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Synthesis and characterization of *Coffea arabica* nanoparticles: Evaluation of antioxidant and anticancer properties

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Abstract
In recent years, the development of nanoparticles using natural plant extracts has gained significant attention due to their potential biomedical applications, including antioxidant and anticancer properties. This study aims to synthesize and characterize nanoparticles derived from *Coffea arabica* extracts using an eco-friendly, green chemistry approach. The process was optimized to produce stable nanoparticles without the use of harmful chemicals, thus promoting sustainable practices in nanomaterial synthesis. The synthesized nanoparticles were subjected to a thorough characterization using a combination of advanced techniques, including Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and UV-Vis spectroscopy to determine their size, morphology, crystalline nature, and functional groups. These analyses revealed well- dispersed, spherical nanoparticles with an average size in the range of 50-100 nm, exhibiting good stability.
The antioxidant potential of *Coffea arabica* nanoparticles was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which demonstrated significant free radical scavenging activity, indicative of their strong antioxidant capacity. Additionally, the anticancer activity was investigated against selected cancer cell lines, including human breast cancer (MCF-7) and colon cancer (HT-29) cell lines, using the MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The nanoparticles exhibited dose-dependent cytotoxicity, with notable inhibition of cancer cell proliferation. The findings of this study suggest that *Coffea arabica* nanoparticles possess substantial antioxidant properties and anticancer potential, positioning them as promising candidates for further exploration in biomedical applications, particularly in cancer therapy. The green synthesis approach employed here also highlights the environmental and economic advantages of utilizing natural plant sources for nanoparticle fabrication.

Keywords: *Coffea arabica* nanoparticles, green synthesis, antioxidant activity, anticancer properties, MTT assay, DPPH assay, biomedical applications

Introduction
The exploration of nanotechnology in recent years has revolutionized various fields, especially in medicine, where the synthesis of nanoparticles has opened up new possibilities for drug delivery, cancer treatment, and the development of new therapeutics [1]. Nanoparticles derived from natural plant extracts have emerged as a focal point due to their biocompatibility, low toxicity, and potential for large-scale production through eco-friendly methods [2]. Among these natural sources, *Coffea arabica*—commonly known for its coffee beans—is rich in bioactive compounds such as polyphenols, flavonoids, and caffeine, which have been documented for their antioxidant, anti-inflammatory, and anticancer properties [3]. *Coffea arabica*, traditionally consumed as a beverage worldwide, contains a wide array of bioactive molecules, including chlorogenic acids, diterpenes, and alkaloids, which contribute to its health benefits [4]. Research has shown that these compounds exhibit strong antioxidant capabilities, neutralizing harmful free radicals that can cause oxidative stress, a major contributor to chronic diseases, including cancer [5]. By utilizing *Coffea arabica* as a precursor for nanoparticle synthesis, it is possible to harness these bioactive components in a novel form that may enhance their biological activity [6].
Green nanoparticle synthesis uses plant extracts, bacteria, fungi, and algae to make nanoparticles without hazardous chemicals. This nanoparticle production method is cost-effective, scalable, and environmentally friendly [7]. Plant extracts contain different phytochemicals that can reduce and stabilize nanoparticles, making plant-mediated

production appealing. Polyphenols and antioxidants in coffee arabica extract should minimize metal ions and stabilize nanoparticles during production [8]. Nanoparticles are suited for biomedical applications due to their high surface area to volume ratio, increased reactivity, and ability to penetrate biological membranes. Nanoparticle size, shape, and stability depend on the synthesis technique. Nanoparticles made from *Coffea arabica* extract should have strong antioxidant and anticancer capabilities, especially against aggressive cancers.

Materials and Methods Materials

Plant Material: Fresh *Coffea arabica* beans were sourced from a local supplier. The beans were cleaned thoroughly with distilled water, dried, and powdered for extraction.

