GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY AGAINST FOODBORNE PATHOGENS

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Abstract

This study aimed to green-synthesize silver nanoparticles (AgNPs) using leaf extracts from Holy basil, Sweet basil, and Tree basil as bioreducing agents, thereby replacing conventional chemical methods. The synthesis successfully yielded spherical AgNPs with a nanometer scale size, exhibiting a maximum surface plasmon resonance (SPR) absorption peak in the 400–480 nm range. The resulting fine, dark-brown powder was then evaluated for its antibacterial efficacy against the foodborne and spoilage bacteria, *E. coli*, *S. aureus*, and *Alcaligenes* sp., using the agar well diffusion method. The results confirmed that all synthesized AgNPs effectively inhibited the growth of all three bacterial strains. Specifically, AgNPs derived from Sweet basil extract demonstrated the highest overall inhibitory effect, followed by those from Holy basil and Tree basil extracts, respectively. Furthermore, across all plant extracts, the prepared AgNPs consistently exhibited the highest antibacterial activity against *S. aureus*, followed by *E. coli* and *Alcaligenes* sp., in descending order.

Keywords: Silver nanoparticles, Green synthesis, Antimicrobial activity

Introduction

Silver and its compounds are widely recognized for their therapeutic and antimicrobial properties, leading to applications in medicine, preservatives, and hygiene products. Notably, silver nanoparticles (AgNPs) have gained significant attention in healthcare due to their effective bactericidal, fungicidal, and anti-yeast capabilities. Conventionally, AgNP production relies on chemical reducing agents, which poses concerns regarding environmental toxicity, potential side effects, and high manufacturing costs. Consequently, green technology, specifically the use of plant extracts as eco-friendly reducing agents, has emerged as a promising and sustainable alternative to circumvent these challenges.

In recent years, global research has focused intensely on the plant-mediated synthesis of AgNPs for microbial control, targeting various pathogens in human, animal, and plant sectors, particularly bacteria and fungi. A prominent example is the recent work by Chommantree, S., et al. (2024), who successfully synthesized biocompatible AgNPs using aqueous guava fruit extract, demonstrating high antioxidant and antibacterial activity against common pathogens like *E. coli* and *S. aureus*. This approach seeks to enhance antimicrobial efficacy while simultaneously reducing the cost and potential toxicity associated with chemical or physical synthesis methods. The application is critical given the increasing incidence of foodborne diseases. These diseases, caused by foodborne bacteria such as *Salmonella*, *Campylobacter*, *Listeria*, and pathogenic *E. coli*, continue to be a major global public health concern. Contributing factors include changes in population lifestyle, industrial livestock practices, microbial virulence evolution, and increased consumption of pre-cooked and ready-to-eat convenience foods. The risk of contamination, especially by heat-resistant toxins like those from *Staphylococcus aureus*, is amplified during various stages of food processing and preservation.

From a global health perspective, foodborne illnesses represent a significant public health burden, especially in developing countries where they cause high mortality, particularly among children and immunocompromised individuals. In developed nations, the economic impact is substantial due to labor absenteeism and loss of productivity. Given that foodborne pathogens can contaminate food at multiple points and proliferate before consumption, developing effective preservation and disinfection strategies is crucial for global food safety. Recent studies highlight that the antimicrobial susceptibility of bacteria to nanoparticles is a vital factor in developing effective, long-shelf-life food packaging and safety protocols.

Against this backdrop, the present research focuses on the green synthesis of AgNPs utilizing extracts from three common Thai indigenous plants: Holy basil, Sweet basil, and Tree basil. The study aims to thoroughly test the synthesized AgNPs' efficacy against various foodborne pathogens and spoilage bacteria relevant to meat products. The ultimate goal is to develop a safe, cost-effective, and easy-to-use disinfectant product that can effectively replace highly toxic chemical agents, thereby significantly reducing the prevalence and spread of foodborne bacterial diseases for consumer safety.

Research Objectives

1. To synthesize silver nanoparticles (AgNPs) via a green synthesis process utilizing extracts from three Thai indigenous plants: Holy basil (*Ocimum tenuiflorum* L.), Sweet basil (*Ocimum basilicum* L.), and Tree basil (*Ocimum gratissimum* L.).

2. To test and evaluate the efficacy of the synthesized AgNPs against key foodborne pathogens and spoilage bacteria relevant to meat products.

Scope of the Research

1. Synthesis Method: The research is limited to the green synthesis of silver nanoparticles (AgNPs) using plant extracts as bioreducing agents. It explicitly focuses on replacing conventional chemical methods.

