

Green Synthesis and Physicochemical Characterization of Copper Nanoparticles Using Citrus *limon* Ethanolic Peel Extract

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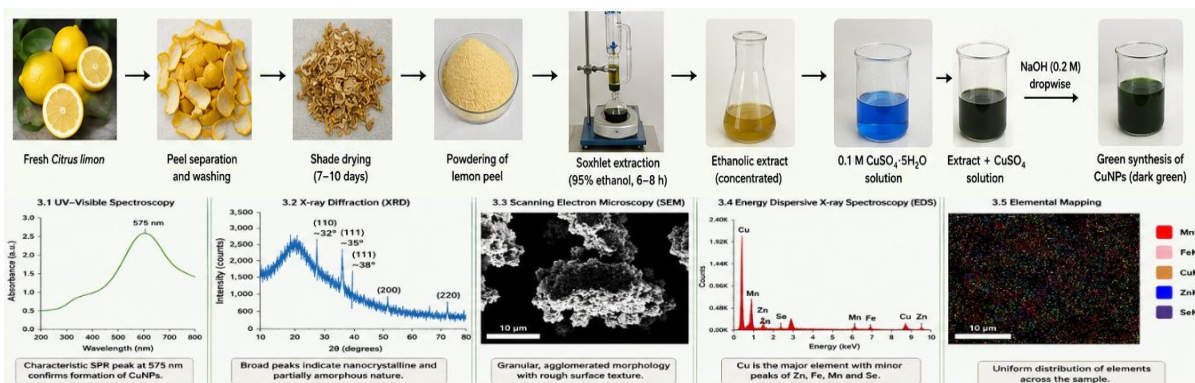
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ABSTRACT

The present study focuses on the green synthesis and characterization of copper nanoparticles (CuNPs) using ethanolic peel extract of Citrus *limon* as a reducing and stabilizing agent. Green nanotechnology has emerged as an environmentally sustainable alternative to conventional chemical and physical nanoparticle synthesis methods, which often involve toxic chemicals and hazardous reaction conditions. In this study, fresh Citrus *limon* peels were shade-dried, powdered, and Soxhlet-extracted with ethanol to obtain a phytochemical-rich extract containing flavonoids, phenolics, citric acid, and ascorbic acid. Copper nanoparticles were synthesized by reacting the ethanolic extract with 0.1 M copper sulfate solution under continuous magnetic stirring, followed by pH adjustment using sodium hydroxide solution. The formation of CuNPs was confirmed by a visible colour change from light blue to dark green. The synthesised nanoparticles were characterised using UV-Visible spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDS), and elemental mapping. UV-Visible spectroscopy confirmed nanoparticle formation through characteristic surface plasmon resonance peaks. XRD analysis revealed the nanocrystalline and partially amorphous nature of the CuNPs. SEM analysis showed granular and agglomerated morphology with rough surface texture, while EDS confirmed copper as the major elemental component. Elemental mapping demonstrated uniform distribution of copper throughout the sample. The study concludes that Citrus *limon* peel extract serves as an efficient eco-friendly reducing and capping agent for the synthesis of stable copper nanoparticles with promising applications in biomedical, catalytic, environmental, and industrial fields.



Keywords- Green synthesis, Copper nanoparticles, Citrus *limon*, Nanotechnology, Characterization of CuNPs.

