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ECO-FRIENDLY SYNTHESIS OF CARBON NANOPARTICLES USING *TERMINALIA CATAPPA* SEED OIL: EVALUATION OF ANTICANCER ACTIVITY IN LUNG CANCER

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Abstract

Recent developments in nanobiotechnology have positioned it as an important approach for present-day disease diagnosis and therapy. In this work, oil extracted from the seeds of *T. catappa* was processed through thermal decomposition to obtain carbon nanoparticles (CNPs). The synthesized particles were verified and studied using multiple analytical techniques, including UV–Vis spectroscopy, FT-IR, scanning electron microscopy (SEM), atomic force microscopy (AFM), and X-ray diffraction (XRD). The anticancer potential of the CNPs was evaluated against the A549 human lung cancer cell line. The half-maximal inhibitory concentration (IC_{50}) was determined to be 148.193 ± 3.099 µg/mL, indicating a moderate level of cytotoxicity. These findings suggest that carbon nanoparticles derived from *T. catappa* seeds may hold promise as a supportive strategy in lung cancer therapy. Furthermore, this study summarizes current progress in the field and highlights the potential of such plant-based CNPs for future anticancer drug development.

Keywords: Carbon nanoparticles (CNPs), anticancer- MTT assay, Anticancer therapy, A549 cell line, Eco-friendly nanoparticles, Biomedical applications

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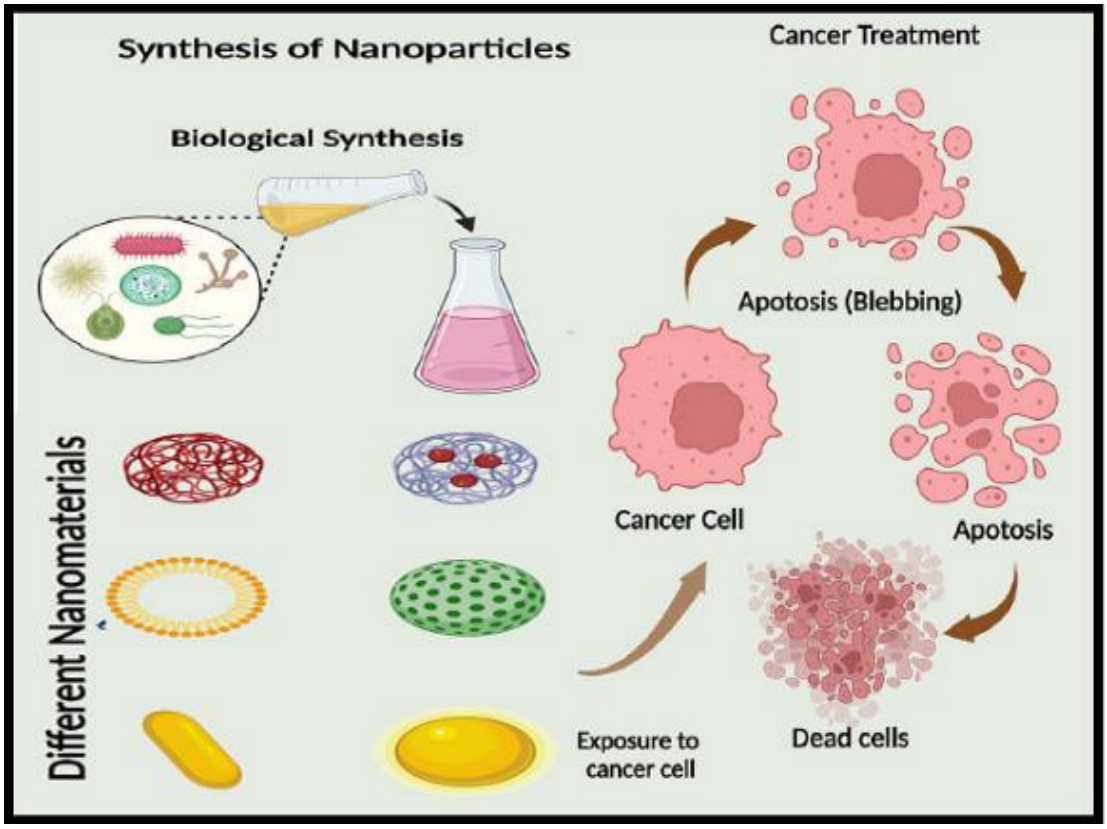
Graphical Abstract:



