

Green Synthesis and Characterization of Copper Nanoparticles Using Blueberry Ethanolic Extract

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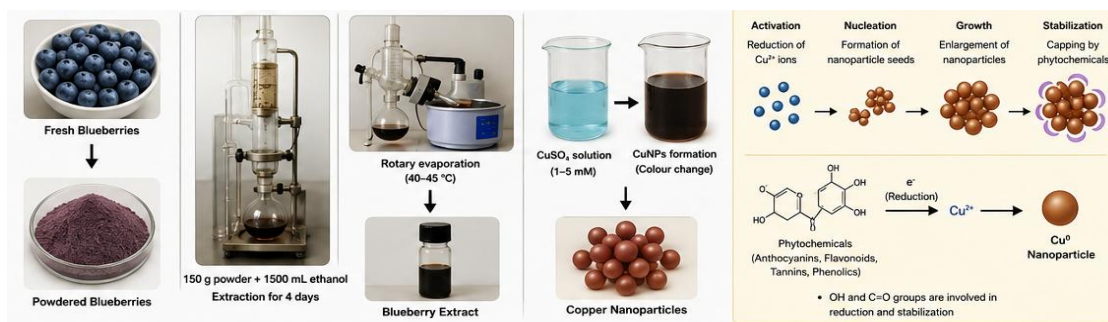
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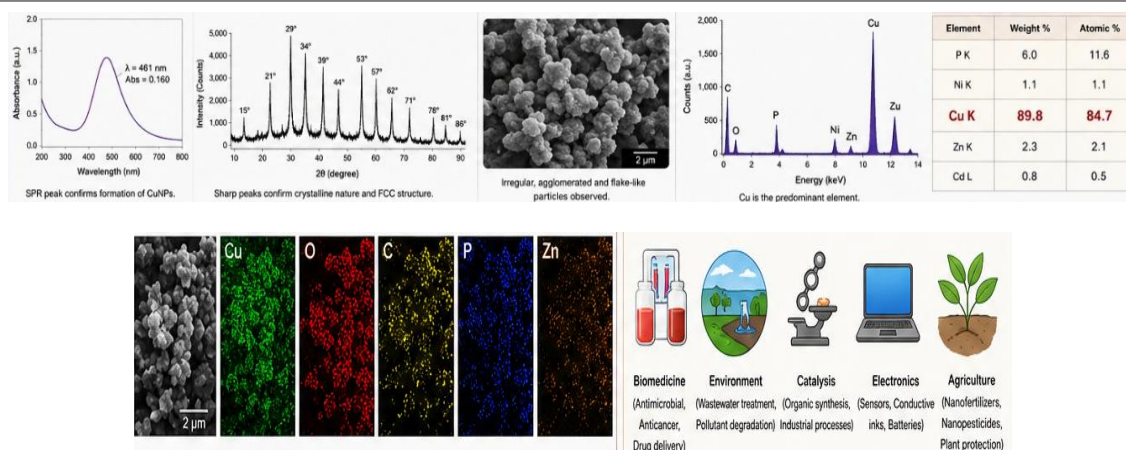
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ABSTRACT

The present study investigates the green synthesis and characterization of copper nanoparticles (CuNPs) using ethanolic extract from blueberries (*Vaccinium* spp.) as a natural reducing and stabilizing agent. Given the unique physicochemical properties of nanoparticles, CuNPs have gained attention for their electrical conductivity, catalytic efficiency, antimicrobial activities, antioxidant properties, and economic viability. Traditional synthesis methods of CuNPs are often fraught with toxic chemicals and environmental risks, highlighting the need for eco-friendly alternatives. In this research, fresh blueberries were dried, powdered, and extracted using ethanol to obtain a bioactive extract rich in anthocyanins, flavonoids, tannins, and phenolic compounds. This extract was employed to reduce copper sulfate solution into CuNPs, indicated by a color change from light blue to dark brown due to surface plasmon resonance (SPR). Characterization of the synthesized nanoparticles included UV-Visible spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), and elemental mapping. UV-Visible spectroscopy confirmed the formation of nanoparticles through characteristic SPR peaks, while XRD established their crystalline nature and face-centered cubic (FCC) structure. SEM revealed an agglomerated and irregular morphology of the nanoparticles, and EDS confirmed high-purity copper as the dominant element. The study concludes that using blueberry extract for CuNP synthesis is a simple, cost-effective, environmentally friendly, and sustainable method, with potential applications across biomedical, environmental, catalytic, agricultural, and industrial fields.





