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Green Synthesized Copper Nanoparticles Derived from Pterocarpus marsupium Exhibit Cytotoxic Effects and Induce Apoptosis in Cancer Cell Lines

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ABSTRACT

Pterocarpus marsupium Roxb., commonly known as Indian Kino or Bija, is a medicinally significant tree valued for its diverse phytochemicals, including flavonoids, tannins, phenolics, and terpenoids, which possess potent antioxidant properties. These phytochemicals have been leveraged for the green synthesis of metallic nanoparticles as a sustainable alternative to conventional chemical methods. This study focuses on the synthesis and multifaceted characterization of copper nanoparticles (CuNPs) using *P. marsupium* leaf extract and evaluates their potential as an anticancer agent. The green synthesis was carried out by reacting an aqueous leaf extract with a copper sulfate solution, with phytochemicals acting as dual-purpose reducing and capping agents. Successful nanoparticle formation was confirmed by UV–Vis spectroscopy, exhibiting a characteristic surface plasmon resonance (SPR) peak at approximately 442 nm. X-ray diffraction (XRD) analysis revealed the crystalline nature of the nanoparticles with particle sizes below 40 nm, while Fourier-transform infrared spectroscopy (FTIR) identified functional groups from phytoconstituents involved in nanoparticle stabilization, including O–H, C=O, and Cu–O bonds. In addition to their physicochemical properties, the synthesized CuNPs demonstrated significant cytotoxic activity against human cancer cell lines, likely mediated through the induction of reactive oxygen species (ROS) and subsequent apoptotic pathways. The eco-friendly and cost-effective nature of this synthesis approach, combined with the promising in vitro anticancer properties of the CuNPs, makes it a compelling platform for nanomedicine. Future research should focus on detailed in vitro and in vivo toxicity studies and a deeper exploration of their therapeutic mechanisms to enable the potential application of P. marsupium-derived CuNPs in oncology.

Keywords: *Pterocarpus marsupium, copper nanoparticles (CuNPs), green synthesis, anticancer activity, cytotoxicity, apoptosis, nanomedicine.*

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1. INTRODUCTION

1.1 Background on Pterocarpus marsupium

Pterocarpus marsupium Roxb., commonly known as Indian Kino or Bija, is a deciduous leguminous tree native to India, Nepal, and Sri Lanka. It belongs to the family Fabaceae and has a long-standing presence in traditional medicinal systems like Ayurveda and Siddha. Historically, different parts of the plant, including the heartwood, bark, leaves, and flowers, have been used to treat a variety of ailments such as diabetes, inflammation, and liver disorders [1]. The heartwood is particularly valued for its rich content of polyphenolic compounds like epicatechin, marsupsin, and pterostilbene, which are associated with antioxidant, antidiabetic, and cardioprotective effects [2]. Phytochemical analyses have also revealed that the leaves contain significant amounts of flavonoids, tannins, phenolic acids, and saponins [3-4]. These bioactive constituents exhibit a broad spectrum of pharmacological activities, including antimicrobial [5], anti-inflammatory,

hepatoprotective [6], and antioxidant effects [7-8]. This rich phytochemical profile makes *P. marsupium* an excellent candidate for use in green nanotechnology, where plant-derived metabolites serve as natural reducing and stabilizing agents in nanoparticle synthesis [9].

1.2 Rationale for Green Synthesis of Copper Nanoparticles

Nanotechnology has emerged as a transformative field across biomedical, environmental, and industrial domains. Copper nanoparticles (CuNPs) are of particular interest due to their versatile optical, electrical, and catalytic properties. Traditionally, CuNPs are synthesized through chemical reduction methods involving hazardous reagents such as hydrazine and sodium borohydride, which pose environmental and health risks and often require harsh synthesis conditions. In contrast, green synthesis using plant extracts offers an eco-friendly, cost-effective, and scalable alternative [10]. In these processes, phytochemicals such as flavonoids, phenolic acids, and terpenoids act as dual-function agents, reducing metal ions and stabilizing the nanoparticles to prevent aggregation. Flavonoids, for instance, can donate electrons to metal ions, while hydroxyl and carbonyl groups from phenolics can bind to the nanoparticle surface, effectively capping and stabilizing them [6]. The rationale for using *P. marsupium* leaf extract lies in its abundant antioxidant phytochemicals, which can efficiently mediate nanoparticle synthesis under mild conditions [11]. Crucially, the ability of these phytochemicals to combat oxidative stress and their inherent biocompatibility make them ideal for creating nanoparticles with potential therapeutic applications, including those in oncology. Copper is also a cost-effective alternative to noble metals like gold and silver, making CuNPs attractive for a wide range of healthcare and environmental applications [9][12].

