Sustainable Finished Leather Preservation: Part I - Myrobalan Capped Copper Nanoparticles

by

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Abstract

Copper nanoparticles were synthesized using myrobalan (tannin) extract as a reducing agent. The synthesized nanoparticles were used in the coating of leather after the finishing process of upper leather to enhance the antimicrobial properties of leather. Primarily, the synthesized myrobalan copper nanoparticles (M-CuNPs) were characterized using UV-Spectroscopy (UV), which resulted in an absorption peak at 412 nm, confirming the presence of CuNPs in the solution. The size distribution and zeta potential values of M-CuNPs were analyzed by Dynamic Light Scattering (DLS) which resulted in an average particle size of 104 nm and zeta potential value of -28mv. These values affirm the stability of the M-CuNPs. Scanning Electron Microscope (SEM) image showed that the particles are spherical in shape. The elemental composition of M-CuNPs was confirmed using Energy dispersive X-ray (EDX) studies. Fourier transform infrared (FTIR) spectra showed peaks at 3173 cm^{-1}, 1727 cm^{-1}, 1147 cm^{-1}, and 624 cm^{-1}, which correspond to -OH, C=O, C-O, and C-H respectively confirming functional groups involved in the synthesis and capping process. The antibacterial activity of the M-CuNPs coated finished leather was evaluated against Gram-positive (*Bacillus subtilis*) and Gram-negative (*Serratia marcescens*) bacteria, which are common pathogenic species that effect the preservation capabilities of the leather. The M-CuNPs coated finished leather exhibits strong antimicrobial activity against both Gram-positive (*Bacillus subtilis*) and Gram-negative (*Serratia marcescens*) bacteria. Hence from the above study, M-CuNPs can be used as a sustainable antibacterial agent for preserving leather and its products.

Introduction

Leather and leather products are highly prone to microbial attacks such as mold, yeast, and bacteria. The leather products have to be preserved, packed, and exported to varied locations with varied atmospheric conditions, they need to have the resistance to microbial attack.[1] Hence, there is always a need to preserve leather and leather products with antimicrobial chemicals to overcome microbial contamination. Antibacterial agents are not exclusively used in the post tanning and finishing process to prevent microbial growth for preservation of final leather and hence, there is always a need for sustainable benign coating for finished leather and its products. These benign products prevent microbial contamination on the grain and flesh side in the final leather thereby increasing the lifespan of finished leather and the products made from them. Various research efforts are taken to create antibacterial coatings to be used on the surface of leather and other materials like medical equipment, clothing, and food preservation, to prevent contamination and spoilage.[2] The criteria for such preservation shall be affordable, non-toxic, and cost-effective for varied applications.[3] Many chemicals and their strategies for imparting antimicrobial properties into leather and its products are not benign.[4] Nanotechnology plays a crucial role in many significant technologies in various fields.[5] Synthesis of nanoparticles using plant extracts is highly eco-friendly, inexpensive, and also safe towards synthesising them. Among the metal nanoparticles, copper nanoparticles are used by researchers for their catalytic, optical, electrical, and antibacterial/antifungal application properties.[6] Various studies were carried out on the antimicrobial properties of copper nanoparticles.[7] Copper nanoparticles are synthesized through different routes such as sol-gel, microwave irradiations, alkoxide-based route, thermal decomposition, etc.[8] There are several techniques used to prepare copper nanoparticles including thermal reduction, sonochemical reduction, microemulsion techniques, laser irradiation, and induced radiation.[9] There is an increasing attention to synthesis nanoparticles using biological compounds because it reduces the production cost, non-toxic to the environment, and also reduces the process time compared to other methods. Plant extracts are effectively used and they act as reducing and capping agents.[10] Copper nanoparticles were synthesised from various plant extracts including Aloe vera, Magnolia kobu, Bifurica bifurcate, Tabernaemontana, Terminalia arjuna.[11-13] Carbohydrates, proteins, phenols, vitamins, flavonoids, and vitamins are major compounds of the plant extracts, which are involved in the reduction process.[14] Copper nanoparticles synthesized using myrobalan were not explored for the preservation of any leather and its products. Hence in the present study, we have selected myrobalan tannin as the reducing agent for the synthesis of copper nanoparticles, coated the leathers with M-CuNPs to investigate their antibacterial properties, and studied the efficacy of its preservation.

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Manuscript received August 7, 2023, accepted for publication October 7, 2023.

