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To cite this article: K. Mrudweeka Balu *et al* 2026 *J. Phys.: Conf. Ser.* **3246** 012038

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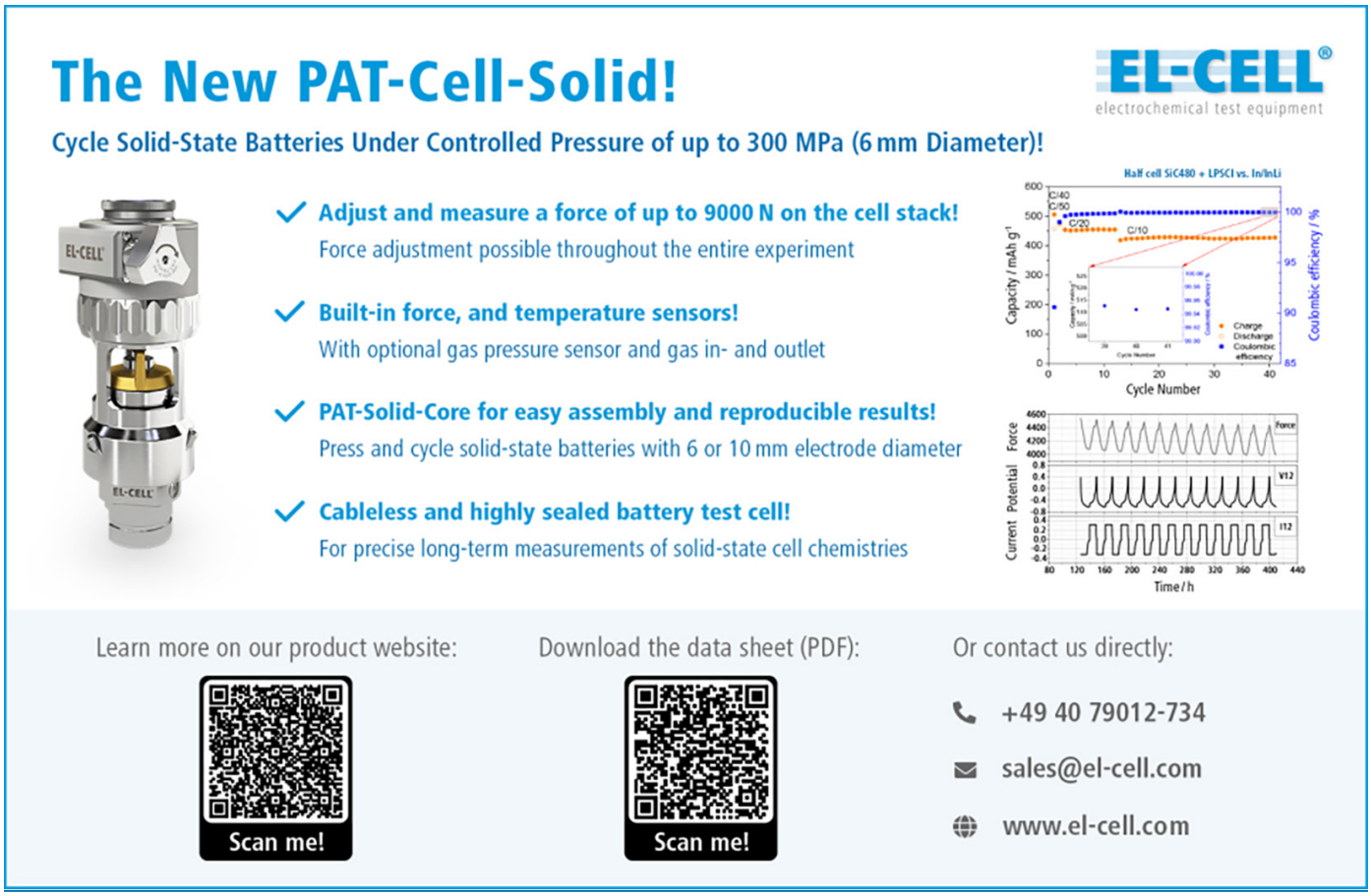

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Journal of Physics: Conference Series **3246** (2026) 012038 doi:10.1088/1742-6596/3246/1/012038

Forensic Utility of CuO Nanoparticles Synthesized via *Cucurbita pepo* Seed Mediated Green Approach for Latent Fingerprint Development