2. Source Materials: The study exclusively uses leaf extracts from three specific plants: Holy basil (*Ocimum tenuiflorum* L.), Sweet basil (*Ocimum basilicum* L.), and Tree basil (*Ocimum gratissimum L.*).

3. Target Microorganisms: The antibacterial evaluation is limited to three specific bacterial strains relevant to food: *E. coli*, *S. aureus*, and *Alcaligenes* sp.

4. Antimicrobial Testing Method: The assessment of antibacterial efficacy is conducted in vitro using only the agar well diffusion method.

Literature Review

Kaviya S. et al. (2011) reported that the biosynthesis of silver nanoparticles (AgNPs) is a novel and highly beneficial method. They successfully synthesized AgNPs using Citrus sinensis peel extract as both a reducing and capping agent. Furthermore, the synthesized AgNPs exhibited antibacterial activity against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the Gram-positive *Staphylococcus aureus*.

Several studies have focused on the green synthesis of AgNPs. For instance, Swarnali M. et al. (2014) employed *Lycopersicon esculentum* (tomato) extract in a simple and eco-friendly process to produce spherical AgNPs. These nanoparticles exhibited clear inhibitory activity against *E. coli* (cultured on LB agar) with a reported Minimum Inhibitory Concentration (MIC) of 50 µg/ml. Similarly, Bandita M. et al. (2015) demonstrated the use of *Citrus limon* extract as both a reducing and capping agent, successfully synthesizing highly stable and concentrated AgNPs. This latter approach was noted for its rapidity and simplicity, suggesting high potential for food, cosmetic, and biomedical applications.

Ghodsieh B. et al. (2017) synthesized silver nanoparticles (AgNPs) using an aqueous extract of saffron (*Crocus sativus* L.) and evaluated their antibacterial activity against six bacterial species. The UV-vis spectrum confirmed AgNP formation, exhibiting a characteristic Surface Plasmon Resonance (SPR) peak at 450 nm. X-ray diffraction (XRD) analysis indicated that the synthesized AgNPs had a particle size of approximately 12-20 nm. Furthermore, electron microscopy revealed a spherical morphology with an average size of 15 nm. The antibacterial assay demonstrated that these AgNPs were effective against all six tested bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella flexneri*, and *Bacillus subtilis*.

Research Methodology

1. Preparation of Plant Extracts

Three Thai indigenous plants were utilized in this study: Holy basil (*Ocimum tenuiflorum* L.), Sweet basil (*Ocimum basilicum* L.), and Tree basil (*Ocimum gratissimum* L.). For each plant species, the leaves were thoroughly washed twice with distilled water and then finely chopped. A 4.0 g portion of the finely chopped leaves was added to a 250 ml beaker containing 40 ml of distilled water. The mixture was homogenized and subsequently boiled for 20 minutes. Following the boiling step, the mixture was filtered through Whatman No. 1 filter paper. The resulting clear filtrate was collected in a 50 ml round-bottom plastic tube and stored at 4 °C until further use.

2. Synthesis of Silver Nanoparticles (AgNPs)

A 10 mM silver nitrate ($AgNO_3$) solution was prepared to serve as the precursor for the synthesis of silver nanoparticles. For the synthesis reaction, a 9 ml aliquot of each plant extract (Holy basil, Sweet basil, and Tree basil), as prepared in Section 1, was added to 120 ml of the 10 mM $AgNO_3$ solution. The synthesis was conducted at 80 °C under constant stirring. The reaction was allowed to proceed until the solution's color visibly changed to brown, indicating the formation of AgNPs. The resulting colloidal solution was then stored in an amber bottle. To collect the nanoparticles, the brown solution was centrifuged at 10,000 rpm for 10 minutes at 37 °C to pellet the AgNPs. The supernatant was subsequently discarded, and the resulting pellet was washed three times with distilled water. Following the washing steps, the purified pellet was dried in a hot air oven at 80 °C for 24 hours. This process yielded a fine powder of silver nanoparticles, which was collected for subsequent experiments.

3. Characterization of Silver Nanoparticles (AgNPs)

The synthesized silver nanoparticles (AgNPs) from all three plant species were characterized using a UV-visible spectrometer. The absorption spectra were recorded over a wavelength range of 300–600 nm.

4. Assessment of Antibacterial Activity by Agar Well-Diffusion Method

The antibacterial activity of the silver nanoparticles (AgNPs), synthesized from the three Thai indigenous plant extracts, was evaluated using the agar well-diffusion method (Cleidson V. et al. 2007).

