

SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLE FROM TWO MEDICINAL PLANTS AND ITS ANTICANCER PROPERTY

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ABSTRACT

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Medicinal plants possess immunomodulatory and antioxidant properties, leading to anticancer activities. *Jatropha curcas*, a tropical shrub plant belonging to the Euphorbiaceae family, is commercially important one as bio diesel is extracted from its seeds on industrial scale. *Jatropha gossypifolia* belongs to the same family, Eurphorbiaceae and the order, Geraniale.

The present study involves the synthesis and their characterization of silver nanoparticles from *Jatropha curcas* and *Jatropha gossypifolia*, using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM) and to evaluate the cytotoxic properties of these silver nanoparticles against lung cancer. The silver nanoparticles (Ag-NPs) were prepared using silver nitrate as silver precursor and the aqueous stem extracts of *Jatropha gossypifolia* and *Jatropha curcas* as reducing agent and stabilizer. The silver nanoparticles isolated from aqueous stem extract of *Jatropha curcus* and *Jatropha gossypifolia* show distinct polydispersity as it shows that synthesized nanoparticles show below 1 μ m size. For the small particles it appear at 93nm and 94nm followed to large particle synthesis at 103nm, which is due to the curcacyline A, curcain and Cyclogossine, present in the stem extract of *Jatropha curcus* and *Jatropha gossypifolia* which is mainly responsible for the reduction of silver ion. Our study showed that A549 lung cancer cell line proliferation were significantly inhibited at a dose dependent manner by AgNPs isolated from *Jatropha* species with an IC₅₀ value of 19.5 μ g/ml (*Jatropha curcus*) and 13.5 μ g/ml (*Jatropha gossypifolia*) of the concentration. The data represented in our study contribute to a novel and unexplored area of nanomaterials as alternative medicine.

KEYWORDS: Silver Nanoparticles, Nanomedicine, UV-Vis Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), *Jatropha curcus*, *Jatropha gossypifolia*, A549 Lung Cancer Cell Line

INTRODUCTION

Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and metastasis [1]. Unfortunately, currently available cancer chemotherapeutic agents insidiously affect the host cells especially bone marrow, epithelial tissues, reticule-endothelial system and gonads [2,3] Because of high death rate associated with cancer and because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment. Metal nanoparticles have tremendous application in the area of catalysis, opto-electronics, diagnostic biological probes and display devices Metallic nanoparticles are mostly prepared from Nobel metals such as Gold, Silver, Platinum and Lead. Among the Nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine [4]. Many attempts have been made to use silver nano particles as an anticancer agent and they have all turned up positive [5]. It has been stressed over the years that size reduction of NPs play an important role in improving their bio availability as well as compatibility for therapeutical applications in diseases like cancer [6]. Many plant-derived products have been reported to exhibit potent antitumour

activity against several rodent and human cancer cell lines [7]. *Jatropha curcas*, a tropical shrub plant belonging to the Euphorbiaceae family contains alkaloids including Jatrophine, Jatropham and curcain with anti-cancerous properties [8]. *Jatropha gossypifolia* belonging to the same family, Euphorbiaceae and the order, Geraniales is antibiotic, insecticidal and used for toothache and as blood purifier [9]. Hence, the present study is conducted to synthesise and characterize silver nanoparticles from the above medicinal plants, and to analyse its anti-microbial activity and cytotoxic properties.

METHODOLOGY

The fresh stem of *Jatropha curcas* and *Jatropha gossypifolia* were identified and collected from the areas of Coimbatore, Tamilnadu and taxonomic identification was made by **Botanical Survey of India**, Coimbatore and the voucher specimen was numbered and kept in our research laboratory for further reference. It is then dried and powdered for the current study.

Preparation of Plant Extract

The stem of *Jatropha curcas* and *Jatropha gossypifolia* were washed with distilled water to remove dirt and soil. The shade-dried materials were powdered. Next, 2.5 g of coarsely powdered material was extracted with 50 mL of double-distilled water. The mixture was filtered through Whatman No 1 filter paper. The extract was stored at 4°C until further use.

