

Anti-Bacterial Power of The Pecut Kuda Plant (*Stachytarpheta jamaicensis* L.) Against Leaf Blight Bacteria (*Xanthomonas oryzae*)

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Abstract: Treating leaf blight in rice, which is caused by the bacterium *Xanthomonas oryzae*, using anti-bacterial agents from synthetic chemical compounds often causes environmental problems, so it is necessary to study the use of natural anti-bacterial agents. The pecut kuda plant (*Stachytarpheta jamaicensis*) has the potential to contain anti-bacterial properties because the leaves are often used by the public as a medicine to heal fresh wounds. This research aims to determine the anti-bacterial power of the *S. jamaicensis* plant against *X. oryzae* bacteria. *S. jamaicensis* leaves were extracted in stages using a series of successive solvents, namely hexane, DCM, and ethanol. Each fraction of *S. jamaicensis* leaf extract was tested for its inhibitory power against the growth of *X. oryzae* on MHA medium using the well method. The inhibitory variable observed was the diameter of the clear zone formed during 5 x 24 hours of incubation. The clear zone diameter data was analyzed to determine the inhibitory power. The results showed that each fraction of *S. jamaicensis* leaf extract had inhibitory power against *X. oryzae*. The *S. jamaicensis* plant can be developed as a source of natural anti-bacterial which are bacteriostatic, especially against *X. oryzae*.

Keywords: Inhibitory power, *Stachytarpheta jamaicensis*, *Xanthomonas oryzae*.

Introduction

Bacterial leaf blight (BLB) is a disease that commonly occurs in rice plants. This disease generally occurs during the rainy season or when the humidity is more than 75%, especially in rice fields which are always flooded with high N fertilization (Fatimah, & Prasetyono, 2020). BLB disease can reduce crop yields to varying degrees, depending on the growth stage of the infected plant, the sensitivity of the rice variety, and environmental conditions (Priamsari & Rokhana, 2020).

HDB disease is caused by the pathogenic bacteria *Xanthomonas oryzae* which can infect rice plants on leaves through leaf wounds or stomata and damage leaf chlorophyll. Efforts made to control bacterial leaf blight include

administering antibiotics. However, the use of anti-bacterial agents from synthetic chemicals often causes resistance so that BLB disease continues to occur (Majida & Nikmah, 2020).

Uncontrolled use of chemicals that exceed requirements can cause environmental pollution and have the potential to harm humans and other living creatures. Pesticides or any disease-fighting materials made from synthetic chemical compounds are generally stable so they are not easily degraded after application in the environment. Thus, these chemical residues accumulate in the food chain. To reduce dependence on the use of antibiotics or bactericides from synthetic chemicals in controlling bacterial leaf blight, it is necessary to study the use of bactericides from natural ingredients. Bactericides or pesticides made from natural ingredients,

whether from plants or animals, generally contain unstable bioactive chemicals, which are easily degraded so that they do not accumulate in the food chain after application in the environment (Suripto *et al.*, 2023).

The pecut kuda plant (*Stachytarpheta jamaicensis* L.), a species of weed plant, is often used by Indonesian people as an ingredient for healing fresh wounds and as an antiseptic agent (Pratiwi, 2021). Thus, it is reasonable to suspect that these plants contain chemicals resulting from secondary metabolism, which can inhibit the growth of pathogenic microorganisms. Secondary metabolites of plant origin that commonly have anti-microbial properties are compounds from the flavonoid, tannin and saponin groups (Illing *et al.*, 2021; Suripto *et al.*, 2023).