I. INTRODUCTION

Nanotechnology is a multidisciplinary branch of science that deals with the synthesis, manipulation, and application of materials at the nanoscale level, generally ranging between 1 and 100 nm¹. At this dimension, materials exhibit unique physicochemical properties such as enhanced catalytic activity, improved optical behavior, superior electrical conductivity, and increased surface area-to-volume ratio². These remarkable characteristics make nanoparticles highly useful in numerous industrial and biomedical applications^{3,4}. From the last few decades, nanotechnology has revolutionized several scientific fields including medicine, environmental science, agriculture, pharmaceuticals, electronics, catalysis, textile engineering, and renewable energy systems⁵. Nanoparticles have been extensively utilized in drug delivery systems, biosensors, imaging techniques, cancer therapy, antimicrobial coatings, water purification systems, and energy storage devices⁶. Among different metallic nanoparticles, copper nanoparticles (CuNPs) have gained substantial attention because of their exceptional physicochemical and biological properties⁷. Copper nanoparticles possess excellent catalytic activity, antimicrobial potential, electrical conductivity, and thermal stability^{8,9}. Copper is relatively inexpensive and abundantly available compared to noble metals such as gold and silver, making CuNPs economically favorable for industrial applications¹⁰. Copper nanoparticles have demonstrated remarkable antimicrobial activity against bacteria, fungi, and viruses¹¹. Their antimicrobial action is mainly attributed to the production of reactive oxygen species (ROS), membrane disruption, and interference with microbial metabolic pathways¹². Due to these properties, CuNPs are widely investigated for applications in medical devices, antimicrobial coatings, wound healing materials, and food packaging industries¹³. CuNPs are also employed in catalytic degradation of dyes, wastewater treatment, sensors, conductive inks, batteries, solar cells, and environmental remediation¹⁴. Their high catalytic efficiency and conductivity make them highly useful in chemical and electrochemical applications¹⁵. Despite the numerous advantages of copper nanoparticles, traditional physical and chemical methods used for their synthesis involve hazardous chemicals, high temperature and pressure conditions, expensive equipment, and toxic reducing agents¹⁶. Such methods can generate harmful by-products that negatively impact human health and the environment¹⁷. Therefore, the development of environmentally friendly and sustainable methods for nanoparticle synthesis has become an important area of research in modern nanotechnology¹⁸. To overcome these limitations, researchers have focused on the development of green synthesis approaches for nanoparticle production¹⁹. Green synthesis involves the utilization of biological materials such as plant extracts,

bacteria, fungi, algae, and biomolecules for nanoparticle synthesis²⁰. Among these methods, plant-mediated synthesis has emerged as one of the most efficient and eco-friendly techniques due to its simplicity, low cost, scalability, and environmental compatibility²¹. Plant extracts contain diverse phytochemicals including flavonoids, phenolics, terpenoids, tannins, proteins, alkaloids, enzymes, sugars, and organic acids that can act as reducing as well as stabilizing agents during nanoparticle synthesis²². These bioactive compounds convert metal ions into nanoparticles without the use of harmful chemicals^{23,24}.

Green nanotechnology has become an important research area because it provides environmentally sustainable alternatives to conventional nanoparticle synthesis methods²⁵. Traditional synthesis methods involve toxic reducing agents such as sodium borohydride and hydrazine, which can produce hazardous waste and environmental pollution²⁶. Green synthesis minimizes toxicity and energy consumption by using natural reducing agents²⁷. Several researchers have reported successful synthesis of metallic nanoparticles using alternative botanical matrices such as gooseberry, strawberry, blueberry, and cranberry extracts²⁸, as well as a diverse selection of medicinal flora²⁹. Plant-mediated synthesis offers advantages such as simplicity, cost-effectiveness, low toxicity, biocompatibility, and improved nanoparticle stability³⁰. Recent evaluations have underlined the efficacy of specific therapeutic trees like *Pterocarpus marsupium* as structural "green nanofactories" for biosynthesizing copper, zinc, gold, and silver nanoparticles³¹. Polysaccharide-based structures are increasingly combined with these systems to act as advanced carrier platforms in nanomedicine³², while detailed characterizations of leaf-derived zinc nanoparticles emphasize their functional versatility³³. Citrus *limon* (lemon) is an important citrus fruit known for its rich phytochemical composition³⁴. Lemon peel contains significant amounts of citric acid, ascorbic acid, flavonoids, limonoids, essential oils, and polyphenolic compounds³⁵. These phytochemicals exhibit strong reducing and antioxidant properties, making lemon extract an excellent candidate for green nanoparticle synthesis³⁶. The use of ethanolic extracts instead of aqueous extracts enhances the extraction efficiency of phytochemicals because ethanol effectively dissolves both polar and semi-polar compounds³⁷. Ethanolic extract of *Citrus limon* peel is expected to provide efficient reduction and stabilization during CuNP synthesis³⁸.