1. Introduction:

One in six people (or 9.6 million people) died from cancer in 2018 alone, making it one of the major causes of death; yet, 70% of cancer-related fatalities take place in middle- and low-income nations (Bray et al., 2018). The most popular methods for managing and treating cancer are hormone therapy, radiation therapy, chemotherapy, and surgery. Nanotechnology-based therapeutic and diagnostic methods have demonstrated promise in recent years to enhance cancer treatment (Vickers, 2004). A more recent frontier of multidisciplinary study encompassing chemistry, medicine, engineering, and biology, with an emphasis on significant advancements in cancer detection, diagnosis, and therapy, was opened by cancer nanotechnology. In recent years, nanoparticles (NPs) have received an amazing scientific attractiveness due to their large surface area to volume ratio and remarkable reactivity with unmatchable features. Recently, nanotechnology-based anticancer medications, such as Abraxane® (Celgene, Summit, NJ, USA), Doxil® (Johnson & Johnson, New Brunswick, NJ, USA) and Myocet (Perrigo, Dublin, Ireland), have been licensed by the US Food and Drug Administration for clinical use(Nguyen, 2011).

Throughout history, plants and their derivatives have been utilised, and as a result of empirical knowledge, they have grown in significance to humanity. People could relate the effects of using certain plants thanks to the observations made about the behaviour of animals that consumed plants, creating a library (Süntar, 2020). When plants are used to treat and cure diseases, they are referred to as medicinal. These plants provide the sole means of treating

particular diseases in various regions of the world (Alasmari, 2020).The usage of medicinal plants has a direct connection to common knowledge that has been widely propagated based on empirical evidence without scientific support. This approach seeks to reduce reliance on the conventional healthcare system, adopt less expensive alternatives, and prevent and manage illnesses (Alcantara et al., 2015). Despite this, each region's traditional knowledge is typically passed through over the years of use and should therefore be taken into consideration and further examined (Fitzgerald et al., 2019).

Indian almond, or *Terminalia catappa* L., is part of the Combretaceae family. The Latin word "terminalis", which describes the tendency of the leaves to be crowded at the extremities of the branches, is the source of the generic name. Large and spreading, this tree species is typically found in coastal communities and tropical climates (Ladele et al., 2016). Along sandy seashores, it offers a variety of functions such as avenue, shade, and ornamental trees in addition to delicious nuts (Phulwaria et al., 2012). Tropical almond is an underutilised fruit that is high in vitamins, antioxidants, and pytochemicals (Weerasekara et al., 2015). *T. catappa's* phytoconstituents' ability to quench free radicals is what gives it its anti-tumorogenic properties (Pandya et al., 2013). Additionally, it contains an extensive amount of tannins that have anti-diabetic effects (Omar et al., 2021) Inhibiting quorum sensing in bacterial populations, which in turn limits biofilm development, is a critical function of a tannin-rich fraction from *T. catappa* (Taganna et al., 2011).

NPs are defined as particles with at least one dimension less than 100 nm (Bensebaa, 2013). Their surface geometry, which establishes the area/volume ratio, gives rise to their chemical (such as catalytic activity augmentation) and physical (such as plasmon resonance and fluorescence enhancement) characteristics (Yan et al., 2019) In comparison to bulk materials with larger dimensions, the surface area of a spherical particle grows proportionately to the square of its diameter when the NPs diameter lowers, leading to an increase in surface activity. In certain instances, a larger area coupled with a smaller size might enhance material biocompatibility and Drug Administration approval for clinical use (Li et al., 2013).

