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Production of wound healing patches containing Iron and Copper nanoparticles synthesized from the leaf extract of *Azadirachta indica.*

Suneeta Panicker*, Sanika Kale, and Shrutika Mali.

Department of Microbiology, Dr. D.Y. Patil Arts, Commerce and Science College, Sant Tukaram Nagar, Pimpri, Pune 411018, India.

ABSTRACT

The study focuses on the preparation of copper nanoparticles(CuNPs) and iron nanoparticles (FeNPs) using neem leaves extract for green synthesis. The nanoparticles were characterized using UV-Visual spectrophotometry, with CuNPs exhibiting absorbance peak at 462 nm and FeNPs at 422 nm. The antibacterial activity of these nanoparticles was tested using *Escherichia coli* and *Staphylococcus aureus*. CuNPs were found to be more potent than FeNPs. *E. coli* was more sensitive than *Staphylococcus aureus* to both the NPs. The wound healing patches with these NPs incorporated in them were prepared and tested for their antibiotic activity against *E. coli* and *S. aureus* to check its efficiency to protect the wound infections. It was observed that these patches retained their antibacterial properties and were able to inhibit both test organisms. This study showcases new developments in hybrid wound dressings, combining gelatin with other materials for improved mechanical strength and antimicrobial agents. These patches can be used to treat infected, oozing, or bleeding wounds, demonstrating the promising potential for future wound care.

Keywords: Copper nanoparticles (CuNPs), Iron nanoparticles (FeNPs), Neem leaves extract, Antibacterial activity, Wound healing, Nanoparticle-incorporated patches.

***Corresponding author**

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INTRODUCTION

Green synthesis is an eco-friendly, sustainable method for producing nanoparticles from natural sources like plants, bacteria, fungi, and algae, demonstrating the potential of green chemistry in various applications. Iron nanoparticles prevent wound infections by generating reactive oxygen species, aiding in clotting, and catalyzing hydrogen peroxide decomposition, improving tissue oxygen levels, cellular metabolism, collagen synthesis, and angiogenesis [1].

Open wounds are at higher risk of bacterial infections, but neem-based CuNPs have strong antibacterial properties, generating reactive oxygen species and preventing microbial replication [2]. CuNPs stimulate angiogenesis, accelerating tissue repair and regeneration. They enhance collagen synthesis, resulting in stronger tissue and more durable skin. Neem extracts act as antioxidants, preventing infections [3].

Plant extract-mediated nanoparticle synthesis is a green, eco-friendly, and cost-effective method using natural compounds like neem extracts, which possess strong antimicrobial properties [4]. Curcumin, previously used for wound healing patches, has limitations due to its inability to dissolve well in the body; making its effectiveness less efficient [5] thus, neem leaf extract was used for the synthesis of nanoparticles.

Neem leaf extract is a promising green method for synthesizing iron and copper nanoparticles due to its rich phytochemical profile, strong reducing capacity, and broad-spectrum antimicrobial properties. Neem's antioxidants enhance nanoparticles' biocompatibility and antimicrobial properties [4, 6]. Wound patches, made from advanced materials, promote healing, protect against infection, and maintan tissue regeneration. Effectiveness depends on wound type, size, depth, location, and patient health. Development is challenging, especially in diabetic patients.

Traditional dressings like cotton, bandages, and plasters don't effectively control bacterial infections, necessitating the use of non-adherent dressings to prevent tissue damage and prolong healing time [7]. Gels offer fluid; easy spread wound protection but may evaporate quickly. Patches form protective barriers, reduce infection risk, require reapplication, and may require secondary dressings. Wound healing patches are cost-effective [8].

Many patches are designed to deliver the drugs for example antibiotics in a controlled condition. This controlled release helps to maintain the therapeutic levels of the medications at the wound site as the gels releases the drugs immediately [9]. Gelatin wound healing patches are gaining attention due to their unique properties and advantages.Derived from collagen, a natural protein found in animal connective tissues, gelatin hashigh biocompatibility and does not trigger immune reactions or inflammation. It is biodegradable,cost-effective, and eco-friendly, making it a viable option for wound management.

