Research Article

A Comparative Study on the *in vitro* Cytotoxicity and Anti-Cancer Properties of Gold Nanoparticles Derived from *Elettaria cardamomum*

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Submission Date: 15-01-2024; Revision Date: 14-03-2024; Accepted Date: 21-04-2024.

ABSTRACT

Aim: This study scrutinizes the anti-cancer potential of gold nanoparticles derived from *Elettaria cardamomum* pods and seeds. **Materials and Methods:** The biosynthesized nanoparticles underwent thorough characterization via UV-vis, FTIR, SEM and EDX analyses. Utilizing the MTT assay, cytotoxicity and anti-cancer efficacy against L929 (normal fibroblast) and Colon cancer cell lines (HT-29 and SW480) cell lines were assessed. **Results:** Notably, the IC_{50} values for pod and seed-based nanoparticles were determined as 156.12 µg/mL and 48.771 µg/mL, respectively, against L929 cells. Strikingly, *Elettaria cardamomum* pod-based nanoparticles demonstrated superior anti-cancer potential against HT-29 and SW480 cell lines compared to seed-based counterparts. **Conclusion:** Therefore, this study suggests the potential therapeutic utility of green-synthesized gold nanoparticles from *Elettaria cardamomum* pods as an innovative approach for treating colorectal cancer.

Keywords: Anti-cancer, Colorectal cancer, Cytotoxicity, *Elettaria cardamomum*, Gold nanoparticles.

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INTRODUCTION

Colorectal Cancer (CRC) is one of the most common diseases in the world, with roughly two million new cases diagnosed each year, making it the third-most common cancer and the fourth-greatest cause of death, accounting for 700,000 fatalities yearly. The probability of developing colorectal cancer ranges from 4% to 5% and the increased risk of developing the condition is associated with a number of factors, such as age, a history of chronic ailments and living habits.[1] It typically begins in the gut lining and can spread to the intestinal wall and underneath muscle tissues if not treated promptly. Moreover, both environmental and genetic factors can combine in a variety of ways to promote carcinogenesis. A vast variety of new and astounding nanomaterials can be used for targeted anticancer drug delivery and therapeutic purpose for colorectal cancer.[2]

Nanotechnology is a rapidly growing, multi-disciplinary area with several possibilities in science and technology. Nanotechnology incorporates essential ideas from numerous fields, including engineering, physics, chemistry and biology, to create new ways of producing and regulating nanoparticles. The nanoparticles are tiny particles with a minimum of one dimension and a size between 1 and 100 nm.[3] Green synthesis is considered a sustainable approach to producing nanoparticles due to the use of affordable and harmless raw materials. Moreover, the green synthesized nanoparticles have excellent bio-availability, low toxicity, therapeutic efficiency, site-specific delivery and target binding. Besides all, this approach has no negative effects on environmental or human health.[4]

Metal nanoparticles are regarded as extremely valuable due to their characteristics like significant surface area and the presence of surface atoms. It has been observed that metal nanoparticles derived from plant extracts and a variety of spices have anti-carcinogenic activity

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DOI: 10.5530/ajbls.2024.13.14

against malignancies of the mouth, the breast, the lung, the cervical, the bladder, the hepatoma and other cancers.[5] Gold nanoparticles are one of many types of metal nanoparticles and they have a particularly broad range of uses in nano-catalysis and hereditary medicine. It has been shown that these nanoparticles' varied properties are influenced by their size.[6]

Elettaria cardamomum ("Queen of Spice") is one of the herbaceous perennial plants belonging to the family Zingiberaceae. It has been reported with various bioactive constituents like myricetin, kaempferol, myricetin, β-carotene, etc. Moreover, numerous researches have evinced the anti-cancer potential of *Elettaria cardamomum*.[7] Hence, in the current investigation, we have compared the cytotoxicity anti-cancer efficacy of the *Elettaria cardamomum* pod and seed-based gold nanoparticles against the normal fibroblast (L929) and human colorectal cancer (HT-29) cell line.

MATERIALS AND METHODS

Sample collection and extract preparation

The seed and pod of *Elettaria cardamomum* were collected separately from Idukki. The seed and pod were shade-dried separately and coarsely ground using a mechanical grinder. The seed and pod powder were stored in separate sealed containers.

The aqueous extract of *Elettaria cardamomum* seed and pod was made by boiling the respective samples (2 g) in distilled water (20 mL) for 1 hr at 70-80ºC. After that, the extract was centrifuged at 5000 rpm for 15 min and the resulting extract was utilized to produce gold nanoparticles.[8]

Synthesis of gold nanoparticles

The aqueous extract of 1 mM HauCl4 (20 mL) was combined with 2 mL of seed and pod extract, respectively and kept for incubation at 40-60ºC for 15 min. The colour shift to dark violet in the reaction solution within the allotted period denoted the formation of gold nanoparticles.[8]

Characterization of nanoparticles

UV-vis Spectroscopy

The absorption spectra of *Elettaria cardamomum* seed and pod extracts were recorded using the UV-Vis Spectrophotometer respectively with wavelength ranges of 300-800nm.