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Abstract. Copper oxide (CuO) nanoparticles were efficiently synthesized by a green synthesis method using pumpkin seed extract. XRD characterisation of the synthesised CuO NPs indicated a crystalline nature, with sharp diffraction peaks corresponding to nanoscale crystallite sizes that matched well with standard JCPDS patterns. Rietveld refinement presented nanoparticles that crystallized in the monoclinic phase with a space group of C2/c, which belongs to the standard tenorite CuO phase. The average crystallite size of CuO NPs, calculated using the Scherrer formula, was 31.39 nm. Besides, the crystallite size determined by the Williamson-Hall method was 37.78 nm for CuO NPs. Further, the forensic applicability of the prepared nanoparticles was assessed using latent fingerprint development studies. Well-defined ridge patterns of enhanced contrast were obtained on both porous and non-porous substrates, which showed that the nanoparticles are efficient fingerprint visualisation agents. These results make pumpkin seed-mediated CuO NPs an eco-friendly and efficient nanoparticle with potential forensic applications.

Keywords: Nanotechnology, green chemistry, CuO nanoparticles, Pumpkin seeds, XRD, latent fingerprint development.

1. Introduction

Nanotechnology is an emerging basic area of interdisciplinary, where the principles of matter engineering are applied to dimensions from 1 to 100nm, where the physicochemical properties are fundamentally different from bulk systems regarding matter size [1]. At this scale, high surface-to-volume ratio, modifications in the electronic structure, and high surface activity impact unique optical, catalysis, and interface properties, providing key applications to several areas of expertise [2].

Out of the broad spectrum of nanomaterials, metal oxide nanoparticles (NPs) remained an area of keen interest owing to their stability, controllable electronic properties, and multifunctional characteristics [3]. CuO (copper oxide) is a p-type semiconducting metal oxide exhibiting a narrow-band gap in the range of 1.2–1.5 eV, has emerged as a particularly attractive material owing to its catalytic activity, antimicrobial properties, chemical robustness, and strong surface

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interaction capability. P-type semiconducting metal oxide - CuO (Copper Oxide), with its low band gap of 1.2-1.5 eV, gained prominence because of its catalysis property, anti-microbial characteristics, resistance to chemicals, and the ability to show strong interactions at the surface level [4,5]. In its nanoscale form, the properties of CuO get improved since its physical and material properties are superior to those of its bulk form, copper oxide, because of the greater number of surface sites [6,7].

The traditional synthesis procedures of CuO nanoparticles involve harsh reaction conditions, dangerous chemicals, and a large energy requirement [8]. This increases the concerns about environmental effects, occupational health, and biocompatibility. This has triggered a growing need for green synthesis techniques, which currently agree with the principles of green chemistry, reducing toxic materials and energy, and thus encouraging sustainability [9].

Biological entities like plant extracts, microorganisms, and biomolecules have been used in green nanoparticle synthesis. They play the major role of reduction and stabilization to enable the easy formation of nanoparticles under reduced conditions [10]. In these methods, especially in plant-mediated synthesis, the availability of an ideal set of phytochemicals in the plant matrix, like polyphenols, flavonoids, terpenoids, proteins, and polysaccharides, acts together to reduce metal ions and control the stabilization of the newly formed nanoparticles without resorting to the use of capping agents [11]. It has also been shown by various studies that plant-mediated CuO NPs have an ideal crystalline structure, nanosize, and uniformly corresponding physicochemical values as indicated by XRD and electron microscopy analyzes [10,12].

The seeds of *Cucurbita pepo,* also known as pumpkin seeds (family *Cucurbitaceae*), represent a promising biological resource for green synthesis of nanoparticles. *Cucurbita pepo* is widely cultivated and its seeds are known to be rich in proteins, unsaturated fatty acids, carbohydrates, and bioactive phytochemicals with antioxidant properties [13]. The biochemical composition of these seeds provides a promising environment for metal ion reduction and stabilization of nanoparticles, making them suitable for sustainable nanomaterial synthesis [14]. The utilization of seed extracts also contributes to agricultural waste utilization, further reinforcing the environmental relevance of this approach.