Keywords- Green synthesis, Copper nanoparticles, Blueberry extract, Ethanolic extraction, Nanotechnology, Phytochemicals.

I. INTRODUCTION

Nanotechnology is one of the fastest-growing and most interdisciplinary branches of modern science and technology, dealing with the design, synthesis, characterization, and application of materials at the nanoscale level, generally between 1 and 100 nm. Materials at this scale exhibit unique physicochemical properties that are significantly different from their bulk counterparts due to their high surface area-to-volume ratio, quantum confinement effects, and enhanced surface reactivity¹. These extraordinary properties include improved optical behaviour, catalytic efficiency, electrical conductivity, thermal stability, magnetic characteristics, and biological activity. Because of these remarkable features, nanotechnology has found extensive applications in medicine, pharmaceuticals, agriculture, environmental science, food technology, cosmetics, textiles, electronics, catalysis, and energy storage systems^{2,3}. Among the different categories of nanomaterials, metallic nanoparticles have gained tremendous scientific and industrial importance owing to their exceptional physicochemical and biological properties. Tailoring metal nanoparticles requires a comparative assessment of gold (Au), silver (Ag), copper (Cu), zinc (Zn), platinum (Pt), and iron (Fe) for targeted applications⁴. Metal nanoparticles have been widely investigated for various technological and biomedical applications⁵, paving the way for nanomedicine to revolutionize healthcare through continuous innovation⁶. Advanced nanostructures such as carbon nanotubes have also expanded the horizon of biomedical applications⁷. The utilization of microfluidic devices integrated with nano-sensors has dramatically enhanced the precision of infectious disease detection⁸.

Copper nanoparticles have attracted considerable attention because copper is comparatively inexpensive, abundantly available, and exhibits excellent

electrical conductivity, catalytic activity, antimicrobial potential, antioxidant behaviour, and optical properties⁹. CuNPs are extensively utilized in conductive inks, sensors, catalysts, antimicrobial coatings, biomedical devices, wastewater treatment systems, drug delivery applications, and electronic industries¹⁰. Copper nanoparticles are synthesized through physical and chemical methods such as chemical reduction, thermal decomposition, electrochemical synthesis, laser ablation, microwave irradiation, and microemulsion techniques¹¹. These methods are effective in producing nanoparticles with controlled size and morphology, they suffer from several disadvantages, including high energy consumption, expensive instrumentation, use of hazardous reducing agents and stabilizers, generation of toxic by-products, environmental pollution, and poor biocompatibility¹². The toxic chemicals used during chemical synthesis may limit the biomedical and environmental applications of the synthesized nanoparticles¹³. There is a growing demand for safer, sustainable, and environmentally benign approaches for nanoparticle synthesis. To overcome the limitations associated with conventional synthesis methods, green synthesis approaches have emerged as an eco-friendly and sustainable alternative for nanoparticle production^{14,15}. Green synthesis involves the utilization of biological entities such as plants, algae, fungi, bacteria, and natural biomolecules for the reduction of metal ions into nanoparticles. These biological systems, plant-mediated synthesis, have gained enormous popularity because it is simple, rapid, cost-effective, environmentally friendly, and suitable for large-scale production¹⁶. Plant extracts contain a wide variety of bioactive phytochemicals that can act simultaneously as reducing, capping, and stabilizing agents during nanoparticle synthesis, thereby eliminating the need for toxic chemicals.

