

## Plant-Mediated Synthesis of Copper Nanoparticles Using *Citrus sinensis* Peel Extract and Their Structural Characterization

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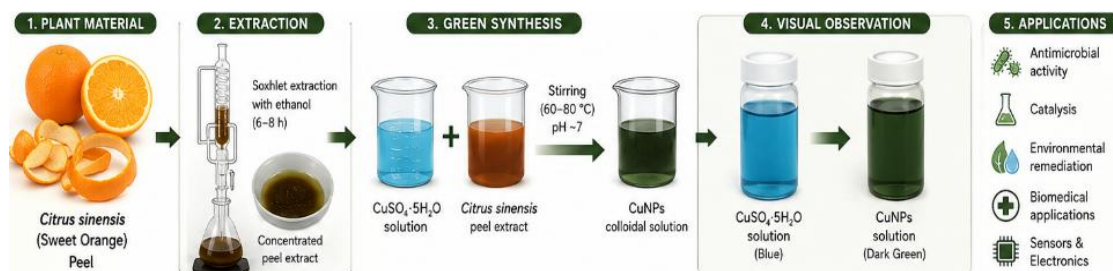
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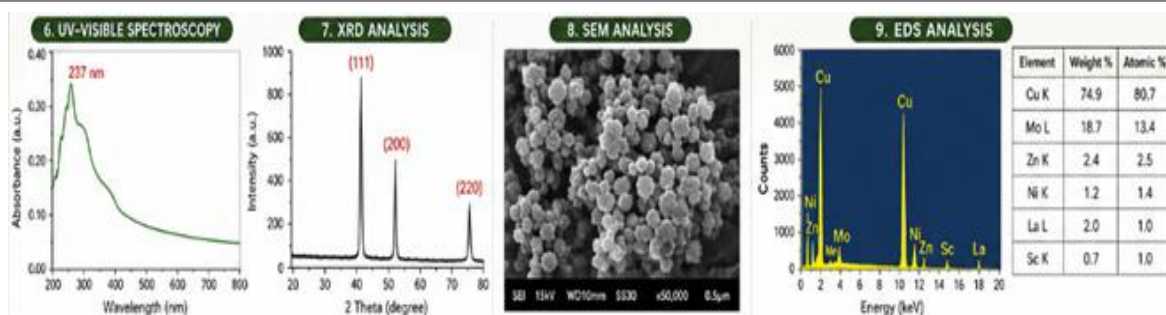
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### ABSTRACT

Nanotechnology has emerged as a rapidly advancing interdisciplinary field with extensive applications in medicine, catalysis, electronics, environmental remediation, agriculture, and material science. Among various metallic nanoparticles, copper nanoparticles (CuNPs) have attracted considerable attention because of their excellent electrical conductivity, catalytic efficiency, antimicrobial activity, optical properties, and cost-effectiveness compared to noble metal nanoparticles. Conventional methods used for the synthesis of copper nanoparticles commonly involve toxic chemicals, hazardous reducing agents, expensive equipment, and high energy consumption, which create environmental and health-related concerns. The present study focuses on the green synthesis of copper nanoparticles using *Citrus sinensis* (orange) peel extract as a natural reducing and stabilizing agent. Orange peel, generally discarded as agricultural waste, contains several bioactive phytochemicals such as flavonoids, phenolic compounds, ascorbic acid, limonene, terpenoids, and pectin that possess strong antioxidant and reducing capabilities. These phytochemicals facilitate the reduction of copper ions into stable copper nanoparticles. The synthesis process was confirmed by a visible color change in the reaction mixture due to surface plasmon resonance. The synthesized nanoparticles were characterized using UV-Visible spectroscopy, X-Ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). Characterization studies confirmed the successful formation, crystalline nature, and surface morphology of the synthesized copper nanoparticles. The study highlights an economical, environmentally friendly, and sustainable approach for nanoparticle synthesis using agricultural waste materials, with potential applications in antimicrobial activity, catalysis, environmental treatment, and biomedical research.