FTIR Studies

The FTIR spectrum was recorded using the Fourier-transform infrared (FTIR) spectroscopy (SHIMADZU, IRSpirit) at the wavelength 4000-600 cm^{-1} with the resolution of 16 cm^{-1}.

SEM Analysis

The SEM analysis was performed with the ZEISS EVO instrument to determine the size and morphology of synthesized gold nanoparticles.

Energy-Dispersive X-Ray studies

The EDX of synthesized gold nanoparticles was measured using an EDS X-ray spectrophotometer, which is commonly incorporated in modern SEMs (ZEISS EVO instrument).

Cytotoxicity Analysis

The cytotoxicity analysis for *Elettaria cardamomum* seed and pod-based gold nanoparticles was carried out by MTT assay. The L929 cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell line was grown MEM medium supplemented with fetal bovine serum and incubated at 37ºC with 5% CO2. After 24 hr, the growth media was replaced with freshly prepared growth media containing varying concentrations of *Elettaria cardamomum* seed and pod-based nanoparticles respectively and followed by further incubation for 24 hr.

After 24 hr of incubation, the complete plate was examined using an Inverted Phase Contrast Microscope and those observations were captured as images. Cytotoxicity is indicated by any observable alterations in the cell's morphology, such as granulation, vacuole formation in the cytoplasm and rounding or shrinking of the cells. Then, the sample content in the wells was removed and 10 µL of MTT solution (mg/mL) was introduced to each test and control well. The supernatant was collected and 200 µL of DMSO (MTT solubilization solution) was mixed well to dissolve the insoluble formazan crystals. A microplate reader was used to measure the absorbance at 570 nm.[9]

The percentages of viability (%V) and inhibition (%I) were calculated as:

Percentage of viability (%V) =100(At/Ac)

Percentage of inhibition (%I) =100 [1-(At /Ac)]

Where, At-absorbance of treated cells; Ac-absorbance of control cells.

Anti-Cancer Activity

The anti-cancer activity for *Elettaria caradmomum* seed and pod-based gold nanoparticles was carried out by MTT assay. The HT-29 and SW480 (Human colorectal

cancer) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell line was grown in the MEM medium supplemented with fetal bovine serum and incubated at 37ºC with 5% CO2. After 24 hr, the growth media was replaced with freshly prepared growth media containing varying concentrations of *Elettaria cardamomum* seed and pod-based nanoparticles respectively and followed by further incubation for 24 hr.

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Where, At-absorbance of treated cells; Ac-absorbance of control cells.

RESULTS

Synthesis of nanoparticles

Gold nanoparticles were successfully synthesized in both reaction solutions containing the seed (Figure 1) and pod (Figure 2) of *Elettaria cardamomum*, respectively. Initially, the seed and the pod extracts of *Elettaria cardamomum* were found to have a pale-yellow colour. The appearance of dark violet colour in both reaction solutions denoted the synthesis of gold nanoparticles. This change in colour is an obvious sign that Au^{3+} ions have been changed into Au nanoparticles.

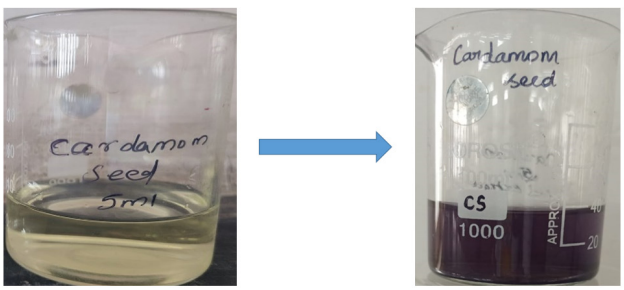

Figure 1: *Elettaria cardamomum* seed-based gold nanoparticles

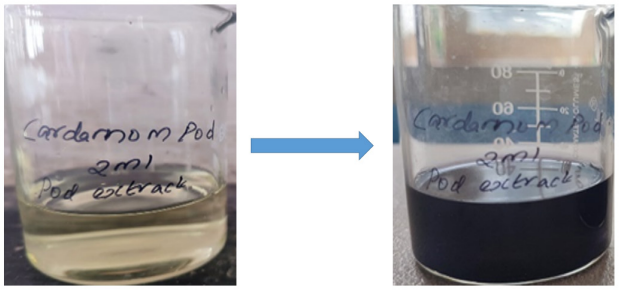

Figure 2: *Elettaria cardamomum* pod-based gold nanoparticles

Characterization of gold nanoparticles

UV Vis Spectroscopy

Both the aqueous extract of seed and pod of *Elettaria cardamomum* do not exhibit the typical colour shift or absorption bands. The strongest band in the UV spectrum of seed-mediated gold nanoparticles (Figure 3) was detected around 550 nm, whereas the highest peak for *Elettaria cardamomum* pod-based gold nanoparticles appeared at the wavelength of 545 nm (Figure 4). The strong Surface Plasmon Resonance (SPR) band intensity has emerged at the wavelength ranges from 530 to 565nm for seed and pod-based nanoparticles, respectively. The mono-dispersion of particles is represented by the expansion of the absorption peak in the UV spectrum.