Similar to advances in sustainable nanomaterial synthesis, forensic science has increasingly incorporated nanotechnology to enhance the visualization of trace evidence [15]. Latent fingerprints, created by the transfer of sweat and sebaceous secretions from friction ridges present on the skin, remain one of the most reliable and widely used means of personal identification in crime investigations. However, visualizing the latent fingerprints, which is mainly challenging on substrates with and without porosity, continues to present practical difficulties due to low contrast, background interference, and surface dependency [16]. Traditional fingerprint developing methods utilize conventional fingerprint powders that are made of chemicals, which may have limitations regarding their sensitivity or could be harmful to the environment owing to their toxic ingredients [17]. Nanoparticle fingerprint powders are more advantageous because of their smaller size and high surface energy, resulting in increased adherence to the organic as well as the inorganic material present in fingerprint residues [18]. Recently, the use of green-synthesized nanoparticles has been shown to be beneficial for fingerprint development, providing superior forensic capabilities without harming the environment [19,20]. In such regard, the use of green-synthesized CuO nanoparticles from the seed extract of Cucurbita pepo provides an innovative method that combines environmentally friendly nanoscience with the practical applications of forensic science in the detection of latent fingerprints.

As a result, the current research aims to utilize green CuO NP synthesis methodologies based on Cucurbita pepo seed extracts and analyze their usages for latent fingerprint detection. The current study hopes to make a positive contribution to green nanomaterial synthesis and advanced eco-friendly approaches for fingerprint visualization technique development.

2. Materials and Synthesis

2.1 Materials required

Materials used are 99% Extra pure cupric nitrate trihydrate (II) ($Cu(NO_3)_2 \cdot 3H_2O$), citric acid ($C_6H_8O_7$), Ethylene Glycol ($C_2H_6O_2$), Ammonia (NH3), Double distilled water, Grade 1 Whatman filter paper.

2.2 Pumpkin seeds extraction

Pumpkin seeds (Cucurbita pepo) were first roasted on a hot plate and followed by milling into a fine powder. In a beaker, 25g of the powdered pumpkin seeds was added to 100 ml of deionized water and maintained on a magnetic stirrer at 60°C for 30 minutes to facilitate the extraction of phytochemicals into the solution. After the extraction process, the mixture was filtered using Grade 1 Whatman filter paper, and 40 ml of the obtained extract was collected. This extract was then mixed with the precursor metal compound solution in a 1:4 ratio, which has been identified as optimal for the formation of the desired nanoparticles.

2.3 CuO nanoparticles synthesis

CuO NPs were synthesized via green synthesis method through pumpkin seeds extract (Cucurbita pepo) which act as a natural reduction and stabilizing agent. Cupric nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) served as the starting material (precursor). In a beaker, $Cu(NO_3)_2 \cdot 3H_2O$ corresponding to a 1 M solution, was dissolved in double-distilled water of 100 ml while being stirred magnetically at 350-450 rpm until the compound completely dissolved. To this solution, 40 ml of pumpkin seed extract was added and stirred for 10-20 minutes to ensure uniform mixing. Separately, Citric acid solution (100 ml) was added to the mixture, where it acted as a chelating agent to stabilize copper ions, control particle size, and enhance homogeneity. The solution was stirred for an additional 20-30 minutes, after which the pH was regulated at 9 using ammonia (pH is adjusted to 9 to create alkaline conditions that promote rapid reduction of metal ions, enhance nucleation, and stabilize the formed nanoparticles, resulting in smaller and more uniform particles). The mixture was then heated with continuous stirring until its volume decreased to roughly one-third. Subsequently, ethylene glycol was added, acting as the solvent, stabilizer, and fuel for influencing nanoparticle morphology. The mixture was left overnight to facilitate gelation by maintaining it at room temperature. The solid-formed gel was gathered in a porcelain crucible, which was then combusted with the help of a burner. This led to the acquisition of a fine powder that was ground for 15 minutes to remove any lumps. The powder was then calcined in the muffle furnace for 2 hours within the temperature range of 600-650 °C in an inert atmosphere to form CuO (Calcination is limited to ~600 °C because it is sufficient to remove organic residues and form crystalline metal oxide nanoparticles, while higher temperatures cause particle growth and loss of nanoscale properties). The calcined product was reground for 15 minutes to achieve a uniform fine powder. The synthesized CuO nanoparticles were subsequently characterized. **Figure 1** shows the synthesis of CuO NPs.

