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RESEARCH ARTICLE

Ecosystem Rehabilitation Approach Using Oriental Herbs to Detour Agricultural Antibiotic Resistance from Antibiotic Overuse

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ABSTRACT: This study reconsiders traditional plant extracts as sustainable alternatives to synthetic antimicrobials in response to ecological concerns and rising resistance in *Agrobacterium tumefaciens* (*A. tumefaciens*), the agent of crown gall. Aqueous extracts of garlic, ginger, cinnamon, and star anise (prepared from 75 g plant material each) were evaluated by Kirby–Bauer disk diffusion across a concentration series using isolates from crown galls. Garlic and ginger produced the largest inhibition zones and were bactericidal at 25% concentration; star anise was bactericidal at 25% with smaller zones, while cinnamon required 50% to show bactericidal effects. In radish assays, all treated seeds germinated, and garlic and ginger—though most effective against bacteria—reduced early seedling vigor at their bactericidal concentrations compared with water controls. These results partially support the hypothesis that herbal extracts can suppress *A. tumefaciens* without preventing germination, but they also reveal potential phytotoxicity at concentrations needed for bacterial control. The study highlights the dual outcomes of antimicrobial efficacy and growth inhibition and recommends optimization of concentration and formulation to maximize crop protection while safeguarding plant growth before field application.

KEYWORDS: Microbiology, Antimicrobials and Antibiotics, Natural Herb Extracts, *Agrobacterium tumefaciens*, Antimicrobial Resistance.

Introduction

The increasing use of synthetic antimicrobials in agriculture and medicine has raised environmental concerns and contributed to the global spread of antibiotic resistance.[1,2] Plant-derived compounds are thus being revisited as eco-friendly alternatives for antimicrobial control.[2,3] In Korean fermented foods like kimchi, lactic acid bacteria play crucial roles in fermentation, preservation, and health-related properties of the final product.[4,5]

Recent studies show that garlic and ginger extracts can inhibit multidrug-resistant bacteria, while cinnamon- and star anise-based essential oils also suppress bacterial growth and biofilm formation.[1,6] Additionally, research on *Agrobacterium tumefaciens* has demonstrated antimicrobial activity from plant essential oils, indicating this pathogen as a suitable model for testing natural antimicrobials.[7]

This study investigates the antimicrobial effects of garlic, ginger, cinnamon, and star anise extracts against *Agrobacterium tumefaciens*.[7] Unlike previous studies focusing mainly on single plant materials or different bacterial targets, this work compares multiple traditional ingredients under identical experimental conditions to evaluate their potential as sustainable alternatives to synthetic antimicrobials.[1,6,7]

Methods

LB Agar Preparation:

Figure 1 shows the LB Agar preparation. (1) Double-distilled water (DDH2O) was prepared. (2) 25 g of LB powder was mixed with DDH2O. (3) 16 g of agar powder was mixed with the solution. (4) The mixed LB agar has been autoclaved at 121 ˚C for 20 minutes for sterilization. After the autoclave, the LB agar solution was stored at 25 ˚C. (5) The solidified LB agar was melted by repeated short microwaves and poured into sterilized Petri dishes (about 10 mL per plate). After solidifying LB agar, the Petri dishes were stored at 4 ˚C for the first two days before the following experiments.

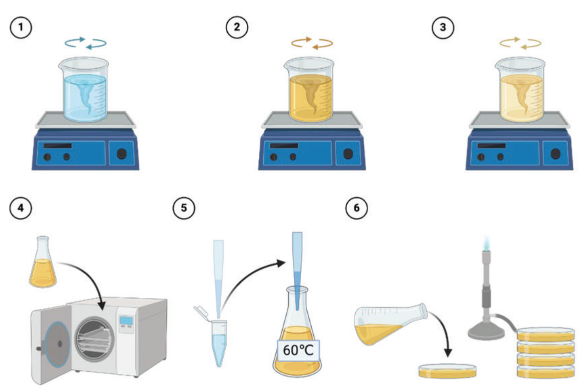

Figure 1: LB Agar preparation. This figure documents the sterile LB agar preparation protocol used to culture Agrobacterium; plates prepared as shown supported subsequent colony growth used throughout the study.

Agrobacterium Collection:

As Figure 2 illustrates, (1) Crown gall was collected from an oak tree in Kowloon City, Hong Kong, by a chopping knife. (2) After soaking them in DDH2O for 24 hours, the crown gall was chopped into 0.5 cm cubes and placed on a fresh LB agar plate. (3) After 72 hours of incubation at 25 ˚C, bacterial col-

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onies from the crown gall were transferred to a new, fresh LB agar plate. After 48 hours of incubation, a few selected colonies were resuspended in DDH2O for further experiments.