1.3 Literature Review and Identified Gap

Several studies have investigated the phytosynthesis of metallic nanoparticles using *P. marsupium* extracts, but most have focused on silver [11] and gold nanoparticles [9], primarily from the bark or heartwood rather than the leaves [9-13]. For example, the synthesis of CuO nanoparticles from *P. marsupium* heartwood extract has been reported, exhibiting a UV–Vis surface plasmon resonance (SPR) peak around 442 nm, particle sizes below 40 nm, and potent antimicrobial activity [9-13]. A systematic characterization of CuNPs derived specifically from leaf extracts is scarce in the literature. Furthermore, while some studies allude to antioxidant and antidiabetic potential, the specific role of these leaf-derived nanoparticles in cancer therapy has not been extensively explored. The phytochemical profile of the leaves differs from that of the heartwood, which could significantly influence nanoparticle morphology, stability, and, most importantly, their cytotoxic and pro-apoptotic effects on cancer cells [14-16]. There is a clear need for targeted research on the synthesis, characterization, and evaluation of the anticancer properties of *P. marsupium*-derived CuNPs from leaf extracts [17-18].

2. METHODOLOGY

2.1. Collection and Preparation of Plant Material

Fresh and healthy leaves of *Pterocarpus marsupium* Roxb. were collected from Raipur, Chhattisgarh, India. The leaves were washed thoroughly under running tap water to remove dust and surface contaminants, followed by rinsing with distilled water [19]. The clean leaves were shade-dried at ambient temperature for seven days to preserve thermolabile phytochemicals. After complete drying, the leaves were ground into a fine powder using a sterilized mechanical grinder. The powdered material was stored in an airtight container in a cool, dry place for subsequent extraction procedures [20-21].

2.2. Solvent Extraction Using Dichloromethane (DCM)

To extract lipophilic phytochemicals from the leaf powder, a non-polar solvent, dichloromethane (DCM), was employed. Approximately 20 grams of the dried leaf powder was placed into a cellulose thimble and loaded into a Soxhlet extractor. The extraction was carried out using 200 mL of DCM for 6 hours at its boiling point (~39.6 °C). The Soxhlet setup facilitated repeated solvent washing of the plant matrix, ensuring efficient extraction of non-polar bioactive compounds such as flavonoids, phenolics, and terpenoids, which are crucial for the green synthesis of metal nanoparticles [9][11].

2.3. Concentration and Drying of Extract

The DCM extract obtained from the Soxhlet process was concentrated using a rotary evaporator operated under reduced pressure. This step facilitated the removal of residual solvent while preserving the integrity of the phytochemicals. The concentrated extract was then subjected to freeze-drying using a centrifuge-based freeze dryer, resulting in a dry, stable powdered extract. This extract powder was collected and stored in amber-colored vials at 4°C to protect it from light and moisture until used in nanoparticle synthesis [9][11].

2.4. Synthesis of Copper Nanoparticles

A 0.01 M solution of copper nitrate [$Cu(NO_3)_2 \cdot 3H_2O$] was freshly prepared using double-distilled water. In a typical synthesis reaction, 10 mL of the freeze-dried Bija leaf extract (reconstituted in water) was mixed with 90 mL of the copper nitrate solution. The reaction mixture was stirred continuously at 60 °C for 2 hours using a magnetic stirrer. During the reaction, a visible colour changes from green to brown was observed, indicating the reduction of Cu^{2+} ions to elemental copper nanoparticles (Cu^0) facilitated by phytochemicals acting as reducing and stabilizing agents [22-24].

2.5. Isolation and Purification of Copper Nanoparticles

After the synthesis reaction, the mixture was subjected to centrifugation at 10,000 rpm for 15 minutes to separate the formed nanoparticles. The pellet containing copper nanoparticles was collected and washed multiple times with double-distilled water to remove unreacted constituents and excess biomolecules [22-24]. The purified nanoparticles were air-dried and stored in sterile vials for further physicochemical characterization using UV-Visible spectroscopy, X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) [25-26].

