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Evaluating the Antibiotic Effectiveness of Green-Synthesized Copper Nanoparticles Against Soft Tissue and Skin Infections

Isha[1]; Pinku Chandra Nath[2]

[1]Graduate Teaching Assistant, College of Medical, Veterinary & Life Sciences, University of Glasgow.
[2]Assistant Professor, Uttaranchal University, Dehradun, Uttarakhand, India.
Email id: **nathpinku005@gmail.com**
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ABSTRACT:

The integration of traditional medicine with nanotechnology presents promising avenues for the development of novel treatments for skin and soft tissue infections (SSTIs) while simultaneously addressing the growing concern of antimicrobial resistance. *Acanthospermum australe.* Kuntze, a medicinal plant traditionally employed by indigenous communities in northeastern Argentina to manage SSTIs, was utilized in this study. Copper nanoparticles (CuNPs), were synthesized. The antimicrobial properties of the CuNP solution were tested against common pathogens responsible for SSTIs, alongside cytotoxicity assessments on peripheral blood mononuclear cells. In comparison to its synthesis precursors, the CuNP solution demonstrated superior antimicrobial efficacy and reduced cytotoxicity. The findings revealed that the antimicrobial effects of the CuNPs were attributed to the copper, rather than the bioactive compounds present in the plant extract. Additionally, the plant extract was crucial in stabilizing the CuNPs and influenced cytotoxic and immune-modulatory responses.

Keywords: Green-Synthesized Copper Nanoparticles; Antibiotic Effectiveness; Soft Tissue Infections

Skin Infections; Antimicrobial Activity; Nanotechnology in Medicine

Introduction:

The skin, the largest organ of the human body, functions as a critical component of the immune system, acting as the first barrier against microbial invasions. Skin and soft tissue infections are one of the most frequent reasons for visits to primary healthcare facilities. These infections encompass a diverse range of clinical conditions, varying in their etiologies and severity, from mild, localized infections to severe, life-threatening complications. The pathogenesis of SSTIs is complex and is driven by a dynamic interplay of microbial invasion, host immune responses, and environmental factors such as inflammation, oxidative stress, and impaired tissue repair. In immunocompetent individuals, SSTIs are primarily caused by bacterial and fungal pathogens, with the most commonly implicated bacteria being *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, including its methicillin-resistant strains (MRSA) (Lee et al. 2022).

Fungal infections, often caused by dermatophytes and yeasts, further complicate the clinical landscape of SSTIs. Dermatophytes, which possess the ability to invade keratinized structures such as the skin, hair, and nails, are mediated by keratinases and proteases, with genera like *Trichophyton*, *Microsporum*, and *Epidermophyton* being the principal culprits. Additionally, yeasts like *Candida* spp. and *Malassezia* spp. are common members of the human skin microbiota but can become opportunistic pathogens under conducive conditions. These fungal species contribute significantly to the overall burden of SSTIs (Song et al. 2022).

The growing prevalence of antimicrobial-resistant strains poses a significant challenge in managing SSTIs, leading to increased morbidity, mortality, and healthcare costs. The emergence of drug-resistant microorganisms, such as MRSA, underscores the urgent need for new antimicrobial agents that are not only effective but also cost-efficient. In recent years, the integration of traditional medicine with advanced technologies like nanotechnology has garnered attention as a promising avenue for developing novel therapeutic strategies. One such approach involves leveraging the antimicrobial properties of copper nanoparticles, which have demonstrated broad-spectrum activity against a wide array of pathogens (Rahman et al. 2022).

In this context, *Acanthospermum australe* (Loefl.) Kuntze, a medicinal plant traditionally used by indigenous communities globally to treat SSTIs, offers a potential natural resource for synthesizing CuNPs. This study aims to explore the synthesis of CuNPs using aqueous extracts of *A. australe* and evaluate their antimicrobial efficacy and cytotoxicity in the context of SSTIs (Ladio et al. 2019).

Nanotechnology has emerged as a critical field in modern research, offering innovative approaches for the manipulation and synthesis of materials at the nanoscale (1 nm = 10^{-9} m). Among its many applications, nanomedicine has become a prominent interdisciplinary science, combining principles

from physics, chemistry, biology, and medicine to develop advanced treatments for human diseases. This rapidly growing field has produced numerous nanoparticle-based products, many of which are already commercially available. Notably, metal nanoparticles, such as those made from copper, exhibit unique physical and chemical properties distinct from their bulk counterparts. As these nanoparticles decrease in size, they demonstrate enhanced surface-to-volume ratios, dispersion properties, and significantly increased antimicrobial activity (Roy et al. 2023).