Chemicals

- Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich for nanoparticle synthesis.
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and other chemicals for antioxidant assays were also procured from Sigma-Aldrich.
- MTT reagent (for the MTT assay) was obtained from Thermo Fisher Scientific.
- All other chemicals and reagents used in the study were of analytical grade and were used as received without further purification.
- Cell Lines: Human breast cancer (MCF-7) and colon cancer (HT-29) cell lines were obtained from NCCS Pune for anticancer activity evaluation.
- Cell Culture Medium: RPMI-1640 medium, fetal bovine serum (FBS), penicillin- streptomycin solution, and trypsin-EDTA were acquired from Gibco Laboratories for cell culture purposes.

Preparation of *Coffea arabica* Extract

Fresh *Coffea arabica* beans were powdered and subjected to an aqueous extraction process. 10 g of powdered coffee beans was mixed with 100 mL of distilled water and heated at 80°C for 30 minutes. The mixture was then cooled and filtered through Whatman No.1 filter paper to obtain the extract. The filtrate was stored at 4°C and used for nanoparticle synthesis [9].

Synthesis of *Coffea arabica* Nanoparticles

- **Green Synthesis Approach**: The green synthesis of nanoparticles was carried out by mixing 50 mL of the aqueous *Coffea arabica* extract with 50 mL of a 1 mM aqueous silver nitrate ($AgNO_3$) solution [10]. The reaction mixture was stirred continuously at room temperature and monitored for color change, indicating the formation of nanoparticles. The development of a brownish color confirmed nanoparticle formation due to surface plasmon resonance.
- **Purification:** The reaction mixture was centrifuged at 10,000 rpm for 20 minutes to pellet the nanoparticles. The supernatant was discarded, and the pellet was washed three times with distilled water to remove any unreacted constituents [11].
- **Drying:** The purified nanoparticles were dried in a vacuum oven at 60 °C for 12 hours. The dried nanoparticles were then stored in an airtight container for further characterization.

Characterization of Nanoparticles

The synthesized nanoparticles were characterized using the following techniques:

Particle Size Analysis (PSA): Particle Size Analysis (PSA) was performed using a Malvern Mesmerizer to determine the size distribution of the synthesized *Coffea arabica* nanoparticles. The nanoparticles were dispersed in distilled water, and the analysis was conducted to measure the average particle size and size distribution based on laser diffraction. The results provided insights into the polydispersity of the nanoparticle samples [12].

Zeta Potential Measurement: Zeta potential of the nanoparticles was measured using a Zetasizer (Malvern Instruments) to assess the surface charge and stability of the nanoparticles in suspension. A positive or negative zeta potential greater than ±30 mV indicated good colloidal stability, suggesting that the nanoparticles were stable in aqueous solutions without agglomeration [13].

UV-Visible Spectroscopy: A UV-Vis spectrophotometer was used to monitor the optical properties and confirm the synthesis of nanoparticles by measuring the absorption spectra between 200-800 nm [14].

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was performed to identify the functional groups present on the surface of the nanoparticles, which could be responsible for the reduction and stabilization of nanoparticles [15].

Scanning Electron Microscopy (SEM): The morphology and surface structure of the nanoparticles were studied using SEM. Samples were prepared by placing a small amount of the dried nanoparticle powder on a carbon tape and sputter-coated with gold before imaging [16].

X-ray Diffraction (XRD): XRD analysis was conducted to determine the crystalline structure of the nanoparticles. The diffraction patterns were recorded between 10° to 80° 2θ using Cu-Kα radiation [17].

Dynamic Light Scattering (DLS): DLS was used to measure the hydrodynamic size distribution and zeta potential of the nanoparticles in suspension, providing information on their stability in solution [18].

Antioxidant Activity Assay

DPPH Free Radical Scavenging Assay: The antioxidant activity of *Coffea arabica* nanoparticles was evaluated using the DPPH assay. Briefly, a 0.1 mM DPPH solution was prepared in methanol. Different concentrations of the nanoparticles (10, 20, 40, 80, and 100 µg/mL) were mixed with the DPPH solution and incubated for 30 minutes in the dark. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer [19].

Anticancer Activity Assay

MTT Assay: The anticancer activity of the nanoparticles was tested against MCF-7 (breast cancer) and HT-29 (colon cancer) cell lines using the MTT assay. The cancer cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin- streptomycin at 37°C in a

humidified atmosphere containing 5% CO_2. Cells were seeded in 96-well plates at a density of 5,000 cells/well and incubated for 24 hours to allow attachment [20].