Materials and Method

Materials

Copper sulphate pentahydrate ($CuSO_4.5H_2O$) was purchased from SRL Chemicals, Chennai. Myrobalan powder was collected from the leather process technology department of CSIR-CLRI. The finished leather for coating was collected from the Tannery Division, CSIR-CLRI, India. All the leather finishing chemicals were procured from Stahl India Pvt. Ltd, (Chrompet, Chennai, India).

Preparation of tannin extraction

Tannin extract was prepared by mixing 1g of myrobalan in 100 ml of distilled water and heated for 30 min at 60°C. The extract was filtered using Whatman No. 1 filter paper and stored.

Synthesis of M-CuNPs

Synthesis of the copper nanoparticles was optimized at different concentrations of copper sulphate (1 mM, 2 mM, 5 mM, and 10 mM). 5 ml of tannin extraction was added dropwise into 95 ml of copper (Cu) solution and kept under shaking conditions (150 rpm) for 24 h for synthesis. The mixture was centrifuged for 10 mins at 6000 rpm, and the pellets were dried overnight in hot air oven at 60°C. Dried particles were fine ground and stored for further use.[6]

Characteristics of M-CuNPs

Absorption spectra of myrobalan-mediated copper nanoparticles (M-CuNPs) were measured using UV-Visible spectrophotometer, 115 VAC (Shimadzu, Kyoto, Japan) at 200 to 800 nm. Functional groups involved in the reduction and capping process were identified using Fourier Transform Infrared (FT-IR) spectroscopy-4200 (JASCO, Japan). The size and distribution of M-CuNPs were determined by Dynamic Light Scattering (DLS) (Malvern instrument Ltd, Malvern, UK). The morphology of copper nanoparticles was examined using Scanning Electron Microscopy (SEM) and Energy- dispersive X-ray spectroscopy (EDAX) was used to analyze the biological composition of M-CuNPs.

Methodology of Leather Nanocoatings

Cow upper crust leather of Indian origin was selected for evaluation. The leather was resin-finished with M-CuNPs. The finishing formulation is presented in Table I. The formulation was sprayed three cross coats on the leather using a spray coating device (HVLP spray gun Bullows 630) followed by drying and pressing at 80°C.[15]

Antibacterial assay of M-CuNPs

The antibacterial activity of synthesized copper nanoparticles was assessed using the agar well diffusion method against Gram-positive

Table I

Resin finish formulation.

Base coat formulation (All the values were expressed in g)		
Adhesion binder	100	1 Coat
Penetrator	100	
Water	1:1	
Season Coat		
Anion Resin compact	100	4 cross Coats
Non-Ionic PU compact	100	
Soft PU	50	
Wax	20	
Penetrator	20	
Pigment	50	
Water	Make up to 1 liter	
Top Coat formulation		
Matt Lac	10	2 cross Coats
Shine Lac	5	
Water	15	
Cross linker	1 %	
M-CuNPs Coat	5 %	3 cross Coats

(*Bacillus subtilis*) and Gram-negative bacteria (*Serratia marcescens*). Fresh bacterial cultures were uniformly spread over the Muller-Hinton agar (MH agar) plates. Wells (6 mm) were made on each petri dish and filled with different concentrations of M-CuNPs (50 µg/ml,100 µg/ml, and 150 µg/ml). Penicillin was used as a positive control.

Effect of copper nanoparticles on growth of bacteria

The growth curve of the bacterial cells treated with synthesized M-CuNPs was evaluated where the fresh cultures were prepared and maintained at a concentration of 10^6 CFU/mL. Experiments have been carried out using Nutrient broth. The fresh cultures were then treated with M-CuNPs at different concentrations (50µg/mL, 100µg/mL,150µg/mL) for 24 h at 37°C. The OD was measured at 600nm in regular time intervals (2,4,6,8,10,12 and 24 h).

Antibacterial evaluation of M-CuNPs coated leather

The antimicrobial properties of the synthesized nanoparticles were determined by the measurement of the zone of inhibitions.[16] The leather samples were treated with 150 µg/ml of M-CuNPs by spraying on the flesh and grain side and were placed on the bacteria spread MH agar plates and incubated for 24 h at 30°C. The zone of inhibition was measured after the inhibition period.[1]

Results and Discussion

Synthesized M-CuNPs were confirmed by change in the color of the solution from light green to dark. The color change of the solution is considered as a primary indication of the formation of copper nanoparticles.[17] When a nanoparticle is much smaller than the wavelength of light, coherent oscillation of the conduction band electrons is induced by interaction with an electromagnetic field, which turns the color from light green to dark. UV-visible absorption was investigated to analyze the optical properties of M-CuNPs. The absorption wavelength of copper nanoparticles was 412 nm (Figure 1).