**Keywords-** Green nanotechnology, Copper nanoparticles (CuNPs), Citrus *sinensis*, orange peel extract, Phytochemical-mediated synthesis, Sustainable nanoparticle synthesis.

## I. INTRODUCTION

Nanotechnology has emerged as one of the fastest growing and most transformative branches of modern science and technology. It involves the design, synthesis, characterization, and application of materials at the nanoscale level, typically ranging from 1 to 100nm<sup>1</sup>. At this dimension, materials exhibit unique physicochemical properties that differ significantly from their bulk counterparts, including enhanced surface area, increased chemical reactivity, superior optical behaviour, improved catalytic efficiency, and remarkable electrical conductivity<sup>2,3</sup>. The concept of nanotechnology was first introduced by physicist Richard Feynman in 1959 during his famous lecture entitled “*There’s Plenty of Room at the Bottom.*” Since then, remarkable advancements in analytical and microscopic techniques such as Scanning Tunnelling Microscopy (STM), Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM) have accelerated research and applications in the field of nanoscience. Among the different classes of nanomaterials, metallic nanoparticles have attracted enormous scientific interest because of their multifunctional applications and unique physicochemical properties<sup>4</sup>. Recent comparative paradigms highlight the vast therapeutic potential of tailoring diverse metal nano-architectures including gold, silver, copper, zinc, platinum, and iron<sup>5</sup>. Copper nanoparticles (CuNPs) are particularly important due to their excellent electrical conductivity, catalytic activity, antimicrobial efficiency, optical properties, and relatively lower cost compared to noble metal nanoparticles such as gold and silver<sup>6</sup>. Copper nanoparticles have found extensive applications in sensors, conductive inks, catalysts, antimicrobial coatings, biomedical devices, wastewater treatment, textile industries, and energy storage systems<sup>7</sup>. Copper nanoparticles possess strong antibacterial and antifungal properties against a wide range of pathogenic microorganisms, making them highly promising for biomedical and environmental applications<sup>8</sup>. Copper nanoparticles are synthesized using physical and chemical approaches such as chemical reduction,

thermal decomposition, electrochemical synthesis, laser ablation, sol-gel methods, and vapor deposition techniques<sup>9</sup>. These methods are capable of producing nanoparticles with controlled size and morphology, they often involve toxic reducing agents, hazardous solvents, expensive instrumentation, and high energy consumption<sup>10</sup>. The generation of toxic byproducts and difficulties associated with waste disposal further limit the sustainability of conventional nanoparticle synthesis methods<sup>11</sup>. These challenges have encouraged researchers to develop environmentally friendly and sustainable alternatives for nanoparticle production. In recent years, green synthesis has emerged as a promising and eco-friendly approach for the synthesis of nanoparticles<sup>12</sup>. Green synthesis utilizes biological resources such as plants, algae, fungi, bacteria, enzymes, and biomolecules as reducing and stabilizing agents<sup>13</sup>. Among these biological systems, plant-mediated synthesis has gained significant attention because of its simplicity, cost-effectiveness, rapid synthesis, scalability, and environmental compatibility<sup>14,15</sup>. Plant extracts contain a wide variety of phytochemicals including flavonoids, terpenoids, tannins, alkaloids, phenolic compounds, proteins, carbohydrates, and organic acids<sup>16</sup>. These bioactive compounds possess strong antioxidant and reducing properties that enable the conversion of metal ions into stable nanoparticles<sup>17</sup>. These phytochemicals also function as capping and stabilizing agents, thereby preventing nanoparticle agglomeration and enhancing stability<sup>18,19</sup>.