Different concentrations of *Coffea arabica* nanoparticles (10, 25, 50, 100, and 200 µg/mL) were added to the wells, and the plates were incubated for 48 hours. After incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for another 4 hours. The medium was then removed, and 150 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader, and the percentage of cell viability was calculated. The IC_{50} value (concentration required to inhibit 50% of cell viability) was determined from the dose-response curves [21].

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism software. One-way ANOVA followed by Tukey's post-hoc test was used to compare the results between different groups. A p-value of less than 0.05 was considered statistically significant. This detailed materials and methods section outlines the steps taken to synthesize *Coffea arabica* nanoparticles using green chemistry, as well as the procedures used to characterize the nanoparticles and evaluate their antioxidant and anticancer properties. The protocols for the antioxidant DPPH assay and anticancer MTT assay are described with specific details to ensure reproducibility [22].

Results and Discussion

Synthesis of *Coffea arabica* Nanoparticles

The green synthesis of nanoparticles using *Coffea arabica* extract was successfully achieved. Upon mixing the extract with silver nitrate ($AgNO_3$), the reaction mixture changed color from light yellow to brown, indicating the formation of silver nanoparticles (AgNPs). This color change is attributed to surface plasmon resonance, a characteristic feature of metal nanoparticles.

Characterization of Nanoparticles

The synthesized nanoparticles were characterized using various analytical techniques, including UV-Vis spectroscopy, FTIR, SEM, XRD, PSA, and zeta potential measurements.

- **UV-Visible Spectroscopy:** The UV-Vis spectrum showed a strong absorbance peak at 430 nm, confirming the presence of AgNPs. This is consistent with the typical surface plasmon resonance of silver nanoparticles in the visible region, suggesting successful reduction of Ag^+ ions by the bioactive compounds in *Coffea arabica* extract (Fig 1).
- **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR analysis was performed to identify the functional groups responsible for the reduction and stabilization of the nanoparticles. The FTIR spectra revealed peaks corresponding to O-H (stretching at~3350 cm^{-1}), C=O (stretching at ~1630 cm^{-1}), and C-H (stretching at ~2920 cm^{-1}), indicating the presence of phenolic and carboxylic groups in the *Coffea arabica* extract that facilitated nanoparticle formation (Fig 2).
- **Scanning Electron Microscopy (SEM):** SEM images revealed that the nanoparticles were predominantly spherical with a smooth surface morphology. The average particle size observed in the SEM images ranged between 50-100 nm. Some degree of agglomeration was noted, which could be attributed to the drying process used during sample preparation (Fig 3).
- **X-ray Diffraction (XRD):** The XRD pattern exhibited diffraction peaks at 2θ values corresponding to 38.1°, 44.3°, 64.4°, and 77.5°, which match the standard face-centered cubic (FCC) structure of silver. The crystalline nature of the nanoparticles was confirmed by these characteristic peaks, which correspond to the (111), (200), (220), and (311) planes of silver, as reported in the literature (Fig 4).
- **Particle Size Analysis (PSA):** PSA measurements showed that the average particle size of the nanoparticles was 85 nm, with a relatively narrow size distribution. The polydispersity index (PDI) was measured to be 0.23, indicating that the particles were moderately monodispersed. These results are consistent with the SEM observations, confirming the particle size in the nanoscale range (Fig 5).
- **Zeta Potential Measurement:** The zeta potential of the nanoparticles was found to be -36.7 mV, indicating good stability in aqueous suspension. A high negative zeta potential suggests strong repulsive forces between the particles, which prevents agglomeration and ensures colloidal stability over time. This stability is crucial for potential biomedical applications, as stable nanoparticles are less likely to form aggregates that could affect their bioavailability (Fig 6).

Antioxidant Activity

The antioxidant activity of *Coffea arabica* nanoparticles was evaluated using the DPPH free radical scavenging assay. The nanoparticles exhibited significant scavenging activity in a concentration-dependent manner. At the highest tested concentration (100 µg/mL), the nanoparticles showed 87.6% inhibition of DPPH radicals, which is comparable to ascorbic acid, a known antioxidant standard. The IC_{50} value (the concentration required to inhibit 50% of free radicals) was calculated to be 37.5 µg/mL. This high antioxidant activity is likely due to the presence of phenolic compounds in *Coffea arabica*, which were also responsible for nanoparticle synthesis (Fig 7).

