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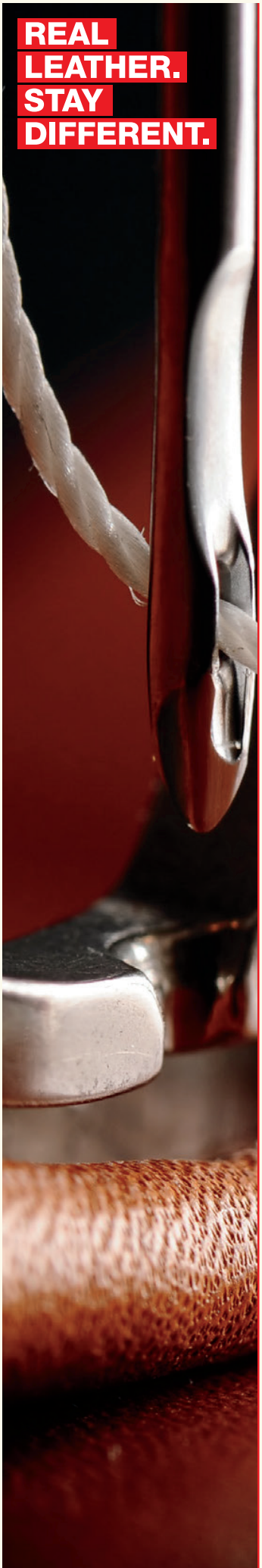

LEATHER BY NUMBERS:

FACTS AND FIGURES FROM THE US LEATHER INDUSTRY AND BEYOND

Note: All figures as of January 2021 or latest available.

ZERO cattle are killed to make US leather. US hides have been valued at JUST 1-2% of a cow's total value for the last two years, which is why they are considered a by-product and often end up as waste. The average price per head of US cattle is $2,000-2.200, while hides vary in price from $5 TO $35 PER PIECE, if sold at all. (1)

330M hides come from the meat and dairy industries around the world. Approximately 34M were processed the US. (2) AS MANY AS 2.4M US HIDES ended up as landfill in 2019, this is 7% of the national total.

Worldwide the waste figure is approximately 40% or 132M hides. With the average hide weighing 25Kg this means that 3M TONNES are thrown away ever year.

Leather production turns more than 4.5M TONNES OF potential waste, every year, into usable, durable goods. This saves 2.7M TONS OF GREENHOUSE GAS EMISSIONS from landfill sites. (3)

Production, processing and distribution of hides and leather products directly employs an estimated 5,486 individuals, who collectively earn more than $384M. US exports of hides and leather was over $1.5BILLION in 2021. (4)

The US exports approximately 95% of all cattle hide and wet blue leather products it produces, worth $2.85BILLION. (5)

Around 45% of global leather production is used to make footwear, 22% for clothing, bags and accessories, 18% for car upholstery, and about 15% for furniture. (6)

Water consumption for the production of leather from cattle hides has fallen by more than 35% in the past 25 years, down from 60 CUBIC-METERS per ton of hides to 38 CUBIC-METERS per ton. US tanneries are required, by law, to connect to effluent treatment plants to prevent pollution. (7)

Leather will biodegrade in LESS THAN 50 YEARS. In contrast, it can take 500 YEARS or more for synthetics, made from petrochemicals, to degrade. (8)

ReFed's conversion rate for food waste is for EACH METRIC TON OF WASTE DISPOSAL there is 9.87MT of CO2 EQUIVALENT emitted. In this case, mostly as methane. (9)

This factsheet is produced by the Leather and Hide Council of America (L&HCA), established to promote the US leather industry which is responsible for a significant proportion of the international trade in hides. The L&HCA works to establish best practice in US leather production and to share this worldwide. Figures quoted refer to the USA unless otherwise stated.

SOURCE:

(1) https://downloads.usda.library.cornell.edu/usda-esmis/files/rx913p88g/w0893q25p/5d86qb66f/lstk0223.pdf
(2) https://downloads.usda.library.cornell.edu/usda-esmis/files/r207tp32d/pg15cj85z/hd76t466z/lsan0422.pdf
(3) 2020 LHCA Infographic
(4) John Dunham & Associates, Economic Impact of the Meat Industry (2016)
(5) https://thesustainabilityalliance.us/wp-content/uploads/2020/04/US-Hide-Skin-and-Leather-Factsheet-0420.pdf
(6) TBC
(7) 2020 LHCA factsheet
(8) https://en.wikipedia.org/wiki/Leather#:~:text=Leather%20biodegrades%20slowly%E2%80%94taking%2025,or%20more%20years%20to%20decompose
(9) https://insights-engine.refed.org/impact-calculator?inputs=%7B%22sector%22%3A%22manufacturing%22%2C%22type%22%3A%22fresh-meat-seafood%22%2C%22unit%22%3A%22tons%22%2C%22alternative%22%3Afalse%2C%22destinations%22%3A%5B%7B%22key%22%3A%22refuse-discards%22%2C%22current%22%3A1%7D%5D%7D

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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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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

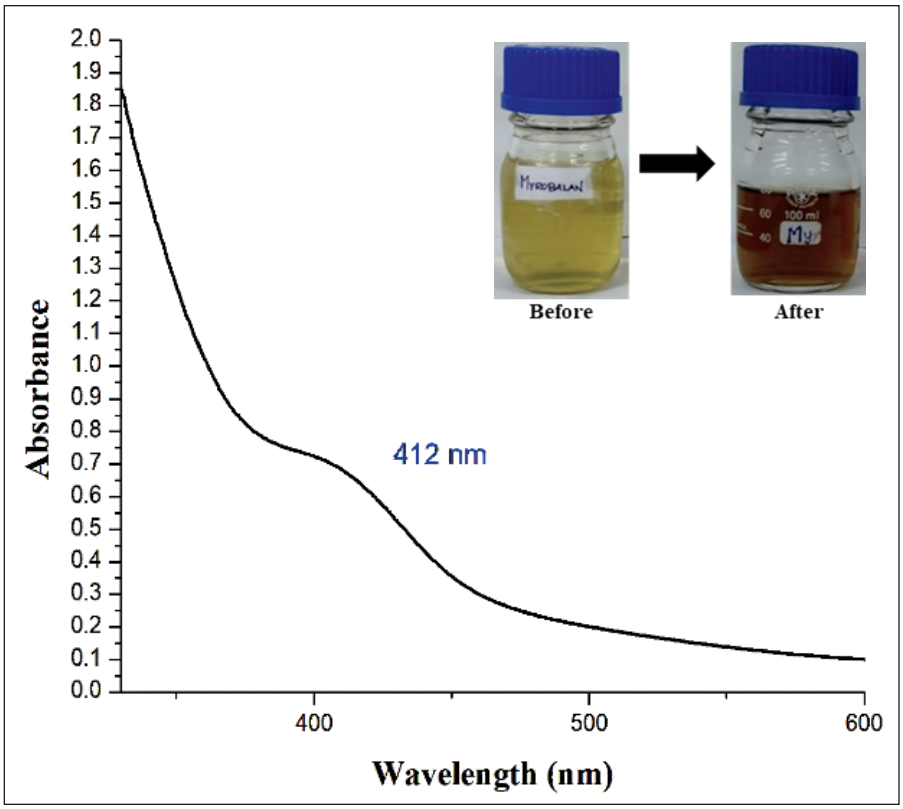

Figure 1. Visible color observation and UV Spectra of M-CuNPs

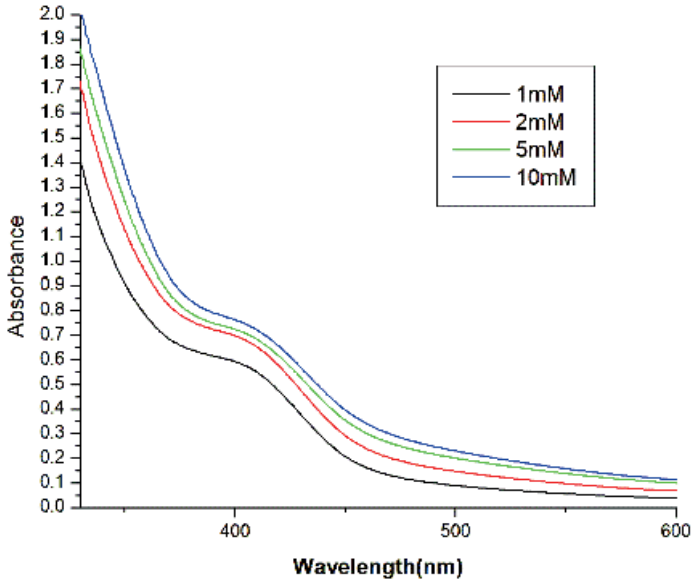

Figure 2. UV spectra of M-CuNPs at different concentrations of Cu solution

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.

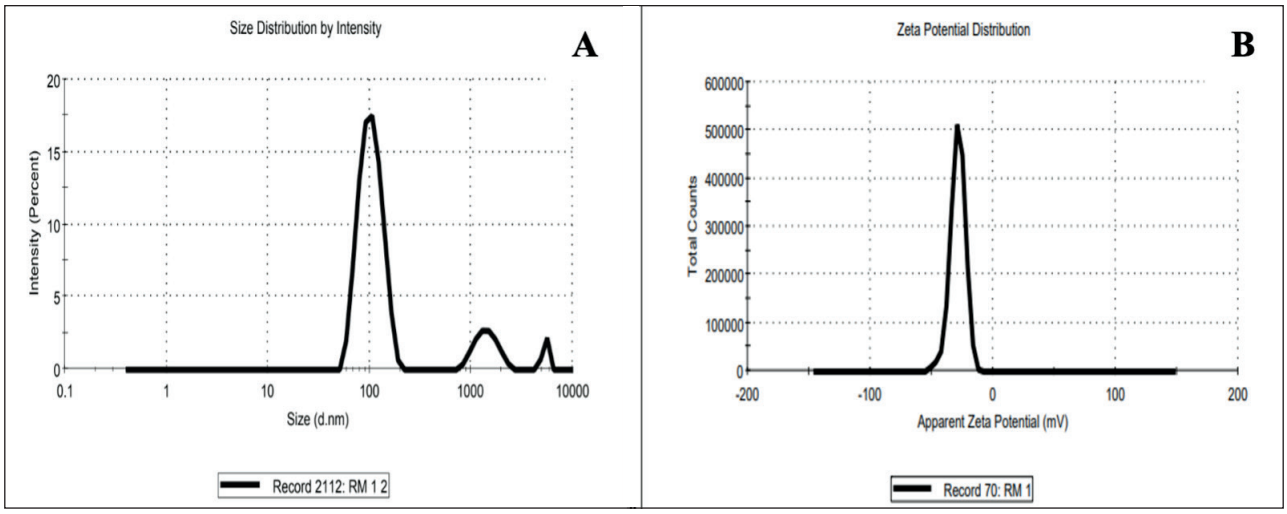

Figure 3. A) Particles Size distribution **B)** Zeta potential analysis of M-CuNPs

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

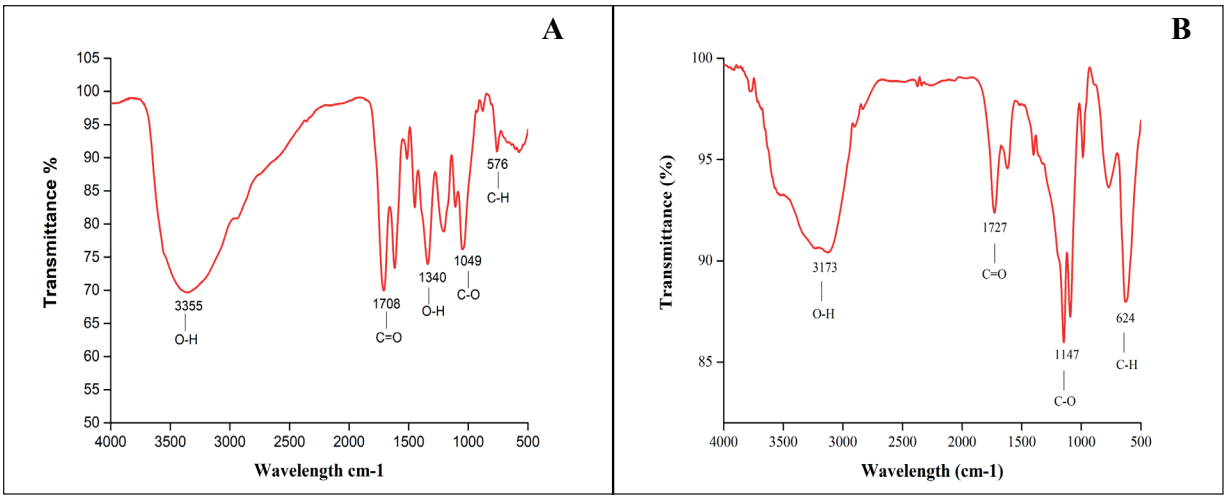

Figure 4. A) FTIR spectrum of Myrobalan **B)** FTIR spectrum of biosynthesized M-CuNPs