FTIR studies

FTIR spectra of *Elettaria cardamomum* pod and seed extract have been assessed before and after the formation of nanoparticles to disclose and comprehend the synthesis mechanism of gold nanoparticles. Different absorption peaks at 1641.09, 3162.10, 3207.85, 3253.59, 3316.49, 3333.65, 3390.83, 3413.70, 3470.88 and 3522.34 cm^{-1} were visible in the FTIR spectra of *Elettaria cardamomum* seed extract (Figure 5). Whereas, the *Elettaria cardamomum* seed-based gold nanoparticles showed the typical IR absorption bands at 1521.01, 1641.09, 3179.26, 3242.16, 3305.05, 3356.52, 3379.39, 3448.01 and 3522.34 cm^{-1} (Figure 6). The absorption peaks for pod-based gold nanoparticles were detected at 1641.09, 3179.26, 3219.28, 3236.44, 3305.05 and 3413.70 cm^{-1} (Figure 8), while the bands for the aqueous extract of *Elettaria cardamomum* pod were found at 1641.09, 3173.54, 3236.44, 3299.34, 3350.80, 3390.83, 3407.98, 3442.29 and 3505.19 cm^{-1} (Figure 7).

The aromatic component is represented by the distinctive IR bands at 1641.09 cm^{-1} (C-H bending). The alcohol (O-H stretching) was illustrated by the absorption maxima at 3236.44 cm^{-1} for pod extract

and their gold nanoparticles. The distinctive peaks for amine (N-H stretching) were detected at 3407.98 and 3413.70 cm^{-1} for pod extract and their gold nanoparticles, respectively. The strong O-H bond is reflected by the absorption peaks at 3522.34 cm^{-1} for seed extract and its gold nanoparticles, respectively. There was just a slight difference in wave numbers between the IR spectrum of the aqueous extracts and their gold nanoparticles.

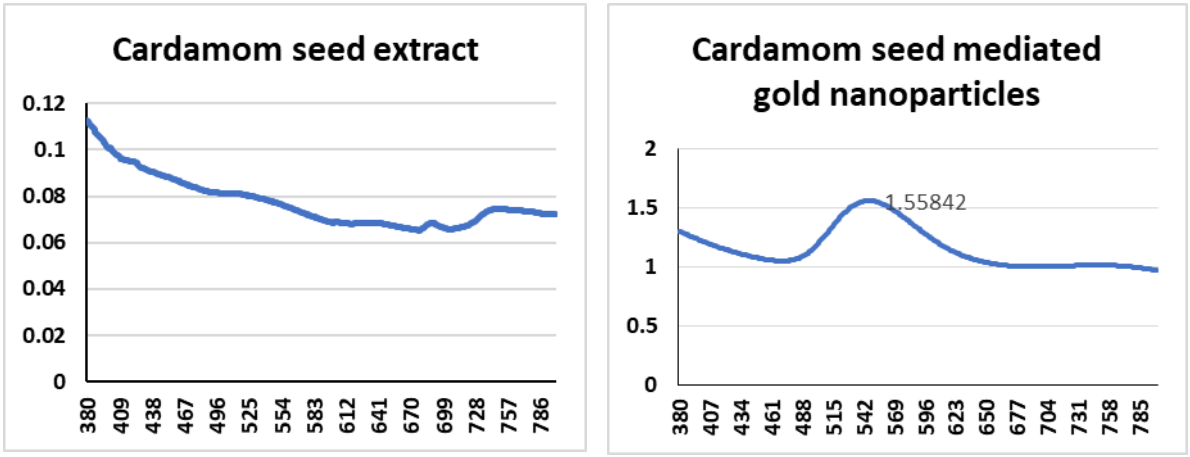

Figure 3: UV Vis spectrum of *Elettaria cardamomum* seed extract and its gold nanoparticles.