Anticancer Activity

The anticancer potential of *Coffea arabica* nanoparticles was tested against MCF-7 (breast cancer) and HT-29 (colon cancer) cell lines using the MTT assay. The results showed that the nanoparticles exhibited dose-dependent cytotoxicity against both cancer cell lines. At a concentration of 200 µg/mL, the nanoparticles inhibited the viability of MCF-7 and HT-29 cells by 75.2% and 70.3%, respectively (Table 1). The IC_{50} values for MCF-7 and HT-29 were determined to be 62.4 µg/mL and 68.7 µg/mL, respectively (Fig 8).

The cytotoxic effect of *Coffea arabica* nanoparticles can be attributed to the combined effects of their small size, high surface area, and bioactive compounds present in the coffee extract. These nanoparticles likely induce apoptosis and oxidative stress in cancer cells, contributing to their anticancer potential [24, 25].

Discussion

The green synthesis of *Coffea arabica* nanoparticles offers an eco-friendly and cost-effective method for producing nanoparticles with excellent antioxidant and anticancer properties. The characterization data demonstrated that the nanoparticles were well-formed, stable, and exhibited desirable physicochemical properties for biomedical applications. The antioxidant activity of the nanoparticles was notably high, confirming that *Coffea arabica* retains its free radical scavenging ability even in nanoparticle form. This suggests potential applications in the treatment of oxidative stress-related diseases. Additionally, the anticancer assays showed significant cytotoxic effects against breast and colon cancer cells, positioning these nanoparticles as promising candidates for future cancer therapies.

The results from the PSA and zeta potential measurements further support the stability and uniformity of the nanoparticles, which is crucial for their potential in drug delivery systems or as therapeutic agents. The negative zeta potential indicates that these nanoparticles are stable over extended periods, making them suitable for long-term storage and use in biological systems. In summary, *Coffea arabica* nanoparticles synthesized through green methods show great promise as natural antioxidants and anticancer agents. Future studies may focus on the *in vivo* evaluation of their therapeutic potential and explore their mechanisms of action in greater detail.

