Journal of Applied Biological Sciences
E-ISSN: 2146-0108 18(1): 118-126, 2024
DOI: https://doi.org/10.71336/jabs.1254
Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *ANDROGRAPHIS PANICULATA* LEAVES EXTRACT AND THEIR ANTIPROLIFERATIVE ACTIVITY IN HUMAN LUNG ADENOCARCINOMA CELLS

Simran Gajbhiye, Kiran Pal, Utpal Dongre*

Dr Ambedkar College, Department of Biochemistry and Biotechnology, Deekshabhoomi, Nagpur, Maharashtra, India.

**Corresponding Author:*
E-mail: utpal24dongre@gmail.com

(Received 22nd November 2023; accepted 31st January 2024; published: 31st January 2024)

ABSTRACT. Cancer is emerging as a global pandemic affecting millions of people. It is often characterised by uncontrolled cell growth due to loss in death mechanism. Conventional anti-cancerous drugs and therapies exhibits other life-threatening side effects. Thus, design and development of new anti-cancer therapeutic agents is the need of time. Metal nanoparticles synthesis via green method have been studying prodigiously worldwide for safe and efficient anti-cancer agents. However, to impart effects of nanoparticles their persistent with the cells are important and for this synthesis of nanoparticles with proper shape and size is important. This study has been focused on the silver nanoparticles synthesis using *Andrographis paniculate* plant leaves aqueous extract and their evaluation for anti-lung cancer activity. Green method synthesised metal nanoparticles were characterised by Scanning Electron Microscopy (SEM), Energy dispersive X Ray analysis (EDAX), Transmission Electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy analysis. FTIR analysis showed presence of varied phytochemical components. SEM analysis of the sample elucidated rough spherical morphological characteristics of the material, while elemental analysis through EDAX technique reported silver (Ag) metal in synthesised sample. TEM analysis confirmed nanoparticles size ranging from 18-52 nm. Result of the study showed decreased cell viability of A549 cells treated over green method synthesised silver nanoparticles.

Keywords: *Phytochemicals, nanoparticles, oxidative stress, cell viability assay, green method.*

INTRODUCTION

In recent past years nanotechnology has been emerged as a panacea against various scientific enigmas. Nanomaterials are small tiny particles ranges in size from 1-100 nm and exists in different shapes like spherical, tube, rods etc [1]. Physical and chemical methods are routinely used for the synthesis of nanomaterials. But these methods are always challenged for their high toxicity, cost and environmental problems. Thus, to overcome these problems, synthesis of nanoparticles using biological sample (Green method) have attended much prominence in the last few decades [2, 3]. Green method to synthesis such metal nanoparticles involve various biological samples like bacteria, fungi, algae and plants. Biochemical compounds present in these samples not only reduces metal ions but also cap them to synthesise nanoparticles. However, use of microbial lysates has been considered as tedious, time consuming and costly procedures as compared to the use

of plant extracts for nanoparticles synthesis. From ancient times, ethanobotany explains the role of plants to treat various ailments [4]. Plant produces secondary metabolites for their benefits, which contain many bioactive compounds able for oxidation and reduction capabilities. This property of plant secondary metabolites is used not only to reduce but also to cap metal ions to synthesise metal nanoparticles [5]. Metal nanoparticles such as silver (Ag) and gold (Au) have been studied prodigiously for their various applications among which anti-microbial and anti-cancer activities are more common [6, 7, 8].

In present scenario cancer is one of the deadliest disease affecting millions of people worldwide. As per recent statistics, approximately 1,958,310 new cancer cases and near about 609,820 deaths due to cancer has been projected to occur in the United States [9]. In India, as compared to the year 2020, a 57.5% rise in cancer patients have been predicted by the year 2040 [10]. However, among various types of cancer increased prevalence of lung cancer has been projected due to high use of tobacco and related products. Tobacco consumption in India has been reported to 28%, in which male confer 42.4% while female confer 14.2% [11]. *A. paniculata* is also known as green chiretta and considered to be a native to India and Shri Lanka. This plant has been reported for their various biological activities [12, 13]. Thus, present study aims to synthesise silver nanoparticles using *A. paniculata* leaves extract and to evaluate their anti-cancer activity in human lung adenocarcinoma A549 cells.