However, whether the bioactive content of the horsewhip plant has a significant inhibitory effect, especially against pathogenic bacteria, such as *X. oryzae*, has never been studied. Likewise, the content of bioactive compounds from *S. jamaicensis* plant that can inhibit bacterial growth, especially the *S. oryzae* bacteria, was not yet known. To obtain a selective antibacterial agent, which only inhibits the growth of pathogenic bacteria, such as *X. oryzae*, it is necessary to carry out extraction in stages using a series of solvents of increasing polarity. The resulting extract fractions can then each be tested for their inhibitory power against the growth of target bacteria. Based on the background of the problem above, this research was carried out with the aim of determining the inhibitory power of various fractions of *S. jamaicensis* plant leaf extracts on the growth of *X. oryzae*, a bacteria that causes bacterial leaf blight in rice plants.

Materials and Methods

Time and place

The research was carried out from June to August 2024. *S. jamaicensis* plant samples were collected from garden land in West Lombok. Extraction of *S. jamaicensis* leaves and the bioassay were carried out at the Biology Laboratory of Mataram University.

Experimental design

Experiments on the inhibitory power of each fraction of *S. jamaicensis* leaf extract against *X. oryzae* bacteria were carried out according to a completely randomized design. There are 4 concentration levels of the extract treatment and 1 concentration level of the positive control treatment as the independent variable. Each treatment was carried out in 3 repetitions. The parameter of bacterial growth inhibition as the dependent variable observed was the diameter of the clear zone formed during 5 x 24 hours of incubation.

Population and research sample

Samples of *S. jamaicensis* plants were taken from populations growing in lowland garden areas in West Lombok. The *X. oryzae* sample was purified from the *X. oryzae* isolate stock at Seedofplant Lumajang Indonesia.

Research procedures

The pecut kuda plant (*S. jamaicensis*) is a herbaceous plant that lives wild in gardens, rice fields, fields or on the side of the road. Leaves of the plants were collected from mature plants that have gone through the generative or flowering phase. The leaves are cut into small pieces then air-dried (without direct sunlight). According to Dharma *et al.* (2020); Suripto *et al.* (2023), drying leaves using direct sunlight can damage the bioactive ingredients they contain. These small pieces of dried leaves were then ground and the resulting dry leaf powder (simplicia) was ready to be extracted (Figure 1).



Figure 1. Habitus of the pecut kuda plant (*S. jamaicensis*)

Simplicia of *S. jamaicensis* leaf was extracted in stages using a modified maceration

technique from Handayani *et al.* (2020); Surtpto *et al.* (2023) used a series of solvents with increasing polarity, namely hexane, DCM and ethanol, respectively (Figure 2).

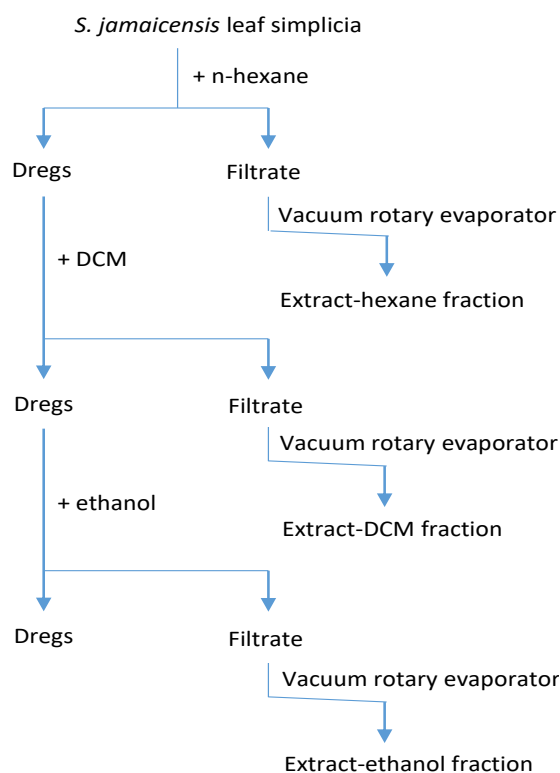


Figure 2. Work flow chart for multilevel extraction of *S. jamaicensis* leaves

S. jamaicensis leaf simplicia was soaked in a glass beaker containing hexane solvent in a ratio of 1:5 (gram/ml) while stirring at 6 hour intervals for 24 hours. The hexane extract solution was filtered using a funnel lined with filter paper and the resulting filtrate was collected in an Erlenmeyer bottle, while the dregs were air-dried and then extracted further in the same way using a solvent at the next extraction level, namely with DCM and then with ethanol. The solvent portion of the filtrate was evaporated using a vacuum rotary evaporator. The resulting thick extract was further compressed using a cup in the evaporation chamber.