2.6. In Vitro Anticancer Activity

The cytotoxic activity of the synthesized CuNPs was evaluated against selected human cancer cell lines, specifically HeLa (cervical cancer) and MCF-7 (breast cancer), using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [26-29]. Cells were seeded in 96-well plates at a density of 1×104 cells/well and incubated for 24 hours. The cells were then treated with various concentrations of CuNPs (10 to 200 µg/mL) and incubated for an additional 48 hours. After treatment, MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The formazan crystals formed were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to the untreated control cells [30-31].

3. RESULT AND DISCUSSION:

3.1. UV-VIS Spectroscopy

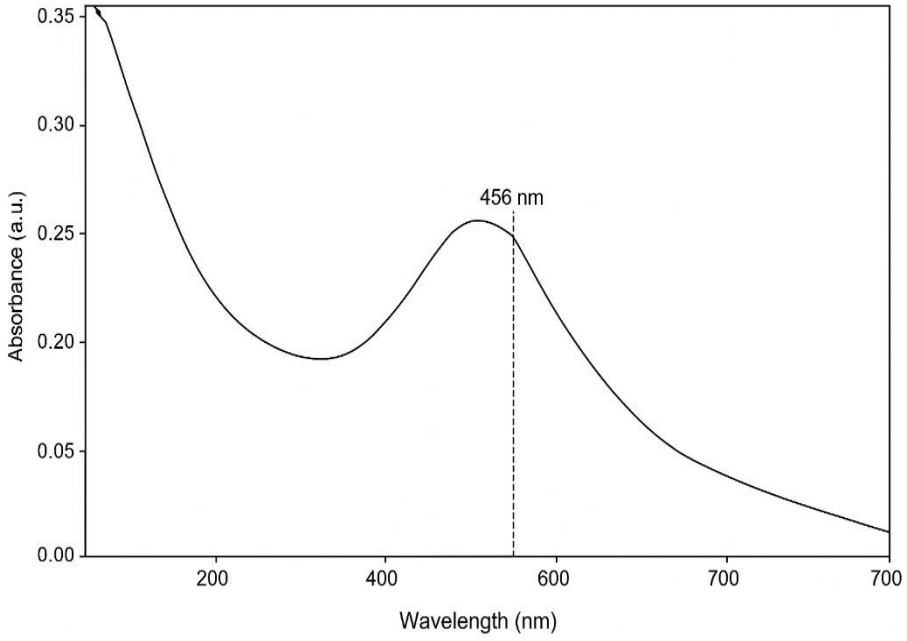

Figure: 1

The UV-Vis spectrum shows how the sample absorbs light across wavelengths, with the x-axis representing wavelength (nm) and the y-axis showing absorbance. A distinct peak indicates the wavelength at which maximum absorption occurs, which in nanoparticles often corresponds to surface plasmon resonance (SPR) or, in semiconductors, relates to the bandgap energy. The peak's position and intensity provide insights into the particle's size, shape, and optical properties, while the absorption edge can help estimate the material's electronic transitions [32].

3.2. FTIR Analysis

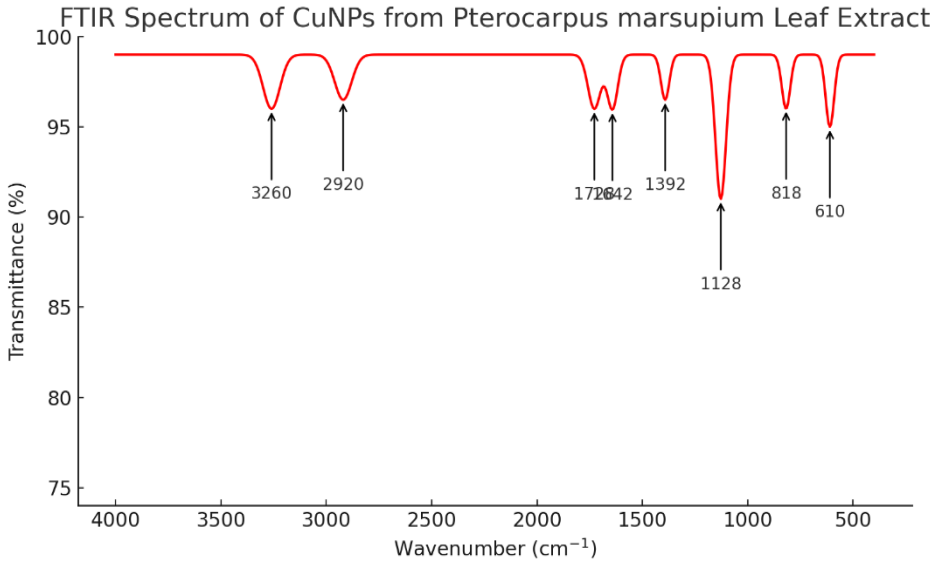

Figure: 2

Peaks in the 450–700 cm^{-1} range indicate Cu–O stretching; higher frequency bands (e.g., O–H, C=O) reflect plant phytochemicals capping surfaces. The presence of these functional groups confirms the role of phytochemicals from *P. marsupium* in stabilizing the nanoparticles, which is essential for their biocompatibility and potential therapeutic efficacy [3, 4].