The US Department of Food and Drug Administration has certified metallic nanoparticles as a safe medicinal ingredient. Because they are harmless and biocompatible (Li et al., 2012) metallic nanoparticles have also been utilised as drug carriers (Kreuter, 1991)cosmetics (Meng, 2024) and medical fillers (HJ et al., 2022)strong chemical stability (Khan et al., 2019), low dielectric constant (Merza & Mousa, 2022), strong catalytic activity (Kvitek et al., 2021), absorption of UV and infrared light (Heath & Poggel, 2021), and above all, antibacterial qualities (Brandelli et al., 2017) are some of the unique characteristics of metallic nanoparticles. This could be a major advancement in cancer treatments if the chemicals' therapeutic and anticancer effects are verified (Borm et al., 2006)

Carbon nanoparticles consist of 2D materials, including graphene, nanotubes, and carbon quantum dots (Moulahoum et al., 2023). Because of their superior ability to carry and deliver a variety of drugs to living cells, carbon nanoparticles have attracted a lot of attention among the different forms of carbon (Jayaprakash et al., 2024). Because they can enter the cellular membrane non-invasively owing to their intrinsic shape. Covalent and non-covalent

bonds typically bind medicinal molecules to the carbon nanoparticles' functional walls (Kadhum et al., 2024).

Numerous methods for the biogenic or biological formulation of nanomaterials from the salts of various metal ions have been discovered (Pandit et al., 2022). Using an environmentally acceptable solvent system with ecologically friendly stabilising and reducing agents allows for the synthesis of nanoparticles under strictly "green" principles (Gutowska et al., 2001)

The current study evaluated the synthesis of carbon nanoparticles using *Terminalia catappa* seed oil extract and their effects on lung cancer cell lines. The biosynthesized nanoparticles were characterized for their size, morphology, and functional groups. The study highlighted their potential cytotoxic effects against lung cancer cells. These nanoparticles demonstrated promising anticancer activity, possibly due to bioactive compounds present in the seed oil. The eco-friendly synthesis method also offers a sustainable approach for nanoparticle production.

2. Methods

2.1 Seed oil preparation:

The seed kernels used to extract oil come from the tropical almond tree, which is widely available in the region. The *Terminalia catappa* seeds were carefully separated from their kernels, cleaned, left to dry in the sun to reduce moisture content, crushed into a fine powder, and then used for extraction.

2.2 Seed Oil Extraction:

To improve oil extraction efficiency and lower moisture content, *Terminalia catappa* seeds are first roasted without oil. Roasting seed facilitates the release of oil by dissolving the cell structure (Arab et al., 2022).After roasting, the seeds are ground into a coarse paste or powder, which increases surface area and boosts the effectiveness of oil extraction. The cell walls are then further broken down by heating the powdered seed paste in a jar with a thick bottom. By softening the mixture, this heating step makes it easier to extract oil. The oil starts to separate from the solid residues as the paste heats up and becomes a coarse mixture. Next, a muslin cloth is placed over the heated mixture to act as a filter, allowing the oil to pass through while retaining the solid residue. The liquid oil is successfully separated from the remaining solids by pressing or squeezing the muslin cloth. After extraction, the oil is collected and examined.

2.3. Preparation of Carbon Nanoparticles

Terminalia catappa seed oil was used for soot collection. This oil was poured into a mud lamp, containing a cotton wick immersed in oil. This wick was left to soak for 2 hours to ensure better absorption of the oil. After this period, the lamp was ignited and allowed to burn. The collected soot was periodically scraped off using a blade and stored in a plastic bag. Once purified and dried, the carbon nanoparticles were ground into a fine powder and stored in air-tight glass vials for further use.

2.4 Instrumentation

Carbon nanoparticles (CNPs) were made from the seed oil of *Terminalia catappa.* The study of synthesized carbon nanoparticles was confirmed by using several methods, including UV-visible spectroscopy (Jasco V 750), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), Atomic force microscopy (AFM), and powder X-ray diffraction (XRD).