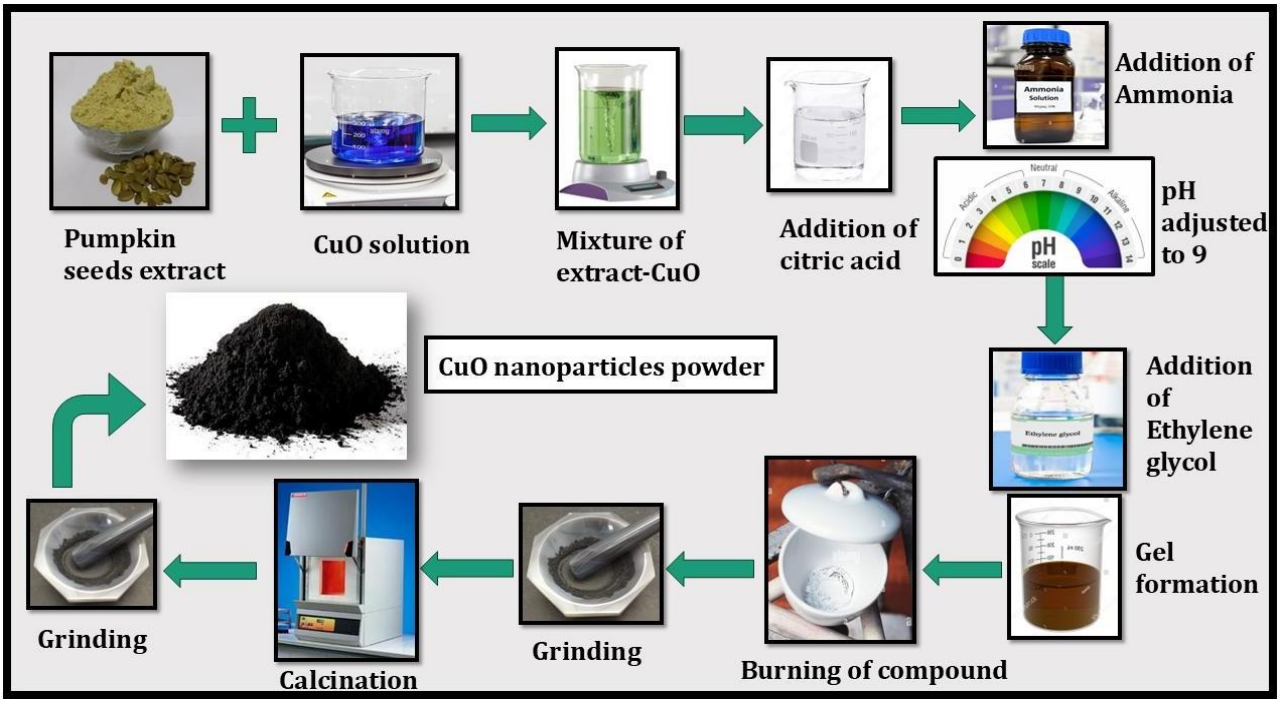

Figure 1. Schematic flow chart depicting synthesis of CuO NPs

2.4 CuO nanoparticles Characterization and Application

To verify the successful production of CuO nanoparticles, a series of structural and optical characterizations were performed. The crystalline phase was analyzed using X-ray diffraction (XRD) on a Rigaku D/MAX 3B diffractometer with a copper target, employing Cu Kα radiation (λ = 1.54056 A˚) as the source. The practical applicability of the synthesized CuO nanoparticles in forensic investigations, particularly for detection of latent fingerprints, was also evaluated. The performance of CuO nanoparticles as a fingerprint development powder was compared with conventional black powder.