Copper, in particular, is renowned for its potent antimicrobial properties coupled with low toxicity to mammalian cells, making it a valuable material in both traditional and modern medicine. Historically, copper compounds have been used to combat skin infections. More recently, copper nanoparticles have garnered considerable attention for their potential in treating skin and soft tissue infections (SSTIs), thanks to their superior antimicrobial efficacy (Parveen et al. 2023).

Traditional synthesis methods for nanoparticles typically rely on physical and chemical processes, which are often expensive and environmentally harmful. In response to these challenges, green synthesis methods have been developed, utilizing plants as eco-friendly alternatives. These plant-mediated techniques harness natural metabolites, primarily phenolic compounds, to facilitate nanoparticle formation without the need for hazardous chemicals. The use of plant-based synthesis is not only sustainable but also aligns with the growing interest in developing biocompatible materials for medical applications (Kumar et al. 2021).

Plants have long played a crucial role in traditional medicine across various cultures, and their therapeutic properties have been documented over centuries. Many medicinal plants are rich in phenolic compounds, such as flavonoids, which possess antimicrobial, anti-inflammatory, and antioxidant properties. These attributes make them particularly valuable for the treatment of SSTIs. *Acanthospermum australe* (Loefl.), is one such medicinal plant. *A. australe* for its ability to disinfect wounds and treat skin ulcers, among other uses. Similarly, copper has been used in traditional Indian medicine (Ayurveda) as a therapeutic agent for various infections, highlighting the deep historical connection between natural remedies and antimicrobial treatments (Li et al. 2017).

The convergence of traditional medicine and nanotechnology presents exciting opportunities for the development of novel therapeutic strategies. This combined approach not only enhances the treatment of SSTIs but also contributes to the global effort to combat antimicrobial resistance. In this study, we aimed to synthesize and characterize copper nanoparticles (AgNPs) using an aqueous extract of *Acanthospermum australe* (TAE), evaluate the phenolic and flavonoid content of the extract, assess its antioxidant activity, and determine the cytotoxicity and antimicrobial efficacy of the resulting AgNP solution and its synthesis components (Zhang et al. 2021).

Materials and Methods:

All experimental procedures adhered to guidelines established in the literature. The plant material, *Acanthospermum australe* (Loefl.), was hand-harvested. C.V. Raman University's experts confirmed the taxonomic identification of the species. University, India. The collected leaves were dried at room temperature, protected from sunlight, and stored under sterile conditions for later use.

The aqueous extract was prepared by boiling 50 g of finely comminuted plant material by following standard literature-reported protocols (Roy et al. 2023). The microbial strains were obtained from the culture collection.

The total phenolic content of TAE was measured using the Folin-Ciocalteu method, with results expressed as mean gallic acid equivalent. Total flavonoid content was also determined using the standard literature reports (Jung et al. 2017).

The copper nanoparticles were synthesized following established green synthesis protocols, with modifications to optimize the stability and size of the nanoparticles. Specifically, an aqueous solution of TAE served as both the reducing and capping agent for the synthesis of CuNPs, using copper nitrate as the precursor. The Scanning Electron Microscopy was performed for characterization (Nagar et al. 2018).

The antioxidant potential of copper nanoparticles was assessed using two assays:

- **Ferric reducing antioxidant power (FRAP):** Antioxidant activity was measured following the standard literature-reported protocol (Contino et al. 2021).
- **DPPH radical scavenging assay:** The antiradical capacity was determined according to the method of Molyneux, with modifications. The effective concentration (EC_{50}) required to reduce the initial DPPH concentration by 50% was calculated, alongside the percentage of DPPH inhibition (Azzaoui et al. 2022).

The cytotoxicity of TAE, $CuNO_3$, and CuNPs was evaluated using peripheral blood mononuclear cells (PBMCs), following established methods with modifications. Paclitaxel (2.5 µg/mL) served as the positive control, while untreated cells served as the negative control (Wang et al. 2010).

The minimum inhibitory concentration (MIC) for TAE, $CuNO_3$, and CuNPs was determined using the broth microdilution method. The Minimum Fungicidal and Bactericidal Concentration (MFC and MBC) of TAE, $CuNO_3$, and CuNPs were assessed according to established protocols, with modifications (Oshim et al. 2016).

Results:

The extraction process for TAE resulted in approximately 13% yield. The extract's total phenolic content was measured at 120.94 ± 2.52 mg GAE/g LE, while its flavonoid content was 21.45 ± 0.28 mg QE/g LE.