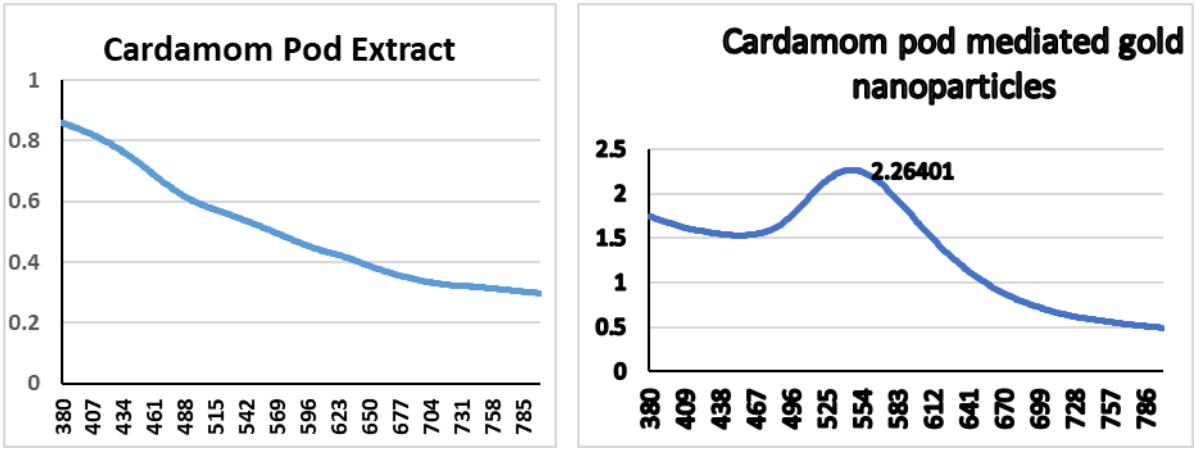

Figure 4: UV Vis spectrum of *Elettaria cardamomum* pod extract and its gold nanoparticles

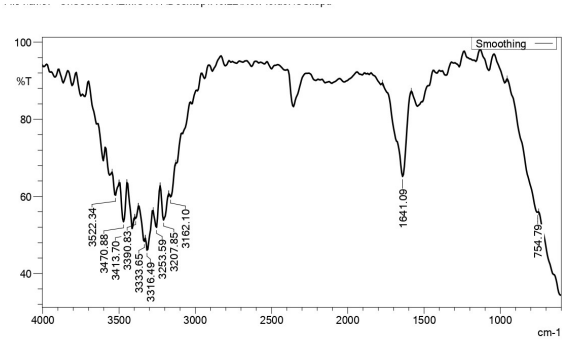

Figure 5: FTIR analysis of *Elettaria cardamomum* seed extract

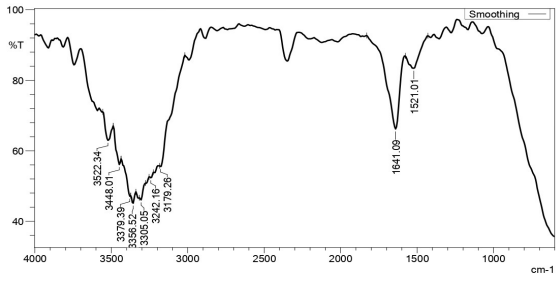

Figure 6: FTIR analysis of *Elettaria cardamomum* seed-based gold nanoparticles

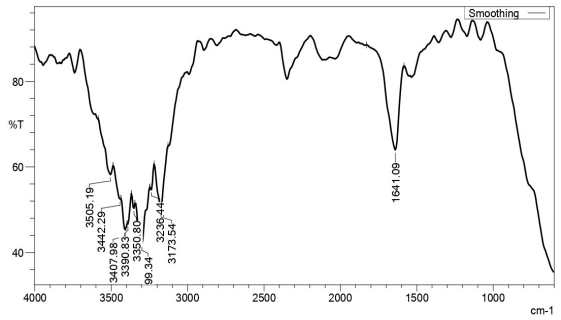

Figure 7: FTIR analysis of *Elettaria cardamomum* pod extract.

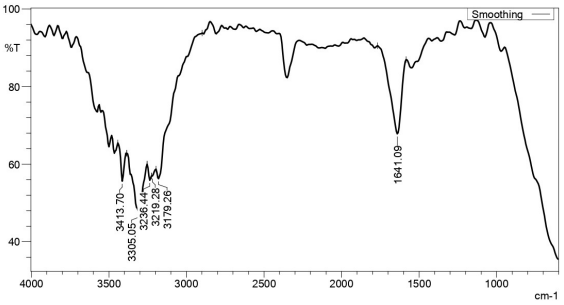

Figure 8: FTIR analysis of *Elettaria cardamomum* pod-based gold nanoparticles

SEM examination

The Scanning Electron Microscopic examination revealed that the gold nanoparticles synthesized from an aqueous extract seed and a pod of *Elettaria cardamomum* (Figure 9) appeared to be spherical in shape with an average size of 20 nm respectively.