3. Results and Discussion

3.1 XRD (X-Ray Diffraction) analysis

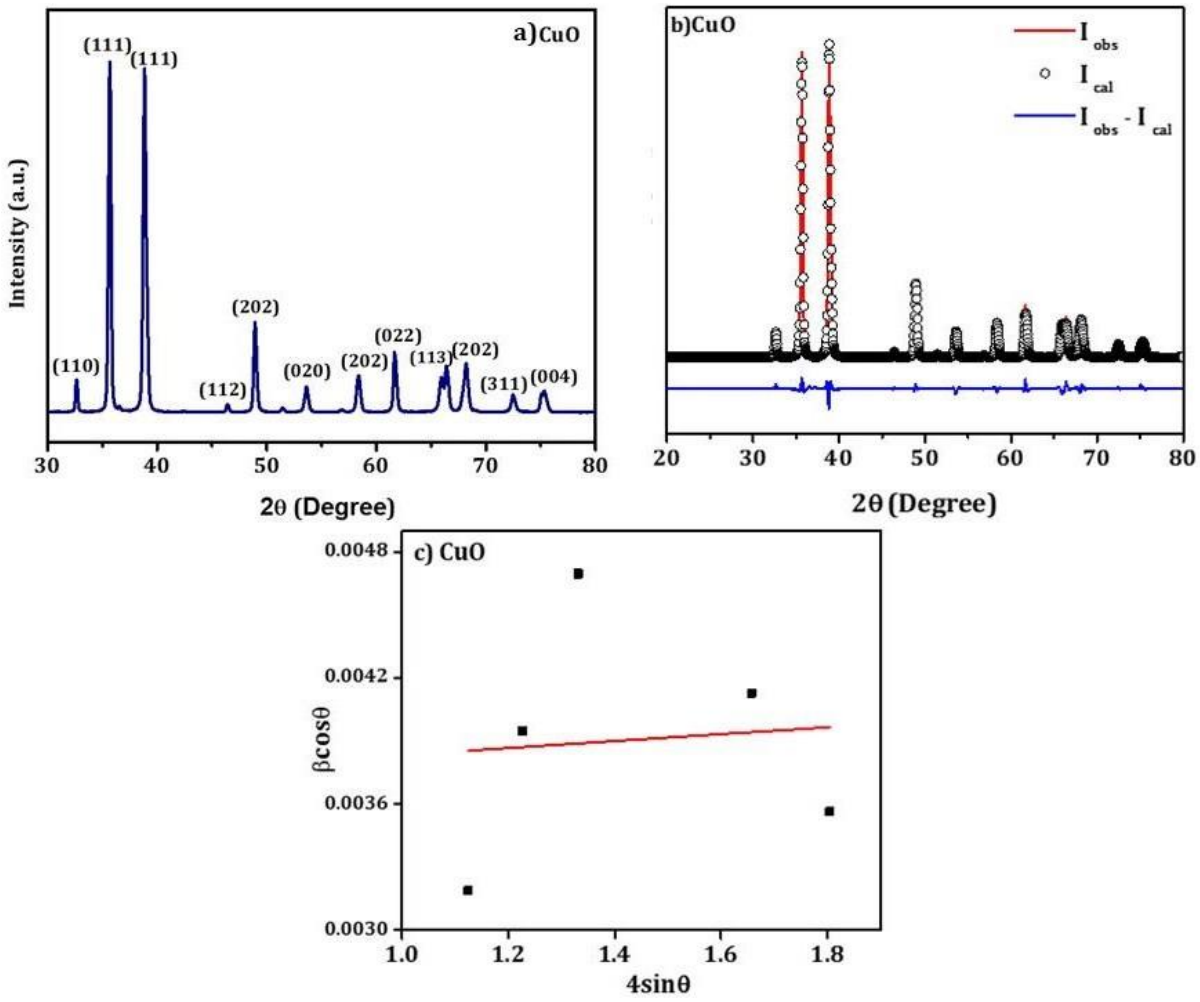

Figure 2. (a) XRD pattern, (b) Rietveld refinement and, (c) W-H plots of synthesized CuO NPs

The phase confirmation, crystallite size and crystallinity of CuO NPs synthesized through the green route were analyzed by XRD. **Figure 2(a)** shows the sharp XRD peaks indicating the crystalline nature of CuO NPs. The XRD pattern exhibits diffraction peaks indexed to the (110), (-111), (111), (112), (202), (020), (022), (113), (-202), (311), (004) planes, which are in accordance with standard JCPDS patterns (No. 45-0937) data for monoclinic CuO. The lack of impurity peaks demonstrates the single-phase of the synthesized CuO nanoparticles. The enhanced intensity of the (−111) and (111) reflections indicate a preferred orientation along these crystallographic directions [21].

Full Prof software was used to carry out Rietveld refinement on the XRD data to investigate the crystallographic features of the green-synthesized CuO nanoparticles. The refinement results

confirm that the CuO NPs crystallize in a monoclinic structure with the C2/c space group, characteristic of the tenorite CuO phase (shown in **Figure 2(b)**). The refined lattice constants were obtained as a = 4.6871 A˚ , b = 3.4266 A˚ , and c = 5.1336 A˚ , with lattice angles α = 90°, β = 99.37°, and γ = 90°, where the deviation of the β angle from 90° validates the monoclinic symmetry.

A strong correspondence between the observed (I_{obs} and calculated (I_{cal}) diffraction intensities is evident across the entire 2θ range, while the difference profile ($I_{obs} - I_{cal}$) remains minimal. The absence of unindexed reflections confirms high phase purity and structural uniformity of the CuO nanoparticles. Furthermore, the smooth background fitting and low residual values indicate the reliability of the refinement process. These observations are in agreement with recent reports on monoclinic CuO nanoparticles synthesized via green and solution-based methods, confirming the formation of single-phase, well-crystallized CuO suitable for subsequent functional applications [22].

