LenteraBio, 2025; Volume 14, Issue 2: 196-203

p-ISSN: 2252-3979 e-ISSN: 2685-7871
https://journal.unesa.ac.id/index.php/lenterabio/index

Antibacterial Activity of Pineapple Leaf Extract Against the Growth of *Ralstonia solanacearum*

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Article History:
Received:
10-August-2024
Revised:
5-May-2025
Available online:
14-May-2025
Published regularly:
31-May-2025

Abstract
Ralstonia solanacearum attacks plants from the Solanaceae family: potatoes, tomatoes, eggplants and chilies. Chemical control using synthetic pesticides which causes soil pollution and negative environmental impacts. Biology control methods have emerged as a safer and more sustainable alternative use compounds from pineapple leaves (*A. comosus* L. Merr). This research aims to determine the effect of pineapple leaf extract in inhibiting the growth of *R. solanacearum* bacteria and to determine the most optimal concentration of pineapple leaf extract in inhibiting the growth of *R. solanacearum* bacteria. The method used was well diffusion using a Completely Randomized Design (CRD) with extract concentrations used, namely 60%, 80%, 100%, a positive control of 0.01% Streptomycin sulfate and a negative control of 10% DMSO for 5 replications. Data analysis used one-way ANOVA and Duncan's test. The research results showed that pineapple leaf extract can inhibit the growth of *R. solanacearum* bacteria. Treatment with a concentration of 100% is the most optimal concentration in inhibiting the growth of *R. solanacearum*, with inhibition zone diameter of 11.4 ± 0.22 mm. Thus, pineapple leaf extract can contribute to the development of environmentally friendly strategies for managing bacterial wilt disease and reducing dependency on synthetic pesticides.

Keywords: Infection; inhibition zone; pesticide reduction.

How to Cite: Aqila NA, Asri MT, 2025. Antibacterial Activity of Pineapple Leaf Extract Against the Growth of *Ralstonia solanacearum*. LenteraBio; 14(2): 196-203

DOI: https://doi.org/10.26740/lenterabio.v14n2.p196-203

INTRODUCTION

Ralstonia solanacearum is a gram-negative bacterium that causes wilt, a devastating disease affecting various stem-bearing crops, particularly those in the Solanaceae family such as potatoes, tomatoes, eggplants and chilies (Choliq *et al*., 2020; Apriyadi and Liestiany, 2019). This pathogen enters plants primarily through wounds caused by agricultural tools, soil, nematodes, or water (Wenas *et al*., 2016), leading to severe wilting and ultimately plant death. The disease significantly impacts agricultural productivity and can result in substantial economic losses for farmers (Setiawan, 2019).

Chemical control using synthetic pesticides such as Stamycin 20 WP and Plantomycin 7 SP is still the predominant approach in managing bacterial wilt (Theresia *et al*., 2023). However, the long-term use of these chemicals poses serious challenges, including the development of bacterial resistance and negative environmental impacts due to residual toxicity affecting non-target organisms (Miller and Spoolman, 2014). In response to these challenges, biological control methods have emerged as a safer and more sustainable alternative. Recent studies have highlighted the potential of microbial and plant-based agents to inhibit pathogenic bacteria with reduced environmental risks (Lestari *et al*., 2023).

One promising natural material is pineapple leaf extract (*Ananas comosus* L. Merr), which has demonstrated antibacterial activity against *Shigella dysenteriae* and *Escherichia coli*, with an optimal inhibitory concentration is 500 mg/mL was observed using the agar diffusion method with perforation technique (Milanda *et al*., 2021). However, no previous research has investigated the effectiveness of pineapple leaf extract (*A. comosus* L. Merr) specially against *R. solanacearum*, leaving an important gap in the current research. Considering the extensive availability of pineapple leaves as agricultural waste in East Java (Setiawan *et al*., 2017), and their content of useful metabolite compounds-such as proteins, terpenoids, flavonoids, glycosides, alkaloids, saponins, and bromelain enzymes (Hartati *et al*., 2020; Sari and Zaini, 2022)- these compounds have potential as a natural antibacterial agent in crop protection.

This study was implemented under controlled laboratory conditions using the well diffusion method, with five treatments and five repetitions to determine the antibacterial activity of pineapple leaf extract against *R. solanacearum*. The objectives of this research are to determine the effect of honey pineapple leaf extract in inhibiting the growth of *R. solanacearum* bacteria and to identify the most optimal concentration of pineapple leaf extract (*A. comosus* L. *Merr)* to inhibit the growth of *R. solanacearum*. By addressing the gaps in previous studies, this research contributes to the development of environmentally friendly strategies for managing bacterial wilt disease and reducing dependency on synthetic pesticides.

