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Mechanism of action of Antimicrobial Activity of Copper-oxide Nanoparticle Synthesized using Root Extract of Gloriosa Superba - An In-vitro Study

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ABSTRACT

Gloriosa superba, a tropical plant known for its medicinal properties, has been utilized traditionally to treat various ailments due to its bioactive alkaloids. Recent advances in nanotechnology have demonstrated the potential of copper oxide nanoparticles (CuONPs) in antimicrobial applications. This study investigates the antimicrobial mechanisms of CuONPs synthesized using the root extract of G. superba. Methods: Copper oxide nanoparticles were synthesized using an aqueous extract of G. superba root tubers. The synthesis involved adding the plant extract to a copper sulfate solution, followed by agitation and filtration. Antimicrobial activities were assessed using the agar well diffusion method, time kill curve assay, and assays for cytoplasmic and protein leakage. Results: CuONPs exhibited significant antimicrobial activity against a range of bacterial and fungal pathogens, including Staphylococcus aureus, Escherichia coli, and Candida albicans. The nanoparticles showed dose-dependent inhibition, with the largest zones of inhibition observed against Pseudomonas sp. The time kill curve assay revealed rapid reduction in microbial counts, and leakage assays indicated that CuONPs disrupt microbial cell membranes, leading to cellular damage. Discussion: The antimicrobial efficacy of G. superba-mediated CuONPs aligns with previous research on green synthesis and antimicrobial properties of copper nanoparticles. The disruption of cell membranes and leakage of cellular contents provide insights into the nanoparticles' mechanism of action. The findings highlight the potential of utilizing G. superba in developing sustainable antimicrobial agents. Conclusion: This study confirms the strong antimicrobial properties of CuONPs synthesized using G. superba root extract. The promising in vitro results suggest that these nanoparticles could be developed into effective antimicrobial agents, offering a sustainable alternative to conventional antibiotics.

1. INTRODUCTION

Gloriosa superba, belonging to the Liliaceae family, is a partially woody herbaceous vine originally from tropical Africa but now widely cultivated as an ornamental plant in tropical Asia and beyond. It is hailed as the state flower in the Southern State of Tamil Nadu India.[1] It sprouts annually from its tuberous underground stems during the rainy season, thriving at altitudes of up to 6000 ft. Alongside G. superba, there are several related species such as G. simplex and G. grandiflora. However, propagating Glory lily poses challenges due to its low seed production and susceptibility to soil-borne diseases. In traditional medicine, the tuberous roots of G. superba are utilized to alleviate conditions like arthritis. Its significance in ethanopharmacology extends to treating ailments such as

ulcers, piles, and certain types of cancer, attributed to the presence of alkaloids, particularly colchicine, throughout the plant.[2] Additionally, Gloriosa superba exhibits antimicrobial properties against various bacteria and fungi. The need for newer and sustainable antimicrobial agents is the need of the hour especially owing to the very serious threat of antimicrobial resistance.

Nanotechnology is a frontier discipline spanning fields such as medicine, electronics, energy, and environmental science.[3,4] It holds considerable promise in revolutionizing wound healing and various positive effects within the medical domain. The distinctive attributes of nanotechnology present a compelling avenue for addressing challenges such as microbial resistance. Among the vast array of nanomaterials, copper oxide nanoparticles (CuONPs) have emerged as exceptionally relevant entities in the arena of wound healing. Copper, acknowledged as an indispensable trace element, assumes pivotal roles in various physiological processes, including angiogenesis, skin regeneration, and immune modulation. CuONPs, endowed with unique physicochemical properties, facilitate the efficient delivery of copper ions to wound sites, thereby fostering the process of wound repair. Moreover, these nanoparticles exhibit robust antimicrobial characteristics, further enhancing their potential in averting wound infections.[4,5]

The clinical efficiency of copperoxide nanoparticles accelerates research toward development of various medication that could prove extremely vital to the future of medicine. The herbal derivatives of the tuber and seed of the Gloriosa superba plant is a well known part of the traditional rural medicine. The combined effect of the Copperoxide Nanoparticles and the extracts from the root tubers of the Gloriosa superba has not been explored in detail in literature. An honest attempt has been made to extract medically relevant information through various invitro studies.

Materials And Methodology

Preparation of Plant extract

Synthesis of G. superba- Mediated Copperoxide Nanoparticles

The root tubers of the G.superba were made into a coarse powder. 2g of the powdered extract was added to 100 mL of distilled water. The extract underwent filtration and was refrigerated at 4oC. Copper sulphate solution was prepared by adding (0.016g in 90mL distilled water). An orbital shaker was employed to subject the mixture to agitation at 120rpm for 24 hours. Nanoparticles were stored in hermetically sealed Eppendorf Tube.