One of the most promising plant materials used for green synthesis is Citrus *sinensis*, commonly known as sweet orange, which belongs to the family Rutaceae. Orange is one of the most widely cultivated citrus fruits in tropical and subtropical regions across the world<sup>20</sup>. Large quantities of orange peels are generated as agricultural, and food-processing waste every year<sup>21</sup>. Despite being discarded as waste, orange peels are rich sources of valuable bioactive compounds such as flavonoids, phenolic acids, limonene, carotenoids, pectin, terpenoids, and ascorbic acid<sup>22</sup>. These compounds exhibit strong antioxidant, antimicrobial, and

reducing properties, making orange peel extract highly suitable for the green synthesis of nanoparticles<sup>2,23</sup>. The phytochemicals present in orange peel extract can effectively reduce copper ions into copper nanoparticles through electron donation mechanisms<sup>24</sup>. Biomolecules such as proteins and polysaccharides adsorb onto the nanoparticle surface and stabilize the synthesized nanoparticles against aggregation<sup>1,25</sup>. The utilization of orange peel waste for nanoparticle synthesis provides dual environmental benefits by reducing agricultural waste accumulation and eliminating the use of hazardous chemicals in nanoparticle production<sup>26,27</sup>. Green synthesized copper nanoparticles are expected to exhibit excellent physicochemical and biological properties suitable for applications in catalysis, antimicrobial treatments, environmental remediation, and biomedical research<sup>28,29</sup>. Despite increasing interest in green nanotechnology, detailed studies focusing on the synthesis<sup>30</sup> and characterization of copper nanoparticles specifically using *Citrus sinensis* peel extract remain limited<sup>31,32</sup>.

## II. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Material

Fresh peels of *Citrus sinensis* (sweet orange), approximately 1.5 kg, were collected from local markets,

ensuring selection of healthy fruits free from fungal infections and visible spoilage for quality control. The peels were manually separated from the pulp using sterile stainless-steel knives and washed thoroughly under running tap water, followed by distilled water to eliminate surface contaminants and prevent interference during extraction and nanoparticle synthesis<sup>32</sup>. The peels were then cut into uniform pieces to enhance drying efficiency and shade-dried at room temperature for 7–10 days, avoiding direct sunlight to preserve heat-sensitive phytochemicals like flavonoids and phenolic compounds<sup>33</sup>. During drying, the peels were periodically turned on clean trays to ensure uniformity and prevent microbial growth, with complete drying validated by a constant weight and brittle texture. After drying, the peels were pulverized into fine powder using a mechanical grinder with intermittent grinding to prevent heat generation and degradation of bioactive compounds. The powder was then sieved for uniform particle size, ensuring consistency for the extraction process. Stored in airtight, labelled glass containers in a cool, dry environment, the prepared peel powder is protected against moisture absorption, oxidation, and microbial contamination, serving as the primary raw material for phytochemical extraction and the green synthesis of copper nanoparticles<sup>34,35</sup> [Figure 1].



Figure 1: Preparation of *Citrus sinensis* Peel Powder

### 2.2 Extraction Process

The extraction of phytochemicals from *Citrus sinensis* peel powder utilized the Soxhlet extraction technique, recognized for efficiently extracting plant bioactive compounds. Ethanol was chosen as the solvent due to its capacity to dissolve a wide range of polar and moderately non-polar compounds, including flavonoids, phenolic compounds, tannins, and terpenoids<sup>36</sup>. Its safety, biodegradability, and alignment with green chemistry principles further supported its selection. Initially, dried peel powder was weighed and placed in a cellulose extraction thimble, which was installed into the Soxhlet apparatus. A round-bottom flask containing ethanol was connected to the extractor and a condenser

and heated to initiate the extraction<sup>37</sup>. The solvent vapors generated during heating traveled through the apparatus, condensed, and percolated through the peel powder, continually extracting soluble phytochemicals. This cyclic process continued for 6–8 hours until the solvent became nearly colorless, signifying exhaustive extraction [Figure 2]. Throughout the extraction, temperature control was critical to maintain extraction efficiency while preventing the degradation of sensitive compounds. Upon completion, the extract in the flask cooled and excess solvent was removed using a rotary evaporator, yielding a concentrated crude extract rich in flavonoids, phenolic acids, ascorbic acid, and terpenoids. Finally, the extract was dried and stored in airtight

containers at 4°C to ensure stability and preserve the antioxidant activity of the phytochemicals<sup>38</sup>. This method proved effective in recovering bioactive compounds suitable for the green synthesis of copper nanoparticles.



