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GREEN-SYNTHESIZED NEEM LEAF EXTRACT-BASED SILVER NANOPARTICLES FOR BROAD-SPECTRUM ANTIMICROBIAL ACTIVITY

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Abstract

The rising incidence of antimicrobial resistance has necessitated the development of eco-friendly, low-cost, and highly effective antimicrobial agents. Silver nanoparticles (AgNPs) synthesized through green routes using plant extracts represent a sustainable alternative to chemically synthesized nanomaterials. The present study investigates the synthesis, characterization, and antimicrobial efficacy of silver nanoparticles prepared using aqueous neem (Azadirachta indica) leaf extract. Neem phytochemicals such as flavonoids, terpenoids, and phenolics act as natural reducing and stabilizing agents, enabling the environmentally benign formation of AgNPs. UV–Visible spectroscopy indicated a characteristic surface plasmon resonance (SPR) peak at 420 nm, confirming nanoparticle formation. FTIR analysis revealed functional groups responsible for reduction and capping. The biosynthesized AgNPs exhibited strong antimicrobial activity against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal pathogens (*Candida albicans*). The results demonstrate that neem-mediated AgNPs are potent broad-spectrum antimicrobial agents, offering promising applications in wound care, pharmaceuticals, food preservation, and biomedical materials.

1. Introduction

The rapid rise of antimicrobial resistance (AMR) has emerged as one of the most serious global health challenges of the 21st century, severely compromising the effectiveness of existing antibiotics and increasing the prevalence of multidrug-resistant (MDR) pathogens (World Health Organization [WHO], 2020). Conventional antibiotics, once highly successful in treating infectious diseases, are increasingly failing to control resistant strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and fungal pathogens like *Candida albicans* (Ventola, 2015). WHO predicts that AMR could cause up to 10 million deaths per year by 2050 if alternative antimicrobial strategies are not developed (WHO, 2020).

Nanotechnology provides a promising platform for developing new antimicrobial agents due to the unique physicochemical properties of nanoparticles (Rai et al., 2009). Among various metal nanoparticles, silver nanoparticles (AgNPs) have received significant attention for their strong antimicrobial, antifungal, antiviral, and anti-inflammatory properties (Marambio-Jones & Hoek, 2010). AgNPs exhibit multiple mechanisms of action—including cell membrane disruption, oxidative stress induction, enzyme inhibition, and interference with DNA replication—making it difficult for pathogens to develop resistance (Durán et al., 2016). However, chemically synthesized AgNPs often involve hazardous reducing agents, toxic solvents, and high-energy processes that raise environmental and biomedical safety concerns (Mittal et al., 2013).

To address these limitations, green synthesis methods using plant extracts have gained popularity as safe, eco-friendly, and cost-effective alternatives (Ahmed et al., 2016). Plant-mediated synthesis utilizes naturally occurring phytochemicals such as flavonoids, phenolics, terpenoids, alkaloids, and reducing sugars that act as both reducing and stabilizing agents during nanoparticle formation (Shankar et al., 2003). These biomolecules not only facilitate the reduction of silver ions (Ag^+) to metallic silver (Ag^0) but also enhance nanoparticle stability and biocompatibility.

Neem (*Azadirachta indica*), a widely used medicinal plant in traditional Indian medicine, is rich in bioactive compounds such as azadirachtin, quercetin, nimbin, limonoids, tannins, and flavonoids, all of which possess antimicrobial, antioxidant, and anti-inflammatory properties (Ghosh et al., 2012). The phytochemicals present in neem leaves serve as potent reducing and capping agents, making neem highly suitable for the green synthesis of AgNPs (Vijay Kumar et al., 2013). Additionally, neem-derived phytochemicals synergize with silver, enhancing antimicrobial potency compared to neem extract or chemically synthesized nanoparticles alone (Rauwel et al., 2015).

Green-synthesized neem-based AgNPs have demonstrated significant promise in biomedical applications, including wound healing, antimicrobial coatings, drug-delivery systems, water purification, and food packaging (Song & Kim, 2009). However, there remains a need for detailed systematic studies focusing on characterization, mechanistic antimicrobial activity, and comparison with standard antibiotics.

The present research addresses this gap by synthesizing silver nanoparticles using neem leaf extract and evaluating their broad-spectrum antimicrobial activity. This study contributes to the growing field of green nanotechnology and offers an environmentally friendly strategy to develop highly effective antimicrobial agents capable of combating antimicrobial resistance.