Each fraction of *S. jamaicensis* leaf extract is ready to be tested for its inhibitory power by treatment at concentration levels of 0, 25, 50, 75 and 100% against the growth of *X. oryzae* bacteria, using 10% DMSO as a solvent and water as a diluent. DMSO functions to increase the solubility of the extract with water (Yuniwati

et al., 2022; Anisaningrum *et al.*, 2023). Before the bioassay, phytochemical screening was carried out on each fraction of *S. jamaicensis* leaf extract using a technique adapted from Nufus (2020); Hanifa *et al.* (2021); Illing *et al.* (2021) to confirm the presence or absence of antibacterial bioactive compounds from the flavonoid, tannin and saponin groups.

Before the bioassay, samples of *X. oryzae* bacteria and growth media were prepared, which included testing the purity of the isolate, rejuvenating and making a suspension of the test bacteria as well as making media for the growth of the test bacteria using a method adapted from Sariasih *et al.* (2020); Hidayati *et al.*, (2022); Ramadhani *et al.* (2023). Testing the purity of bacterial isolates was carried out by Gram staining, namely by using a standard Gram dye solution. Pure isolates are characterized by a red color and a uniform bacilli cell shape.

Pure isolates of the *X. oryzae* bacteria were rejuvenated by taking 1 oas of pure isolate from the stock, scraping it on a petri dish containing MHA media, then incubating for 24 hours at 30°C. Making test bacterial suspensions was done by taking 1-3 rejuvenated test bacterial oases, taken and transferred using an oase needle, then suspended in a tube containing 9 mL of NaCl solution and 0.9% sterile physiological salt solution, then vortexed until the turbidity is homogeneous.

Making Mueller Hinton Agar (MHA) media for the growth of *X. oryzae* bacteria was done by dissolving 15.2 grams of MHA media powder in 400 mL of distilled water on a hot plate, stirring it until it is homogeneous until it boils, then covering it tightly with aluminum foil. This media was sterilized using an autoclave at 121°C at 1 atm pressure for 15 minutes. 20 mL of sterile media was poured into each sterile petri dish and ready to be used in bioassays.

The anti-bacterial power of various fractions of *S. jamaicensis* leaf extract against *X. oryzae* bacteria was tested using the well method, which was modified from Henaulu & Kaihena (2020); Hidayati *et al.*, (2022); Anisaningrum *et al.* (2023). Take 100 µL of the *X. oryzae* bacterial suspension that has been prepared using a micropipette and pour it into a petri dish containing solid MHA medium. Spread the bacterial suspension evenly on the surface of the medium using a spreader and dry it by leaving it

at room temperature for 15 minutes. Make a 9 mm diameter well in the *X. oryzae* bacterial growth medium in a test petri dish using a blue tip. Fill the well with 100 µL of *S. jamaicensis* leaf extract solution using a micropipette according to the predetermined treatment concentration.