Figure 2: Soxhlet extraction of *Citrus sinensis* peel

### 2.3 Green Synthesis of Copper Nanoparticles (CuNPs)

The synthesis of copper nanoparticles was achieved using *Citrus sinensis* peel extract as a natural reducing and stabilizing agent, with copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) as the metal precursor [Figure 3]. A solution of copper sulfate was prepared, and the peel extract was diluted before being added to the solution under continuous stirring<sup>39</sup>. Optimized temperature and pH conditions led to a color change from blue to dark green, indicating successful nanoparticle formation due to surface plasmon resonance. Phytochemicals in the extract aided in reducing and stabilizing the nanoparticles, which were then isolated through centrifugation and washed to remove impurities<sup>40</sup>. The final product, purified copper nanoparticles, underscores the method's eco-friendliness and cost-effectiveness, avoiding hazardous chemicals.



Figure 3: copper Sulfate pentahydrate solution and *Citrus sinensis* peel extract

## III. RESULT AND DISCUSSION

### 3.1 Visual Observation

The synthesis of copper nanoparticles was initially confirmed by a visible color change in the reaction mixture. The blue color of the copper sulfate solution gradually changed to dark green due to the reduction of  $\text{Cu}^{+2}$  ions into copper nanoparticles<sup>41</sup>. This color change occurs because of surface plasmon resonance [Figure 4]. The shift in visual appearance is a well-established indicator of metal reduction and subsequent capping by plant-derived compounds, as seen in similar green synthesis experiments using alternative fruit matrices<sup>42</sup>.

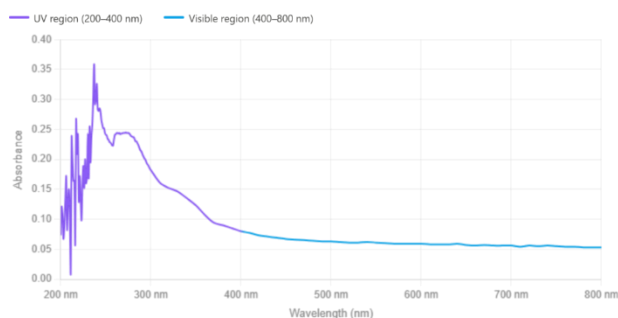


Figure 4: Reduction of  $\text{Cu}^{2+}$  ions into copper nanoparticles

### 3.2 UV-Visible Spectroscopy Analysis

The optical properties and formation of the synthesized copper nanoparticles (CuNPs) were investigated using UV-Visible spectroscopy in the wavelength range of 200–800 nm. The obtained spectrum revealed a strong absorption band in the ultraviolet region, particularly between 220 and 280 nm, with a prominent absorption peak centered around 237 nm [Graph 1]. This absorption peak confirms the presence of bioactive phytochemicals from the plant extract that participated in the reduction and stabilization of copper ions during nanoparticle synthesis<sup>43</sup>. The absorption observed near 237 nm may be attributed to  $\pi \rightarrow \pi^*$  electronic transitions associated with phenolic compounds, flavonoids, tannins, and other aromatic biomolecules present in the plant extract. These phytochemicals act as both reducing and capping agents, facilitating the green synthesis and stabilization of CuNPs. Similar UV absorption bands in the range of 220–270 nm have been reported in previous studies on green synthesized copper and copper oxide nanoparticles<sup>44</sup>. The spectrum further showed a gradual decrease in absorbance with increasing wavelength from 300 to 800 nm, indicating the absence of significant aggregation of nanoparticles in the colloidal solution<sup>45</sup>. A weak and broad tail extending into the visible region suggests the formation of nanosized copper particles with relatively stable dispersion<sup>46</sup>. Chemically synthesized metallic copper nanoparticles that often

