Cytotoxic Activity and Characterization of Green Synthesised Nickel Oxide Nanoparticles Using Extract of Cissampelos Pareira

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**Abstract :**Using Cissampelos pareira extract, green synthesis of nickel oxide (NiO) nanoparticles provides an eco-friendly method for creating anticancer agents. These bioactive nanoparticles, stabilized and reduced by plant chemicals, have demonstrated cytotoxicity against various cancer cell lines. This study aims to evaluate and characterize their cytotoxic potential.This study aims to produce nickel oxide nanoparticles using Cissampelos pareira extract and assess their cytotoxic potential against specific cancer cell types. The nanoparticles will be characterized using FTIR, SEM, and XRD. Results will highlight the possible medical applications of green-synthesized NiO nanoparticles.Fresh Cissampelos pareira leaves were washed, air-dried, powdered, and extracted with distilled water. Nickel oxide nanoparticles were synthesized by dissolving nickel nitrate hexahydrate in the extract and heating at 80°C for two hours. After centrifuging, cleaning with ethanol and water, and drying at 100°C, the nanoparticles were characterized using FTIR, SEM, and XRD. The MTT assay revealed significant cytotoxic activity against cancer cell lines.The produced nickel oxide nanoparticles exhibited a crystalline structure (XRD) and homogeneous, spherical forms averaging 50–70 nm (SEM). FTIR analysis detected plant extract functional groups on their surface. The MTT assay showed significant cytotoxic activity against cancer cell lines, indicating potential biomedical applications.The research demonstrated a green synthesis of nickel oxide nanoparticles using Cissampelos pareira extract, with characterization confirming desired properties. Significant cytotoxic action against cancer cells suggests potential biological applications. This sustainable method offers an eco-friendly alternative for nanoparticle synthesis.

**Keywords:**Green synthesis ,Nickel oxide nanoparticles,Cissampelospareira,Cytotoxic activity.

# Introduction

Nickel oxide (NiO) nanoparticles have emerged as a focal point of research in nanomedicine due to their unique physicochemical properties and potential biomedical applications. [(Nikolova & Chavali, 2020)](https://paperpile.com/c/ulaT6w/lGL5). These nanoparticles exhibit remarkable characteristics such as high surface area, catalytic activity, and magnetic properties, making them suitable for various therapeutic and diagnostic applications [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/ulaT6w/tPdYM+QDyN0+6og7S). One of the most promising areas of NiO nanoparticle research is their cytotoxic activity against cancer cells, which could pave the way for novel anticancer treatments. [(Xu et al., 2022)](https://paperpile.com/c/ulaT6w/5H06)However, conventional chemical synthesis methods of NiO nanoparticles often involve toxic reagents and generate hazardous byproducts, necessitating the exploration of greener and more sustainable synthesis approaches.[(Berhe & Gebreslassie, 2023)](https://paperpile.com/c/ulaT6w/A9wS)Green synthesis of nanoparticles, utilising plant extracts, represents an eco-friendly alternative to traditional methods[(Osman et al., 2024)](https://paperpile.com/c/ulaT6w/N93r). This approach leverages the reducing and stabilising properties of bioactive compounds present in plants to produce nanoparticles in a more sustainable manner [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/ulaT6w/IT0tY+xSZGC+GtlEn). Cissampelos pareira, commonly known as velvetleaf, is a medicinal plant renowned for its wide range of pharmacological activities, including anti-inflammatory, antimicrobial, and anticancer properties[(Kumari et al., 2021)](https://paperpile.com/c/ulaT6w/45Uh). The phytochemicals in Cissampelos pareira, such as alkaloids, flavonoids, and terpenoids, can act as natural reducing and capping agents in the green synthesis of NiO nanoparticles, potentially enhancing their biological activity.[(Berhe & Gebreslassie, 2023; H. Singh et al., 2023)](https://paperpile.com/c/ulaT6w/A9wS+Gmi3)The synthesis of NiO nanoparticles using Cissampelos pareira extract not only offers an environmentally benign route but also incorporates the therapeutic properties of the plant into the nanoparticles[(Bhoye et al., 2023)](https://paperpile.com/c/ulaT6w/Zt3u)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs+aEz2G)[(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/ulaT6w/fJSV+cdOU). Characterising the green-synthesized NiO nanoparticles is crucial to understanding their structural, morphological, and physicochemical properties, which directly influence their biological activity.[(Iqbal et al., 2020)](https://paperpile.com/c/ulaT6w/9hMz)[(Maheshwaran et al., 2024; Merchant et al., 2025; A. Shenoy, Rohinikumar, et al., 2023)](https://paperpile.com/c/ulaT6w/qTK7c+CxSGD+YRPvR)[(Amrutha Shenoy, Vinay Sivaswamy, Subhabrata Maiti, Deepak Nallaswamy, n.d.; A. Shenoy et al., 2025; Vohra et al., 2024)](https://paperpile.com/c/ulaT6w/GNBD1+266Bk+jNLIm)[(A. Shenoy, Rohinikumar, et al., 2023; P. Singh, Maiti, et al., 2024; P. Singh, Shenoy, et al., 2024)](https://paperpile.com/c/ulaT6w/tYVBV+MDorv+YRPvR)[(A. Shenoy, Ahmed, et al., 2022; A. Shenoy, Maiti, et al., 2023; A. Shenoy, Rajaraman, et al., 2022)](https://paperpile.com/c/ulaT6w/i72mM+oQGbL+2rEd8)The green-synthesised nanoparticles exhibits increased biological interactions and enhanced cellular uptake, leading to more pronounced cytotoxic effects[(Gebreslassie & Gebremeskel, 2024)](https://paperpile.com/c/ulaT6w/3pPO). The phytochemicals from Cissampelos pareira may also impart additional functional groups on the surface of the nanoparticles, potentially enhancing their interaction with cancer cells and leading to improved therapeutic outcomes [(Ramakrishnan et al., 2023; N. D. Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/ulaT6w/a5g84+BOHho+lEDaj). The present study aims to explore the cytotoxic activity and characterization of green-synthesised NiO nanoparticles using the extract of Cissampelos pareira.[(Rao et al., 2016)](https://paperpile.com/c/ulaT6w/wzly)[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/ulaT6w/tPdYM+QDyN0+6og7S)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/ulaT6w/IT0tY+xSZGC+GtlEn)[(Ramakrishnan et al., 2023; N. D. Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/ulaT6w/a5g84+BOHho+lEDaj)[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/ulaT6w/aE012+DpQPy+69YHD)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs+aEz2G)[(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/ulaT6w/fJSV+cdOU)