2.5 Measurement of cell toxicity of CNPs

The cytotoxic effect of the synthesized carbon nanoparticles (CNPs) was assessed on the A549 lung cancer cell line following the procedure described by Mosmann (1983), using the MTT colorimetric assay. For the assay, viable A549 cells were harvested, counted with a hemocytometer, and adjusted in Dulbecco's Modified Eagle Medium (DMEM) to a density of 1×10^4 cells/ml. The cells were seeded into 96-well plates and incubated for 24 h to permit adherence. After this period, the cells were exposed to varying concentrations of CNPs (50–175 µg/ml) and incubated again under controlled conditions (37 °C, 5% CO_2, and humidified atmosphere) for 24 h.

Following treatment, the culture medium was replaced, and MTT solution (5 mg/ml in phosphate-buffered saline) was added to each well. After 4 h of incubation at 37 °C, the resulting purple formazan crystals were solubilized with 100 µl of dimethyl sulfoxide (DMSO). Absorbance was then recorded at 540 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls.

$$Inhibitory\ of\ cell\ proliferation\ (\%) = \frac{Mean\ absorbence\ of\ the\ control - Mean\ absorbence\ of\ the\ sample}{Mean\ absorbence\ of\ the\ control}\ X\ 100$$

The IC_{50} values were obtained from the dose–response curve, corresponding to the concentration that inhibited 50% of cell viability compared to vehicle control cells. All experiments were conducted in triplicate to ensure reproducibility.

2.6 Measurement of apoptotic induction using acridine orange/ethidium bromide (AO/EB)

Apoptotic cell death was evaluated using fluorescence microscopy, following the procedure of Baskic et al. (2006).

A549 cells (5×10^4 cells/well) were seeded in 6-well culture plates and allowed to adhere for 24 h. After this period, cells were treated with CNPs at concentrations of 125 and 150 µg/ml for 24 h. Post-treatment, the cells were gently detached, rinsed with chilled phosphate-buffered saline (PBS), and stained with a freshly prepared mixture of acridine orange (AO, 100 µg/ml) and ethidium bromide (EB, 100 µg/ml) in a 1:1 ratio. The staining was performed at room temperature for 5 min. The labeled cells were then immediately examined under a fluorescence microscope at 20× magnification. For further confirmation, the cells were washed three times

with PBS, restained with AO/EB (1:1 ratio, 100 µg/ml), and directly visualized using fluorescence microscopy to distinguish live, apoptotic, and necrotic populations.

3. Results and discussions

3.1. Characterization of CNPs:

The optical characteristics of the synthesized sample were analysed using a Jasco V-750 UV–Visible spectrophotometer. Absorption signals in the region of 278–290 nm corresponded to single-walled carbon nanotube (SWCNT) features, while the overall spectrum confirmed the reduction of carbon nanoparticles.

FTIR analysis provided information on surface functional groups associated with the nanoparticles. Prominent absorption bands were detected in the hydroxyl (O–H) stretching region at 3691.37, 3631.85, 3749.94, 3732.80, and 3724.87 cm^{-1}, representing alcohol-related functionalities. Additionally, C–H stretching vibrations typical of alkanes were observed as a medium peak at 2925.49 cm^{-1}.

The crystalline structure and particle size of the carbon nanoparticles were further evaluated by powder X-ray diffraction (XRD). Analysis confirmed that the biosynthesized nanoparticles originated from *Terminalia catappa* seed oil and exhibited an average size of approximately 14.83 nm, with nearly all particles falling within this dimension.

Morphological features were visualized using scanning electron microscopy (SEM). The micrographs demonstrated predominantly spherical particles, with particle clusters ranging between 300–500 nm when viewed under magnifications of 30 kx, 25 kx, and 20 kx at scales of 300, 400, and 500 nm, respectively.

Elemental composition was determined by energy-dispersive X-ray spectroscopy (EDX). The theoretical stoichiometric distribution of elements was estimated as carbon (91.90%), oxygen (7.50%), aluminum (0.24%), chlorine (0.15%), and rubidium (0.21%), confirming the elemental profile of the synthesized nanoparticles.

3.2 Measurement of cell toxicity of CNPs

The cytotoxicity of the sample on A549 cells was determined by the method of Mosmann, (1983).