The present study aims to synthesize copper nanoparticles using the ethanolic extract of Citrus *limon* peel through an eco-friendly and sustainable approach³⁹. The major objectives of this research include the preparation of ethanolic extract rich in phytochemicals, the synthesis of copper nanoparticles using green synthesis methodology, and characterization of the synthesized nanoparticles using UV-Visible

Spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM)⁴⁰. The study also aims to evaluate the morphology, stability, and crystalline nature of synthesized CuNPs and to analyze the role of phytochemicals in the reduction and stabilization process⁴¹. The successful completion of this work is expected to contribute significantly to the field of green nanotechnology and sustainable nanoparticle synthesis⁴².

II. METHODOLOGY

2.1 Collection and Preparation of Plant Material

Fresh lemons (*Citrus limon*) were procured from a local market and carefully selected for uniform ripeness and absence of spoilage⁴³. The fruits were thoroughly washed with tap water to remove dust, debris, and possible microbial contaminants, followed by rinsing with distilled water⁴⁴. The peel and pulp were separated, and the peel was shade-dried at room temperature for 7–10 days to preserve thermolabile bioactive compounds⁴⁵. After drying, the peel was finely powdered using a sterile grinder and stored in an airtight container to prevent moisture absorption and degradation of phytochemicals until the time of extraction⁴⁶ [Figure 1].

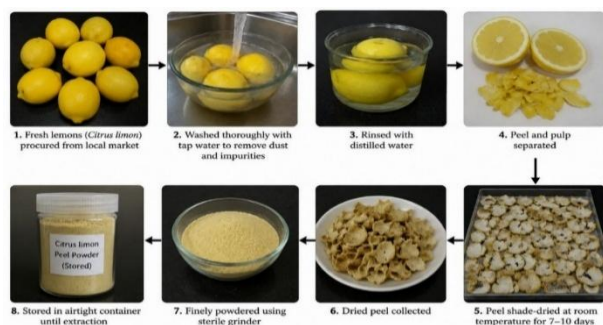


Figure 1: Preparation of *Citrus limon* Peel Powder for Extract Preparation

2.2 Preparation of *Citrus limon* Ethanolic Extract

The powdered lemon peel was subjected to Soxhlet extraction using 95% ethanol as the solvent, which efficiently extracts both polar and semi-polar phytochemicals such as flavonoids, polyphenols, and ascorbic acid⁴⁷. Approximately 50 g of powder was placed in a thimble, and extraction was performed for 6–8 hours at 60–65°C⁴⁸. The extract was then filtered using Whatman No. 1 filter paper to remove solid residues⁴⁹. The filtrate was concentrated under reduced pressure using a rotary evaporator at 45 °C to obtain a viscous, dark yellow ethanolic extract⁵⁰. The extract was stored at 4 °C in amber bottles to prevent photodegradation until further use [Figure 2].



Figure 2: Soxhlet Extraction of *Citrus limon* Peel Using Ethanol

2.3 Synthesis of Copper Nanoparticles

Copper nanoparticles (CuNPs) were synthesized through a green synthesis method using *Citrus limon* ethanolic peel extract as both reducing and stabilizing agents. Initially, a 0.1 M aqueous solution of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was prepared by dissolving the required amount of copper sulfate in 50 mL of distilled water. The solution was subjected to continuous magnetic stirring for 30 minutes at room temperature until complete dissolution and uniform mixing were achieved. Subsequently, 5 g of *Citrus limon* peel ethanolic extract was taken separately, and the prepared copper sulfate solution was added slowly into the plant extract under continuous stirring conditions. The reaction mixture was further stirred continuously until both solutions were completely mixed. To maintain the pH of the reaction medium, a 0.2 M sodium hydroxide (NaOH) solution was prepared and added dropwise as required. The reaction mixture was carefully observed for a visible colour change from light blue to dark green, indicating the reduction of Cu^{2+} ions into zero-valent copper nanoparticles (Cu^0) and confirming nanoparticle formation⁵¹. The reaction was allowed to proceed for 4–6 hours under constant stirring to ensure complete reduction of copper ions and the formation of stable nanoparticles (Khane et al., 2022) [Figure 3].

