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Green synthesis of silver nanoparticles using *Andrographis paniculata* (Burm. f.) Nees stem extract and their evaluation for anti-cancer activity

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Abstract

Cancer is often characterised by uncontrolled cell growth due to loss in cell death mechanisms. It is emerging as a global pandemic affecting huge number of people across the globe. Design and development of new anticancer agents using conventional methods are always challengeable due to their life-threatening side effects. These limitations may overcome by the use and green method synthesised nanomaterials. But to impart their effects in biological system by retaining in intracellular environment a proper shape and size of nanoparticle is essential. Regarding the same, present study aims to synthesise silver metal nanoparticles using *Andrographis paniculata* stem extract and to evaluate their anti-lung cancer activity. Material characterisation were done through Scanning Electron Microscopy (SEM), Energy dispersive X Ray analysis (EDAX), Transmission Electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy analysis. EDAX and SEM analysis showed presence of silver nanoparticles in synthesised material with rough spherical morphology respectively. TEM analysis showed nanoparticles with size ranged from 13-27 nm. Decreased A549 cell viability was reported when cells were treated with synthesised silver nanoparticles.

Key words : Phytochemicals, nanoparticles, oxidative stress, cell viability assay.

In recent past years nanotechnology has been emerged as a panacea against various scientific sphinx. Nanomaterials are exhibit different shapes like tubes, rod spheres etc. and size usually ranges from 1-100 nm.[11] Physical, chemical and biological methods are commonly employed for nanomaterial synthesis. But for biological applications of nanoparticles

physical and chemical methods are not considered safe and eco-friendly due to high requirement of toxic chemicals, high pressure, temperature etc. On the other hand, nanomaterial synthesized using biological samples are considered as safe, cost effective and environment friendly, hence it is considered as a green method.[12,28] Synthesis of metal nanoparticles using green method shows varied applications due to plasmon resonance and thus could exhibit different intracellular functions. Bacterial culture, algae, fungi, plant extract etc. are most often used for the green method-based nanomaterial synthesis. However, nanoparticles synthesize using bacterial cultures, algae and fungi are considered to be costly, tedious and time-consuming methods as compared to the use of plant extracts.[17] Use of plants to cure different ailments have been practicing since ancient times. Plant secondary metabolites are rich in different functional groups usually synthesized by plants as a part of their defense mechanisms. During reaction of plant extracts with metal solution, phytochemicals not only reduce metal ions but also cap them to form nanoparticles with different shapes and sizes.[26] Thus, each plant phytochemicals could synthesize unique and different nanoparticles. Metal nanoparticles such as silver (Ag) and gold (Au) have been studied colossally for their various applications; among which study of anti-microbial activity and anti-cancer activity are more common.[6]

Cancer is considered as a deadliest disease affecting millions of people around the world. As per recent statistics on cancer, in United States it has been projected that cancer may responsible for approximately 1,958,310 new cancer cases and near about 609,820 deaths.[23] In developing countries like India, situation will be worst if urgent precautions are not taken. In India, it has been predicted that by the year 2040, new cancer incidences may increase to 2.08 million. However, among various types of cancer, prevalence of lung cancer may increase due to high (approximately 28%) use of tobacco.[22] Also, a rise of approximately 6.9% of new lung cancer cases and approximately 9.3 % deaths have been predicted in male and female.[13] *Andrographis paniculata* (Burm. f.) Nees is also called as green chiretta and reported as a native plant of India and Shri Lanka. Different parts of this plants have been reported for different biological activities due to varied phytochemicals.[1,5] Thus, present study aims to synthesise silver nanoparticles using *Andrographis paniculata* stem extract and to evaluate their anti-cancer activity in human lung adenocarcinoma A549 cells.

