

Bioreduction of silver nanoparticles from aerial parts of *Euphorbia hirta* L. (EH-ET) and its potent anticancer activities against neuroblastoma cell lines

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Euphorbia hirta L. (Family: Euphorbiaceae) is a versatile medicinal plant and enriched with novel bioactive molecules and possess broad-spectrum pharmacological actions. Present work is aiming to synthesis and characterize of silver nanoparticles (AgNPs) by bioreduction method an using ethanolic extract of aerial parts of *Euphorbia hirta* L. (EH-ET). The synthesized AgNPs observed by a color change of source solution (as AgNPs) and further confirmed by the UV-Visible spectroscopic technique. The AgNPs synthesized were characterized by Scanning Electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and Zeta potential analysis. The synthesized AgNPs are polydispersed and some places it's agglomerated. The particle size EH-ET silver nanoparticles were analyzed using Beckman coulter particle size analyzer (Delsa™ Nano common). The average size of the particles size noted in 2.9-206.3 nm. Anticancer activity of EH-ET Silver nanoparticles was tested against neuroblastoma cells (SH-SY5Y) and breast cancer cells (SH-SY5Y) and cytotoxicity were tested in vero cells by MTT assay. The preliminary confirmation of the synthesized AgNPs by the present method was made by the appearance of reddish brown color and the visible absorption peak at 429.5 nm. SEM image revealed that AgNPs synthesized were spherical in shape and silver nanoparticles were in the size range of 2.9 to 206.3 nm. FT-IR spectra showed the peaks corresponding to functional groups C=O, -C=C and -OH, which actively participated in bio-reduction and subsequent stabilization reactions in the synthesis of AgNPs. The obtained nanoparticles showed promising anticancer activity against neuroblastoma cells (SH-SY5Yc) and breast cancer cells (MCF-7) with IC₅₀ values of 29.85 and 335 µg/mL, respectively. Whereas, the nanoparticles did not show any activity against vero cell lines. The synthesized silver nanoparticles using an ethanolic extract of *Euphorbia hirta* L. would be helpful for the preparation of potent cytotoxicity agents to destroy cancer cells.

Keywords: Anticancer activity, Breast cancer cell lines, *Euphorbia hirta* L., Neuroblastoma cell lines, Silver nanoparticles, Vero cells

Euphorbia hirta L. (Family: Euphorbiaceae) is a naturally gifted medicinal plant an excellent source for a variety of novel bioactive molecules such as alkaloids, triterpenoids, steroids, flavonols, glycosides and anthraquinones, which is mainly responsible for board-spectrum of pharmacological actions including anticancer activity^{1,2,11,17}. Review of literature revealed that the *Euphorbia hirta* L. exhibits the antibacterial and antifungal activity³, antiviral activity^{4,5}, hepatoprotective⁶, antioxidant¹¹ and anticancer activity^{7,8}. Biosynthesized silver nanoparticles from medicinal plants have excellent biological activity against bacteria¹², fungi, virus, cancer and viral infections⁹. The ethanolic extracts of *Euphorbia hirta* L. mainly consist of flavanoids^{15,17} and polyphenols such as Quercetin, Kaempferol¹⁶ and act as

a reducing agent for the bioreduction of silver nanoparticles. Based on the above facts, the present work was designed to synthesize and characterize biogenic silver nanoparticles from ethanolic leaf extract of *Euphorbia hirta* L. (EH-ET). Biosynthesized silver nanoparticles were studied for the anticancer activity against neuroblastoma cells (SH-SY5Yc cells), breast cancer cells (MCF-7 cells) and cytotoxicity was also tested in normal vero cells by MTT assay⁷. In the review, the literature shows methanolic leaves extract of silver nanoparticles and their larvicides activity^{11,13} but in our studies reported the anticancer activities of ethanolic leaves to extract of AgNPs.

