Sustainable Finished Leather Preservation: Part II - Wattle Tannin Capped Copper Nanoparticles

by

D. Ruby Shekinah, Saranya Kailasam, Sujata Mandal, Swarna V. Kanth*
Centre for Human Organisational Resources Develpment (CHORD),
CSIR-Central Leather Research Institute, Adyar, Chennai-600020, India

Abstract

Part 1 of the current paper described the green synthesis of myrobalan capped copper nanoparticles for the preservation of the finished leather. In Part II of the current study, Copper Nanoparticles (CuNPs) were synthesized using green synthesis with wattle (tannin) extract as a reducing agent. Green synthesis is the preferred approach for the synthesis of metal nanoparticles due to its eco-friendly and cost-effectiveness for the preservation of finished leather. To improve the antibacterial qualities of leather with different types of finishes, the resin finished leather was coated with synthesized wattle copper nanoparticles (W-CuNPs). The chemical constituents of wattle induce the reduction of Cu^{2+} ions to CuNPs with the capping process and also remain as a stabilizing agent for the capping process. The formation of W-CuNPs was confirmed by UV-Spectroscopy at an absorbance of 467 nm. The identified functional groups such as -OH, C=C, and C-N were analyzed by Fourier transform infrared spectrum (FT-IR) which are responsible for the reduction and stabilization of copper nanoparticles. The spherical shape of the nanoparticles was ascertained by Scanning Electron Microscope (SEM), whereas Energy dispersive X-ray (EDX) studies confirmed the presence of copper with 65%. The average particle size of W-CuNPs was found to be 96 nm. The zeta potential value of -24 mv affirmed the stability of the W-CuNPs. The maximum zone of inhibition was 12 mm against Gram-positive (*Bacillus subtilis*) and 11 mm against Gram-negative (*Serratia marcescens*) bacteria on W-CuNPs coated finished leather, which exhibits strong antimicrobial activity. Hence, CuNPs synthesized by wattle extract have the potential to preserve the finished leather and its products as an antibacterial agent.

Introduction

Tannins are polyphenolic compounds and are used for converting the hide and skin into leather.[1] Wattle is widely used as the tanning agent for making leather from the raw hide or skin.[2] These tanned leathers are further processed post-tanning using retanning chemicals[3] (poly phenols, melamine, acrylics), fatliquors[4] (oils and fat), dyes (acid and basic dyes), and finishing processes using acrylic binders[5] and cellulose acetate butyrate and nitro cellulose lacquers. All these chemicals have the potential to further preserve leather. Despite the stabilization effect of wattle as tannins and other chemicals from the post-tanning and finishing processes of the leather, the finished leather and its products are further susceptible to microbial attack due to its large surface area and ability to retain moisture. To prevent microbial contamination of the entire range of finished leathers, it is necessary to protect the leather and leather products with antimicrobial properties in a sustainable way.[6] Wattle is widely used as the tanning material for vegetable tanned leather. Nanotechnology has gained prominence and is gaining significant technological advances in varied fields of science and technology. To overcome the problem of toxicity, nanotechnology, and green chemistry merge to produce eco-friendly nanomaterials using plants, microbes, etc.[7] The green synthesis of nanoparticles has gained prominence due to its reliable, sustainable, eco-friendly, cost-effective, and non-toxic protocols. Copper nanoparticles have significant physical and chemical properties with low production costs. According to green synthesis, the tannin extract acts as a strong natural reducing agent and stabilizer for copper nanoparticles. Wattle tannins are responsible for reducing copper metal salts into copper nanoparticles and capping agents. Tannins are also responsible for the size and morphology of the nanoparticles. In Part II of the present study, we have studied wattle from the commercial vegetable tannin as the reducing agent for the synthesis of copper nanoparticles and evaluated their antimicrobial properties in the finished leather.

Materials and Methods

Preparation of tannin extraction

Approximately 1 g of wattle was mixed with 100 ml of de-ionized water and boiled at 60°C for 15 min. The extract was cooled and filtered through Whatman No. 1 filter paper. The filtrate was used for the synthesis of copper nanoparticles.