Selection and authentication of Andrographis paniculata plant stem and preparation of distilled water extract:

Andrographis paniculata stem was collected in the month of July from Nagpur city and authenticated from taxonomists. Soxhlet apparatus and Rotary evaporator have been considered to maintain good phytochemicals quality and quantity during extraction procedures, therefore we selected these two apparatuses for the preparation of plant stem aqueous extract. For this, fresh plants were dried in shed for 20 days and later on collected stems. 20 grams of stem were subjected to prepare aqueous extract. Dry mass of extract was used to prepare silver nanoparticles.

Preparation of silver nanoparticles:

Two hundred ml solution of 100 mM silver nitrate ($AgNO_3$) was taken in a beaker and kept in water bath at 50°C. To this, 1 gram of plant stem extract was added and kept for 30 minutes with continuous stirring and observed change of $AgNO_3$ (colourless solution) to brownish colour. After this, the solution was centrifuged at 10000 RPM for 10 minutes. Pellet was collected and dried at 80°C in oven. The dried mass was then grinded by pestle and mortar for 2 hours. This sample was used for characterisation and evaluation for anticancer activity.

Characterisation of synthesised sample: Sample characterisation was done using SEM, EDAX, TEM and FTIR analysis from Sophisticated Test and Instrumentation Centre (STIC) Cochin University of Science and Technology, India.

Cell Lines: Efficacy of the green method synthesised silver nanoparticles was assessed on A549 cells. A549 cell line was purchased from National Centre for Cell Science (NCCS) Pune, India. Cell line was maintained in Ham's F12 nutrient mixture media (Himedia) with 1% penicillin streptomycin antibiotic solution (Hyclone) and 10% Fetal Bovine Serum (FBS) (Hyclone) using 5% CO_2 at 37°C in CO_2 incubator. Experiments were done on 4th passaged cells.

Cell viability assay: Cell viability assay using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was performed on A549 cell line. Cells were seeded in 96 well plate at the concentration of 2×10^4 cells. Later on, cells were treated with green method synthesised silver nanomaterial sample (Experimental) and without sample (Control) and incubated at 37°C in CO_2 incubator for 24 hours. Experiment was done in triplicate at the different concentrations of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL of the sample. After 24 hours, media from each well was removed and 50 µL MTT (5mg/mL media) was added. Plate was again incubated for 4 hours in CO_2 incubator. After incubation media was removed and purple coloured farmazan crystals were dissolved in 100µL dimethyl sulphoxide solution. Absorbance was measured for each well using Elico ELISA plate reader at 570 nm[7]. Results were expressed in percentage using following formula:

Cell Viability= OD of Sample/OD of Control $\times$100.

Green synthesis of silver nanoparticles and its morphological and elemental analysis :

Plant phytochemicals reduces Ag+ ions to form nanoparticles. Further, these neutral ions aggregated with each other and capped by phytochemicals to form nanoparticles of sizes ranging from 1-100 nm. Change in colour during reaction of plant extract and metal ions has been considered as a preliminary test for the synthesis of nanoparticles.[9] In present study change in brown colour when $AgNO_3$ solution reacted with *Andrographis paniculata* plant stem aqueous extract was observed, which indicated the synthesis of silver nanoparticles (Figure 1). This preliminary colour change has also been reported by previous other studies.[27] Further, SEM coupled with EDAX techniques were used to study morphological characteristics and elemental analysis respectively for the synthesised