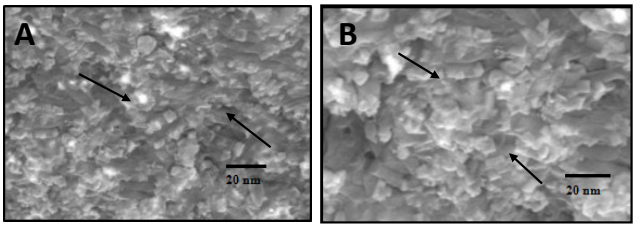

Figure 9: (A) SEM analysis of *Elettaria cardamomum* seed-based synthesis of gold nanoparticles; (B) SEM analysis of *Elettaria cardamomum* pod-based synthesis of gold nanoparticles. Energy-Dispersive X-Ray studies

Data on the chemical composition of samples were gathered using Energy Dispersive X-ray Spectroscopy (EDXS) for elements with atomic numbers (Z)>3. Gold nanoparticles synthesized from the seeds and pods of Elettaria cardamom demonstrated high absorption band peaks at 2.2 keV, which is indicative absorption of gold; however, few weak signals were also detected. Figure 10 represents the EDX data of *Elettaria cardamomum* seed and pod derived gold nanoparticles.

Cytotoxicity Activity

The cell line was treated with increasing concentrations of synthesized gold nanoparticles like 20-100 µg/mL for 24 hr (Figures 11 and 12). The *Elettaria cardamomum* pod-based gold nanoparticles showed Slight cytotoxicity, whereas the seed-based gold nanoparticles exhibited slight to mild cytotoxicity to L929 cells after 24 hr (Tables 1 and 2). The control showed no cytotoxicity. The IC_{50} value for pod and seed-based gold nanoparticles was found to be 156.12 µg/mL and 48.771 µg/mL respectively. The highest cell viability was found to be 82 for pod-based gold nanoparticles and 57 for seed-based gold nanoparticles

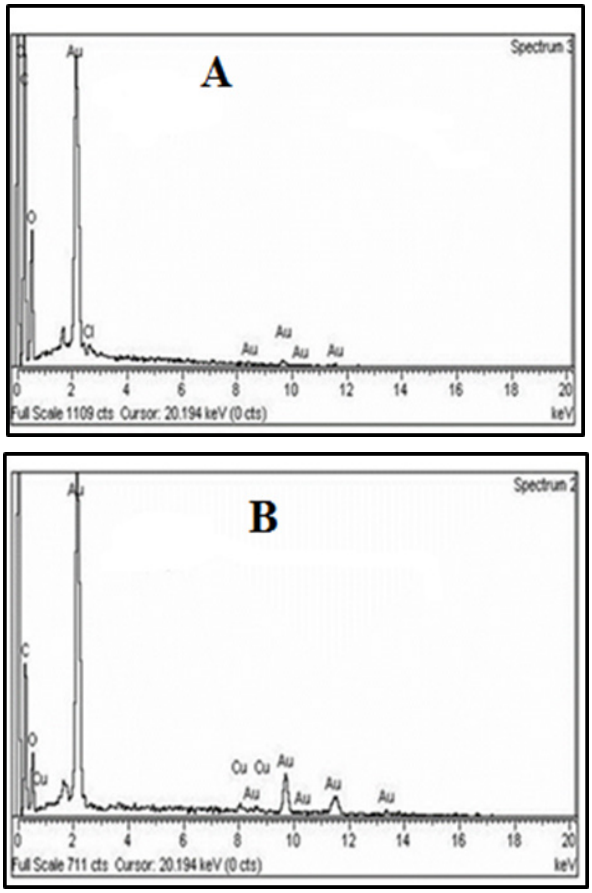

Figure 10: (A) EDX analysis of *Elettaria cardamomum* seed-based gold nanoparticles; (B) EDX analysis of *Elettaria cardamomum* pod-based gold nanoparticles.

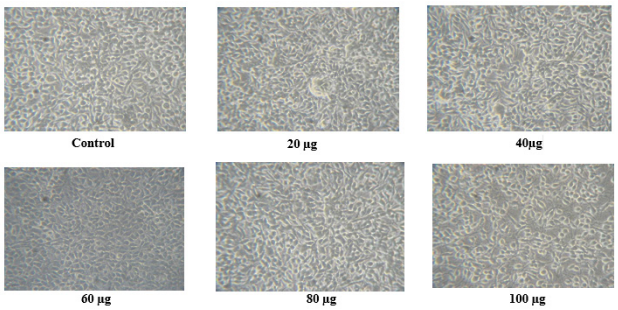

Figure 11: Cytotoxicity analysis of *Elettaria cardamomum* pod-based gold nanoparticles.