Synthesis of W-CuNPs

In the typical synthesis of copper nanoparticles, 10 ml of wattle extract was added into 90 ml of 10 mM copper sulphate solution and the mixture was kept under shaking conditions (150 rpm) for 24 h at 37°C. The pellet was collected by centrifugation at 6000 rpm for 10 min and washed with double distilled water to remove all the debris. Supernatant was removed and pellets were dried overnight in a hot air oven at 60°C. Wattle synthesised copper nanoparticles were collected and stored at room temperature for further use.[8]

*Corresponding author email: swarna@clri.res.in
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Characterization of W-CuNPs

UV-Visible spectrophotometer

The reduction of copper nanoparticles was observed using UV-Visible spectrophotometer (115 VAC, Shimadzu, Kyoto, Japan). For the analysis, tannin extract was used as blank, and synthesized copper nanoparticles were scanned at the wavelength range between 200–700 nm.

Fourier Transform Infrared Spectroscopic

The FT-IR analysis was performed by the potassium bromide (KBr) pellet method within a fixed spectral range. It analyses the chemical bonds of nanoparticles by their absorbance and transmittance value in the ranges of 400 to 4000 cm^{-1} by Fourier transform infrared spectroscopy (JASCO, Japan).

Scanning Electron Microscopy and Energy Dispersive X-ray

The morphology of copper nanoparticles was analyzed using SEM. Micrograph imaging was carried out at a voltage of 10 keV.[8] The elemental composition of synthesized copper nanoparticles was examined using EDX.

Dynamic Light Scattering

The size, distribution of synthesized copper nanoparticles, and zeta potential values were determined by DLS using Zeta Sizer Nano-ZS90 (Malvern Instrument Ltd, Malvern, UK).

Methodology of Leather Nanocoatings

Cow upper crust leather of Indian origin was selected for the evaluation. The leather (dimension 10 cm × 10 cm) was resin finished with W-CuNPs. All the details about finishing formulations were discussed in Part I paper. 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.

Antibacterial assay of W-CuNPs and W-CuNPs coated leather

The antibacterial activity of synthesized copper nanoparticles was assessed using the agar well diffusion method against Gram-positive (*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 were filled with different concentrations of W-CuNPs (300 µg/ml, 400 µg/ml, and 500 µg/ml). Penicillin was used as a positive control. The antimicrobial properties of the synthesized nanoparticles were determined by the measurement of the zone of inhibitions. The leather samples (10 mm diameter) were treated with 500 µg/ml of W-CuNPs on the grain side and were placed on the pathogens spread MH agar plates and incubated for 24 h at 30°C. Zone of inhibition (ZOI) was measured after the incubation period.[6]

Effect of copper nanoparticles on growth of bacteria

The growth curve of the bacterial cells treated with synthesized W-CuNPs was evaluated where the fresh cultures were prepared using Nutrient broth and maintained at a concentration of 10^6 CFU/mL. The fresh cultures were then treated with W-CuNPs at different concentrations (300µg/mL, 400µg/mL,500µg/mL) for 24 h at 37°C. Growth pattern of the bacterial cultures in the presence of W-CuNPs have been monitored at 600nm in regular time intervals (2,4,6,8,10,12 and 24 h).