Antimicrobial Activity:

Antibacterial and Antifungal Activity

The agar well diffusion technique was employed to effect the antimicrobial activity of copperoxide nanoparticle synthesized using root extract of Gloriosa superba. The preparation of the Mueller Hinton agar plates was followed by sterilization in an autoclave at 1210C for 20 minutes. Sterile petri plates were filled and allowed to reach the room temperature.[6] Bacterial suspensions of the species selected for the study were carefully inoculated in an even fashion over the agar plates. Wells were prepared over the agar plates using polystyrene tips of 9mm diameter. The copperoxide nanoparticles extract of the root extract was added to the wells in different concentration.[7,8] The control well was filled with an standard antibiotic (Fungi- Flucanazole and Bacterial – Amoxyrite). Incubation of the plates occurred at 37oC for 24 hours (bacterial) and 48 hours (fungal). Antimicrobial activity was fathomed by measuring the zone of inhibition effected by the extract in the well and documented in millimeters.

Time Kill Curve Assay

The bactericidal property of the extract is analyzed using the time kill curve assay. Here the microorganisms selected in the study were subjected to cultivation in the Mueller Hinton broth with different concentrations of copperoxide nanoparticles (25µg/mL, 50µg/mL and 100µg/mL). Their growth was monitored over specific intervals of time namely 1-5 hours. In order to achieve reliable data a pre-incubatory period of 5 hours was provided for each microorganism so as to reach the stable mid-log bacterial growth phase. For this study an inoculum of 0.5 McFarland pathogen was created in the sterile phosphate buffered saline. This inoculum was diluted in 15mL of antimicrobial free Mueller Hinton Broth that's preheated to 37oC. 90 µL of the resultant mixture is spread evenly in a 96 well ELISA plate and 10 µL of the extract is added to each well at different concentrations.

Cytoplasmic Leakage Analysis

The cell membrane integrity of the bacterial cells were tested using the cytoplasmic leakage. 10ml of the bacterial suspensions of the bacteria analyzed werecentrifuged for 15minutes at 3500 RPM. A phosphate buffer with pH of 7.0 was introduced and shaken for 24 hours. Various concentrations of the copperoxide nanoparticles extract of the root extract of G.superba was added and centrifuged again at 3500 rpm for 15mins. The supernatant is extracted and analysed.

Protein Leakage Analysis

The protein leakage analysis was performed using the Bradford Assay. The selected bacterial species were subjected to the gradient of concentrations of the extract for 24 hours namely 25µg/mL, 50µg/mL and 100µg/mL.

Centrifugation of the bacterial suspension at 6000 rpm was performed for a total of 15 mins thereby yielding a supernatant phase. 200µL of the yield was added to the 96 well ELISA along with 800µL of Bradford agent. The sample is maintained in incubation for 10 mins at a dark environment. Amoxicillin was employed as the standard against which the samples were tested. The sample was tested at an optical density of 595 nm.

Results: Antibacterial activity of G.superba mediated copperoxide nanoparticles

The antibacterial activity of copperoxide nanoparticles through the agar well diffusion test has yielded promising results. The organisms against which the tests were carried out are those which are predominantly present in the oral wounds. The zone of inhibition reveals good antibacterial activity against S. mutans, S. aureus, Lactobacillus sp., E. faecalis, E. coli, Pseudomonas sp.. C. albicans also displayed a zone of inhibition comparable to standard and control. Table 1 shows the antimicrobial activity of the G.superba mediated copperoxide nanoparticles. Zone of inhibition appreciated using each organism at various concentrations have been displayed in Figure 1 image pallet. A dose-dependant effect was observed when used against Pseudomonas sp.