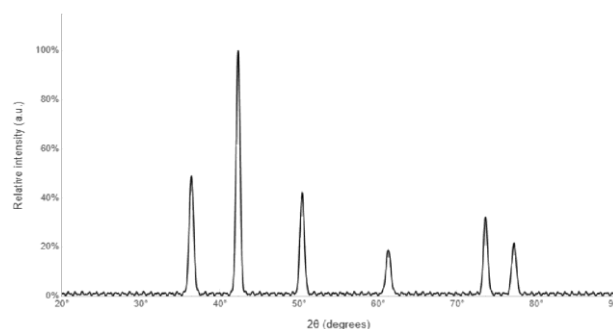
exhibit a sharp surface plasmon resonance (SPR) peak around 560–600 nm, the present biosynthesized nanoparticles displayed broad and less intense absorption behavior, which may be due to smaller particle size, partial oxidation, phytochemical surface coating, and polydispersity of the nanoparticles. The broad spectral nature also indicates strong interaction between copper nanoparticles and plant-derived metabolites adsorbed on the nanoparticle surface. These biomolecules enhance colloidal stability and prevent excessive agglomeration<sup>46</sup>.



Graph 1: UV-VIS spectrum of *Citrus sinensis* peel extract

### 3.3 XRD Analysis

The XRD pattern, shown in Graph 2, exhibited distinct diffraction peaks at approximately 36°, 43°, 50°, 61°, 74°, and 78° (2θ), confirming the crystalline nature of the synthesized material. The intense peak observed near 43° indicates the dominant crystal orientation. The sharp and well-defined diffraction peaks suggest good crystallinity and phase purity of the nanoparticles. Absence of significant impurity peaks further confirms the successful synthesis of the desired crystalline phase<sup>47</sup>.



Graph 2: XRD Analysis of *Citrus sinensis* peel extract

### 3.4 SEM Analysis

The SEM micrograph revealed that the synthesized copper nanoparticles were predominantly irregular to nearly spherical in morphology with noticeable agglomeration. The nanoparticles appeared clustered together, forming dense aggregates, which is commonly observed in biologically synthesized nanoparticles due to their high surface energy and strong interparticle attractive forces such as van der Waals interactions [Figure 5]. The aggregation may also result from the presence of phytochemical residues acting as capping and stabilizing agents on the nanoparticle surface. The particles exhibited rough and heterogeneous surface texture, indicating successful nucleation and growth during the green synthesis process. The nanoparticles are composed of fine granular structures assembled into larger clusters. The granular appearance suggests the formation of nanosized copper particles with relatively high surface area, which is advantageous for catalytic, antimicrobial, and adsorption-related applications<sup>48</sup>.