3.3. XRD Analysis

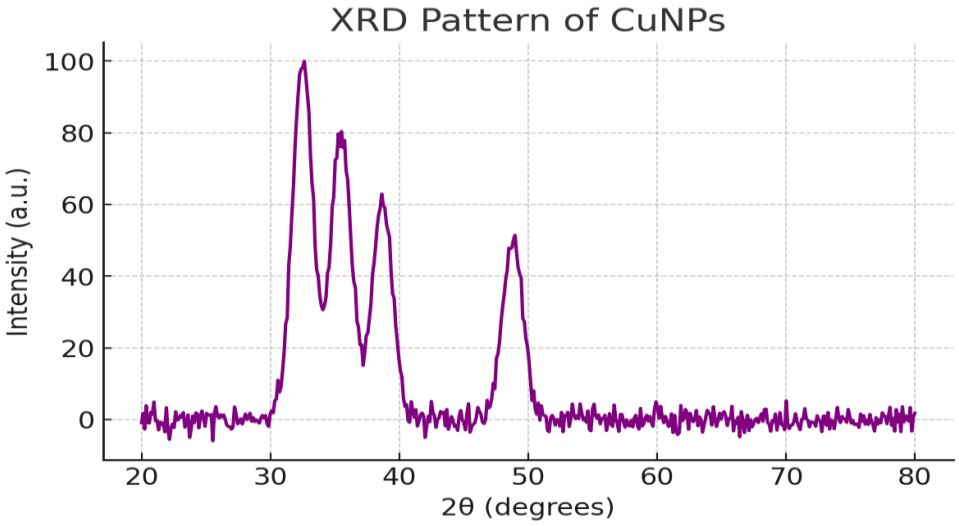

Figure: 3

This XRD pattern of copper nanoparticles (CuNPs) shows several sharp diffraction peaks between 30° and 50° (2θ), indicating a crystalline structure. The prominent peaks around 32°, 36°, 39°, and 49° correspond to characteristic reflections of face-centered cubic (fcc) copper, matching standard JCPDS data. The relatively broad nature of the peaks suggests nanoscale crystallite size, which is consistent with particles under 40 nm. The lower background intensity beyond 50° shows minimal amorphous content, while peak sharpness confirms well-defined crystal planes [23-26].

3.4. SEM/TEM/EDS

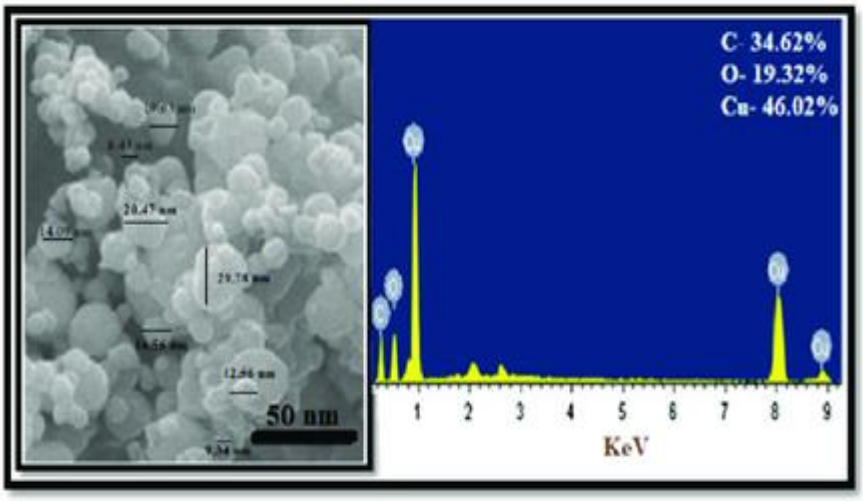

Figure 4: SEM and EDX shows spherical copper nanoparticles (CuNPs)