Results and Discussion

UV- Visible Spectroscopy of W-CuNPs

In the synthesis of metallic nanoparticles, the color change of the solution is considered as a primary indication.[9] The color change of the solution from light brown to dark confirmed the presence of synthesized W-CuNPs. The color change of W-CuNPs in the solution is due to the excitation of surface plasmon vibration in copper nanoparticles.[10] The absorbance of copper nanoparticles appears at 467 nm (Figure 1). Different concentrations of copper solution (1 mM, 2 mM, 5 mM, and 10 mM) were used to attain maximum production of copper nanoparticles. The concentration of wattle was fixed constant at 5%. The UV spectrum of the W-CuNPs with different concentrations of copper solution

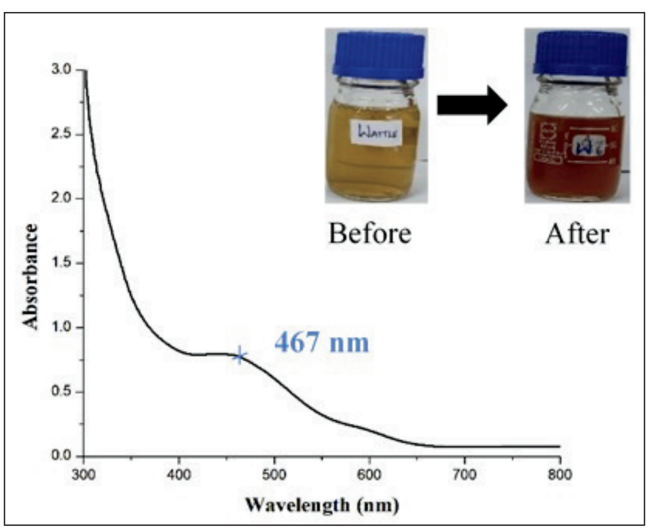

Figure 1. Visible color observation and UV Spectra of W-CuNPs

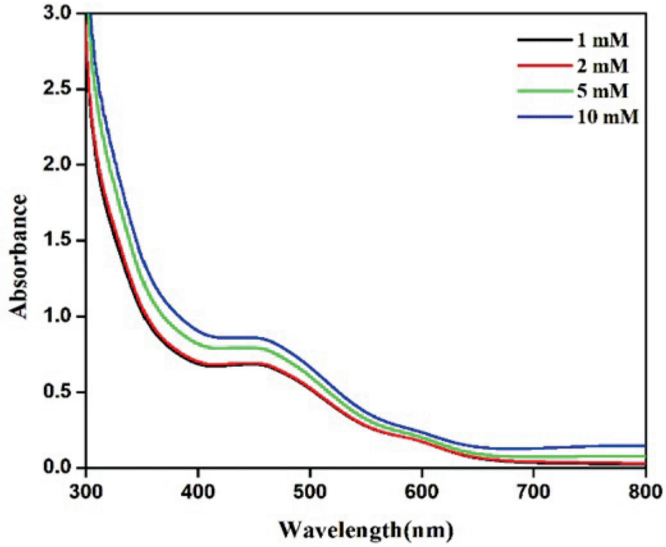

Figure 2. UV spectra of W-CuNPs at different concentrations

(Figure 2) showed that, while increasing the concentration of the copper solution, the intensity of absorbance peaks also increased. The absorption peak was observed at the blue shift region, which is due to the electrons of the W-CuNPs that are thermally excited to the conduction band.

Particle size distribution of W-CuNPs

DLS analysis was carried out to find out the size and surface charge of the nanoparticles. The average particle size of W-CuNPs was found to be 96 nm with a polydispersity index of 1.330. In this study (Figure 3), the zeta potential value was -24 mV. The high negative potential value indicated the high stability of W-CuNPs. Further analysis related to the particle size was carried out using SEM examination that also resulted in average particle size much less than 100 nm.

Functional group analysis of W-CuNPs

FTIR analysis was carried out to find the functional groups, that are involved in the synthesis and capping process. The wavenumber modifications in the functional groups in the wattle tannin and W-CuNPs were analyzed. The FTIR spectrum