Thus, the present study aimed to make wound patches containing copper and iron nanoparticles, synthesized using neem leaf extract.

MATERIALS AND METHODS

Preparation of Neem leaf extract

The uninfected neem leaves were collected and washed with water for several times to remove impurities. 300gm of sliced neem leaf was boiled in 1000ml of distilled water for 1 hour and 40min. The volume of the extract was reduced to 300ml which was then filtered through Whatman filter paper1. The filtrate was stored in refrigerator until further use [10].

Synthesis of iron nanoparticles from the leaf extract

1mM of ferric chloride solution was prepared and mixed with the neem leaf extract in 1:1 ratio. This mixture incubated at 55˚C for 1hour and then at room temperature in dark for 24 hours. Formation of iron nanoparticle was indicated by colour change from brown to black [11]. The synthesized nanoparticles were centrifuged at 6000rpm for 15min. The pellet was washed with ethanol and distilled water twice to remove any unreacted plant components. After washing, the pellet was transferred to a clean petri dish, allowed to dry at room temperature and a fine dry powder was obtained.

Synthesis of CuNPs from the leaf extract

10mM solution of copper sulfate was prepared and mixed with the prepared leaf extract in 3:1 ratio. This mixture was then incubated in dark for 24 hours. The colour change of the mixture from blue to green and darker green after incubation indicated the formation of CuNPs [12]. The synthesized nanoparticles were centrifuged at 6000rpm for 15min. The pellet was washed with ethanol and distilled water twice to remove any unreacted plant components. After washing, the pellet was transferred to a clean petri dish and allowed to dry at room temperature and a fine dry powder was obtained.

Synthesis of Nanoparticles with polyvinyl alcohol - as a stabilizer

0.01gm of polyvinyl alcohol (PVA) in 10ml of water was dissolved by boiling for about 5min then mixed with 10mM (10ml) of copper sulphate or 1mM (10ml) of ferric chloride solution. For iron nanoparticles synthesis, neem leaf extract was added in a 1:1 ratio and for CuNPs, it was added 3:1 ratio [13]. Similar protocol as mentioned above was followed after these additions.

Characterization of synthesized FeNPs and CuNPs by UV-Vis spectrophotometer analysis

The NP solutions were scanned for a spectra range of 200nm to 700nm to obtain the lambda max using a UV visible spectrophotometer, which indirectly confirms the synthesis of the nano sized particles [10].

Antibacterial activity of the Nanoparticles and Nano patches

The nanoparticles were resuspended in various solvents like DMSO, methanol, and water to check its antibacterial activity against *S. aureusand E.coli*[14]. Antibiotic amoxicillin (100mg/ml) was used as a positive control. Well diffusion method, also known as the Kirby-Bauer technique, was employed to assess the susceptibility of the bacterial strains. Pure cultures of *S. aureus* and *E. coli* were first inoculated in nutrient broth and incubated at 37°C for 18-20 hours to obtain log phase culture. The turbidity was matched to 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/mL. The standardized inoculum was uniformly spread over the surface of the Mueller-Hinton agar (MHA) plates using a sterile spreader. Wells were bored in the plates using a 6mm borer. 100µl of nanoparticles and the antibiotic solutions were added to their respective wells. The plates were placed at 4ºC for pre-diffusion for 15mins and then incubated at 37°C for 18–24 hours. After incubation, the plates were examined for zones of inhibition around the wells [11], and the diameter of zone was measured in mm using a ruler. To determine the antibacterial activity of the patches, similar protocol was followed with slight modification; the patches were directly placed on the plates spread with the test organisms.

Preparation of wound healing patches

A mixture of agar (1.25g), gelatin (0.185g), glycerine (1.8ml), PVA (2.5g) and water (50ml) was boiled to get a homogenous solution [15]. This mixture was cooled to 45ºC and 1ml of nanoparticles was added to it [16]. After mixing thoroughly it was poured onto sterile petri plates or glass slides and sundried for 24 hours. The obtained dried patches were then tested for their antibacterial activity.

RESULTS

Neem leaf extract

The neem leaf extract was successfully prepared using the aqueous extraction method. After filtration, the resulting extract appeared deep brownish green in color, as seen in Figure 1 with a slightly bitter odor characteristic of neem. The extract was clear with no particulate matter after filtration, indicating effective removal of solid debris.