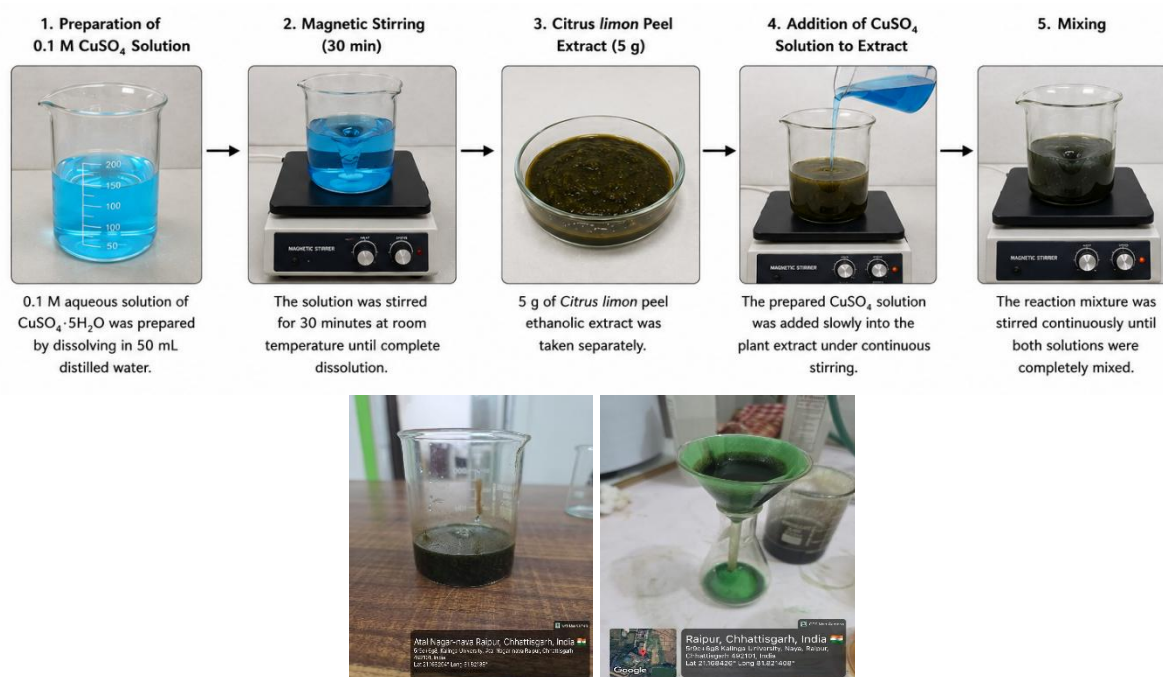
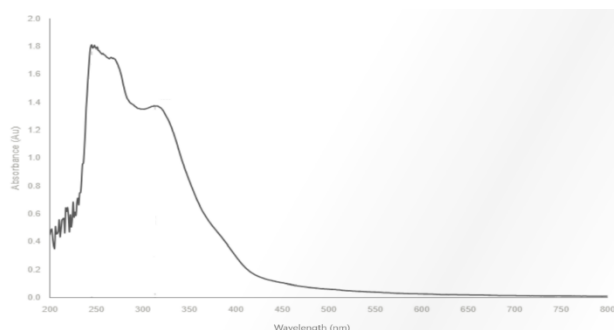


Figure 3: Green Synthesis Procedure of Copper Nanoparticles (CuNPs) Using Citrus *limon* Peel Extract

III. CHARACTERIZATION OF COPPER NANOPARTICLES

3.1 UV-Visible Spectroscopy

The UV-Vis absorption spectrum exhibited a strong absorption peak at approximately 250 nm in the UV region, confirming the formation of the synthesized material/nanoparticles. A broad absorption band between 280–340 nm indicates surface interaction and stabilization by phytochemical constituents. The absorbance gradually decreased toward the visible region with negligible absorption above 500 nm, suggesting good particle dispersion and reduced agglomeration⁵². The spectral profile confirms the successful synthesis and stability of the prepared sample [Graph 1].