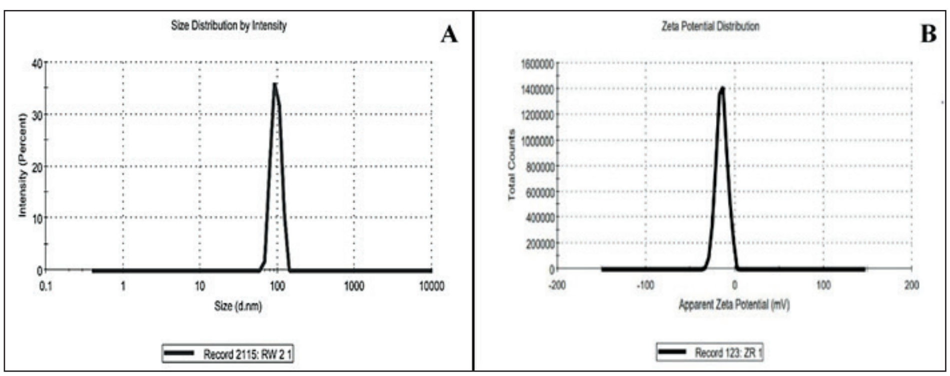

Figure 3. A) Particles Size distribution **B)** Zeta potential analysis of W-CuNPs

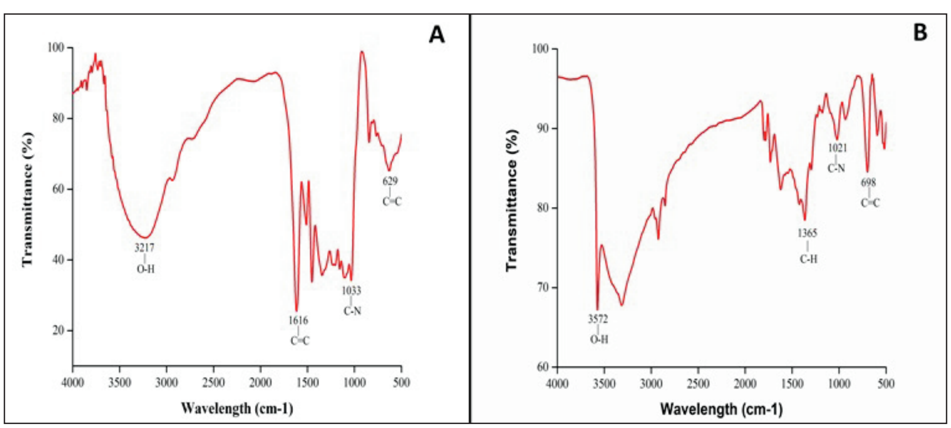

Figure 4. A) FTIR spectrum of wattle **B)** FTIR spectrum of biosynthesized W-CuNPs