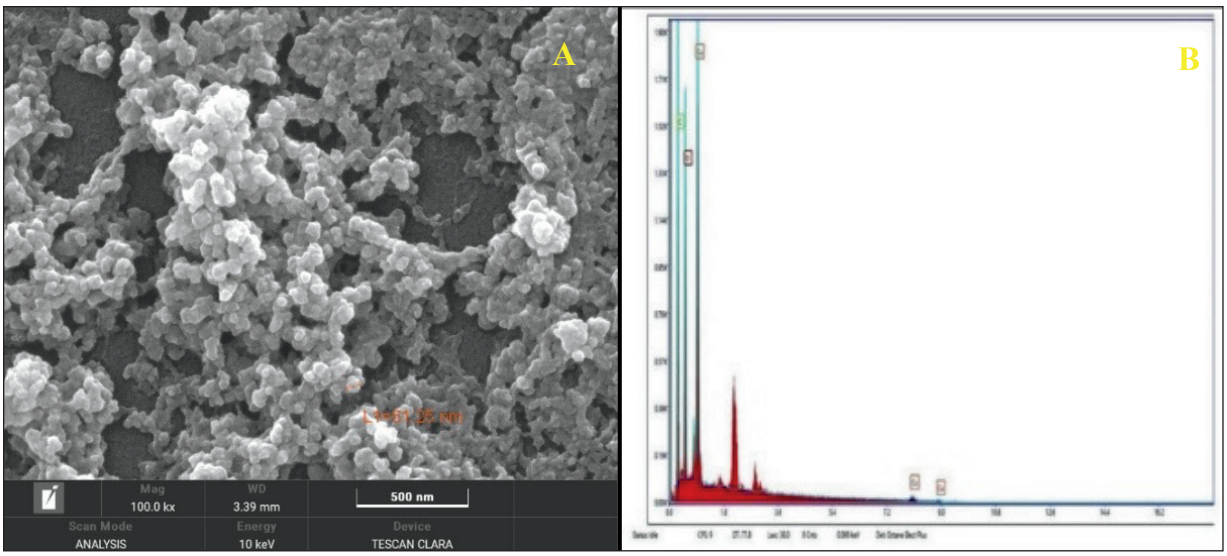

Figure 5. A) SEM micrograph of M-CuNPs **B)** Elemental analysis of M-CuNPs

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

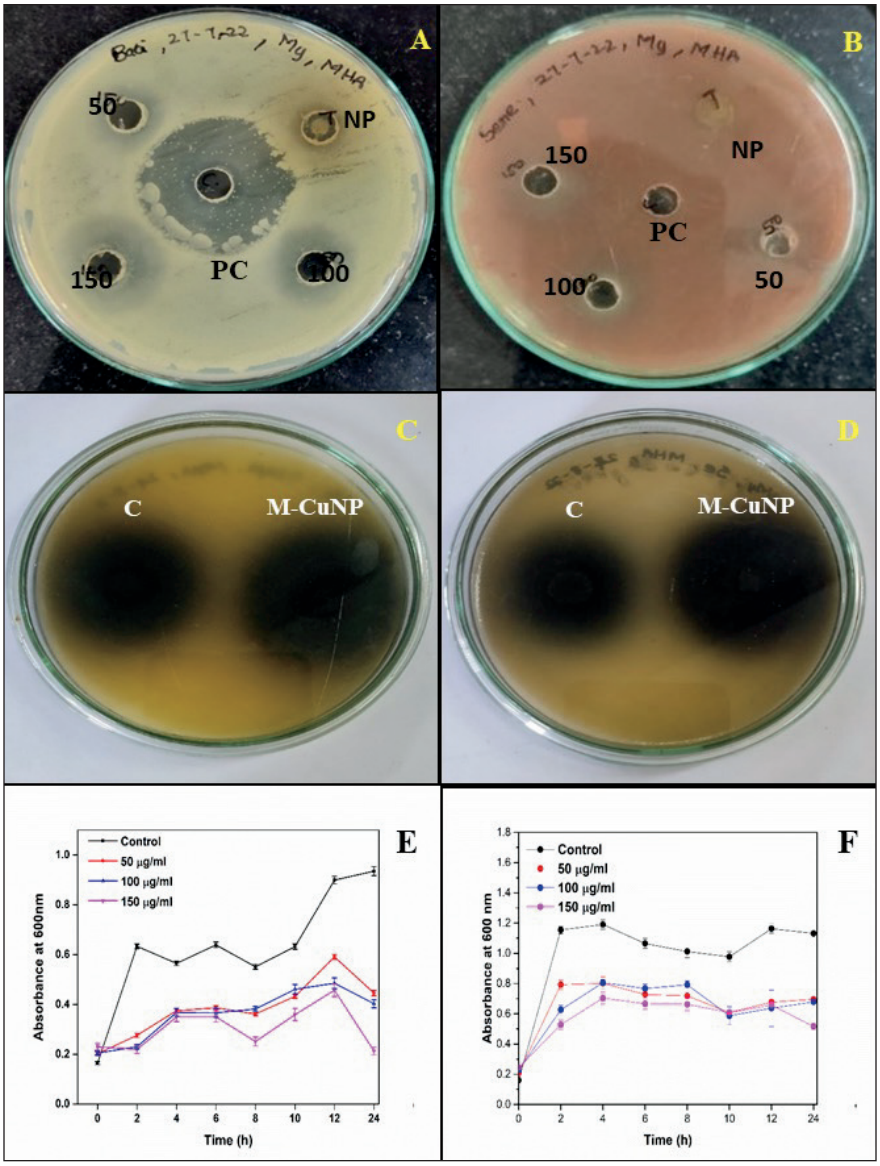

Figure 6. Antibacterial activity of M-CuNPs against **A)** *Bacillus subtilis* **B)** *Serratia marcescens,* and Antibacterial activity of M-CuNPs coated leather against **C)** *Bacillus subtilis* **D)** *Serratia marcescens* **E)** Growth pattern of *Bacillus subtilis* against M-CuNPs **F)** Growth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

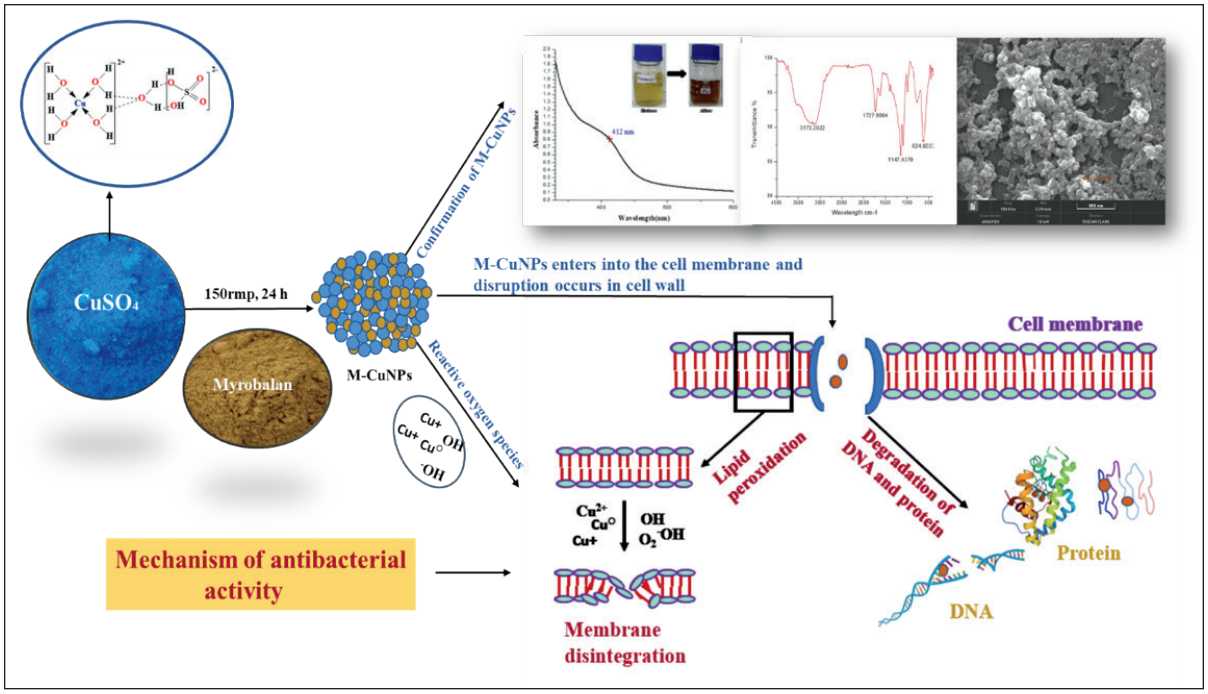

Figure 7. Synthesis and antibacterial activity mechanism of M-CuNPs

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

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Conflict of Interest

The authors declare no conflict of interest.

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

by

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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]

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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

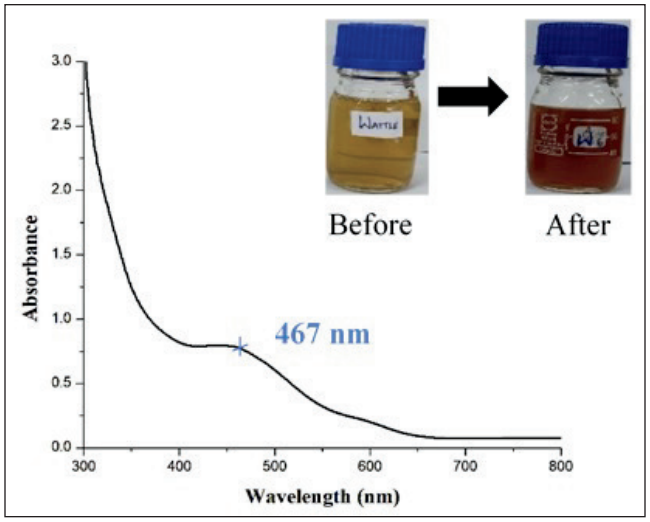

Figure 1. Visible color observation and UV Spectra of W-CuNPs

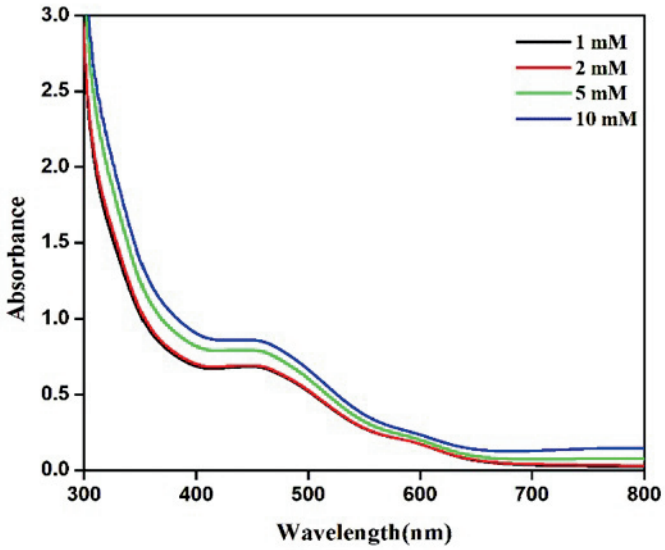

Figure 2. UV spectra of W-CuNPs at different concentrations

(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

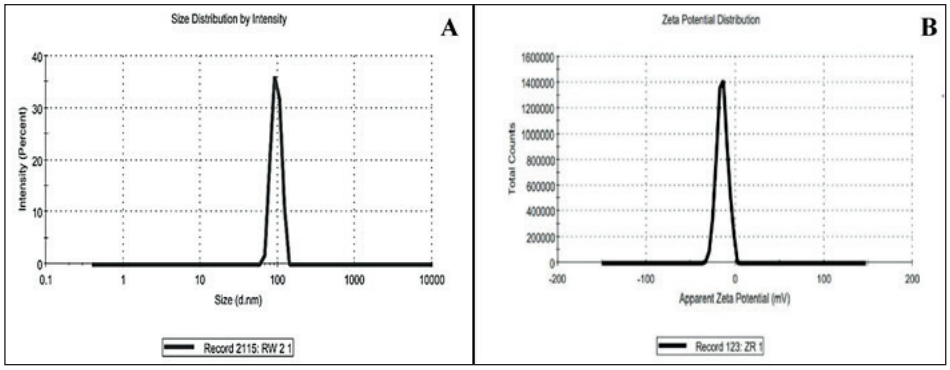

Figure 3. A) Particles Size distribution **B)** Zeta potential analysis of W-CuNPs

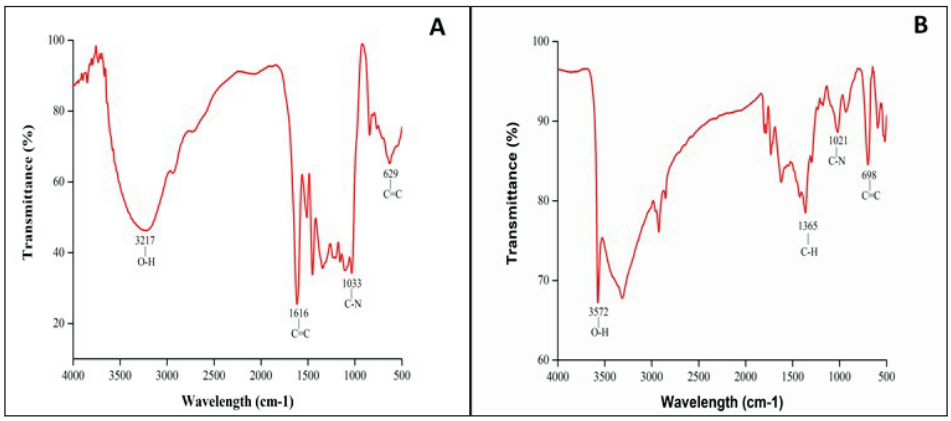

Figure 4. A) FTIR spectrum of wattle **B)** FTIR spectrum of biosynthesized W-CuNPs

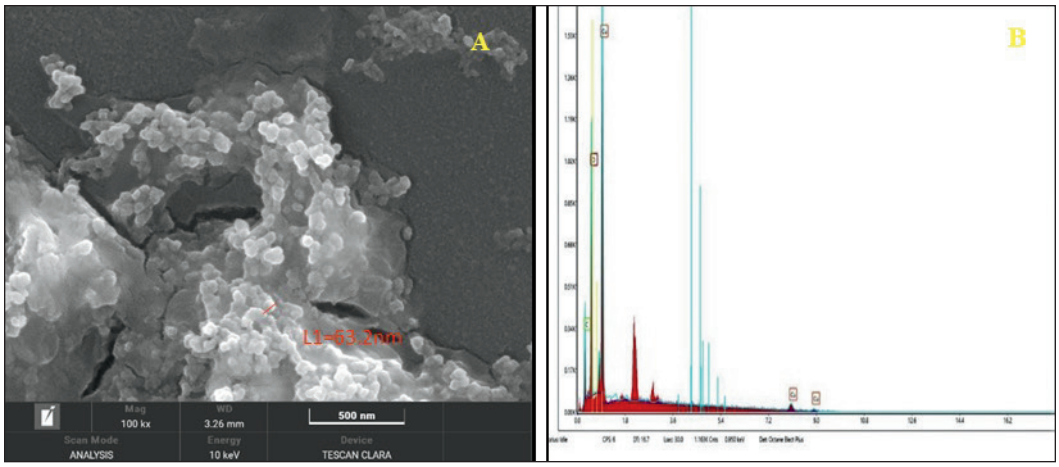

Figure 5. A) SEM micrograph of W-CuNPs **B)** Elemental analysis of W-CuNPs

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

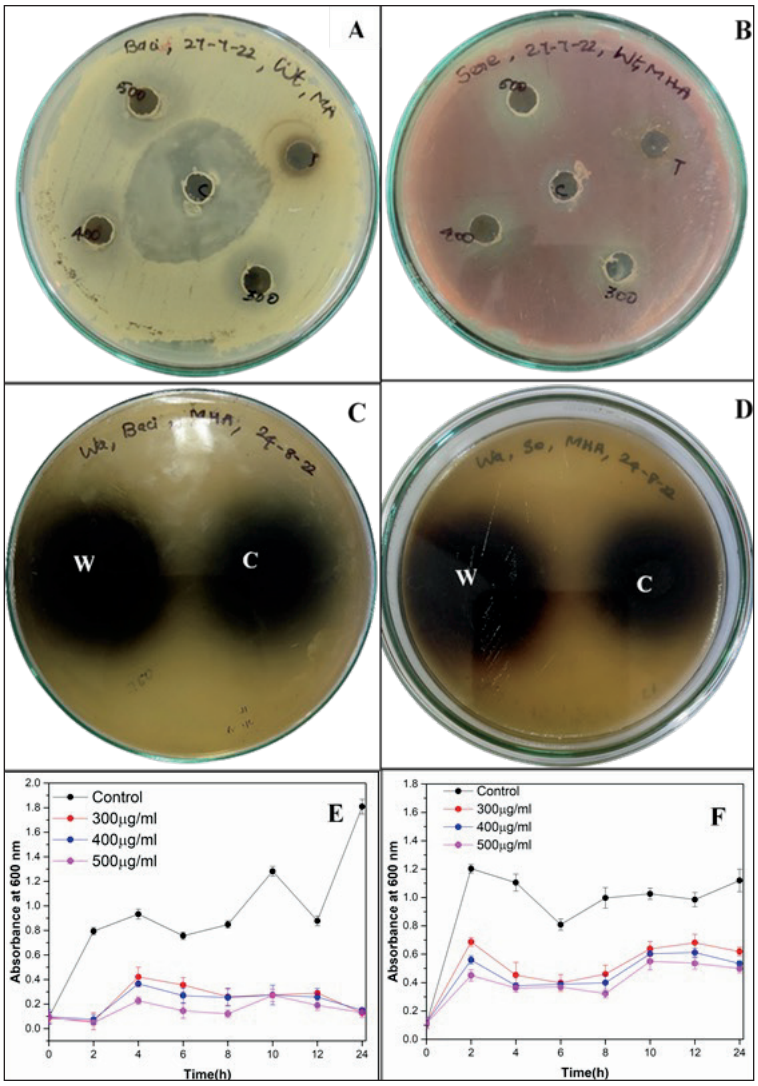

Figure 6. Antibacterial activity of W-CuNPs against **A)** *Bacillus subtilis* **B)** *Serratia marcescens* and Antibacterial activity of W-CuNPs coated leather against **C)** *Bacillus subtilis* **D)** *Serratia marcescens* **E)** Growth pattern of *Bacillus subtilis* against W-CuNPs **F)** Growth pattern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