The CuO particle size at the crystalline level was determined by the Debye-Scherrer equation-

$$D = \frac{\lambda}{\beta \cos\theta} \quad (1)$$

where D represents the crystallite size, λ denotes X-ray wavelength, k is the shape factor (0.94), β denotes full width at half maximum (FWHM), and θ is the Bragg's angle. The CuO crystallites were determined to have an average size of 31.39 nm [23].

To consider the effect of lattice strain, the Williamson Hall (W–H) method was performed,

$$Cos\theta\ \beta = \frac{K\lambda}{D} + \varepsilon(4\sin\theta) \quad (2)$$

where ε denotes the microstrain. In this approach, a plot of βcosθ against 4sinθ yielded a linear relationship, with the intercept providing the crystallite size and the slope representing the strain. The obtained crystallite size from W-H is 37.78 nm, whereas the lattice strain was derived from the slope, which is 0.000164, shown in **Figure 2(c)**. Crystallite sizes determined from W-H analysis were slightly higher compared to the Scherrer method calculation, likely due to lattice distortions and minor particle aggregation induced by phytochemicals in the pumpkin extract.

3.2 Forensic Application-Latent fingerprint development

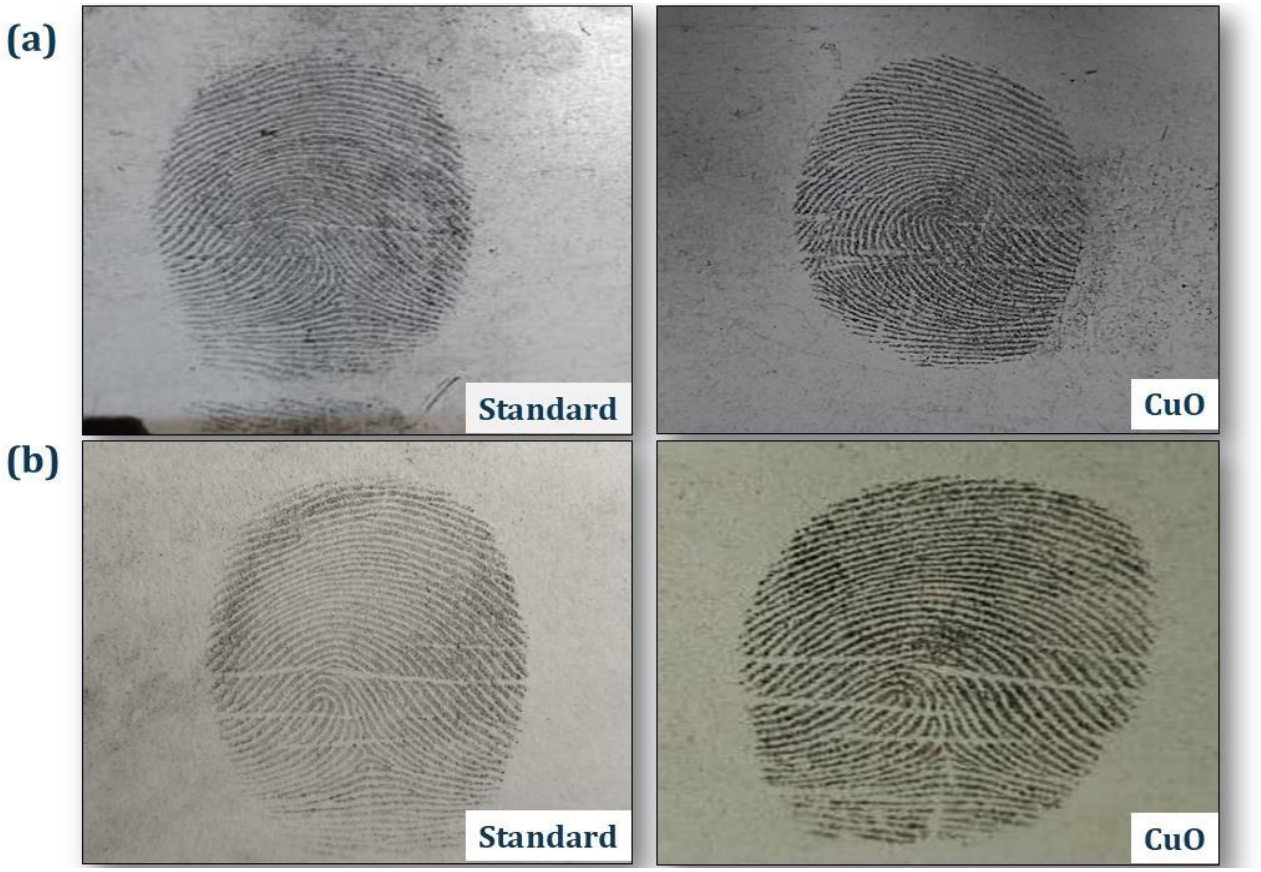



Figure 3. Latent fingerprints development using CuO NPs on non-porous (a. switch board, b. sun board) and porous (c. paper, d. wall) surfaces