MATERIALS AND METHODS

Selection and Authentication of A. paniculata Plant Leaves and Preparation of Distilled Water Extract

A. paniculata leaves were 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 during extraction procedures, therefore we selected these two apparatuses for the preparation of aqueous extract of the selected plant leaves. Fresh leaves were dried in shed for 20 days. 20 grams of leaves were subjected to prepare extract. Dry mass of extract was used to react with silver nitrate (AgNO3) solution to synthesize nanoparticles.

Preparation of Silver Nanoparticles

200 mL solution of 100 mM $AgNO_3$ (Himedia) was taken in a beaker and kept at 50 ℃ in water bath. To this, 1 gram of extract was added and kept for 30 minutes with continuous stirring. Colourless solution of $AgNO_3$ was changed to brownish colour. After this, the solution was centrifuged at 10000 RPM for 10 minutes. Pellet was collected and dried at 80℃ in oven. The dried mass was then grinded by pestle and mortar for 2 hours. This sample was used for characterisation and their evaluation for anti-lung cancer activity.

Characterisation of Synthesised Sample

SEM, EDAX, TEM and FTIR analysis were done to characterise the biosynthesised material from Sophisticated Test and Instrumentation Centre (STIC) Cochin University of Science and Technology, India.

Cell Lines

Human lung adenocarcinoma A549 cells was used to assess the efficacy of green synthesised silver nanoparticles. A549 cell line was procured from National Centre for Cell Science (NCCS) Pune, India. Cells were maintained and grown in Ham's F12 nutrient mixture media (Himedia) with 1% penicillin streptomycin antibiotic solution (Hyclone) and 10% Fetal Bovine Serum (FBS) (Hyclone) using 5% CO2 at 37˚C in CO2 incubator. Experiments were done on 4th passaged cells.

Cell Viability Assay

Cell viability assay was performed on A549 cell line using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). Cells were seeded at the concentration of 2×10^4 cells in 96 well plate. Later, cells were incubated at 37°C in CO2 incubator for 24 hours with sample (Experimental) and without (Control) sample. Experiment was done in triplicate at the concentrations of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL. After 24 hours, media from each well was removed and 50 µL MTT (5 mg/mL media) was added. This plate was again incubated for 4 hours in CO2 incubator. After incubation media was removed and purple coloured farmazan crystals were dissolved in 100 µL dimethyl sulphoxide solution. Absorbance was measured for each well using Biorad ELISA plate reader at 570 nm [14]. Results were expressed in percentage using following formula:

Cell Viability= OD of Sample/OD of Control ×100.

RESULTS AND DISCUSSION

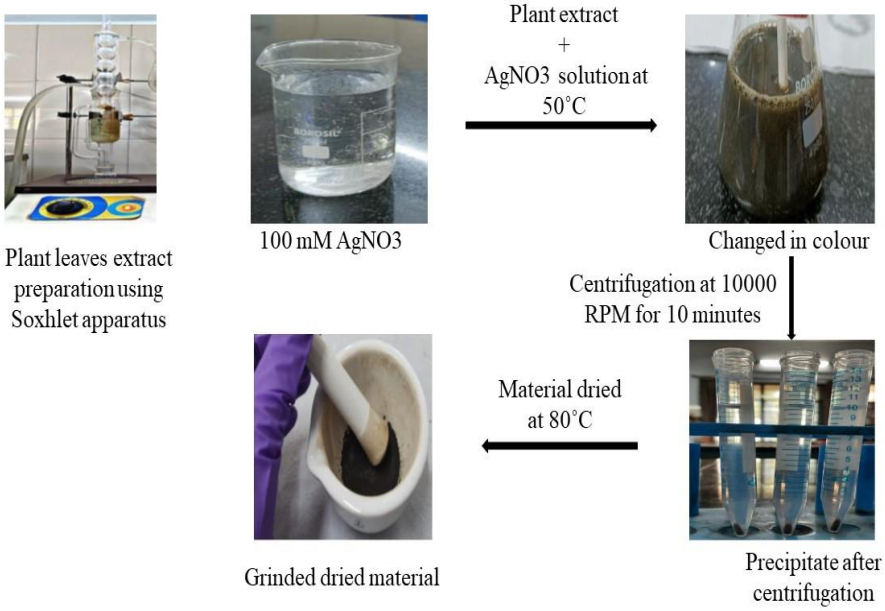

Fig. 1. *Synthesis of silver nanoparticles using A. paniculata plant leaves extract.*