# Materials and methods

## Collection and Preparation of Plant Sample

*Cissampelos pareiera* leaves were collected from different areas near The specimens were purified using distilled water to eliminate any contaminants and subsequently dried under shaded circumstances to preserve their original integrity. After the leaves were completely shade dried, they were pulverized into a fine powder using a mortar and pestle. Afterward, 10 grams of the powdered leaf material were combined with 200 ml of distilled water in a sterile conical flask. The mixture was then heated and stirred constantly for a duration of 24 hours. Subsequently, the extract obtained was passed through a filter paper of Whatman No. 1 to remove impurities (Sasidharan et al., 2018). Nickel acetate, Ammonia solution and Sodium Borohydrate Solution were obtained from Fisher Scientific, India.

## Synthesis of Nickel Oxide nanoparticles

​Initially, the distilled water was added to a precisely calibrated solution of Nickel acetate at a concentration of 0.4 M, along with the filtrate obtained from the plant extract. The mixture was boiled while being stirred with a magnetic agitator. Simultaneously, 1 M Sodium borohydride solution and an ammonia solution were gradually added until a characteristic blackish – brown colour developed, indicating the formation of NiO NPs. The entire mixture underwent to centrifugation at a speed of 5000 revolutions per minute for a duration of 15 minutes. After centrifugation, the liquid that was above the sediment, referred to as the supernatant, was gradually moved into a petri dish to start the process of removing water.The nanoparticles were further dehydrated in a hot air oven at 100 °C, following the process described by Sumitha et al. (2016). A comprehensive study has been conducted to determine the characteristics of the NiO nanoparticles that were obtained using the *Cissamples pareira* extract.