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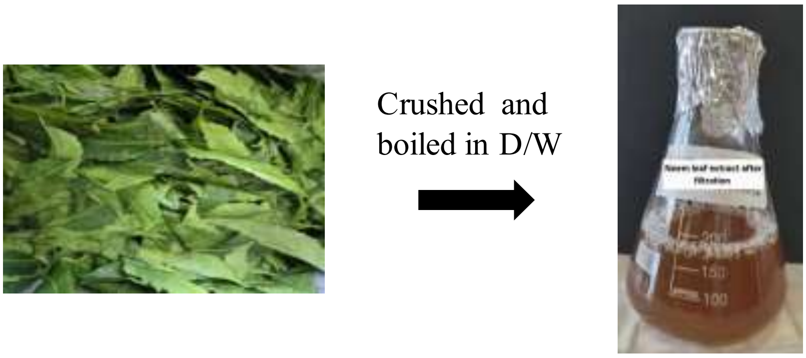

Figure 1: Neem leaf extract preparation

Synthesis of iron nanoparticles from the leaf extract

The synthesis of iron nanoparticles was indicated by colour change from brown to black upon mixing neem leaf extract with iron salt solution and incubation, suggesting the reduction of iron ions as seen in Figure 2.

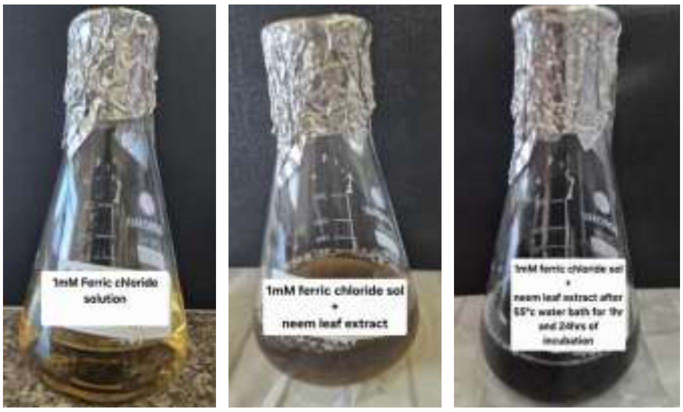

Figure 2: Synthesis of iron nanoparticles

Synthesis of CuNPs from the leaf extract

The synthesis of CuNPs was indicated by colour change from blue to green and darker green upon mixing neem leaf extract with copper salt solution and incubation indicating the reduction of copper as seen in Figure 3.

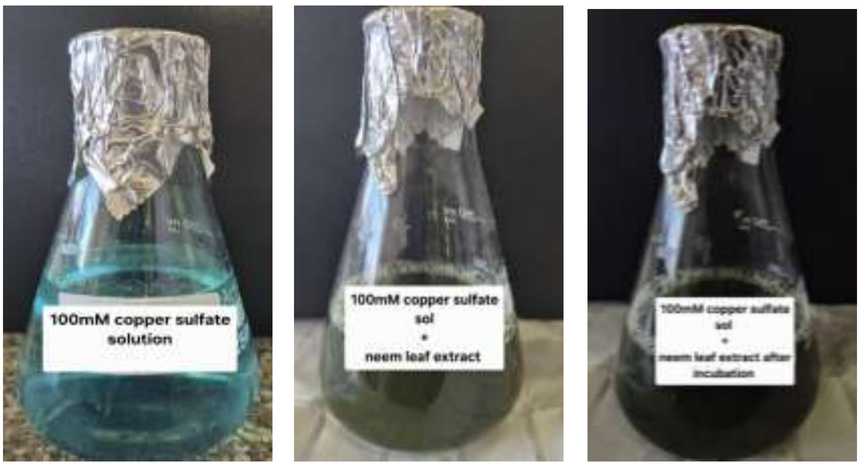

Figure 3: Synthesis of copper nanoparticles

UV visible spectrophotometric characterization of FeNPs **and** CuNPs

The synthesized NPs were characterized using UV – spectrophotometer. To determine the absorbance peak the NP solution was scanned for a range of 200nm to 700nm. The lambda max obtained for CuNPs was 462 nm and that for FeNPs was 422nm as seen in the Figure 4. This confirmed the synthesis of nanoparticles.