Latent fingerprints are the invisible marks left by touch when sweat, oils, and residues from the skin deposit on a surface. They are not visible with the unaided eye, so special visualization methods are needed to make the fingerprints visible. Later the prints can be photographed and compared for the identification. Traditional methods include powders and chemical reagents, which can be limited in sensitivity, surface compatibility, or safety. Nanoparticles have been emerged as a promising alternative due to their enhanced surface properties and interaction with fingerprint residues [18,24].

CuO nanoparticles have several unique features that make them useful for the development of latent fingerprints. They have a high surface area which allows them to bind with lipids and amino acids present in the residues. The functional groups on CuO nanoparticles surface can form electrostatic and hydrogen bonds with the organic materials in fingerprints [25].

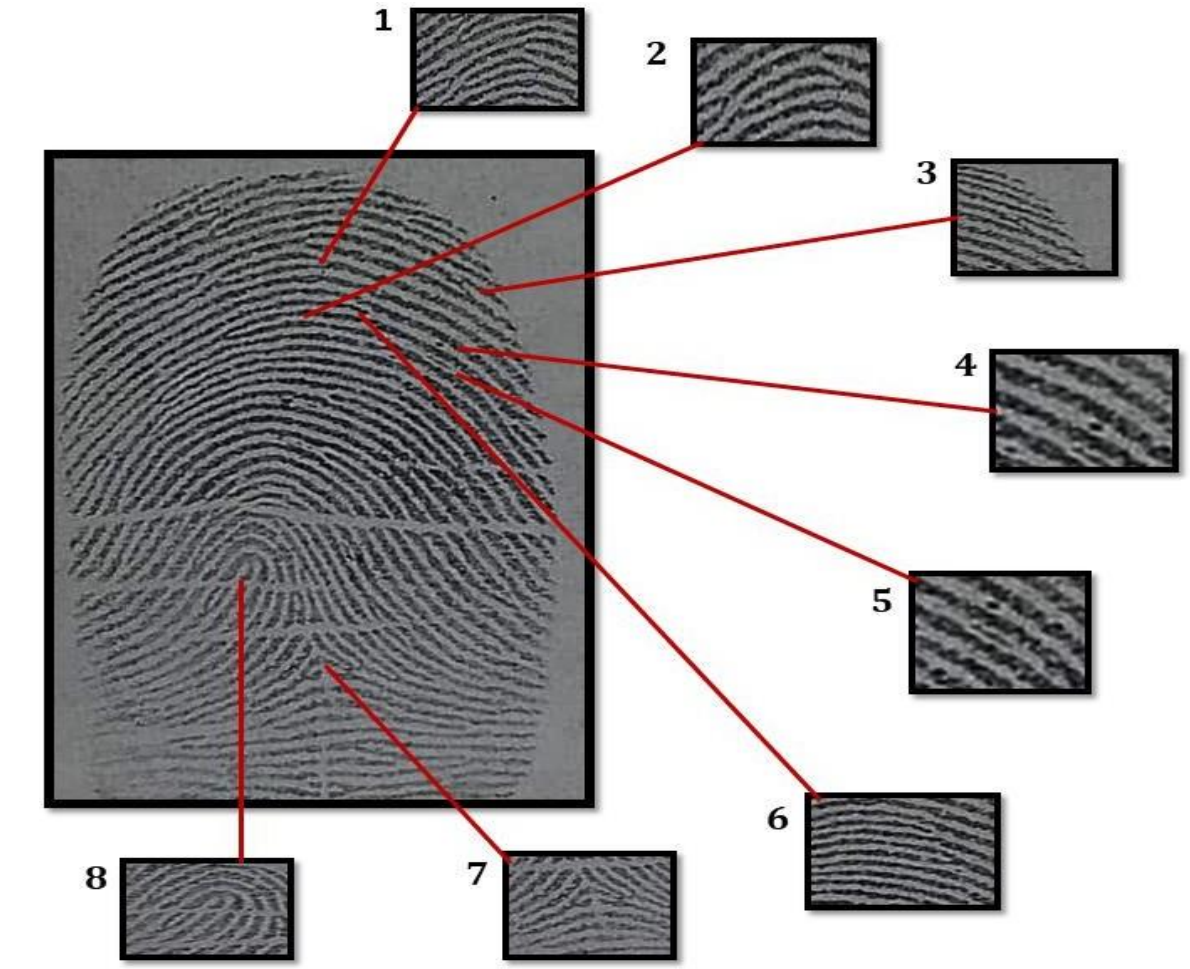

Figure 4. Minutiae characteristics of developed Fingerprint using CuO NPs

In the present study, fingerprints of a volunteer were collected on different porous and non-porous substrates such as switchboard, sun board, paper and wall. Then, synthesized CuO nanoparticles powder is lightly dusted on the latent fingerprints present on both porous and non-porous surfaces using a brush. The CuO powder adhered to ridge residues, and the stained fingerprints were photographed. Later, analyzed for the ridge characteristics of the fingerprints. The CuO NPs powder synthesized via *Cucurbita pepo* was black in color. **Figure 3** shows the comparison photographs of CuO NPs Powder and traditional black powder.

Copper (II) oxide NPs demonstrated noticeably improved performance when compared with conventional black powder on surfaces with and without porosity. CuO nano powder showed stronger and more selective adhesion to the fingerprint ridge residues, resulting in clearer ridge impressions with minimal background staining. Ridge characteristics such as core, delta,

bifurcation, enclosure, dot, island, bridge and ridge endings were clear without using any illumination sources for the fingerprints developed by CuO nanoparticles powder (**Figure 4**).
Several studies have explored CuO NPs for latent fingerprint development. Renuka et al [26], reported successful development of latent fingerprints using ZrO_2/CuO nanocomposite powder on both porous and non-porous surfaces; however, the ridge enhancement was achieved through the ultraviolet (UV) illumination at 254 nm. But, in the present study without any use of UV, the ridge characteristics came out clear.