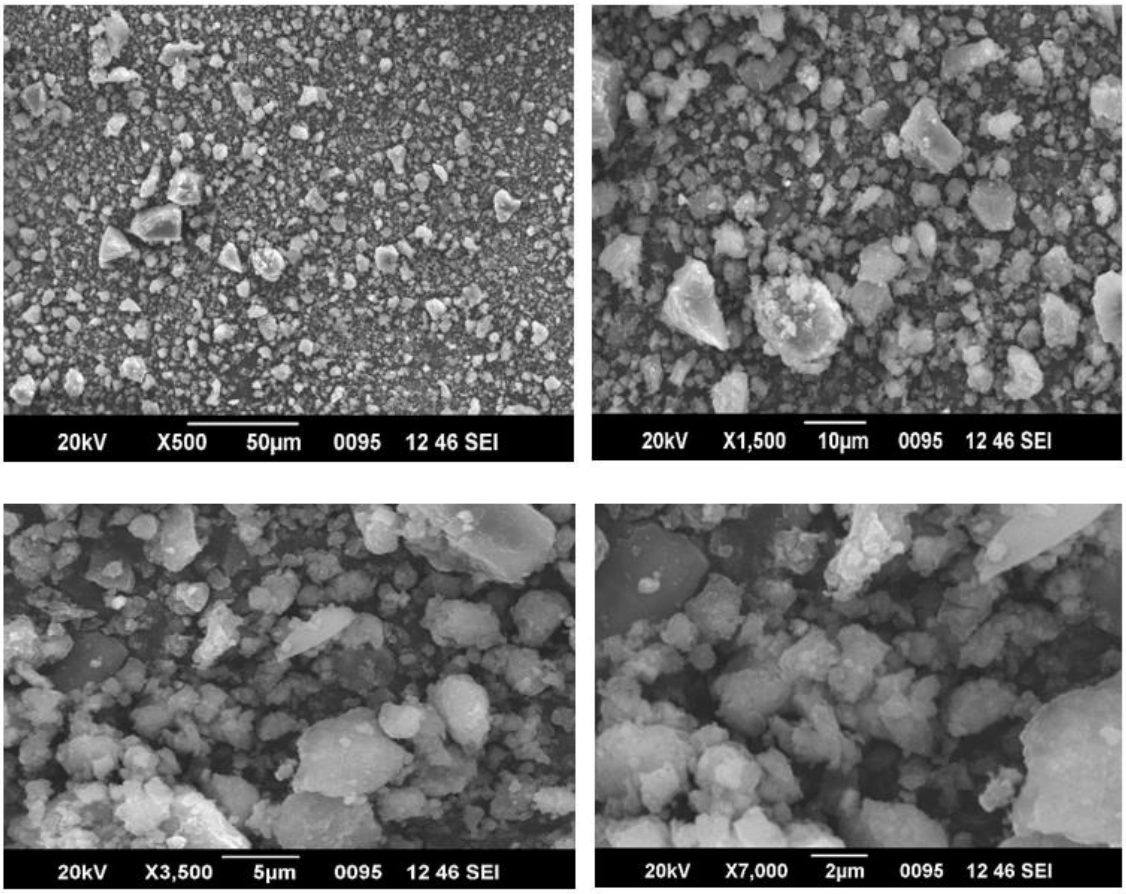

Fig. 2. *SEM analysis of A. paniculata plant leaves extract derived silver nanoparticles at 500X, 1500X, 3500X and 7000X.*

