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Characterizations and Evaluation of Anti-Cancer Property of Silver Nano-Particles of *Adhatoda vasica* Leaf and *Saraca indica* in A-549, a Human Lung Adenocarcinoma Cell Line



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ABSTRACT

Adhatoda vasica and *Saraca indica* are widely distributed in Indian continent. These plants have great biological significance and widely used in many pathological conditions. Silver has been used in nanotechnology as size reduction could be achieved, results in reduction of toxicity. This concept was adopted to formulate the silver nanoparticles of herbal extract viz *ashoka* and *A. vasica*. In the present study, the anti-cancer properties of these drugs were explored in A-549, a human lung adenocarcinoma cell line. The nanoparticles were characterized by scanning electron microscopy and UV. Study showed that silver nanoparticles of these drugs inhibited the A-549 cell growth and decreased the cell viability monitored at 24 h.



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INTRODUCTION

Lung cancer is a major threat to both developing and developed nation. According to GLOBOCON 2012, it is estimated 13 percent of lung's cancer total new cases diagnosed in 2012.¹ However in respect to India, lung cancer contributes 6.9 percent of all new cancer cases were recorded.² The overall 5 year survival rate of lung cancer is dim with approximately 15 percent and 5 percent in developed and developing nations respectively.³ A number of factors are responsible for the lung cancer induction in human body that include age, family history, smoking and occupation exposure to asbestoses and radon during the decay of uranium.

Adhtoda vasica, belongs family *Acanthaceae*, commonly known as Adoosa, evergreen shrub. In Ayurveda, it has been used for the treatment of respiratory disorders, expectorant and tuberculosis therapy and mentioned drug in Indian Pharmacopeia.⁴ It possess numerous pharmacological activity like bronchodilator activity, wound healing activity, anti-bacterial activity, anti-ulcer activity, abortifacient and uterotonic activity.⁵ *A.vasica* is rich in alkaloids, flavonoids, tannins, terpenes and glucosides. It mainly consists of quinazoline alkaloid also known as vasicine. Other alkaloids present in *A. vasica* are 1-vasicinone, deoxyvasicine, maintone, vasicinoline and vasicinol.⁶

Ashoka is one of the most sacred plant after tulsi in India. *Saroca asoca* belongs to family *fabaceae*. Ashoka is found to have anti-microbial activity, anti-cancer activity, anti-menorrhagic activity and anti-oxytotic activity.⁶⁻¹¹ Chemical constituent may differ to specific aerial part i.e. stem, root, etc. Bark contained primarily ecatechin, procyanidin, p2,11'-deoxyprocyanidin B, (+) catechin, (24, £)- 24- methyl-cholesta-5-en-3p-ol (22 E, 21£)-24- ethycholesta-5,22 dien-33-ol,(24 £)-24-ethylcholesta-5-en-3-p-ol,leucopelargonidin-3-O-p-D glucoside, leucopelargonidin and leucocyanidin.¹²

In spite of advancement in treatment, the cured ratio is still poor. In recent times, new chemotherapeutic agents have been developed but, not with broad spectrum range along with severe adverse effects.

Nanoparticles (NP's) are defined as engineered structures having diameter < 100 nm. Nanoparticles have gained attention such as drug carrier system and to pass organ barrier i.e.

blood brain barrier. NP's possess unique characteristics that allow catalytic reactions as well as their ability to absorb. Chemically, NP's surface are more reactive to find their analogue.¹³ The adverse effects at therapeutic range induced the researcher to search for newer anti-cancer compound or to find a solution from the previously established formulation. Hence it was aimed to characterize and evaluate the anti-cancer activity of *A. vasica*'s leaf and *ashoka bark*.

MATERIALS AND METHODS

Preparation of silver Nanoparticles

Crude drug samples of *A. vasica* leaves family *Acanthaceae* and *Ashoka bark* family *fabaceae*. Were authenticated at CSIR-(NISCAIR) against the reference number NISCAIR/RHMD/Consult/2015/2817/10-1 and 2824/17/1 respectively. 10 g of each drug was weighed and poured into the beaker containing 100 ml double distilled water. Volume of the beaker after adding water was marked. Heated individually on the water bath for one hour; maintained constant volume of flask to the constant volume. After one hour, content was filtered and residue was discarded. Extract was cooled and filtered thrice to remove the undesirable particles. Silver nitrate (AgNO_3) solution was prepared by the following method: 0.017 gm of silver nitrate was weighed and dissolved in double distilled and autoclaved water to produce 10^{-3} molar solution. 5 ml pure extracts of *A. vasica* was transferred to 45 ml of previously prepared the silver nitrate solution. The procedure is adopted for ashoka extract, to prepare the AgNPs and subjected to U.V spectrometry.