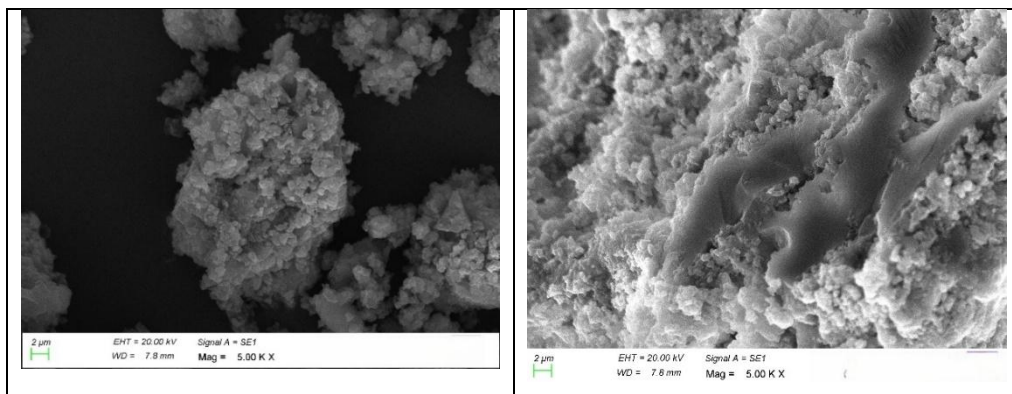


Figure 5: SEM Image of *Citrus sinensis* peel extract

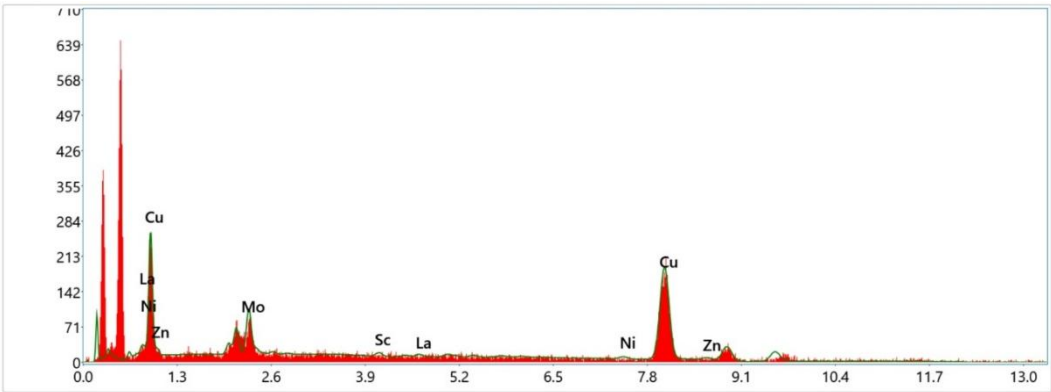
### 3.5 EDS Analysis

The obtained EDS spectrum exhibited a strong and intense peak for copper (Cu), particularly around the energy region of approximately 0.9 keV and 8.0 keV, which are characteristic signals of copper atoms. The high intensity of the copper peak indicates that copper is

the major constituent element present in the synthesized nanoparticles. The highest weight percentage copper of approximately 74.9% and an atomic percentage of 80.7%, confirming the successful reduction of  $\text{Cu}^{2+}$  ions into metallic copper nanoparticles [Graph 2]. Minor peaks corresponding to zinc (Zn), nickel (Ni), scandium

(Sc), molybdenum (Mo), and lanthanum (La) were also observed in the spectrum. Zinc was detected with a weight percentage of 2.4% and an atomic percentage of 2.5%, while nickel showed 1.2 wt.% and 1.4 wt.%<sup>49</sup>. These trace elements may originate from instrumental background signals, sample holder contamination, precursor impurities, or environmental contamination during sample preparation and analysis. The presence of molybdenum (Mo) with a relatively higher weight percentage of 18.7% could be attributed to the metallic sample holder or coating material used during SEM-EDS analysis. Lanthanum and scandium peaks may arise from instrumental components or substrate materials rather than the synthesized nanoparticles themselves. The EDS results clearly demonstrate that copper is the

predominant element in the synthesized sample, indicating successful synthesis of copper nanoparticles through the green synthesis approach using *Citrus sinensis* peel extract<sup>50</sup>. The absence of significant unwanted impurity peaks further confirms the relatively high purity of the synthesized nanoparticles. The elemental composition obtained from EDS analysis supports the findings of UV-Visible spectroscopy, FTIR, XRD, and SEM characterization techniques. While SEM provided information regarding surface morphology and particle shape, EDS analysis confirmed the elemental composition and purity of the nanoparticles. Therefore, EDS served as an important complementary characterization technique in validating the successful biosynthesis of copper nanoparticles.



