Development of Nano-Biopesticides from Mature Mango Leaf and Rambutan Peel Extracts for the Inhibition of *Xanthomonas* sp., the Causal Agent of Citrus Canker in 'Tubtim Siam' Pomelo.

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Abstract

'Tubtim Siam' pomelo is a Geographical Indication (GI) product and a major export commodity of Nakhon Si Thammarat Province. Growers currently face citrus canker caused by *Xanthomonas axonopodis*, which produces lesions on the fruit surface and reduces market value. The current control strategies rely on repeated applications of copper-based compounds, leading to pathogen resistance and chemical residue accumulation. This study compared the antibacterial activity of mature mango leaf extract, rambutan peel extract, their combination, and silver nanoparticles synthesized from the most effective extract against *Xanthomonas* sp. Antibacterial activity was evaluated using the disc diffusion method, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Rambutan peel extract at 50 mg/ml exhibited the highest antibacterial activity, with a statistically significant difference at the 0.05 level, producing an inhibition zone of 11.76 ± 0.02 mm. The MIC and MBC values were 6.25 mg/ml and >50 mg/ml, respectively. The combination of mature mango leaf and rambutan peel extracts at a 2:1 ratio showed the highest antibacterial activity among the combinations, producing an inhibition zone of 6.18 ± 0.02 mm ($p < 0.05$). Biogenic silver nanoparticles synthesized from rambutan peel extract exhibited the strongest antibacterial activity, producing an inhibition zone of 15.70 ± 0.02 mm. The MIC decreased to 0.3125 mg/ml, while the MBC was >20 mg/ml. These findings suggest that plant-derived biogenic silver nanoparticles provide an environmentally friendly alternative to copper-based compounds for controlling citrus canker in pomelo orchards.

Keywords: Canker disease**,** Mangiferin, Phenolic compounds, Silver Nanoparticles

Introduction

Tubtim Siam pomelo (Geographical Indication: GI) is a distinctive economic fruit of Pak Phanang District, Nakhon Si Thammarat Province. It currently experiences high demand in both domestic and international markets. However, farmers face a critical challenge from citrus canker disease, a severe disease affecting citrus crops globally, caused by the Gram-negative bacterium *Xanthomonas* sp. This pathogen can infect all parts of the plant, primarily affecting the above-ground parts. Outbreaks typically occur during periods of new leaf flush and fruit development. The pathogen enters plant tissues through natural openings, such as stomata and cuticle fissures, as well as wounds caused by pests like leaf miners. The bacteria multiply within intercellular spaces, leading to the formation of hard, brown cankerous lesions on the fruit peel and leaves. This notably causes lesions on the fruit peel, which degrades produce quality and market acceptability, reduces photosynthetic efficiency, and causes premature fruit drop, posing a major barrier to domestic and international trade. In severe outbreaks, the infection can cause stunted growth or plant death.

Currently, disease control primarily relies on disrupting the pathogen's life cycle. This is done through the eradication of infected plant parts and the continuous application of copper compounds, such as copper oxychloride and cupric hydroxide (Kanchanaburi Provincial Agricultural Extension Office, 2024). However, research by Marco and Stall (1983) confirmed that prolonged chemical usage has been shown to induce pathogen resistance to copper ions, significantly reducing the control effectiveness of these compounds. It also leads to the accumulation of chemical residues in soil and water, negatively impacting the ecosystem.

To address these challenges, exploring alternatives through plant extracts focuses on antimicrobial phenolic compounds with specific phytochemistry and biological mechanisms. For the mechanism of rambutan peel, tannins, specifically ellagitannins, can disrupt the stability of bacterial cell membranes and bind to the bacterial DNA gyrase enzyme, halting the replication process of genetic material. For the mechanism of mango leaves, mangiferin, a xanthone glycoside, disrupts the phospholipid bilayer structure, causing leakage of intracellular components, and inhibits ribosomal function in synthesizing essential proteins for survival. Furthermore, integrating these with nanoparticles presents a powerful approach; silver nanoparticles (AgNPs), ranging from 1-100 nanometers in size, exhibit high reactivity with their environment due to a massive surface-area-to-volume ratio. AgNPs possess bactericidal properties against both Gram-positive and Gram-negative bacteria by disrupting the cell membrane and penetrating bacterial structures.

Therefore, this research aims to investigate the performance of extracts from mature mango leaves and rambutan peels to develop a bio-nanotechnology foliar spray for inhibiting *Xanthomonas* sp. This study seeks to mitigate disease outbreaks and reduce reliance on synthetic chemicals, while enhancing crop quality and promoting environmentally friendly agriculture. The specific research objectives are:

1. To compare the inhibitory activity of mature mango leaf and rambutan peel extracts against the canker-causing bacterium in Tubtim Siam pomelo.

2. To develop the most effective plant extract into a bio-nanotechnology agent for the inhibition of the canker pathogen in Tubtim Siam pomelo.