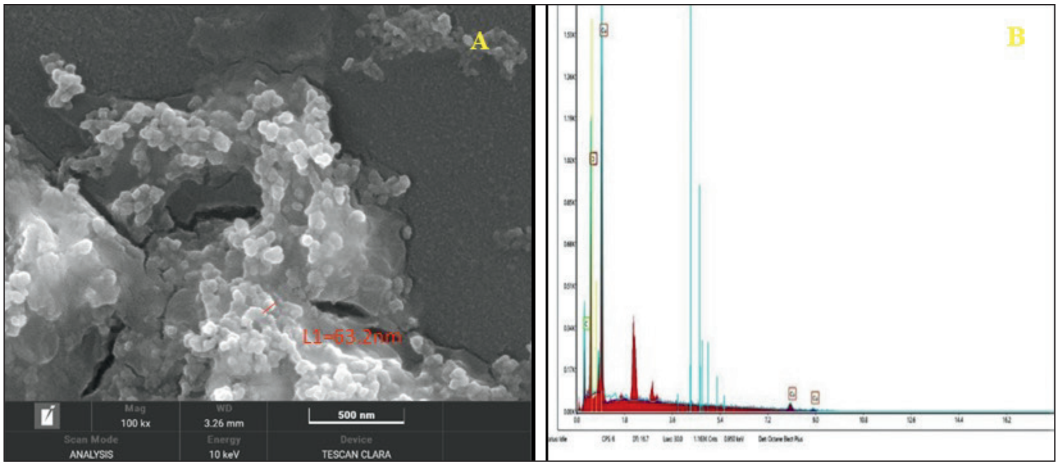

Figure 5. A) SEM micrograph of W-CuNPs **B)** Elemental analysis of W-CuNPs

of both wattle extract and W-CuNPs is shown in Figure 4. The broad peak at 3218 cm^{-1} of wattle extract shifted to 3572 cm^{-1} in W-CuNPs, which represents the hydroxyl (-OH) functional groups in alcohol and phenolic compounds. The C=C stretching was located at 1616 cm^{-1} in wattle was not found in the W-CuNPs. The peak at 1034 cm^{-1} in tannin shifted to 1021 cm^{-1} in W-CuNPs, which represents the C-N stretching amine. The wavelength of 630 cm^{-1} shifted to 698 cm^{-1}, which represents C=C bending alkene. Hence, the FTIR results indicate the functional groups of -OH, C-N, and C=C that are responsible for the capping and reduction of W-CuNPs.[11]

Morphology and size of W-CuNPs

The surface morphology and the elemental analysis of W-CuNPs were determined by SEM microscopy and EDX. As shown in Figure 5, SEM analysis revealed that W-CuNPs are spherical in shape, and the average size was found to be 63.2 nm. The strong signal of copper atoms in EDX confirmed the formation of copper nanoparticles. The results showed a high amount of copper content present in the W-CuNPs with a percentage of 65% at 10 keV. In addition to the copper, oxygen, and carbon elements were also recorded with the percentages of 21.42% and 13.13%, which could be due to the presence of biological compounds of tannins.[12]

Antibacterial Activity of W-CuNPs

The zone of inhibition of W-CuNPs against two pathogenic bacteria such as *Bacillus subtilis,* and *Serratia marcescens* confirmed that W-CuNPs have potential antibacterial activity. The measured values of the zone of inhibition are presented in Table I. The results showed that W-CuNPs are highly active against gram-positive bacteria (*Bacillus subtilis*) when compared to gram-negative bacteria (*Serratia marcescens*). As shown in Figure 6, the zone of inhibition was observed as 3 mm at 300 μg/ml, 5 mm at 400 μg/ml, and 6 mm at 500 μg/ml concentration against *Bacillus subtilis* and 2 mm at 300 μg/ml, 3 mm at 400 μg/ml and 5 mm at 500 μg/ml against *Serratia marcescens*. In the present study, increased concentration of W-CuNPs also increased the inhibition of bacterial growth. Whereas, the effect of W-CuNPs on growth of bacterial cells was evaluated. The changes in the growth patterns of W-CuNPs treated cultures were observed. With the increase in W-CuNPs concentration, the bacterial growth declined in both the species (*Bacillus subtilis* and *Serratia marcescens*). The growth pattern of each strain (*Bacillus subtilis and Serratia marcescens*) are shown in Figure 6 (E and F). The maximum death rate of W-CuNPs treated cultures were observed at 500 μg/ml of *Bacillus subtilis* and *Serratia marcescens*. When compared to the controls, the results show that the W-CuNPs

Table I

Antibacterial activity of the synthesized W-CuNPs

Concentration of W-CuNPs (μg/ml)	**Zone of inhibition (mm)**	
	Bacillus subtilis	***Serratia marcescens***
300	3 ± 0.2	2 ± 0.1
400	5 ± 0.1	3 ± 0.2
500	6 ± 0.22	5 ± 0.3
Penicillin	19 ± 0.1	3 ± 0.1