Graph 2: EDS Analysis of Citrus *sinensis* peel extract

3.6 EDS Parameters

The results indicate that copper (Cu) is the major element present in the synthesized material, with a weight percentage of 74.9% and an atomic percentage of 80.7%. The high net intensity value of copper further confirms its dominant presence, indicating the successful synthesis of copper-based nanoparticles. The low error percentage (5.3%) associated with copper suggests high reliability and accuracy of elemental detection. Molybdenum (Mo) is the second major constituent, showing a weight percentage of 18.7% and an atomic percentage of 13.4%. The significant presence of molybdenum suggests its successful incorporation into the nanoparticle matrix, potentially contributing to enhanced catalytic activity, structural stability, and

electronic properties of the synthesized nanomaterial. Zinc (Zn) is present at 2.4 wt.%, confirming the incorporation of zinc species within the sample. The coexistence of copper and zinc may indicate the formation of mixed-metal or composite nanoparticle systems with improved physicochemical properties. Lanthanum (La), nickel (Ni), and scandium (Sc) are present in smaller quantities with weight percentages of 2.0%, 1.2%, and 0.7%, respectively. These trace elements may enhance surface reactivity, thermal stability, and catalytic performance of the nanoparticles. The comparatively higher error percentages observed for Sc, Ni, and La indicate their presence in lower concentrations within the sample [Table 1].

Table 1: EDS Parameters of Citrus *sinensis* peel extract

| Element | Weight % | MDL  | Atomic % | Net Int. | Error % | R      | A      | F      |
|---------|----------|------|----------|----------|---------|--------|--------|--------|
| Sc K    | 0.7      | 1.16 | 1.0      | 2.2      | 64.4    | 0.8474 | 0.7813 | 1.0667 |
| Ni K    | 1.2      | 2.03 | 1.4      | 2.2      | 64.0    | 0.8885 | 0.9515 | 1.1706 |
| Cu K    | 74.9     | 2.94 | 80.7     | 95.9     | 5.3     | 0.8955 | 0.9607 | 1.0496 |
| Zn K    | 2.4      | 3.19 | 2.5      | 2.5      | 23.1    | 0.9031 | 0.9574 | 1.0576 |
| Mo L    | 18.7     | 2.44 | 13.4     | 25.6     | 15.4    | 0.8219 | 0.4915 | 1.0049 |
| La L    | 2.0      | 4.49 | 1.0      | 1.7      | 60.5    | 0.8609 | 0.8538 | 1.0454 |

### 3.7 Elemental Mapping Analysis

The SEM-EDS elemental mapping analysis was performed to investigate the elemental composition and spatial distribution of the synthesized nanoparticles<sup>33</sup>. The presence of multiple metallic elements distributed across the sample surface is confirmed by Figure 6. In the elemental map, magenta represents molybdenum (MoL), cyan indicates scandium (ScK), green corresponds to lanthanum (LaL), yellow represents nickel (NiK), brown/orange indicates copper (CuK), and blue corresponds to zinc (ZnK)<sup>5,48</sup>. The black background represents areas with no detectable elemental signal, while the scale bar of 10  $\mu\text{m}$  indicates micrometre-scale analysis. The elemental mapping

reveals a heterogeneous but well-dispersed distribution of metallic constituents throughout the sample. Molybdenum signals appear dominant and widely distributed, indicating successful incorporation within the nanoparticle matrix<sup>49</sup>. Copper and zinc signals are also clearly observed, confirming the formation of Cu–Zn-based nanostructures. Partial overlap between copper and zinc regions suggests possible composite formation or interfacial interaction between the metallic components. Nickel, lanthanum, and scandium signals are comparatively weaker, indicating their presence in trace or moderate amounts<sup>50</sup>. These elements may contribute to improved catalytic activity, structural stability, and surface reactivity of the nanoparticles.