A

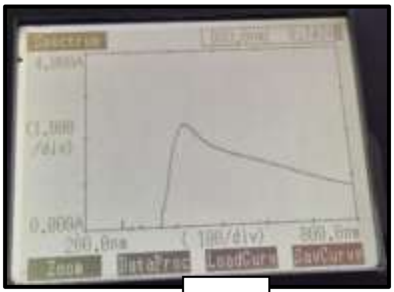
B

Figure 4: UV spectrophotometric characterization of NPs A) CuNPs-Absorbance peak at 462nm B) FeNPs-Absorbance peak at 422nm

Antibacterial activity using well diffusion method

Antibacterial activity of nanoparticles was checked with each solvent and the zone of inhibition was measured in order to conclude sensitivity of the test organisms. The both crude NPs exhibited the maximum antibacterial activity, with clear zones of inhibition observed around the wells, indicating strong antimicrobial potential (Table 1). In contrast, NPs in methanol and water showed no antibacterial activity, as there were no visible zones of inhibition. This suggests that the active components responsible for antimicrobial effects may not be effectively soluble or active in these solvents. The NPs in DMSO showed lower antibacterial activity, producing small inhibition zones, indicating limited effectiveness compared to the crude NPs.

FeNPs and CuNPs were synthesized with polyvinyl alcohol (PVA), a biopolymer known to enhance nanoparticle stability and bioactivity. The antibacterial activity of both NPs was increased when PVA was used as stabilizer. *E. coli* was more sensitive than *S. aureus* to both the NPs. CuNPs showed higher antibacterial activity than FeNPs (Table 2). PVA modification appeared to improve the overall antibacterial potential, as indicated by increased zones of inhibition in most treated samples. The zone of inhibition was notably larger in these samples compared to the PVA-free counterparts, indicating enhanced activity due to the presence of PVA [5].

Table 1: Antimicrobial activity of CuNPs and FeNPs without PVA

Test organisms	Control	CuNPs		FeNPs	
		Pellet	Supernatant	Pellet	Supernatant
S. aureus	23	12	10	11	10
E. coli	21	19	13	12	10

Table 2: Antimicrobial activity of CuNPs and FeNPs with PVA

Test organisms	Control	CuNPs with PVA		FeNPs with PVA	
		Pellet	Supernatant	Pellet	Supernatant
S. aureus	25	15	11	13	10
E. coli	22	27	15	15	11

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Wound healing patches

Wound healing patches were successfully developed using a blend of glycerine, polyvinyl alcohol (PVA), gelatin, and water, in which the NPs were incorporated. After the drying period, the formulation formed a clear, flexible, and uniform patch. The resulting patch exhibited good film-forming ability, structural integrity, and elasticity as seen in Figure 5, indicating effective cross-linking and interaction among the biopolymeric components. The presence of glycerine contributed to the flexibility and moisture-retention capacity of the patch, while gelatin and PVA provided strength and stability. The successful formation of the patch under ambient conditions demonstrates the feasibility of using simple, cost-effective drying methods for wound dressing production.

Figure 5: Wound patch with NPs

Antibacterial activity of patches

The antibacterial activity of the NPs incorporated wound healing patches was evaluated against *S. aureus* and *E. coli*. All nanoparticle loaded patches demonstrated inhibition of both *S. aureus* and *E. coli*, confirming their antibacterial potential (Figure 6). Among them, patches containing CuNPs exhibited the highest antibacterial activity, in both bacterial strains. Iron nanoparticle-incorporated patches also showed effective antibacterial action however, their activity was slightly lower compared to the copper-loaded patches. These results indicate that metal nanoparticles can be incorporated in the wound healing patches to significantly improve their antimicrobial properties.