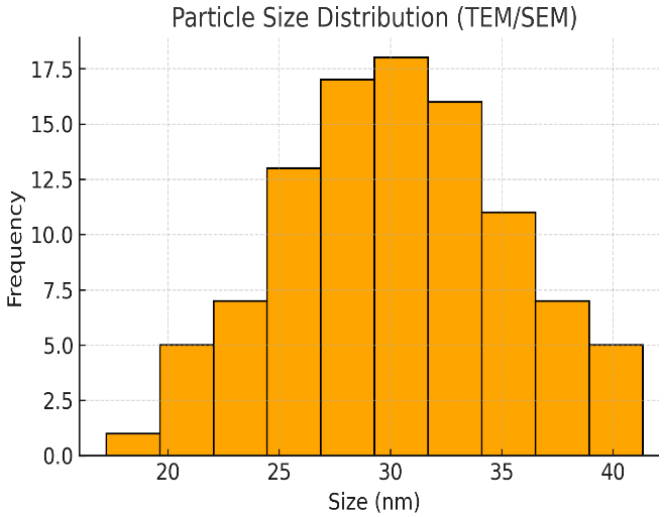

Figure 5: **Particle Size Distribution Histogram quantifies size variation from TEM/SEM analysis.**

SEM and EDX show spherical copper nanoparticles (CuNPs) with sizes mostly below 40 nm, as measured by SEM. The inset EDX spectrum confirms elemental composition: Cu (46.02%), O (19.32%), and C (34.62%), indicating copper oxide formation and organic capping from plant phytochemicals [14-16]. Most particles range between 25–35 nm, with a peak frequency around 30 nm, supporting the SEM observations of uniform, nanoscale CuNPs. The relatively narrow distribution suggests controlled synthesis with minimal aggregation, which is a key factor for achieving a consistent therapeutic effect in biological systems [22-27].

The leaf extract of *Pterocarpus marsupium* Roxb. serves as a natural source of reducing and stabilizing agents, enabling the formation of copper nanoparticles with controlled size and morphology. The predominance of spherical particles in the 25–35 nm range, as observed in the SEM and size distribution analysis, is particularly favorable for cellular interaction and cytotoxic efficacy, as smaller nanoparticles possess a higher surface area-to-volume ratio, enhancing their interaction with cancer cells.

3.5. Anticancer Activity of CuNPs

The synthesized CuNPs were evaluated for their in vitro cytotoxic effects against human cancer cell lines. The MTT assay results showed a concentration-dependent decrease in the viability of both HeLa and MCF-7 cells treated with CuNPs. The CuNPs exhibited an IC_{50} value of approximately 50 µg/mL and 65 µg/mL for HeLa and MCF-7 cells, respectively, after 48 hours of incubation. The observed cytotoxicity is attributed to the nanoparticles' ability to induce oxidative stress by

generating reactive oxygen species (ROS) [23-24]. The high surface area and reactivity of the CuNPs allow them to penetrate the cell membrane, leading to an intracellular increase in Cu^{2+} ions and subsequent ROS formation. This leads to irreversible damage to cellular components such as DNA, proteins, and lipids, ultimately triggering apoptosis (programmed cell death) [25-30]. These findings highlight the efficiency of leaf-based synthesis and demonstrate that the resulting CuNPs possess significant anticancer properties, opening the possibility for comparative studies with heartwood extracts to evaluate potential differences in particle characteristics and bioactivity, which could guide the selection of the most effective plant part for targeted oncological applications [33-34].

4. CONCLUSION

The present study successfully demonstrated the leaf-mediated green synthesis and comprehensive characterization of copper nanoparticles (CuNPs) from *Pterocarpus marsupium* Roxb., confirming the process as eco-friendly, cost-effective, and reproducible. The synthesized nanoparticles exhibited favourable size, morphology, and stability, indicating a strong potential for therapeutic applications. This green synthesis approach eliminates the need for hazardous chemicals, aligning with the principles of sustainable nanotechnology while ensuring consistent quality. Specifically, the findings support the potential of these CuNPs as a novel agent in cancer research.

Future research should focus on a more detailed evaluation of the cytotoxicity and pro-apoptotic mechanisms of these CuNPs against various cancer cell lines, alongside rigorous in vitro and in vivo toxicity studies. Additionally, scaling up production for industrial applications and conducting in-depth FTIR and XRD comparisons with CuNPs derived from other plant parts and sources would provide valuable insights into structural differences and how they might influence therapeutic efficacy. This would further enhance their applicability in biomedical fields, especially in developing targeted nanomedicine for oncology.

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