Materials and Methods

Preparation of extracts

Fresh leaves of *Euphorbia hirta* L. collected from Kalasalingam University, Krishnankoil, 65 Km southwest from Madurai Tamilnadu, India. Healthy

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leaves are dried under shade and powdered. 100 g of leaf dried powder packed in soxhlet apparatus for 48 h by using hot continues extraction process with 500 mL ethanol (98%) to achieve complete extraction. A solvent is collected and subjected to distillation to concentrate the extract. An extract is dried under vacuum and stored under 5°C until further use.

Synthesis of silver nanoparticles

Analytical grade Silver nitrate (AgNO_3) was purchased from Hi-Media (Mumbai, India). An aqueous solution of 1 mM AgNO_3 was used for the synthesis of silver nanoparticles. For the preparation of silver nanoparticles, 5 mL of *Euphorbia hirta* L. extract (100 mg vacuum dried ethanol extract dissolved in 5 mL distilled water) with 95 mL of AgNO_3 (1 mM) solution in a conical flask and kept in a magnetic stirrer for 1 h. It was analyzed in UV-Visible double beam spectrophotometer in the range of 200-800 nm.

Characterization of nanoparticles

UV-Visible spectra of the silver nanoparticles obtained from *Euphorbia hirta* L. were measured for prediction of surface plasma resonance band (SPR) by Shimadzu UV-Visible spectrophotometer. The scanning electron microscopy (SEM) analysis was analyzed by a Carl Zeiss EVO-18 electron microscope. For SEM imaging to visualize the morphological size and shape of the EH-ET AgNPs a sample of a nanoparticles solution was placed on a carbon strip attached to an SEM brass, the extra solution was detached using blotting paper and then allowed to dry by putting it under a mercury lamp for 5 min. The Fourier transform infrared spectra of EHET AgNPs were recorded using IR Tracer-100 Shimadzu FT-IR Spectrophotometer using the spectral range 4000-400 cm^{-1} with a resolution of 4 cm^{-1} . Malvern Zetasizer Nano series compact scattering spectrophotometer version 7.2 (Malvern Instruments Ltd, Malvern, UK) was used to analyse the Zeta potential of the EH-ET AgNPs. The particle size of silver nanoparticles was determined by using Beckman coulter particle size analyzer (Delsa™ Nano common).

Anticancer activity

The anticancer activity of different concentrations of EH-ET AgNPs was studied against Neuroblastoma cell (SH-SY5Y cells) and breast cancer (MCF-7 cell), the vero cells cytotoxicity also tested by MTT assay¹⁰. The parameters such as inhibitory concentration

(concentration required to inhibits the growth of 50% cancer cells IC_{50}) and cytotoxic concentrations (concentration required to inhibits the growth of 50% normal cells CC_{50}) were measured for the analysis of anticancer and cytotoxicity potential of EH-ET AgNPs, respectively. The results were mentioned in the graphs 6 & 7 and Figs. 8 & 9.

Results and Discussion

Synthesis and characterization of Silver nanoparticles

In the present work, we synthesized AgNPs by bio-reduction method using silver nitrate solution as a source and ethanol leaf extract of *Euphorbia hirta* L. as a reducing agent. (Fig. 1) represented the generation of AgNPs. In brief, the ethanolic leaf extract added to the source solution and the mixture was stirred on a magnetic stirrer till color changed to reddish brown. The color change indicates the reduction of silver nitrate to AgNPs. The synthesis of the AgNPs was also further indicated by the presence of surface plasma resonance (SPR) at 429.5 nm in the UV-Vis spectrum (Fig. 1). The FTIR spectrum of AgNPs is shown in (Fig. 2). It is evident that the spectrum had absorption peaks at 3452 cm^{-1} , 2885 and 2835 cm^{-1} , 1639 cm^{-1} , 1514 cm^{-1} , 1080 cm^{-1} , 462 cm^{-1} . Of those, a band at 1639 cm^{-1} indicated the presence of (C=O) and peak at 3452 cm^{-1} are due to a hydroxyl group (-OH), peak 1514 cm^{-1} for C=C and 2885 and 2835 cm^{-1} for c-alkyl stretch. SEM image was recorded and is presented in (Fig. 3). It is evident that the AgNPs synthesized were spherical in shape. EH-ET silver nanoparticles (Fig. 4) had the particle size from