MATERIALS AND METHODS

This research was conducted from January to May 2024. Testing the antibacterial activity was conducted in IDB Genetics and Molecular Laboratory, Department of Biology, Universitas Negeri Surabaya. Samples of this research were pineapple (*A. comosus* L. Merr) leaves of Queen variety collected from Balai Penyuluh Pertanian (BPP) in Tegalrejo village, Babadan, Ngancar District, Kediri Regency, East Java. Making pineapple leaf extract was carried out in the Basic Biology Laboratory, C10 Department of Biology, State University of Surabaya. Antibacterial activities of pineapple leaf extract (*A. comosus* L. Merr) were tested using a well diffusion method.

Making pineapple leaf extract was carried out by harvesting pineapple leaves, which were then cut into small pieces of ± 2 cm and dried. After dried, pineapple leaves were crushed into simplicia powder. 500 grams of pineapple leaf simplicia were macerated using 96% ethanol solvent for 2 times 18 hours (Erturk *et al*., 2018) with ratios of 1:10 and 1:5 (Yasacaxena *et al*., 2023). In the process of making the extract, it produced the filtrate, which was then evaporated using a rotary evaporator at 50°C (Milanda *et al*., 2021) to produce a thick extract.

The Completely Randomized Design (CRD) method, with 5 replications and 5 treatments, was used in this research. The treatments were comprised of pineapple leaf extract (*A. comosus* L. Merr) at concentrations of 60%, 80%, and 100%, positive control of streptomycin sulfate 0.01%, and negative control of DMSO 10%.

The NA media was made by dissolving 20 grams of NA powder in 1.000 ml of distilled water (Samsuar *et al*., 2021). The NB media was made by dissolving 8 grams of NB powder with 1.000 ml of distilled water (Rosmania and Yuniar, 2021). The media was homogenized using a stirrer, heated on a hot plate, and then sterilized in an autoclave at 121°C at 1 atm pressure for 15 minutes (Rezi *et al*., 2014; Tille, 2017).

The suspension of *R. solanacearum* bacteria was made using 1 ose needle of rejuvenated bacteria on NA media into a test tube containing 5 ml of NB media and incubated at 30°C for a day. 1 ml suspension of bacteria was diluted using sterile 9 mL of sterile 0.9% NaCl (sodium chloride) solution. The turbidity was adjusted using a uv-vis spectrophotometer at a wavelength 600 nm until matched the 0.5 McFarland I standard, with an absorbance value of 0.1 or approximately 2×10^8 CFU/ml (Nuria, 2010; Chávez *et al*., 2012).

The well diffusion method was used for antibacterial activity. One ml (100 µL) suspension of *R. solanacearum* bacteria was poured into 20 ml of molten NA media. Then, it was homogenized until it solidified. After the NA media was solidified, three wells were made in the petri dish using a 6 mm cork borer. In each petri dish,the wells were filled with 50 µL of extract solution (60%, 80%, and 100%), positive control streptomycin sulfate 0.01% (Yudha and Ngadiani, 2018), and negative control DMSO 10% (Katrin *et al*., 2015) using a micropipette. Then, they were incubate for one day (24 hours) at 30°C. Inhibition zone observation was carried out after 24 hours, and measurement was conducted using a ruler (mm). Inhibition zone (clear zone) measurement refers to Formula 1 (Toy *et al*., 2015).

$$D = \frac{(D1-D3) + (D2-D1)}{2} \quad (1)$$

Description: D: average diameter of inhibition zone; D1: vertical diameter (mm); D2: horizontal diameter (mm); D3: well diameter (mm).

Data from the results of the research were analyzed using a normality test to determine whether the data were normally distributed. Then, a one-way analysis of variance (ANOVA) was followed to determine the effect of treatment. Duncan test was also carried out to observe the differences in the effect of each treatment.

RESULTS

The bacteria used in testing the antibacterial activity of pineapple (*A. comosus* L. Mer) leaves were *R. solanacearum* bacteria. The results of testing antibacterial activities were in the form of inhibition zone measured using a ruler (mm). The results of testing antibacterial activities proved that there was a significant difference in treatment of extract concentration, positive control, and negative control. The results of the research showed that the most optimum concentration in inhibiting the growth of *R. solanacearum* bacteria was a concentration of 100% pineapple leaf extract because a concentration of 100% was able to form the largest inhibition zone compared to other concentrations, with the results of the inhibiton zone diameter of 11.22 ± 0.22 mm (Table 1).