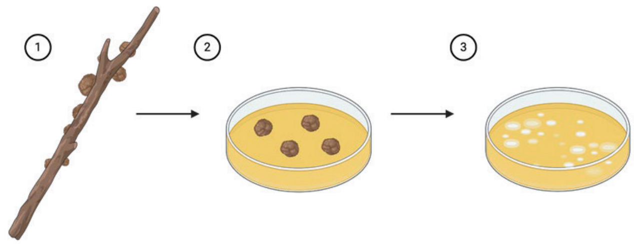

Figure 2: Agrobacterium collection methods. The procedure yielded colonies morphologically consistent with Agrobacterium, which were used for the antimicrobial assays described later.

Herb Extract Preparation:

Figure 3 below shows the herb extract preparation methods for herb extracts. (1) 75 g each of garlic, ginger, cinnamon, and star anise powders was prepared. (2) Each of the powders was mixed separately with 300 mL of DDH2O. (3) The mixtures were incubated at 4°C for 24 hours for the extraction of active compounds. (4) After incubation, each mixture was filtered. (5) The extracts were stored at -20°C until later use in the experiment.

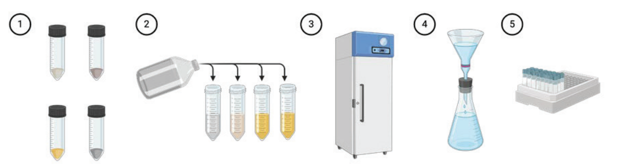

Figure 3: Herb extract preparation. Standardized aqueous extracts (75 g plant material in 300 mL DDH2O) were produced and stored at −20°C for use in antibiotic selection and seed assays.

Antibiotic Selection Test:

Figure 4 shows the Antibiotic Selection Test procedure. (1) Bacterial cultures were spread on LB agar plates using the spread plate method. (2) Antimicrobial discs were prepared by soaking sterile paper discs (6 mm in diameter) in solutions of different herb extract concentrations (100%, 50%, 25%, 10%, 5%, 1%). An additional paper disc was left unsoaked in extracts and served as a 0% concentration control. Antimicrobial discs were placed onto the surface of each plate using sterile tweezers. (3) The plates were incubated at 25 °C for 24 hours, and a zone of inhibition was observed.

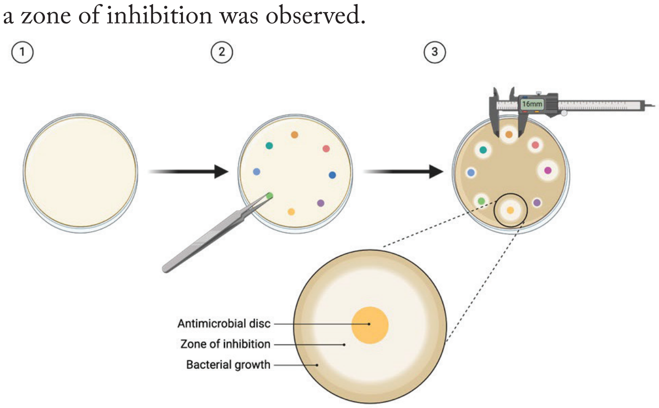

Figure 4: Antibiotic selection test. The disk-diffusion method and concentration series (100–0%) shown here generated the inhibition-zone data used to compare antibacterial potency among extracts.

Plant Seed Germination Test:

Radish seeds were soaked in DDH2O for 12 hours. Approximately 70 seeds were evenly spaced on the surface of sterile plastic containers. The containers were divided into three treatment groups: one to be sprayed with 25% garlic extract, one with 25% ginger extract, and the other with sterile distilled water as a control. The groups were sprayed at consistent time points (6:00 and 18:00 daily) for 7 days and incubated at 25°C between sprayings.

Data Analysis:

To collect the quantitative data, ImageJ software was used to measure the zone of inhibition diameter in millimeters and root and shoot lengths in centimeters.

Results

Agrobacterium Selection from Wild Woods:

Agrobacterium is known to form white to cream-colored, smooth, convex, glistening, and circular colonies with entire edges that appear mucoid or sticky. The bacterial colonies collected from the wild wood sample also showed similar characteristics, as shown below in the image (Figure 5). The matching characteristics observed suggest that the bacteria sample we obtained was Agrobacterium.

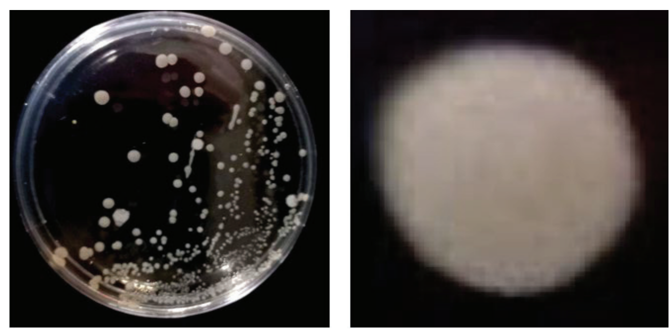

Figure 5: Images of Agrobacterium. Representative colony morphology (white/cream, smooth, convex, mucoid) supported the identification of the isolated strain as Agrobacterium used in the experiments.