Cell cultures

A-549, human lung adenocarcinoma was purchased from NCCS, Pune. A- 549 cell line was maintained in DMEM high glucose (Dulbecco's modified eagle media) (Genetix Biotech Asia, New Delhi) supplemented with 10% Fetal bovine serum (FBS) (High Media, Mumbai, India) 100 units/ml penicillin and 100 $\mu\text{g/ml}$ streptomycin (Himedia, Mumbai, India). The cell was cultured and incubated in 5% CO_2 humidified at 37°C for growth.

RESULTS AND DISCUSSION

CHARACTERISATION OF SILVER NANOPARTICLE.

UV Absorption peak

The progress for AgNPs formation was monitored using the UV vis Shimadzu double beam spectrophotometer 1800, Columbia USA, operated at a resolution of 1 nm with optical path length of 10 nm.

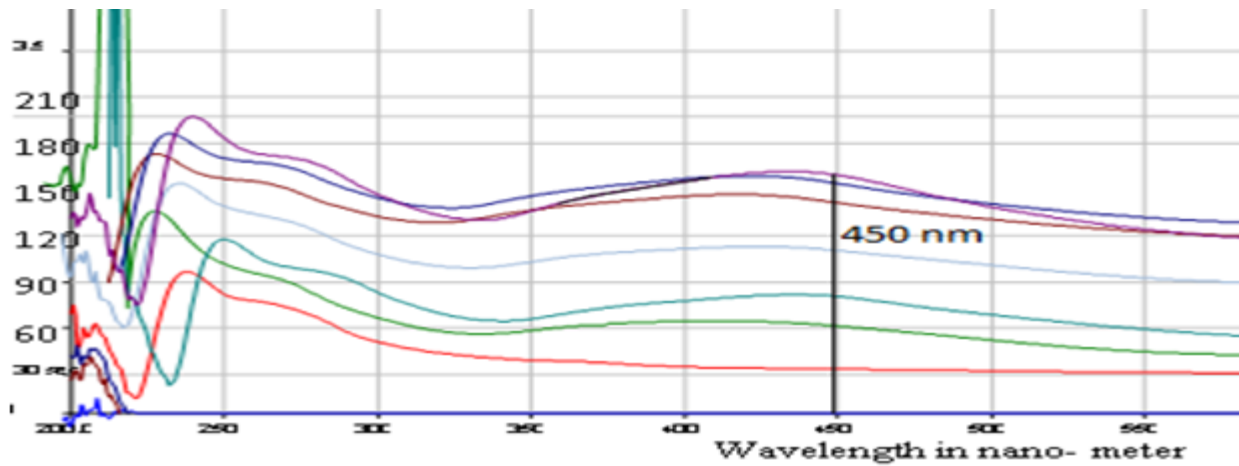


Figure:1 UV of Ashoka silver nanoparticles

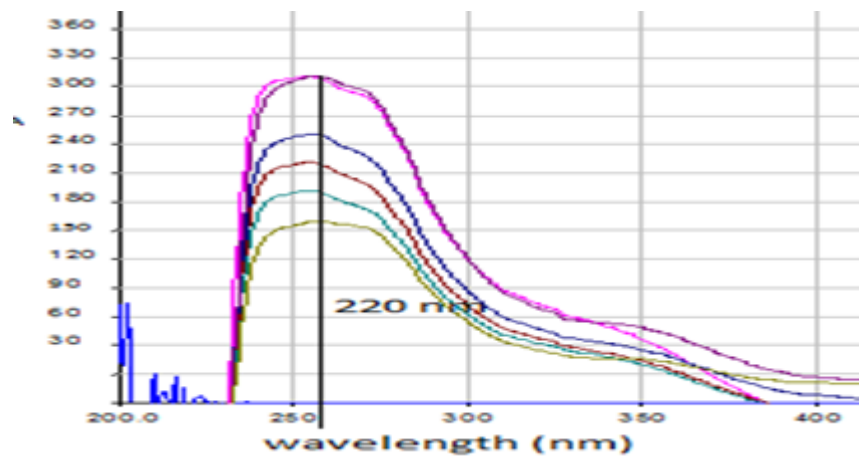


Figure 2: UV of silver nanoparticles of *adhatoda vasica*

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed on gold-platinum coated samples that were previously air dried on silicon wafers and analysis was done using an analytical scanning electron microscope (SEM ZEISS EVO® HD, Germany).