Recent advancements in plant-powered nanotechnology have highlighted the efficiency of green synthesis approaches using medicinal flora¹⁷. The therapeutic potential of *Pterocarpus marsupium* as a "green nanofactory" has been widely established through comparative reviews of biosynthesized gold, silver, copper, and zinc nanoparticles¹⁸. Specific characterization of zinc nanoparticles from Bija (*Pterocarpus marsupium* Roxb) leaf extract confirms the highly stable nature of plant-stabilized metallic cores¹⁹. Botanical resources from unique ecological zones, such as the green synthesis of zinc nanoparticles using phenolic acids from edible mushrooms of the Chhattisgarh region, offer excellent reduction matrices²⁰. Beyond zinc, the efficacy of *Swertia chirayita* as a green source for copper nanoparticles has underscored the adaptability of local medicinal flora for copper reduction²¹. Comparative studies on citrus-mediated green synthesis of zinc nanoparticles highlight how specific organic acid distributions direct the final structural properties of metallic nanomaterials²².

Plant-derived phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, phenolic acids, anthocyanins, sugars, proteins, and organic acids possess strong antioxidant and reducing properties. These compounds facilitate the conversion of metal ions into stable nanoparticles through electron donation mechanisms. The biomolecules adsorb onto the surface of nanoparticles and prevent their aggregation, thereby enhancing nanoparticle stability and biocompatibility. The use of plant extracts in nanoparticle synthesis also aligns with the principles of green chemistry by minimizing environmental hazards and reducing energy consumption^{22,23}. Berry fruits have recently attracted significant interest in green nanotechnology due to their exceptionally high antioxidant and phytochemical content²⁴. Various berries, blueberry (*Vaccinium* spp.), is considered one of the richest natural sources of anthocyanins, flavonoids, catechins, tannins, quercetin, chlorogenic acid, and other phenolic compounds^{26,27}. These phytochemicals possess remarkable antioxidant activity and strong reducing capability, making blueberries highly suitable for green nanoparticle synthesis. The hydroxyl and carbonyl functional groups present in blueberry phytochemicals play a crucial role in electron transfer reactions during reduction of copper ions into copper nanoparticles²⁸. These compounds act as natural stabilizing agents by forming a protective layer around the nanoparticles²⁹, thereby preventing oxidation and aggregation. The extraction solvent used for phytochemical isolation significantly influences nanoparticle synthesis efficiency³⁰. Ethanol extraction is considered more effective than aqueous extraction because ethanol efficiently dissolves phenolic compounds, flavonoids, and antioxidant biomolecules³¹. Ethanol extracts generally contain higher concentrations of phytochemicals, resulting in improved reducing efficiency, better nanoparticle stability, smaller

particle size, and enhanced uniformity. Ethanol is relatively safe, biodegradable, and environmentally acceptable, making it suitable for green synthesis applications³². To broaden the safety and delivery profiles of such plant-derived agents, the development of polysaccharide-based nanocarriers has emerged as a paradigm shift in biopharmaceutical transport, particularly for the oral delivery of proteins and peptides³². Incorporating these natural matrices ensures that the synthesized metallic systems retain exceptional biocompatibility.

In the present study, copper nanoparticles are synthesized using the ethanolic extract of blueberry through a plant-mediated green synthesis approach. The blueberry extract serves as a natural reducing and stabilizing agent during nanoparticle formation. The synthesis process is monitored visually through color change associated with surface plasmon resonance (SPR) phenomenon. The synthesized copper nanoparticles are characterized using advanced analytical techniques, including UV-Visible spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive Spectroscopy (EDS), and elemental mapping to investigate their optical, structural, crystalline, morphological, and elemental properties. The present research contributes toward the development of sustainable nanotechnology by demonstrating an economical, eco-friendly, and efficient method for the synthesis of copper nanoparticles. The synthesized CuNPs may possess significant potential for applications in antimicrobial agents, environmental remediation, catalysis, biomedical sciences, agriculture, and nanotechnology-based industrial systems. Furthermore, the study highlights the importance of plant-based green synthesis approaches as promising alternatives to conventional nanoparticle production techniques.

II. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh and mature 1kg blueberries (*Vaccinium* spp.) were procured from a certified local market to ensure the quality and authenticity of the plant material. The fruits were visually inspected to eliminate damaged, diseased, or over-ripened samples. Selected blueberries were thoroughly washed under running tap water to remove surface dust, soil particles, and other adhering impurities, followed by rinsing with distilled water to eliminate any remaining contaminants. The cleaned blueberries were then shade-dried at room temperature for 7–10 days under hygienic conditions to preserve thermolabile phytochemicals such as anthocyanins, flavonoids, and polyphenols³³. Direct exposure to sunlight was avoided to prevent degradation of bioactive compounds. The dried blueberries were subsequently ground into a fine powder using a sterile mechanical

grinder. The powdered material was sieved to ensure uniform particle size and stored in airtight, moisture-free containers at room temperature until further use for ethanolic extraction. This preparation step was essential

to enhance solvent penetration and maximize the extraction efficiency of phytochemicals responsible for the reduction and stabilization of copper nanoparticles²⁵ [Figure 1].

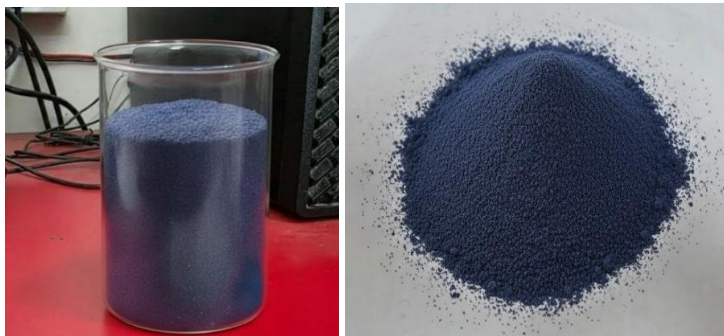


Figure 1: Blueberries in Powdered form

2.2 Preparation of Blueberry Ethanolic Extract

The powdered blueberry material was subjected to Soxhlet extraction using analytical-grade ethanol as the solvent due to its efficiency in extracting polyphenols, flavonoids, and anthocyanins²¹. Approximately 150 g of powdered blueberry material

was loaded into a thimble and placed inside the Soxhlet extractor. 1500 mL of ethanol was used as the extraction solvent. The extraction process was carried out for 4 days until the solvent in the siphon tube became colourless, indicating complete extraction of phytochemicals¹⁹ [Figure 2].



Figure 2: Soxhlet extraction of Blueberry

After extraction, the ethanolic extract was filtered using Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40–45 °C

to remove excess solvent and obtain a semi-solid crude extract [Figure 3]. The concentrated extract was transferred to sterile glass vials and stored at 4 °C for further nanoparticle synthesis²⁵ [Figure 4].



Figure 3: Removal of Solvent from blueberries extract

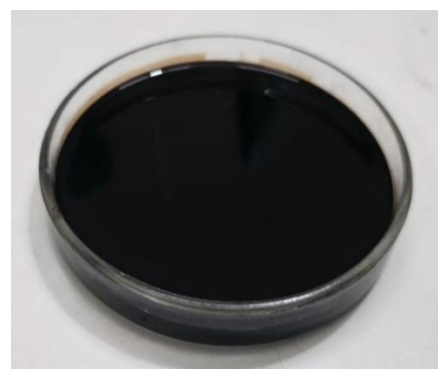


Figure 4: Blueberries extract after removal of solvent

2.3 Green Synthesis of Copper Nanoparticles

Copper nanoparticles were synthesized using a plant-mediated green synthesis approach, following standardized metal salt reduction parameters³⁴. An aqueous solution of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was prepared at a known molarity (typically 1–5M) using distilled water. A measured volume of blueberry ethanolic extract was added dropwise to the copper sulfate solution under constant magnetic stirring at room temperature. The reaction mixture was maintained at 60–80 °C for 1–2 hours to facilitate reduction of Cu^{2+} ions. A visible color change from light blue to dark brown or reddish-brown indicated the formation of copper nanoparticles. The phytochemicals present in the blueberry extract acted as reducing agents, converting Cu^{2+} ions to Cu^0 nanoparticles, and simultaneously served as capping agents, preventing aggregation and enhancing nanoparticle stability [Figure 5].