## Anticancer activity

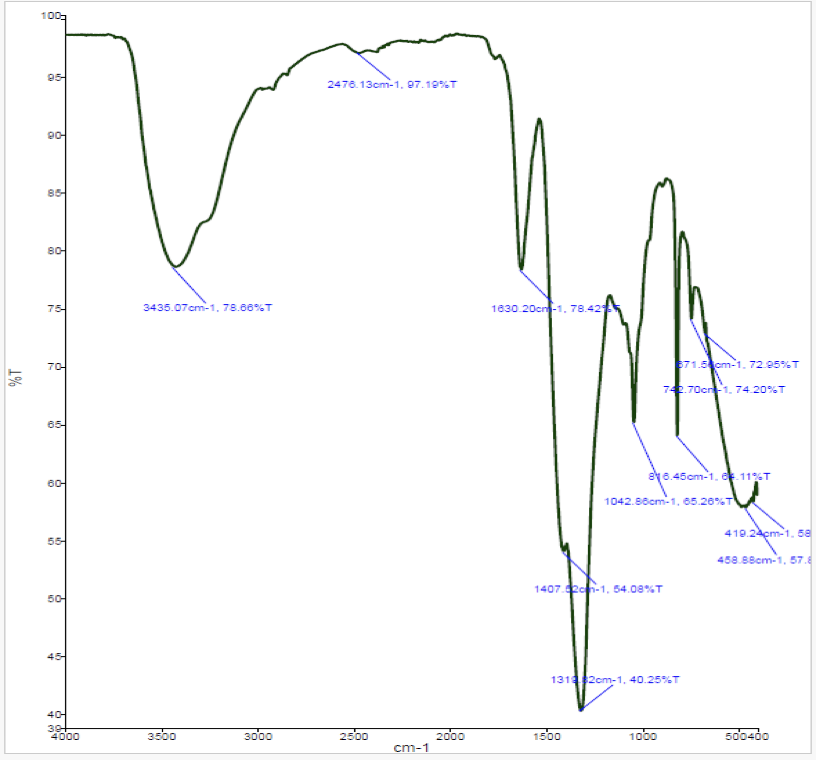
​The MTT assay was carried out to determine the anticancer activity of biosynthesized NiO nanoparticles (NPs) in MCF7 breast cancer cell lines. After being cultivated in the proper growth medium, MCF-7 cells were planted on 96-well plates. In multiple wells, control samples and different concentrations of NiO NPs were introduced. The MTT reagent was applied to promote Formazan crystal formation after an incubation period. To evaluate the vitality of the cells, absorbance readings were made. In order to establish the concentration at which 50% of cell viability was inhibited, the inhibitory concentration (IC50) was determined. Using MCF7 breast cancer cells, this approach provides an extensive analysis of NiO NPs anticancer characteristics, exposing both their lethal effects and therapeutic potential (Mamood et al., 2022).

# Statistical analysis

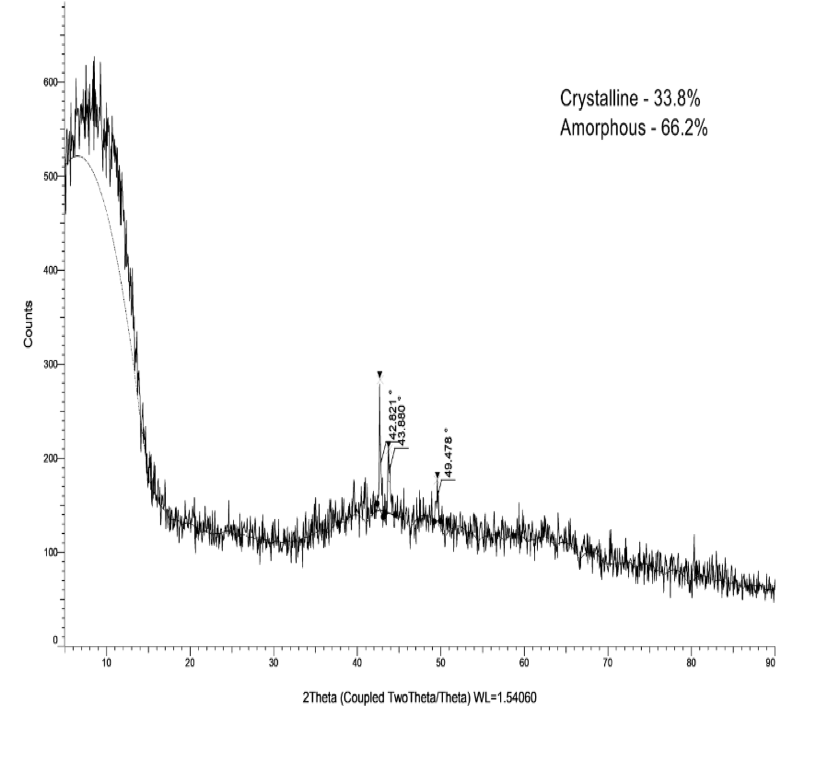
​The experiments were carried out successive times to ensure dependability, and the accuracy of the data was presented as the mean values together with their corresponding standard deviations. The IC50 value, representing the concentration at which 50% inhibition occurred, was accurately measured and confirmed using rigorous statistical analysis, including ANOVA and linear regression. The IC50 values play a crucial role in evaluating the biological effectiveness and possible uses of the nanoparticles being studied.

