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Green Synthesis of Silver Nanoparticles Using *Nigella sativa* Seeds and Apple Peel Extracts and Their Antimicrobial Activity Against *Escherichia coli*

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ABSTRACT

In nanotechnology, synthesizing silver nanoparticles (AgNPs) with plant-based extracts has emerged as an eco-friendly and sustainable method. **Objectives:** To focus on the green synthesis and characterization of AgNPs using extracts from *Nigella sativa* seeds (black seed) and *Malus domestica* (apple) peels, both rich in bioactive phytochemicals that serve as natural reducing and stabilizing agents. **Methods:** The synthesis process was verified by UV-Vis spectroscopy using typical surface plasmon resonance (SPR) peaks (~410 nm), which means that the AgNPs were formed successfully. Dynamic light scattering (DLS) analysis was used to determine the hydrodynamic size (117 nm) and uniformity of the AgNPs, and the zeta potential analysis showed the low negative surface charges because of capping using plant biomolecules. The antimicrobial activity of the synthesized AgNPs was tested against *Escherichia coli*, a common pathogenic bacterium. **Results:** Results showed significant antibacterial effects, with a zone of inhibition of 27 mm. The previously stated mechanisms, such as ROS generation and apoptosis-like responses, were removed, as they were not experimentally verified. The use of *N. sativa* and apple peel extracts provided a cost-effective and environmentally benign synthesis route, enhancing nanoparticle stability and bioactivity. **Conclusions:** These findings highlight the potential of green-synthesized silver nanoparticles as effective antimicrobial agents specifically against E. coli, without extending claims to untested biomedical or environmental applications.

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative bacterium and is a member of the family Enterobacteriaceae [1]. *E. coli* is also a pathogen that causes several common bacterial infections in humans and animals [2]. It is a leading cause of broad-spectrum infection, urinary tract infection (UTIs), enteritis, septicemia, as well as other clinical infections such as neonatal meningitis [3]. *E. coli* is worth studying since it is a component of the intestinal microbiota and may become pathogenic as well [4]. *E. coli* strains are either classified as pathogenic or non-pathogenic. The pathogenic strains can further be categorized in terms of virulence factors and related diseases, and the non-pathogenic strains, like *E. coli* K-12, are extensively utilized in laboratory research. The *E. coli* O157:H7 can cause severe disease that causes approximately 63,000 incidences of hemorrhagic colitis in the U.S annually [5]. Since antimicrobial resistance (AMR) is gaining momentum in most countries, it is a growing concern for human health, and therefore alternative antimicrobial mechanisms are urgently required. Plants were utilized as early as ancient times in the treatment of human diseases caused by microorganisms, as they contain a considerable amount of phytochemicals [6]. Nigella sativa is a medicinal plant, and the seeds are very useful health-wise. It is reported to

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prevent and control various diseases [7]. The seeds are anti-inflammatory, anticancer, antibacterial, antifungal, and antiviral, hence a miracle plant [8]. They have significant bioactive properties, which include thymoquinone, dithymoquinone, thymol, carvacrol, phellandrene, 4-pinene, and 4-pinene [9]. These compounds have shown potential in treating a variety of diseases, and thymoquinone has been said to contribute to DNA repair. Antimicrobial effects of black seed oil have been reported against bacteria, including *E. coli*, Salmonella, and Shigella, as well as Vibrio [10]. The other fruit that people often eat is the apple, which has plenty of health-promoting phytocompounds [11]. Apple peels are rich in procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and quercetin conjugates [12], with major flavonoids including quercetin-3-O-β-d-glucopyranoside, quercetin-3-O-β-d-galactopyranoside, quercetin, catechin, epicatechin, and quercetin-3-O-α-l-arabinofuranoside [13]. Classical medicine has been based on utilizing plants and more recent uses, such as green methods of synthesizing nanoparticles [14]. We have employed black seed and apple peel extracts to prepare silver nanoparticles by a green synthesis method in this work. It is postulated that the synergistic effect of these two plant extracts on antibacterial activity against E. coli is related to complementary phytochemical profiles. Also, the application of the apple peels assists in decreasing the amount of waste in the environment, and points out the therapeutic benefits of the apple peels.

The study aims to examine the antimicrobial synergies between the silver nanoparticles that were produced using the N. sativa seeds and apple peels against E. coli, giving a new and environmentally friendly antimicrobial approach.