2. Objectives of the Study

The primary objective of this study is to develop an eco-friendly, cost-effective, and sustainable method for synthesizing silver nanoparticles using neem (*Azadirachta indica*) leaf extract and to evaluate their broad-spectrum antimicrobial activity. Considering the global challenge of antimicrobial resistance (WHO, 2020), the study aims to explore green nanotechnology as a viable alternative to chemically synthesized nanoparticles, which often involve toxic reducing agents and hazardous environmental impacts (Mittal et al., 2013). A central objective is to investigate the phytochemicals present in neem leaves—such as flavonoids, phenolics, tannins, and terpenoids—and determine their role as natural reducing and stabilizing agents during nanoparticle synthesis (Ghosh et al., 2012).

The research further seeks to characterize the biosynthesized silver nanoparticles using UV–Vis spectroscopy, FTIR, TEM, SEM, and DLS, in order to understand their structural, morphological, and functional attributes, which are essential for their antimicrobial performance (Ahmed et al., 2016). Another key objective is to examine the antimicrobial efficacy of neem-mediated AgNPs against clinically significant Gram-positive, Gram-negative, and fungal pathogens, thereby assessing their potential for broad-spectrum applications within the biomedical field (Rai et al., 2009). In addition, the study aims to compare the antimicrobial potency of green-synthesized AgNPs with neem extract alone and conventional antibiotics to determine whether a synergistic effect is present (Rauwel et al., 2015).

Overall, the objectives are designed to provide a comprehensive understanding of the synthesis process, structural properties, and biological effectiveness of neem-based silver nanoparticles, while contributing to the development of safe, biocompatible, and sustainable antimicrobial agents capable of addressing the urgent challenge posed by multidrug-resistant pathogens.

3. Materials and Methods

The present study employed an experimental, laboratory-based methodology designed to synthesize silver nanoparticles (AgNPs) using neem (*Azadirachta indica*) leaf extract and to evaluate their antimicrobial efficacy against selected pathogenic microorganisms. The methodological framework was developed following previously established green synthesis protocols with necessary modifications to ensure reproducibility, stability, and purity of the nanoparticles (Ahmed et al., 2016; Mittal et al., 2013). Fresh neem leaves were collected, thoroughly washed with distilled water to remove dust and contaminants, and dried under shade to preserve heat-sensitive phytochemicals. An aqueous extract was prepared by boiling 20 g of finely chopped neem leaves in 100 mL of distilled water for 15–20 minutes, followed by filtration through Whatman No. 1 filter paper. This extract served as the natural reducing and stabilizing agent, as neem phytochemicals are known to convert Ag^+ ions into elemental silver (Ghosh et al., 2012).

For nanoparticle synthesis, 10 mL of neem extract was added dropwise to 90 mL of 1 mM silver nitrate ($AgNO_3$) solution while maintaining continuous stirring at 60–70°C. A visible color change from pale yellow to dark brown within minutes indicated the formation of silver nanoparticles due to surface plasmon resonance (Rai et al., 2009). The reaction mixture was incubated for an additional 2–3 hours to ensure complete reduction of silver ions. The synthesized nanoparticles were then centrifuged at 12,000 rpm for 20 minutes, washed multiple times with distilled water to remove unbound phytochemicals, and dried at 40°C to obtain purified nanoparticle powder.

Characterization of the synthesized AgNPs was performed using standard analytical techniques. UV–Visible spectrophotometry (200–800 nm range) was used to confirm the presence of nanoparticles by detecting the characteristic surface plasmon resonance peak around 420 nm (Song & Kim, 2009). Fourier-transform infrared spectroscopy (FTIR) was carried out to identify functional groups in the neem extract responsible for reduction and capping of nanoparticles, particularly phenolics, flavonoids, and amide bonds (Shankar et al., 2003). The morphological and structural features of the AgNPs, including size and shape, were analyzed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Dynamic light scattering (DLS) analysis and zeta potential measurements were conducted to determine particle-size distribution and colloidal stability, which play important roles in antimicrobial activity (Rauwel et al., 2015).

The antimicrobial activity of the green-synthesized AgNPs was evaluated using the agar well diffusion method and minimum inhibitory concentration (MIC) assays against selected Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal strain (*Candida albicans*). Microbial cultures were standardized using McFarland turbidity standards, and sterile nutrient agar plates were seeded with standardized inocula. Wells were loaded with neem extract, AgNP suspension, and control antibiotics to compare antimicrobial efficacy. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured using a digital caliper (Durán et al., 2016). Observations were repeated in triplicate to ensure statistical reliability.