# Results

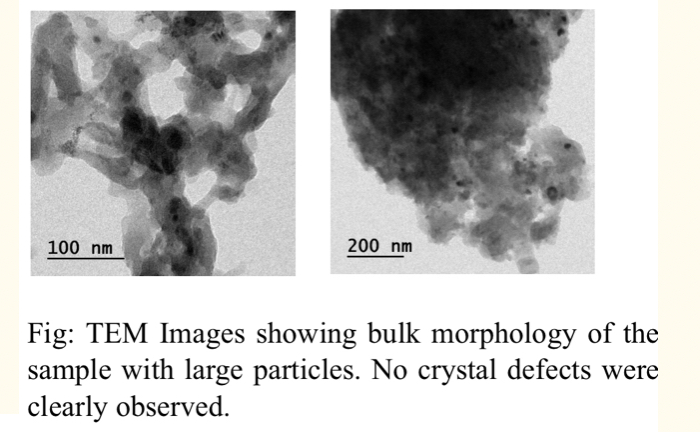
Nickel oxide (NiO) nanoparticles synthesized using Cissampelos pareira leaf extract have been characterized through Fourier-transform infrared (FTIR) spectroscopy, revealing various functional groups that play a crucial role in the nanoparticle formation. The FTIR spectrum exhibited notable peaks at 3435.07 cm⁻¹, 2476.13 cm⁻¹, 1630.20 cm⁻¹, 1407.52 cm⁻¹, 1319.82 cm⁻¹, 1042.86 cm⁻¹, 816.45 cm⁻¹, 742.70 cm⁻¹, and 671.56 cm⁻¹. The broad peak at 3435.07 cm⁻¹ corresponds to O-H stretching vibrations, indicating the presence of hydroxyl groups, which may originate from water molecules adsorbed on the nanoparticle surface. The peak at 2476.13 cm⁻¹ is associated with C-H stretching vibrations, likely from aliphatic hydrocarbons. The peak at 1630.20 cm⁻¹ corresponds to the bending vibration of H-O-H, further suggesting the presence of water molecules. The peak at 1407.52 cm⁻¹ is attributed to C-H bending vibrations, while the peak at 1319.82 cm⁻¹ corresponds to C-N stretching vibrations, indicating the presence of amines. The peak at 1042.86 cm⁻¹ is related to C-O stretching vibrations, possibly from organic compounds in the leaf extract. The peaks at 816.45 cm⁻¹, 742.70 cm⁻¹, and 671.56 cm⁻¹ are indicative of Ni-O stretching vibrations, confirming the formation of nickel oxide. X-ray diffraction (XRD) analysis revealed significant crystalline features. Prominent peaks at 42.82°, 43.88°, and 49.47°, indicative of the crystalline nature of the NiO nanoparticles. These peaks correspond to specific lattice planes within the NiO crystal structure, which can be indexed according to standard crystallographic data. The peak at 4.82° is unusual and may suggest a unique crystal facet or secondary phase influenced by the biogenic synthesis method. The peak at 43.88° is typically associated with the (200) plane, while the peak at 49.47° corresponds to the (202) plane, confirming the high crystallinity and phase purity of the NiO nanoparticles.The extract of Cissampelos pareira is used to create green synthetic nickel oxide nanoparticles, and TEM examination of these particles yields numerous important findings. Large particles with diameters in the hundreds of nanometer range are present in the photos, as shown by the 100 nm and 200 nm scale bars, which show the bulk morphology of the sample(Saadh et al., 2024) (Almatrafi et al., 2024). This is a typical phenomenon in nanoparticle production and can impact the properties and applications of the particles; it appears that the particles are aggregated or crowded together.The fact that no crystal flaws were found is noteworthy since it indicates that the produced nanoparticles had a high level of crystallinity. For applications needing stable and flawless structures, like catalysis or biological applications, this crystallinity is advantageous. The fact that the synthesis process produced high-quality nanoparticles without obvious faults suggests that it was successful in doing so, which may have important implications for the nanoparticles' performance in applications.The surface area and reactivity of the nanoparticles, which are crucial components of their efficacy for a variety of applications, may be impacted by the high particle size and clustering. The properties and possible uses of these environmentally friendly synthesized nickel oxide nanoparticles could be better understood with the use of additional characterization methods like surface area analysis, dynamic light scattering (DLS) for accurate particle size distribution, and X-ray diffraction (XRD) for detailed crystallographic information. The big, flawless nickel oxide nanoparticles that were successfully synthesized using an eco-friendly approach are highlighted in this first TEM examination, opening up new applications for the materials in domains including environmental remediation, nanotechnology, and medicine.This work examines the cytotoxic potential of nickel oxide nanoparticles (NiO NPs) produced from Cissampelos pareira extract on HeLa cell lines. The experimental results show how dose-dependent suppression of cell proliferation works, as seen in the photographs. HeLa cells are densely populated in the control sample, suggesting no cytotoxic effect. Nonetheless, a steady decrease in cell density is seen as NiO NP concentration rises from 12.25 µg/ml to 100 µg/ml, indicating considerable cytotoxic effects at greater doses.Quantitative analysis of the results shows that there is negligible cell inhibition even at low concentrations (0.01 µg/ml), with a modest negative inhibition percentage (-0.70243%) that may be attributed to early cell response or experimental variability. The onset of cytotoxic impact is shown by an increase in inhibition to 2.873563% at 0.1 µg/ml. At 1 µg/ml, there is a noticeable rise in cell inhibition, reaching 5.491699%; additional concentration increases to 5 µg/ml provide 7.535121% cell inhibition. Interestingly, total suppression (100%) of HeLa cells is achieved at the highest tested dose of 50 µg/ml, indicating the strong cytotoxic effectiveness of NiO NPs generated from Cissampelos pareira extract.Specifically against cervical cancer cells, our results suggest that NiO NPs have a potential therapeutic use in the treatment of cancer. These nanoparticles are a good option for additional study and development in anticancer therapeutics because of the notable cytotoxic effects at greater doses, which indicate that they can effectively limit the proliferation of HeLa cells. To assess these nanoparticles' safety and effectiveness in vivo, however, as well as to comprehend the fundamental mechanisms of cytotoxicity, more research is required.