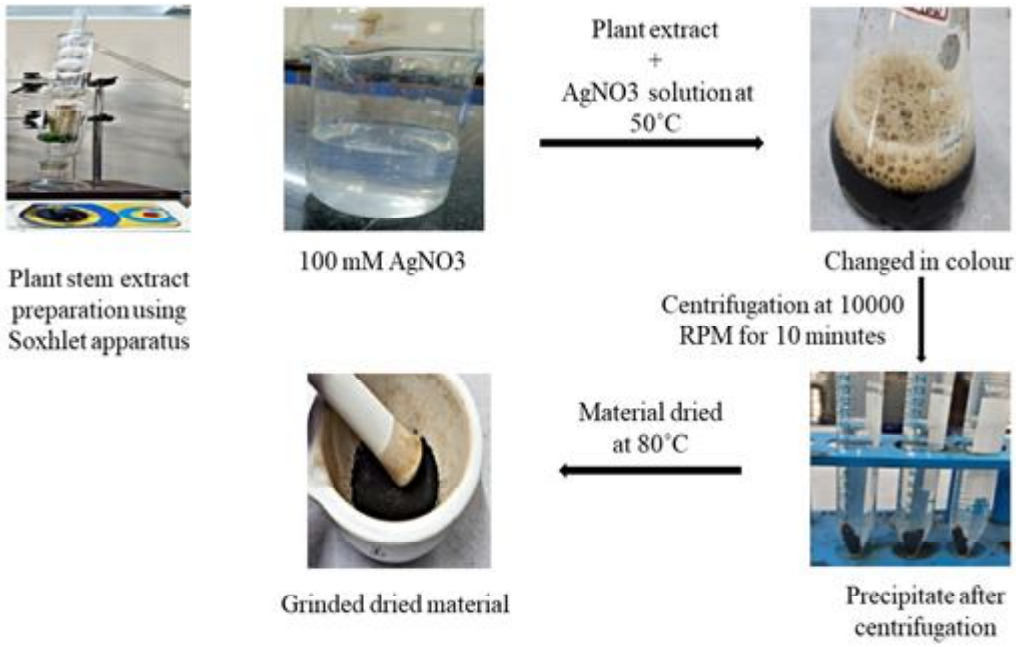

Figure 1. Synthesis of silver nanoparticles using *Andrographis paniculata* plant stem extract.

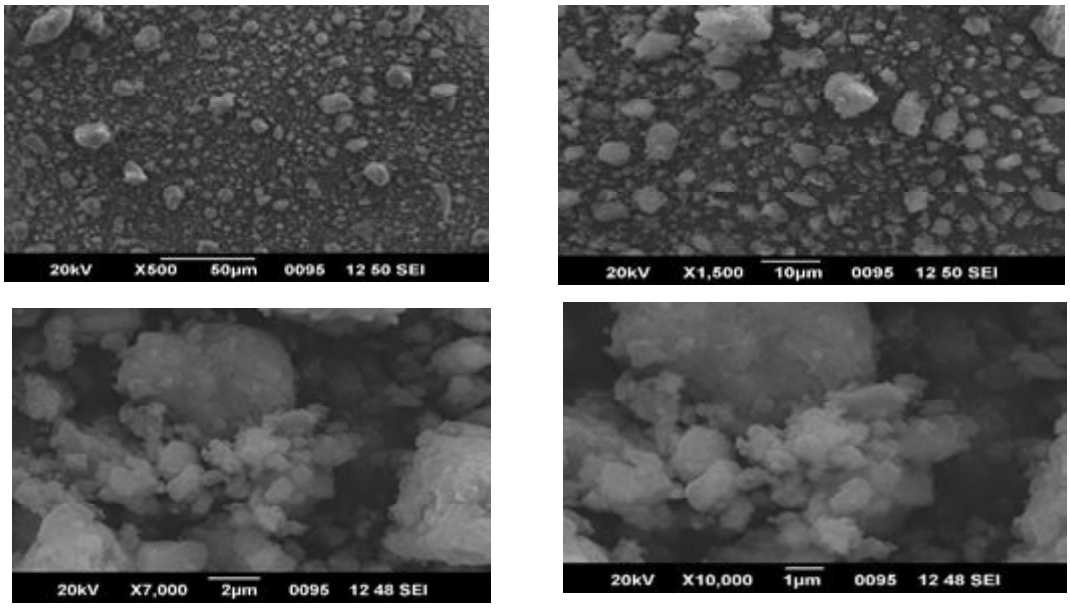

Figure 2. SEM analysis of *Andrographis paniculata* plant stem extract derived silver nanoparticles at 500X, 1500X, 3500X and 7000X.