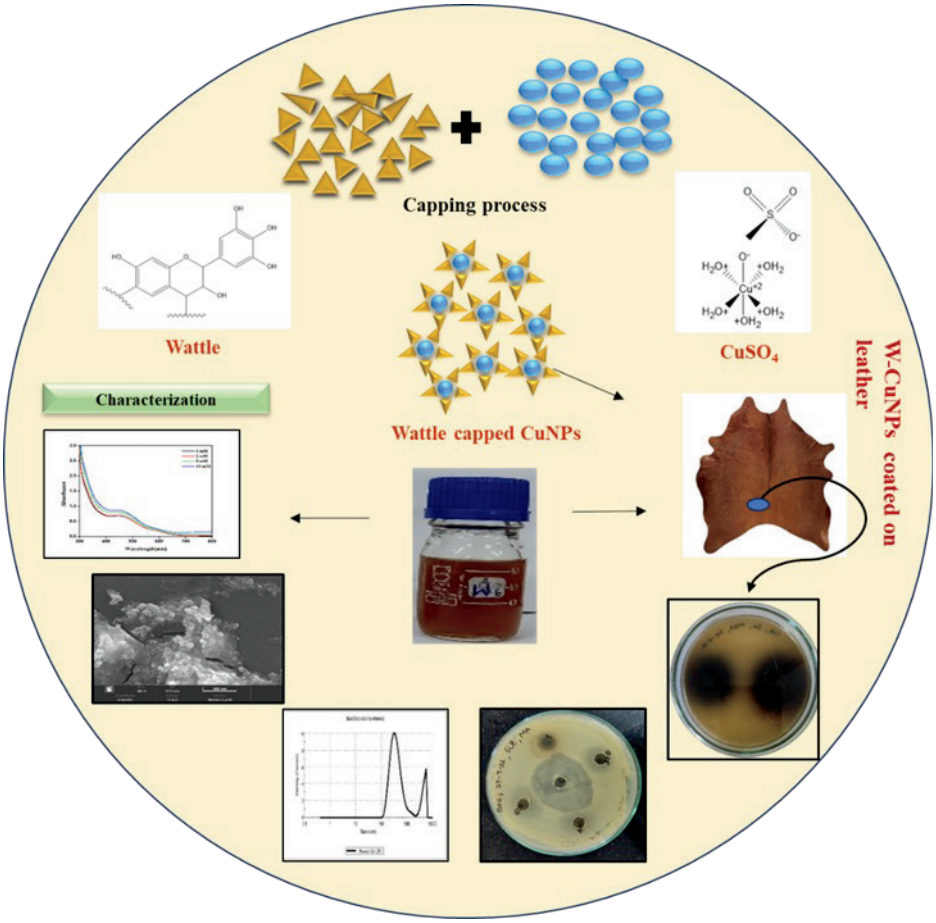

Figure 7. Schematic illustration of synthesis, characterization, and antibacterial effect of W-CuNPs coated on leather

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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Reduction of Cr (VI) Formation in Leather with Herbal Extracts

by
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Abstract

This study investigates the effects of extracts from natural products such as oak bark, and onion peel, on the formation of Hexavalent Chromium (chromium (VI)) or (Cr (VI)) in leather during the finishing process. To enable chromium (VI) formation, finished leather samples were aged under various conditions, including exposure to UV light. The amount of chromium (VI) in each leather sample was measured using a PC UV-Visible spectrophotometer at 540 nm based on the ISO/FDIS 17075 standard method. The results showed that extracts from *Quercus cortex,* and *Allium cepa* significantly reduced chromium (VI) formation in the leather under all aging conditions.

Introduction

Chrome tanning is still the most important and widely used method of tanning in the leather industry. The presence of Cr (VI) in leather has attracted great attention in the modern world as environmental protection requirements and safety regulations became increasingly strict. There are risks when converting Cr(III) to Cr (VI) form in the production, storage, transportation and use of chrome-tanned leather, however more than 90% of the leather in the world is processed using chromium salts because of the facile technology, process reliability and high technological and operational properties of the finished leather.[1,2] However, since 1994 when traces of hexavalent chromium were first detected in leather products, chromium-tanned leather attracted increasing attention[3]as hexavalent chromium can cause cancer, skin allergies, liver and kidney necrosis.[4] The European Union(EU) countries even have zero tolerance policies.[5] Nevertheless, chromium salt in its trivalent form (basic chromium sulfate, $Cr(OH)SO_4$ is used in tanning. Chromium-tanned leather imparts excellent hydrothermal stability due to the stable Cr^{3+} complexes. The stable cross-linked structure is formed through covalent bonds between Cr^{3+} and the side-chain ionized carboxyl groups of collagen proteins.[6] Cr (VI) is not used in any step of leather manufacture; therefore, the emergence of hexavalent chromium in leather products should be explained. Numerous studies focused on processing, auxiliaries,[7] temperature,[8] photoaging,[9] humidity,[10] and other factors in the leather manufacturing process were performed to understand the formation of hexavalent chromium. It was found that peroxide and free radicals (ROO·, RO· and·OH) generated by unsaturated fatliquoring agents, photoaging and temperature, play important roles in promoting the chromium (III) oxidation reaction.[11] Therefore, many antioxidants and reducing agents are used in leather processing with the goal of blocking the free-radical chain oxidation and reducing the produced Cr (VI). Taking into account that antioxidative chemicals in leather production can be a solution to prevent the formation of Cr (VI), it is important to investigate the content of these products and their effect on the leather. Every extra chemical used in leather processing has a different effect on the environment and brings extra costs to production. Within the framework of sustainable production, the use of herbal products at various stages of leather treatment is becoming increasingly important.[12-20] Vegetable tannins (condensed and hydrolysable) have a strong inhibitory effect on the Cr^{3+} oxidation in leather. The greater number of hydroxyl groups on the single-ring polyhydroxy phenol results in a stronger inhibitory effect of α-H oxidation. When the same number of hydroxyl groups are on the single-ring polyhydroxy phenols, o-hydroxy phenols has a stronger inhibition than that of p-hydroxy phenols.[21]

Although there are many studies investigating the effect of herbal extracts on Cr (VI) formation in the wet-end treatment, the extracts used in this research were applied to the leather during the finishing stage. It is important to use natural products as an alternative to chemical products to ensure sustainability in the leather industry.

EXPERIMENTS

Materials and Methods

For the study, bovine leather processed by the Turan-Skin factory, located in Kazakhstan in the Shymkent city, was used. The tanning recipe for leathers is shown in Table I.

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Table I

The recipe for leathers

Process	%	Product	Temperature	Time (min.)	pH
Washing	300	Water	30		
	0,3	Degreasing agent		60	
Drain & Wash					
	300	Water	35		
	2	Chromium salt			
	2	Synthetic tanning agent			
	2	Chrome syntan		60	
Neutralization	1	Sodium formate		60	
	2	Sodium bicarbonate		2×10+60	5,5-6,0
Drain & Wash					
Dyeing-fatliquoring-Retanning	150	Water	40		
	3	Dyestuff		35	
	+70	Water	70		
	6	Natural fatliquor agent			
	4	Synthetic fatliquor agent		60	
	3	Resin retanningagent		30	
	1	HCOOH		30	3,8-4,0
Drain & Wash					
Drying					

Two plants materials were used in this research during the finishing process: Oak bark (*Quercus cortex*) and Onion peel (*Allium cepa*), as shown in Table II.

Extraction Process

The plants were first dried and ground then 100 grams of dried plant material were mixed with 3000 grams of distilled water and boiled for 3 hours over low heat. The resulting liquid was cooled and filtered.[13,22]

Processes with Plant Extracts

During the finishing process of this recipe, a herbal extract made from oak bark (*Quercus cortex*), onion peel (*Allium cepa*), were used instead of chemical pigments. For the control sample, the basic finishing recipe with the use of a chemical pigment or water were used.

The recipe for the finishing of the leather is shown in Table III.

Table II

The plants used in the study and picking regions.

Plant type		Picking Regions	Extract's pH
Common Name	Botanical Name		
Oak bark	*Quercus cortex*	Kazakhstan	3,5
Onion peel	*Allium cepa*	Kazakhstan	4,5

Table III

Recipe for finishing process.

CHEMICALS	APPLICATION Coat (gramme)	EXPLANATIONS
Stage 1		
CPT 2350	150	Acrylic Binder (Alpa Chemistry)
CPT 2345	150	Binder (Alpa Chemistry) Acrylic Polymer
CPU 1641	150	Polyurethane Binder (Stahl)
CRE 1036	200	Acrylic Binder (Alpa Chemistry)
CST 6760	200	StukoWax (Alpa Chemistry)
CW 171	50	Synthetic Wax (Alpa Chemistry)
CW 159	50	StukoWax (Stahl)
CST HD	50	Polyurethane Binder (Stahl)
Dyestuff	2000	Plant Extracts (oak bark, onion peel) or chemical pigment
1) 3× spray – RotoPress (80°C, 150 Bar)-3× spray – RotoPress (80°C, 70 Bar)-3× spray (80°C, 70 Bar)		
Stage 2		
CK 1622	150	Polyurethane lacs (Stahl)
Dyestuff	300	Plant Extracts (oak bark, onion peel) or chemical pigment
1) 2× spray – RotoPress (90°C – 70 Bar)		

All leathers were conditioned for reproducible testing in the laboratory under the same conditions (20 ± 2 °C 65 ± 2% RH).

Aging Process

In many studies, the aging process is implemented for 24 hours at 80°C, and the Cr (VI) amount which occurs in leather is identified. In this study, in addition to the aging condition mentioned, tougher conditions were instituted to facilitate different aging processes with new treatment regimes, in order to form a higher amount of Cr(VI) in the leather and more clearly examine the effect of using herbal extracts. Leathers were aged in a heat-adjustable UV cabinet (UV light of 254 nm) at 80°C and under UV for 24h and 72h periods and Cr (VI) formation was thus enabled in the sample leather. In order to conduct comparative analysis and determine how the herbal extracts influence Cr (VI) formation in leather, subsequent aging processes on the leather were evaluated. Also, in the control group, leather samples were tanned into finished products, with no treatment with any extracts, and were compared with other treated samples to determine significant experimental effects.

Chromium (VI) Analysis on Leather Samples

Cr (VI) analysis of leather samples was conducted at 540 nm with Shimadzu UV-1601 PC UV-Visible brand spectrophotometer according to the IUC 18 (EN ISO 17075) standard method. The analysis was repeated three times.

Statistical Evaluation

When evaluating the results of the study, statistical analysis used NCSS (Number Cruncher Statistical System) 2023, Statistical Software (NCSS LLC, Kaysville, Utah, USA), descriptive statistics (mean, standard deviation, median, frequency and ratio).

Between groups of abnormal distribution of parameters Kruskal Wallis, Post HocDunn tests were used to identify differences between groups; Mann Whitney U test was used in the evaluation according to two groups. The results were evaluated at 95% confidence interval and $p<0.05$; $p<0,01$ significance levels.

Results and Discussions

In this investigation, onion peel and oak bark extracts were used in the finishing process. In the control groups, instead of these two extracts, the processes were carried out by using normal pigment in the factory or just water. After the finishing process, all leathers were conditioned for reproducible testing in the laboratory under the same conditions (20 ± 2 °C & 65 ± 2% RH).

In this study, Chromium(VI) content of leather samples after aging process (80 °C, 24h) is shown in Table IV.

Table IV

Chromium (VI) content of leather samples after aging process (80°C, 24h)

Type of sample	Cr (VI) mg/kg
control group	
chemical pigment 1	12.6
chemical pigment2	12.6
chemical pigment 3	12.6
water 1	12.07
water 2	12.1
water 3	12.01
experimental group	
oak bark 1	< 3
oak bark 2	< 3
oak bark 3	< 3
onion peel 1	< 3
onion peel 2	< 3
onion peel 3	< 3

Table V

Evaluation of chromium (VI) analysis measurements (80°C, 24h)

		Cr (VI) mg/kg
Control	Median (min-max)	12.3 (12.0 -12.6)
	Ort+SD	12.3±0.29
Experimental	Median (min-max)	2.0 (2.0-2.1)
	Ort+SD	2.05±0.05
Test Value		9.7
[c]p		0.007**
control group		
chemical pigment	Median (min-max)	12.6 (12.6-12.6)
	Ort+SD	12.6±0
water	Median (min-max)	12.1 (12.0-12.1)
	Ort+SD	12.1±0.01
experimental group		
oak bark	Median (min-max)	2.0 (2.0-2.0)
	Ort+SD	2.0±0
onion peel	Median (min-max)	2.0 (2.0-2.1)
	Ort+SD	2.05±0.05
Test Value		17
[c]p		0.004**
Post Hoc		oak bark - onion peel p:0.007** water - oak bark p:0.022*

[c]MannWhitney U test [c]Kruskal Wallis &Post HocDunn test *p<0.05 **p<0.01

Statistical evaluation of chromium (VI) analysis measurements (80°C, 24h) is shown in Table V.

When the research results are examined, a statistically significant difference between control group and experimental group was identified; chromium (VI) contents ($p< 0.05$); 12.3mg/kg Cr (VI) in control group, <3 mg/kg Cr (VI) in experimental group. In our study, a statistically significant difference was found between the chromium (VI) contents of chemical pigment and oak bark ($p< 0.05$); chemical pigment -12.6 mg/kg, oak bark <3 mg/kg. The content of onion peel was significantly lower in the experimental group than that of chemical pigment. There was no significant difference between the content of oak bark and onion peel ($p<0,01$). The content of water was significantly higher in the control group than that of onion peel. There was a statistically significant difference between the content of water and oak bark.

It can be clearly seen in Figure 1 that Cr (VI) formation is considerably reduced in leathers treated with onion peel and oak bark extracts.

Chromium(VI) content of leather samples after aging process (80°C, 24h) is shown in Table VI.

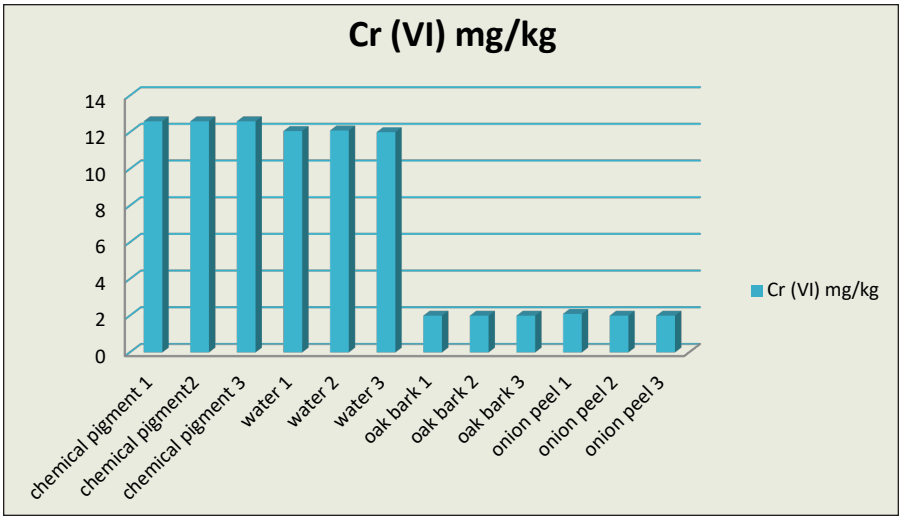

Figure 1. Chromium (VI) content of leather samples after aging process (80°C, 24h)

Table VI

Chromium(VI) content of leather samples after aging process by UV(80°C/UV, 24h).

Type of sample	Cr (VI) mg/kg
control group	
chemical pigment 1	13.2
chemical pigment 2	13.2
chemical pigment 3	13.2
water 1	12.4
water 2	12.4
water 3	12.4
experimental group	
oak bark 1	< 3
oak bark 2	< 3
oak bark 3	< 3
onion peel 1	< 3
onion peel 2	< 3
onion peel 3	< 3

Table VII

Evaluation of chromium (VI) analysis measurements(80 °C/UV, 24h).

		Cr (VI) mg/kg
Control	Median (min-max)	12.8(12.4-13.2)
	Ort+SD	12.8±0.4
Experimental	Median (min-max)	2.4(2.3-2.4)
	Ort+SD	2.35±0.05
Test Value		9.8
ᶜp		0.008**
control group		
chemical pigment	Median (min-max)	13.2(13.2 -13.2)
	Ort+SD	13.2±0
water	Median (min-max)	12.4(12.4-12.4)
	Ort+SD	12.4±0
experimental group		
oak bark	Median (min-max)	2.3(2.3-2.3)
	Ort+SD	2.3±0
onion peel	Median (min-max)	2.4(2.4-2.4)
	Ort+SD	2.4±0
Test Value		17
ᶜp		0.004**
Post Hoc		oak bark - onion peel p:0.007**
		water - oak bark p:0.022*

ᶜMannWhitney U test ᶜKruskal Wallis &Post HocDunn test *p<0.05 **p<0.01

Evaluation of chromium (VI) analysis measurements(80°C/UV, 24h) is shown in Table VII.

No significant difference was found between chromium (VI) content after aging at 80°C, 24h and chromium (VI) after aging at 80°C/UV, 24h. The use of chemical pigment found the highest chromium (VI) content in the control group($p<0.05$), chemical pigment – 13.2 mg/kg and water – 12.4 mg/kg.