Figure 1 illustrates morphological alterations in A549 lung cancer cells following exposure to CNP sample L1 (125 and 150 µg/ml, 24 h). Compared to untreated controls, which retained normal healthy morphology, treated cells exhibited features typical of apoptosis and cell damage, including reduced size, partial detachment, membrane irregularities, and distorted cellular outlines. Images were captured using a Bio-Rad fluorescence microscope.

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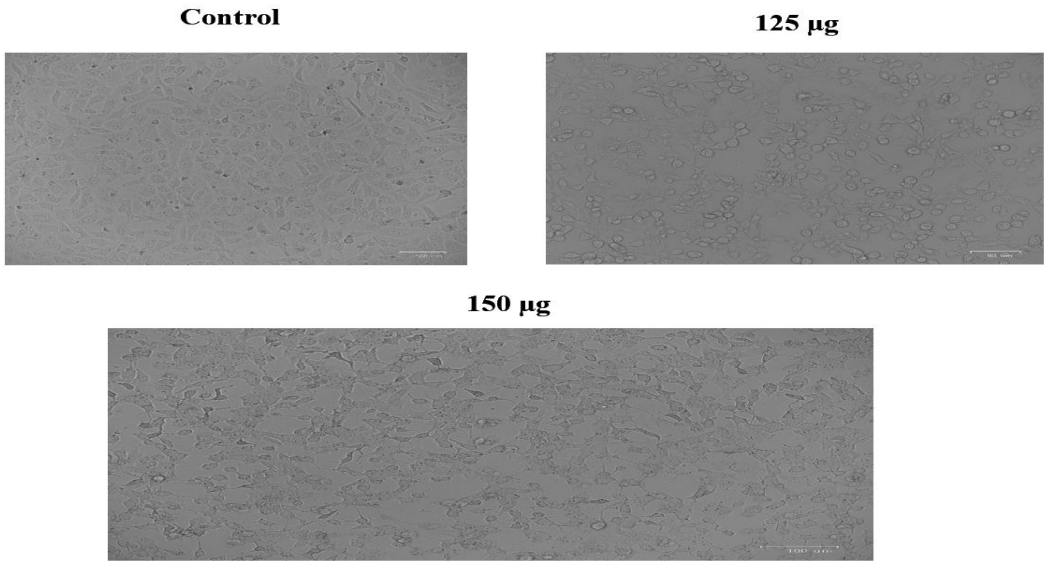


Figure 1: Morphological changes in control and sample-treated Lung Cancer (A549) cells for 24 h.

Similar observations were reported by(Maurya et al., 2011), where A549 cells exposed to gallic acid (100–400 µM) for 24 h displayed progressive shrinkage and loss of cell integrity. Their study also confirmed that total cell counts and viability decreased in a dose-dependent manner.

Cell viability testing in the present work further demonstrated that survival decreased as CNP concentration increased. The untreated control retained close to 100% viability, while treatment at the highest dose (175 µg/ml) reduced viability to approximately 29.55%, reflecting pronounced cytotoxicity.

The inverse measure, cell growth inhibition, showed a steady rise with dose, starting at ~10% inhibition for 50 µg/ml and reaching ~73.6% at 175 µg/ml. This confirms a strong dose-dependent anticancer effect of the nanoparticles.

Figure 2 presents the bar chart of cell viability, showing a progressive decline from control to the highest dose.

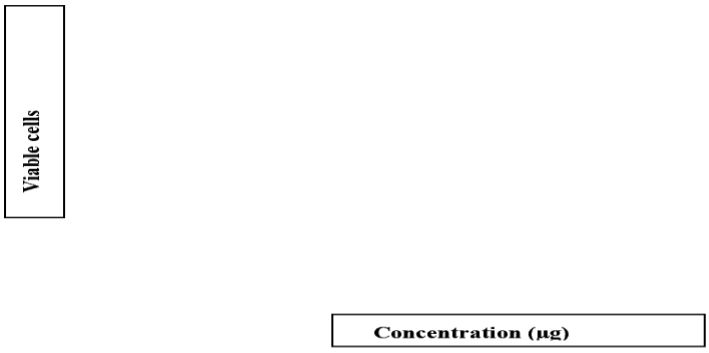


Figure: 2 Cell viability

Figure 3 demonstrates a linear increase in inhibition percentage with rising nanoparticle concentration.