During the green synthesis process, the solution exhibited a rapid color change from pale yellow to dark brown, occurring within a few minutes, signifying the successful formation of copper nanoparticles. Scanning Electron Microscopy (SEM) images revealed that the nanoparticles were spherical and non-agglomerated. Using ImageJ software, the diameters of at least 100 nanoparticles were measured, and statistical analysis performed via Origin software indicated an average size of 17 ± 3 nm (indicated in Figure 1) (Saha et al. 2017).

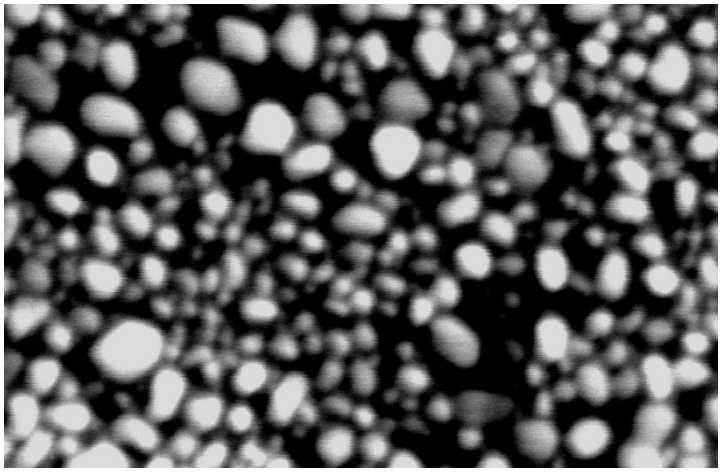

Figure 1: SEM Image of the synthesized copper nanoparticles.

Table 1 presents the findings from the FRAP and DPPH assays conducted on TAE, $CuNO_3$, and CuNPs.

Table 1: Results of FRAP and DPPH assays conducted on TAE, $CuNO_3$, and CuNPs.

Specimen	**DPPH**		**FRAP**
	EC_{50}	**% Inhibition**	
TAE	310.48 ± 0.56	78.32 ± 0.19	598 ± 8
CuNO3	Not Determined	3.72 ± 0.11	00
CuNPs	Not Determined	36.42 ± 0.16	281 ± 3

Figure 2 illustrates the cytotoxic effects of TAE, $CuNO_3$, and CuNPs on PBMCs following 24 and 72 hours of incubation.