To attain maximum production of M-CuNPs, Cu solution was optimized using the concentrations of 1 mM, 2 mM, 5 mM, and 10 mM. Myrobalan extraction (5%) was used as a constant for the above varied concentrations of Cu solution. The UV-visible spectrum of the M-CuNPs with different concentrations of the copper solution is shown in Figure 2. As shown in Figure 2, increasing the concentration of the Cu solution, increased the intensity of absorption peaks. A 5 mM concentration of Cu solution showed optimum production of M-CuNPs within the nano range. A 10 mM of Cu solution showed higher production of M-CuNPs than 5 mM; however, the size of these particles was high and not in the nano range. Both1 mM and 2 mM concentrations resulted in low production of CuNPs. Hence

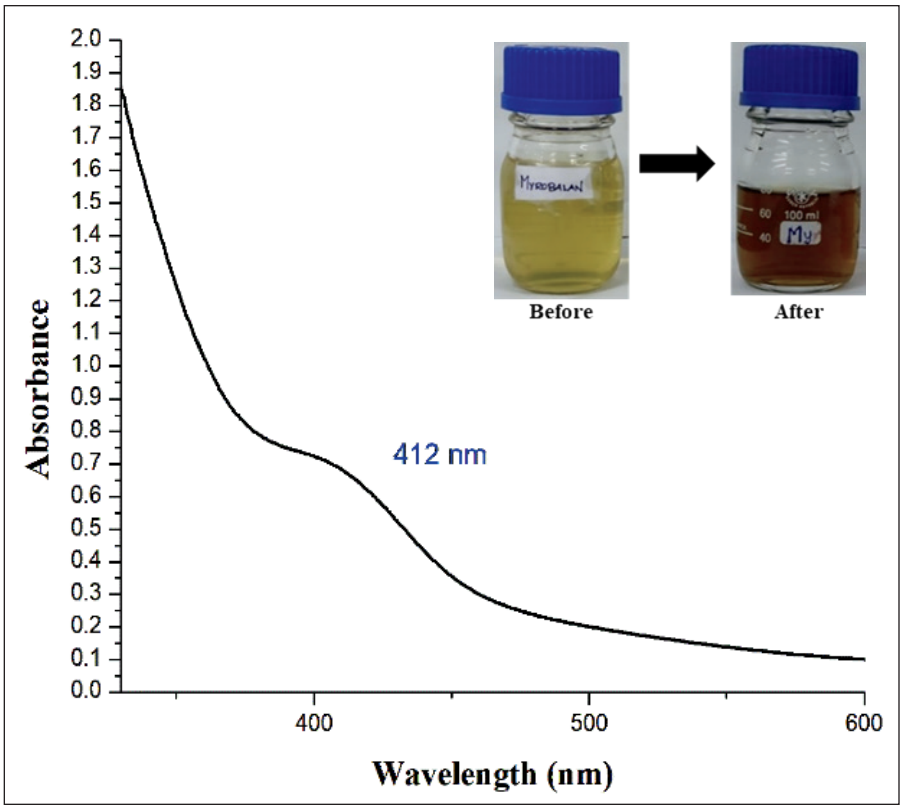

Figure 1. Visible color observation and UV Spectra of M-CuNPs

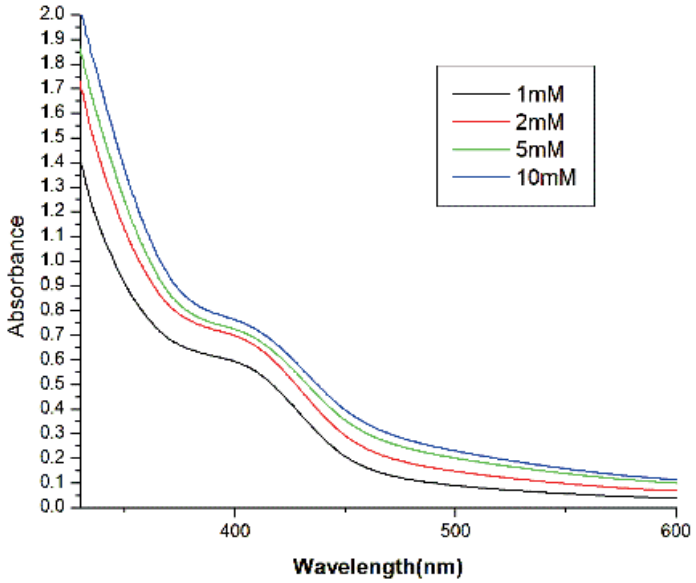

Figure 2. UV spectra of M-CuNPs at different concentrations of Cu solution

5 mM concentration of Cu solution was taken as the optimum concentration for the M-CuNPs production.