Chromium (VI) obtained by using onion peel was found to be significantly lower than chemical pigment ($p<0.05$). No significant difference was found between oak bark and onion peel; oak bark

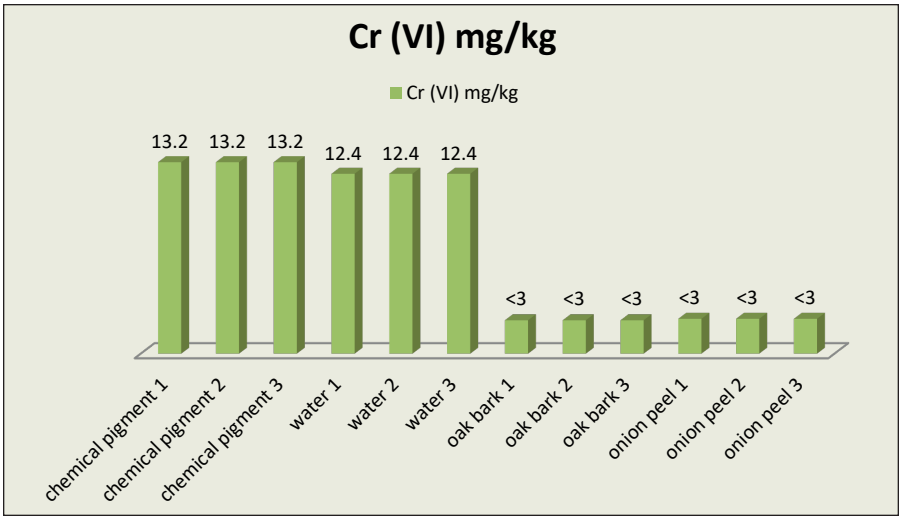

Figure 2. Chromium(VI) content of leather samples after aging process (80°C/UV, 24h)

<3mg/kg and onion peel <3 mg/kg ($p<0.01$). Figure 2 shows that the first six samples finished with the chemical pigment and water are significantly higher, the last six samples finished with the herbal extracts show that the amount of chromium (VI) decreased sharply.

Chromium(VI) content of leather samples after aging process (80°C/UV, 72h) is shown in Table VIII.

Evaluation of chromium (VI) analysis measurements (80°C/UV, 72h) is shown in Table IX. Chromium(VI) content in leather samples after aging process (80°C/UV, 72h)is shown in Figure 3. In this figure, a statistically significant difference was found between chromium (VI) content in the control and experimental groups.

Table VIII

Chromium(VI) content of leather samples after aging process (80°C/UV, 72h).

Type of sample	Cr (VI) mg/kg
control group	
chemical pigment 1	14.4
chemical pigment 2	14.4
chemical pigment 3	14.4
water 1	12.9
water 2	12.9
water 3	12.9
experimental group	
oak bark 1	< 3
oak bark 2	< 3
oak bark 3	< 3
onion peel 1	< 3
onion peel 2	< 3
onion peel 3	< 3

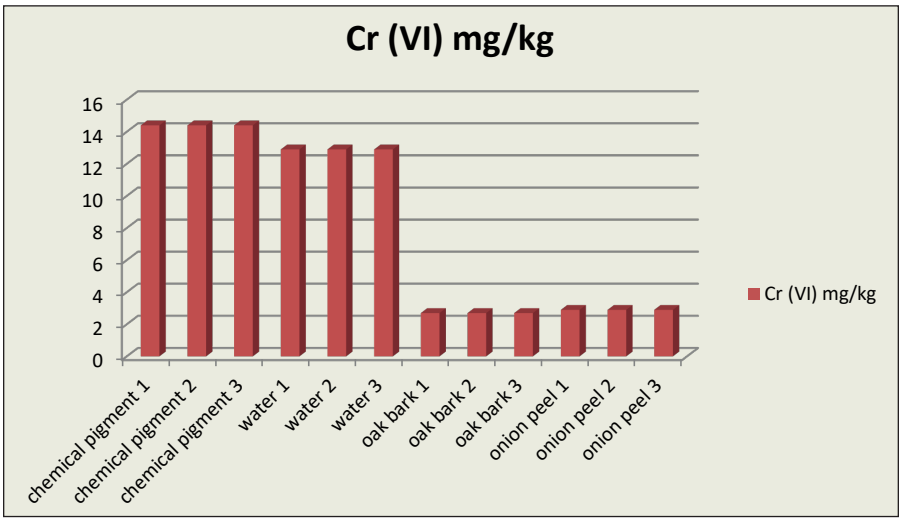

Figure 3. Chromium(VI) content of leather samples after aging process (80°C/UV, 72h)

Table IX

Evaluation of chromium (VI) analysis measurements(80°C/UV, 72h).

		Cr (VI) mg/kg
Control	Median (min-max)	13.6(12.9-14.4)
	Ort+SD	13.6±0.8
Experimental	Median (min-max)	2.8(2.7-2.9)
	Ort+SD	2.8±0.1
Test Value		2.7
[c]p		0.007**
control group		
chemical pigment	Median (min-max)	14.4(14.4-14.4)
	Ort+SD	14.4±0
water	Median (min-max)	12.9(12.9-12.9)
	Ort+SD	12.9±0
experimental group		
oak bark	Median (min-max)	2.7(2.7-2.7)
	Ort+SD	2.7±0
onion peel	Median (min-max)	2.9(2.9-2.9)
	Ort+SD	2.9±0
Test Value		17
[e]p		0.004**
Post Hoc		oak bark - onion peel p:0.007**
		water - oak bark p:0.022*

[c]Mann Whitney U test [e]Kruskal Wallis &Post Hoc Dunn test *p<0.05 **p<0.01

In this study, a statistically significant difference was found between chromium (VI) content in the control and experimental groups (p <0.05); chemical pigment was significantly higher in the control group, chromium (VI) content in the control group was 13.6 mg/kg; chromium (VI) content in the experimental group was <3 mg/kg. Chromium (VI) content obtained using onion peel was significantly lower than using chemical pigment(p<0.05). A dramatically significant difference was found between chemical pigment and oak bark (p <0.05). No significant difference was found between oak bark and onion peel(p<0.01), chromium(VI) content in oak bark was <3 mg/kg, chromium (VI) content in onion peel was <3 mg/kg.

A statistically significant difference was found between water and oak bark (p<0.05). Onion peel content was significantly lower in the experimental group than water.

Conclusion

In this study, the possibility of using some plant extracts as dyes in the finishing process of the leather was investigated. The recipe was provided according to the finished formula, replacing the chemical pigment and water with plant extracts such as oak bark and onion peel. For this reason, chrome-tanned crust leathers were dyed in the finishing process. These processes were repeated 3 times and compared with the leather control, which was made according to the main recipe, where the chemical pigment and water were used.

Three types of aging processes were carried out for the leather:1) the aging process was implemented for 24 hours at 80°C; 2) leather was aged in a heat-adjustable UV cabinet (UV light of 254 nm) at 80°C and under UV for 24h and 72 h periods and chromium (VI) formation was thus activated in the leather sample; 3) leather was aged in a UV cabinet (UV light of 254 nm) at 80°C and under UV

for 72h period. In three cases, it was noticeable that chromium(VI) was significantly reduced in leather treated with natural extracts. In the first case, chromium (VI) in the chemical pigment trial showed 12.6 mg/kg; water 12.1 mg/kg; oak bark <3 mg/kg; onion peel < 3 mg/kg. In the second case, chromium(VI) in the chemical pigment 13.2 mg/kg; chromium(VI) in water 12.4 mg/kg; chromium(VI) in oak bark < 3mg/kg; chromium(VI) in onion peel < 3 mg/kg. In the third case, chromium(VI) in the chemical pigment– 14.4 mg/kg; chromium(VI) in water 12.9 mg/kg;chromium(VI) inoak bark < 3 mg/kg; chromium(VI) in onion peel < 3 mg/kg.

On this basis, it can be concluded that onion peel and oak bark extracts are able to reduce heavy metal like chromium (VI) in leather and are recommended to be used in the finishing process of leather. We believe that, this study represents is one of the most important research projects for the leather industry.

Acknowledgment

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Eco-friendly Chrome Tanning of Leather using Ultrasound Technique

by
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Abstract

This article describes the development of an environmentally friendly chrome tanning process of leather using ultrasound. Most of the leathers are tanned by the conventional method using basic chromium sulfate. It is one of the most polluting and time-consuming steps in leather processing. Investigations were carried out on ultrasound assisted eco-friendly tanning process so that the chrome tanning agent could provide better quality leather. Effects of using ultrasound in chrome tanning process were studied at different pH, tanning time, tanning agent dosage, and then compared with that of conventional method. Tanned leathers were characterized by scanning electron microscopy (SEM), photomicrographic analysis, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and energy dispersive X-ray (EDX). SEM analyses of the surface and cross-section of the tanned leather showed that fiber structures were not affected by ultrasound. It was also found that the shrinkage temperature of leather tanned with ultrasound was increased by about 5-29 °C. Chrome uptake and content were found to increase by 30-50% and 1-7%, respectively. Tanning time was shortened from 6 hours to 2 hours and the quantity of leachable chromium in ultrasound assisted chrome tanned leather was also decreased significantly. The noteworthy enhancing effects have been attributed largely because of the increased penetration of tanning agents into pickled leather. Photomicrographic analysis of the cross-section of the tanned leather also showed a higher penetration of tanning agents in presence of ultrasound.

Introduction

Leather industry is one of the most ancient and potential industries in Bangladesh that supplies the necessary materials such as leather goods, shoes, and garments using by-products of the meat industry. But, leather processing generates huge number of by-products and wastes.[1, 2] It is reported that only about 20% of wet salted hides and skins are transformed into commercial leather generating a significant amount of chromium tanned leather waste (CTLW).[3]

The real problem, however, is that the leather industry presently faces many challenges due to environmental regulations. In Bangladesh, there are about 165 leather industries, and 93% of them are located in the western part of the capital city Dhaka and others are located at different locations of the country.[4-6] In Bangladesh, 240 MT hides or skins are processed per day which generates 8.47 million liters of wastewater and 98 MT solid wastes.[7] Leather and Leather Goods (LLG) are the second-largest export product of Bangladesh just after the ready-made garments. Bangladesh aims a target to achieve a total of $60 billion export earnings by 2021 of which $5 billion is expected to be achieved from LLG.[8]

Tanning is the process of converting hides or skins into non-putrescible leather having certain physical, chemical and biological properties.[9] The common feature of hides/skins is that they are composed primarily of collagen and can be made from the skin of any vertebrate. In tanning, raw hides/skins are treated with inorganic and organic tanning agents such as chromium, aluminum, titanium, iron, and zirconium basic salts as well as high molecular weight vegetable substances, aldehydes, oils, and other substances.[10, 11] More than 85-90% of leather is processed by chrome tanning, and it has been used for over 100 years.[12]

Due to diffusion limitations, a significant amount of chromium remains in spent liquor during conventional chrome tanning processes.[13] This excess amount of chrome increases the suspended solids and dissolved solids of the wastewater resulting in an increase in environmental pollution and pollution load of existing water bodies.[14] Therefore, it is a crucial need to develop a process to speed up tanning without impairing the quality of finished products.

Ultrasound is one of the novel techniques for assisting the tanning process. Ultrasound is a sound wave with a frequency of 20 kHz-10 MHz that is generally used to enhance physical and chemical processes.[15,16] The use of ultrasound over the last few decades has led to tremendous advances in the provision of versatile equipment for testing, detection, imaging, chemical processing and research.[17] Sonochemistry deals with the effects of sonic waves and wave properties on chemical systems. Sonochemical activity arises from acoustic cavitation in liquid media.[18,19] Acoustic cavitation involves nucleation, growth and explosive collapse of microbubbles on a

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microsecond timescale.[20] In the liquid phase high micro mixing will increase the heat and mass transfer and diffusion of species inside the pores of the solid.[21]

Several research groups reported the applications of ultrasound or the synergetic effect of cavitation and capillary action in leather processing such as soaking and liming,[22,23] degreasing,[24,25] chrome tanning,[13,26-28] vegetable tanning,[29-33] dyeing,[34,35] and fatliquoring.[36-38] Some researchers concluded that penetration and fixation of chrome tanning agents were separated due to cavitation effect.[28] During the research, it was observed that ultrasound assisted tanned leather showed higher uniform distribution of chromium throughout the samples than that of conventionally tanned leather. Moreover, the fiber structure of tanned leather was monitored with SEM. It was shown that the collagen fibers were intact upon tanning with ultrasound. Effects of various parameters such as tanning time, tanning agent dose, concentration of tanning agent were studied in detail. The thermal stability of the tanned leather in presence of ultrasound and without ultrasound was also reported. Thus, this study is aimed at developing an eco-friendly chrome tanning process using ultrasound.

Experimental

Materials

Raw materials used in chrome tanning were divided into two categories: primary raw materials and auxiliary raw materials. The primary raw materials were cow pickled pelt and basic chromium sulfate (basicity 33%). To operate the tanning process, different types of chemicals (laboratory reagent grade) were used besides the primary raw materials. The percentage of used chemicals was based on the weight of the pickled pelt. The auxiliary chemicals were 150% pickling liquor, 1.5% sodium formate (HCOONa), 0.25% oxidized chitosan (OCS), 1.5% sodium bicarbonate ($NaHCO_3$), 0.25% busan 30L. The percentage of all chemicals mentioned here are based on the weight of cow pickled pelt.

Experimental set-up

Ultrasonic bath SONOREX DIGIPLUS DL 514 BH of BANDELIN electronic GmbH & Co. KG, GERMANY with a power generation of 860W and 35 kHz frequency was used in this study. Leather samples were taken in a glass beaker clamped inside the ultrasonic bath containing water. Conventional tanning was carried out using a drum in absence of ultrasound with provisions of controlling temperature and rotation per minute (rpm). In this study all experiments were carried out in triplicate and the presented results are their averages.

Tanning procedure of cow pickled pelt

Sample preparation: The samples were cut into a size of 2 inch × 2 inch taking from the equivalent lateral position of the cow pickled pelt corresponding to the line of the backbone of the animal and weighed with an electronic balance (Shimadzu ATY224). The weights and areas of the samples used in this study were almost similar (4.0 g).

Chrome tanning: Chrome tanning was performed using ultrasound and conventional techniques. Details of the tanning process are described in Table I. All samples were divided into two parts for carrying out experiments with ultrasound and conventional methods. In the first group, the pickled pelt samples were taken in a beaker that was placed in a water bath to maintain the temperature exposed to ultrasound. Then, the pelts were chrome tanned with

Table I

Details of chrome tanning operation

Sample	Tanning condition (For penetration)	Tanning condition (For basification)
1	6% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 1 hour
2	8% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 1 hour
3	10% basic chrome sulfate, 1.5% HCOONa and OCS for1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 1 hour
4	12% basic chrome sulfate, 1.5% HCOONa and OCS for1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 1 hour
5	6% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 2 hours
6	8% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 2 hours
7	10% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 2 hours
8	12% basic chrome sulfate, 1.5% HCOONa and OCS for 1 hour	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 2 hours
9	6% basic chrome sulfate, 1.5% HCOONa and OCS for 2 hours	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 4 hours
10	8% basic chrome sulfate, 1.5% HCOONa and OCS for 2 hours	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 4 hours
11	10% basic chrome sulfate, 1.5% HCOONa and OCS for 2 hours	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 4 hours
12	12% basic chrome sulfate, 1.5% HCOONa and OCS for 2 hours	50% water, 0.25% busan and 1.5% $NaHCO_3$ for 4 hours

6%, 8%, 10%, and 12% of basic chromium sulfate (BCS) for 2, 3, and 6 hours. At the beginning of tanning half of the basic chromium sulfate $Cr(OH)SO_4$ was added to the pickling liquor (150%) and the other half was added after 30 min and was sonicated. Then sodium formate (1.5% of sample) was added as a masking agent. The tanning bath was then basified using sodium bicarbonate which was divided into three equal portions and was added within an hour. A pH of 2.5 - 4.0 of the liquor was maintained while adding sodium bicarbonate.

The second part of the sample was tanned by conventional method where a stationary glass vessel and rotary drum were used instead of ultrasound. A hotplate stirrer was used to control the temperature. In chrome tanning an initial pH of 2.5 to 4.0 and the temperature of 30°-50°C was maintained.