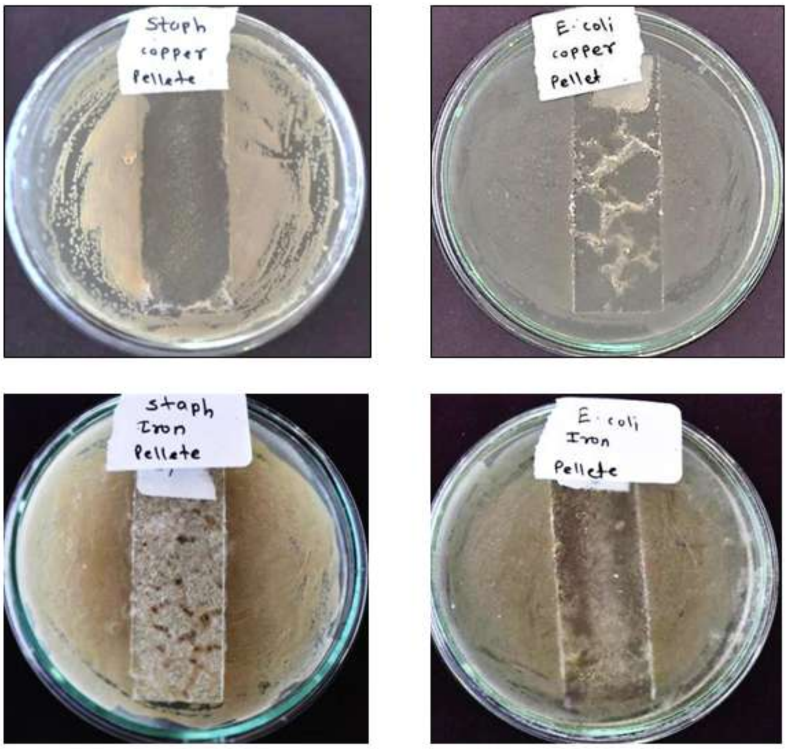

Figure 6: Antibacterial activity of NP incorporated wound healing patches

DISCUSSION

The study showcases the green synthesis of FeNPs and CuNPs using *Azadirachta indica* leaf extract, demonstrating their antimicrobial potential and therapeutic benefits. The biogenic agent, neem extract, converts ions into nanoparticles, stabilizing their structure and providing additional biological properties, making the nanoparticles more biocompatible and active. The synthesized nanoparticles, characterized using UV-Vis spectroscopy were found to have significant antibacterial activity against common pathogens like *S. aureus* and *E. coli,* with CuNPs showing stronger inhibition capacity.

The composite film, made of PVA, glycerol, and agar, is flexible, breathable, and moist. When loaded with neem extract-derived FeNPs and CuNPs, acts as a barrier, antimicrobial, and therapeutic platform, promoting wound closure and healing.

This study promotes environmentally sustainable nanotechnology in biomedical applications, using neem for low-cost, eco-friendly synthesis. FeNPs and CuNPs offer a safer, more affordable, and multifunctional alternative to traditional silver nanoparticles.

A group of researcher proposed that wound healing patches can be ideal for diabetic patients due to their high blood sugar levels, damaged blood vessels, and reduced oxygen and nutrient delivery. The incorporation of nanoparticles in these patches, make them more suitable for such patients [17].

Another group [18] carried out a similar study in which they incorporated copper NPs in a hydrogel and showed its antibacterial activity against *E. coli* and *Staphylococcus*.

One more study constructed Gelatin-Based Hydrogels blended with Gellan and tannic acid, as an Injectable Wound Dressing and demonstrated accelerated wound healing in mice [19]. The current used NPs for antibacterial action instead of tannic acid. The incorporation of nanoparticles into a gelatin matrix allowed for the controlled release of nanoparticles over time by providing sustained and long lasting antibacterial effects.

CONCLUSION

In this study, FeNPs and CuNPs were successfully synthesized using neem leaf extract, PVA as stabilizer and characterized through UV spectrophotometer. These NPs were incorporated into wound healing patches formulated with gelatin, glycerol, agar powder, and PVA. The patches were evaluated for their antibacterial activity. Copper NPs incorporated NPs were more potent than Iron NPs. The findings highlight the potential of neem-mediated FeNPs and CuNPs in enhancing the antibacterial efficacy of wound healing materials. Further research on their stability, release kinetics, and in vivo wound healing performance could pave the way for their biomedical applications.

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