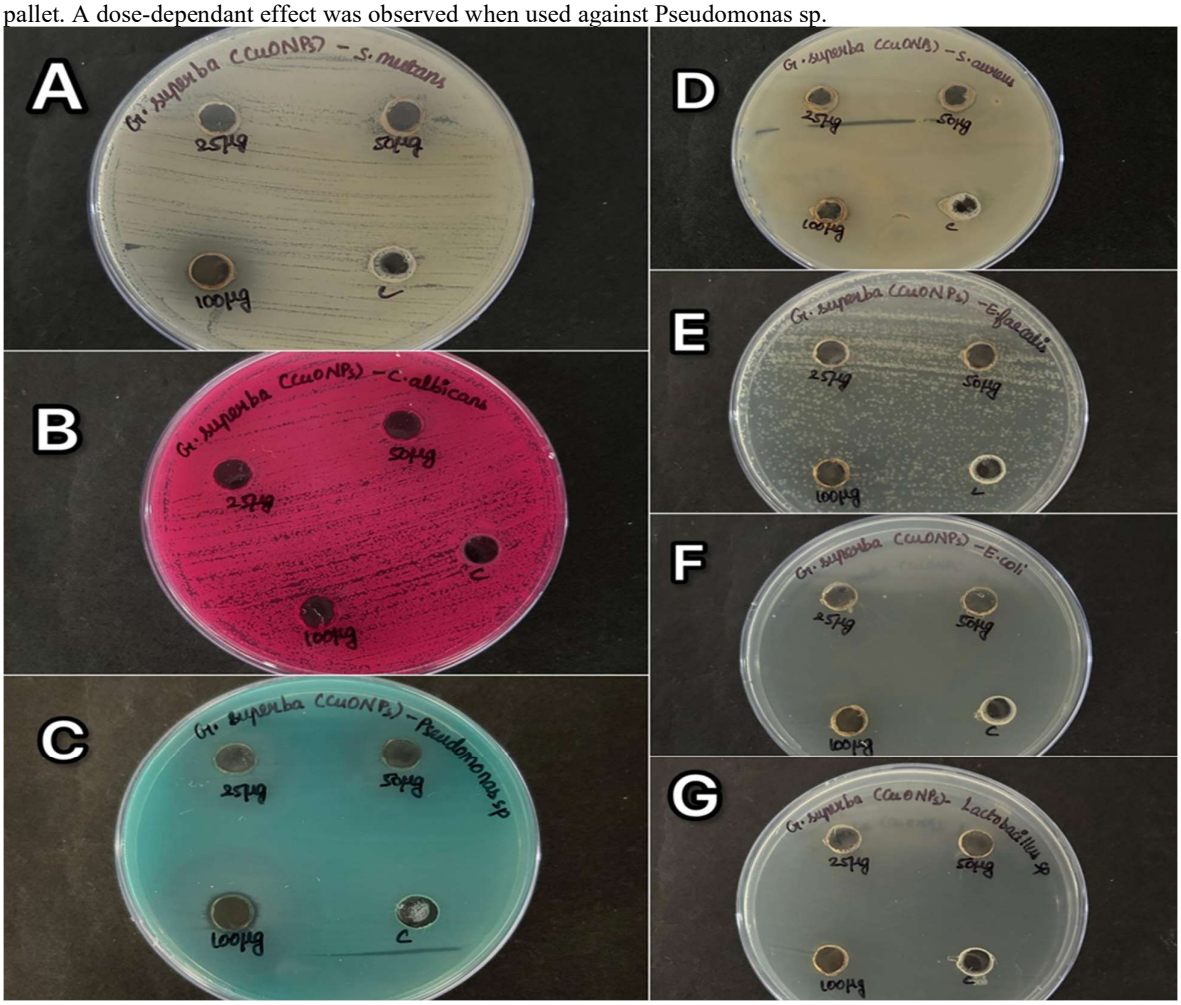

Figure 1- Zone of inhibition expressed by G.superba mediated copperoxide nanoparticles
(A) S. mutans (B) C. albincans (C) Psuedomonas sp. (D) S. aureus (E) E. faecalis (F) E. coli (G) Lactobacillus sp.

Time-kill curve assay

The time kill curve assay was carried out for 6 wound pathogens namely S. mutans, S. aureus, Lactobacillus sp., E. faecalis, E. coli, Pseudomonas sp.. The results attest a concentration dependent antimicrobial activity as compared to the control. The microbial counts rapidly diminished after the 1 hour mark in all the organisms. The extract proves very effective in reducing the counts within 1 hour against the Pseudomonas sp. Group. Figure 2 portrays the time-kill curve assay for each microorganism. A dose dependent decrease of the microbial count was consistent across all organisms.