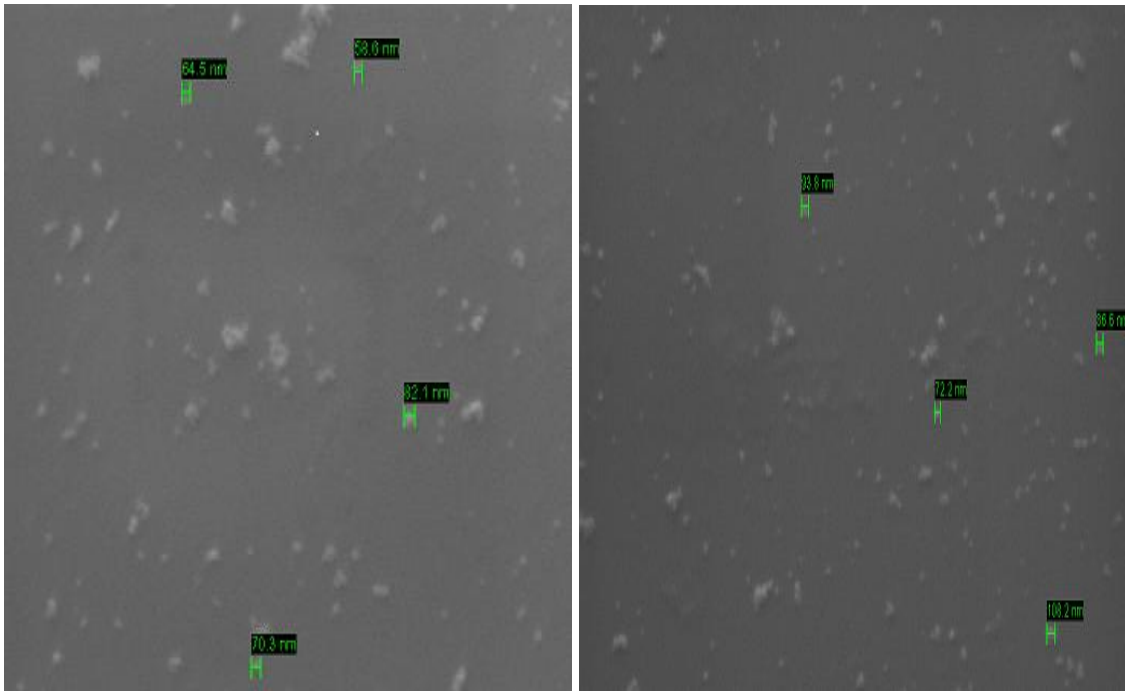


Figure 3: SEM Images of Biosynthesized Silver nanoparticles *A. vasica*

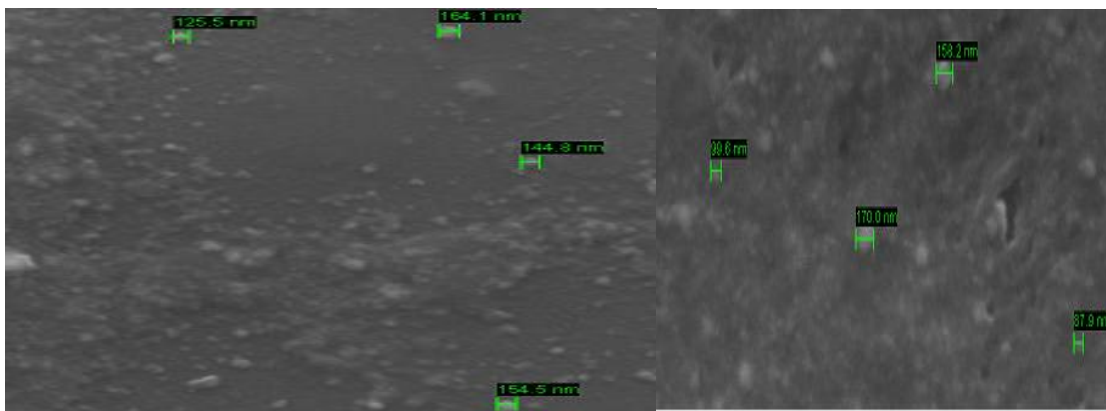