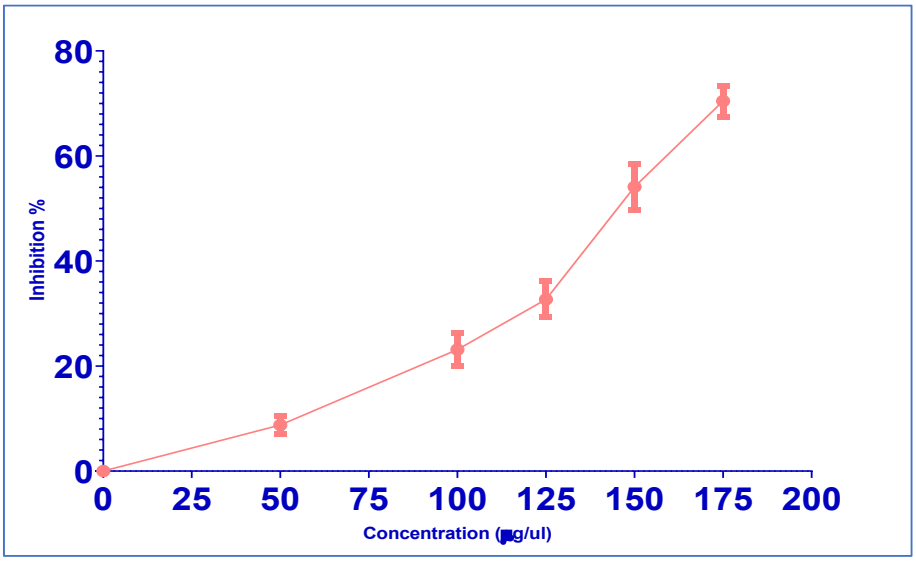


Figure: 3 Dose dependent relationship

Figure 4 highlights the IC_{50} value, calculated as 148.193 ± 3.099 µg/ml, representing the concentration required to suppress 50% of cell viability. This value indicates that the biosynthesized CNPs exhibit moderate anticancer potential, since lower IC_{50} values generally reflect higher potency.

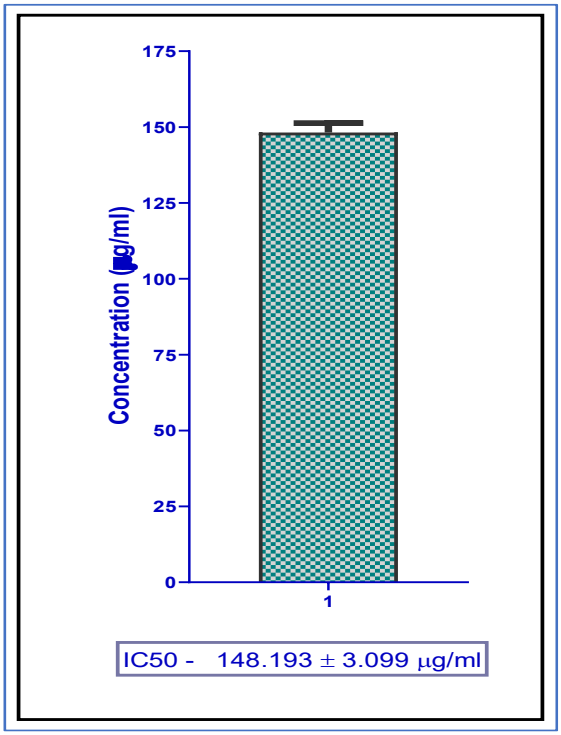


Figure:4 CNP IC50 value

The carbon nanoparticles synthesized from *Terminalia catappa* seed oil exhibited a clear, dose-dependent inhibitory effect on A549 lung cancer cells. Increasing concentrations led to progressive loss of viability and higher inhibition rates, confirming their cytotoxic potential. The calculated IC_{50} value further substantiates their anticancer efficiency by identifying the concentration required to suppress half of the cell population(Warnasih et al., 2024).