The size and distribution of nanoparticles were determined by DLS. The results of DLS showed a mean size distribution of M-CuNPs at 104 nm with a polydispersity index of 1.000 (Figure 3 A). The size distribution could also be due to the aggregation of particles with the solution. However, we have found that the particle size from the SEM analysis is much lower than, and hence they fall under the nano range. The present study findings are similar to the previous study of Mali et al. (2020), who reported that the average particle size of CuNPs synthesized by *Celastrus paniculatus* was 290 nm with a polydispersity index of 1.00 and aggregated.[6] The zeta value of M-CuNPs was -28 mV (Figure 3 B). The negative zeta potential value shown by synthesized M-CuNPs might be due to the presence of other bio-organic components in the extract as capping agents.[18]

FTIR analysis was carried out to identify the functional groups involved in the synthesis process of M-CuNPs. The comparative FTIR spectra of myrobalan and M-CuNPs are shown in Figure 4. The FTIR spectrum of myrobalan extracts showed a broad peak at 3355 cm^{-1} in myrobalan and a peak at 3173 cm^{-1} in M-CuNPs. These peaks indicate the hydroxyl (-OH) functional group in alcohols and phenolic compounds. The shift in the peak of the OH group in M-CuNPs is due to the interaction between tannin and the Cu molecules. The peak at 1708 cm^{-1} in tannin and 1727 cm^{-1} in M-CuNPs represent C=O stretching and carboxylic acid with dimer. The shift also confirms the interaction between the myrobalan and Cu molecules. The aliphatic ether of the C-O stretching peak at 1049 cm^{-1} shifted to 1147 cm^{-1} confirming the tannin and Cu interactions.

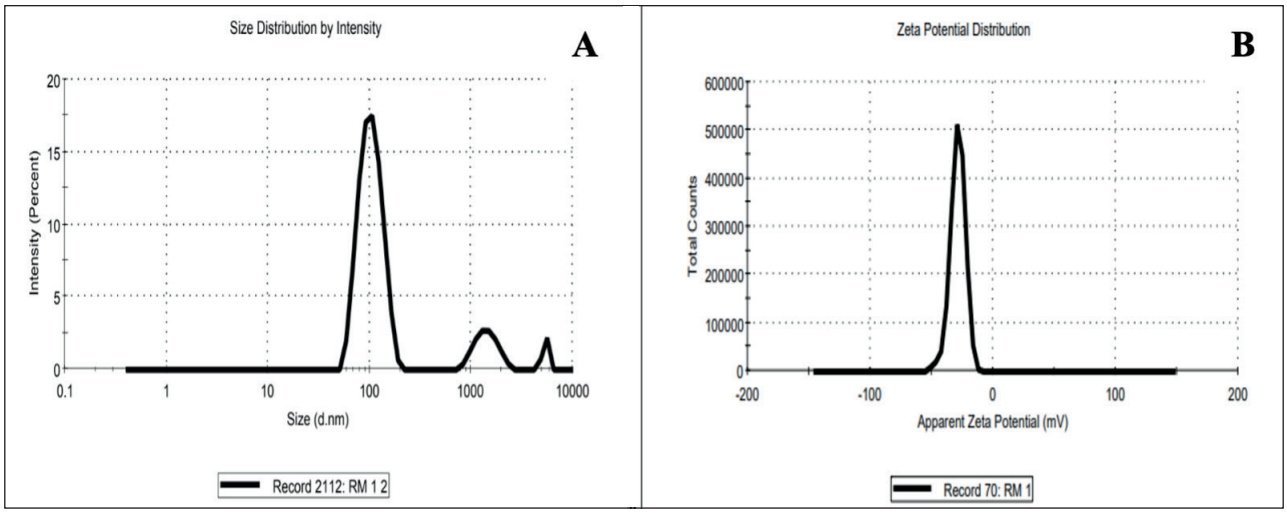

Figure 3. A) Particles Size distribution **B)** Zeta potential analysis of M-CuNPs

The band at 576 cm^{-1} shifted to 624 cm^{-1}, which confirms that the C-H of the alkyne group is also involved in the interaction of myrobalan with Cu. Peak shifts in the specific functional group of M-CuNPs have predominantly transpired due to the capping of myrobalan over Cu metals. Hence, the study confirms that the -OH, C=O, C-O, and C-H are responsible for the reduction and capping of M-CuNPs. Saranya, et al., also reported that the functional groups of the aldehyde, alcohols, amines, and carboxylic compounds of tannins are involved in the capping process of the nanoparticles.[19]