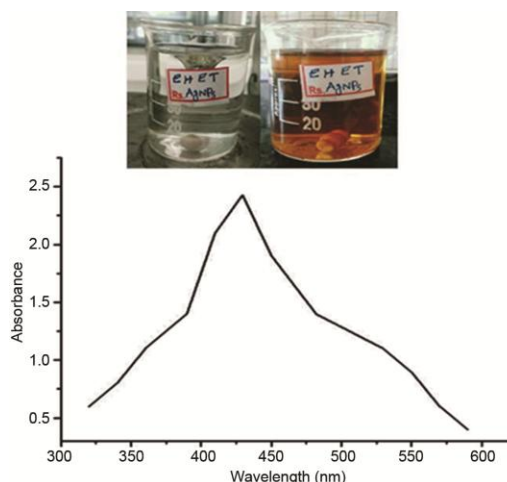


Fig. 1 — UV Visible spectra of biosynthesized silver nanoparticles from ethanolic extracts of *Euphorbia hirta* L. aerial parts (EH-ET AgNPs)

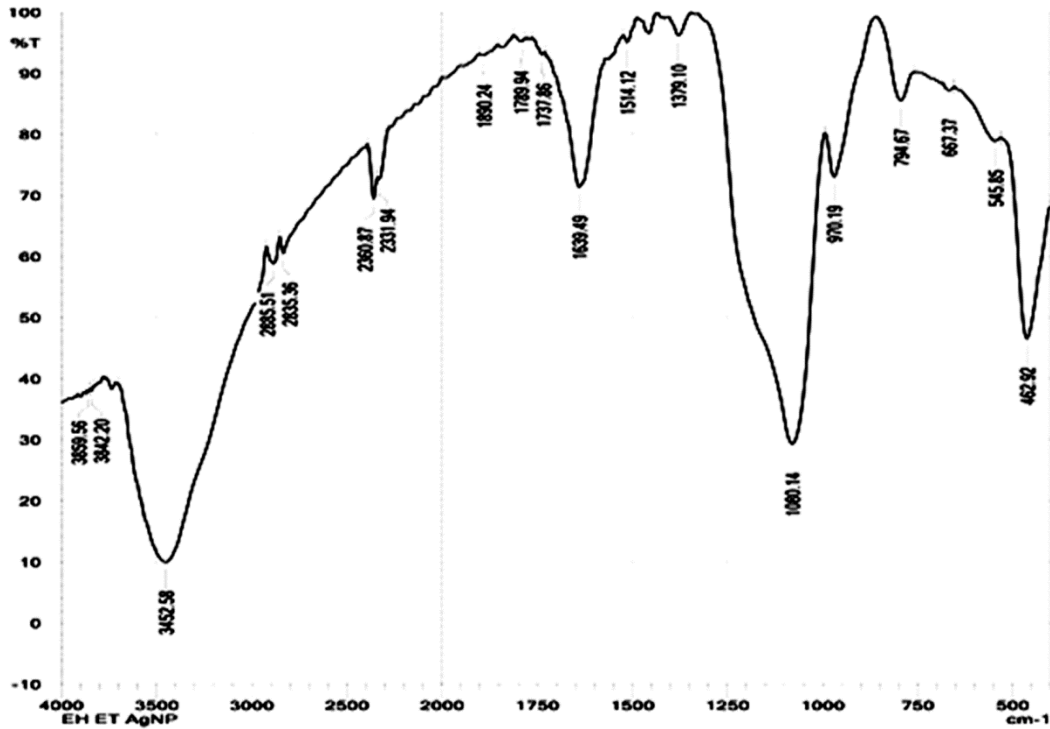


Fig. 2 — FTIR spectra of the biosynthesized EH-ET AgNPs

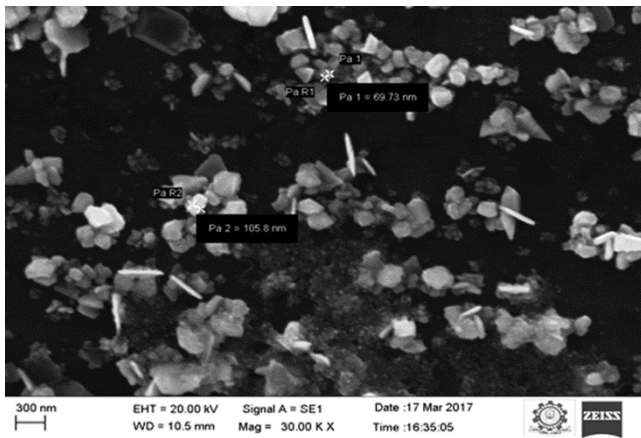


Fig. 3 — SEM micrograph of biosynthesized EH-ET AgNPs

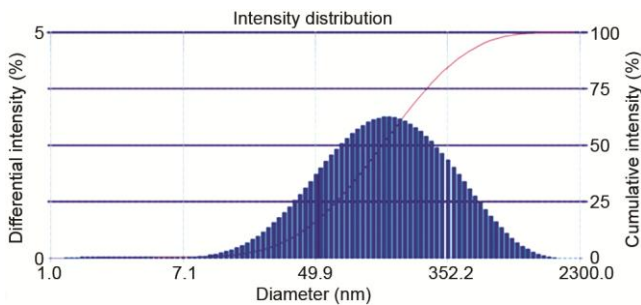


Fig. 4 — Particle size analysis of the biosynthesized EH-ET AgNPs

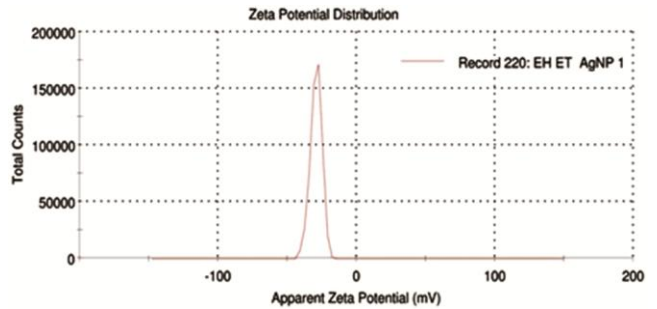


Fig. 5 — Zeta potential of the biosynthesized EH-ET AgNPs

2.9-206.3 nm polydisperse index was found to be 0.330, which indicates the uniform distribution of AgNPs in the medium.