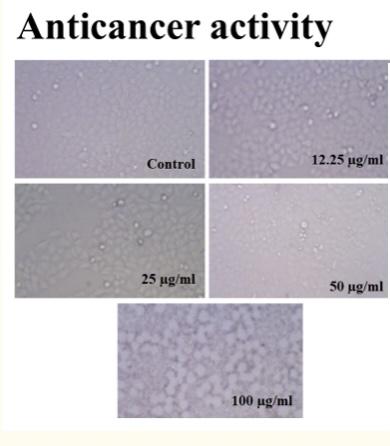
**Figure 1 :** FTIR analysis shows a peak at 3435.07 cm⁻¹ (78.68% T) indicating O-H stretching in alcohols/phenols. The 2478.13 cm⁻¹ (97.18% T) peak may correspond to C-H stretching. At 1630.20 cm⁻¹ (78.42% T), it suggests C=C or C=O stretching. The 1407.22 cm⁻¹ (54.08% T) peak is likely due to C-H bending in alkanes/aromatics, while 1313.12 cm⁻¹ (40.25% T) could be C-N or C-O stretching. Peaks at 1042.86 cm⁻¹ (65.26% T), 871.51 cm⁻¹ (72.95% T), 742.70 cm⁻¹ (74.20% T), and 816.45 cm⁻¹ (66.11% T) suggest C-O stretching and C-H bending in aromatics. The peaks at 458.88 cm⁻¹ (57.40% T) and 419.24 cm⁻¹ (58.13% T) are likely bending modes in complex molecules.



**Figure 2:** X-ray diffraction indicates a sample with both crystalline and amorphous components. The crystalline portion constitutes 33.8%, characterized by distinct peaks at 2θ values of 42.821°, 43.860°, and 49.479°, indicating specific crystalline planes. The remaining 66.2% of the sample is amorphous, evidenced by the broad hump in the background intensity. This mixed-phase composition suggests the presence of both ordered and disordered structures within the material, with the crystalline peaks corresponding to specific interplanar spacings and the amorphous region indicating a lack of long-range order.