Table 1: Cytotoxicity of *Elettaria cardamomum* pod-based gold nanoparticles on L929 cell line

Concentration (µg)	Toxicity	Cell Viability	Cytotoxic reactivity
20	9	91	Slight
40	11	89	Slight
60	11	89	Slight
80	15	85	Slight
100	18	82	Slight

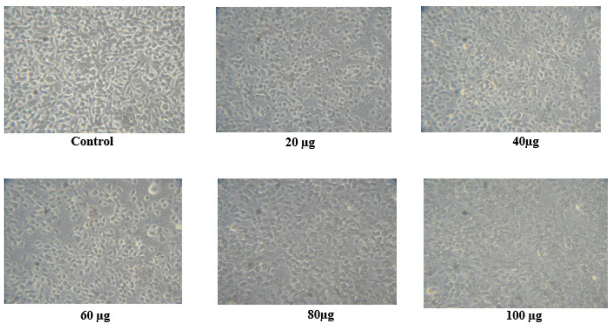

Figure 12: Cytotoxicity analysis of *Elettaria cardamomum* seed-based gold nanoparticles.

Table 2: Cytotoxicity of *Elettaria cardamomum* seed-based gold nanoparticles on L929 cell line

Concentration (µg)	Toxicity	Cell Viability	Cytotoxic reactivity
20	14	86	Slight
40	24	76	Slight
60	35	65	Mild
80	37	63	Mild
100	43	57	Mild

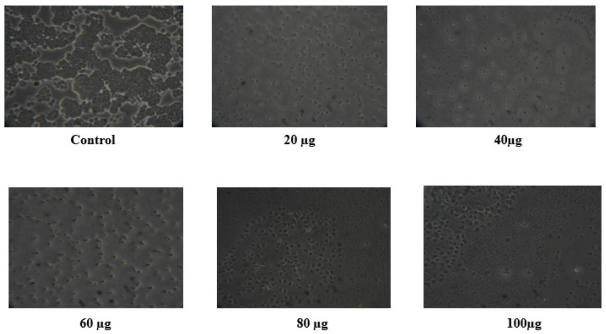

Figure 13: Anti-cancer activity of *Elettaria cardamomum* pod-based gold nanoparticles.

Table 3: Anti-cancer activity of *Elettaria cardamomum* pod-based gold nanoparticles on HT29 cell line

Concentration (µg)	Toxicity	Cell Viability	Anticancer Activity
20	67	33	Moderate
40	78	22	Severe
60	84	16	Severe
80	86	14	Severe
100	89	11	Severe

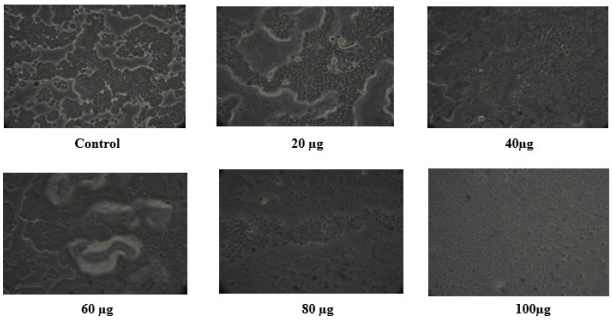

Figure 14: Anti-cancer activity of Elettaria cardamomum seed-based gold nanoparticles.

Table 4: Anti-cancer activity of *Elettaria cardamomum* seed-based gold nanoparticles on HT29 cell line.

Concentration (µg)	Toxicity	Cell Viability	Anticancer Activity
20	21	79	Mild
40	32	68	Mild
60	40	60	Mild
80	51	49	Moderate
100	60	40	Moderate

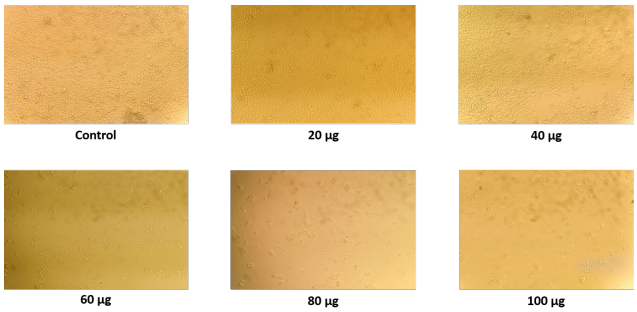

Figure 15: Anti-cancer activity of *Elettaria cardamomum* seed-based gold nanoparticles.

Table 5: Anti-cancer activity of *Elettaria cardamomum* seed-based gold nanoparticles on SW-480 cell line.

Concentration (µg)	Toxicity	Cell Viability	Anticancer Activity
20	15	85	Moderate
40	29	71	Moderate
60	41	59	Moderate
80	58	42	Moderate
100	70	30	Severe

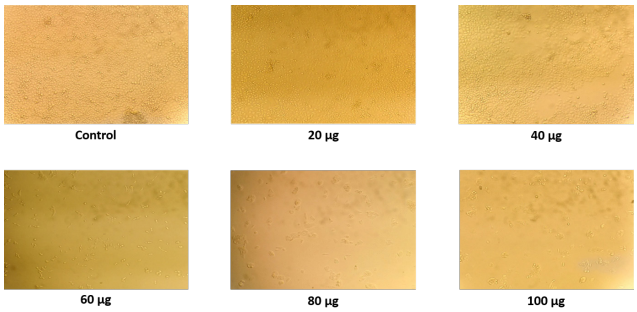

Figure 16: Anti-cancer activity of *Elettaria cardamomum* pod-based gold nanoparticles.