The morphological characterization of copper nanoparticles was carried out using SEM. As shown in Figure 5 A, spherical shaped nanoparticles are observed at 100 kx magnification. The size of the M-CuNPs as observed from the SEM micrograph is 61.25 nm. The results confirm that the M-CuNPs are in the nano range. EDX spectroscopic study was also carried out to determine the elemental analysis of the M-CuNPs. EDX spectrum showed a strong Cu signal at 8 eV along with carbon and oxygen, which confirmed the formation of copper nanoparticles. Cu at 44.5%, oxygen at 21.25%, and carbon at 44.46% are the compositions of M-CuNPs. There are two components present in the M-CuNPs. One is the metal and the other is the organic component of the tannin. It is observed that there is a maximum percentage of Cu in the M-CuNPs, which is due to the metal component of nanoparticles. The carbon and oxygen component of the M-CuNPs is due to the presence of organic compounds in myrobalan which act as the capping agent of the nanoparticles.[20]

Antibacterial activity of M-CuNPs

The antibacterial activity of M-CuNPs was evaluated against two pathogenic bacteria *Bacillus subtilis,* and *Serratia marcescens*. Penicillin was used as the positive control (PC) and myrobalan extract was used as the negative control (NC). The measured values of the zone of inhibition are presented in Table II and the photographic images of the plates are presented in Figure 6 (A, B). The values of the zone of inhibition show that M-CuNPs have high antibacterial activity against *Bacillus subtilis* and *Serratia marcescens*. The zone of inhibition of the bacterial strains increased with an increase in the concentration of M-CuNPs. In the present study, M-CuNPs showed

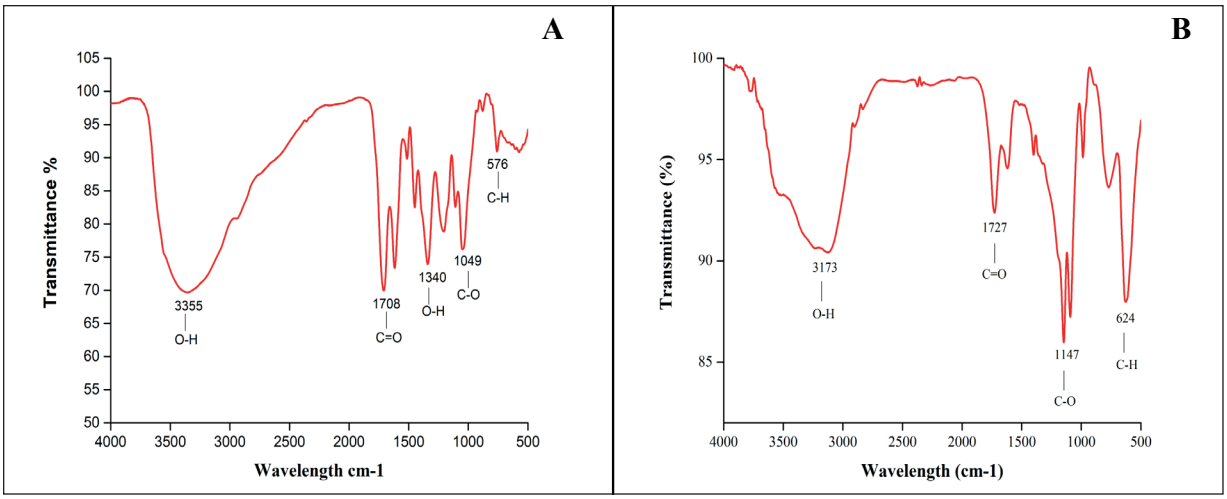

Figure 4. A) FTIR spectrum of Myrobalan **B)** FTIR spectrum of biosynthesized M-CuNPs