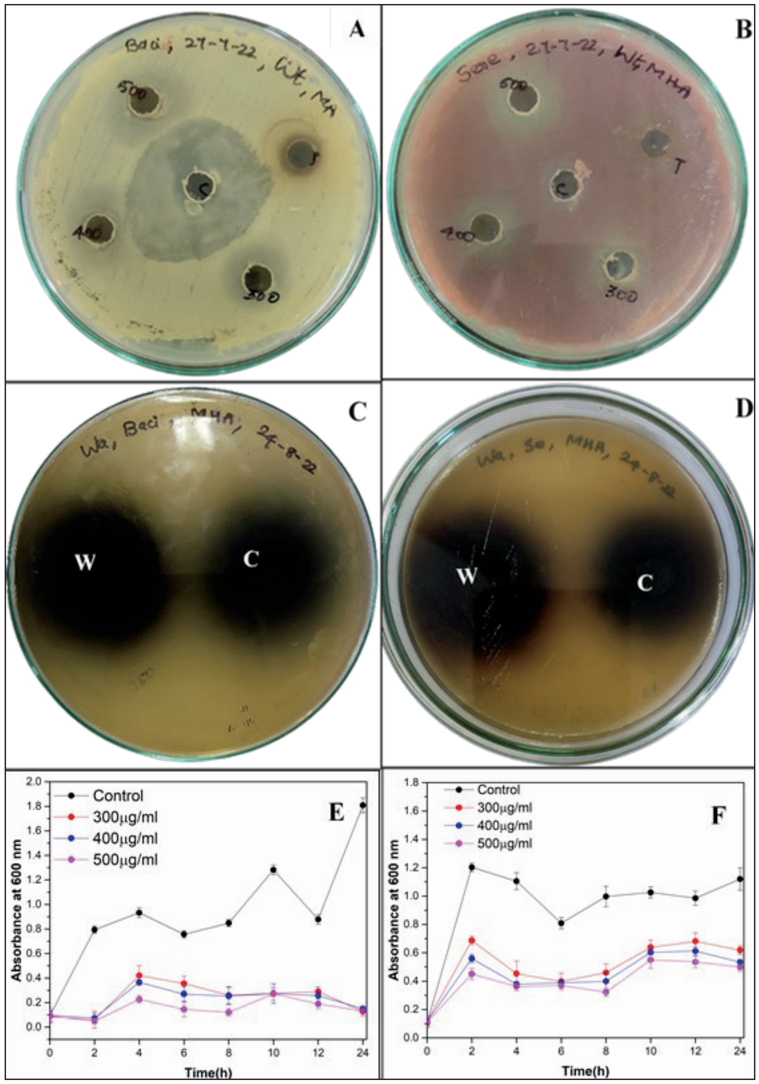

Figure 6. Antibacterial activity of W-CuNPs against **A)** *Bacillus subtilis* **B)** *Serratia marcescens* and Antibacterial activity of W-CuNPs coated leather against **C)** *Bacillus subtilis* **D)** *Serratia marcescens* **E)** Growth pattern of *Bacillus subtilis* against W-CuNPs **F)** Growth pattern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

exhibited decline in growth of bacterial species by increasing the incubation period. The experiments trials were repeated thrice for the standard deviation.

The bacterial growth inhibition of W-CuNPs coated leather was observed around 12 mm at 500 µg/ml against *Bacillus subtilis* and 11 mm at 500 µg/ml concentration against *Serratia marcescens.* W-CuNPs coated leather also exhibited a higher zone of inhibition against *Bacillus subtilis* compared to *Serratia marcescens*. The higher antibacterial activity against gram-positive bacteria is due to the structure of the bacterial membrane which contains a higher quantity of amines and carboxyl groups on its surface and copper has a high affinity for these functional groups.[13] The interaction may cause cell wall lysis and cell organelles disruption, which impedes the growth of bacteria. The plausible antibacterial mechanism of W-CuNPs is based on the reactive oxygen species generation (ROS) which is due to oxidative stress. It releases highly unstable oxygen radicals such as hydroxyl (OH), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2), which have strong disrupting potential that leads to lipid peroxidation, protein oxidation, and DNA degradation in the bacterial cell.[14-15] W-CuNPs also enhance the cellular ROS level that influences lipid peroxidation, protein oxidation, and DNA destruction and finally kill the microorganism cells. The illustration of synthesized wattle copper nanoparticles and characterization along with their antibacterial activity of W-CuNPs coated on leather is given in Figure 7.