Synthesis of Silver Nanoparticles

90ml filtrate was treated with 10ml of aqueous 1mM silver nitrate solution in a conical flask. Also 80 ml filtrate was treated with 20ml of aqueous 2mM silver nitrate solution in a conical flask. The bio reduction of silver nitrate occurred within 12hrs at 121°C. The color change to yellowish brown of the medium was noted by visual observation indicating the formation of AgNPs (silver nanoparticles), which remain stable for more than 3 months without any changes in the absorption spectrum. The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into distilled water.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Visible Spectroscopy Analysis

Change in color was visually observed in the silver nitrate solution incubated with aqueous stem extract of *Jatropha curcas* and *Jatropha gossypifolia*. The bio reduction of precursor silver ions was monitored by sampling of aliquots (2 ml) at different pH. Absorption measurements were carried out on Elico SL159 UV-Visible Spectrophotometer at a resolution of 1 nm. UV-Vis. analysis is starting from 200nm to 600nm.

FTIR Spectroscopy Analysis

For Fourier transform infrared (FTIR) spectroscopy measurements, the bio-transformed products present in cell-free filtrate were freeze-dried and diluted with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on Shimadzu IR Prestige-21 FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 400– 4000 cm⁻¹ at a resolution of 4 cm⁻¹.

SEM Analysis

Scanning electron microscopy (SEM) micrographs were obtained using a Hitachi scanning electron microscope (model S-2600 N, Tokyo, Japan) operating in the high-vacuum mode with an acceleration voltage of 20 kV.

The FTIR and SEM analysis is done at Sophisticated Analytical Instrument Facility (SAIF), STIC Cochin University of Science and Technology, Cochin.

CELL CYTOTOXIC ASSAY

Cell Culture

Lung Cancer cell line **A549** (Michigan cancer foundation) was obtained from NCCS, Pune.

The cells were plated in 75 cm² tissue culture flasks in RPMI-1640 medium supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were grown at 37° C under humidified 5% CO₂ and 95% air. The media were replaced every 2 days. At 85% confluence, cells were washed twice with phosphate-buffered saline (PBS, pH 7.3).

Cells were then trypsinized and centrifuged at 1500 rpm for 4 mins at room temperature. The pellet was suspended in media and subcultured further. The ratio of subculture has to be 1:3 or 1:4.

MTT Assay

Cytotoxicity was evaluated using MTT Survival Assay. The cleavage of the tetrazolium salt MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] into a blue colored formazan by the mitochondrial enzyme succinate dehydrogenase is potentially very useful for assaying cell survival and proliferation.

The conversion takes place only in living cells and the amount of formazan produced is proportional to the number of cells present. Thus, the MTT assay detects living but not dead cells and the signal generated is dependent on the degree of activation of the cells (X. Ye et al., 1999).

RESULTS

Synthesis of Silver Nanoparticles

Figure 1 shows the photographs of *Jatropha gossypifolia* stem extract as control, aqueous solution of 1mM AgNO₃ with *Jatropha gossypifolia* stem extract and aqueous solution of 2mM AgNO₃ with *Jatropha gossypifolia* stem extract. The first tube, Control (plant extract without AgNO₃) does not show any colour change.

The second tube (1mM concentration) shows the change in colour of the reaction medium as an effect of presence of silver nano particles, whereas the third tube (2mM concentration) shows the slight change in colour of the reaction medium as an effect of presence of silver nano particles.

Figure 2 shows the photographs of *Jatropha curcus* stem extract as control, aqueous solution of 1mM AgNO₃ with *Jatropha curcus* stem extract and aqueous solution of 2mM AgNO₃ with *Jatropha curcus* stem extract. The first tube, Control (plant extract without AgNO₃) does not show any colour change The second tube (1mM concentration) shows the change in colour of the reaction medium as an effect of presence of silver nano particles, whereas the third tube (2mM concentration) shows the slight change in colour of the reaction medium as an effect of presence of silver nano particles.

This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. There is always an essential need for reducing entities in the reaction medium to bring such conversions.