METHODS

The study was an experimental study, and it was carried out at the University of Lahore between October 2024 and December 2024. The seeds of N. sativa and apple were bought at the local market, Lahore, Pakistan. The two were washed with double-distilled water and kept at 20°C awaiting further processing. The pH of the extracts was determined, and it was 6.5-7.0. The major phytochemical content and total phenolic content were identified to standardize the extracts, making them reproducible. To prepare extracts of N. sativa seeds and apple peels, 10 g of N. sativa and 10 g of apple peels were crushed and added to 400 mL of distilled water. Then this was boiled on a hot plate. The mixture was collected when it reduced to 150 mL, after which it was filtered using Whatman No.1 filter paper and stored at 20°C for further processes. Extracts were prepared in three independent batches to ensure reproducibility, and each batch was used for subsequent nanoparticle synthesis. Silver nanoparticles were prepared from Aldrich silver salt ($AgNO_3$). A 0.1 mM solution was prepared by adding the silver salt to 90 mL of distilled water. A total of 10 mL of plant extract was added dropwise. Colour change indicated the formation of silver nanoparticles. Synthesis was performed in triplicate for each extract, and NP suspensions were labeled and stored separately. Negative control (distilled water) and positive control (standard $AgNO_3$ without plant extract) were included for comparison. To check the therapeutic potential of these nanoparticles, antimicrobial activity was performed using *E. coli*. All *E. coli* experiments were conducted under BSL-2 conditions following institutional biosafety guidelines. It was tested on the *E. coli* strain ATCC 25922, which was cultured on nutrient agar and then sub-cultured. The agar well diffusion method was used to determine the effectiveness of these nanoparticles [15]. Wells that had a diameter of 6 mm were used, and 50 µL of the NP solution was added to the well. Favourable management: ampicillin 10 µg/mL. Lentil seed: negative control. Three times were used to perform all these tests. Plates were incubated at 37 o C and mm areas of inhibition by measured using a digital caliper. To determine the reproducibility and validation, the duplicate tests were performed with sets of independently prepared extracts. Nutrient agar was prepared according to the instructions provided by the manufacturer and was inoculated in the sterile Petri dishes to a depth of approximately 4mm. Agar was allowed to dry at room temperature. A sterile cotton swab was used to transfer the prepared *E. coli* inoculum (0.5 McFarland standard) onto the agar plate in a uniform manner, covering the entire plate. It was necessary to aseptically punch four 6 mm in diameter wells in the agar surface after a few minutes of drying the surface. One hundred and fifty microliters of each of the concentrations were gently transferred to different wells on the agar plates that had been inoculated. Incubation at 37 o C was then conducted on the plates for 24 hours. The distance in millimeters that the zone of inhibition of each well was developed was measured with a digital caliper after incubation. This was repeated 3 times, and the mean standard deviation of the inhibition zones was determined. One-way ANOVA with the post hoc test of Tukey in SPSS version 27.0 was used to conduct statistical analysis, and $p<0.05$ was the significant value. Synthesized silver nanoparticles were characterized using several techniques to confirm their formation, determine their physicochemical properties, and assess their purity. DLS identifies the size of a nanoparticle in solution through the measurement of the time scale of Brownian motion. Measurements were performed using a Zeta sizer (Malvern Instruments) at 25°C, scattering angle 90°, with AgNP suspensions diluted 1:10 in deionized water. Instrument calibration was performed using standard polystyrene latex particles [16]. The formation of silver nanoparticles

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was primarily confirmed using a Shimadzu UV-1800 UV-Vis spectrophotometer by observing the characteristic surface plasmon resonance (SPR) peak. The calculation of the nanoparticles suspensions in sterile distilled water was obtained, and the absorbance spectra were recorded between 300 nm to 700 nm. The emergence of an absorption peak that is normally within the 400-500 nm area is a great sign of the presence of silver nanoparticles. The high, sharp, and narrow peak typically indicates the presence of smaller, monodisperse nanoparticles, whereas a broader structure indicates larger or polydisperse nanoparticles. The production of silver nanoparticles was mainly verified with a Shimadzu UV-1800 UV-Vis spectrophotometer with the characteristic surface plasmon resonance (SPR) peak. The nanoparticle suspensions were diluted using sterile distilled water, and the absorbance spectra were recorded within 300 700 nm. The emergence of an absorption peak around 400-500 nm can be regarded as a good sign of the existence of silver nanoparticles. The intensity and the wavelength of the SPR peak can give information regarding the size, shape, and concentration of the produced nanoparticles. A sharp, skinny summit usually implies the creation of smaller, monodisperse nanoparticles, whereas a bigger peak might represent bigger or polydisperse nanoparticles.