Bhagat et al. [27] employed green-synthesized, biomolecule-capped CuO nanoparticles and reported high-quality ridge detail on selected non-porous surfaces, with fingerprints visible under natural light. Atri et al. [28], investigated rectangular CuO nanosheets, observed sharp ridge patterns and excellent reproducibility on multiple non-porous substrates. Similarly, Dubey et al. reported the use of CuO nanorods for visualizing fingerprints on smooth, non-absorbent surfaces like glass and steel, where high contrast and clean ridge patterns were achieved. Unlike several earlier reports that focused predominantly on non-porous materials, the present CuO sample exhibited effective ridge adherence and contrast even on porous surfaces, indicating broader applicability.
Grover et al. [29] reported SiO_2/CuO nanocomposite demonstrated excellent ridge clarity on a variety of non-porous surfaces. However, the method was not successful on porous substrates such as paper, where fingerprint development failed. But, in the present study, clear ridge patterns with well-defined level 1 characteristics and visible level 2 minutiae were observed on both permeable and impermeable surfaces.
Overall, in the present study, CuO nanoparticles have the capability to develop fingerprints on both porous and non-porous substrates without UV assistance, while maintaining good ridge clarity and minimal background interference, which highlights the useful advantage of the present CuO nano powder for routine forensic applications.

Conclusion

In the present study, copper oxide (CuO) NPs were successfully prepared using *Cucurbita pepo* (pumpkin) seed extract through a green, eco-friendly synthesis route and thoroughly evaluated for latent fingerprint detection. Structural analyzes, including XRD and Rietveld refinement, validated the formation of single-phase, well-crystallized monoclinic CuO with lattice parameters matching standard tenorite, indicating high crystallinity and phase purity, highlighting the success of this sustainable synthesis route. These nanoparticles, when applied to latent fingerprint detection, on both non-porous and porous substrates, provided clean ridge details with high contrast and minimal background noise, allowing for clear viewing of critical minutiae such as delta, core, bifurcations, and ridge endings without any illumination source. These results show that the *Cucurbita pepo*-mediated CuO nanoparticles can be used in an environmentally friendly, cost-effective, and highly efficient substitute for classical fingerprint powder and thus have a great potential in real-life forensic applications.

Acknowledgements

The authors take this opportunity to thank with gratitude the Head, Department of Sciences & Humanities, the Principal, and the Management of Matrusri Engineering College (Autonomous), Saidabad, Hyderabad, Telangana, India, not only for the facilities provided but also for the financial support given to perform this research work.

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