Upon completion of tanning operations, the tanned leathers were covered by polythene and were piled up for several days to complete the fixation of tanning chemicals and were dried in air. Tanned leathers obtained in both ultrasound and conventional methods were then used for analysis and characterization.

Characterization of tanned leather

Tanned leathers were characterized with field emission scanning electron microscopy (JSM-7600F, JEOL, Japan), photomicrographic analysis (Nikon, K16976, Japan), thermogravimetric analysis (TGA-50, SHIMADZU, Japan), differential scanning calorimetry (NETZSCH STA 499 F3 Jupiter, Germany), energy dispersive x-ray spectrophotometry (JED-2300 Analysis Station Plus, JEOL, Japan), boiling test, and toxicity characteristic leaching procedure (TCLP) test.

UV-Visible spectrophotometry

The chromium content was determined by UV/visible spectrophotometry. The principle of this method is based on Lambert-Beer law, which shows that absorbance (A) is directly proportional to concentration for dilute solution. A SHIMADZU UV-1700 spectrophotometer at 540 nm wavelength was used for the analysis. Once the characteristic peak was determined, the subsequent testing and calibration of this sample were conducted on this spectrophotometer. The data obtained from UV spectrophotometers was used to characterize the kinetics of tanning and to calculate the percentage of chrome uptake.

% chromium uptake at time 't' was calculated using the following equation:

$$\%\ Cr_{uptake} = \frac{Cr_i - Cr_f}{Cr_i} \times 100$$

Where Cr_i is the initial concentration of chromium in the tanning liquor and Cr_f is the final concentration of chromium in the tanning liquor.

Boiling test

The boiling test was used to determine the percentage of shrinkage of the tanned leather. A piece of tanned leather (1 inch × 1 inch) was boiled at 100°C for 10 minutes. Then the distinction from the initial to the final surface area of the tanned leather was measured and the percentage of shrinkage was calculated by the following equation:

$$\%\ \text{Shrinkage} = \frac{(A_i - A_f)}{A_i} \times 100$$

where A_i and A_f are the initial and final area of the tanned leather after boiling.

Toxicity Characteristic Leaching Procedure (TCLP)

TCLP is commonly used to measure the quantity of leachable chromium in tanned leather. TCLP extraction solution was prepared by titrating 40 g citric acid in 400 mL of distilled water by 1 M NaOH. The pH was maintained to 5.0 ± 0.1. Air-dried chrome tanned leather powder (1 g) was added to 50 mL TCLP extraction solution. The mixture was turned at ambient temperature for 48 hours at a speed of 30 rpm. After 48 hours extraction, the reaction composition was filtered. In this experiment, UV-Vis Spectrophotometry was also used to determine the amount of leachable chromium from tanned leather.

Results and Discussion

Effect of ultrasound on percent chromium uptake of leather

Chromium uptake was studied during the tanning process for different tanning conditions. In this study tanning was carried out using 6%, 8%, 10% and 12% basic chromium sulfate for 120 minutes. Cr uptake of leather tanned with ultrasound and conventional methods are shown in Figure 1. From this figure, it is evident that chromium uptake in the case of tanning with ultrasound was faster than tanning without ultrasound. Ultrasound improves the penetration of chromium through the pores of collagen fibers and hence enhances the percentage of chromium uptake by leather sample. Thus 87%, 98%, 91% and 90% chromium uptakes were obtained in the case of tanning with ultrasound for 120 minutes using 6%, 8%, 10% and 12% basic chromium sulfate, respectively while only 17%, 29%, 33% and 39% chromium uptake was observed in the case of conventional method under same tanning conditions.

The results showed that chromium uptakes of the leather samples tanned with ultrasound increased up to the concentration of basic chromium sulfate at 8% and then decreased. Because pickled pelts can't absorb all the chromium present in tanning liquor if excessive amount of tanning agent is used.[39,40] Thus, the use of 8% basic chromium sulfate for 2 hours is the best condition for ultrasound assisted chrome tanning to get maximum percentage of chromium uptake.

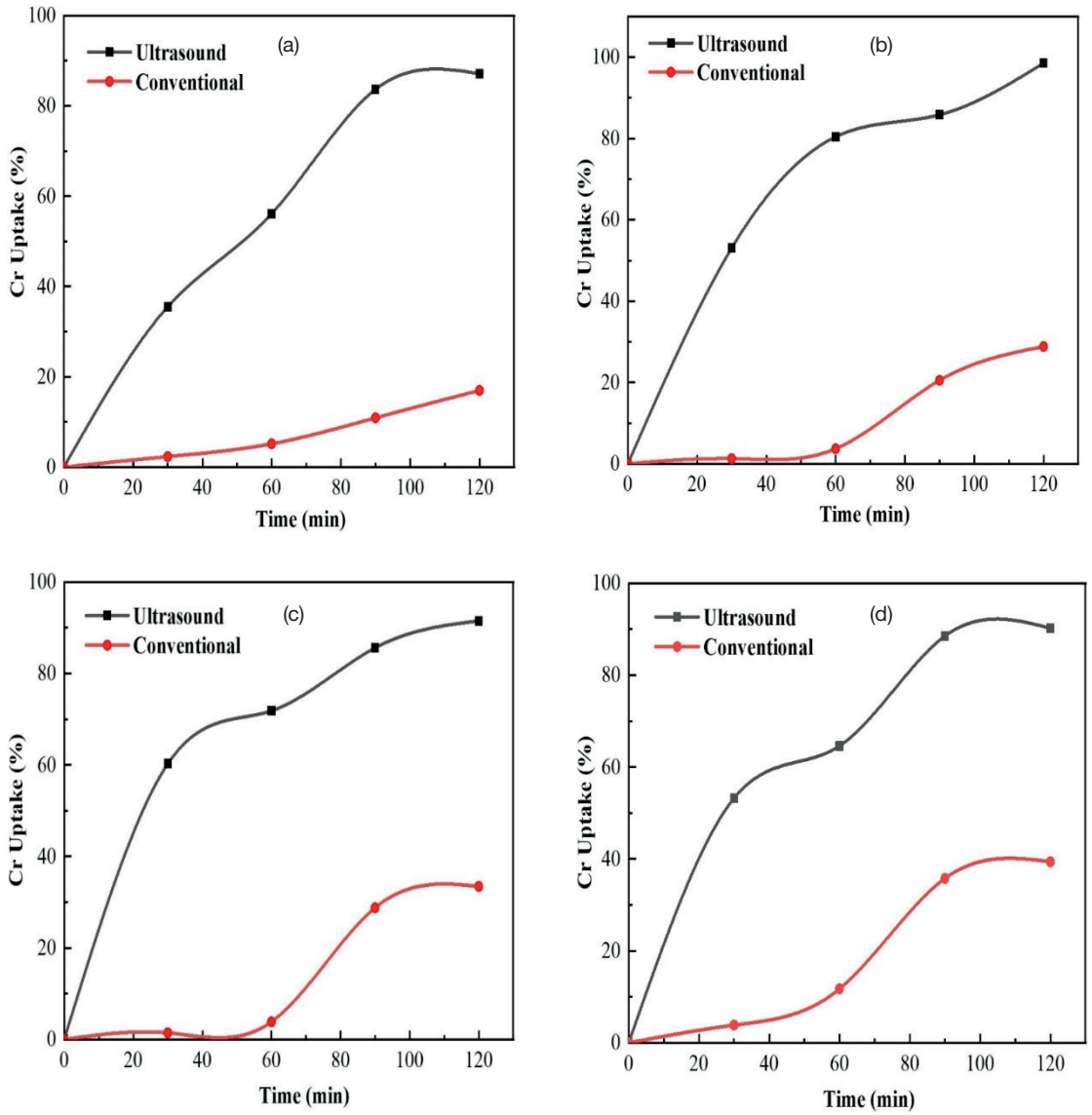

Figure 1. Cr uptake of leather (%) as a function of time **(a)** Sample 1, **(b)** Sample 2, **(c)** Sample 3, and **(d)** Sample 4

Effect of time on chromium uptake

Cr uptake reached 98% when tanning was carried out with ultrasound for 180 minutes while only 33% Cr uptake was observed in the case of conventional method under the same conditions (Figure 2a). A further increase in tanning time proved to be detrimental for ultrasound process as was evidenced that 79% chromium uptake was observed with ultrasound for 360 minutes. However, it was increased to 63% with conventional process (Figure 2b). This reduction of Cr uptake might be due to desorption of some non-chemically bonded chromium ions by means of the effect of excessive sound waves which were generated in the sonicator.

Chromium content in the tanned leather

Chromium content in tanned leather was determined by energy dispersive X-ray spectroscopic (EDX) method. From EDX spectra of tanned leather, elemental composition of the leather was derived. Tanned leather contained several elements e.g., C, H, N, O, Cr, S, P, Na, Ca, Cl; but amount of H, Na, Ca, P were ignored in EDX analysis. EDX spectra of leather tanned in presence of ultrasound (a) and in absence of ultrasound (b) are shown in Figure 3. The spectra of other samples also exhibited similar trends. Cr-uptake of ultrasound assisted tanned leather with 8% BCS for 2h, 3h and 6 h were 6.16, 5.26 and 4.7 while those of the conventionally tanned leather were 0.82, 0.89 and 3.66, respectively.

Elemental analysis of chrome tanned leather with ultrasound and conventional method have been done. Chromium uptake of ultrasound assisted tanned leather was higher than that of conventionally tanned leather. The uptake of other elements also showed similar trends.

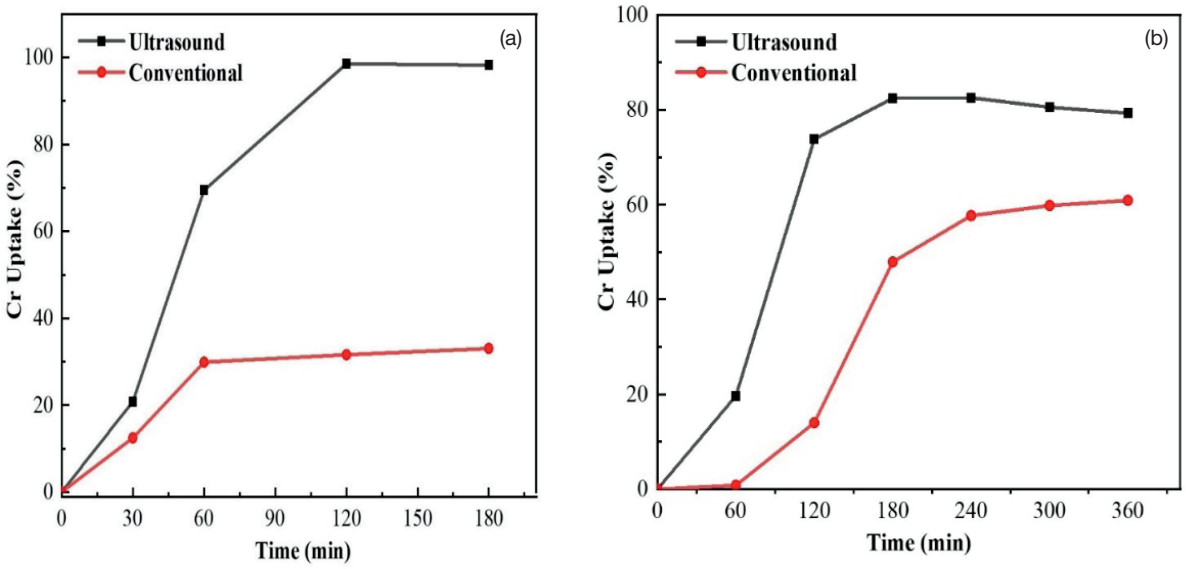

Figure 2. Cr uptake of leather (%) as a function of time **(a)** Sample 6 **(b)** Sample 10

Thermogravimetric analysis of tanned leather

Thermal stability of leather tanned with and without ultrasound were compared to investigate the effect of ultrasound on leather tanning. Thermal degradation studies of tanned leathers were performed using thermogravimetric analyzer under N_2 atmosphere.

Figures 4 (a) and (b) represent the thermograms of leathers tanned with 8% basic chromium sulfate for 2 hours using ultrasound and conventional methods, respectively. Temperatures required for 10%, 30%, 50%, 70% and 80% weight loss of leather tanned with ultrasound were 74°, 302°, 382°, 523° and 715°C, respectively while same weight loss occurred at 60°, 171°, 335°, 497° and 550°C for leather tanned with conventional method.

The temperature needed for a fixed percentage of weight loss was increased for leathers tanned using ultrasound technique. The improvement of the thermal stability of ultrasound assisted tanned leathers was due to higher penetration of basic chrome sulfate through the pores of leather leading to the formation of chromium-collagen complexes which acted as a barrier to the decomposition of leather.[41]

Differential scanning calorimetric analysis of tanned leather

Differential scanning calorimetry (DSC) comes into prominence in recent years for evaluation of thermal behaviors (e.g., denaturation and shrinkage temperatures) of the raw skin, pickled, tanned, and finished leathers. Several important temperatures such as peak temperature, onset temperature, extrapolated onset temperature, and end set temperature can be determined from a typical DSC

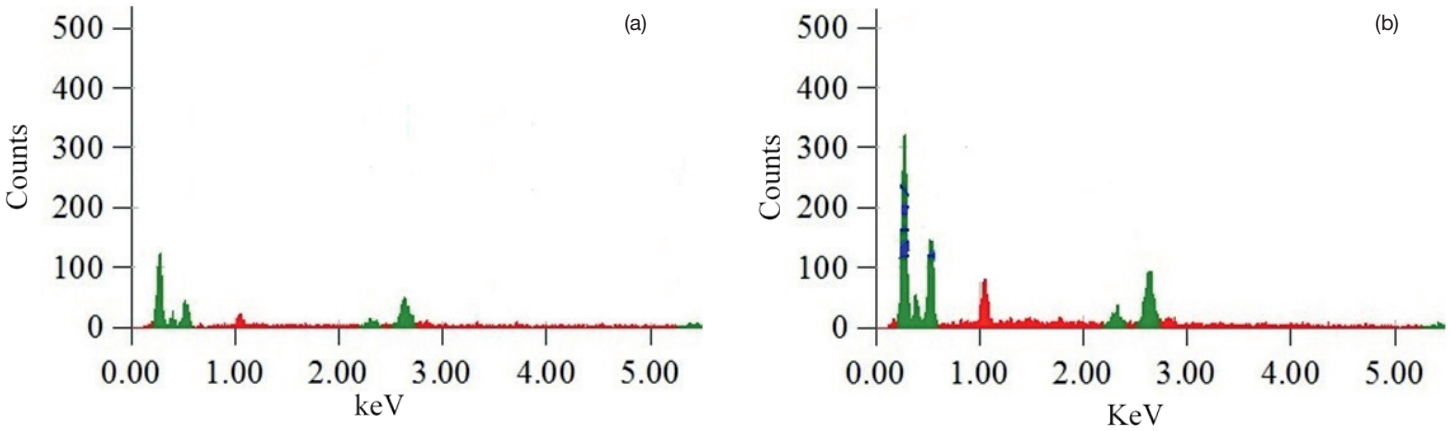

Figure 3. EDX spectra of leather tanned with ultrasound **(a)** and without ultrasound **(b)**

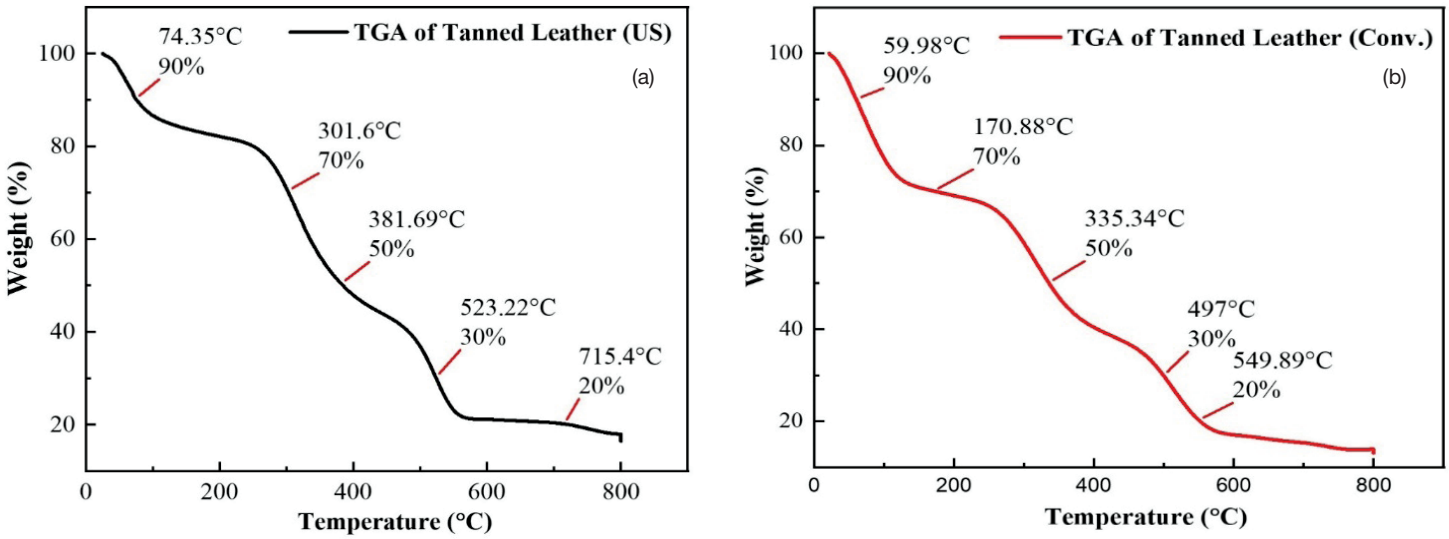

Figure 4. TGA thermograms of leather tanned with 8% of basic chromium sulfate for 2 hours **(a)** with ultrasound **(b)** conventional method

thermogram. In this study, the onset temperature was considered as the shrinkage temperature. The onset temperature is the temperature at which the slope of the thermogram first deviates from the baseline.

