GREEN BIOSYNTHESIS OF SILVER NANOPARTICLES FROM
ELETTARIA CARDAMOMUM (SEED) AND ITS IN VITRO
CYTOTOXIC ACTIVITY

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ABSTRACT
The green synthesis of silver nanoparticles (AgNPs) using Elettaria cardamomum (seed) extract and its cytotoxicity activity against Hep-2 cell line were reported. The synthesized AgNPs using Elettaria cardamomum extract was preliminarily confirmed by UV–visible spectroscopy and it was further characterized by FT-IR. The UV–visible spectrum showed an absorption peak at 456 nm which reflects surface plasmon resonance (SPR) of AgNP. The synthesized SNPs exhibited a dose-dependent cytotoxicity activity against Human Larynx Carcinoma cancer(Hep-2) and the inhibitory concentration (IC50) were found to be 51µg/ml. Furthermore, the synthesized SNPs shows significant anticancer activity against Hep-2 cancer cell line.

KEYWORDS: Silver Nanoparticle, Anticancer, Elettaria cardamomum.

INTRODUCTION
Nanoparticles are the basics for nanotechnology. Nowadays nanoparticles are made from noble metals, in particular Ag, Pt, Au and Pd. The development of new materials with nanometer size including nanoparticles, nanotubes, nanowires, etc. Among all, nanoparticles with the unique properties in chemistry, optics, electronics, and magnetics have
led to an increasing interest in their synthesis.\[7\] The most widely used and known applications of silver nanoparticles include topical ointments and creams containing silver to prevent infection of burns and wounds. Physical and chemical methods may also successfully get the pure nanoparticles but these methods are costly and potentially dangerous to the surroundings. The use of biological organisms such as microorganisms, plant extract could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner.\[1\] The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-pharmacology. The advantages of using plant and plant-derived materials for biosynthesis of metal nanoparticles researchers to understand the possible mechanism of metal nanoparticle formation and the plant.\[4,12\] Recently, silver nanoparticles are emerging as promising agents for drug delivery cancer therapy. The anticancer actions of nano-sized silver particles have been evaluated against a variety of human cancer cells,\[9,10\]

**MATERIALS AND METHODS**

**Materials**

Silver nitrate (AgNO3) and MTT were purchased from Hi Media Laboratories Pvt. Ltd. India. The Hep-2 cancer cell line was purchased from King Institute of Preventive Medicine, ICMR, Chennai, India.

**preparation of the Plant extract**

The *Elettaria cardamomum* (seed) were collected from the local market. The E. Cardamomum (seed) was powdered finely using mortar and pestle. 20 g of the leaf powder was dissolved in 100 ml of Millipore water and the mixture was boiled at 80°C for 10 min and then filtered through Whatman Grade No.1 FilterPaper(11µm)and used for the further study.\[6,14\]

**Purification of the extract**

The extract was further centrifuged at 3500rpm for 10 minutes to remove the heavy biomaterials and stored at 4°C.

**Synthesis of silver nanoparticles**

10 ml of S. aromaticum extract as added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag+ ions. Various temperatures were maintained at RT, 40, 60, and
80°C using water bath for optimization. The solution stirring magnetically at 1000 rpm for 10 min.\textsuperscript{[13]} The colour change was observed at various temperatures and silver nanoparticle was formed. The extract is used as reducing and stabilizing agent for 1mM of Silver nitrate.\textsuperscript{[8,18]}

**Purification of silver nanoparticles**

To remove excess silver ions, the silver colloids were centrifuged at 10,000 rpm for 15 minutes and washed three times with deionized water. A dried powder of silver nanoparticles was obtained by freeze-drying for further characterization studies.

**UV–Vis spectra analysis**

The preliminary characterization of silver nanoparticles was carried out using UV-visible spectroscopy (11, 16). UV–Vis spectral analysis was done by using nanodrop 2000r in a scanning range of 200 nm to 800 nm. Deionized water was used as blank. The spectra recorded were then re-plotted using Origin 6.0 version.

**FTIR analysis**

The interaction between protein-silver nanoparticles were analyzed by Fourier transform infrared spectroscopy (FTIR) and the spectra were recorded in the wavelength interval of 4000 to 400nm-1.

**Cell culture**

Human Larynx Carcinoma cancer (Hep-2) cell lines were obtained from King Institute of Preventive Medicine, ICMR, Chennai, India. It was cultured in Dulbecco’s modified Eagle’s medium (DMEM: Hi media Laboratories Mumbai, India), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Hi Media Laboratories Mumbai, India).