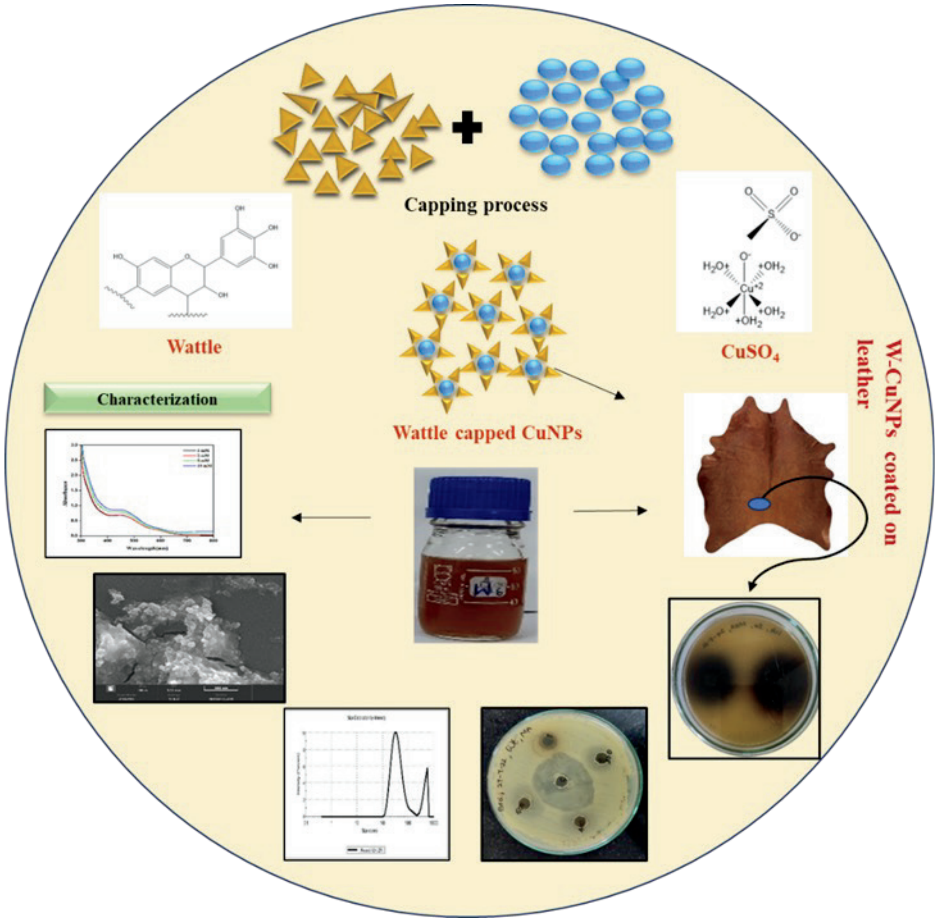

Figure 7. Schematic illustration of synthesis, characterization, and antibacterial effect of W-CuNPs coated on leather

Conclusion

In the present study, Copper Nanoparticles (CuNPs) were synthesized using wattle extract to provide a cost-effective and proficient means for the synthesis of CuNPs. The characterization studies using UV-Vis spectroscopy, DLS, FTIR, SEM, EDX, and antibacterial activity revealed the morphological parameters and the role of wattle as a stabilizing agent during CuNPs synthesis. The particles were spherical in shape. The role of wattle in the formation of CuNPs bending vibrations and stretching bonds was confirmed by FTIR studies. The antibacterial activity performed against *Bacillus subtilis* demonstrated that the maximum ZOI was higher in gram-positive bacteria compared to gram-negative bacteria. Hence, W-CuNPs can be used as potential antibacterial agents for leather and leather products. Wattle and Myrobalan as commercial tanning chemicals can be modified as nano tannins with its application in finished leather or leather products as antimicrobial agents with enhanced properties for green preservation.

Acknowledgement

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Conflict of Interest

The authors declares no conflict of interest.

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