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EVALUATION OF BIOSYNTHESIZED COPPER OXIDE (CUO) NANOPARTICLES USING CYPERUS ROTUNDUS LEAF - AN IN VITRO ANTI-CANCER STUDY IN LUNG CANCER CELL LINE

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ABSTRACT:
The biological synthesis of copper nanoparticles using *Cyperus rotundus* extracts represents a pivotal shift toward sustainable nanotechnology, merging ancient ethnomedicinal practices with high-tech industrial requirements. This perennial herb is densely packed with secondary metabolites, including polyphenols, flavonoids, and essential oils, which serve as a sophisticated biochemical laboratory for the reduction of copper ions. To prepare the aqueous extract, 20g of dried and powdered *Cyperus rotundus* fruit, sourced from Chennai, was combined with 100 ml of distilled water and filtered. The resulting filtrate was then reacted with dropwise copper sulphate on a magnetic stirrer to facilitate the green synthesis of nanoparticles. These particles were isolated via centrifugation and subsequently characterized using UV-Vis, SEM, FTIR, and XRD analysis to confirm their structural and chemical properties. Beyond the synthesis itself, the primary therapeutic interest in these nanoparticles lies in their role as potent modulators of the cellular redox environment. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, is the fundamental driver of DNA damage, protein carbonylation, and the initiation of various chronic inflammatory pathologies. The resulting CuO nanoparticles are distinguished by their optimized physical architecture, characterized by a diminished particle size and a vastly expanded surface-area-to-volume ratio. Such structural refinements are critical, as they dictate the material's reactivity and performance efficiency in diverse technological and biomedical settings.
Keywords: health, cancer, public health, health risk, medicine.

1. Introduction :

The biological synthesis of copper nanoparticles using *Cyperus rotundus* extracts represents a pivotal shift toward sustainable nanotechnology, merging ancient ethnomedicinal practices with high-tech industrial requirements.(1) This perennial herb is densely packed with secondary metabolites, including polyphenols, flavonoids, and essential oils, which serve as a sophisticated biochemical laboratory for the reduction of copper ions(2). Unlike traditional chemical synthesis, which often relies on toxic reducing agents and stabilizing surfactants, the "green" approach leverages these plant-based compounds to encapsulate the nanoparticles, preventing agglomeration and ensuring a uniform size distribution.(2,3) This natural "capping" process is crucial because the efficacy of copper nanoparticles in applications like electrochemical sensing and field emission is directly dependent on their structural integrity and high surface-area-to-volume ratio.(4) By maintaining these precise dimensions,(5) the nanoparticles exhibit superior catalytic properties, allowing for the rapid degradation of inorganic pollutants and more efficient electron transport in next-generation lithium-ion batteries and supercapacitors.(4,6)
The production of nanoscale materials is accelerating due to their many uses in everything from bioengineering and aerospace to agriculture and medicine.(7) It is also observed that nanoscale molecules and particles, whether metal or non-metal, have remarkable characteristics in comparison to their parent enormous materials.(8) Because of its novel methods for identifying and controlling a wide range of biological processes that take place at the nanoscale, nanotechnology is anticipated to have a major influence on biology and medicine. Metal-based nanoparticles show great promise for use in drug delivery, imaging, medicine, and antibacterial agents. (9)As a result, several aspects of nanomaterials, such as size, shape, solubility, aggregation, and optical properties, that may influence toxicity have been studied. Apart from these potential uses, metal nanoparticles may disrupt several other biological processes, including autopsy and angiogenesis.(8,10) Among these, copper nanoparticles are particularly notable for their high yield and low cost, which makes them perfect for use in biomedical and environmental applications.(1) Because of their unique properties, which make them useful for a variety of applications like sensors, catalysts, optics, solar cells, environmental remediation, antimicrobial treatments, and more, researchers have shown a great deal of interest in nanomaterials made from copper and copper oxide.(2) However, conventional techniques for producing metal oxide nanoparticles (NPs), like CuONPs, frequently entail hazardous chemicals and severe physical conditions.(3)
Furthermore, the integration of *C. rotundus*-derived nanoparticles into the energy sector addresses the urgent need for cost-effective and eco-friendly materials.(4,6,11) In the fabrication of solar cells and photodetectors, these copper-based nanomaterials offer an affordable alternative to noble metals like gold or silver while maintaining high conductivity and optical sensitivity. (4,6,11,12)Their role extends into thermal engineering as well; when suspended in carrier liquids to create nanofluids, these particles significantly boost the heat transfer capabilities of industrial cooling systems. This multifaceted utility—ranging from the remediation of contaminated water via photocatalysis to the enhancement of magnetic storage media—highlights the extraordinary versatility of copper nanoparticles.(4,6,11–14) Ultimately, by refining the bioactive potential of a plant used in Ayurveda for centuries, modern science is creating a bridge between biological conservation and technological advancement, proving that the solutions for future electronics and environmental sustainability may very well be found in our prehistoric botanical heritage.(4,6,11–13)