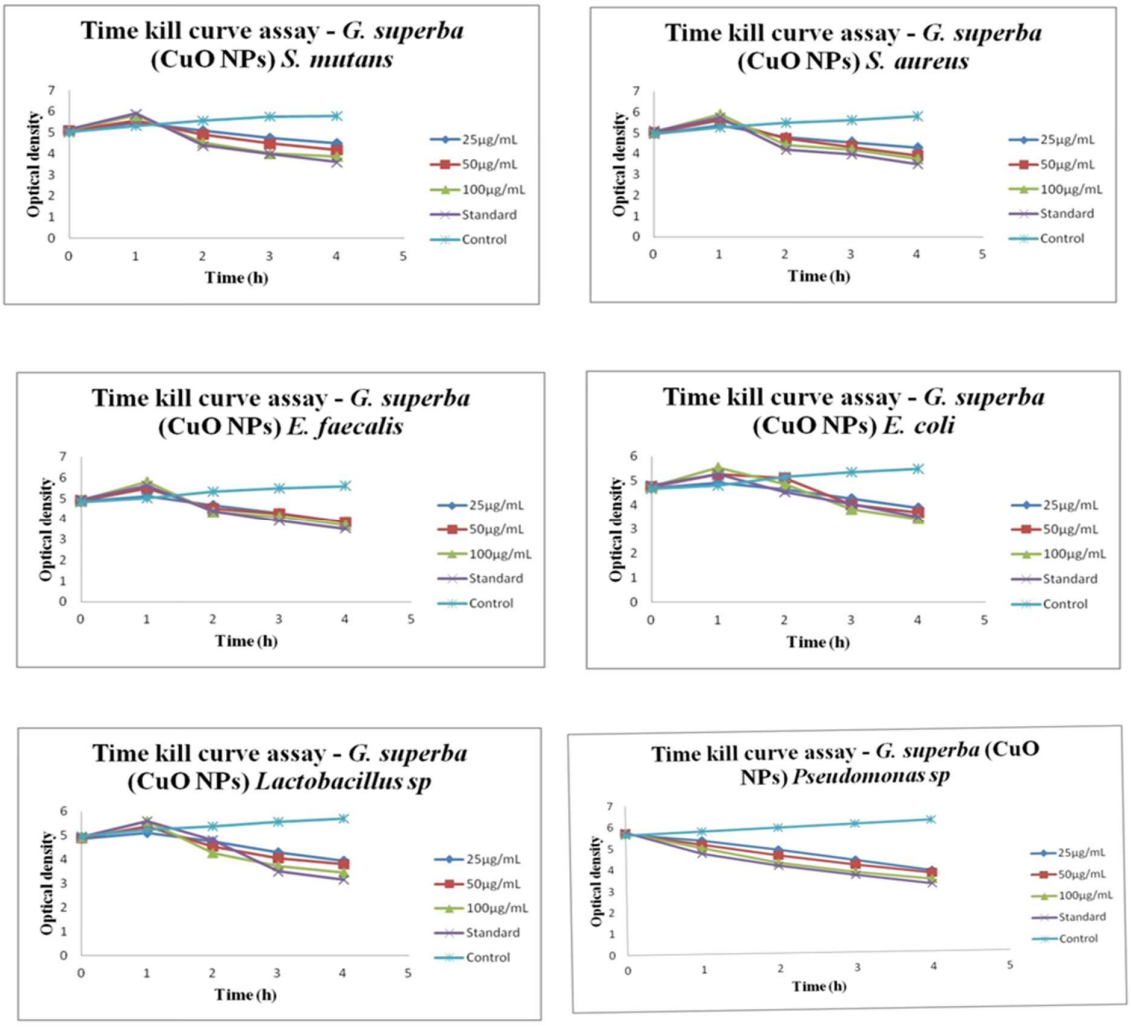

Figure 2- The time-kill curve assay of G.superba mediated copperoxide nanoparticles against wound pathogens.

Protein and cytoplasmic leakage analysis

The integrity of the plasma membrane after being subjected to the green- synthesised nanoparticles of G. superba has been studied extensively using the protein and cytoplasmic leakage assay. This reveals the ability of the membrane disruption that causes solid proof of microbial cell destruction thereby contributing to the antibacterial property. The test was carried out against S. mutans, S. aureus, Lactobacillus sp., E. faecalis, E. coli, Pseudomonas sp. A concentration dependant destruction and leakage of both cytoplasm and protein is a testament to the antibacterial properties of the product.Figure 3 and 4 reveal the results graphically.