**Cell line maintenance**

Hep2 cell lines were purchased from King Institute of preventive medicine, chennai, India. The cell lines were maintained at 5% CO2 in CO2 incubator (19). Cultures were viewed using an inverted microscope to evaluate the quality of confluency and the absence of bacterial and fungal contaminants were confirmed(17).
MTT assay
To determine the cytotoxic effect of silver nanoparticles, cell viability study was done with the MTT reduction assay. Hep-2 cells were seeded in a 96-well plate at the density of $5 \times 10^3$ cells/well. The cells were allowed to attach and were grown in 96-well plate for 24 h, in 200 µl of EMEM with 10% FBS (3). After that the media was removed and replaced with suspension of various concentrations of silver nanoparticles 10 to 100 mg/ml (minimum 4 wells were seeded with each concentration) the cells were incubated for 48 h (2). After the addition of MTT (10 ml, 5 mg/ml), the cells were incubated at 37°C for another 4 h. The medium was then removed, and 200 µl of DMSO was added to each well. Optical density of the formazan product was read at 620 nm using multi well spectrophotometer(17, 20). The results were given as mean of four independent experiments. OD value was subjected to sort-out percentage of viability by using the following formula.

\[
\text{Percentage of cell viability} = \frac{\text{OD value of treated sample (AgNPs)}}{\text{OD value of control sample}} \times 100
\]

Statistical analysis
The grouped data were statistically evaluated using GRAPHPAD PRISM 6 software. Values are presented as the mean ±SD of the four replicates of each experiment.

RESULTS
Nanoparticle synthesis using *Elettaria cardamomum* plant extract

![Images](A) (B)
UV–visible analysis of synthesized AgNPs from *Elettaria cardamomum* extract at different temperature conditions

**UV-Vis spectra analysis**

The color change shows the presence of Ag nanoparticles in the *S. aromaticum* extract and it was characterized by UV-Vis spectrophotometer and monitored by taking readings at different temperature. Fig. 1a shows the UV–vis spectra of silver nanoparticle formation at different temperature of using *Elettaria cardamomum* extract of (i)RT, (ii) 40°C, (iii)60°C, (iii) 80°C aqueous medium. The SPR bands of silver colloid for different temperature were
appeared at 426 to 456nm. The strong broad peak located at 456 nm was observed at 80°C for Ag nanoparticles. (15, 21) Our results are similar to Shalini Chauhan et.al (22).

FTIR analysis

FT-IR spectra of green synthesized silver nanoparticles from *Elettaria cardamomum* (AgNPs). Typical wavenumber range by infrared spectrum is 4000–400 cm⁻¹

The FT-IR transmission spectra of silver nanoparticles from *Elettaria cardamomum* are represented in Figure. FTIR spectra of AgNPs *Elettaria cardamomum* showed that the peaks expected at 3368, 2963, 1722, 1621, 1377, 1221, 1032 and 610 cm⁻¹. The band 3368 corresponds Normal’polymeric’OH stretch. 2963 corresponds Methyl C–H asym stretch. 1377 corresponds Methyl C–H sym bend. 1621 corresponded to Alkenyl C = C amide stretch. 1722 shows Carboxylic acid. 1221 corresponds Aromatic phosphates (P–O–C stretch). 1032 Aliphatic phosphates (P O C stretch). 610 shows Disulfides (S S stretch). This evidence suggests that the protein molecules could possibly perform the function of the formation and stabilization of AgNPs in the aqueous medium (6, 18).

Cytotoxicity assay

The cytotoxicity of the silver nanoparticle was studied against the Hep2 cell line by MTT assay. The cytotoxicity effect of synthesized silver nanoparticle cancer cell was studied at different concentration (10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg, 100 μg).
µg). The IC50 value as calculated for the silver nanoparticle. It was found to be 51 µg for Hep2 cell line. This study shows that the dose required was less for the cancer cell line.

(A)

Bright field microscopic images of morphological characterization of Hep-2 cells
(a) Normal cells (b) Treated cells
The cytotoxicity of the silver nanoparticle was studied against the Hep2 cell line by MTT assay. This is the first study to report the cytotoxicity of AgNPs synthesized using *Elettaria cardamomum* against Hep-2 cancer cell lines. The cytotoxicity effect of synthesized silver nanoparticle cancer cell was studied at different concentration (10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg, 100 µg). The IC50 value was calculated for the silver nanoparticle. It was found to be 51 µg for Hep2 cell line. This study shows that the dose required was less for the cancer cell line.

**CONCLUSION**

We report a simple, quick and efficient green synthesis of silver nanoparticles from the Elettaria cardamomum. The characterization with UV–vis spectroscopy and Fourier transmission infrared (FT-IR) is the preliminary evidence for the formation of nanoparticles. The synthesized silver nanoparticles showed promising anticancer activity against human pharynx cancer cell line. From the study, it can be concluded that the silver nanoparticles synthesized using Plant possess high anticancer activity against cell lines which further suggest the potential therapeutic use of these nanoparticles.

**REFERENCE**