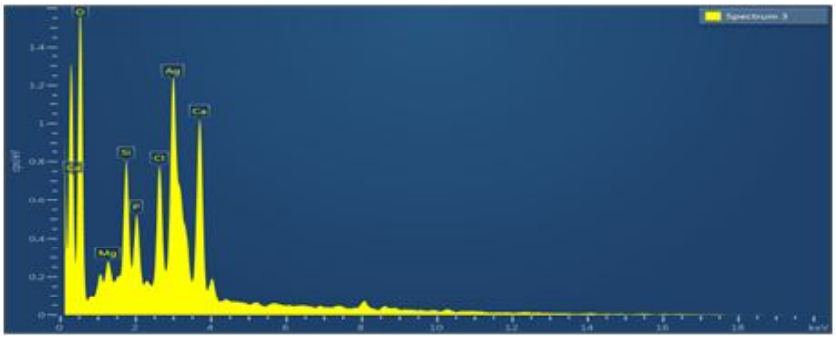

Figure 3. EDAX analysis of *Andrographis paniculata* plant stem extract derived silver nanoparticles confirming synthesis of silver (Ag) nanoparticles.

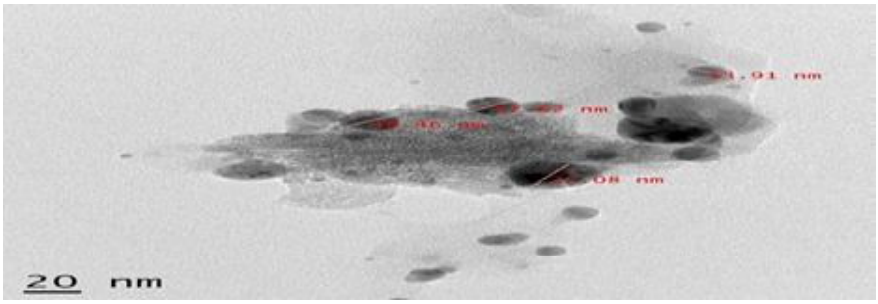

Figure 4. TEM analysis of *Andrographis paniculata* plant stem extract derived silver nanoparticles showing nanoparticles ranging from 13-27 nm in size.

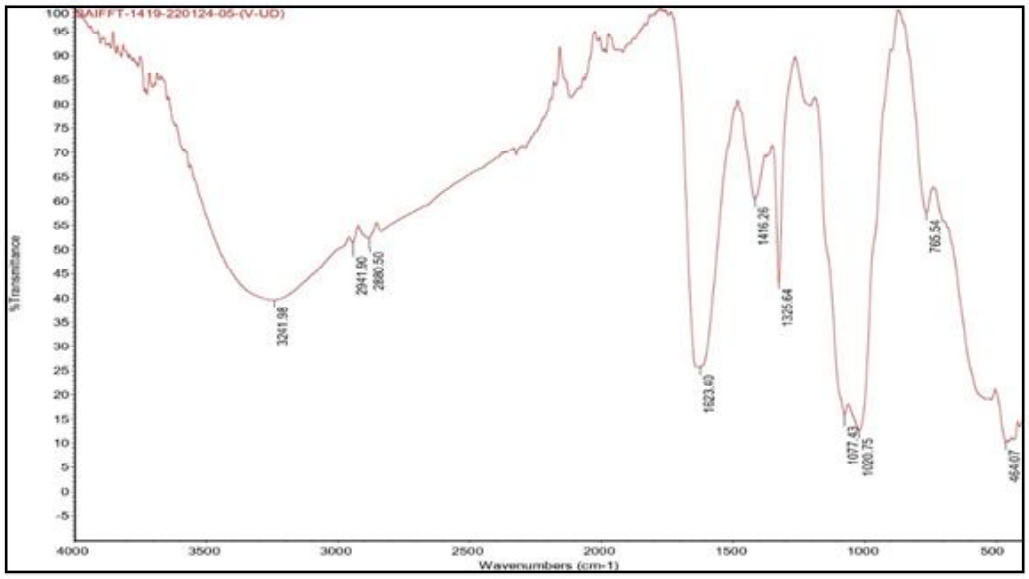

Figure 5. FTIR spectrum of *Andrographis paniculata* plant stem extract derived silver nanoparticles.