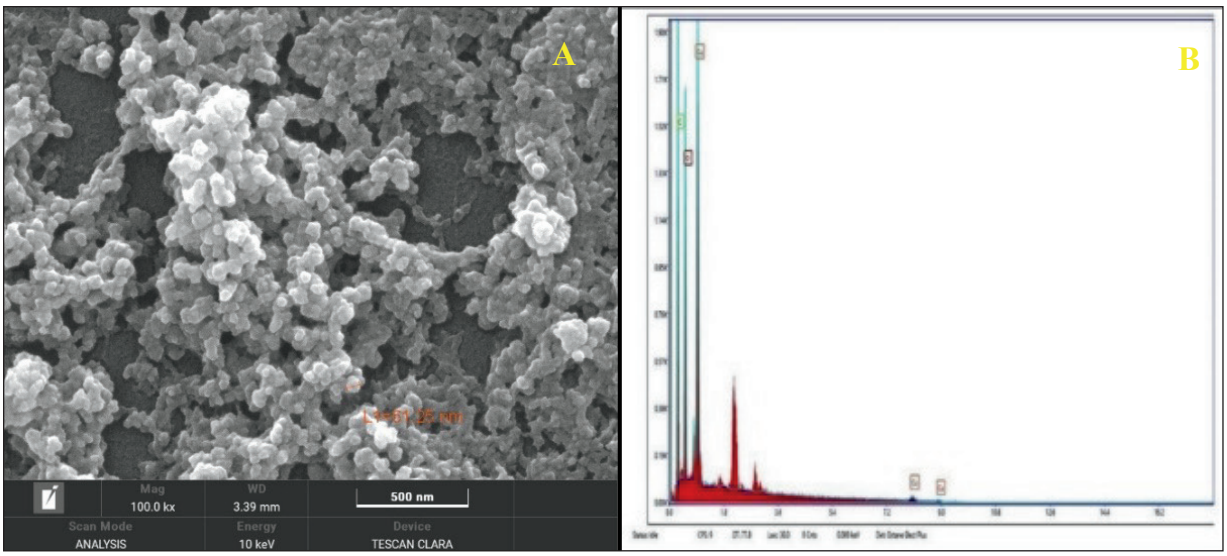

Figure 5. A) SEM micrograph of M-CuNPs **B)** Elemental analysis of M-CuNPs

Table II

Antibacterial activity of the synthesized M-CuNPs.

Concentration of M-CuNPs (µg/ml)	Zone of inhibition (mm)	
	Bacillus subtilis	*Serratia marcescens*
50	6 ± 0.2	2 ± 0.1
100	8 ± 0.21	5 ± 0.2
150	12 ± 0.32	6 ± 0.2
Penicillin	16 ± 0.1	1 ± 0

maximum inhibition against *Bacillus subtilis* compared to *Serratia marcescens*. However, for *Serratia marcescens,* the M-CuNPs showed much higher antibacterial activity than penicillin.

The growth of bacterial cells was evaluated in the presence and absence of M-CuNPs. Controls show normal growth patterns, whereas changes were observed in the growth patterns of M-CuNPs treated cultures. With the increase in M-CuNPs concentration, the bacterial growth declined in both the species (*Bacillus subtilis* and *Serretia marcescens*). The growth patterns of each strain (*Bacillus subtilis and Serretia marcescens*) are shown in Figure 6(E and F). The maximum death rate of M-CuNPs treated cultures were observed at 150 µg/ml of