Positive control treatment (streptomycin sulfate antibiotic) resulted in the largest diameter of inhibition zone of 12.88 ± 0.36 mm. A concentration of 100% resulted in the largest average inhibition zone diameter than other concentrations of 11.4 ± 0.22 mm, followed by a concentration of 80% of 5.4 ± 0.41 mm, and a concentration of 60% of 4.7 ± 0.75 mm. Meanwhile, for negative control (DMSO 10%) there was no antibacterial activity that inhibited the growth of *R. solanacearum* bacteria (Table 1).

The percentage of inhibition zone diameter for 60% concentration was 36.49%, 80% concentration was 41.92%, 100% concentration was 88.50%, positive control (streptomycin sulfate) was 100%, and negative control (DMSO 10%) did not produce a percentage of inhibition (0%). This showed that the positive control had a higher success rate in inhibiting the growth of *R. solanacearum* bacteria, but the success of pineapple leaf extract treatment with various concentrations could also inhibit the growth of *R. solanacearum* bacteria.

Based on the test results, it was shown that the antibacterial activity of pineapple leaf extract had an effect on the growth of *R. solancearum* bacteria on Nutrient Agar (NA) media, which was indicated by the presence of an inhibition zone (clear zone) that appeared around the well holes (Figure 1). The Duncan test results showed that the treatment of pineapple leaf extract at a concentration of 60%, 80%, 100%, positive control streptomycin sulfate 0.01% and negative control DMSO 10% was significantly different. This showed that pineapple leaf extract affected the growth of *R. solanacearum* bacteria.

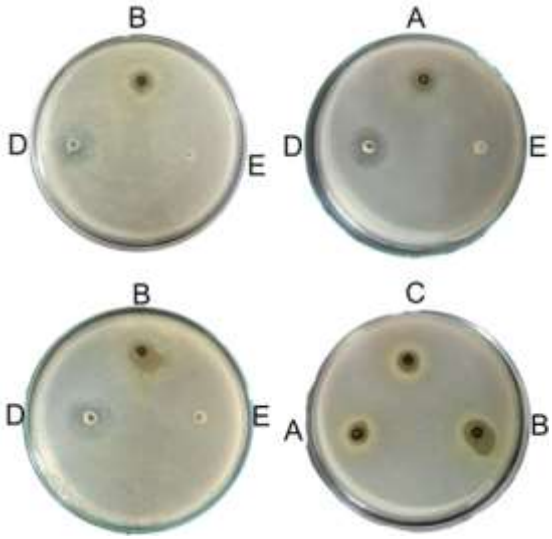

Figure 1. Antibacterial activity test results of pineapple leaf extract against *Ralstonia solanacearum*: (A) concentration 60%, (B) concentration 80%, (C) concentration 100%, (D) positive control Streptomycin sulfate, (E) negative control DMSO 10%.

Table 1. Antibacterial activity of pineapple leaf extract against *R. solanacearum*

Treatments	Diameter *Clear Zone* (mm)*	Percentage of Inhibition Zone Diameter (%)
Negatif control (DMSO 10%)	0.00 ± 0.00[a]	0%
Extract concentrations 60%	4.70 ± 0,75[b]	36.49%
Extract concentrations 80%	5.40 ± 0.41[c]	41.92%
Extract concentrations 100%	11.4 ± 0.22[d]	88.50%
Positif control (streptomycin sulfat) 0.01%	12.88 ± 0.36[e]	100%

Notes: Different notations a, b, c, d and e indicate significant differences based on Duncan's test with a significance level of α = 0.05.

DISCUSSION

Testing the antibacterial activity of pineapple leaf extract on the growth of *R. solanacearum* bacteria used a well diffusion method. A well diffusion method was chosen because this method is more effective than using a disc to test antibacterial effectiveness (Haryati *et al*., 2017), the application of this method is easier and more practical (Nadi *et al*., 2020), and measuring the diameter of the clear zone can be carried out more easily because bacteria can grow to the bottom of the growth media, not only on surface (Retnaningsih *et al*., 2019). The well method is more recommended than the use of test media because when the extract enters the well hole, an osmolarity phenomenon occurs. This phenomenon occurs when the concentration of extract is high in the well hole compared to the test media so that osmolarity occurs uniformly, is more consistent and is more effective in inhibiting the growth of the test bacteria (Hoque and Ratilla, 2011).