RESULTS

Using the agar well diffusion technique, the antibacterial activity of Silver nanoparticles (AgNPs) biosynthesized against *Escherichia coli* was tested. A clear zone of inhibition with a diameter of 27.0 mm was observed, demonstrating significant antimicrobial activity. Diameter measured in millimeters; wells of 6 mm diameter; incubation: 24 h at 37°C. (Figure 1)

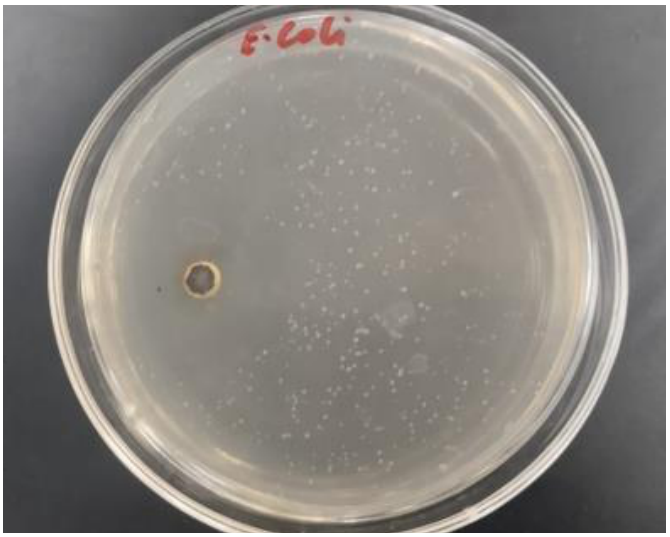

Figure 1: Zone of Inhibition of Synthesized Nanoparticles Against *E. coli*

The hydrodynamic diameter of AgNPs was measured using DLS. The intensity-weighted size distribution showed a primary peak at 117.4 nm with a standard deviation of 33.96 nm, indicating moderate polydispersity. The peak accounted for ~100% of the measured population, suggesting the absence of significant secondary populations or large aggregates. Hydrodynamic diameter expressed in nanometers (Figure 2).

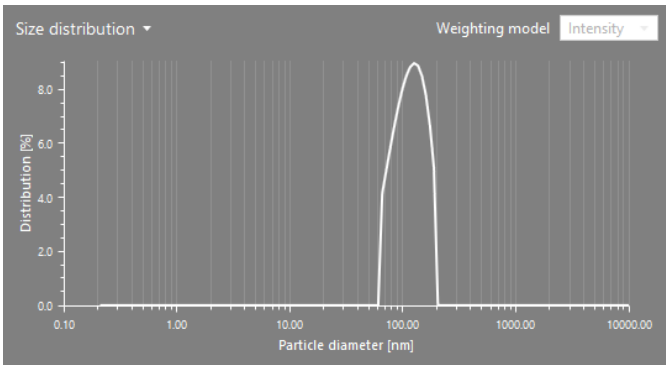

Figure 2: Intensity-Weighted Size Distribution of Green-Synthesized Silver Nanoparticles Measured by DLS

The surface charge and colloidal stability of the nanoparticles were determined by measuring the Zeta potential. The zeta potential average was -0.3 -0.4 mV with a periodic mobility of -0.0206 2-1 cm -1 V-1 sls and the conductivity of 0.000 mS/cm. The near-neutral zeta potential indicates low electrostatic repulsion, suggesting that the nanoparticles may be prone to aggregation over time (Figure 3).

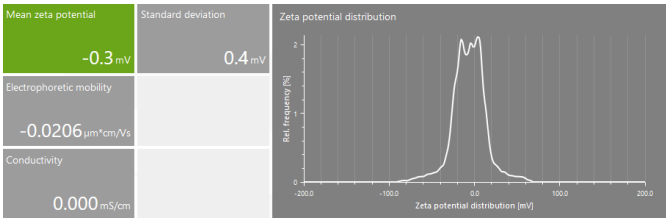

Figure 3: Zeta Potential Distribution of Green-Synthesized Silver Nanoparticles

UV–Vis spectroscopy showed a sharp shoulder at ~220 nm, attributed to π–π* transitions of phenolic compounds and aromatic proteins from the plant extract, and a prominent SPR peak at ~410 nm, characteristic of AgNP formation. The sharp SPR peak indicates predominantly small, dispersed nanoparticles with minimal aggregation. SPR peak at 410 nm confirms nanoparticle formation (Figure 4).