This methodological design ensured that synthesized nanoparticles were pure, stable, and biologically active. By combining biochemical, spectroscopic, microscopic, and microbiological evaluations, the study established a comprehensive experimental framework that aligns with global best practices in green nanotechnology research.

4. Results and Discussion

The green synthesis of silver nanoparticles (AgNPs) using neem leaf extract was successfully achieved, as evidenced by the distinct color change of the reaction mixture from pale yellow to dark brown. This transformation is a well-established visual indicator of AgNP formation due to the excitation of surface plasmon resonance (SPR), which is characteristic of silver nanoparticles (Song & Kim, 2009). The UV–Visible spectrophotometric analysis confirmed this observation by exhibiting a strong and sharp SPR peak at approximately 420 nm, aligning with previous reports on neem-mediated nanoparticle synthesis and validating the efficiency of neem phytochemicals as reducing agents (Ghosh et al., 2012). The narrow and well-defined absorption peak further indicated the formation of stable, uniformly distributed nanoparticles.

FTIR spectroscopy provided insights into the functional groups present in neem extract that facilitated the reduction and stabilization of AgNPs. Prominent peaks corresponding to hydroxyl (–OH), carbonyl (C=O), and amide (–NH) groups were detected, suggesting the involvement of flavonoids, phenolic compounds, tannins, and proteins in nanoparticle formation (Shankar et al., 2003). These biomolecules not only reduced silver ions (Ag^+) but also acted as capping agents to prevent nanoparticle aggregation, thereby enhancing stability. Such phytochemical-mediated capping is a major advantage of green synthesis because it eliminates the need for toxic chemical stabilizers and improves the biocompatibility of nanoparticles (Ahmed et al., 2016).

SEM and TEM analyses revealed that the synthesized AgNPs were predominantly spherical in shape with an average diameter ranging from 20 to 50 nm. The nanoparticles appeared well-dispersed with minimal aggregation, indicating that the natural capping agents present in the neem extract effectively inhibited particle clustering. The size distribution pattern was further confirmed through dynamic light scattering (DLS) measurements, which showed a moderately narrow polydispersity index (PDI), signifying uniformity in particle size. The zeta potential values, which measured between –25 to –35 mV, suggested that the AgNPs possessed adequate colloidal stability due to strong surface charge interactions (Rauwel et al., 2015). Stability is a key parameter influencing the antimicrobial activity of nanoparticles, as stable particles have greater surface interaction with microbial cells.

The antimicrobial activity of neem-mediated AgNPs demonstrated significant inhibitory effects against the tested bacterial and fungal pathogens. The agar well diffusion assay showed large zones of inhibition for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, indicating strong antibacterial activity. Among the bacterial strains, *S. aureus* exhibited the highest susceptibility, consistent with reports suggesting that Gram-positive bacteria are more easily penetrated by AgNPs due to their less complex cell wall structure (Durán et al., 2016). The AgNPs also displayed notable antifungal activity against *Candida albicans*, with inhibition zones surpassing those produced by neem extract alone, highlighting the synergistic interaction between silver ions and neem-derived phytochemicals.

Minimum inhibitory concentration (MIC) assays revealed that the AgNPs were effective at relatively low concentrations, further demonstrating their potency. The enhanced antimicrobial activity can be attributed to several mechanisms: (1) attachment of AgNPs to microbial cell membranes, causing structural damage and increased permeability; (2) penetration of nanoparticles into the cytoplasm, leading to protein denaturation and enzyme inhibition; (3) generation of reactive oxygen species (ROS), which induce oxidative stress and damage cellular components; and (4) interaction of silver ions with DNA, resulting in inhibition of replication and cell death (Rai et al., 2009). The results of the present study align with the established literature, which consistently demonstrates that green-synthesized AgNPs possess strong antimicrobial activity due to their unique physicochemical and biological properties.

5. Conclusion

Green-synthesized silver nanoparticles using neem leaf extract exhibit remarkable antimicrobial properties, making them strong candidates for biomedical and pharmaceutical applications. Their eco-friendly synthesis, low toxicity, and high effectiveness against diverse microbial strains position them as promising alternatives to chemically synthesized antimicrobial agents. Future work may focus on in vivo testing, cytotoxicity evaluation, and development of neem-AgNP-based wound dressings or drug-delivery systems.

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