Table 6: Anti-cancer activity of *Elettaria cardamomum* pod-based gold nanoparticles on SW-480 cell line.

Concentration (µg)	Toxicity	Cell Viability	Anticancer Activity
20	46	54	Moderate
40	52	48	Moderate
60	58	42	Moderate
80	68	32	Moderate
100	76	24	Severe

DISCUSSION

All spices include a significant quantity of the bioactive substances needed to generate nanoparticles; therefore, these nanoparticles can be applied in various disciplines as they increase the nutritional value and overall health benefits. These spice-based nanoparticles have the potential to have a variety of positive health effects, including anti-carcinogenic, antioxidant, anti-inflammatory, anti-diabetic, enzyme retardation and antimicrobial action.[10] To the best of our knowledge, this study is the first report on the comparison of cytotoxicity and anticancer activity of the Elettaria cardamomum pod and seed-based gold nanoparticles against the normal fibroblast cell line (L929) and Human colorectal cancer cell line (HT-29).

The UV Visible spectra of gold nanoparticles produced using HAuCl4 and black cardamom extract (1:1) indicated the rapid synthesis of gold nanoparticles as the absorption peaks overlap with each other after the time interval of 10 min. The absorption maxima were detected at the wavelength of 526nm due to the interaction of surface plasmons of gold nanoparticles with the electromagnetic spectrum. Absorption maxima for gold nanoparticles were recorded at different wavelength for solutions with varying pH values.[11] Likewise, the UV Visible spectroscopic examination revealed a peak at 550 nm for gold nanoparticles of Amomum villosum (5 years old), which confirmed the bio-reduction of the metal ions to nanoparticles.[12] The findings of Pattanayak and Nayak[8] revealed that the UV spectrum of gold nanoparticles synthesized from Elettaria cardamomum with a ratio of 1:1 (aqueous extract of cardamom: gold solution) showed Surface Plasmon Resonance at the wavelength of 550 nm.

Before reduction, the black cardamom extract's FTIR spectra clearly display a significant number of peaks between 4000 and 3200 cm^{-1}. There was a large peak of about 3200 cm-1 that was attributed to -OH. After the bioreduction, this radical vanished from the spectrum, which showed that -OH was used during the bio-reduction of HAuCl4.[11] The absorption peaks at 3375.45, 2928.54, 1608.89 and 1022.17 cm^{-1} region in the FTIR spectrum of the dried fruits of Amomum villosum extract before and after synthesis are attributable to the stretching bonds of the main -OH alcohol group, aliphatic -CH, C=C and alcohol C-O groups, respectively.[12] The spectra of AuNPs were obtained in the 500-4000 cm^{-1} region range. There are noticeable intensity peaks at 3200 cm^{-1}, 2100 cm^{-1} and 1600 cm^{-1}. At different wave numbers, functional groups such as C-H stretch (aromatics), CH_3-R, C-O-C, N-H and C=O stretching were found.[13]

The SEM examination revealed that the gold nanoparticles were effectively produced from the plant extracts of Delphinium uncinatum and Erythrophyleum guineense respectively were appeared to be below 100 nm in size.[14] However, the study of Shah *et al.*,[15] concluded the presence of 36 nm-sized gold nanoparticles from the Sageretia thea leaves extract. Similarly, the Scanning Electron Microscope (SEM) examination of Gold Nanoparticles synthesized using Lasiosiphon Eriocephalus Decne Extract confirmed the formation of hexagonal and crystalline-shaped nanoparticles of size ranging from 20-60 nm.[16]

Soshnikova *et al.*,[12] showed that the EDX analysis had significant signals of gold atoms in the synthesized nanoparticles from the dried fruits of Amomum villosum at 2.3 keV, which are typical of metallic gold nanoparticles. The result was related to our findings. The EDX studies showed substantial Gold (Au) signal was found in the gold nanoparticles of Etlingera elatior extract, as well as weak signs for Nitrogen (N), Oxygen (O) and Carbon (C). The weak signals might be due to the binding of biomolecules to the surface of synthesized nanoparticles.[17] Likewise, the distinctive 2 keV signals confirmed the presence of gold, along with that weak signal corresponding to the carbon and oxygen were also detected in the EDX analysis of gold nanoparticles derived from Ephedra extract.[18]