Antibiotic Selection Test:

Figure 6 below shows Representative images showing inhibition zones produced by garlic, ginger, star anise, and cinnamon extracts at various concentrations. The diameter of each inhibition zone was measured to evaluate antibacterial effects.

Figure 6: The antibacterial activity of the four herbal extracts. Representative plates show larger inhibition zones for garlic and ginger versus smaller zones for cinnamon and star anise, visually illustrating relative antibacterial potency.

Overall, garlic and ginger solutions were the most effective at killing bacteria, as larger zones with no growth were observed. In contrast, the zones formed by star anise and cinnamon solutions were smaller.

Garlic and ginger had the largest inhibition zone, and they killed bacteria from 25% concentration. Star anise also killed bacteria from 25% concentration, but the size of the inhibition zone was smaller than that of garlic and ginger. Cinnamon showed the smallest inhibition zone, and it could only kill bacteria from 50% concentration (Figure 7).

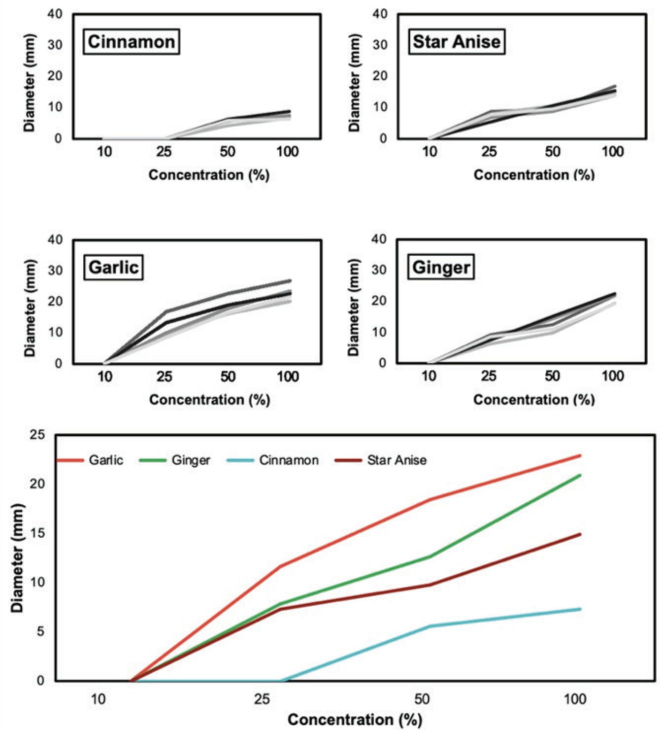

Figure 7: Results of the antibiotic selection test. Quantified inhibition-zone diameters show that garlic and ginger were bactericidal at 25% concentration, star anise was bactericidal at 25% but with smaller zones, and cinnamon required 50% to be bactericidal.

Plant Germination & Growth Test:

In the plant seed germination test, seeds treated with distilled water showed the best overall growth (Figure 8). Seeds treated with the 25% garlic solution were also able to successfully germinate. However, the plant growth was visibly smaller in size compared to that watered with just distilled water. Seeds treated with the 25% ginger solution germinated as well, but showed the smallest growth visually.

Plants grown with distilled water were the longest, with a root length average of approximately 6.08 cm and a shoot length average of approximately 8.55 cm. Next, plants grown with garlic solution were shorter, with a root length average of approximately 3.85 cm and a shoot length average of 4.13 cm. Plants grown with a ginger solution were the shortest, with a root length average of approximately 2.38 cm and a shoot length average of 2.79 cm (Figure 9).

Figure 8: Germination and early growth of seeds under different treatments. Photographs of seedlings reveal that all seeds germinated, but seedlings treated with 25% garlic or 25% ginger displayed visibly reduced growth compared with distilled-water controls.

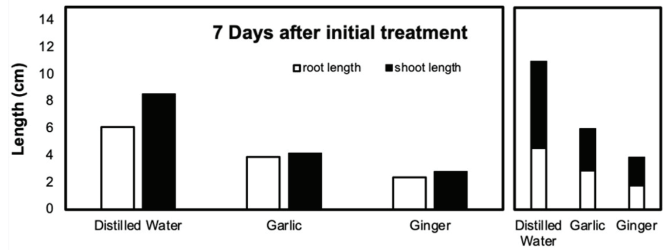

Figure 9: Quantitative comparison of root and shoot lengths. Measured root and shoot lengths confirm that distilled-water controls had the greatest growth (root ~6.08 cm, shoot ~8.55 cm), while 25% garlic and especially 25% ginger produced significantly shorter roots and shoots.