Methodology

1. Preparation of Crude Extracts Using Solvent Extraction

Mature mango leaves and fresh rambutan peels were thoroughly washed, chopped into small pieces, and dried in an oven at 60°C for 48 hours. The dried materials were then finely ground into powder and macerated in 90% ethanol at a ratio of 1:10 (w/v) for 3 days. The resulting solutions were filtered, and the solvent was subsequently removed using a rotary evaporator to obtain the crude extracts.

2. Isolation and Purification of Bacterial Culture

Xanthomonas sp. was isolated from infected pomelo fruits using the streak plate method on Nutrient Agar (NA) and incubated at 28–30°C for 48 hours. Pure bacterial suspensions were prepared (4 replicates per isolate) and adjusted to match the 0.5 McFarland standard (1.5×10^8 CFU/mL) prior to testing.

2.1 Gram Staining: Performed to examine the Gram reaction, cellular morphology, and bacterial arrangement.

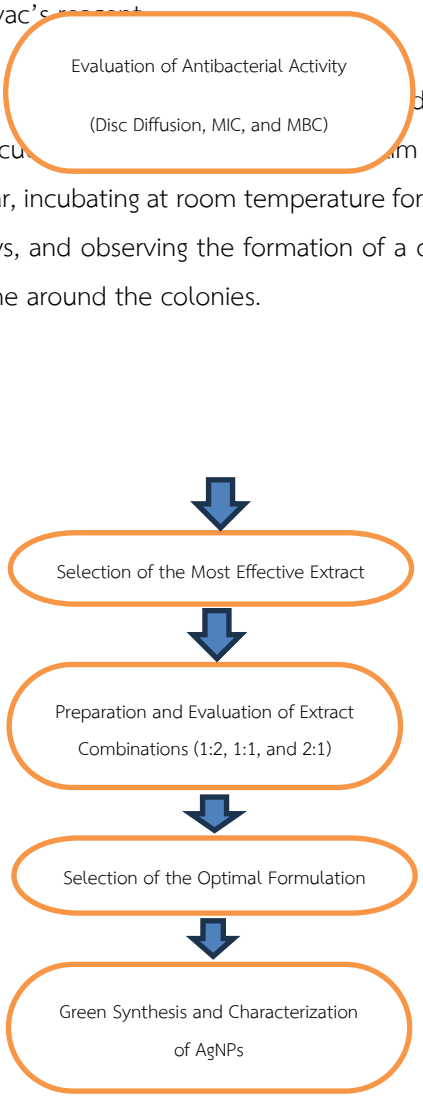
2.2 Starch Hydrolysis Test: Conducted by adding 2–3 drops of iodine solution onto the culture medium.

2.3 KOH String Test: Executed by placing a drop of KOH solution on a glass slide, mixing it with the bacterial colony, and slowly lifting the inoculating loop.

2.4 Catalase Test: Performed by adding a drop of H_2O_2 solution and mixing the bacterial culture with the solution on a glass slide to observe gas bubble formation.

2.5 Oxidase Test: Conducted by using an inoculating loop to transfer the bacterial colony onto a filter paper saturated with 1% Kovac's reagent.

2.6 [illegible]d by inocu[illegible]m Milk Agar, incubating at room temperature for 2–3 days, and observing the formation of a clear zone around the colonies.



Evaluation of Antibacterial Activity of AgNPs

3. Determination of Antibacterial Activity Against *Xanthomonas* sp.

The antibacterial activity against *Xanthomonas* sp. was evaluated across three experimental groups:

1. Crude extracts of mature mango leaves and rambutan peels at concentrations of 50, 100, and 200 mg/ml.
2. Combined extracts of mature mango leaves and rambutan peels at ratios of 1:2, 1:1, and 2:1.
3. The most effective extract synthesized into silver nanoparticles (AgNPs).

The inhibitory efficiency was evaluated using the disc diffusion method, and both the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined.

3.1 Disc Diffusion Assay

The bacterial suspension of *Xanthomonas* sp. was adjusted to the 0.5 McFarland standard and lawn-cultured onto Nutrient Agar. Sterile filter paper discs were placed onto the agar surface and loaded with 20 µL of each test substance. Control groups included distilled water, 95% ethanol, Tetracycline antibiotic, and $AgNO_3$ solution (specifically for AgNPs evaluation). The plates were incubated at 35±2°C for 18–24 hours, after which the diameters of the inhibition zones were measured. The experiments were performed in triplicate, and data were analyzed. *(Note: You statistical analysis).*

3.2 Determination of MIC and MBC

The MIC and MBC of each test substance were determined using the broth dilution method. A serial dilution of the substances was prepared in test tubes, followed by the inoculation of *Xanthomonas* sp. suspension adjusted to the 0.5 McFarland standard, along with appropriate controls. The tubes were then incubated at 37°C for 24 hours.

The MIC was defined as the lowest concentration that completely inhibited visible bacterial growth. To evaluate the MBC, aliquots from the tubes showing no visible turbidity were subcultured onto agar media, and the MBC was recorded as the lowest concentration that showed no colony growth. The experiments were performed in triplicate, and data were analyzed.