The DSC thermograms of the leather tanned with ultrasound and conventional method using 8% basic chromium sulfate for 2 hrs. is shown in Figure 5 (a, b). The thermograms of the leather showed that the shrinkage temperature of sample tanned in presence of ultrasound was 111°C while that of conventionally tanned leather was 82°C. The shrinkage temperature of sample treated under ultrasound was 29°C higher than that of conventionally tanned leather. The main reason for the improvement of shrinkage temperature is that a very small amount of chromium fixation happened within two hours in the conventional tanning method whereas most of the chromium present in tanning liquor was fixed with collagen fiber in ultrasound-assisted tanning process. Therefore, the fixation of chromium increased with ultrasound.

Boiling test of tanned leather

The influence of ultrasound on tanned leather was also measured by evaluating the results of the boiling tests. In the present study, the percentage of area loss or area shrinkage was determined by the boiling test according to the ISO standard procedure (ISO 3380:2015).

Table II shows that the reduction in the area of the samples tanned with ultrasound were less than that of the samples tanned by the conventional method. Shrinkage areas of leather (Sample 2) tanned with ultrasound and conventional method are 31.25%

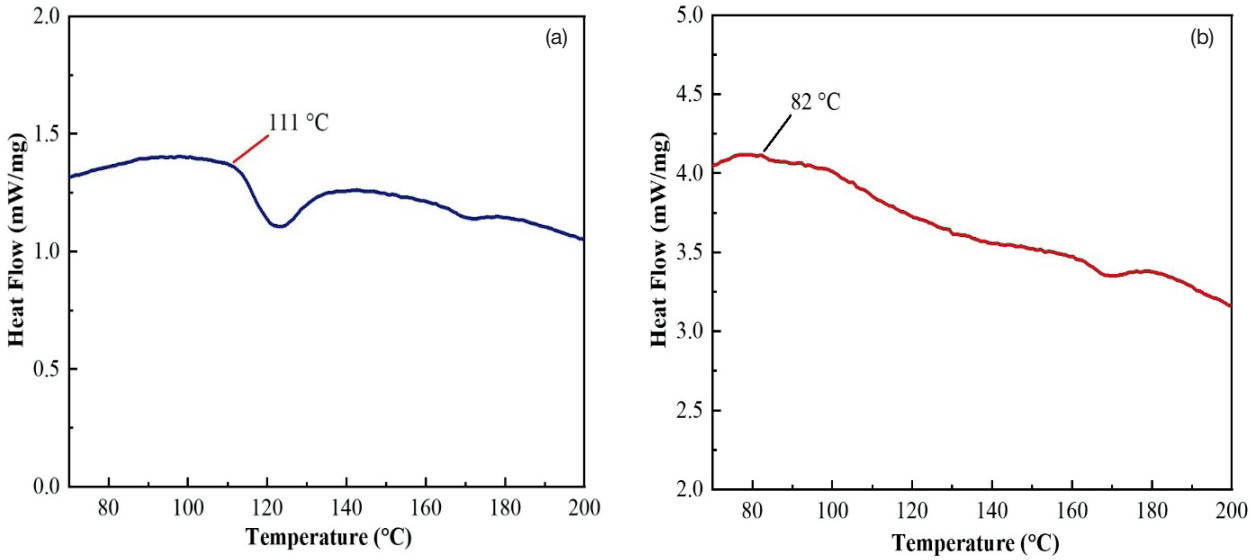

Figure 5. DSC thermograms of leather tanned **(a)** with ultrasound and **(b)** without ultrasound.

Table II

Percentage of area loss of chrome tanned leather by boiling test

Sample No.	Experimental Technique	Initial area (cm^2)	Final area (cm^2)	Area loss (cm^2)	Area loss or shrinkage (%)
1	With Ultrasound	4	2.90	1.10	27.50
	Conventional	4	2.64	1.36	34.00
2	With Ultrasound	4	2.75	1.25	31.25
	Conventional	4	2.20	1.80	45.00
3	With Ultrasound	4	2.54	1.46	36.50
	Conventional	4	2.15	1.85	46.25
4	With Ultrasound	4	2.58	1.42	35.50
	Conventional	4	2.14	1.86	46.50
5	With Ultrasound	4	2.88	1.12	28.00
	Conventional	4	2.63	1.37	34.25
6	With Ultrasound	4	2.72	1.28	32.00
	Conventional	4	2.21	1.79	44.75
7	With Ultrasound	4	2.64	1.36	34.00
	Conventional	4	2.10	1.90	47.50

and 45%, respectively. Conventionally tanned leather has a lower amount of fixed chromium and consequently, higher shrinkage occurred.

Field emission scanning electron microscopic (FESEM) analysis

The surface morphology of the wet-blue leathers produced by tanning under ultrasound and conventional techniques were observed by FESEM as shown in Figure 6.

The micrographs show the chromium penetration across the cross-sectional view of the tanned leather with 8% basic chromium sulfate for 2 hours using both ultrasound and conventional techniques. In the micrographs, the darker region represents the concentration of chromium. It was observed that ultrasound assisted tanned leather showed a higher uniform distribution of chromium throughout the samples than that of conventionally tanned leather. Therefore, FESEM further confirmed that the tanning agent completely penetrates the full cross-section of leather in the process of ultrasound-assisted chrome tanning.

The fibril bundles of the tanned leather were also analyzed from FESEM micrographs which showed that the fibrils and fibril bundles of collagen in the flesh, corium, and grain layers of the chrome tanned leather and observed that the collagen fibrils are

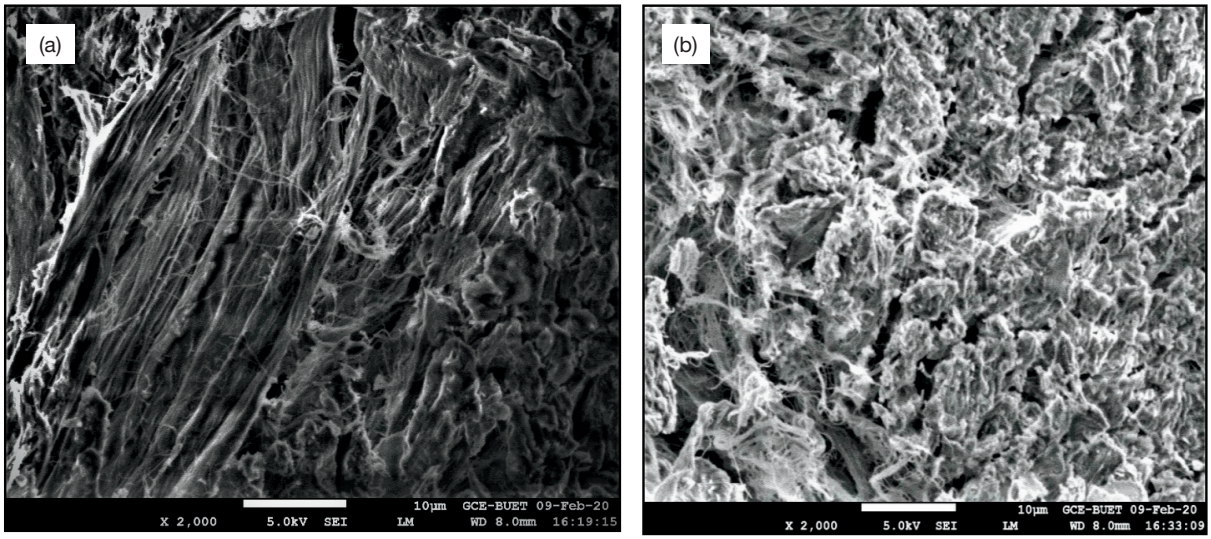

Figure 6. FESEMs of the cross-sectional view of the tanned leather **(a)** with ultrasound and **(b)** without ultrasound at magnification ×2000

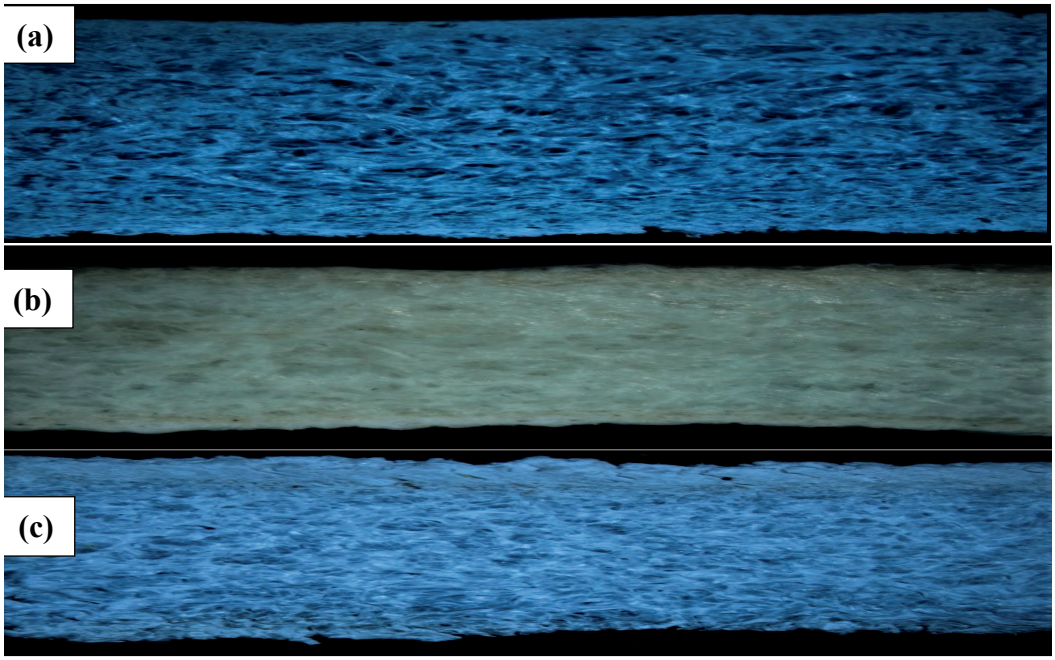

Figure 7. Microscopic images of the cross-section of **(a)** leather tanned with ultrasound, **(b)** pickled pelt and **(c)** leather tanned with conventional method.

intact for the leather tanned with both ultrasound and conventional techniques. These results revealed that collagen fiber structures were not damaged because of the usage of ultrasound in the tanning for 2 hours. But it was observed that in the case of the tanning process for 6 hours, the distance between fibrils is more with ultrasound than that of the conventional process. It means exposure to ultrasound for longer period is harmful for the structure of leather. Thus, in ultrasound-assisted chrome tanning process, 2-3 hours tanning time is optimum to complete the tanning operation.

Diffusion of tanning agent

Ultrasound enhances the penetration of the tanning agent and the degree of penetration was examined with a stereomicroscope. The cross-sectional images of the tanned leather obtained from the stereo microscope are shown in Figure 7. The depth of penetration of the tanning agent depends on the type of sample and the tanning agent used.

It was observed that the tanning agent distribution in the leather samples treated with ultrasound was about 30-70% higher than that of the conventional method. The principal reason behind this is the effect of acoustic cavitation which enhances the diffusion rate of tanning agents through the pores of collagen fibers.[21] Almost 98% penetration of tanning agent was observed for tanned leather after 2 hours of tanning with ultrasound whereas only 29% penetration was observed for that sample after 2 hours of tanning without ultrasound. Besides this, uniform distribution of tanning agents throughout the whole cross-section of the pelt, even the inner portion of the pelt is possible in the case of ultrasound-assisted chrome tanning, which is quite impossible in the conventional tanning process. Hence, ultrasound can improve the penetration or diffusion of the tanning agent and impart better-quality leather.

Determination of leachable chromium content

All the absorbed chromium present in tanned leather is not chemically bonded to the collagen chains. The chemical bonding within the carboxyl groups of collagen fibers and chromium ion is supposed to happen. In this study, chromium physically attached to the fibers was extracted by the TCLP. It helps in determining the quantity of free chromium that can be leached out from the tanned leather to the environment when it is disposed. The leached chromium present in TCLP liquor was measured by UV-Vis spectrophotometric analysis (Table III).

These results indicated that the amounts of leachable chromium with ultrasound-assisted tanned leathers are lower than that of conventionally tanned leather. It is assumed that chemically bonded chromium in the tanned leather is not easily leached out.[28] Thus, higher hydrothermal stability for the tanned leather might be obtained for ultrasound assisted tanning.

Estimation of environmental benefits

Ultrasound may be applied to reduce the effluent load to the environment. Ultrasound can improve diffusion, penetration and fixation of chromium even at a lower concentration of tanning chemicals. As a consequence, Cr content in the spent liquor is reduced (Table IV) resulting a lower pollution load to the environment.

Table III

Leachable chromium of tanned leather

Sample No.	Sample specification	Amount of leachable chromium (mg/gm)		Improvement (%)
		With ultrasound	Without ultrasound	
1	8% BCS, 2h	0.027	0.047	42.55
2	10% BCS, 2h	0.128	0.165	22.42
3	8% BCS, 3h	0.113	0.140	19.29
4	8% BCS, 6h	0.241	0.247	2.43

A significant reduction in the amount of unused chromium in the spent tanning liquor due to the use of ultrasound was observed. The reductions were 69%, 65%, and 16% for samples 1, 2, and 3 that were tanned with 8% BCS for 120 minutes, 180 minutes, and 360 minutes, respectively.

Conclusion

Ultrasound showed significant effects on both penetration and basification of chromium ion through hide or skin by enhancing the tanning operation. Ultrasound decreased chrome tanning time from 6 hours to 2 hours. In presence of ultrasound, the percentage of Cr uptake was 70% for leather tanned with 8% basic chromium sulfate in 2 hours while it was only 35% for conventionally tanned leather. These results revealed that the use of 8% basic chromium sulfate and duration of 2 hours are the optimum conditions for ultrasound-assisted chrome tanning to get the maximum percentage of chromium uptake. The amount of unused chromium in spent liquor was also determined. A reduction of about 69% of unused chromium in the tanning effluent liquor was obtained by using ultrasound. EDX studies of tanned leather showed that chromium contents of ultrasound assisted tanned leather were about 7% higher than that of conventionally tanned leather. Thermal characterization of ultrasound assisted chrome tanned leather was carried out by DSC and TGA. The shrinkage temperature of ultrasound assisted leather was 5°-29°C higher than that of conventionally tanned leather. TGA analysis revealed that the thermal stability of ultrasound assisted tanned leather was better than that of conventionally tanned leather. Thus, it can be concluded that application of ultrasound in leather processing might be a potential alternative to develop an eco-friendly leather manufacturing technique.