The concentration treatment for each extract fraction was 25, 25, 75 and 100% using 10% DMSO as a solvent and water as a diluent. In this study, the antibiotic chloramphenicol 250 mg was also used as a positive control treatment. Each treatment was carried out in 3 repetitions. Incubate the growth of the test bacteria at 30°C for 5 x 24 hours. The inhibitory variable observed was the diameter of the inhibition zone or clear zone which formed after 24, 48, 72, and 120 hours of incubation (Figure 3). The growth inhibition power of the test bacteria was assessed based on the diameter of the clear zone or commonly called the inhibition zone, which was formed during observation. The inhibitory power was calculated using the following formula (Equation 1):

$$ID = \frac{(VD - WD) + (HD - WD)}{2} \quad \text{----- (1)}$$

Where,

- ID = Inhibition zone diameter
- HD = Horizontal diameter
- VD = Vertical diameter
- WD = Well diameter

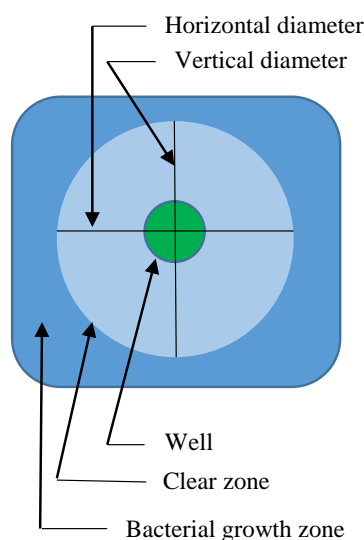


Figure 3. Schematic of observing the bacterial growth inhibition zone

Data analysis

The inhibitory power of each *S. jamaicensis* leaf extract fraction treatment on the growth of *X. oryzae* bacteria was determined quantitatively based on the diameter of the clear zone or inhibition zone as described above, which was formed during 5 x 24 hours of incubation. Inhibitory zone diameter data, expressed as inhibitory power data, were then analyzed descriptively to compare the antibacterial power between *S. jamaicensis* leaf extract fractions and their concentrations on the growth of *X. oryzae* during 5 x 24 hour incubation. Inhibitory power data between observation times, namely 24, 48, 72, 96, and 120 hours of incubation were also compared.

The results of descriptive analysis are comparisons between extract fractions, between concentrations and between observation times. Comparisons of the overall inhibitory power data were presented in the table. Meanwhile, visualizations of resistance comparison data which are the average of 3 repetitions were presented in the form of bar graphs. In general, the work flow chart for research on the antibacterial power of various *S. jamaicensis* leaf extract fractions on the growth of *X. oryzae* bacteria can be seen in Figure 4.

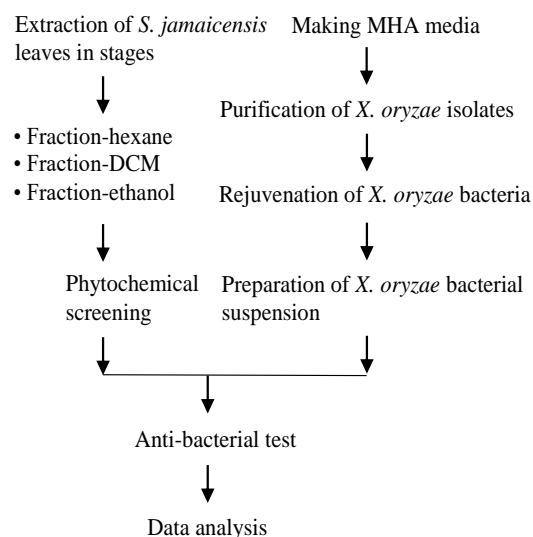


Figure 4. Flow chart of the inhibitory power test of various fractions of *S. jamaicensis* leaf extract on the growth of *X. oryzae* bacteria

Results and Discussion

Results

Based on the results of phytochemical screening, each fraction of the *S. jamaicensis* leaf extract was qualitatively confirmed to contain bioactive compounds from the flavonoid, tannin and saponin compound groups, generally known to have anti-bacterial properties. The characteristics of the content of these three groups of compounds from each fraction of *S. jamaicensis* leaf extract were in accordance with the respective standard compounds (Table 1).