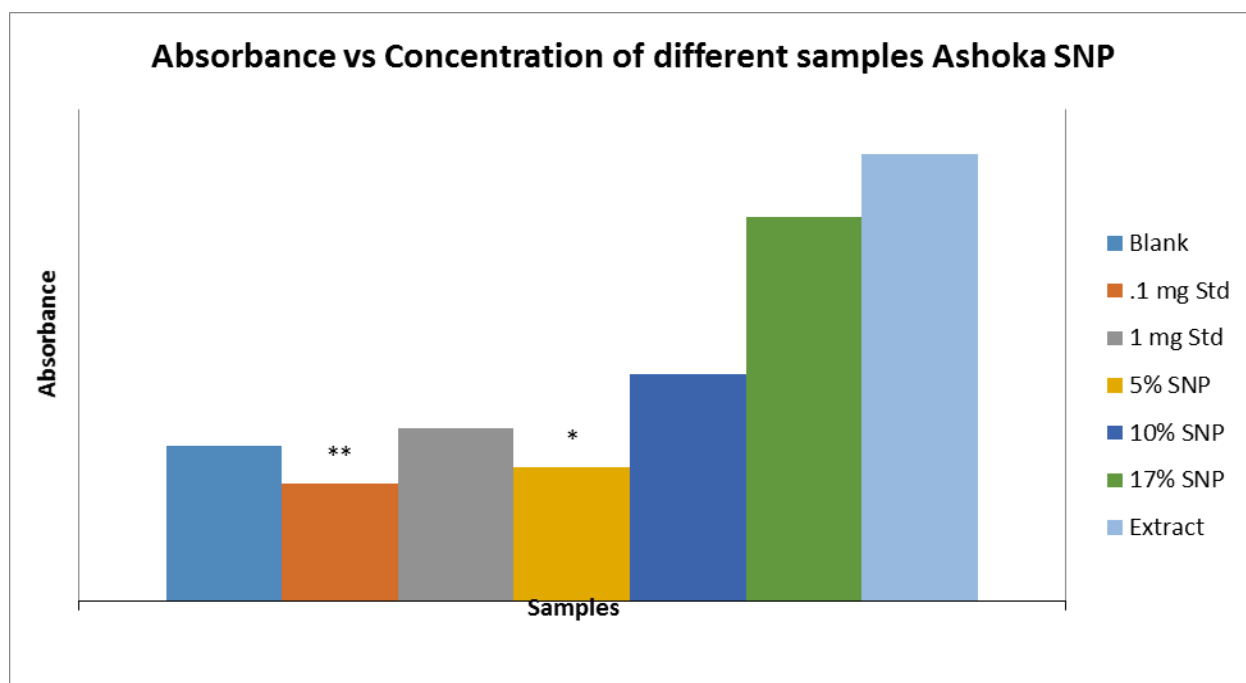


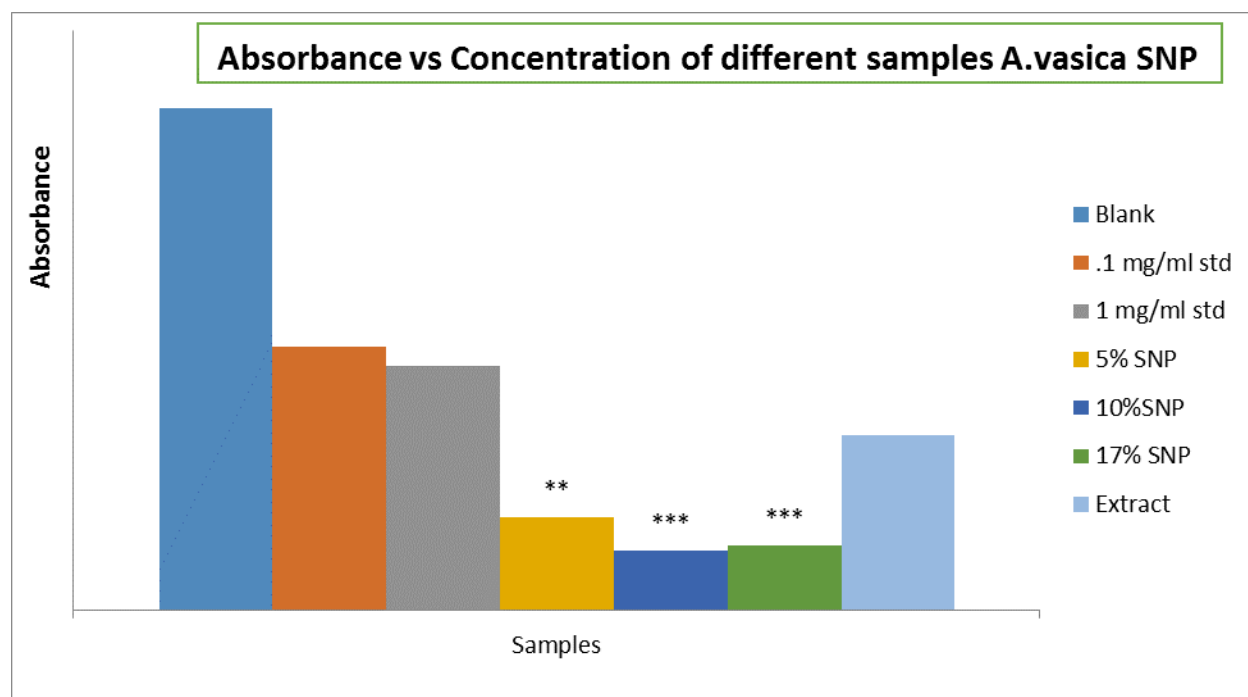
Figure 4: SEM Images of Biosynthesized Silver nanoparticles of *Ashoka*