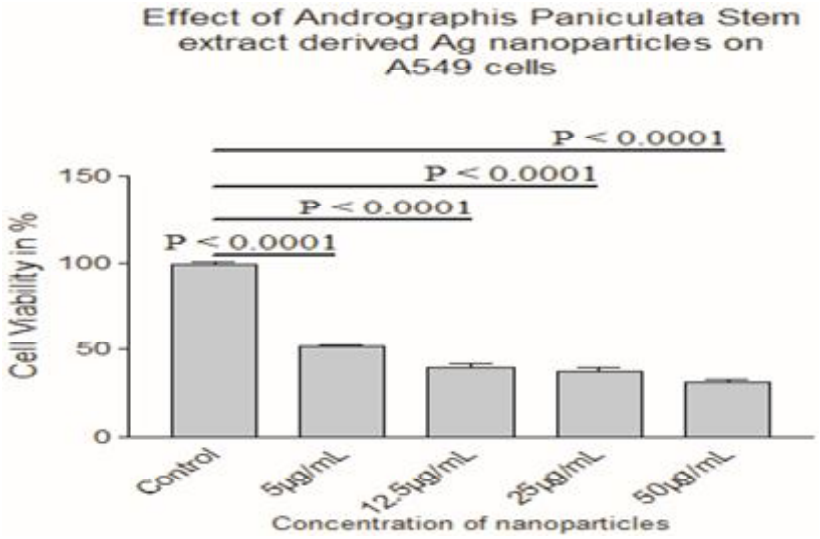

Figure 6. Effect of *Andrographis paniculata* stem extract derived Ag nanoparticles for anti-lung cancer activity. Control: without nanoparticle sample (Cells+ Media). Experimental (Cell+ Media+ Plant derived nanoparticle in concentration of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL).

nanoparticles. SEM analysis revealed massive aggregation of material with some rough spherical characters (Figure 2). EDAX based elemental analysis showed presence of silver (Ag) ion confirms the synthesis of silver nanoparticles (Figure 3). Further, TEM showed spherical nanoparticles with size ranging from 13-27 nm (Figure 4). These results are consistent with other findings. Suriyakalaa U et al. synthesised silver nanoparticles using *Andrographis paniculata* plant extract and reported nanoparticles size ranging from 13-27 nm, as reported in the present study.[27] Anantharaman *et al*.[2] synthesised nanoparticles using *Andrographis paniculata* plant extract and reported nanoparticles of the sizes ranging from 18-43nm.

FTIR analysis :

FTIR analysis of the synthesised silver nanoparticle was performed to analyses possible involvement of *Andrographis paniculata* plant stem extract phytochemicals. FTIR spectrum (Figure 5) showed role of the plant phytochemicals for the reduction and capping of Ag nanoparticles. The FTIR spectrum of Ag nanoparticles ranges from 464.07 CM^{-1} to 3241.98 CM^{-1}. The peaks in the spectrum are 464.07 CM^{-1}, 765.54 CM^{-1}, 1020.75 CM^{-1}, 1077.43 CM^{-1}, 1325.64 CM^{-1}, 1416.26 CM^{-1}, 1623.40 CM^{-1}, 2880.50 CM^{-1}, 2941.90 CM^{-1} and 3241.98 CM^{-1}. 464.07 CM^{-1} represents presence of alkyl halides, 1020.75 CM^{-1} corresponds to C-N stretching represent amine group, 1077.43 CM^{-1} corresponds to C-O stretching represents primary alcohol, 1325.64 CM^{-1} corresponds to C-N stretching represents aromatic amines, 2880.50 CM^{-1} and 2941.90 CM^{-1} corresponds to C-H stretching represents alkanes and 3241.98 CM^{-1}

corresponds to O-H stretching represents carboxylic acid. Components observed through FTIR analysis in this study represents reported phytochemicals such as flavonoids, alkaloids, tannins, triterpenoids, polyphenols etc. in *Andrographis paniculata* plant.[8] The result of the study is consistent with the results of other previous findings.[8,24]