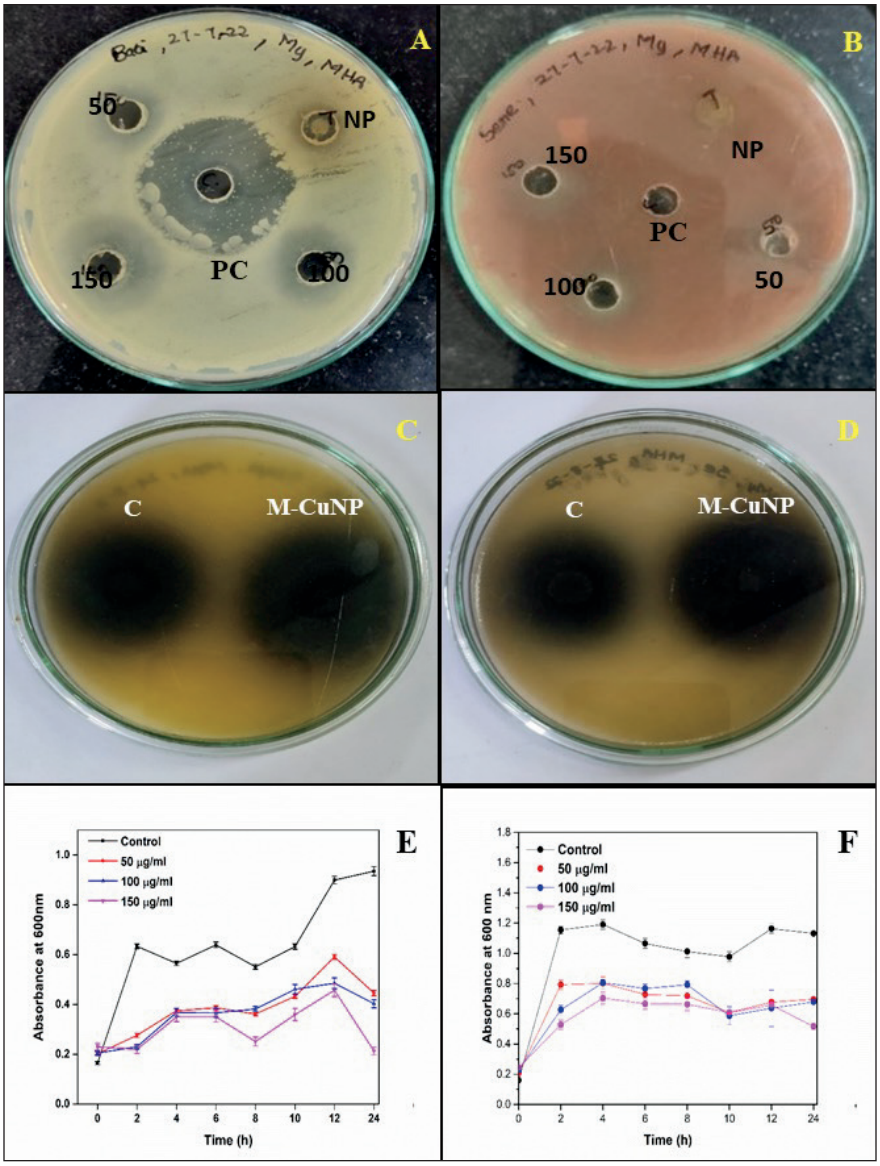

Figure 6. Antibacterial activity of M-CuNPs against **A)** *Bacillus subtilis* **B)** *Serratia marcescens,* and Antibacterial activity of M-CuNPs coated leather against **C)** *Bacillus subtilis* **D)** *Serratia marcescens* **E)** Growth pattern of *Bacillus subtilis* against M-CuNPs **F)** Growth pattern of *Serratia marcescens* against M-CuNPs.

Bacillus subtilis and 150 µg/ml of *Serretia marcescens*. When compared to the controls, the results show that the M-CuNPs exhibited decline in growth of bacterial species by increasing the incubation period. The experimental trials were repeated thrice for the standard deviation.

M-CuNPs concentration of 150 µg/ml showed the maximum antibacterial activity and therefore a concentration of 150 µg/ml of M-CuNPs has been fixed for leather finishing application. Cow upper leathers were finished with a resin-finishing formulation along with the optimized concentration of M-CuNPs. Figure 6 (C, D) shows the zone of inhibition of M-CuNPs coated leathers. The zone of inhibition of M-CuNPs coated leather was measured as 13±0.21 mm and 11±0.2 mm against *Bacillus subtilis* and *Serratia marcescens*, respectively. Antibacterial activity of the M-CuNPs coated leathers also exhibited a much higher zone of inhibition against *Bacillus subtilis* as compared to *Serratia marcescens*.

Maximum inhibition of *Bacillus subtilis* might have occurred due to their higher quantity of amines and carboxyl groups (present in the Bacillus membranes) on its surface and copper has a higher affinity to these functional groups.[21] This interaction may lead to rapid cell wall lysis and cell organelle disruption resulting in the inhibition of bacteria.[21] The zone of inhibition of non-coated leather was observed around 7 mm at 150 µg/ml against *Bacillus subtilis* and 6 mm at 150 µg/ml concentration against *Serratia marcescens.* From the results of the antibacterial activities of leather with and without the coating of M-CuNPs, it was found that the zone of inhibition was highest in leather due to M-CuNPs. The uncoated leather also exhibited a zone of inhibition for the two species, which itself conforms that it is a tanned and finished leather and resists microbial attack. However, the leather coated with M-CuNPs resulted in much higher values than that of non-coated leather which can be attributed to enhanced preservation capabilities of M-CuNPs. Based on the results of the above study, the packing materials used for the preservation of the finished leathers and leather products can be coated with these sustainable green nanomaterials for enhanced preservation during packaging and transit for logistics and export.

Anti-bacterial mechanism of copper nanoparticles

The antibacterial properties of M-CuNPs can be related to their capability to break the bacterial cells and cause multiple noxious effects by the generation of reactive oxygen species in the bacterial cells.[22] M-CuNPs have the property of a high surface area to volume ratio, which permits copper ions to effectively bind to the surface of bacterial cell walls through electrostatic force, and consequently, damage cell walls in Gram-positive bacterial strains, whereas in Gram-negative bacteria, intercellular constituent leakage and cell death take place. The surface attached Cu nanostructures promote the generation of reactive oxygen species, which is crucial in the antibacterial effect of copper caused by oxidative stress. The imbalance in cells occurs due to excess reactive oxygen species over the capability of the cell to create an antioxidant response. It releases highly unstable oxygen radicals such as superoxide (O_2^-), hydroxyl (OH), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2), which are highly reactive and have strong disrupting potential, leading to lipid peroxidation, protein oxidation, and DNA degradation in the bacterial cell. M-CuNPs may also possibly invade the bacterial cells, and M-CuNPs can disrupt the whole cytoplasmic cellular components affecting the enzyme functions. M-CuNPs can also bind with the DNA molecules and lead to the disordering of the helical structure by cross-linking nucleic acid strands.[23-25] The antibacterial mechanism of M-CuNPs is given in Figure 7.