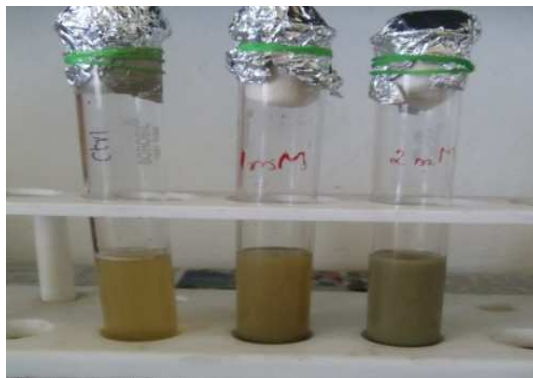


Figure 1 & 2: Isolated Silver Nano Particles from *Jatropha curcus* and *Jatropha gossypifolia*



Figure 3: UV-Vis Absorption Spectra of Ag Nanoparticles Synthesized from *Jatropha gossypifolia* at 1mM Silver Nitrate

The appearance of a yellowish-brown color confirms the existence of silver nanoparticles in the solution. The time duration of change in colour was 12 hrs. This change in color of the reaction mixture was taken as a primary evidence for the formation of silver nanoparticles. The time duration of change in colour and thickness of the colour varies from plant to plant. The reason could be that the quantitative variation in the formation of silver nanoparticles (or) availability of H⁺ ions to reduce the silver. The aqueous silver ions when exposed to herbal extracts reduced in solution, there by leading to the formation of silver hydrosol [10].

CHARACTERIZATION OF SILVER NANO PARTICLES

UV-Vis Spectroscopy

The formation of silver nanoparticles by reduction of the aqueous silver ions during exposure of *Jatropha gossypifolia* and *Jatropha curcus* aqueous stem extract may be easily followed by UV-Vis spectroscopy. The test samples (stem extract treated with 1mM and 2mM silver nitrate) were collected in aliquots from the reaction mixture and analyzed to record their absorbance by UV-Vis spectrophotometer.

The control (without AgNO₃) shows no evidence of absorption in the range 200-600nm. The samples exposed to silver nitrate solution show the wide spectrum range around 300- 410nm. The UV-Vis spectrum of colloidal solutions of silver nano particles synthesized from stem extracts of *Jatropha gossypifolia* have the characteristic absorbance peaks at 351.40 nm at 1mM AgNO₃ (Figure 4) and 325.40 nm at 2mM AgNO₃. The UV-Vis spectrum of colloidal solutions of silver nano particles synthesized from stem extracts of *Jatropha curcus* have the characteristic absorbance peaks at 349nm at 1mM AgNO₃ and 314 nm at 2mM AgNO₃ (Figure 5). No other peak was observed in each spectrum which confirms that the synthesized products are Ag only. For the highest nanometer of synthesis nanoparticles was absorbed at 1mM concentration.

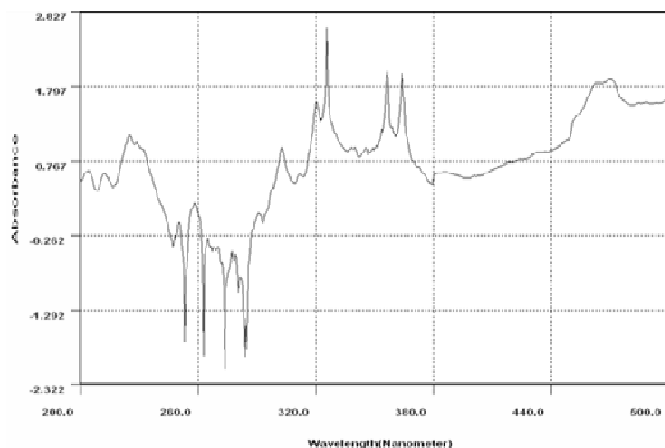


Figure 4: UV-Vis Absorption Spectra of Ag Nanoparticles Synthesized from *Jatropha gossypifolia* at 1mm Silver Nitrate

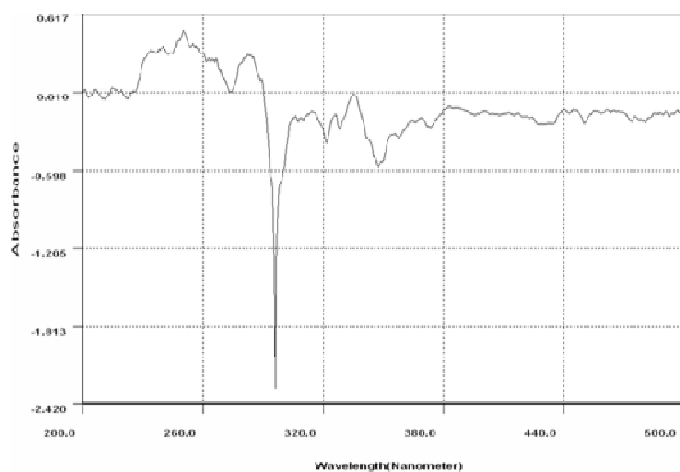


Figure 5: UV-Vis Absorption Spectra of Ag Nanoparticles Synthesized from *Jatropha curcas* at 1mm Silver Nitrate

The position and the number of peaks in the absorption spectra are dependent on the shape of the particles: for an ellipsoidal particle there are two peaks whereas for spherical particle there is only one peak centered at 420 nm, indicating the formation of silver nanoparticles. The absorption maximum at 420 nm is attributed to the Mie scattering by silver metal [11].