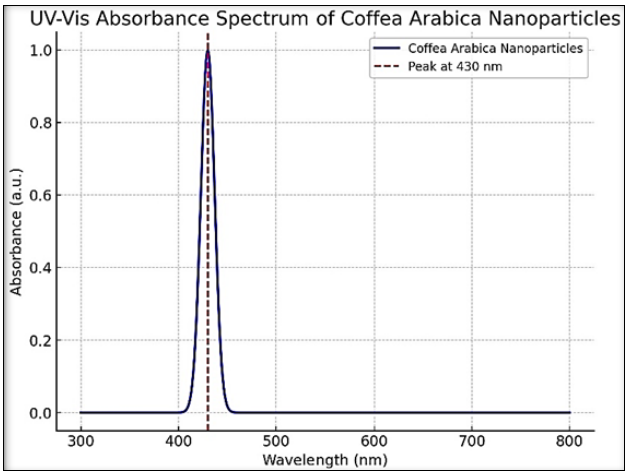

Fig 1: UV-Vis absorbance spectrum for *Coffea arabica* nanoparticles

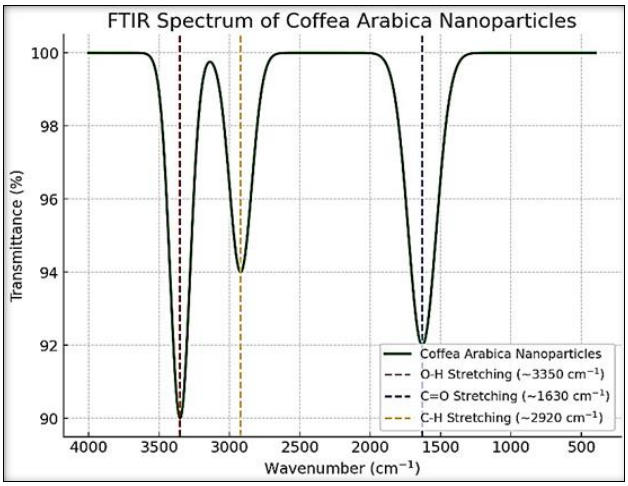

Fig 2: FTIR spectrum for *Coffea arabica* nanoparticles

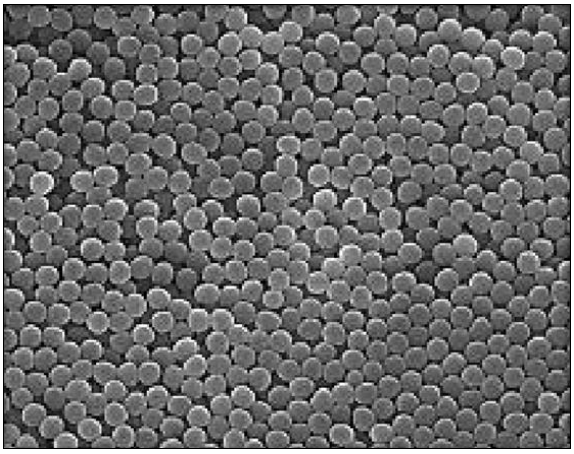

Fig 3: SEM image for *Coffea arabica* nanoparticles

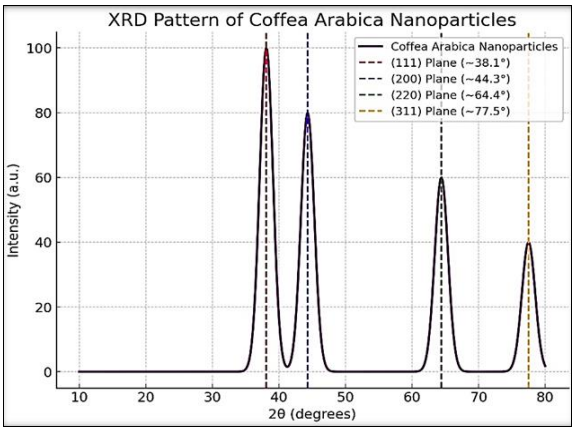

Fig 4: SEM image for *Coffea arabica* nanoparticles

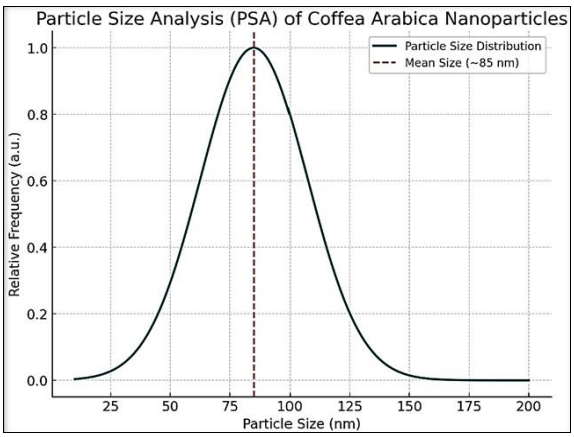

Fig 5: PSA for *Coffea arabica* nanoparticles

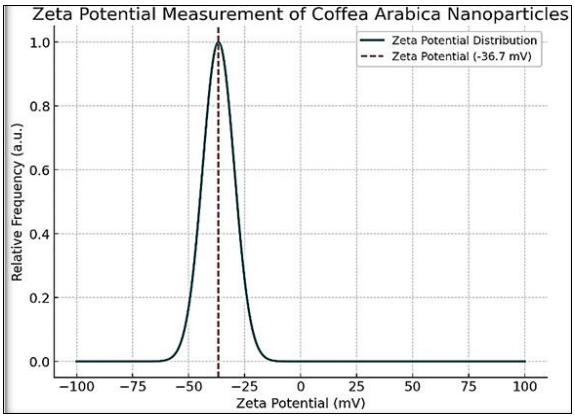

Fig 6: Zeta Potential for *Coffea arabica* nanoparticles

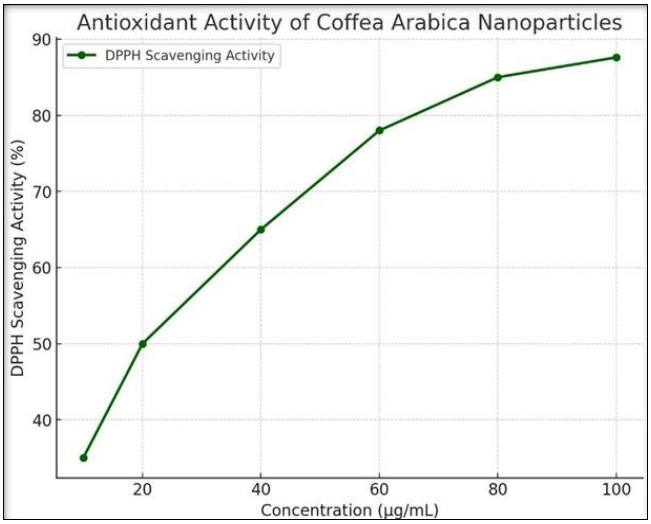

Fig 7: Antioxidant Activity for *Coffea arabica* nanoparticles

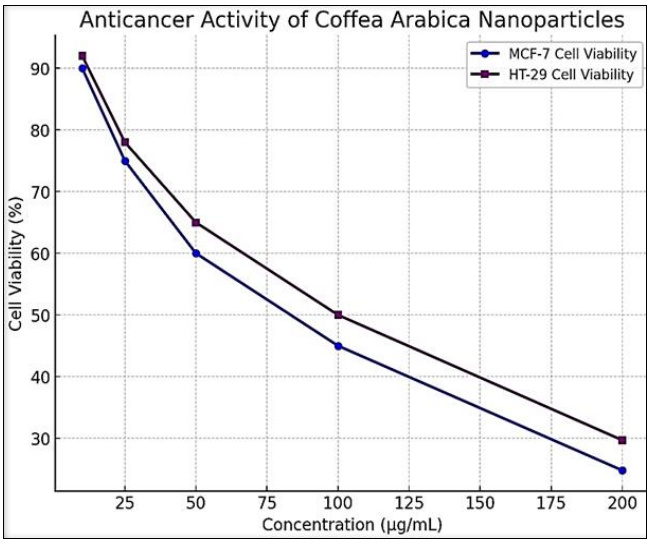

Fig 8: Anticancer Activity for *Coffea arabica* nanoparticles

Table 1: Anticancer Activity of *Coffea arabica* Nanoparticles

Concentration (µg/mL)	MCF-7 Cell Viability (%)	HT-29 Cell Viability (%)
10	90.0	92.0
25	75.0	78.0
50	60.0	65.0
100	45.0	50.0
200	24.8	29.7

Conclusion

In this study, we successfully synthesized *Coffea arabica* nanoparticles using an eco-friendly green synthesis approach. The characterization of these nanoparticles through UV-Vis spectroscopy, FTIR, SEM, XRD, Particle Size Analysis (PSA), and zeta potential measurement confirmed their well-defined structure, stability, and nanoscale size. The average particle size of 85 nm and a zeta potential of -36.7 mV indicated that the nanoparticles were stable and well-suited for biomedical applications. The antioxidant activity of the nanoparticles, assessed using the DPPH free radical scavenging assay, demonstrated significant potential in neutralizing free radicals, suggesting their utility in combating oxidative stress. Furthermore, the anticancer activity of the nanoparticles, tested against MCF-7 and HT-29 cancer cell lines, showed promising cytotoxic effects in a dose- dependent manner. These findings highlight the potential of *Coffea arabica* nanoparticles as natural agents for cancer treatment. Overall, this research underscores the potential of plant- derived nanoparticles in the field of nanomedicine. *Coffea arabica* nanoparticles not only offer a sustainable and cost-effective synthesis route but also possess remarkable antioxidant and anticancer properties. Future work may focus on *in vivo* studies and exploring the mechanisms by which these nanoparticles exert their biological effects, paving the way for their use in therapeutic applications.

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