2. Materials and method:

2.1 Green synthesis of copper nanoparticles

2.1.1 Phase I: Preparation of the Botanical Reductant: The process begins with the procurement of high-quality *Cyperus rotundus* (Nut Grass) specimens, sourced locally from vendors in Chennai, India. To isolate the essential bioactive compounds, the plant's fruits are subjected to a thorough drying process to remove moisture, followed by mechanical grinding into a fine, homogenous powder.
An aqueous extraction is then performed by integrating **20g** of the pulverized sample with **100 ml** of deionized distilled water. This mixture is typically heated or agitated to facilitate the leaching of phytochemicals—such as polyphenols and flavonoids—into the solvent, creating a concentrated "green" reducing broth.
2.1.2 Phase II: Bio-Reduction and Nanoparticle Synthesis: Once the extract is prepared, it is refined through filtration to remove any remaining particulate debris. The resulting clear filtrate serves as the reaction medium. For the synthesis of the nanoparticles, the extract is placed on a **magnetic stirrer** to maintain constant kinetic energy and uniform temperature.
A precursor solution of **Copper Sulphate (CuSO4)** is introduced dropwise into the stirring extract. During this stage, the phytochemicals in the *C. rotundus* act as natural electron donors, reducing the copper ions (Cu2+) into metallic copper nanoparticles (Cu0). The visible transition in the solution's color often serves as the first indicator of successful nanoparticle formation.
2.1.3 Phase III: Isolation and Purification: To isolate the synthesized materials, the colloidal mixture undergoes **centrifugation**. This high-speed rotation separates the solid copper nanoparticles from the liquid supernatant. The resulting pellet is typically washed and dried to ensure that any unreacted precursors or plant residues are removed, yielding purified copper nanoparticles ready for analysis.
2.2 Characterization and Analytical Validation: To confirm the structural and chemical integrity of the synthesized nanoparticles, a suite of advanced analytical techniques is employed:
2.2.1 **UV-Vis Spectroscopy:** Used to confirm the formation of nanoparticles by identifying the characteristic **Surface Plasmon Resonance (SPR)** peak unique to copper.
2.2.2 **Scanning Electron Microscopy (SEM):** Provides high-resolution imaging to determine the **morphology**(shape) and size distribution of the particles.
2.2.3 **Fourier Transform Infrared Spectroscopy (FTIR):** Identifies the specific functional groups from the plant extract that are responsible for reducing and capping the nanoparticles.
2.2.4 **X-Ray Diffraction (XRD):** Analyzes the **crystalline structure** and phase purity of the copper, confirming that the material is indeed in a nano-crystalline state.