FTIR ANALYSIS

FT-IR analysis was carried out to identify the possible bio-molecules and cell-metal ions interaction responsible for formation and stabilization of silver nanoparticles. FT-IR spectrum of AgNPs synthesized from *Jatropha curcas* is shown in Figure 6 and FT-IR spectrum of AgNPs synthesized from *Jatropha gossypifolia* is shown in Figure 7. Mukerjee and co-workers have reported that the reduction process of Ag^+ to Ag nanoparticles occurs possibly in the presence of enzyme NADPH-dependent dehydrogenase. The exact route in which the electrons are shuttled is matter of investigation. Also the information regarding environment responsible for high stability of metal nanoparticles is not comprehensively available. FTIR analysis of isolated silver nanoparticles free from proteins and water soluble compounds was done in this direction. The analysis of IR spectra gives an idea about biomolecules bearing different functionalities which are present in underlying system. Representative spectra for plant extract at 24hrs manifest-The major IR bands at 3480, 3342, 3231, 2115 cm^{-1} and minor bands at 1635, 685, 655 cm^{-1} (*Jatropha gossypifolia*). For other plant (*Jatropha curcas*) extract at 24hrs major IR bands at 3453, 3353, 3276 and 2111 cm^{-1} and minor bands at 1634, 1411, 1063 and 687 cm^{-1} .

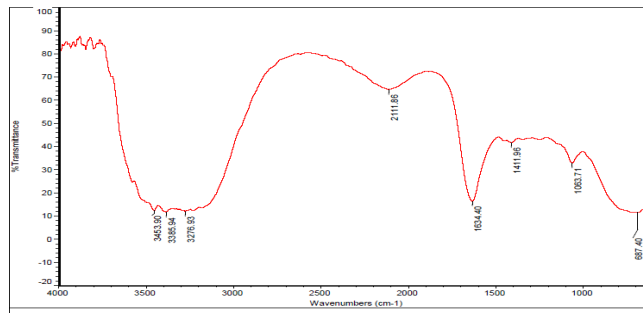


Figure 6: FTIR Spectrum of AgNPs (1mM Concentration) Synthesized by *Jatropha gossypifolia* Extract

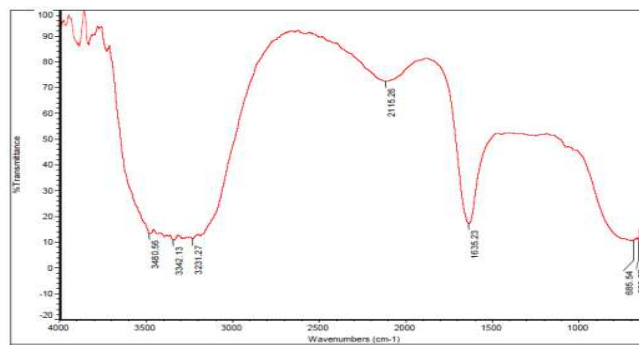


Figure 7: FTIR Spectrum of AgNPs (1mM Concentration) Synthesized by *Jatropha curcus* Extract

SEM ANALYSIS

SEM images show similar appearance for the presence of nanoparticles synthesized from *Jatropha curcus* and *Jatropha gossypifolia*. The synthesized nanoparticles show below 1 μ m size. For the small particles it appear at 93nm and 94nm followed to large particle synthesis at 103nm. The SEM image shows cluster of nanoparticles at two different size of 24hrs incubation time of plant product.