Cell viability analysis in A549 cells :

Due to unique physical and chemical properties metal nanoparticles have been widely used in the field of biological sciences to resolve scientific enigmas. The prime advantage of nanomaterials over conventional drug delivery systems and therapeutic agents includes their target specificities and nearly negligible treatment side effects. Thus, nanomaterials are widely used in design and development of new therapeutic agents against different ailments, bioimaging system, in-vitro diagnostics, novel biomaterial design and active implants.[15,20] Among varieties of nanomaterials metal nanoparticles synthesised using metal compound or metal salts could implement their effect in biological systems more accurately and specifically due to their unique metallic characteristics like plasmon resonance, Rayleigh scattering, Raman scattering etc.[3] Plant phytochemicals reduces metal ions to metal nanoparticles, however its capping by plant phytochemicals makes metal nanoparticles a better choice for biological studies. Each plant is unique for their phytochemical contents hence they can reduce metal ions in such a way to produce different size and shape of nanoparticles. However, due to low surface area spherical nanoparticles are considered more effective in biological systems.[18] Present study aims to study effect of biogenic silver nanoparticles synthesised using *Andrographis paniculata* plant stem extract in the concentrations of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL to assess cell viability in A549 cell line. Result of the study corroborated a significant decreased cell viability when A459 cells were treated with synthesised Ag nanoparticles at the concentrations of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL as compared to control ($P<0.0001$) (Figure 7). The decreased cell viability of A549 cells were possibly due to high oxidative stress caused by Ag+ ions. Oxidative stress has been considered as an imbalance between the levels of free radicals and antioxidants. It has been reported that Ag+ released from metal nanoparticles due to plasmon resonance could trigger intracellular Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) levels through Fenton reaction. In presence of Ag+ ions Fenton reaction breaks hydrogen peroxide into hydroxyl free radical (OH•) which reacts with intracellular proteins, enzymes, DNA, cell membrane and disrupt them, resulting in cell death.[4,19] Cancer cell death mechanism reported to be governed by mitochondrial intrinsic and extrinsic pathways. Thus, reduction in activation of apoptosis process through oxidative stress induced inactivation of mitochondrial membrane potential (MMP) has also been reported for decreased cancer cell viability.[29] Another possible mechanism that could be associated with decreased cell viability of A549 cells include, up and down regulation of certain intracellular proteins. Under oxidative stress intracellular proteins modulates the mselves in such a way that cells are getting certain exogenous or endogenous signalling stimuli and thus triggers intracellular alterations

such as altered gene expression, metabolic activities, membrane alterations, cell signalling etc leading to cell death through abrupt cell cycle progression.[16] Such Ag + ion dependent induced oxidative stress killing mechanisms are also reported by other studies. In A549 cells, AgNPS induced free radical generation are reported to be responsible for increased apoptosis process through sub G1 phase arrest, S phase arrest during cell cycle progression, release of cytochrome c from mitochondria, altered Bax and Bcl2 gene expression and activation of caspase 3 and 9, which results in cell death.[14] Result of this study is consisted with the other finding.[10]

A well-known literature explains bioactive components and ethnobotany of *Andrographis paniculata* plant. The results of the present study showed the silver nanoparticles synthesis using studied plant stem aqueous extract. SEM analysis showed rough spherical morphology of material. Presence of silver in EDAX analysis confirmed synthesis of silver nanoparticles, while TEM analysis showed nanoparticle size ranging from 13-27 nm. FTIR analysis elucidated nanoparticle capping by plant phytochemicals components such as alkyl halides, amine group, primary alcohol, aromatic amines, alkanes and carboxylic acid. Biological efficacy of synthesised nanomaterial showed decreased cell viability of A549 cells, suggesting their anti-cancer activity. Further molecular, biochemical and immunological studies are required to validate the findings.

Conflict of interest :

Authors declares no conflict of interest.

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