Ahmed *et al.*,[19] performed an MTT assay to determine the toxicity rate for the gold nanoparticles synthesized

from cardamom husks and the result was found to be 19.637% in the normal fibroblast cell line derived from Human stem cells (HDFn). The cell viability of L929 fibroblast cells treated with Dragon fruit mediated gold nanoparticles was found to be 80% at the concentration of 100 μg/mL,[20] which was comparable to our result. The cytotoxicity of gold nanoparticles synthesized using the aqueous extract of *Limonia acidissima* leaves was examined using the L929 cell line. [21] MTT assay was used to measure the cell viability of the L929 fibroblast cells treated with gold nanoparticles of Nepenthes khasiana at the doses of 4.39, 8.75, 17.25, 35.15 and 7.30 μg/mL after 2, 4, 8, 12 and 24 hr.[22] Interestingly, the findings of Sett *et al*., (2016), revealed that the green synthesized gold nanoparticles from the Dillenia indica fruits showed very minimal cytotoxicity effects to the L929 cell line and the cell viability was reported as almost 90% after 24 hr.

Rosmarinus officinalis leaves derived gold nanoparticles were able to stop the growth of HT-29 colon cancer cells, which caused cancer cells to undergo apoptosis.[23] The gold nanoparticles synthesized using the *Abutilon indicum* leaves extract exhibited anti-cancer action against the HT-29 colon cancer cells and the IC_{50} values of 210 and 180 g/mL after 24 and 48 hr respectively.[24] The gold nanoparticles of Allium sativum inhibited the growth of HT-29 cells with an IC_{50} value of 269 μg/mL, which thereby proving its anti-cancer property.[25] Similarly, Miri *et al*.,[26] used the MTT assay to examine the anti-cancer effect of Prosopis farcta extract mediated gold nanoparticles on the colon cancer cell line HT-29. The study revealed that as the Au-NPs concentration reached 200 g/mL, the effects of apoptosis on cells were intensified. The IC_{50} value was found to be 419.7 μg/mL for gold nanoparticles against the HT 29 cells after 72 hr. The findings of Anadozie *et al*.,[27] also supported the anti-cancer activity of gold nanoparticles against the HT-29 cell line. Moreover, dose-related anti-cancer activity was reported for the gold nanoparticles derived from the aqueous extract of Garcinia kola seed in the MTT assay. Likewise, the ethanolic extract of the Terminalia catappa plant inhibited the growth of SW480 cells in the MTT assay.[28] The anti-cancer activity of the gold nanoparticles synthesized by the Haloxylon salicornicum against the SW480 cell line.[29]

CONCLUSION

In the current investigation, the gold nanoparticles were synthesized from the aqueous extract of Elettaria cardamomum seed and pod respectively. The different characterization studies like UV Vis spectroscopy, FTIR, SEM and EDX studies confirmed the formation of 20 nm spherical-shaped gold nanoparticles. The minimal cytotoxicity was exhibited by the Elettaria cardamomum pod-based gold nanoparticles against the L929 cell line than the seed-based gold nanoparticles. Moreover, the significant anti-cancer potential was revealed by the cardamomum pod-based gold nanoparticles against the HT-29 as well as SW480 cell lines. This method of biosynthesis of gold nanoparticles was simple, scalable and environmentally beneficial. Moreover, these produced gold nanoparticles will be much more beneficial in colorectal cancer treatment due to their significant anti-cancer activity.

ACKNOWLEDGEMENT

The authors are grateful to Kongunadu Arts and Science College (Autonomous), G.N. Mills P.O, Coimbatore, Tamil Nadu, India and Genolites Research and Development Laboratory, Coimbatore, Tamil Nadu, for providing the facilities to carry out the present study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

The authors contributed equally for concept making, data acquiring, investigating and writing the manuscript. All authors have read and approved the manuscript.

ABBREVIATIONS

UV-vis Spectroscopy: Ultraviolet Visible Spectroscopy; **FTIR:** Fourier-transform infrared; **SEM:** Scanning Electron Microscopy; **EDX:** Energy-Dispersive X-ray; **MEM:** Minimum Essential Medium; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; **DMSO:** Dimethyl sulfoxide; **SPR:** Surface Plasmon Resonance; **IR:** InfraRed.

SUMMARY

The present study used *Elettaria cardamomum* seed and pod to synthesis the gold nanoparticles, which was charcacterized using UV-vis, FTIR, SEM and EDX analysis. The *Elettaria caradmomum* pod derived gold nanoparticles showed more promising anticancer activity against the colon cancer cell lines such as HT-29 and SW480. The results of this study demonstrate the potential of *Elettaria caradmomum* pod derived Gold nanoparticles as novel and effective agents for colon cancer treatment.

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Cite this article: Meladom SK, Narendhirakannan RT. A Comparative Study on the *in vitro* Cytotoxicity and Anti-Cancer Properties of Gold Nanoparticles Derived from *Elettaria cardamomum*. Asian J Biol Life Sci. 2024;13(1):100-8.