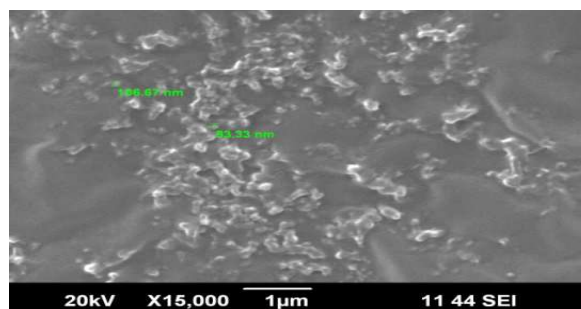


Figure 8: SEM Micrographs of the Synthesized Silver Nanoparticles (1mM Concentration) using Aqueous Stem Extracts of *Jatropha curcus*

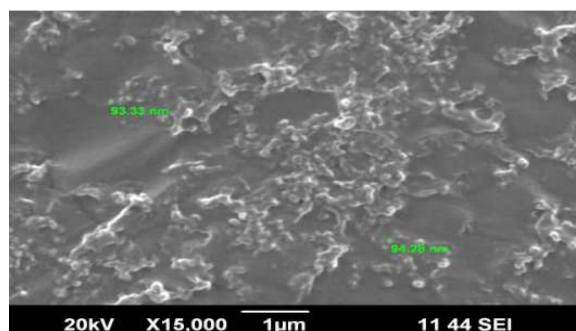


Figure 9: SEM Micrographs of the Synthesized Silver Nanoparticles (1mM Concentration) using Aqueous Stem Extracts of *Jatropha gossypifolia*

CYTOTOXICITY ASSAY

Invitro cytotoxic activity against Lung Cancer cell line **A549** was evaluated at different concentration (5µg, 10µg, 15µg, 20µg, 25 µg, 30 µg, 35 µg, 40µg, 45 µg and 50 µg) by MTT assay. The invitro screening of the AgNPs showed potential cytotoxic activity against the lung cancer cell lines. The results obtained are shown in Table 2 & Figure. Our cytotoxicity analysis of the sample shows a direct dose-response relationship; cytotoxicity increased at higher concentrations. After 4 hours of treatment, the silver nanoparticles were found to more toxic to cancer cell than the normal cell

Table 1: Showing the % of Cytotoxicity of AgNPs Isolated from *Jatropha curcus*

Sl. No.	Concentration (µl)	Mean ± SD
1	Control	-
2	5	30.01 ± 2.59
3	10	38.31 ± 3.19
4	15	20.48 ± 2.67
5	20	53.13 ± 2.64
6	25	53.54 ± 2.81
7	30	54.66 ± 1.22
8	35	75.93 ± 0.57
9	40	79.19 ± 0.35
10	45	81.51 ± 0.65
11	50	81.94 ± 1.63

Table 2: Showing the % of Cytotoxicity of AgNPs Isolated from *Jatropha gossypifolia*

Sl. No.	Concentration (µl)	Mean ± SD
1	Control	-
2	5	31.95 ± 1.35
3	10	36.68 ± 3.08
4	15	56.65 ± 1.21
5	20	58.14 ± 0.88
6	25	60.89 ± 0.35
7	30	67.23 ± 0.54
8	35	71.84 ± 1.35
9	40	76.63 ± 1.03
10	45	80.20 ± 0.94
11	50	83.08 ± 0.47

Renukadevi *et al.*, [12] showed that silver nanoparticles isolated from leaf extracts of *Baliospermum montanum* has shown the cytotoxicity effect on Vero and Hep2 cell line. This preliminary cytotoxicity study of silver nanoparticle might contribute to the comprehensive of this compound in cancer. Almost 60% of drugs approved for cancer treatment are of natural origin [12].

SUMMARY AND CONCLUSIONS

Synthesis of metallic nanoparticles using green resources like aqueous extracts of *Jatropha curcus* and *Jatropha gossypifolia* is a challenging alternative to chemical synthesis, since this novel green synthesis is pollutant free and eco-friendly synthetic route for silver nanoparticles. Characteristics of silver nanoparticles such as shape and size are important not only for augmenting the antimicrobial activity, but also for reducing tissue and eukaryotic cell toxicities.

The reduction of the metal ions through aqueous stem extracts leading to the formation of silver nanoparticles of

fairly well-defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications make this method potentially exciting for the large-scale synthesis of other inorganic materials (nanoparticles). Toxicity studies of silver nanoparticles on human pathogen and on cancer cell opens a door for a new range of antibacterial agents and anticancer agents.

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