Table 1. The results of phytochemical screening of various fractions of *S. jamaicensis* leaf extract

Compound	Reagent	Results		
		Standard	The extract	Inf.*
Flavonoids	1 mL extract + 0.1 mg Mg powder + 5 drops concentrated HCl	A red or orange color was formed	Forms a pink color	+
Tannin	1 mL extract + FeCl ₃ 10%	Forms a dark blue or bluish green color	Forms a dark blue color	+
Saponin	1 mL of extract was shaken + 2N HCl	A stable foam was formed, the foam did not disappear after dropping 2N HCl	Foam forms and did not disappear after dropping 2N HCl	+

* Information

Each *S. jamaicensis* leaf extract fraction with various concentration treatments had an inhibitory power in the weak category, namely an inhibitory zone diameter (D) of less than 5 mm against the growth of *X. oryzae* bacteria. The results also show that the higher the treatment concentration, which applies to all *S. jamaicensis*

leaf extract fractions, the larger the diameter of the inhibition zone produced, although it is still in the weak inhibition category. As a comparison or positive control, the antibiotic chloramphenicol 250 mg showed very strong inhibitory power, with the resulting inhibition zone diameter being 25 mm or more against the growth of *X. oryzae* bacteria (Table 2).

Table 2. Diameter of inhibition zone of *X. oryzae* growth during 120 hour incubation in various treatments of *S. jamaicensis* leaf extract fractions (average of 3 replicates in mm)

Extract fractions	C* (%)	Incubation time (hours)				
		24	48	72	96	120
Hexane	25	2.07	2.41	2.4	2.1	1.81
	50	2.53	2.51	2.35	2.05	1.89
	75	2.26	2.49	2.49	2.46	2.47
	100	3.32	3.2	2.67	2.54	2.82
DCM	25	2.52	2.36	2.29	2.21	2.18
	50	3.66	3.5	3.4	3.37	2.75
	75	4.35	4	3.47	3.45	2.93
	100	4.8	4.55	4.55	4.43	3.8
Ethanol	25	2.53	2.4	2.36	2.34	1.87
	50	3.61	3.42	2.75	2.6	2.29
	75	4.17	3.88	3.73	3.55	3.37
	100	4.46	4.38	4.35	4.21	3.3
Control +		25.98	24.57	23.9	23.69	23.5

* concentration

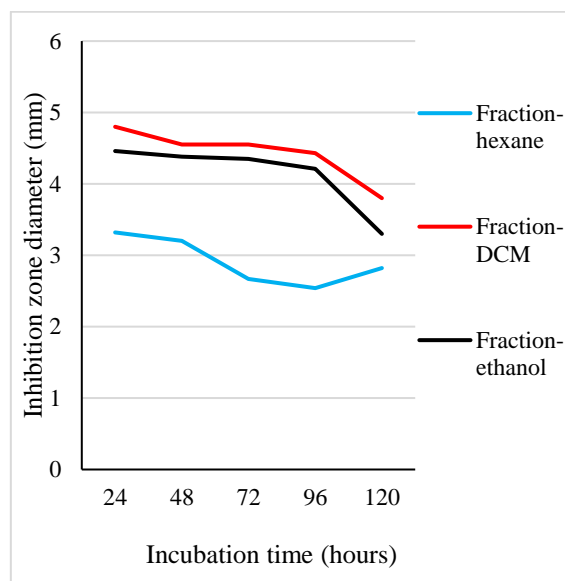


Figure 5. Diameter of the growth inhibition zone of *X. oryzae* during 5 x 24 hours of incubation when treated with various fractions of *S. jamaicensis* leaf extract.

The results of the negative control, namely using 0% extract but using DMSO as a solvent and water as a diluent, are not shown in the table because they did not produce an inhibition zone. The results also showed, that in general, the highest inhibitory power of *S. jamaicensis* leaf extract on the growth of *X. oryzae* was shown by the DCM-extract fraction, followed by lower inhibitory power respectively by the ethanol-extract fraction and the hexane-extract fraction. The maximum inhibition zone diameter was generally reached after 24 hours of incubation. Next, after 48, 72, 96 and 120 hours, the diameter of the inhibition zone became smaller (Figure 5).