The electrostatic repulsion between the biosynthesized silver nanoparticles is a key parameter for the investigation of nanoparticles stability, AgNPs are negatively charged in nature will prevent the particles association, subsequently reduces the agglomeration in the medium and increase the stability of AgNPs (Fig. 5). The EH-ET AgNPs in the present investigation were negatively charged with a Zeta potential of -29.4 mV. Which provide the support that the particles were uniformly dispersed in the medium and excellent indicator for AgNPs stability.

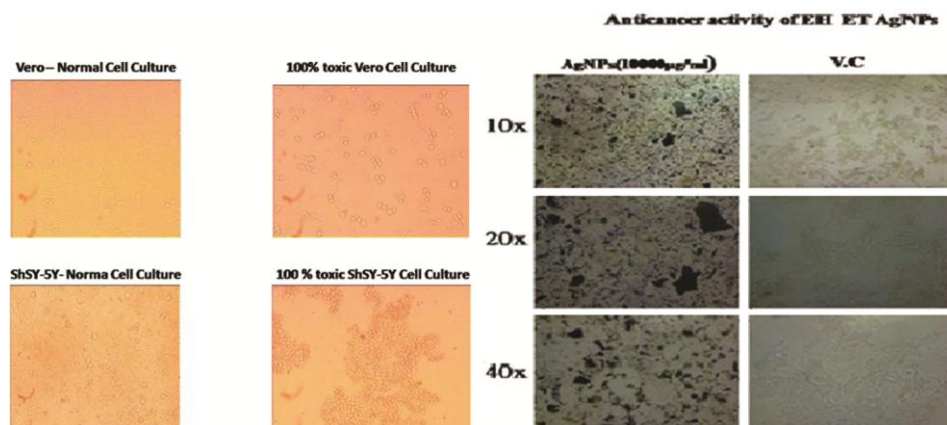


Fig. 6 — Cytotoxicity of EH-ET AgNPs in breast cancer cell (MCF-7 cells) and vero cells