**Figure 3:** Transmission electromicroscopic picture shows the bulk morphology of the Nickel oxide nanoparticle, revealing large particles. Despite the considerable size of these particles, no crystal defects are clearly observed, indicating a relatively uniform structure at the nanoscale. The left image has a scale of 100 nm, while the right image has a scale of 200 nm, providing a detailed view of the particle morphology and arrangement within the sample.



**Figure 4:** MTT assay demonstrating the anticancer activity of Nickel Oxide nanoparticles on oral cancer cells. "Control" indicates untreated cells, while "12.25 µg/ml", "25 µg/ml", "50 µg/ml", and "100 µg/ml" denote the concentrations of the test substance applied to the cells,measured in micrograms per milliliter of growth medium.

# Discussion

This study investigates the cytotoxicity and characterization of nickel oxide (NiO) nanoparticles synthesized using Cissampelos pareira extract. The nanoparticles' crystalline structure and morphology were confirmed through X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The FTIR analysis identified key functional groups, including hydroxyl (O-H) and carbonyl (C=O) groups, and confirmed the presence of nickel-oxygen bond stretching vibrations, which suggest high crystallinity and effective green synthesis. The XRD results revealed significant crystalline features, indicating phase purity with prominent peaks corresponding to the face-centered cubic (FCC) structure of NiO. [(Vandanjon et al., 2023)](https://paperpile.com/c/ulaT6w/JAEa)Transmission electron microscopy (TEM) showed that the synthesized NiO nanoparticles had a bulk morphology with larger particles in the hundreds of nanometers, indicating aggregation but high crystallinity. These findings suggest that the nanoparticles, while having a lower surface area compared to smaller particles, might offer stability and cost-effectiveness [(Maheshwaran et al., 2024; Merchant et al., 2025; A. Shenoy, Rohinikumar, et al., 2023)](https://paperpile.com/c/ulaT6w/qTK7c+CxSGD+YRPvR). The observed particle clustering could influence surface area and reactivity, which are crucial for their effectiveness in various applications.[(Sagadevan & Podder, 2015)](https://paperpile.com/c/ulaT6w/hjzt)Cytotoxicity assays using HeLa cell lines demonstrated a dose-dependent inhibition of cell growth, with significant cytotoxic effects observed at higher concentrations of NiO nanoparticles [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/ulaT6w/aE012+DpQPy+69YHD). The results indicate that NiO nanoparticles induce cytotoxicity through the generation of reactive oxygen species (ROS) and the release of nickel ions, which lead to apoptosis in cancer cells [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs). This study highlights the promising anticancer activity of green-synthesized NiO nanoparticles, suggesting their potential therapeutic applications. Future research should focus on further particle characterization, exploring in vivo efficacy, and optimizing synthesis conditions to enhance their application in cancer therapy and environmental remediation[(Abudayyak et al., 2020)](https://paperpile.com/c/ulaT6w/cyWB)[(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/ulaT6w/fJSV+cdOU)[(Abudayyak et al., 2020)](https://paperpile.com/c/ulaT6w/cyWB)Thus, this study demonstrates the potent cytotoxic effects of nickel oxide (NiO) nanoparticles synthesized using *Cissampelos pareira* extract against HeLa cell lines [(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/ulaT6w/Dq0QI+4LruU+WZBKs+aEz2G). The cytotoxicity observed is primarily attributed to the nanoparticles' ability to generate reactive oxygen species (ROS) and release nickel ions, which together induce apoptosis in cancer cells. The findings underscore the potential of these green-synthesized NiO nanoparticles as effective therapeutic agents for cancer treatment. Moving forward, further research is needed to validate these findings in vivo, optimize synthesis parameters, and explore their full potential in both cancer therapy and environmental applications.

# Conclusion

This study successfully synthesized nickel oxide nanoparticles using Cissampelos pareira extract, demonstrating a green and sustainable approach. Characterization techniques (FTIR, SEM, XRD) confirmed the desired crystalline and spherical properties of the nanoparticles, with plant extract functional groups present on their surfaces. The MTT assay revealed significant cytotoxic activity against cancer cell lines, indicating potential biomedical applications. Overall, this green synthesis method offers an eco-friendly and effective alternative for producing biologically active nanoparticles.

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