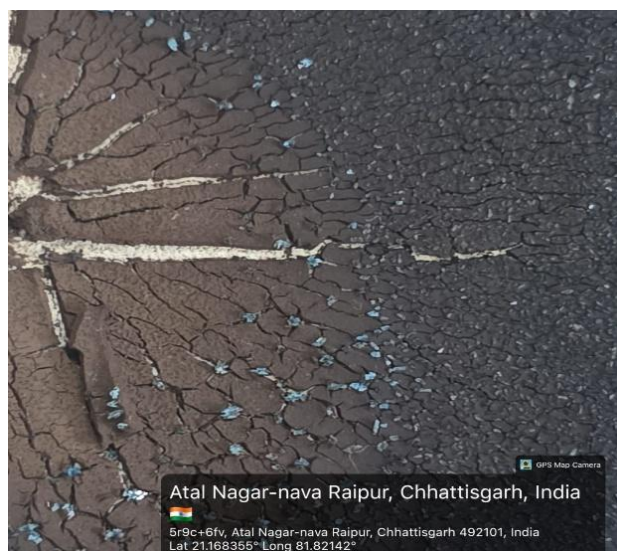
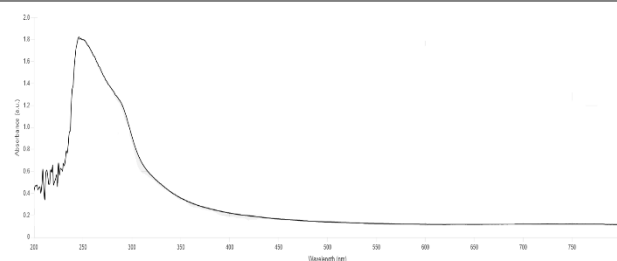


Figure 5: Blueberry Copper nanoparticles

III. RESULTS AND DISCUSSION

3.1 UV-Visible Spectroscopy Analysis

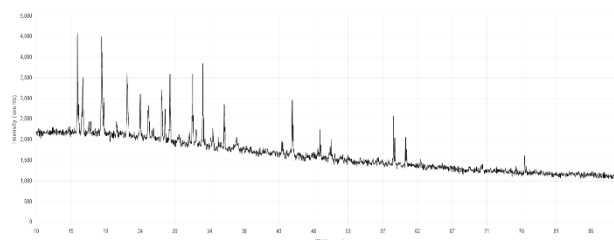
UV-Visible spectroscopy confirmed the formation of copper nanoparticles through the appearance of a characteristic surface plasmon resonance (SPR) peak. The obtained absorption peak confirmed the successful synthesis of CuNPs. Copper nanoparticles generally exhibit SPR peaks within the range of 230–600 nm, depending on particle size and synthesis conditions³⁵. The obtained absorption peak confirmed the successful synthesis of CuNPs. The broadness of the peak suggested a nanoscale particle distribution and confirmed the presence of phytochemical capping layers on the nanoparticle surfaces [Graph 1], which aligns with structural properties observed in similar plant-mediated colloidal metal systems³⁶.



Graph 1: UV-VIS Spectrum of Blueberry copper Nanoparticles

3.2 XRD Analysis

The X-ray Diffraction (XRD) analysis was carried out to investigate the crystalline nature and structural properties of the synthesized copper nanoparticles prepared using blueberry ethanolic extract. The XRD pattern exhibited characteristic diffraction peaks at different 2θ values, confirming the formation of crystalline copper nanoparticles. Prominent diffraction peaks were observed approximately at 2θ values of 15°, 21°, 29°, 34°, 39°, 44°, 53°, 57°, 62°, 67°, 71°, 78°, 81°, and 86° with varying diffraction intensities ranging up to nearly 5000 counts. The highest-intensity peak was observed at a mid-range diffraction angle, indicating the dominant crystalline orientation of the nanoparticles. The presence of sharp diffraction peaks demonstrates the crystalline nature and good structural ordering of the synthesized CuNPs, while slight peak broadening confirms the nanoscale dimensions of the particles, matching standard crystal structures established for bio-reduced copper³⁷. The diffraction peaks correspond to the characteristic crystal planes of copper nanoparticles with a face-centered cubic (FCC) crystalline structure³⁸. The absence of significant impurity peaks indicates the high purity of the synthesized nanoparticles. The nanoscale crystallite size of the particles may be estimated using the Debye–Scherrer equation, which relates peak broadening to crystallite dimensions. The XRD analysis confirms the successful green synthesis of crystalline copper nanoparticles using blueberry-mediated phytochemical reduction and stabilization [Graph 2].