Evaluation of cell proliferation by MTT Assay

The number of viable A-549 cells after the silver nanoparticles of various herbal extracts treatment was evaluated by MTT (3-[4,5-methylthiazol-2-yl] 2,5diphenyltetrazolium bromide) assay. In brief, A-549 cells (1×10^3 cells /well) were seeded in a 96- well plate and kept overnight for attachment. The next media was replaced with the various concentrations of silver nanoparticles of herbal extracts (1%, 5% and 17%) and cells were allowed to grow for 24 h. Four hours before the completion of incubation, 10 μ l of MTT (10 mg/ml) was added to each well. After completing the incubation, 200 μ l of DMSO was added to each well. Color developed after 30 minutes of the reaction was measured at 550 nm. Evaluation of A-549 cell count and viability was carried out using by trypan blue dye.



Graph 1.1: Showing Cytotoxicity activity of Ashoka SNP with A-549 cell line



Graph 1.2: Showing Cytotoxicity activity of *A.vasica* SNP with A-549 cell line

As soon as *Saraca indica* leaf extract was mixed in an aqueous solution of silver nitrate, the reduction of pure Ag^+ ions to AgO was monitored by measuring UV-vis spectrum of the reaction media at regular intervals. The color of silver nanoparticles was seen dark brown, may due to the excitation of the surface plasmon vibration in metal nanoparticles. UV-vis spectra were recorded as function of reaction time. The metal ions reduction occurs very rapidly and most of the reduction of Ag^+ ions was completed in between 200 to 210 sec. The intensity of the color of reaction mixture increases evenly with time of microwave exposure. Absorbance intensity increases steadily as a function of reaction time and it was observed that the surface plasmon peak occurs at 420 nm with a slight shift in the vertex of the peak towards shorter wavelength (blue shift) and fixed at 405 nm. The peak height gradually increases from 0 to 210 sec which shows the gradual formation of nanoparticles and blue shift reflects the formation of smaller nanoparticles. The microwave assisted method is much faster than the earlier conventional studies with other biological routes. The particle size and shape is confirmed with drop coated TEM grids. The particles of *A.vasica* are almost in spherical shape with diameters in the range of 50 to 100 nm; *Saraca indica* with diameter range 100-200nm and are well dispersed. Graph 1.1 shows that 5% SNP of Ashoka showed the marked cytotoxicity activity, however

graph 1.2 showed that 10%, 17% and 5% showed the prominent cytotoxic activity. We have noticed that silver nanoparticles synthesized using *Saraca indica* bark and *A. vasica* extract produce sensitivities towards A-549 human lung adenocarcinoma Cell Line.

CONCLUSION

Rapid green synthesis of silver nanoparticles from *Saraca indica* bark and *A. vasica* extract using a simple, fast and efficient microwave-assisted route of spherical shaped, fcc structure with diameter range of 50 to 100 nm has been envisaged. The formation of silver nanoparticles using the microwave as thermal energy is the fastest methodology available till today. Synthesis of silver nanoparticle does not require much chemical or surfactant. Change in color occurs due to surface plasmon resonance during the reaction with the ingredients present in the bark and leaf extract results in the formation of silver nanoparticles which is confirmed by UV-Vis, and TEM. Silver nanoparticles, so obtained, are stable for more than 3 months. Investigation of the cytotoxic effect of nano sized silver colloidal solution against A-549 cell line *reveals* high efficacy of silver nanoparticles as a strong cytotoxic agent which can be useful in pharmaceuticals and in cosmetic industry.

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