3. Result :

3.1 SEM:

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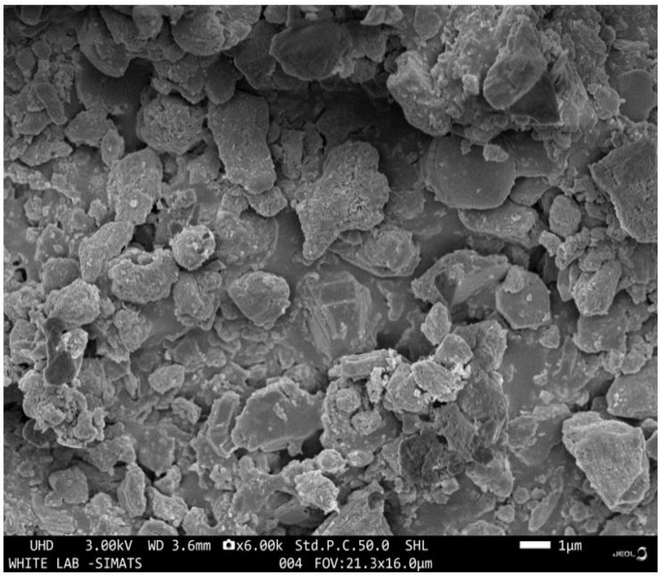
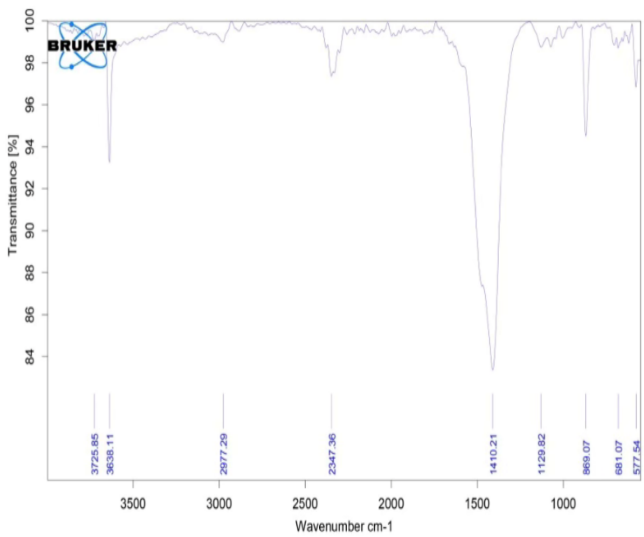


Figure 1: SEM image of ZnO NPs of leaf extracts of piper nigrum leaf



3.2 FTIR: Figure 2: FT-IR spectrum of green synthesised ZnO NPs

3.3 X ray differaction (XRD) :

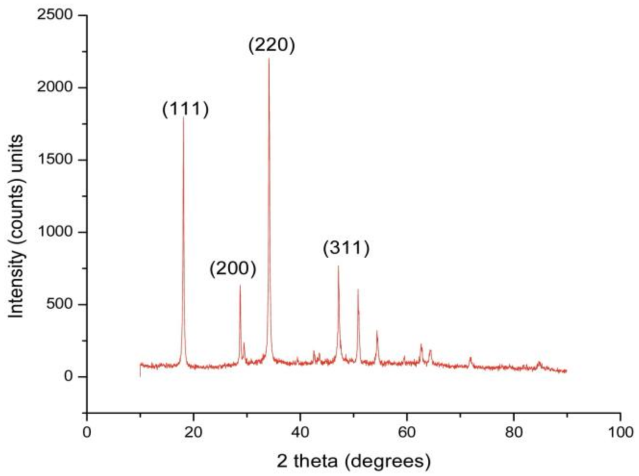
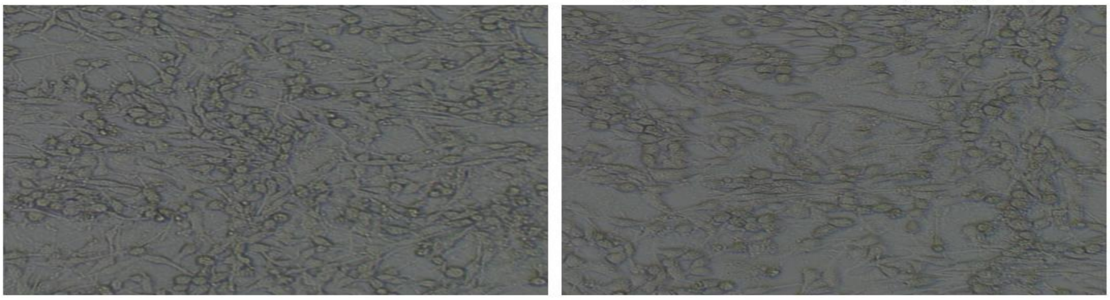


Figure 3: X ray differaction (XRD) cures of synthesised copper oxide

3.4: Inverted Phase Contrast Microscopy

Control **_Cyperus rotandus_ + CuO (100 µg/ml)**



Cells were treated with *Cyperus rotandus* + CuO (100 µg/ml) for 24 h along with control group.
Images were obtained using an inverted phase contrast microscope (10 x)

Figure 4: After a 24-hour incubation period with 100µg/ml of the *Cyperus rotundus*-mediated CuO nanoparticles, the cellular architecture was compared against an untreated control group to identify any significant structural deviations or cytotoxic indicators."