Graph 2: XRD Analysis of Blueberry copper Nanoparticles

3.3 SEM Analysis

The SEM micrograph of the blueberry-mediated copper nanoparticles reveals the formation of irregularly shaped agglomerated particles distributed

over the surface. The particles appear predominantly flake-like and clustered, with non-uniform morphology and varying particle dimensions in the micrometre range. The observed agglomeration may be attributed to the high surface energy and strong interparticle interactions of the synthesized nanoparticles during the drying process³⁹. The surface morphology exhibits rough and uneven textures, indicating the successful nucleation and growth of copper-based nanostructures. Some particles possess layered or plate-like appearances, which may result from the adsorption of phytochemical constituents from the blueberry extract acting as capping and stabilizing agents⁴⁰. The presence of these biomolecules on the nanoparticle surface likely contributed to particle stabilization and controlled growth during the green synthesis process. The synthesized particles are densely packed with slight porosity between aggregates, suggesting effective particle formation with partial coalescence. The broad size distribution observed in the micrograph indicates heterogeneous growth kinetics during synthesis. The particle aggregation behaviour is commonly reported in biologically synthesised copper nanoparticles due to their oxidative tendency and intermolecular hydrogen bonding among plant-derived organic compounds [Figure 6].

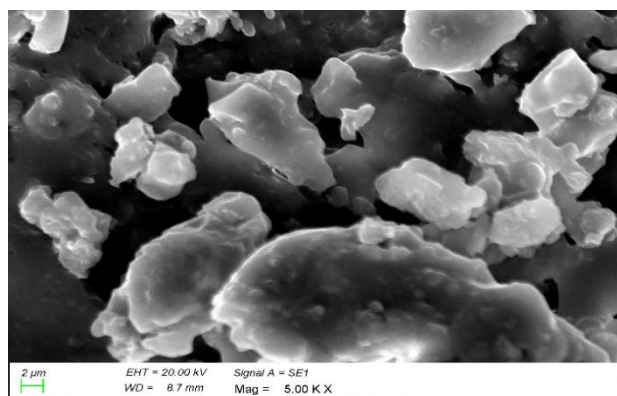
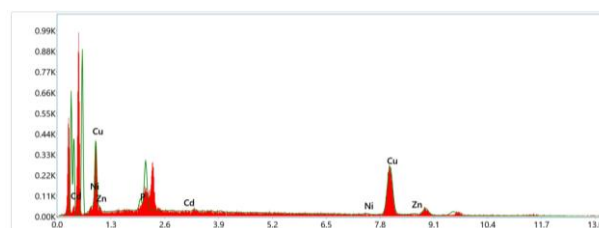


Figure 6: SEM Analysis of Blueberry copper Nanoparticles

3.4 EDS Analysis

The EDX spectrum of the blueberry-mediated copper nanoparticles confirms the elemental composition and successful formation of copper-based nanostructures. The spectrum exhibits prominent characteristic peaks corresponding to copper (Cu), indicating the presence of copper as the major constituent in the synthesized sample. The intense Cu peaks observed around the higher energy region (~7.8 keV) are attributed to the characteristic Cu K α emission, confirming the formation of copper nanoparticles⁴¹. Additional peaks corresponding to elements such as oxygen (O), carbon (C), phosphorus (P), nickel (Ni), zinc (Zn), and cadmium (Cd) are also observed in the spectrum. The oxygen peak suggests the possible presence of oxidized copper species such as CuO or Cu₂O formed during synthesis or exposure to air⁴².