Negative control in this research used 10% DMSO. The results of the test with negative control DMSO 10% did not show the presence clear zone and antibacterial activity. Negative control DMSO 10% is used to compare the diameter of bacteria's inhibition zone and to see the effect of the solution used on antibacterial activity (Zahra, 2021). DMSO 10% does not form a clear zone around the well, indicating no antibacterial activity (Andriyawan, 2015). The reason for using DMSO 10% is because DMSO solvent can dissolve almost all non-polar and all polar compounds (Rahmi and Putri, 2020).

This study used a positive control of streptomycin sulfate as an antibiotic. Streptomycin sulfate is an aminoglycoside antibiotic used as an effective antibacterial for gram-negative bacteria (Diyasti and Lizarmi, 2021). Fajarusshidiq *et al*., (2020) research shows that *R. solanacearum* is a gram-negative bacteria, so it is to use suitable to use in testing *R. solanacearum* bacteria. The antibiotic streptomycin sulfate was found to be ideal for this research. Streptomycin sulfate inhibited *R. solanacearum* bacteria with an average clear zone diameter of 21.30 ± 3.17 mm (Nabilla and Asri, 2021). Streptomycin is bactericidal antibiotic that acts by irreversibly binding to bacterial ribosomes and inhibiting protein synthesis (Raini, 2015).

This study pineapple leaf extract at concentration of 60%, 80%, and 100%, which indicated the antibacterial activities of *R. solanacearum* in the form of a clear zone around the well. Antibacterial compounds in pineapple leaves are alkaloids, flavonoids, tannins (Sahu *et al*., 2020), terpenoids, phenols, steroids and saponins (Raphael and Thomas, 2019).

According to Septiani *et al*. (2017), the mechanism action of inhibiting bacterial growth is by changing cell permeability, inhibiting enzyme activity, changing protein and nucleic acid molecules, damaging cell walls, and inhibiting the synthesis of nucleic acids and protein in bacteria. Phenolic compounds disrupt the permeability of the cytoplasmic membrane in bacteria. Moreover, they also can denature and inactivate proteins (Indarto *et al*., 2019). Alkaloids act as antibacterials by damaging peptidoglycan components of bacterial cells, causing damage to the cell wall and causing cell death (Darsana *et al*., 2012). Alkaloids has a role as a compound or molecule that can insert between DNA bases and is able to inhibit cell topoisomerase enzymes, which cause cahnges in amino acid structure and genetic balance in the bacterial DNA chains (Purbaya *et al*., 2018). Alkaloid compounds contain nitrogen that can interact with amino acid compounds that make up bacterial DNA. This interaction caused changes the structure and composition of amino acids, which changed the genetic balance in the DNA chain, causing damage and encouraging the lysis process so that the bacteria would die (Rinawati, 2010).

Flavonoids act as antibacterials by forming complex compounds against extracellular proteins. This causes bacterial cell proteins to denature, causes damage to cell membranes, and is followed by the release of intracellular compounds (Amalia *et al*., 2018). The mechanism of flavonoids act as antibacterials is divided into 3: disrupting cell membrane function, influencing energy metabolism, and inhibiting nucleic acid synthesis. The formation of nucleic acids is restrained by the accumulation of nucleic acid bases, resulting in inhibition of DNA and RNA formation (Cushnie and Lamb, 2005). The mechanism flavonoids inhibit cell membrane function involves producing complex compounds dissolved in extracellular proteins, leading to the release of intracellular components from bacteria (Nuria *et al*., 2009). Furthermore, flavonoids can inhibit energy metabolism occurring in the cytoplasm of bacterial cells and also inhibit bacterial motility (Hartini and Mursyida, 2019).

By changing the bacterial cell membrane, steroids function as antibacterials (Monalisa *et al*., 2011). By interacting with the phospholipid in the cell membrane, steroids cause changes in its morphology and compromise of integrity (Zulita, 2018). The sensitivity of bacteria to steroid components causes the destruction of liposomes (Ningsih *et al*., 2018). The mechanism of tannins as antibacterials is by inhibiting cell wall synthesis, resulting in a less perfect cell wall (Purwanto and

Irianto, 2022). Mechanism tannin compounds have to denature proteins and coagulate proteins (Wiartini, 2023). Antibacterial effect of tannin by disrupting protein transport in the inner layer of cells, deactivates enzymes, disrupts polypeptides in the cell wall, and prevents the development of cell walls. This causes bacterial cells to start lysis due to osmotic and physical pressure (Rijayanti, 2014). Furthermore, tannins can inhibit reverse transcriptase and DNA topoisomerase enzymes, preventing bacterial DNA replication (Purwantiningsih and Surnindyah, 2014).