The assay was performed against three foodborne pathogen strains. A standardized inoculum (approximately 1×10^8 CFU/ml) of each pure bacterial culture was prepared. A 100 µl volume of the inoculum was then uniformly spread onto the surface of Nutrient Agar (NA) plates.

Subsequently, wells with a 6 mm diameter were punched into the agar using a sterile cork borer. Aliquots (10 µl and 20 µl) of each of the three synthesized AgNP solutions were pipetted into their respective wells.

Chloramphenicol (20 mg/ml) and sterile distilled water were used as the positive control and negative control, respectively. The plates were then incubated at 37 °C for 24 hours. Following incubation, the antibacterial efficacy was determined by measuring the diameter (in mm) of the clear zone of inhibition surrounding each well.

Research Results

1. Synthesis of Silver Nanoparticles using Plant Extracts

The synthesis of silver nanoparticles (AgNPs) was conducted using leaf extracts from Holy basil, Sweet basil, and Tree basil, which functioned as reducing agents in the reaction. These agents facilitate the reduction of Ag^+ to Ag^0, resulting in the formation of silver nanoparticles (AgNPs), as shown in the following equation:

$$Ag^+ \ (aq)^+ \ e^- \longrightarrow Ag^0 \ (s)$$

A progressive color change in the reaction mixture was observed, transitioning from an initially clear solution to light yellow, dark yellow, and finally to a deep brown within a 50-minute period.

This chromatic transition (color change) is indicative of the synthesis of silver nanoparticles (AgNPs), which caused the solution to darken. When compared at the same time interval, the reaction utilizing Sweet basil extract exhibited the most intense (darkest) color, followed by the Holy basil and Tree basil extracts, respectively.

2. Spectroscopic Confirmation of AgNP Synthesis

The synthesis of silver nanoparticles (AgNPs) was verified using UV-visible (UV-Vis) spectroscopy, with spectra scanned over the 300–600 nm wavelength range. The formation of AgNPs was confirmed by the detection of a maximum absorption peak (Surface Plasmon Resonance, or SPR) within the 400–480 nm range. This absorption is a characteristic optical property specific to silver nanoparticles (Kanika S. et al., 2020).

At a wavelength of 440 nm, the AgNPs synthesized using Sweet basil extract exhibited the highest absorbance (A = 2.542), followed by those from Holy basil (A = 1.719) and Tree basil (A = 1.597), respectively.

Following synthesis, the colloidal AgNP solution was centrifuged at 5,000 rpm for 10 minutes to pellet the nanoparticles. The supernatant was then discarded, and the resulting pellet was washed three times with distilled water. The purified pellet was subsequently dried in a hot air oven at 80 °C for 24 hours. This process yielded a fine, dark-brown powder.

3. Antibacterial Activity Assessment by the Agar Well-Diffusion Method

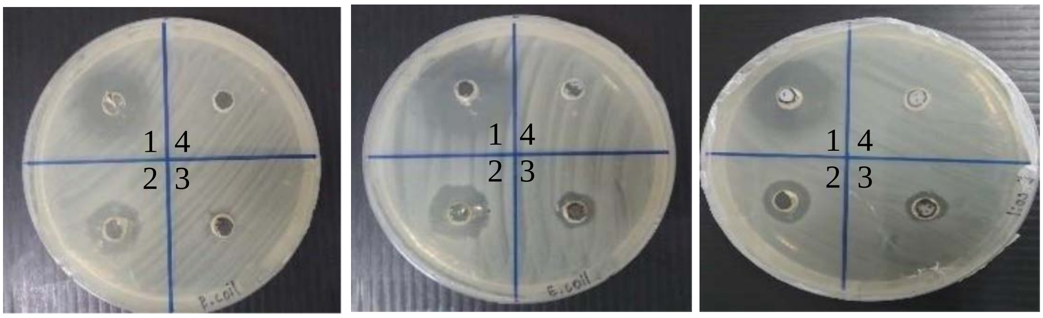
The antibacterial efficacy of the synthesized silver nanoparticles (AgNPs) was evaluated using the agar well-diffusion method. The assay was conducted against three bacterial strains: *E. coli*, *S. aureus*, and *Alcaligenes* sp.

The results demonstrated that the AgNPs synthesized using all three plant extracts effectively inhibited the growth of all three bacterial strains (Figures 1-3). Furthermore, all synthesized AgNPs exhibited the highest antibacterial activity against *S. aureus*, as evidenced by the largest (or widest) inhibition zones observed for this bacterium.