Discussion

Antibiotic Selection Test Analysis:

The results of the antibiotic selection test showed the antimicrobial effects of garlic, ginger, cinnamon, and star anise. These herbal extracts were able to kill bacteria due to their antimicrobial compounds.

Garlic contains allicin, which is an antimicrobial compound that works when raw garlic is crushed or chopped.

Allicin interferes with thiol-containing enzymes in bacteria, disrupting microbial processes such as RNA synthesis and acetyl-CoA formation. Previous studies have shown that garlic is effective against pathogenic bacteria, particularly gram-negative bacteria like Grobacterium that are resistant to multiple antibiotics.

Ginger contains compounds like zingiberene, α-curcumene, and zingerone, which can attack the cell membrane of bacteria. This destroys the cell membrane and ultimately leads to leakage of cellular components, inhibiting the growth of bacteria.

Cinnamon contains compounds like trans-cinnamaldehyde and eugenol, which have been shown to have strong antimicrobial properties. Trans-cinnamaldehyde damages the cell membrane of bacteria and disrupts cell division. Eugenol can inhibit bacterial growth by altering the lipid profile and inhibiting the activity of ATPases.

Star anise contains phenolic compounds like shikimic acid, quinic acid, flavonoids, and eugenol. They have antimicrobial properties by damaging bacterial cell membranes and disrupting intracellular enzymes. Specifically, these compounds extracted from star anise have been shown to inhibit biofilm formation and damage pathogenic bacteria.

Additionally, the zones of inhibition were observed for garlic and ginger extracts starting at a concentration of 25%, and these extracts showed larger inhibition zones compared to cinnamon and star anise. This suggests that garlic and ginger are more effective in suppressing bacterial growth than cinnamon and star anise. Garlic and ginger showed superior inhibitory effects on bacteria, likely due to the higher effectiveness of their compounds in damaging bacterial cell membranes.

Plant Seed Germination Test Analysis:

In our experiment, plant growth was significantly lower in treatments with 25% concentrations of garlic and ginger extracts compared to the control group watered with distilled

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water. Root and shoot lengths of the plants were all lowest with the garlic and ginger extracts tested.

This result is likely because the concentrations of the garlic and ginger solutions may have caused osmotic stress in the plants. Plant cells need to maintain appropriate turgor pressure and water balance for growth processes like cell division and elongation. However, the high solute levels outside cells from compounds in the extracts would have created an osmotic gradient that draws water out of cells. This dehydration would have disrupted turgor pressure and impaired water and nutrient transport. To re-establish equilibrium, plants would have diverted energy into osmoregulation instead of growth.

Additionally, chemicals in garlic and ginger extracts may have interfered with nutrient uptake.

Conclusion

In summation, this study demonstrated both the antimicrobial activity of natural herbal extracts as well as their potential inhibitory effects on plant growth. Our hypothesis that natural herb extracts would suppress bacterial growth without negatively affecting plant growth was partially supported.

The results from the antibiotic selection test confirmed that all extracts - garlic, ginger, cinnamon, and star anise - inhibited the growth of Agrobacterium as expected. This validated their antimicrobial properties against pathogenic bacteria.

The plant seed germination test revealed that garlic and ginger extracts, which were the most effective in the antibiotic selection test, did allow successful germination of the plants. However, when compared to the plants treated with distilled water, the minimum concentrations of these extracts required to inhibit bacterial growth also hindered plant growth.

In the field of antibiotics research, this study's results significantly contribute to the existing body of evidence regarding the potential of natural herbal extracts as alternatives to antimicrobial chemicals. The confirmed antimicrobial activity observed in garlic, ginger, cinnamon, and star anise extracts is consistent with previous research about the effectiveness of these natural compounds in treating bacterial infections. Specifically, this study demonstrated the natural herbs' efficacy against Agrobacterium, a bacterium known to cause plant diseases.

However, this study also uncovered the inhibitory effects of natural herbal extracts on plant growth. These findings emphasize the need for further exploration and optimization of natural herbal extracts to develop a balance between their antimicrobial properties and their impact on plant growth.

Furthermore, this study addresses the issue of overuse and environmental damage caused by chemically synthesized antimicrobials. Shifting toward natural extracts can help reduce the release of residual antimicrobials into the environment, mitigate ecological disruptions, and combat the development of antimicrobial resistance.

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Taehee is currently a 12th grader at Cheongna Dalton School, South Korea, and enjoys learning about science, math, and engineering. She has developed an interest in biological research on antimicrobials in modern agriculture and medicine. She further pursues her passion for science by leading the Citizen Science Club, which carries out collective volunteer activities to support researchers in multiple fields, including biology, environmental science, and physics.