The mechanism of phenol compounds in destroying bacterial cell walls incorporates breaking the cross-linking of bacterial peptidoglycan (Lingga *et al*., 2016). Phenolic compounds can cause damage to the cytoplasmic membrane and denature proteins, this results in damage to the cell wall due to decreased permeability of the cytoplasmic membrane. Changes in the permeability of cytoplasmic membranes can disrupt important organic ions in bacterial cells, which results in the inhibition of cell growth and cell death (Purwantiningsih and Surnindyah, 2014).

Bromelain enzyme destroys peptidoglycan in bacterial membranes and denatures proteins, resulting in bacteria lysing (Liliany *et al*., 2018). Bromelain enzyme damages bacterial cytoplasm by preventing DNA topoisomerase and reverse transcriptase enzymes, additionally disrupting enzyme function and protein transport processes in bacterial cells (Nurnaningsih and Laela, 2022).

Based on the results of the Duncan's test, significant difference were observed between the negative control, positive control, and pineapple leaf extract concentration treatments. Duncan's test also indicated that the antibiotic streptomycin sulfate was more effective than the extract treatment. However, the antibacterial activity resulting from the pineapple leaf extract concentration treatment still inhibit the growth of *R. solanacearum* bacteria. Therefore, it can be concluded that pineapple leaf extract (*A. comosus* L. Merr) is able to inhibit the growth of *R. solanacearum* bacteria.

Data analysis produces groupings based on categories of the inhibition zone of an antibacterial substance/compound, indicating that the treatment with 100% pineapple leaf extract concentration falls is into the strong category, as the response to bacterial growth inhibition zone is 11.4 mm in diameter. The 80% concentration produces a clear zone diameter (inhibition zone) of 5.4 mm, placing in the medium category, while 60% concentration results in a clear zone diameter (inhibition zone) of 4.7 mm, categorizing is as weak. The inhibition zone test aims to demonstrate that a particular compound possesses antibacterial properties (Sartika *et al*., 2013). The antibacterial inhibition zone category is divided into four: very strong, strong, moderate and weak. Antibacterial activity is considered weak when the inhibition zone is less than 5 mm in diameter, moderate between 5-10 mm, strong between 10-20 mm, and more than 20 mm is classified as very strong (Davis and Stout, 2009). According to Milanda *et al*. (2021), the ethanol extract of pineapple skin leaves at a concentration of 500 mg/mL against Shigella dysenteriae produced an inhibition zone of 16.8 mm, while the same concentration against Escherichia coli resulted in an inhibition zone of 19.6 mm. These research results indicate that the antibacterial activity falls into the strong category. Pineapple leaf extract produced a clear zone in antibacterial activity testing, suggesting that the compounds in pineapple leaf extract may work synergistically to inhibit the growth of *R. solanacearum* bacteria. The combination of these compounds can provide better results in inhibiting the growth of bacteria because these compounds can work synergistically to disrupt various important biological processes of bacteria (Tilarso *et al*., 2021; Zaini *et al*., 2024; Dewatisari and Hariyadi, 2024). This research is an alternative innovation of plant bactericides as an effort to eliminate plant wilt caused by *R. solanacearum*, bacteria so it can replace the role of chemical pesticides that have negative effects.

CONCLUSION

Pineapple (*A. comosus* L. Merr) leaf extract has effects on the growth of *R. solanacearum* bacteria. The most optimal concentration of pineapple (*A. comosus* L. Merr) leaf extract in inhibiting the growth of *R. solanacearum* bacteria is a concentration of 100% with a diameter of the clear zone in the media of 11.4 ± 0.22 mm. Meanwhile, positive control of streptomycin sulfate results in a clear zone (inhibition zone) of 12.88 ± 0.36 mm. These results imply that pineapple leaf extract has potential as a natural antibacterial agent against *R. solanacearum*.

ACKNOWLEDGEMENTS

The author would like to thank Balai Penyuluhan Pertanian (BPP) Ngancar District, Kediri Regency and Brawijaya University for providing research materials.

CONFLICT OF INTEREST

There is no conflict of interest.

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