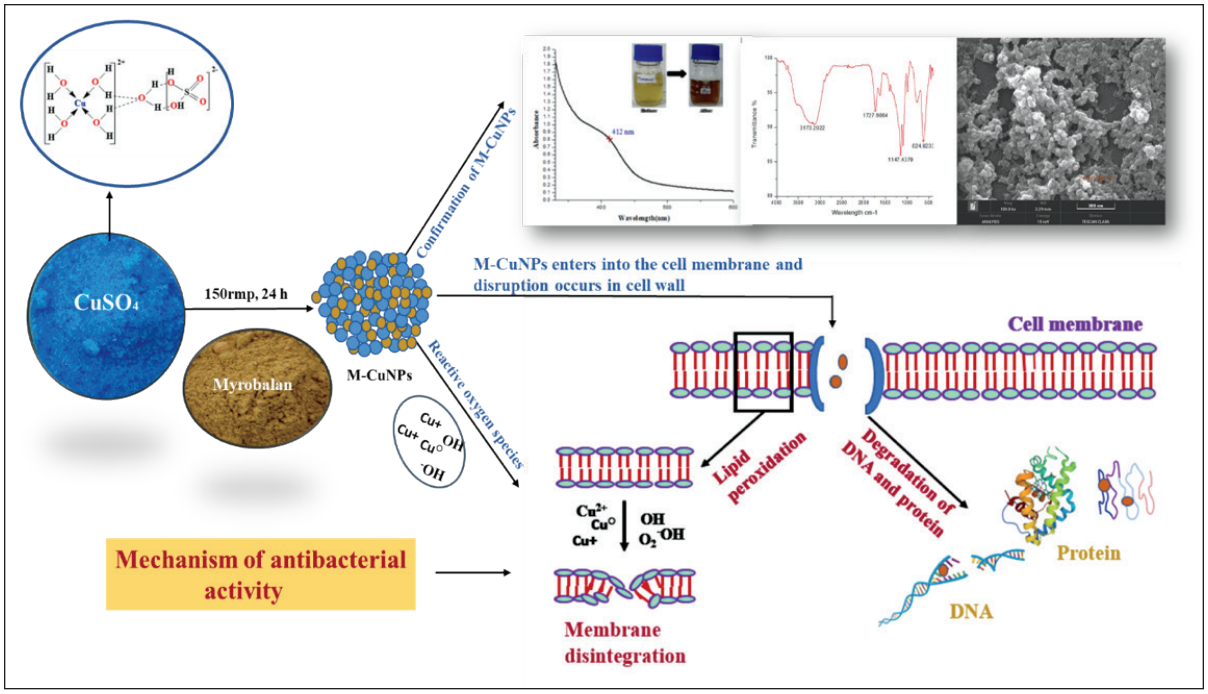

Figure 7. Synthesis and antibacterial activity mechanism of M-CuNPs

Conclusion

M-CuNPs were found to be effective in the enhanced preservation of leather by coating on the finished leather. The preservation properties were established by characterization of M-CuNPs with nano size distribution, zeta potential, SEM, and EDX studies. The spectroscopic studies established the interactions between myrobalan and the Cu ions in nano form confirming functional groups involved in the synthesis and capping process. The synthesized and capped M-CuNPs facilitate the preservation abilities of the leather, which was confirmed by subjecting the leather to common pathogenic species. The enhanced zone of inhibition observed from M-CuNPs coated leather in comparison to non-coated leather established that M-CuNPs can be used as a sustainable antibacterial agent for preserving the finished leather. These M-CuNPs can also be used for coating the packaging material (Paper sheets) that are generally used for the preservation of finished leather and leather products. The antibacterial properties will be enhanced in the leather and leather products during transit while exporting the leather and its products through the sea route, which generally takes longer duration and has higher moisture content in shipment.

Acknowledgement

The authors gratefully acknowledge the financial support provided by the CSIR-Integrated Skill Initiative Program, Project Code - NWP0100, CSIR-CLRI Communication number is 1875.

Conflict of Interest

The authors declare no conflict of interest.

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