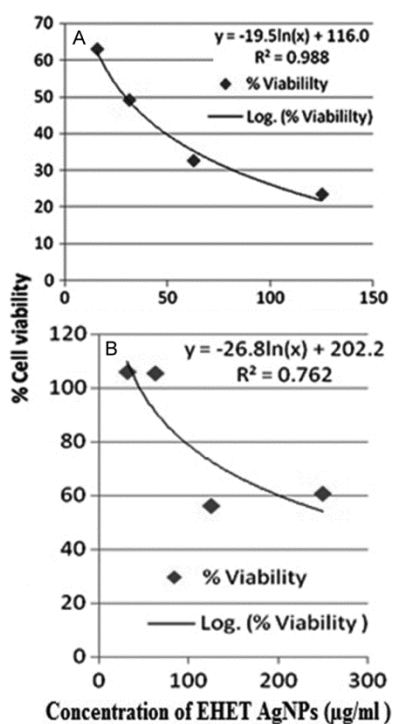


Fig. 7 — (A) Cytotoxicity of EH-ET AgNPs in neuroblastoma cell (SH-SY5Y cells); & (B) Cytotoxicity of EH-ET AgNPs in vero cell

Anticancer activity and cytotoxicity of silver nanoparticles

Anticancer activity and cytotoxicity of silver nanoparticles tested against neuroblastoma cell (SH-SY5Y cells), breast cancer (MCF-7 cell) and vero cells, respectively. The obtained silver nanoparticles showed anticancer activity against Neuroblastoma cells (SH-SY5Y cells) with IC_{50} value of $29.86 \mu\text{g/mL}$ and breast cancer (MCF-7 cell) is $335 \mu\text{g/mL}$, whereas, Cytotoxicity (CC_{50}) of EH-ET AgNPs in vero cells was found to be $292.69 \mu\text{g/mL}$ (Figs. 6-8). The synthesized silver nanoparticles using an ethanolic extract of *Euphorbia hirta* L. would be

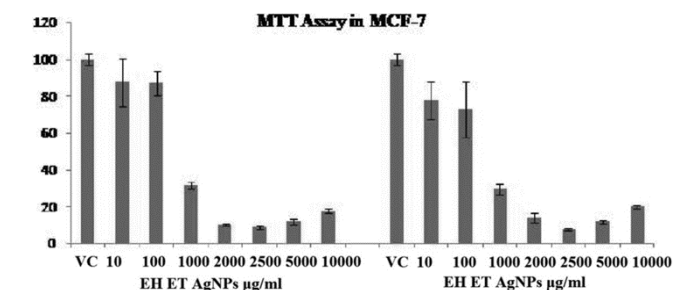


Fig. 8 — Cytotoxicity of EH-ET AgNPs in breast cancer cell (MCF-7 cells) graph

helpful for the preparation of potent cytotoxic agents to destroy cancer cells.

Conclusion

In addition to possessing anticancer potential, EH-ET has the capacity to reduce metal salts into metal nanoparticles such as AgNPs. The present study proved that the silver nanoparticles of ethanol extract of *Euphorbia hirta* L. showed significant anticancer activity against Neuroblastoma cells (IC_{50} : $29.86 \mu\text{g/mL}$; CC_{50} : $292.69 \mu\text{g/mL}$; $SI > 10$) and Breast cancer cells (IC_{50} : $335 \mu\text{g/mL}$). Furthermore, this is the first study which revealed that AgNPs synthesized from *Euphorbia hirta* L. The present work describing the anticancer activity of EH-ET AgNPs against Neuroblastoma cell and without affecting normal vero cells.

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