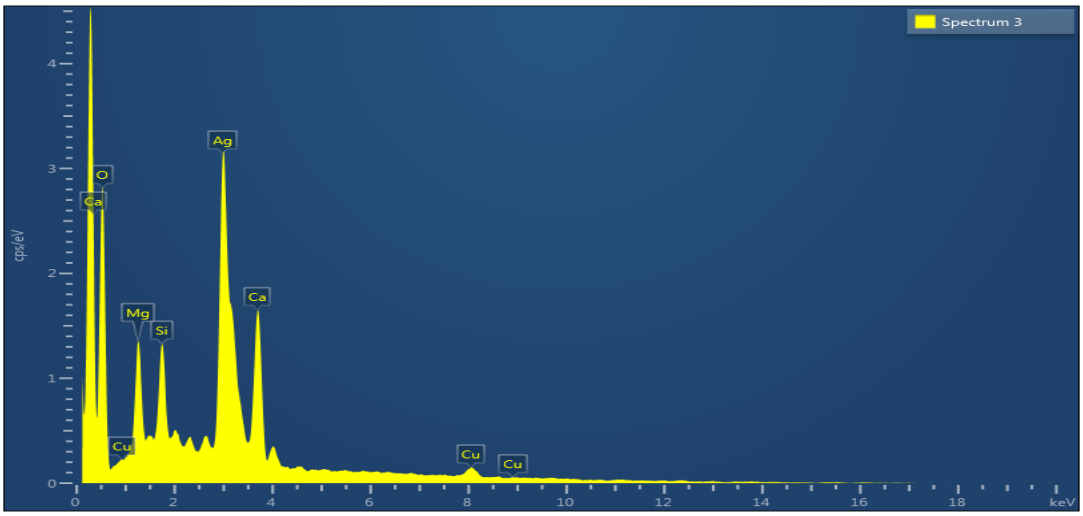

Fig. 3. *EDAX analysis of A. paniculata plant leaves extract derived silver nanoparticles confirming synthesis of silver (Ag) nanoparticles.*

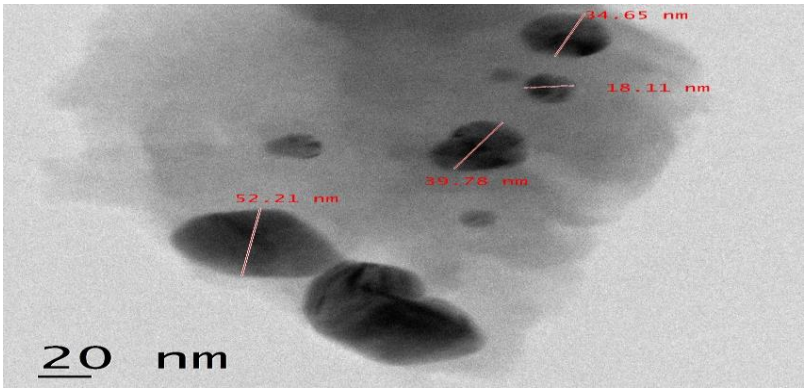

Fig. 4. *TEM analysis of A. paniculata plant leaves extract derived silver nanoparticles showing nanoparticles ranging from 18 to 52 nm in size.*