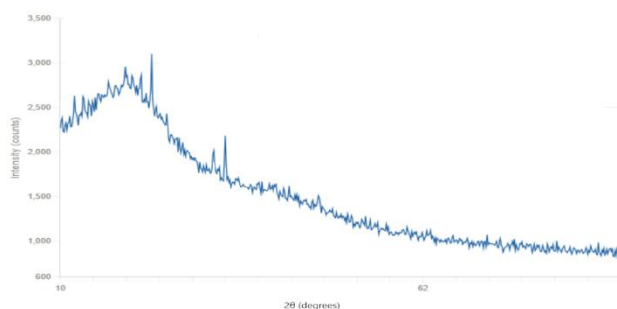


Graph 1: UV-VIS Spectrum of Citrus *limon* Peel Extract Copper Nanoparticles

3.2 X-ray Diffraction (XRD)

X-ray Diffraction (XRD) analysis was performed to determine the crystalline structure, phase

purity, and crystallite nature of the synthesized copper nanoparticles (CuNPs). The obtained XRD diffractogram exhibited a broad diffraction pattern with low- to moderate-intensity peaks, indicating the nanocrystalline and partially amorphous nature of the synthesised material. A broad hump observed between approximately 15° and 30° (2θ) suggests the presence of phytochemical compounds from the Citrus *limon* ethanolic extract, which acted as reducing and capping agents during nanoparticle synthesis. The broadness of this region also confirms the formation of nanoparticles with very small crystallite size. Distinct diffraction peaks were observed around 32°, 35°, and 38° (2θ), which may correspond to the characteristic crystalline planes of copper and copper oxide nanoparticles⁵³. The peak near 32° can be indexed to the (110) plane, while the diffraction peak around 35° corresponds to the (111) plane of monoclinic copper oxide structures. The peak observed near 38° is associated with the (111) crystalline plane of face-centered cubic (FCC) metallic copper nanoparticles. Additional weak peaks at higher 2θ values may correspond to planes such as (200) and (220), further supporting nanoparticle formation. The comparatively broad and less intense peaks indicate nanoscale particle dimensions and moderate crystallinity, which are common characteristics of green synthesized nanoparticles due to phytochemical stabilization. The absence of additional sharp impurity peaks confirms the relatively pure nature of the synthesized CuNPs⁵⁴. Overall, the XRD results confirmed the successful formation of nanosized copper-based nanoparticles synthesized using Citrus *limon* ethanolic extract [Graph 2].



Graph 2: XRD Analysis of Citrus *limon* Peel Extract Copper Nanoparticles

3.3 Scanning Electron Microscopy (SEM)

The SEM image showed that the synthesized CuNPs possessed predominantly granular and quasi-spherical surface features with uneven particle distribution. The agglomeration observed in the micrograph may be attributed to the presence of phytochemical constituents from the Citrus *limon* ethanolic extract acting as capping and stabilizing agents during nanoparticle formation. These phytochemicals promote adhesion between particles, leading to cluster formation during the drying process. The micrograph was recorded at a magnification of 1.00 KX with an accelerating voltage of 15.00 kV and a working distance of 8.7 mm. Based on the scale bar of 10 μ m, the aggregated structures appear in the micrometre range; however, these larger structures are composed of numerous nanosized primary particles⁵⁵. The rough and highly textured morphology indicates a high surface area, which may enhance the catalytic and antimicrobial efficiency of the synthesized nanoparticles (Khane et al., 2022). The dark background with bright surface contrast confirms effective electron interaction with the copper-based material⁵⁶[Figure 4].