Carbon signals may originate from organic phytochemicals present in the blueberry extract, which act as reducing and stabilizing agents during nanoparticle formation. The presence of phosphorus and other trace elements may be associated with naturally occurring biomolecules and mineral constituents of the plant extract⁴³. Minor peaks of Ni, Zn, and Cd may arise from instrumental background, substrate contribution, environmental contamination, or trace impurities during sample preparation and analysis. The relatively high intensity of copper peaks compared to other elements confirms the dominance of copper within the synthesized material [Graph 3].



Graph 3: EDS Analysis of Blueberry Copper Nanoparticles

The EDX quantitative analysis of the blueberry-mediated copper nanoparticles demonstrates that copper (Cu) is the predominant element present in the synthesized sample, with a weight percentage of 89.8% and an atomic percentage of 84.7%. The high copper content confirms the successful synthesis of copper-based nanoparticles through the green reduction process mediated by blueberry extract. The strong Cu signal intensity further indicates the purity and effective formation of the nanomaterial. Phosphorus (P) was detected with a weight percentage of 6.0% and an atomic percentage of 11.6%. The presence of phosphorus may be associated with phosphate-containing biomolecules and organic constituents naturally present in the blueberry extract, which may contribute to nanoparticle stabilization and surface functionalization⁴⁴. Trace amounts of zinc (Zn), nickel (Ni), and cadmium (Cd) were also identified. Zinc was present at 2.3 wt%, while nickel and cadmium showed relatively lower concentrations of 1.1 wt% and 0.8 wt%, respectively. These minor elemental signals may arise from residual impurities, precursor contamination, environmental exposure, instrumental artifacts, or substrate-related interference during SEM-EDX analysis⁴⁵. Their comparatively low concentrations indicate that they are not major constituents of the synthesized nanoparticles. The relatively low error percentage observed for copper (4.7%) indicates reliable quantitative detection of Cu, whereas higher error values for Ni and Cd suggest that these elements are present only in trace amounts. The dominance of copper along with the presence of biologically derived elements supports the successful green synthesis and stabilization of copper nanoparticles using blueberry extract [Table 1].

Table 1: EDS Parameter of Blueberry Copper Nanoparticles

Element	Weight %	MDL	Atomic %	Net Int.	Error	R	A	F
P K	6.0	27.22	11.6	16.2	10.2	0.8329	0.3760	1.0096
Ni K	1.1	69.90	1.1	2.4	64.0	0.9022	0.9670	1.2154
Cu K	89.8	106.96	84.7	132.5	4.7	0.9085	0.9734	1.0409
Zn K	2.3	136.73	2.1	2.8	25.6	0.9153	0.9665	1.0475
Cd L	0.8	68.91	0.5	1.1	60.3	0.8543	0.7048	1.01

3.5 Elemental Mapping

Elemental mapping analysis demonstrated an uniform distribution of copper throughout the nanoparticle sample [Figure 7]. The homogeneous

elemental distribution indicated efficient synthesis and stabilization of nanoparticles. The mapping images also confirmed purity and proper dispersion of the synthesised nanoparticles⁴⁶.