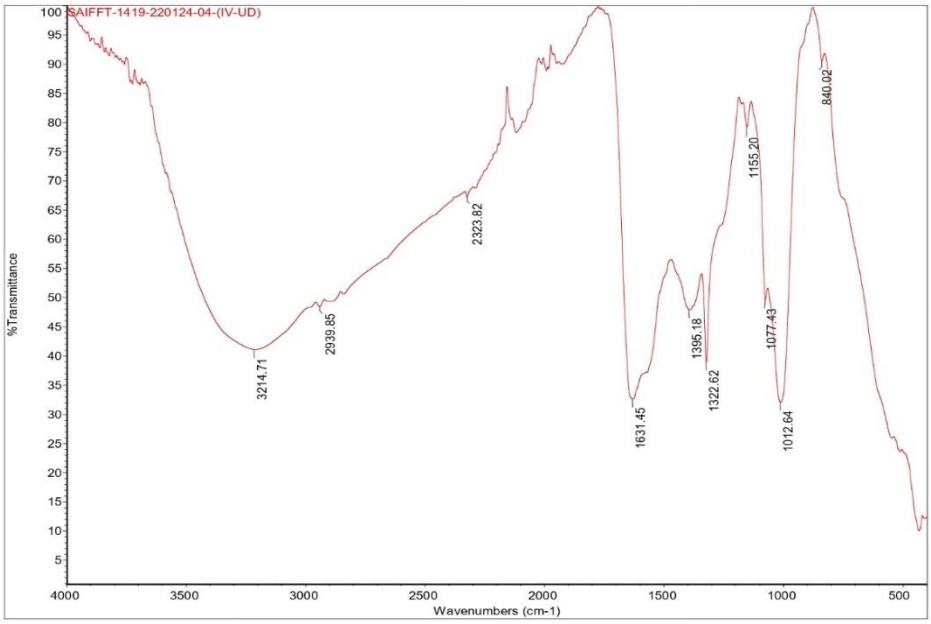

Fig. 5. *FTIR spectrum of A. paniculata plant leaves extract derived silver nanoparticles representing peaks ranging from 840.02 CM^{-1} to 3214.71 CM^{-1}.*

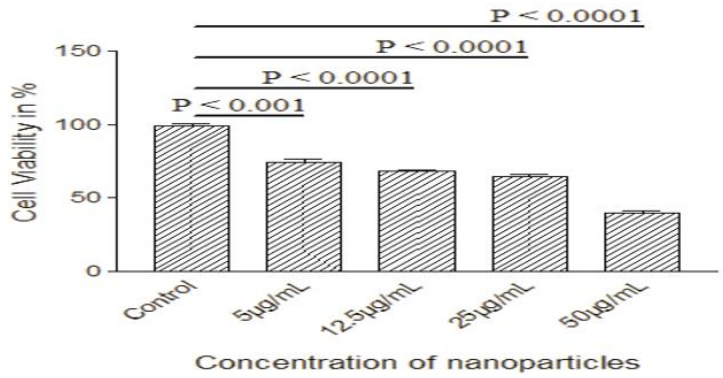

Fig. 6. *Effect of A. paniculata leaf extract derived Ag nanoparticles for anti-lung cancer activity. Control: without nanoparticle sample (Cells+ Media). Experimental (Cell+ Media+ Plant derived nanoparticle in concentration of 5 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL).*

Green Synthesis of Silver Nanoparticles and Its Morphological and Elemental Analysis

Change in colour when plant extract mixed with $AgNO_3$ solution is considered as a preliminary observation for green synthesis of metal nanoparticles. In present study, Ag nanoparticles was successfully synthesised using *A. paniculata* plant leaves aqueous extract and 100 mM $AgNO_3$ solution (Figure1). Plant phytochemicals reduces metal ions into their neutral form which then aggregates to form metal nanoparticles. This reduction process has been considered for colour change. This result is consistent with other previously reported findings [15, 16]. Further, SEM coupled with EDAX analysis were used to study morphological characteristics and elemental analysis respectively. SEM analysis showed massive material with some spherical aggregations (Figure 2). EDAX based elemental analysis showed presence of silver (Ag) ion, which confirmed the synthesis of silver nanoparticles (Figure 3). Further, TEM showed spherical nanoparticles with 18-52 nm size (Figure 4). These results are consistent with other previously reported findings. Kotakadi V S et al. synthesised silver nanoparticles using *A. paniculata* extract and reported nanoparticles of size 54±2 nm. Similarly, Sinha S N et al. synthesised silver nanoparticles using *A. paniculata* leaf extract and reported nanoparticle size ranging from 40-60 nm [15, 16].

FTIR Analysis

FTIR analysis of the synthesised biogenic nanoparticle was performed to analyses possible involvement of *A. paniculata* plant leaves 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 ranged from 840.02 CM^{-1} to 3214.71 CM^{-1}. The peaks in the spectrum are 840.02 CM^{-1}, 1012.64 CM^{-1}, 1077.43 CM^{-1}, 1155.20 CM^{-1}, 1322.62 CM^{-1}, 1395.18 CM^{-1}, 1631.45 CM^{-1}, 2323.82 CM^{-1}, 2939.85 CM^{-1} and 3214.71 CM^{-1}. Peak at 1077.43 CM^{-1} corresponds to C-O stretching representing presence of primary alcohol, 1155.20 CM^{-1}, 1322.62 CM^{-1} corresponds to S-O stretching representing presence of sulphone group, 1631.45 CM^{-1} corresponds to C=C stretching represents presence of conjugated alkenes, 2939.85 CM^{-1} corresponds to C-H stretching represents presence of alkane and 3214.71 CM^{-1} corresponds to O-H stretching represents presence of carboxylic acid. Phlobatannin, flavonoid compounds, polyphenols, alkaloids, terpenoids, tannins and saponins are reported to be present in *A. paniculata*

plant extract [17]. Compounds observed through FTIR analysis in this study represents reported phytochemicals of this plants. The result of the study is consistent with the results of other previous findings [15, 16, 17].