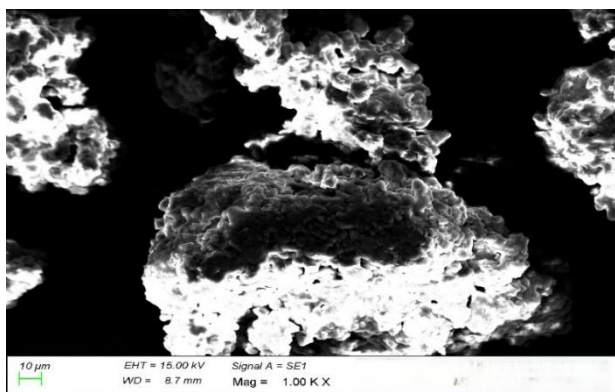
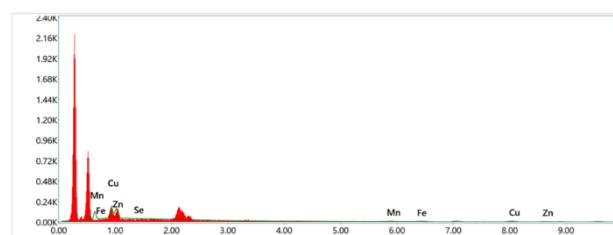


Figure 4: SEM Image of Citrus *limon* Peel Extract Copper Nanoparticles

3.4 Energy Dispersive X-ray Spectroscopy (EDS)

Energy Dispersive X-ray Spectroscopy (EDS) analysis was carried out to determine the elemental composition and purity of the synthesized copper nanoparticles (CuNPs). The EDS spectrum confirmed the successful formation of copper-based nanoparticles

by displaying characteristic elemental peaks corresponding to copper (Cu) as the major constituent in the sample. A prominent copper peak was observed in the region around 0.9–1.0 keV, along with additional Cu signals at higher energy regions near 8.0 keV, which are characteristic binding energies of copper and confirm its presence within the synthesized nanoparticles⁵⁷. The spectrum also exhibited minor peaks corresponding to elements such as zinc (Zn), iron (Fe), manganese (Mn), and selenium (Se). The Zn peak observed near 1.0 keV and around 8.6 keV may indicate trace elemental impurities or possible environmental contamination during sample preparation and analysis. Similarly, small Fe and Mn peaks detected near 0.6–0.8 keV and in the higher energy region may have originated from instrumental components, substrate materials, or residual phytochemical-associated mineral content present in the plant extract. The weak selenium (Se) signal observed around 1.3–1.5 keV was comparatively low in intensity, indicating its trace-level presence in the sample⁵⁸. The significantly higher intensity of the copper peaks compared to other elemental signals demonstrates that copper was the dominant element present in the synthesized nanomaterial. The absence of strong unwanted impurity peaks indicates relatively good purity of the synthesized CuNPs [Graph 3].



Graph 3: EDS Analysis of Citrus *limon* Peel Extract Copper Nanoparticles

Copper (Cu) was the predominant element in the sample with a weight percentage of 56.8% and an atomic percentage of 55.8%, confirming the successful synthesis of copper-based nanoparticles [Table 1]. The strong Cu signal indicates efficient reduction of Cu^{2+} ions into copper nanoparticles during the green synthesis process using Citrus *limon* ethanolic extract. The EDS spectrum also showed the presence of zinc (Zn) with a weight percentage of 25.4% and atomic percentage of 24.2%. The presence of Zn may be attributed to trace mineral constituents naturally present in the plant extract or possible instrumental or substrate-associated impurities during analysis. Iron (Fe) was detected with a weight percentage of 9.3% and atomic percentage of 10.3%, while manganese (Mn) exhibited 8.4% weight percentage and 9.5% atomic percentage⁵⁹. These minor elemental signals may arise from residual phytochemical-associated micronutrients, environmental contamination, or sample holder interactions during SEM-EDS examination. A very small amount of selenium (Se) was detected with only 0.1% weight and

atomic percentage, indicating trace-level presence within the sample. The low net intensity and high error percentage associated with selenium suggest that it is present in negligible quantity and does not significantly

contribute to the nanoparticle composition⁶⁰. The relatively high copper content compared to other detected elements confirms the purity and successful formation of CuNPs.