4. Green Synthesis of Silver Nanoparticles (AgNPs) from the Potent Extract

The extract exhibiting the highest efficacy was mixed with a 1 mM $AgNO_3$ solution. The mixture was continuously stirred at 40°C, and color changes of the solution were visually observed at specific time intervals: 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours. To confirm the synthesis of AgNPs, characterization was performed by measuring the optical absorbance using a UV-Vis spectrophotometer within the wavelength range of 350–700 nm, followed by

Fourier Transform Infrared Spectroscopy (FT-IR) analysis.

Results and Discussion

Table 1: Results of Antibacterial Activity against *Xanthomonas* sp. using Disc Diffusion Method

Table 2: Results of Mixed Extracts on the Inhibition of Canker-Causing Bacteria using Disc Diffusion Method

Test Substance	Diameter of Inhibition Zone (mm)		
	Rep 1	Rep 2	Rep 3
Rambutan peel extract (50 mg/mL)	12.42	10.77	12.10
Mango leaf extract (100 mg/mL)	8.24	8.60	8.00
Rambutan peel extract+$AgNO_3$	15.65	15.75	15.70
Mango leaf extract+$AgNO_3$	14.12	14.00	14.18
$AgNO_3$ solution	15.05	14.90	15.05
Tetracycline	37.50	37.68	37.50

The negative controls (95% ethanol and distilled water) exhibited no zones of inhibition across all experiments.

Evaluation of the antibacterial activity of the crude extracts revealed that the rambutan peel extract (Rong Rien variety) at a concentration of 50 mg/ml exhibited the highest effectiveness in inhibiting *Xanthomonas* sp., the causative agent of citrus canker in Tubtim Siam pomelo. It yielded an average inhibition zone of 11.76 ± 0.02 mm, which was greater than the mature mango leaf extract (Nam Dok Mai variety). Additionally, both treatments demonstrated high data precision, with coefficients of variation (CV) under 10%. Furthermore, the findings demonstrated that the rambutan peel extract substantially outperformed the mango leaf extract, presenting an MIC of 6.25 mg/ml compared to the mango leaf extract's MIC of 50 mg/ml.

When the optimal rambutan peel extract was advanced into a bio-nanotechnology agent via Green Synthesis with silver nitrate ($AgNO_3$), the resulting silver nanoparticles (AgNPs) substantially elevated the antibacterial performance. The inhibition zone expanded to 15.70 ± 0.02 mm, and the MIC decreased to 0.3125 mg/ml. This indicates that the nano-synthesis process enhanced the antimicrobial activity by 20-fold compared to the standard crude extract. Characterization via UV-Vis technique confirmed a maximum absorption peak at 428.2 nm, and

Test Substance	Concentration (mg/ml)	Diameter of Inhibition Zone (mm)		
		Rep 1	Rep 2	Rep 3
Mango leaf	50	7.10	6.88	7.70
	100	8.24	8.60	8.00
	200	6.80	6.68	6.90
Rambutan peel	50	12.42	10.77	12.10
	100	6.41	6.93	6.95
	200	7.35	6.98	6.40
Tetracycline	100	28.00	27.66	26.96

FT-IR analysis confirmed the role of hydroxyl (-OH) groups acting as reducing agents.

Additionally, the mechanism of action of the synthesized AgNPs was found to be predominantly bacteriostatic rather than bactericidal, which is advantageous as it reduces the evolutionary pressure for the pathogen to develop resistance.

However, investigating the synergistic potential of combining rambutan peel and mango leaf extracts revealed an antagonistic effect, which reduced the inhibition zone to 6.16 – 6.40 mm. Therefore, it is concluded that utilizing AgNPs synthesized solely from rambutan peel extract was the most effective treatment evaluated in this study.

Conclusions

This study compared the efficacy of crude extracts from mature mango leaves (*Mangifera indica* cv. Nam Dok Mai) and rambutan peel (*Nephelium lappaceum* cv. Rong Rien) in inhibiting *Xanthomonas* sp., the causal agent of citrus canker in Tubtim Siam Pomelo. The results indicated that the rambutan peel extract exhibited substantially higher inhibitory activity than the mango leaf extract, with a Minimum Inhibitory Concentration (MIC) of 6.25 mg/ml compared to 50 mg/ml for mango leaves. Consequently, the highly potent rambutan peel extract was utilized to develop a bio-nanotechnology product via green synthesis with silver nitrate ($AgNO_3$). The synthesized silver nanoparticles (AgNPs) remarkably enhanced antibacterial efficacy, drastically reducing the MIC to just **0.3125 mg/ml**, which represents a **20-fold increase** in efficiency compared to the original crude extract. Interestingly, the mode of action primarily focused on growth inhibition (bacteriostatic) rather than direct eradication (bactericidal), a mechanism that potentially reduces selection pressure for pathogen resistance. In conclusion, these findings demonstrate the strong potential of rambutan peel-derived AgNPs as a safe, eco-friendly, and highly effective bio-nanospray for controlling citrus canker, offering a sustainable alternative for modern pomelo cultivation.

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