Discussion

Phytochemical examination showing qualitative characteristics of the flavonoid, tannin and saponin content of horsehip plants was previously carried out by Illing *et al.* (2021). Similar qualitative characteristics for flavonoid, tannin and saponin content have also been observed in phytochemical examinations of other plant species (Handayani *et al.*, 2020; Madiabu *et al.*, 2023; Rizki & Suparno, 2023). Several reports have stated that compounds from the flavonoid, tannin and saponin groups of plant origin generally have anti-bacterial properties, with varying inhibitory power depending on the plant species used, the extraction method and the bacterial species being tested (Priamsari & Rokhana, 2020; Laraswati *et al.*, 2021; Pratiwi, 2021).

The weak bactericidal power of *S. jamaicensis* leaf extract against *X. oryzae* may be due to the fact that very little anti-bacterial content such as flavonoids, tannins and saponins can be extracted from *S. jamaicensis* leaf simplicia through maceration techniques. Extraction of bioactive materials using cold maceration techniques is generally weaker than hot maceration or soxhletation. Most of the active anti-bacterial content is still left in the dregs (the bioactive content is still stored in the cells in the unground leaf tissue), even though the extraction is carried out in stages (Wibowo *et al.*, 2020; Nahor *et al.*, 2022; Ramadhani *et al.*, 2023).

The weak anti-bacterial power of *S. jamaicensis* leaf extract on the growth of *X. oryzae* is not only due to the low content of anti-bacterial compounds which can be extracted through maceration techniques, as described

above, but also because *X. oryzae* is classified as a Gram negative bacterium which is resistant to contains anti-bacterial ingredients which are bacteriostatic. According to Sariasih *et al.* (2020); Rizki & Suparno (2023), *X. oryzae* bacteria are pathogenic microbes from the group of Gram-negative bacteria that are resistant to bioactive ingredients that are bacteriostatic. However, several reports have stated that bioactive ingredients from the *S. jamaicensis* plant can inhibit the growth of other types of Gram negative bacteria, such as *Streptococcus mutans* and *Mycobacterium smegmatis* with moderate to high inhibitory power (Pratiwi, 2021; Ramadhani *et al.*, 2023).

Inhibition of the growth of *X. oryzae* in the *S. jamaicensis* leaf extract treatment reached a maximum after 24 hours of incubation, which was indicated by the appearance of a round and clear zone. After that the clear zone became less clear, and even on the fifth day (120 hours) of incubation, including the control + treatment (using the antibiotic chloramphenicol), the clear zone became smaller. This may be caused by the growth of *X. oryzae* bacteria around the previously formed baning zone, which causes the size of the clear zone to decrease. Giving each fraction of *S. jamaicensis* leaf extract does not seem to kill the *X. oryzae* bacteria but only inhibits the growth of the bacteria. This fact shows that the anti-bacterial mode of action of *S. jamaicensis* leaf extract is more as a bacteriostatic agent than bactericidal. According to Majida & Nikmah (2020), bacteriostatic agents have an effect by inhibiting the growth of organisms but not killing them. Once the effects of the bacteriostat wear off, the bacteria usually begin to grow rapidly. This is different from bactericides which kill bacteria.

Conclusion

Various fractions of *S. jamaicensis* leaf extract, namely the extract fractions: -hexane, -DCM, and -ethanol, have been confirmed to contain anti-bacterial compounds from the flavonoid, tannin and saponin groups. Each of various extract fractions of *S. jamaicensis* leaf was proven to be bacteriostatic and showed weak inhibitory power against the *X. oryzae* bacteria. The highest inhibitory power was shown by the fraction-DCM, then the weaker inhibitory power

was shown by the fraction- ethanol and the fraction-hexane.

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