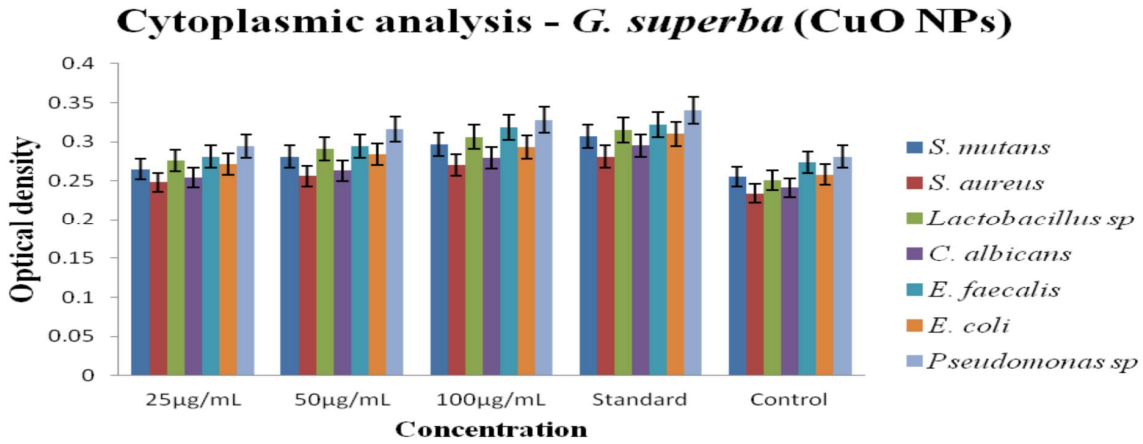

Figure 3: Represents the cytoplasmic leakage encounter with the use of copperoxide nanoparticles produced by green synthesis using G. superba

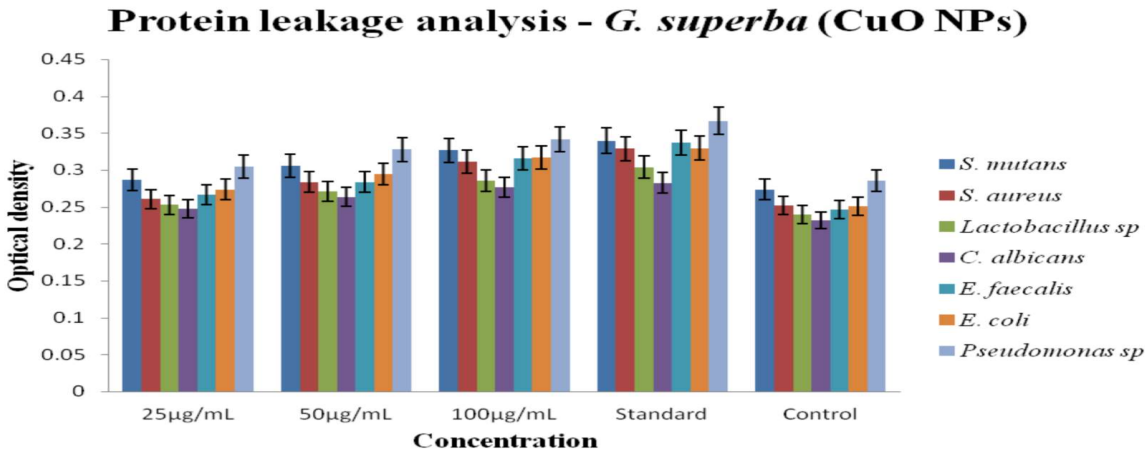

Figure 4 : Represents the findings of the Protein leakage analysis demonstrating optimal protein leakage to act as a potent antimicrobial

Discussion:

The copperoxide nanoparticles synthesis assisted by extracts of *Gloriosa superba* is very efficient. Numerous studies exist in literature that test the green synthesis and methodology mentioned in our study. The focus of the present study is to extract the effects of Gloriosa superba in a reliable and stable concoction thereby the providing medicinally relevant data in terms of antibacterial activity.

The agar well diffusion studies performed reveals a strong antibacterial dose dependant effect on Pseudomonas sp. and comparable antibacterial effects against S. mutans, S. aureus, Lactobacillus sp., E. faecalis, E. coli . The findings of the present study are in line with the findings of many well cited literature pieces. [9-12]

The use of protein and cytoplasmic leakage in the current study has proved to establish the exact mechanism of action of the extract. This combined with the time kill curve study proves to establish the antimicrobial property of the root extract assisted synthesis of the copper oxide nanoparticles, on solid evidence. These studies have been performed earlier to obtain scientific evidence in earlier studies using copperoxide nanoparticles.[13-14]

Limitations of study: The study has assessed the wound pathogens closely associated to Maxillofacial infections. The expanse of pathogens that affect humans should be included to provide a holistic medication.[15-16] The results of the study could be more clinically relevant had it been an invivo type study. However promising invitro study could further research in this arena.

Conclusion:

The copper oxide nanoparticles synthesised using extracts from *G. superba* have astonishing antibacterial property established through the various invitro studies performed in this study. With further research the plant can be harnessed to provide a sustainable antimicrobial product.

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