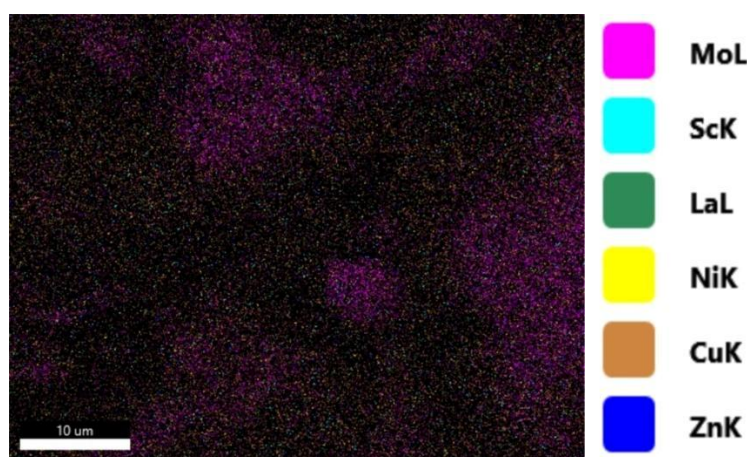
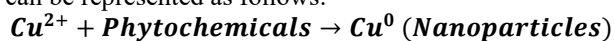


Figure 6: Elemental Mapping Analysis of Citrus *Sinensis* peel extract

## IV. MECHANISM, APPLICATIONS, AND ADVANTAGES OF GREEN SYNTHESIZED COPPER NANOPARTICLES

The green synthesis of copper nanoparticles using Citrus *sinensis* peel extract involves the reduction of  $\text{Cu}^{2+}$  ions into metallic copper nanoparticles through the action of phytochemicals such as flavonoids, phenolic compounds, terpenoids, and ascorbic acid<sup>5,51</sup>. These bioactive compounds donate electrons to copper ions, leading to nanoparticle formation, while proteins and polysaccharides present in the extract act as natural stabilizing and capping agents that prevent aggregation and enhance nanoparticle stability. The synthesis process can be represented as follows:



Green-synthesized copper nanoparticles possess diverse applications due to their unique physicochemical and biological properties<sup>29</sup>. They exhibit strong antimicrobial activity against bacteria and fungi by disrupting microbial cell membranes and generating reactive oxygen species. Additionally, copper nanoparticles are widely used as catalysts in oxidation and reduction reactions, photocatalytic degradation of dyes, and wastewater treatment processes. In the biomedical field,

they show promising applications in drug delivery, wound healing, antioxidant activity, and cancer therapy<sup>16</sup>. Copper nanoparticles are incorporated into textile materials to produce antimicrobial fabrics and are also utilized in sensors, electronics, and energy-related applications<sup>5</sup>. Green synthesis offers several advantages over conventional physical and chemical synthesis methods. It is an eco-friendly, cost-effective, and energy-efficient approach that avoids the use of hazardous chemicals and toxic reducing agents<sup>16</sup>. The process is simple, rapid, and suitable for large-scale industrial applications. The utilization of agricultural waste, such as orange peel, contributes to sustainable waste management and environmental protection<sup>30</sup>. Green synthesized nanoparticles are generally more biocompatible and safer for biomedical applications due to the absence of harmful chemical residues, making green nanotechnology an important approach for sustainable scientific development.

## V. CONCLUSION AND FUTURE PERSPECTIVES

The present study successfully demonstrated the green synthesis of copper nanoparticles using Citrus *sinensis* peel extract as a natural reducing and stabilizing

agent. The phytochemicals present in orange peel, including flavonoids, phenolic compounds, terpenoids, and ascorbic acid, played a significant role in the reduction and stabilization of copper nanoparticles. The synthesis process was found to be simple, eco-friendly, cost-effective, and sustainable. Successful nanoparticle formation was confirmed through visible color change and characterization techniques such as UV-Visible spectroscopy, FTIR, XRD, SEM, and EDS analysis, which verified the optical properties, functional groups, crystalline structure, morphology, and elemental composition of the synthesized nanoparticles. The study also highlighted the effective utilization of agricultural waste for advanced nanotechnology applications. The synthesized copper nanoparticles possess promising potential in antimicrobial, catalytic, environmental, biomedical, textile, and industrial applications. Future research may focus on optimization of synthesis conditions, evaluation of antimicrobial and anticancer activities, toxicological and biosafety studies, wastewater treatment applications, large-scale industrial production, and development of nanoparticle-based drug delivery systems to further expand the practical applications of green-synthesised copper nanoparticles.

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