies which stated that, anti-bacterial ingredients that are bacteriostatic do not kill bacteria but only inhibit their growth. In contrast to these bacteriostatic agents, bactericidal anti-bacterial agents have a lethal toxic effect, which is observed by the relentless increase in the area of the inhibition zone (Ramalho et al., 2018; Abarca et al., 2019; Kusampudi et al., 2023; Ravikumar et al., 2022).

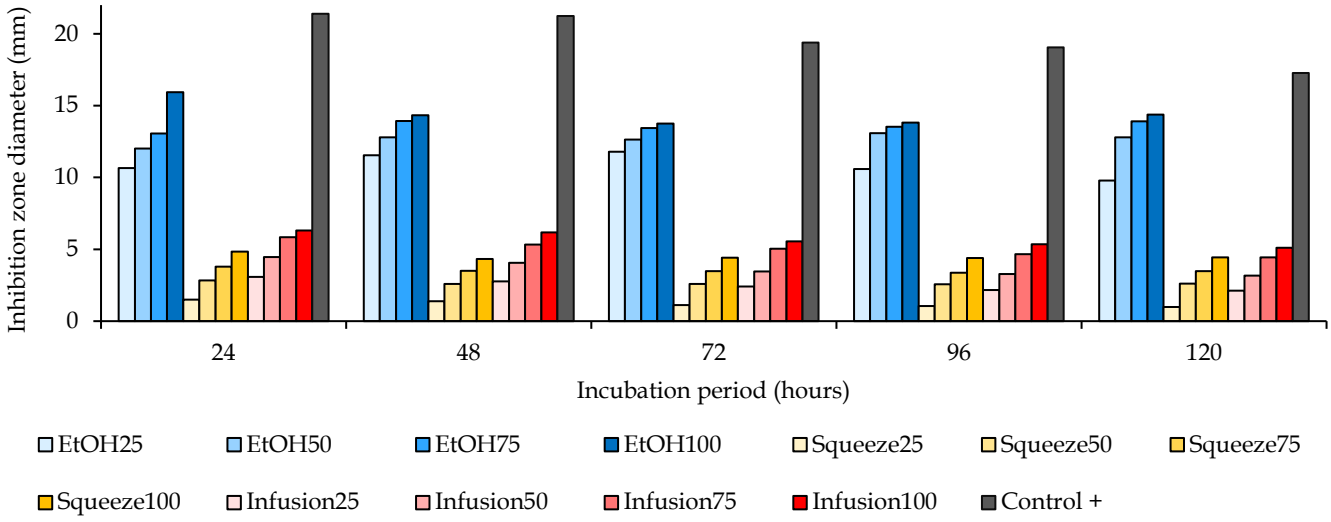

Figure 4. Diameter of the inhibition zone in the treatment of various extracts of *E. hirta* plant against *X. oryzae* bacteria during 5 x 24 hours of incubation (average in mm from 4 replications)

The decrease in antibacterial activity of the *E. hirta* plant mentioned above is likely due to the degradation of active compounds in the extract, in this case flavonoids, tannins and saponins during the observation period. Bioactive compounds, such as flavonoids, tannins, and saponins, degrade when exposed to the environment for several days, reducing their inhibitory power against bacterial growth. This mode of antibacterial action is often characteristic of bacteriostatic agents (Asysyuura et al., 2017).

The mode of action of antibacterial compounds from the flavonoid, tannin and saponin groups from the *E. hirta* plant against *X. orzae* bacteria may occur in the same way as bacteriostatic antibiotics, namely inhibiting protein and folic acid synthesis and disrupting DNA replication. Bacteriostatic agents such as Tetracycline and Erythromycin work by inhibiting protein synthesis in bacteria by binding to bacterial ribosomes, thereby inhibiting the production of proteins essential for bacterial growth. Bacteriostatic agents such as Sulfonamides work by inhibiting the synthesis of folic acid in bacteria, which is necessary for DNA and RNA synthesis. Bacteriostatic agents such as Quinolones (although some quinolones are bactericidal) can inhibit DNA replication in bacteria by inhibiting the enzymes required for DNA replication (Dwicahyani et al., 2018; Azzahra, & Trimulyono, 2024).