4. Discussion :

The green synthesis of copper oxide (CuO) nanoparticles using *Cyperus rotundus* represents a sophisticated convergence of eco-friendly chemistry and advanced pharmacology.(15) At its core, this process utilizes the complex botanical matrix of the plant—specifically its high concentration of secondary metabolites such as cyperene, flavonoids, and polyphenolic acids—to function as a multifaceted biochemical reactor(16). Unlike conventional chemical reduction,(17) which often necessitates high temperatures and the use of toxic agents like sodium borohydride, this "green" route operates under ambient conditions. The plant's phytochemicals act as natural capping agents, adhering to the surface of the growing nanoparticles.(18) This biological "envelope" is crucial; it not only dictates the final size and morphology of the CuO particles but also prevents their agglomeration, ensuring they remain stable and reactive within a biological system.(9)

Beyond the synthesis itself, the primary therapeutic interest in these nanoparticles lies in their role as potent modulators of the cellular redox environment.(7,8,10,19) Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, is the fundamental driver of DNA damage, protein carbonylation, and the initiation of various chronic inflammatory pathologies.(8) CuO nanoparticles synthesized via *C. rotundus* exhibit a remarkable capacity to mimic natural antioxidant enzymes. By acting as electron donors or catalysts, they directly scavenge superoxide radicals and hydroxyl ions, effectively "quenching" the oxidative fire that fuels inflammation.

To scientifically validate these properties, researchers employ comprehensive *in vitro* screening protocols. These include the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to measure radical-neutralizing efficiency and thiobarbituric acid reactive substances (TBARS) assays to quantify the inhibition of lipid peroxidation.(8,19) These tests confirm whether the nanoparticles can protect the fatty acid components of cell membranes from being degraded by oxidative attacks. Furthermore, because these particles are synthesized using natural extracts, they carry a lower risk of "off-target" toxicity compared to their chemically derived counterparts. This bio-synergy not only improves the safety profile of the nanomaterials but also potentially enhances their bioavailability, making them a formidable candidate for future applications in targeted drug delivery, wound healing, and the treatment of systemic inflammatory disorders.(8,10,19)

5. Conclusion:

The utilization of *Cyperus rotundus*, a mainstay in traditional pharmacopeia, as a biological scaffold for the synthesis of copper oxide (CuO) nanoparticles represents a significant stride in sustainable nanotechnology. This perennial herb is prized for its dense concentration of bioactive secondary metabolites, which possess innate antioxidant, anti-inflammatory, and antimicrobial characteristics. By repurposing these natural compounds as both reducing and capping agents, researchers can effectively orchestrate the transition of metal ions into stable, high-performance nanostructures. This biogenic approach not only aligns with the principles of green chemistry by bypassing the need for hazardous synthetic reagents but also offers a scalable and economically viable pathway for large-scale production.

The resulting CuO nanoparticles are distinguished by their optimized physical architecture, characterized by a diminished particle size and a vastly expanded surface-area-to-volume ratio. Such structural refinements are critical, as they dictate the material's reactivity and performance efficiency in diverse technological and biomedical settings. Furthermore, the "green" synthesis process ensures that the nanoparticles are naturally functionalized; the surface of each particle remains coated with a thin layer of the plant's organic constituents. This biological "capping" does more than just prevent particle clumping; it synergistically enhances the nanomaterial's intrinsic properties, potentially imparting superior free-radical scavenging abilities and potent antimicrobial action. Consequently, *Cyperus rotundus*-mediated nanoparticles stand as a multi-functional interface where ancient botanical wisdom meets cutting-edge material engineering.

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