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Authors' contributions

Professor Dr. Md. Zahangir Alam, Professor Dr. Md. Nurnabi and Dr. Md. Abu Sayid Mia planned and designed the research. Dr. Md. Abu Sayid Mia and Shamima Yeasmin conducted the experiments and wrote the manuscript. Professor Dr. Md. Zahangir Alam and Professor Dr. Md. Nurnabi supervised the whole research and revised the manuscript.

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Table IV

Amount of chromium present in the spent tanning liquor

Sample No.	Sample specification	Amount of chromium in the spent liquor (%)		Difference (%)
		Ultrasound	Conventional	
1	8% BCS, 2h	2	71	69
2	8% BCS, 3h	2	67	65
3	8% BCS, 6h	21	37	16

Availability of data and materials

All data from this study are presented in the paper.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tambaqui (*Colossoma Macropomum*) Leather Tanning: A Study on the Influence of Skin Morphology on the Physical-Mechanical Properties of Leather

by
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Abstract

The fish production chain presents losses of up to 35%, generating a large amount of waste. Using these residues to produce value-added materials boost fish production towards a sustainable path. The use of fish skin to obtain leather is increasingly attracting the market for fashion and luxury products. Besides considered an exotic material with a unique design, fish leather also has good mechanical properties. Due to the morphology of fish skin, different mechanical responses can be observed, depending on the leather cutting direction. However, there are no specific technical standards for sampling this material. In this scenario, 45 Tambaqui skins were tanned with chromium associated with oxazolidine, and the mechanical properties of the leather were evaluated in four cutting directions: parallel, perpendicular, dorsal-ventral and ventral-dorsal, all concerning the cephalocaudal line of the fish. Skin and leather micrographs revealed layers of parallel collagen fiber bundles distributed in superimposed layers oriented obliquely to the preceding one. Insertions of collagen fibers perpendicular to the leather surface were also observed, joining the deeper layers to the superficial layers. Tensile strength (TS) and elongation (E) results indicated statistical differences, with higher TS results for the perpendicular cut and greater elongation for the parallel cut. The tear strength was higher for the ventral-dorsal cut, statistically differing from the parallel cut. The data indicate that the cutting direction of the specimen can influence the physical-mechanical behavior of the leather, reinforcing the importance of standardization for the qualitative evaluation of fish leather.

Introduction

Fish farming is one of the main branches of aquaculture and has been intensified worldwide. In Brazil, production surpassed the mark of 860 thousand tons in 2022, with Tilapia (*Oreochromis niloticus*) as the main cultivated species.[1] Among the native fish, Tambaqui (*Colossoma macropomum*) stands out. Native to the Amazon and Orinoco river basins, the Tambaqui is considered a "round" fish with small scales and protective lamellae, featuring a typical design.[2,3]

According to the Food and Agriculture Organization of the United Nations (FAO), the amount of loss in the fish production chain reaches 35%, double the losses in the processing of meat products[4]. The use of these residues in value-added products contributes to boosting the fish chain towards a sustainable path. Among fish waste, the skin is of great interest for reuse in the form of leather or as a source of collagen and its peptides. Each skin has a specific natural pattern, giving the leather a unique character that appeals to the fashion and luxury goods industries.[5] FAO considers the use of fish skins in fashion design articles one of the actions that contribute to the Blue Growth Initiative[4], fulfilling the agenda of the Sustainable Development Goals (SDGs) elaborated by the UN. The initiative brings together strategies and actions to improve the use of water resources, seeking economic, environmental, and social benefits.

In addition to the exclusive and differentiated appearance, fish leather has good resistance due to the skin morphology and the organization of collagen fibers.[6,7] The dermis is made up of layers of overlapping collagen fibers bundles. These layers are composed of continuous and parallel fibers superimposed at different angles along the body, varying from 50 to 70° concerning the preceding layer.[8] Such an arrangement gives the skin/leather of fish a highly organized structure that influences its qualitative properties.

The growing interest in fish leather promotes the need for sampling standardization methods to evaluate intrinsic quality. So far, there are no specific standards for sampling fish leather. However, due to the morphology and organization of collagen fiber bundles in the skin, the specimens' cutting direction can influence the leather's physical-mechanical behavior. In this sense, the present work aims to investigate the structure of the Tambaqui skin and the influence of the cutting direction on the mechanical properties of the leather.

Material and Methods

Material

Tambaqui (*Colossoma macropomum*, n=45), a species registered in the National System for the Management of Genetic Heritage

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and Associated Traditional Knowledge under N° A3B1887, were purchased from the Mar & Rio Pescados (São José do Rio Preto, SP). Busan® 7600 bactericide (Buckman, USA) was used for skin preservation. Other reagents used in the tanning process were of commercial analytical grade.

Methods

Fish skin composition

Skin samples (200 g) were analyzed for their centesimal composition. Crude protein contents were determined by method 45 (Protein - Dumas Method), and ash contents by method 5 (Ashes or Mineral Matter)[9]. Lipids were determined following standard methodology.[10]

Histological analysis of Tambaqui skin

Skin fragments were fixed in 10% neutral buffered formalin for 24 hours. The fixed samples were cut to a thickness of 6 µm, stained using Hematoxylin/Eosin and Masson's Trichrome techniques, and mounted on glass slides with Entellan®[11]. The slides of histological sections were observed and photographed under a Leica DM 2500P Optical Microscope with Polarized Light, positioning the rotating circular stage of the microscope at 45°.

Tanning process

The fish were stunned by thermal shock in an ice bath. The cut lines were made in the fish's gill, dorsal, ventral and caudal regions. Subsequently, with the aid of pliers, the skins were removed, fleshed, and preserved in salt and bactericide (NaCl and Buzan® 7600) until processing.

In the tanning process, the steps of soaking, liming (Table I), deliming, bating, pickling, tanning (Table II), retanning I, basification, retanning II, and fat liquoring (Table III) were carried out. Factors such as pH, water temperature, drum rotation speed, and time were monitored throughout the process and are shown in Tables (I-III).

Table I

Pre-tanning process conditions of Tambaqui skin.

Process	Water (%) m.m^{-1}	Reagents	Reagent (%) m.m^{-1} *	°C	Run time (min)	pH	Remarks
Soaking	200	Water		25			
		Bactericide	0.1		1440		Drain
	200	Water		25	10		Drain
	100	Water		25	10		Drain
	100	Water		25			
		surfactant**	0.3		10		Drain
	100	Water		25			
		surfactant**	0.3		10		Drain
	200	Water		25			
		Sodium carbonate	0.2				
		surfactant**	0.3		240		Run 10'/h Drain
Liming	100	Water		25	10		pH = 8.5 - 9.5 < 2.0° Be*** Drain
	100	Water		25			
		Sodium sulfide	1.5				
		surfactant**	0.4				
		Lime powder ($Ca(OH)_2$)	2.5		240		
	100	Water		25	840		Run 10'/h (14 h) Drain
	200	Water		25	10		Drain

*The percentage of reagent corresponds to the initial mass of the skins.

**anionic + non-anionic surfactants, free of nonylphenol ethoxylated

***Baume degrees.

Table II

Tanning process conditions of Tambaqui skin.

Process	Water(%) m.m-1	Reagents	Reagent (%) m.m-1*	°C	Run time	pH	Remarks
Deliming	200	Water		25			
		Ammonium chloride	0.5		10		Drain
	200	Water		25			
		Ammonium chloride	2.0				
		Deliming agent**	1.0				
		surfactant	0.6		20	8.2	Phenolphthalein; pH < 8.2
Bating	100	Water		37	5		Drain
	200	Water		37	5		Drain
	200	Water		30-34			
		Pancreatic enzyme	0.05				
		surfactant	0.6		60		Fingerprint test
	100	Water		25			
		Formic acid	0.1		60	6.8	Drain
	100	Water		25			
		Ammonium chloride	1.0				
		surfactant	1.0		60	7.3	Drain
Pickling	100	Water		25			
		Sodium chloride	10.0		10		
		Formic acid	2.0		10	3.2	
					840		Run 10'/h (14 h)
Tanning		Water		25			Pickling water
		Chromium powder (33% basic)	10.0		60	2.8	
		Oxazolidine	3.0				
		Acrylic retanning agent	5.0				
		Sulphited fish oil	0.5				
		Fatty alcohol-based	0.5		40		
		Glycol esters based	0.2		90		
		Sodium formate	1.0		20	3.2	
		Sodium bicarbonate	0.5		30	3.3	
		Sodium formate	1.0		20	3.5	
		Sodium bicarbonate	1.5		20	4.1	
					840		Drain and horse up overnight

*The percentage of reagent corresponds to the initial mass of the skins.

**Combination of organic acids and inorganic salts.

Table III

Retanning and finishing process conditions of Tambaqui leather.

Process	Water (%) m.m^{-1}	Reagents	Reagent % m.m^{-1} *	°C	Run time	pH	Remarks
Retanning I/ Pre lubrification	200	Water		35			
		Formic acid	0.2				
		Oxalic acid	0.2				
		surfactant	1.0		20	3.4	Drain
	100	Water		35			
		Chromium powder (33% basic)	3.0				
		Sulphited fish oil	1.0		30		
		Polymer retanning agent	3.0		30		
		Acrylic retanning	6.0		30		
Basification		Sodium formate	2.0		20	4.5	
		Sodium bicarbonate	1.5	40	90	5.9	Drain
	200	Water		25	10		Drain
Retanning II/ Dyeing	60	Water		25			
		Mimosa powder	4.0				
		Phenolic retanning agent	3.0				
		Oxazolidine	0.5				
		Neutralizing agent**	3.0				
		Blue aniline	3.0				
		Turquoise aniline	1.0		45		
		acrylic retanning	5.0		25		Cutting Check
	150	Water		50			
		Formic acid	2.5		30	4.3	Drain
	200	Water		55			
		Formic acid	0.2		10		Drain
Fat liquoring/ Fixation	200	Water		55			
		Synthetic oil-based	5.0				
		Fatty alcohol-based	3.0				
		Sulphited fish oil	2.0				
		Glycol esters based	0.5		45		
		Formic acid	2.9	55	50	3.2	4 × 10' + 20'
		Cationic oil***	1.0		15	3.0	

*The percentage of reagent corresponds to the initial mass of the skins.

**Based on aromatic sulfonic acids and aliphatic dicarboxylic acids.

***Aliphatic Esteramide /Polyglycol Ether

Tambaqui leather scanning electron microscopy (SEM) analysis

For scanning electron microscopy (SEM) evaluation, the leather was cut with a razor blade and fixed in a semicircular stub using carbon tape. Images were obtained using a scanning electron microscope (FEI Quanta 250), operated in low vacuum (LV) mode and with an accelerating voltage of 15 kV.

Tensile and tear strength tests

In this study, the side of the fish was not considered an influencing factor, and random distribution was performed to remove the specimens. One of the sides was used to obtain the samplings for the tensile strength test (3 samples), and the other for the tests referring to the tear strength (3 samples).

The physical-mechanical behavior of the Tambaqui leathers was evaluated according to the technical standards for tensile strength (ISO 3376:2020) and tearing strength (ISO 3377-2:2016). The determination of thickness and conditioning of the samples followed the guidelines described in the technical standards ISO 2589:2016 and ISO 2419:2012, respectively.

The Tambaqui leathers were cut in four different directions: parallel (Figure 1A), perpendicular (Figure 1B), dorsal-ventral (Figure 1C), and ventral-dorsal (Figure 1D), all concerning the cephalocaudal line of the fish.

Statistical analysis

The experiments were carried out following a completely randomized experimental design, in which 45 fish were randomly selected for each test specimen direction. The variance analysis of the tensile and tear strength results was performed using the least squares method with a statistical model that included the fixed effect of the cutting direction (parallel, perpendicular, dorsal-ventral, and ventral-dorsal).

Results and Discusion

Tambaqui skin composition

Tambaqui skin showed 69.7% moisture, 27.1% crude protein, 1.2% lipids, and 0.2% ash (wet basis). Factors such as type of feed, sex, and age at slaughter can influence skin composition, explaining the differences in the literature.[3,12]. Regarding the protein content, this difference may also be related to the nitrogen conversion factor used.

Considering the lipid content, ideally, they should be below 4%.[13] At higher lipid levels, the tanning process becomes more difficult. Therefore, paying attention to the first stages of the process is critical for the success of the procedure.

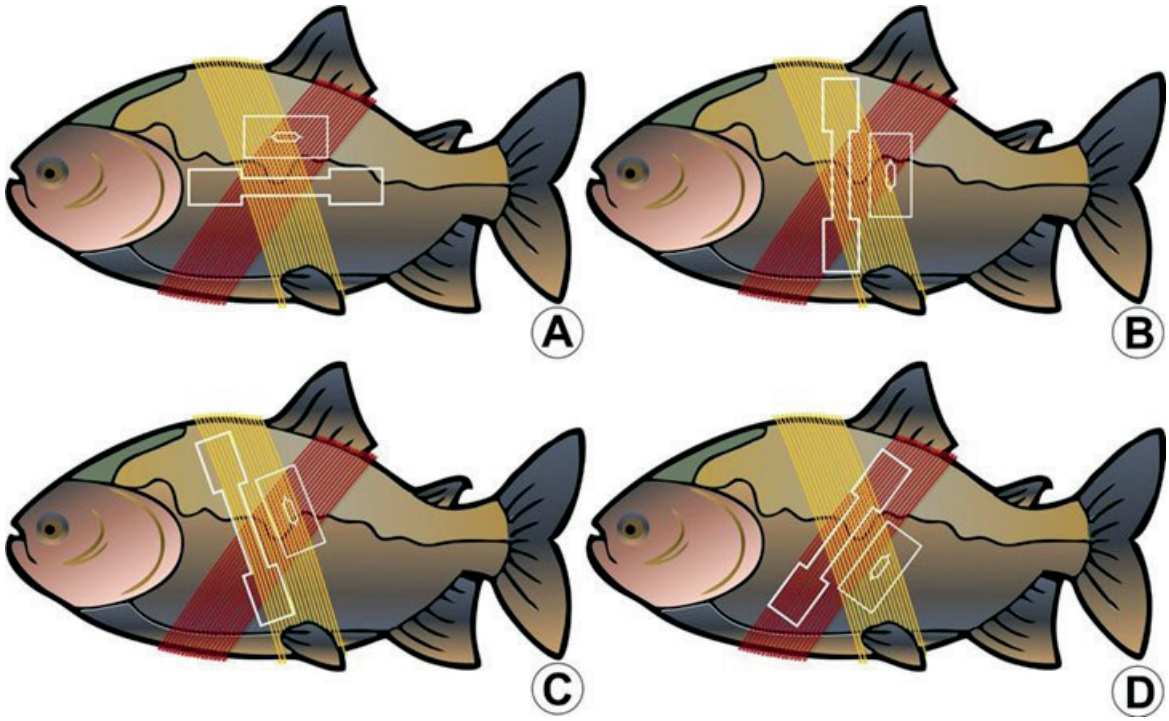

Figure 1. Graphical representation of the Tambaqui leather cuts obtained in the parallel **(A)**, perpendicular **(B)**, dorsal-ventral **(C)**, and ventral-dorsal **(D)** directions concerning the cephalocaudal line.

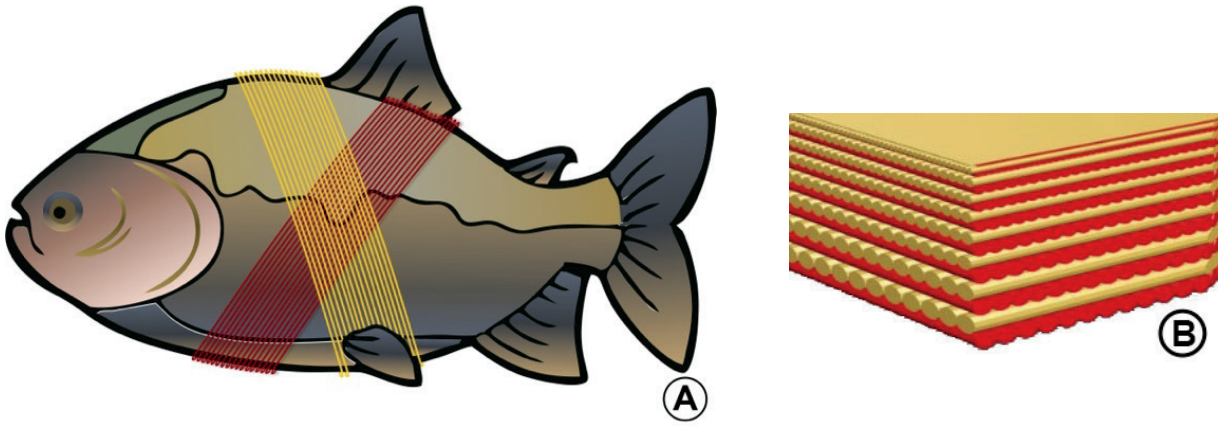

Figure 2. Parallel collagen fibers bundles distributed in a helical fashion around the Tambaqui body **(A)**, arranged in superimposed layers, oriented in an oblique direction to the preceding one **(B).** (Adapted from Nadol et al [15]).