Comparable evidence has been reported by (Chu et al., 2007), who examined the biological effects of *T. catappa* water extract. While the extract (0–100 µg/ml) did not significantly affect the viability of A549 cells, it displayed cytotoxicity against Lewis lung carcinoma (LLC) cells, with an IC_{50} of 14.5 µg/ml. Invasion and motility assays revealed a dose-dependent reduction in A549 cell migration, with only 24.8% and 28.8% of cells retaining invasive and motile capacity at 100 µg/ml after 24 h.

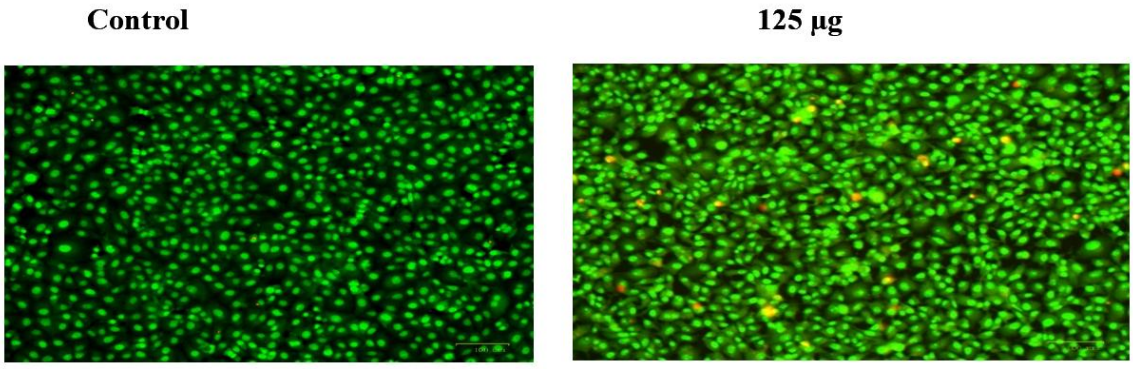
Animal studies reinforced these findings: in LLC-bearing C57BL/6 mice, oral administration of the extract reduced lung metastasis by 68% relative to untreated controls. After 30 days, treated mice showed a 2.6-fold decrease in small solid tumors, a 2.3-fold reduction in tumor mass, and no observable systemic toxicity, as indicated by stable body weight. Together, these findings highlight the potential of *T. catappa-derived* preparations, both seed oil-based nanoparticles and aqueous extracts, as promising candidates for lung cancer therapy and metastasis prevention.

3.3 Measurement of apoptotic induction using acridine orange/ethidium bromide (AO/EB) dual staining method

Apoptotic changes in A549 cells were examined using the AO/EB dual-staining approach described by Baskic et al. (2006).

Cells treated with CNPs (125 and 150 µg/ml) for 24 h were stained and visualized under a fluorescence microscope (ZOE Fluorescent Cell Imager, Bio-Rad). The staining patterns revealed distinct stages of cell death: viable cells emitted green fluorescence with intact nuclear morphology; early apoptotic cells appeared yellow due to chromatin condensation and nuclear fragmentation; and late apoptotic or necrotic cells displayed intense orange-to-red fluorescence with disrupted nuclear integrity.

Figure:4 CNP IC50 value



150 µg

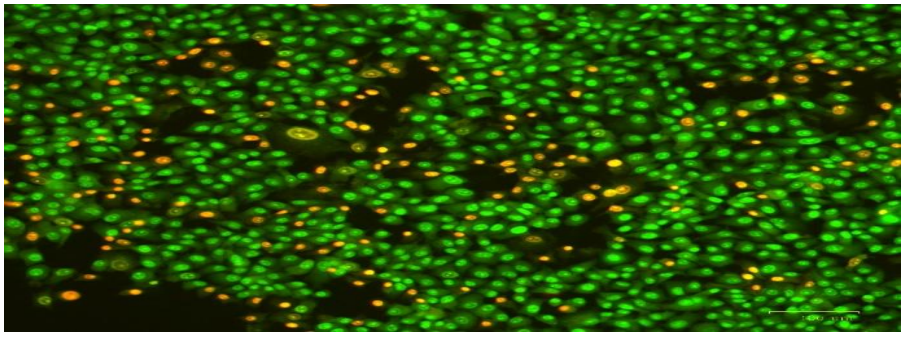


Figure 5 Apoptotic Staining – Control & sample treated Lung Cancer A549 cells for 24 h.