When comparing the volumes of AgNPs applied, a dose-dependent effect was observed. The 20 µl volume demonstrated superior antibacterial efficacy against all three bacterial strains (*E. coli*, *S. aureus*, and *Alcaligenes* sp.) compared to the 10 µl volume. This was evidenced by significantly wider zones of inhibition, as illustrated in Figures 1-3.

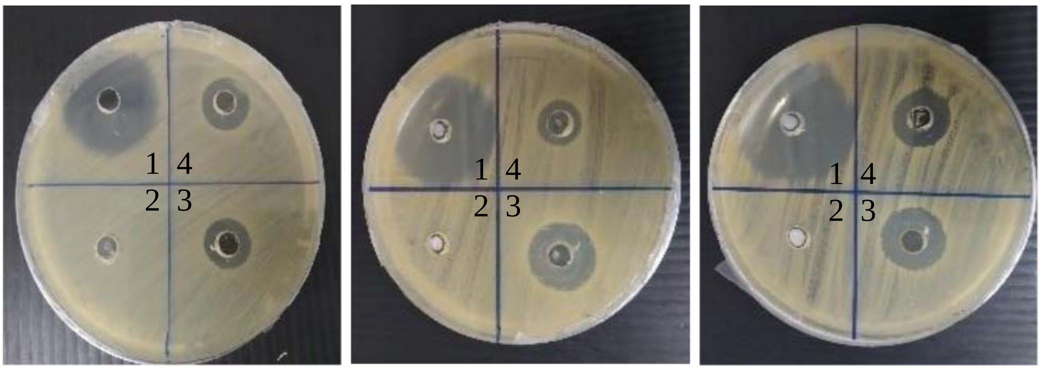
A comparison of the plant extracts (at a uniform 20 µl volume) revealed that AgNPs synthesized from Sweet basil extract exhibited the strongest overall inhibitory activity against all three bacterial strains. The effect was most pronounced against *S. aureus*, followed by *E. coli* and *Alcaligenes* sp., yielding inhibition zone diameters of 1.74, 1.52, and 1.39 cm, respectively (Figures 1-3).

AgNPs synthesized from Holy basil extract demonstrated the second-highest efficacy. Similarly, these nanoparticles showed the greatest inhibition against *S. aureus*, followed by *E. coli* and *Alcaligenes* sp., with zone diameters of 1.46, 1.35, and 0.87 cm, respectively. Likewise, AgNPs derived from Tree basil extract were also most effective against *S. aureus*, followed by *E. coli* and *Alcaligenes* sp., resulting in inhibition zones of 1.63, 1.18, and 1.26 cm, respectively (Figures 1-3).



A. B. C.

Figure 1: Growth inhibition of *E. coli* was demonstrated by silver nanoparticles (AgNPs) synthesized from A: Holy basil, B: Sweet basil and C: Tree basil leaf extracts.



A. B. C.

Figure 2: Growth inhibition of *S. aureus* was demonstrated by silver nanoparticles (AgNPs) synthesized from A: Holy basil, B: Sweet basil and C: Tree basil leaf extracts.

A. B. C.

Figure 3: Growth inhibition of *Alcaligenes sp.* was demonstrated by silver nanoparticles (AgNPs) synthesized from A: Holy basil, B: Sweet basil and C: Tree basil leaf extracts.

Conclusion and Discussion

This research successfully demonstrated the eco-friendly (or green) synthesis of silver nanoparticles (AgNPs) utilizing leaf extracts from Holy basil, Sweet basil, and Tree basil as bioreducing agents, replacing conventional chemical methods. The synthesis was evidenced by a visual color change in the solution to dark yellow or light brown within a 50-minute period. When analyzed with a UV-Vis spectrophotometer (300-600 nm), the sample exhibited its highest absorbance in the 400-480 nm wavelength range. This result indicates the successful formation of silver nanoparticles (AgNPs), as this absorption is characteristic of their optical properties.

The synthesized AgNPs were obtained as a fine, dark-brown powder. When tested for antibacterial efficacy against foodborne pathogens and spoilage bacteria relevant to pork (*E. coli*, *S. aureus*, and *Alcaligenes* sp.), the AgNPs derived from all three plant sources demonstrated effective inhibition against all three bacterial strains.

These findings conclude that Thai indigenous plant extracts can serve as viable and environmentally benign reducing agents for AgNP synthesis, offering an alternative to hazardous chemical methods. This study provides a foundation for further investigation into the safety profile of these AgNPs, specifically their cytotoxicity. Pending safety confirmation, these green-synthesized AgNPs hold significant potential for development into effective products for the prevention and eradication of other pathogenic bacteria in the future.

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