By inhibiting important processes in bacteria, bacteriostatic agents can slow or stop bacterial growth, thus giving the host's immune system a chance to fight the infection. Because the bacteriostatic material from the *E. hirta* plant does not kill the *X. oryzae* bacteria directly, as explained above, controlling this bacterial leaf blight disease requires cooperation with the host's immune system to eliminate the infection, in this case the use of rice cultivars that are superior in immunity. The method of controlling bacteria with bacteriostatic agents is similar to controlling pathogenic fungi, such as controlling rice stem rot disease, namely only inhibiting or stopping the growth of fungal hyphae (Akinbobola, 2022; Dayasagar et al., 2025).

E. hirta plant extracts may be bactericidal when extracted with organic solvents less polar than ethanol, such as dichloromethane. This solvent is semipolar and will be able to attract more flavonoids, tannins and saponins (which are a group of semipolar compounds). These three compounds are bacteriostatic at low concentrations, but can become bactericidal at higher concentrations (Suripto et al., 2023b; Marbun et al., 2024; Mashitah et al., 2024). With a high content of anti-bacterial compounds, the inhibition of protein and folic

acid synthesis and the disruption of DNA replication that occurs not only causes growth inhibition but also kills bacterial cells.

This is in accordance with previous research, which reported that dichloromethane extract of *E. Hirta* plants at lower concentrations was bacteriostatic, but at higher concentrations it was bactericidal against *X. campestris*. These differences may be caused by differences in bioactive components (variations in extraction techniques and application concentrations) and/or differences in species or even strains of target bacteria (Monon et al., 2024; Ouédraogo et al., 2025). Differences in the type of antibacterial effect, bacteriostatic or bactericidal, can also be caused by differences in the species or strains of target bacteria. The sensitivity and response factors of cells to antibacterial compounds vary between different bacterial species. Even differences in strains cause differences in responses, especially in enzyme production, production of various virulence factors, production of extracellular polysaccharides which play a role in biofilm formation (Liu et al., 2014; Ambang et al., 2023; Naqvi, 2019; Nugrahani et al., 2020; Rohmatin & Suparno, 2023).

The ethanol extract, infusion and juice of the *E. hirta* plant contain chemical compounds of the flavonoid, tannin and saponin group. Flavonoids as antibacterial compounds have three mechanisms of action, namely blocking nucleic acid synthesis, membrane function and energy metabolism. The formation of nucleic acids is inhibited through the accumulation of nucleic acid bases resulting in inhibition of the formation of DNA and RNA. Flavonoids produce complex compounds with extracellular proteins which are then dissolved, causing damage to bacterial cell membranes. Flavonoids also cause permeability disorders of cell walls, microsomes and lysosomes, due to the interaction of flavonoids with bacterial DNA. Inhibition of energy metabolism by flavonoids is carried out as an effort to inhibit bacterial respiration mechanisms. Energy inhibition affects the absorption of metabolites and the biosynthesis of bacterial macromolecules (Asha & Thirunavukkarasu, 2015; Ardiansyah et al., 2018; Panche et al., 2016; Donadio et al., 2021; Simanungkalit et al., 2020; Amaechi et al., 2024).

Tannin as an antibacterial will denature proteins in bacterial cells. Tannin will inhibit the reverse transcriptase and DNA topoisomerase enzymes, as a result bacterial cells cannot form. The antibacterial activity of tannins is related to its performance in inactivating the survival of microbial cells as well as inactivating enzymes and disrupting protein transport in the inner layers of cells. Tannins also target cell wall polypeptides, resulting in incomplete cell wall formation, which can trigger bacterial cells to lyse and die. Saponin acts as an antibacterial by reducing surface tension, resulting in increased cell permeability or leakage and causing intracellular compounds to come out. Saponins can cause severe damage to the tested bacteria through cell wall degradation followed by disruption of the cytoplasmic membrane and membrane proteins, resulting in leakage of cell contents (Anuzar et al., 2017; ; Sariwating et al., 2022; Dong et al., 2020).

Conclusion

This study aimed to evaluate the antibacterial activity of *E. hirta* ethanol extract, infusion, and juice against *X. oryzae*. All preparations were confirmed to contain flavonoids, tannins, and saponins and demonstrated inhibitory effects. Among them, the ethanol extract showed the strongest inhibition, followed by the infused and juice, indicating that ethanol is more effective in extracting antibacterial constituents. These findings highlight the potential of *E. hirta*, particularly the ethanol extract, as a natural alternative for controlling bacterial leaf blight.

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Author Contributions
Conceptualization, E.H., M.P., and S.; methodology, validation, formal analysis, E.H. and S.; software, writing—review and editing, visualization, S.; investigation, resources, data curation, writing—original draft preparation, project administration, M.P.; supervision, funding acquisition, E.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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