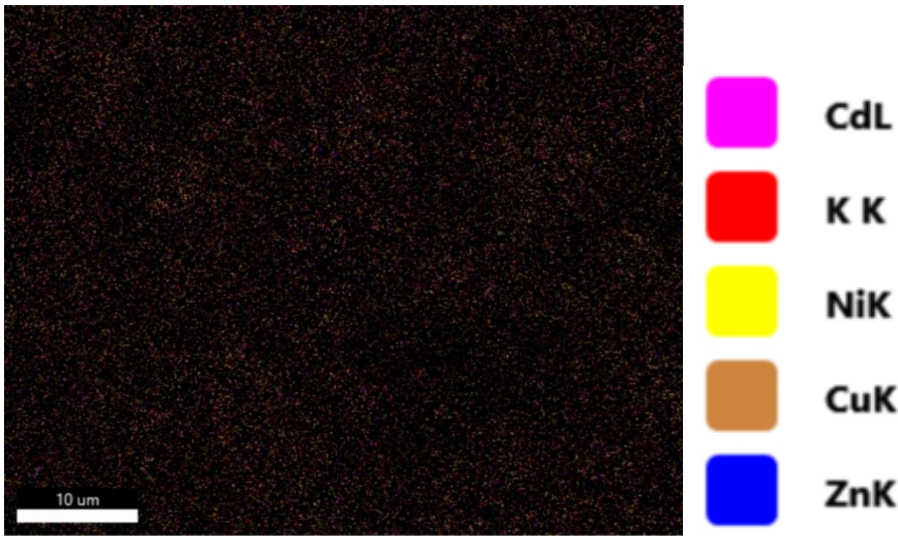


Figure 7: EDS Elemental Mapping of Blueberry Copper Nanoparticles

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

The green synthesis of copper nanoparticles (CuNPs) utilizing blueberry ethanolic extract involves a process where phytochemicals in the extract facilitate the reduction of copper ions (Cu^{2+}) into stable copper nanoparticles (Cu^0). Blueberries are noted for their rich content of antioxidant biomolecules, including anthocyanins, flavonoids, tannins, and polyphenolic compounds, which contribute significantly to the reducing and stabilizing processes⁴⁷. The synthesis unfolds through four principal stages:

- a) **Activation:** Copper ions are initially reduced by phytochemicals⁴⁸.
- b) **Nucleation:** Reduced copper atoms aggregate to form nanoparticle seeds⁴⁹.
- c) **Growth:** These seeds expand as more copper is deposited⁵⁰.
- d) **Stabilization:** Phytochemicals adsorb on the surface of the nanoparticles, preventing aggregation and enhancing stability⁵¹. Functional groups such as

hydroxyl and carbonyl play critical roles in these processes.

This eco-friendly and sustainable green synthesis method presents multiple advantages over traditional techniques, such as reduced environmental toxicity, lower energy consumption, and the utilization of renewable plant resources⁵². The incorporation of blueberry extract not only enhances the biocompatibility and stability of the nanoparticles but also improves the reduction efficiency due to its high antioxidant content. Copper nanoparticles produced through this method hold extensive applications across various fields. In biomedicine, they display antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory properties, making them suitable for wound healing, drug delivery, and antimicrobial formulations. In environmental contexts, CuNPs are effective in wastewater treatment and the degradation of harmful pollutants. Their catalytic efficiency also makes them invaluable in industrial processes. CuNPs excellent electrical conductivity facilitates their use in electronics, including conductive inks, sensors, and energy storage devices. In agriculture, they find applications in nanofertilizers, nanopesticides, and plant disease

management. Thus, the green synthesis of copper nanoparticles signifies an important advancement with promising potential across scientific, industrial, and environmental domains.

V. CONCLUSION AND FUTURE PERSPECTIVES

The study demonstrates an eco-friendly and cost-effective method for synthesizing copper nanoparticles (CuNPs) using ethanolic extract of blueberry (*Vaccinium* spp.). Key phytochemicals in the extract, such as anthocyanins and flavonoids, facilitate the reduction of copper ions and stabilize the nanoparticles. Confirmation of nanoparticle formation was indicated by a color change due to surface plasmon resonance, while further characterization through UV-Visible spectroscopy, FTIR, X-ray Diffraction (XRD), SEM, and TEM validated their properties, confirming crystalline nature and uniform morphology. This green synthesis approach is advantageous compared to traditional methods, delivering environmental safety, low toxicity, reduced energy use, and enhanced biocompatibility, thanks to the phytochemical-rich blueberry extract. The synthesized CuNPs showcase potential for applications in biomedical, catalytic, environmental, agricultural, and industrial fields, contributing significantly to sustainable nanotechnology. Future research directions include optimizing synthesis conditions like pH and temperature for better control over nanoparticle characteristics. Investigations into the antimicrobial and anticancer properties of the CuNPs, as well as their catalytic efficiencies, will enhance their biomedical and industrial utility. Long-term stability studies, large-scale synthesis, and commercialization efforts should also be pursued, alongside comparative analyses of different berry extracts for nanoparticle production. Additionally, exploring the development of nanoparticle-based systems for drug delivery, biosensing, and pollutant management could expand their application in contemporary science and technology.

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