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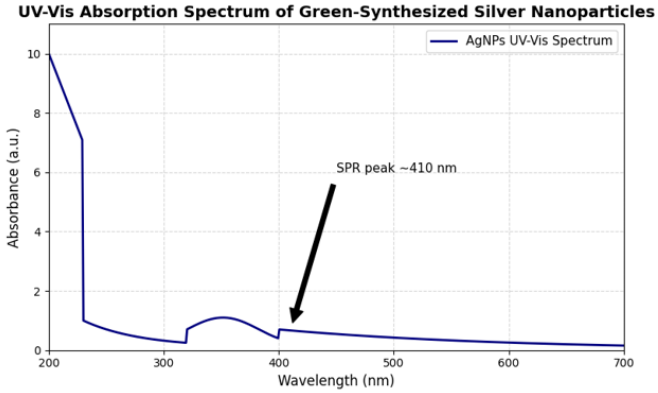

Figure 4: UV–Vis Absorption Spectrum of Green-Synthesized Silver Nanoparticles

DISCUSSION

The synthesis process involved combining extracts of *Nigella sativa* seeds and apple peels with silver nitrate, which resulted in the reduction of silver ions to form AgNPs. *N. sativa* seeds contain thymoquinone and other bioactive compounds, which likely contribute to efficient silver ion reduction and nanoparticle stabilization. Apple peels, rich in polyphenols and flavonoids, serve as effective reducing agents while utilizing agricultural waste, promoting sustainability (Fruit Peel Synthesis). The variability in nanoparticle size and shape between the two extracts may be attributed to differences in phytochemical profiles, influencing their antimicrobial efficacy. The antimicrobial activity of the synthesized AgNPs was assessed against *E. coli* using standard well diffusion assays. Both *Nigella sativa*- and apple peel-derived AgNPs showed significant inhibition of *E. coli* growth, aligning with literature findings by Arsène *et al.* [17]. Silver nanoparticles synthesized using Nigella sativa alone typically show zones of inhibition ranging from 14–20 mm against various bacterial strains [18]. The enhanced antimicrobial activity observed in this study suggests potential synergistic effects between the bioactive compounds from both plant sources." – previously implied but now more precise. Zeta potential measurements showed a near-neutral surface charge around -0.3 mV. Typically, zeta potential values greater than ±30 mV are required to ensure stable dispersions by preventing aggregation through electrostatic repulsion. The low zeta potential here suggests that the nanoparticles may tend to aggregate over time, which could contribute to the observed high PDI in DLS results. This phenomenon has been reported in other green synthesis studies where capping agents from plant extracts provide steric rather than strong electrostatic stabilization. Despite the promising results, the high polydispersity and low zeta potential indicate that further optimization of synthesis parameters, such as extract concentration, reaction time, temperature, and pH, could improve nanoparticle uniformity and stability. Techniques such as sonication or the addition of natural stabilizers might also reduce aggregation. The antimicrobial efficacy of AgNPs is influenced by their size, shape, and surface chemistry [19]. Smaller nanoparticles, with higher surface area-to-volume ratios, typically show greater antimicrobial activity due to increased interaction with bacterial cells (Shape-Dependent Antibacterial Activity) [20]. Our study produced nanoparticles within the optimal size range, contributing to their effectiveness against *E. coli*. The use of *Nigella sativa* seeds and apple peels for AgNP synthesis offers multiple advantages. Green synthesis avoids toxic chemicals, making it suitable for biomedical applications where biocompatibility is critical. *Nigella sativa*, a traditional medicinal plant, imparts bioactive compounds that may enhance the therapeutic potential of AgNPs beyond their antimicrobial effects, such as antioxidant or anti-inflammatory properties (*Nigella Sativa* AgNPs) [21]. Apple peels, as agricultural waste, provide a cost-effective and sustainable resource, aligning with circular economy principles(Fruit Peel Synthesis)[22]. Future research must consider the optimization of synthesis parameters (temperature, pH, and concentration of extract)to regulate nanoparticle size and morphology and increase antimicrobial effectiveness. The stability of AgNPs must also be studied for practical applications. Investigation of synergism between plant-derived bioactive compounds and silver may provide opportunities for developing multifunctional nanomaterials with improved therapeutic properties. Co-formulation with other antimicrobial agents or incorporation into composites, such as polymers or textiles, could expand the applications of AgNPs.

CONCLUSION

E. coli is a significant pathogen affecting humans and animals, and the rise of antimicrobial resistance emphasizes the need for alternative control strategies. In this study, using an environmentally friendly, green synthesis method, *Nigella sativa* seeds and apple peel extracts were effectively used to create silver nanoparticles (AgNPs). Clear zones of inhibition demonstrated the potent antibacterial activity of the biosynthesized AgNPs against *E. coli.* According to these results, green-synthesized silver nanoparticles (AgNPs) show great promise as antibacterial agents, providing a biocompatible and sustainable substitute for traditional chemical or physical techniques. Future research should concentrate on maximizing the stability of nanoparticles and assessing their efficacy in in vivo settings.

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Authors Contribution

Conceptualization: MUR
Methodology: AB, SAK, JB
Formal analysis: SAK, JB
Writing review and editing: JB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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