In **figure 5** the control group, cells exhibited uniform green fluorescence, confirming healthy, intact morphology with negligible apoptosis. At 125 µg/ml, a mixed population of viable (green), early apoptotic (yellow), and a few late apoptotic (orange/red) cells was observed, suggesting the initiation of programmed cell death at this dose. Treatment with 150 µg/ml led to a marked reduction in green cells and a pronounced increase in orange/red-stained nuclei, indicative of advanced apoptosis or necrosis. These findings demonstrate a clear dose-dependent cytotoxic effect of CNPs, with progressive apoptotic induction at higher concentrations.

The results are consistent with earlier studies. (Zarredar et al., 2021), reported that TCE exposure altered apoptosis-associated genes and microRNAs in A549, MCF-7, and MDA-MB-231 cells. Similarly, (Shanehbandi et al., 2021) showed that TCE inhibited proliferation and promoted apoptosis in SW480 cells, as confirmed by MTT assay, Annexin V/PI staining, and gene expression profiling. (Majoumouo et al., 2020) further demonstrated that *T. catappa*-derived endophytic fungal extracts induced apoptosis in HeLa cells, characterized by late apoptotic markers detected via flow cytometry.

Collectively, the present study aligns with these findings, reinforcing that biosynthesized CNPs induce apoptosis in lung cancer cells through a dose-dependent mechanism.

4. Conclusion

The present study demonstrates the successful synthesis and characterization of carbon nanoparticles (CNPs) derived from *Terminalia catappa* seed oil. Spectroscopic analysis confirmed the formation of single-walled carbon nanotubes (SWCNTs), with UV–Vis absorption observed in the 200–600 nm region. X-ray diffraction (XRD) patterns displayed a broad peak at $2\theta = 20.47$, indicating their nanocrystalline nature. Scanning Electron Microscopy (SEM) images showed predominantly spherical nanoparticles with an average diameter of 14.83 nm, while Energy Dispersive Spectroscopy (EDS) confirmed a high carbon content (91.90%) along with traces of oxygen, aluminum, chlorine, and rubidium. Atomic Force Microscopy (AFM) revealed considerable surface roughness, characterized by negative skewness (valley-dominated features) and high kurtosis (sharp surface features). These results highlight the eco-friendly synthesis of pure carbon nanoparticles from T. catappa seed oil,

suggesting their potential for applications in sustainable electronics, biomedicine, and advanced materials.

In addition, the biosynthesized CNPs exhibited significant anticancer potential, which can be attributed to the inherent pharmacological activity of *T. catappa.* Notably, the nanoparticles displayed anticancer efficacy without the need for external molecular doping, emphasizing their intrinsic therapeutic value. This study highlights the potential of developing nanomaterials influenced by ethnopharmacology, linking traditional medicinal wisdom with contemporary nanoscience to formulate innovative anticancer solutions.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used chat gpt / quill bot in order to proof read English sentences and crosscheck the results. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- Tropical almond oil was successfully extracted from *Terminalia catappa* seeds, offering a sustainable source for nanomaterial synthesis.
- Carbon nanoparticles (CNPs) were synthesized using a traditional mud lamp method, ensuring an eco-conscious and cost-effective fabrication process.
- The synthesized CNPs were thoroughly analyzed using UV-Vis spectroscopy, FTIR, XRD, SEM with EDAX, and AFM, confirming their structural, morphological, and elemental properties.
- The synthesized carbon nanoparticles showed significant cytotoxic effects against human lung cancer cell lines (e.g., A549), indicating promising anticancer activity.
- The findings support further exploration of CNPs as drug delivery systems or therapeutic agents in cancer treatment.

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