Table 1: SEM–EDX Elemental Analysis of Synthesized Cu-Based Nanoparticles

Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
Mn K	8.4	13.51	9.5	1.7	63.7	0.9021	0.9640	1.1864
Fe K	9.3	13.09	10.3	1.6	37.9	0.9100	0.9710	1.2267
Cu K	56.8	24.32	55.8	4.0	32.5	0.9371	0.9752	1.0535
Zn K	25.4	35.34	24.2	1.3	50.2	0.9473	0.9768	1.0468
Se L	0.1	37.15	0.1	0.0	100.0	0.8239	0.2437	1.0011

3.5 Elemental Mapping Analysis

Elemental mapping analysis was carried out using SEM-EDS mapping techniques to investigate the spatial distribution and dispersion of elements present in the synthesized copper nanoparticles (CuNPs). A relatively uniform distribution of elemental signals throughout the analyzed region indicates homogeneous nanoparticle formation [Figure 5]. Different colors were assigned to individual elements for identification, where manganese (Mn) was represented by red, iron (Fe) by light pink, copper (Cu) by orange, zinc (Zn) by blue, and selenium (Se) by purple. Among the detected elements, copper (Cu) exhibited the most prominent and uniformly distributed signal across the sample surface, confirming that copper was the major constituent of the synthesized nanoparticles. The homogeneous dispersion of copper signals indicates effective reduction of Cu²⁺ ions and successful formation of copper nanoparticles throughout the reaction matrix. The widespread distribution of Cu

further suggests that the nanoparticles were synthesized uniformly without significant phase segregation. Minor distributions of manganese (Mn), iron (Fe), and zinc (Zn) were also observed within the mapped region. These elements appeared in lower intensity compared to copper and may be associated with trace phytochemical mineral contents from the Citrus *limon* extract or minor impurities introduced during sample preparation and instrumental analysis. Selenium (Se) showed extremely low intensity and sparse distribution, indicating its negligible presence in the synthesized sample. The mapping image also revealed fine dispersion of elemental particles without the formation of large, isolated domains, suggesting good elemental integration within the nanoparticle matrix. The scale bar of 10 μm confirms that the elemental distribution was analyzed over a microscale region containing aggregated nanosized particles⁶¹.



Figure 5: Elemental Mapping Analysis of Citrus *limon* Peel Extract Copper Nanoparticles

IV. APPLICATIONS

The green synthesized copper nanoparticles (CuNPs) derived from Citrus *limon* extract have extensive applications across various fields owing to their unique properties. In biomedicine, they demonstrate strong antibacterial, antifungal, and antiviral activities, making them ideal for wound dressings and medical devices^{5,62}. They are also significant in cancer

research for inducing oxidative stress in cancer cells. CuNPs effectively treat wastewater and degrade pollutants and are used in water purification. In catalysis, they function as efficient catalysts in various reactions²⁷. Their excellent electrical and thermal conductivity enables use in electronics, while their potential in agriculture as nano-pesticides and nano-fertilizers is gaining interest.

V. CONCLUSION

The study successfully demonstrated the green synthesis of copper nanoparticles (CuNPs) using *Citrus limon* peel extract as a reducing and stabilizing agent, offering an eco-friendly and cost-effective alternative to traditional methods. Color change from light blue to dark green indicated the reduction of Cu^{2+} ions and formation of nanoparticles. Characterization through UV-Visible spectroscopy, XRD, SEM, and EDS confirmed the synthesis and properties of nanosized copper particles, revealing their nanocrystalline nature and characteristic morphology. Phytochemicals in the extract played a key role in stabilizing and capping the nanoparticles. The findings highlight the effectiveness of plant-mediated green synthesis for environmentally friendly CuNPs with potential industrial and biomedical applications.

Future research on green synthesized copper nanoparticles (CuNPs) should concentrate on optimizing synthesis parameters like pH, temperature, extract concentration, and reaction time to enhance particle size control, stability, and crystallinity. Studies on the catalytic efficiency of CuNPs in degrading industrial dyes and environmental pollutants, along with toxicological and biocompatibility assessments for medical applications, are essential. Advanced characterization techniques such as TEM, DLS, and zeta potential analysis will deepen understanding of nanoparticle morphology and stability. Future efforts may also explore large-scale production and CuNPs-based nanocomposites for applications in electronics, renewable energy, sensors, food packaging, and agriculture, utilizing agricultural waste and medicinal plant extracts to promote sustainable nanotechnology development.

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