

Green Copper Oxide Prepared by *Annona squamosa*: Synthesis, Characterization and Its Biomedical Application

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Abstract

Introduction:

Green synthesis of nanoparticles has emerged as an eco-friendly and sustainable alternative to conventional chemical methods. Copper oxide nanoparticles (CuO NPs) are widely recognized for their antimicrobial and anti-inflammatory potential. Plant-based synthesis using *Annona squamosa* provides an efficient approach due to its rich phytochemical composition.

Aim:

To synthesize copper oxide nanoparticles using *Annona squamosa* extract and evaluate their anti-inflammatory and antimicrobial activities.

Materials and Methods:

The plant material was processed to obtain a crude extract, which acted as a reducing and stabilizing agent for nanoparticle synthesis. The synthesized CuO nanoparticles were subjected to anti-inflammatory and antimicrobial evaluation using standard experimental methods.

Results:

The nanoparticles demonstrated moderate anti-inflammatory activity compared to the standard drug diclofenac. Significant antimicrobial activity was observed against

Staphylococcus aureus, *Escherichia coli*, and *Candida albicans*, with the highest inhibition against the fungal strain.

Discussion & Conclusion:

The study confirms that green synthesized CuO nanoparticles possess promising antimicrobial and moderate anti-inflammatory properties. These findings highlight their potential application in biomedical fields, though further studies are required to enhance their efficacy and validate clinical use.

Introduction

Nanotechnology has significantly transformed the field of biomedical science by enabling the development of nanoscale materials with enhanced physicochemical and biological properties. Among these, metal oxide nanoparticles, particularly copper oxide nanoparticles (CuO NPs), have gained considerable attention due to their broad-spectrum antimicrobial activity, chemical stability, and cost-effectiveness (1,2). Their unique properties, such as high surface area-to-volume ratio and enhanced reactivity, contribute to their effectiveness in various biomedical and environmental applications.

Conventional methods of nanoparticle synthesis, including physical and chemical approaches, often involve toxic reagents, high energy consumption, and environmental hazards. These limitations have led to the growing interest in green synthesis techniques that utilize biological resources such as plant extracts, microorganisms, and enzymes (3,4). Plant-mediated synthesis is especially advantageous due to its simplicity, scalability, and eco-friendly nature. Phytochemicals such as flavonoids, phenolic acids, terpenoids, and alkaloids act as natural reducing and stabilizing agents, facilitating nanoparticle formation while enhancing their biological functionality (5,6).

Annona squamosa, commonly known as custard apple, is a medicinal plant rich in bioactive compounds with known antimicrobial, antioxidant, and anti-inflammatory properties (7). The use of *Annona squamosa* extract in nanoparticle synthesis not

only promotes green chemistry but also improves the therapeutic potential of the resulting nanoparticles.

Copper oxide nanoparticles exhibit potent antimicrobial effects against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi. Their mechanism of action involves the generation of reactive oxygen species (ROS), disruption of microbial cell membranes, and interference with cellular metabolic processes (8,9). Furthermore, their potential to modulate inflammatory responses makes them valuable in the development of anti-inflammatory agents (10).

In this context, the present study aims to synthesize CuO nanoparticles using *Annona squamosa* extract and evaluate their anti-inflammatory and antimicrobial activities, thereby exploring their potential as alternative therapeutic agents.

Materials and Methods

Fresh plant material of *Annona squamosa* was collected and thoroughly washed with running tap water to remove impurities. The material was then shade dried for a period of 30 days to preserve its phytochemical constituents. The dried plant material was ground into a fine powder using a mechanical grinder, and a crude extract was prepared for nanoparticle synthesis. This extract was utilized as both a reducing and stabilizing agent in the green synthesis process.

The synthesis of copper oxide nanoparticles was carried out by reacting the plant extract with an appropriate copper precursor under controlled laboratory conditions, resulting in the formation of CuO nanoparticles. The synthesized nanoparticles were then subjected to biological evaluation. Anti-inflammatory activity was assessed using standard in vitro methods and compared with a reference drug, diclofenac. Antimicrobial activity was evaluated against selected microbial strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, using agar well diffusion techniques. The zones of inhibition were measured to determine the antimicrobial efficacy of the synthesized nanoparticles.

Results

The synthesized copper oxide nanoparticles exhibited notable biological activity in both anti-inflammatory and antimicrobial assays. In the anti-inflammatory evaluation, the standard drug diclofenac demonstrated a significantly higher inhibition rate of approximately 85–90%, whereas the CuO nanoparticles showed moderate inhibition ranging from 35–40%. Although lower than the standard, the nanoparticles still exhibited appreciable anti-inflammatory potential.

In antimicrobial studies, the nanoparticles displayed effective inhibitory activity against all tested microorganisms. The zones of inhibition for *Staphylococcus aureus* and *Escherichia coli* were approximately 23 mm, indicating significant antibacterial activity. Notably, *Candida albicans* exhibited the highest sensitivity, with a zone of inhibition reaching up to 35 mm. At lower concentrations, the antimicrobial activity decreased, with inhibition zones ranging between 11–16 mm. These results collectively demonstrate the promising antimicrobial potential of green synthesized CuO nanoparticles.

Discussion

The present study highlights the effectiveness of green synthesis in producing biologically active copper oxide nanoparticles using *Annona squamosa* extract. The phytochemical constituents present in the plant extract play a crucial role in the reduction and stabilization of nanoparticles, thereby influencing their size, morphology, and biological activity (5,7). These naturally derived compounds enhance the functionality of nanoparticles and contribute to their improved therapeutic potential.

The moderate anti-inflammatory activity observed in this study, when compared to diclofenac, may be attributed to differences in mechanism of action, dosage, and bioavailability. However, the presence of flavonoids and phenolic compounds in the plant extract likely contributes to the inhibition of inflammatory mediators, thereby

imparting measurable anti-inflammatory effects (10,11). This suggests that further optimization of synthesis parameters could enhance their anti-inflammatory efficacy.

The antimicrobial activity demonstrated by CuO nanoparticles is consistent with previously reported studies. The nanoparticles exert their effects through multiple mechanisms, including the generation of reactive oxygen species, disruption of microbial cell membranes, protein denaturation, and interference with DNA replication (8,12). The pronounced activity against *Candida albicans* indicates strong antifungal potential, which is particularly significant given the increasing prevalence of fungal infections and resistance to conventional antifungal agents.

Additionally, green synthesized nanoparticles offer several advantages over chemically synthesized counterparts, including reduced toxicity, enhanced biocompatibility, environmental sustainability, and cost-effectiveness (3,4,13). These characteristics make them highly suitable for applications in nanomedicine, drug delivery, and environmental remediation.

Despite these promising findings, further research is required to evaluate the long-term stability, toxicity, and in vivo efficacy of these nanoparticles. Advanced characterization techniques and clinical studies will be essential to fully establish their potential for therapeutic applications.

Conclusion

The study successfully demonstrates the green synthesis of copper oxide nanoparticles using *Annona squamosa* extract and confirms their significant antimicrobial and moderate anti-inflammatory activities. The strong inhibitory effect against *Candida albicans* highlights their potential as effective antifungal agents. The eco-friendly synthesis approach further enhances their applicability in biomedical research. However, further studies are necessary to optimize their performance and validate their clinical utility.

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