Histological analysis of Tambaqui skin

The dermis of fish has overlapping layers of parallel collagen fibers bundles. These bundles are helically distributed along the body (Figure 2A). Each layer is oriented obliquely to the previous one, also differing in thickness (Figure 2B). The overlap between the layers can present different angulations depending on the body region (head, center, tail).[8,14]

Through the histological analysis of Tambaqui skins, evidenced by optical microscopy of polarized light, it is possible to verify the overlapping layers, with deeper regions presenting thicker layers of collagen fiber bundles (Figure 3A). Furthermore, collagen fibers arranged vertically can be noted, joining the superficial layers to the deeper ones (Figure 3 A and B, arrow 4). They are distributed in intervals and have the function of restricting the sliding movement of the layers.[8] These structures were also identified in the skin of other aquatic species such as eels[16] and frogs[17]. In the study by Allen et al.[18] the structure of *Monacanthus tuckeri* skin, a small fish that inhabits Atlantic corals, is presented in the form of micrographs and schematic drawings, showing the presence of collagen fibers inserted perpendicularly to the layers. Eastman & Hikida[19] describe the stratum compactum of the Ploughfish dermis (*Gymnodraco acuticeps*) as a thick matrix formed by collagen fibers regularly arranged in parallel layers, united by vertical columns of collagen fibers.

Scanning electron microscopy (SEM) analysis of Tambaqui leather

In this study, the organization of collagen fiber bundles in Tambaqui leather is shown in Figure 4A. Like in skin, collagen fiber bundles are organized in layers (Figure 4 A and B, arrow 1) interspersed

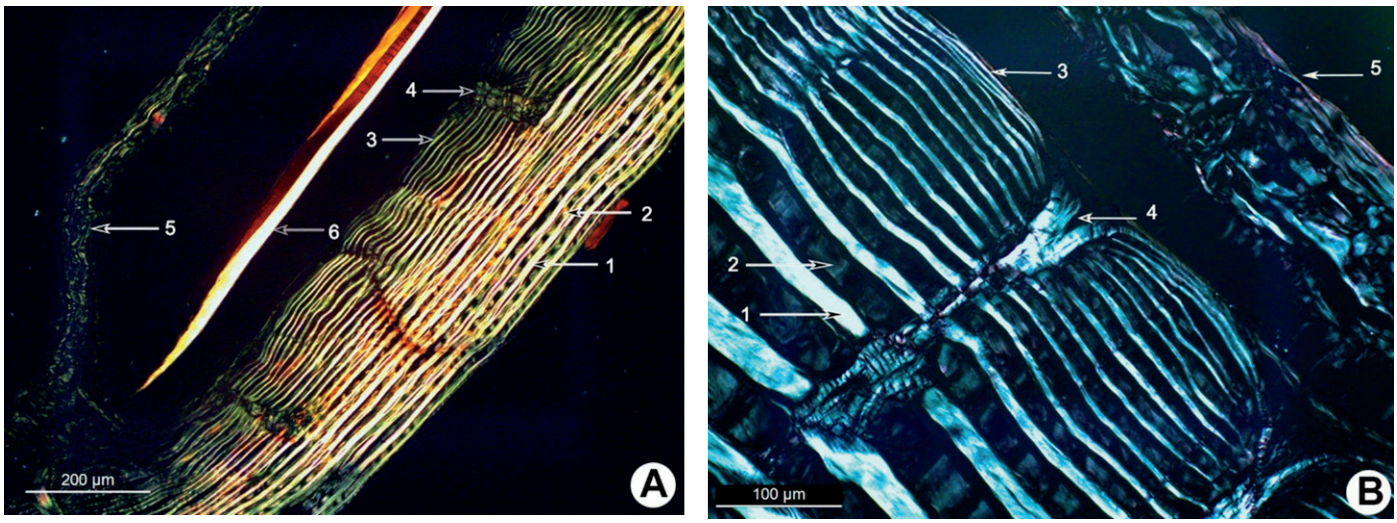

Figure 3. Images of the histological section of Tambaqui skin in the central region (between head and tail, and back and belly), evidenced by optical microscopy of polarized light. Layers with shiny collagen fibers bundles with the same vibration direction **(1)**, alternating with layers of collagen fibers bundles with different vibration directions **(2)**, collagen fibers bundles of the superficial dermis **(3)**, vertically inserted collagen fibers **(4)**, lamellae of the scale **(5)** and scale **(6)**. Staining: Masson's Trichrome (MT) (Figure **3A**) and hematoxylin and eosin (Figure **3B**).

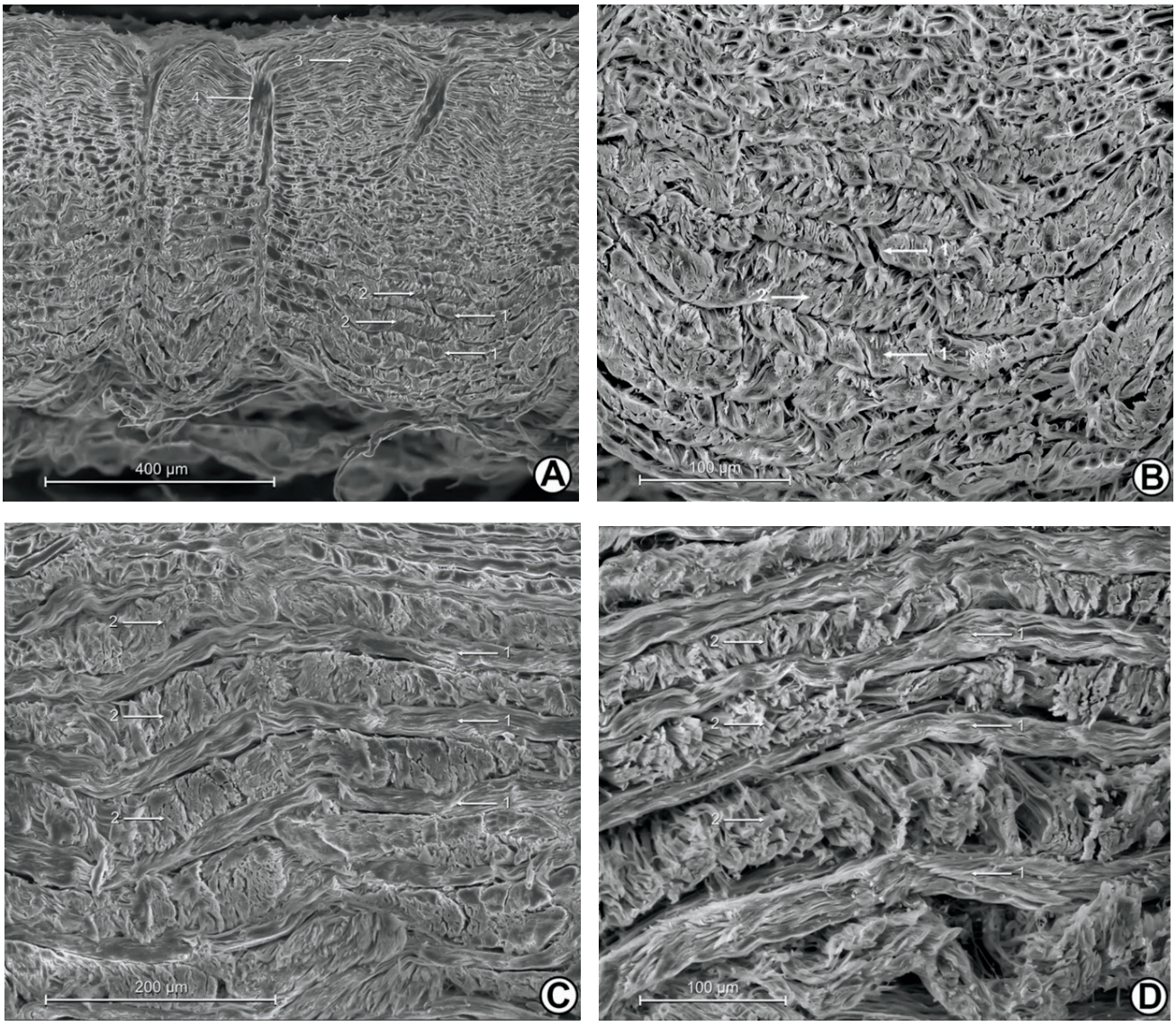

Figure 4. SEM image of the perpendicular section to the Tambaqui leather surface. Parallel cutting direction **(A** and **B)**, dorsal-ventral **(C)**, and ventral-dorsal **(D)** concerning the cephalo-caudal line of the fish. Layers with collagen fibers bundles organized in the same direction (arrow 1), alternating with layers of collagen fibers bundles with an oblique direction (arrow 2), collagen fibers from the superficial dermis (arrow 3), and perpendicular collagen fibers projections to the leather surface (arrow 4). Author: Luciana dos Reis Fernandes.

by collagen fiber bundle arrangements with different orientations (Figure 4 A and B, arrow 2). These layers are present throughout the leather structure, thinner when close to the surface (Figure 4A, arrow 3). Collagen fibers arranged vertically are also presented (Figure 4 A, arrow 4). Other works have also identified perpendicular projections for different species of fish.[8,14,20]. In the case of Tambaqui (Figure 4A, arrow 4), these joints appear throughout the entire structure of the leather, which may favor the material's resistance as they prevent slipping between layers.

When cut in a parallel direction (Figure A and B), the layers are composed of collagen fiber tips, oriented in oblique directions to each other. As for the dorsal-ventral and ventral-dorsal directions, the collagen fibers appear as tops, alternated by layers of more elongated collagen fibers. Collagen fiber bundles aligned and oriented towards the traction force axis can provide better resistance to the leather.[21,22] Observing the dorsal-ventral cut (Figure 4C), fiber tops (arrow 2) appear oriented to the left, while in the ventral-dorsal (Figure 4D), they appear slanted towards to the right (arrow 2). Considering the difference in the collagen fiber arrangements, different mechanical responses can be observed depending on the cutting direction.

Tensile and tear strength tests

In this study, four cutting directions were chosen to evaluate the physical-mechanical properties of Tambaqui leather: parallel (PL),

perpendicular (PP), dorsal-ventral (DV), and ventral-dorsal (DV) directions, considering the cephalocaudal line.

The graphs in Figure 5A-C show a significant difference ($p \leq 0.05$) between treatments. As reported in other studies the perpendicular cut direction showed greater tensile strength (Figure 5A).[20,23,24] Cuts in the ventral-dorsal direction come in second with 15.13 N/mm^2. Such results are attributed to the greater alignment of the fibers (Figure 4D), oriented to the tension force axis. The specimens cut in the parallel direction showed the lowest tensile strength (8.38 N/mm^2) but the highest elongation percentage (Figure 5B).

Other authors also observed the effect of collagen fiber arrangements on the mechanical properties of fish skins and leather.[25,26] Kirt & Khora[27] observed differences between the concentration, distribution, and orientation of collagen fibers in the ventral and dorsal regions on the mechanical behavior of puffer skin (*Lagocephalus gloveri*). The results indicate greater tensile strength for the skin in the ventral area, attributed to the higher density and alignment of collagen fibers.

A minor effect of collagen fiber orientation was observed for tear strength (Figure 5C). A statistically significant difference was observed only between the ventral-dorsal and parallel cuts ($p \leq 0.05$). In this case, the test evaluates the material's resistance by applying a force from the high-stress concentration (tear) point. According to Maina et al.[6] the leather resistance to tearing is more related to the softness and plasticity, characteristics acquired during the tanning process. However, the authors also suggest a positive correlation between tensile strength and tear strength, which was not observed in the present experiment.

Our results corroborate previous studies that also identified differences in the mechanical properties of fish skins and leather cuts in different directions.[25,26] This reinforces the need to develop adequate methodologies for sampling and qualitative evaluation of fish leather.

Conclusion

Chrome-tanned Tambaqui leathers were evaluated concerning morphology and mechanical properties. Collagen fibers distributed in layers, observed in scanning electron microscopy (SEM) images, showed the organization of the fish dermis, as reported in the literature. Physical-mechanical analyzes of Tambaqui leathers indicate that different cutting directions of the specimens influence the qualitative properties. Samples cut perpendicular to the cephalocaudal line of the fish showed greater tensile strength. The parallel cuts presented the highest values in assessing the elongation capacity until failure. Regarding tearing strength, cuts in the ventral-dorsal direction showed greater resistance. Thus, the anisotropy of collagen and its influence on the mechanical properties of leather was demonstrated, indicating the need for specific technical standards for the sampling and qualitative evaluation of fish leather.

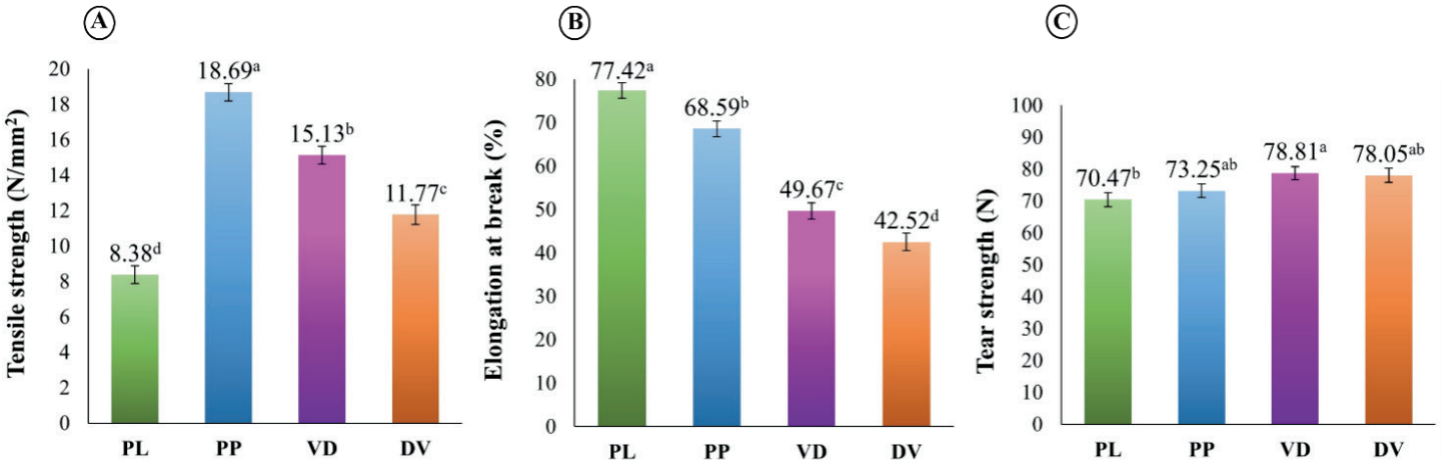

Figure 5. Tensile strength, elongation at break and tear strength of Tambaqui leather cut in the parallel (PL, green), perpendicular (PP, blue), ventral-dorsal (VD, purple), and dorsal-ventral directions (DV, orange). Different lowercase letters indicate statistically significant differences between samples (Tukey, $p<0.05$).

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Statements and Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Credit authorship contribution statement

Jessica Valéria de Campos: Conceptualization, Investigation, Methodology, Data curation; Fernanda Ramalho Procopio: Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing – original draft; Waldomiro Barioni Júnior: Formal analysis; Ana Rita de Araujo Nogueira: Supervision, Validation, Writing – review & editing; Manuel Antonio Chagas Jacintho: Conceptualization, Supervision, Methodology, Investigation, Validation, Writing – review & editing.

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