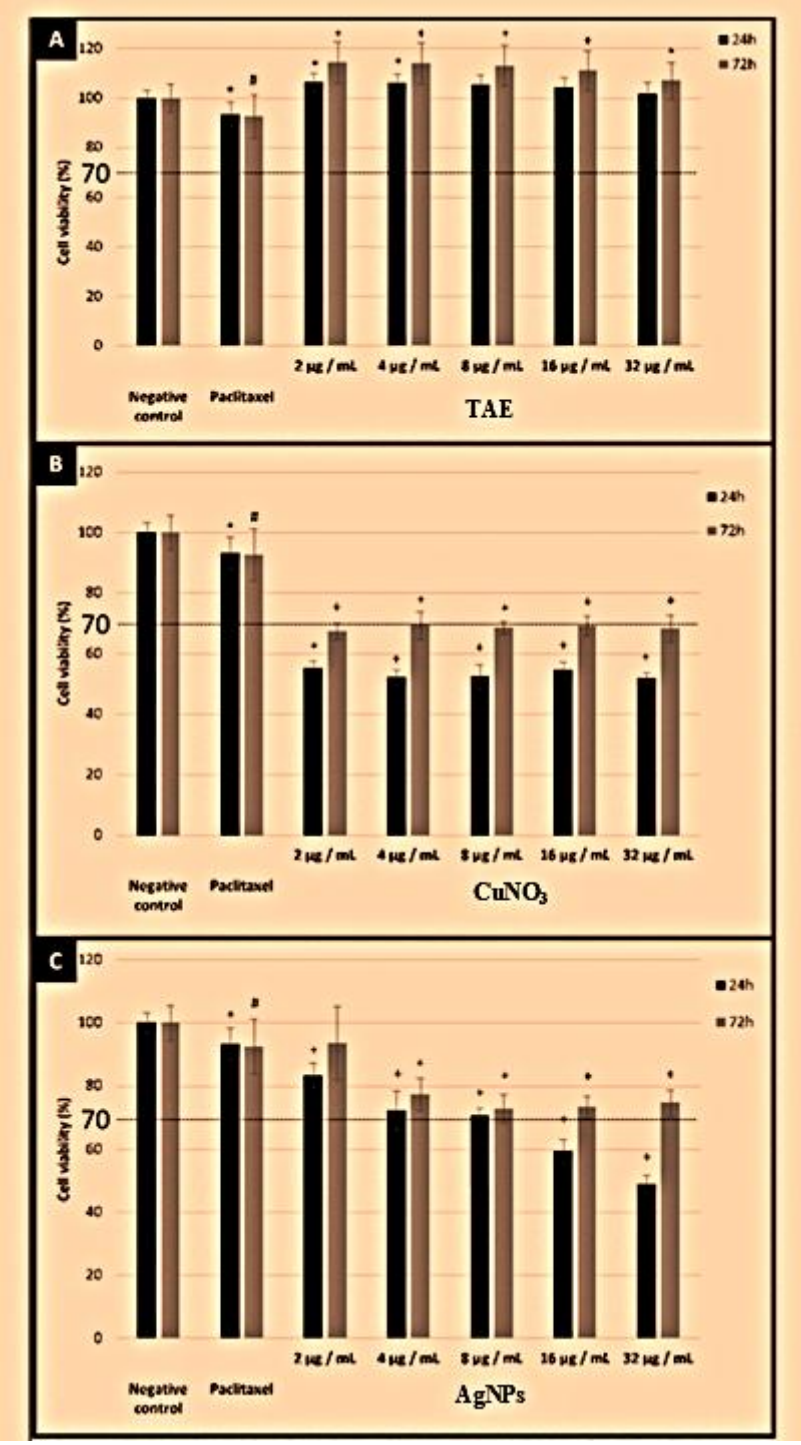

Figure 2. Percent cell viability after 24 and 72 hours of incubation. Results are presented as follows: **(A)** µg LE/mL for TAE; **(B)** µg copper/mL for CuNPs; and **(C)** µg copper/mL for AgNO3. The negative control group consisted of untreated cells, while the positive control group was treated with paclitaxel (2.5 µg/mL). Each treatment and control group had at least 12 determinations. Symbols indicate statistically significant differences from the negative control: * (P between 0.0137 and 0.0436), # (P between 0.0051 and 0.0067), and + (P < 0.0001).

Discussion:

In recent years, the World Health Organization (WHO) has strongly emphasized the urgent need for research and development of new antimicrobial agents, driven by the escalating threat of drug-resistant strains and the corresponding rise in healthcare costs. As a result, nanoparticles and medicinal plants have gained significant attention as promising alternatives for treating skin infections. Additionally, advancements in environmentally sustainable green synthesis methods have become a key focus for researchers. The biological synthesis of copper nanoparticles (CuNPs) using plant extracts has emerged as a highly cost-effective and eco-friendly alternative for large-scale production (Angulo et al. 2009).

In ancient Indian medicine, Ayurveda was a pioneer in utilizing metallic herbal preparations, known as "bhasmas," to treat various ailments. Copper bhasma, a well-documented remedy dating back to the 7th century BCE, was produced through a series of physicochemical processes involving medicinal plants. Today, these bhasmas are believed to be biologically produced nanoparticles, highlighting the deep-rooted historical use of metallic compounds in traditional medicine (Sinha et al. 2021).

For this study, plant selection was based on the traditional knowledge of indigenous communities who have long used medicinal plants for skin infection treatments. TAE (plant extract) served as both a reducing and capping agent for CuNP synthesis. Several factors influence the efficacy of the synthesis process, including the type of plant material, extraction method, geographical and seasonal factors, and the specific chemical metabolites present in the plant. Studies have shown that variations in growth conditions significantly affect plant composition, ultimately influencing extraction yield and nanoparticle synthesis (Bhat et al. 2014).

Chemical analysis of TAE revealed the presence of phenolic compounds, particularly flavonoids, which play a crucial role in reducing copper ions and forming stable nanoparticles. In this study, TAE demonstrated substantial antioxidant activity. In contrast, copper nitrate ($CuNO_3$) solution displayed no antioxidant activity, while CuNPs exhibited lower activity (>50%) compared to TAE in both DPPH and FRAP assays. The observed antioxidant activity of CuNPs can be attributed to the presence of TAE-derived phenolic compounds on the nanoparticle surface, which serve as antioxidants and stabilizers (Tavan et al. 2023).