Cell Viability Analysis in A549 Cells

In medical field nanotechnology has proven a better choice in design and development of new drug delivery systems and therapeutic agents. The main advantage of nanotechnology over conventional therapeutic system includes target specificity, cost effectiveness, long efficacy, eco-friendly etc. and therefore research and use of nanotechnology has been extensively increased for bioimaging system, in-vitro diagnostics, novel biomaterial design, active implants etc. Metal nanoparticles due to their vast metallic properties may exhibit different shapes and sizes after synthesis and hence show their wide applications [18, 19]. Each plant is unique for phytochemistry. Even phytochemicals are varied geographically among same or different plants. Functional groups present in phytochemicals are used to synthesised metal nanoparticles. Thus, phytochemicals may synthesise nanoparticles with different shapes and sizes which could impart their different roles differently in biological systems [20]. Present study aims to study effect of biogenic silver nanoparticles synthesised using *A. paniculata* plant leaves 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 cells using MTT assay. 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, as compared to control ($P<0.001$). Again, a significant decreased cell viability was reported when A459 cells were treated with synthesised Ag nanoparticles at the concentrations of 12.5 µg/mL, 25 µg/mL and 50 µg/mL as compared to control ($P<0.0001$) (Figure 6). This decreased cell viability is possibly due to the different biochemical effects that could impart the green method synthesised nanoparticles in A549 cells. Colossal literature explains different mechanisms for such decreased cell viability with respect to green method synthesised silver nanoparticles. Among these mechanism, oxidative stress induced due to Ag+ ions within the studied cells have been reported as one of the main reasons for cancer cell death. Ag+ ions triggers Fenton reaction which generates more amount of hydroxyl (OH•) free radicals. Also, metal ions have been reported for elevated Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Alterations in free radical and anti-oxidant level leads to oxidative stress. Indeed, hydroxyl radicals are reported for the maximum cellular damages amongst all other free radicals [21]. Elevated levels of free radicals cause DNA damage, enzyme inactivation, lipid peroxidation, mitochondrial modulations which releases cytochrome c responsible for caspase 3 and 9 activations. All these intracellular alterations cause cell death [22]. Up and down regulation of intracellular proteins could be the second reason for decreased viability of A549 cells. Under oxidative stress cellular proteins represent a situation where cells mimics responses to any endogenous or exogenous stimuli. These alterations cause altered cell signalling, metabolic activities and gene expressions resulting in cell death via decreased cell cycle progression and membrane aberrations [23, 24]. Such possible mechanism to kill cancer cells have already been reported by other findings. Piao M J et al. reported that in cancer cells silver nanoparticles triggers formation of apoptotic bodies, DNA fragmentation and mitochondria-based apoptosis pathways through Bax and Bcl-2 gene expression modulations, leads to cell death [25]. Result of this study is consisted with the other finding [26].

CONCLUSION

Silver nanoparticles using *A. paniculata* plant leaves aqueous extract was synthesised successfully. SEM analysis showed spherical material with rough morphology of the synthesised material. Presence of silver in EDAX analysis confirmed synthesis of silver nanoparticles, while TEM exhibited nanoparticle size ranging from 18-52 nm. FTIR analysis elucidated various plant phytochemicals components such as primary alcohol, sulphone group, conjugated alkenes, alkane and carboxylic acid for reduction and capping of metal ions to synthesise nanoparticles. Also, green method synthesised nanoparticles showed decreased cell viability in A549 cells. Further molecular and immunological studies are required to validate the findings.

Acknowledgements. Authors are thankful to STIC Cochin University India for characterising the green methods synthesised nanoparticles.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: U.D., Design: S.G., U.D., Data Collection or Processing: K.P., S.G., Analysis or Interpretation: U.D., Literature Search: S.G., K.P., U.D., Writing: S.G., K.P., U.D.

Financial Disclosure. This research received no grant from any funding agency/sector.

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