The antioxidant properties of a plant are primarily governed by the composition and concentration of phenolic compounds, which are key players in neutralizing free radicals and stabilizing metals. These compounds function as electron donors, with polyphenols being particularly effective due to their ability to donate hydroxyl groups and stabilize unpaired electrons within their aromatic structure. The results of this study confirm that TAE is rich in phenolic compounds, explaining its potent antioxidant activity and its role as both a reducing agent and a capping agent for CuNPs (Flora et al. 2013).

The cytotoxicity of TAE, $CuNO_3$, and CuNPs was evaluated on PBMCs (peripheral blood mononuclear cells), which consist of immune cells such as T cells, B cells, NK cells, and monocytes. Cytotoxicity tests on PBMCs are critical for predicting immune responses and assessing immunotoxicity during preclinical safety evaluations. These assays also provide insights into the interaction between nanomaterials and immune cells. The study revealed that CuNO3 was cytotoxic, while TAE exhibited no toxicity at the tested concentrations. Furthermore, the plant extract demonstrated low cell proliferation compared to untreated cells, suggesting that the metabolites in TAE, primarily phenolic compounds, enhance non-specific immune responses by promoting macrophage and neutrophil activity (Zhu et al. 2016).

The absence of toxicity in TAE is of paramount importance in the context of CuNPs for medical applications, particularly for their potential use in topical treatments for skin infections. However, the CuNP solution showed variable cell viability depending on dosage and exposure time. While CuNO3 exhibited cytotoxicity, CuNPs, and TAE were non-toxic, with TAE even demonstrating a slight proliferative effect. These findings suggest that the reduction in cell viability associated with CuNPs is related to copper content, but the TAE metabolites coating the nanoparticles mitigate cytotoxicity and modulate immune responses. This implies that copper ions in their free form are more toxic than copper nanoparticles coated with phytochemicals, offering a safer alternative for therapeutic use (Kim et al. 2024).

Although $CuNO_3$ exhibited antimicrobial activity, its higher minimum inhibitory concentration (MIC) and cytotoxicity rendered it unsuitable as an antimicrobial agent. In contrast, CuNPs showed more effective antimicrobial properties. Variations in MIC values among isolates of the same species and between species of the same genus highlight the necessity of testing multiple isolates for accurate evaluation of antimicrobial efficacy. While $CuNO_3$ may have bacteriostatic properties, its toxicity precludes its use as an antimicrobial agent (Mallick et al. 2021).

To fully validate the fungicidal or bactericidal potential, further research is essential, particularly through methods like time-kill assays to directly correlate findings. Additionally, it is crucial to consider that the selection of endpoint readings for clinical drugs depends on the distribution of their minimum inhibitory concentration, pharmacokinetic and pharmacodynamic properties, animal study models, and ultimate therapeutic outcomes (Mohsin et al. 2022).

While the precise mechanisms underlying the superior antimicrobial efficacy of copper nanoparticles remain somewhat elusive, current evidence suggests that their mode of action is the result of multiple simultaneous processes. CuNPs appear to bypass many of the resistance mechanisms that microbes develop against conventional antimicrobial treatments. Consequently, modern strategies to combat multidrug-resistant pathogens are increasingly focused on exploring the synergistic potential between conventional antimicrobial agents and metallic nanoparticles, such as CuNPs (Wang et al. 2021).

Conclusion:

This study successfully demonstrated the synthesis of copper nanoparticles (CuNPs) using the green-synthesis method involving *Acanthospermum australe* extracts. The synthesized CuNPs exhibited significant antimicrobial activity against common pathogens associated with soft tissue and skin infections (SSTIs), showcasing their potential as an alternative treatment, particularly in the fight against antimicrobial resistance. Moreover, the reduced cytotoxicity of the CuNP solution compared to its synthesis precursors emphasizes its safety profile for potential therapeutic applications.

The findings underscore the importance of integrating traditional medicinal knowledge with modern nanotechnology to create innovative, eco-friendly, and effective treatments. While the antimicrobial action of the CuNPs was attributed primarily to the copper itself, the role of *A. australe* in stabilizing

the nanoparticles and influencing immune responses cannot be overlooked. This synergy between plant extracts and metallic nanoparticles opens new avenues for developing biocompatible nanomaterials with enhanced therapeutic efficacy.

Further research should focus on elucidating the detailed mechanisms of CuNP action and exploring their clinical applications in SSTIs. As antimicrobial resistance continues to rise globally, the development of nanotechnology-based strategies, particularly those utilizing green synthesis, holds great promise in overcoming current therapeutic challenges and improving patient outcomes.

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