Green Synthesis of Silver Nanoparticles Using *Ledebouria Revoluta* Bulb Extractand Its Biological Activity

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Abstract. In this present examination explores the viability of silver nanoparticles (AgNPs) green synthesis from bulb extract of *Ledebouria revoluta* as an antitumor specialist utilizing human lung cancer cell line (A549). The AgNPs synthesis was determined by UV-Visible range and it was additionally described by field X-beam diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) investigation High Resolution Transmission electron microscope (HRTEM) and EDS. The level of dictated by MTT examine. The outcomes demonstrated that green synthesized silver nanoparticles inhibited proliferation of human lung disease cell line A549 with an IC_{50} esteem 72.65 µg/ml. The antimicrobial exercises of silver nanoparticles were checked against gram positive and gram negative strains utilizing in well diffusion technique. The antibacterial chlorophenicol as the positive control was completed. The outcome demonstrated the silver nanoparticles indicated effective movement against bacterial pathogens and cancer growth action.

Keywords: A549 cell line · MTT assay · Gram positive and gram negative bacteria · *Ledebouria revoluta* bulb extract

1 Introduction

Nanotechnology ways to deal with keep the sicknesses in people and regular products have as of late been expanding enormously and select the physiochemical exercises of nanosized metal particles make it fruitful in normal science and medication [1]. The different potential natural uses of nanoparticles have assumed a noteworthy job in the look for ecofriendly procedure of creating nanoparticles utilizing different biomaterials as the traditional synthetic union includes the utilization of dangerous solvents vitality and high weight which might be hurtful to the ecological fields [2]. Nanoparticles combination by restorative plants demonstrates more preferred standpoint they may improve the antibacterial action of silver nanoparticles in light of the fact that the therapeutically vital bioactive particles present stuck the plants may sticky situation on the outside of the nanoparticles and lessen the silver particles to AgNPs [3, 4]. The phytochemicals present in plant removes have been accounted for to make decrease of metal particles nanoparticles and in the long run devastate the utilization of poisonous synthetic

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substances high weight temperature vitality and support of microbial societies [5, 6]. This green union of NPs utilizing phytocompounds as bio-reductants is accomplishing a more noteworthy catalyst [7].

Green combination of nanoparticles is a rising part of nanotechnology in which naturally generous materials like plant leaf concentrate, microbes and organisms these utilized for the synthesis [8]. Silver nanoparticles (AgNPs) have pulled in critical enthusiasm among the developing nanoproducts on account of their exceptional properties and expanding use for different applications in nanomedicine [9]. The silver nanoparticles have pulled in with significant enthusiasm because of their broad pertinence in various research fields, for example, science vitality, medication and catalysis. AgNPs were discovered the size extending from 30 to 60 nm. A silver nanoparticle at various focus was assessed for its antibacterial impact, against different pathogens. Hence silver NPs as an antimicrobial operator appear to be contrasted with anti-microbial, also, few reports show synergistic upgrade of action of anti-microbial with AgNPs. Presently the incorporated silver nano particles from natural items have been generally contemplated by different analysts [10].

The *Ledebouria revoluta* Jessop [Syn: Scillaindica (wight) baker or *Scilla hyacinthina* (Roth) J.F. Macbr and Drimiopsisbotryoids baker [syn: Drimiopsiskirkii baker] are Leafaceous species of the family Asparagaceae The Plant List 2013. Traditionally used medicinal plants have recently attracted the attention of pharmaceutical and scientific communities [11]. Cancer is a standout amongst the most life threating ailments in which deregulating expansion of strange cells attacks and upsets encompassing tissues [12]. Cancer is an all out malady in which the cell development is forceful and intrusive and metast ordinarily prompting demise [13]. Today caner is the biggest single reason for death is people and chemoprevention has been a promising anticancer methodologies went for lessening the dismalness and mortality of malignant growth by deferring the procedure of carcinogenesis [14].

The present examination was expected to green combination of silver nanoparticles utilizing bulb extract of *L. revoluta* against cytotoxicity A549 malignant growth cell lines. We have researched the antibacterial movement of integrated nanoparticles fundamental by well dispersion strategies. The impacts of nanoparticles on bacterial development have been additionally broke down by utilizing the base inhibitory fixation strategy.

2 Materials and Methods

2.1 Preparation of Plant Extract

The fresh bulb of *Ledebouria revoluta* gathered from Periyar University grounds. 20 gm of collected fresh bulb was added to 250 ml of refined water and bubbled for 15 min at 60 °C. The prepared aqueous extract was separated through Whatman No. 1 channel paper. The sifted concentrate was put away in cooler at 4 °C.

2.2 Synthesis of AgNPs

For synthesis of AgNPs, 50 ml of the aqueous bulb extract was added to 450 ml of 1 mm silver nitrate (AgNO3) solution. The blend was kept into dim condition at 24 h. The improvements of AgNPs were considered by visual perception of the shading change from light yellow to deep brown color.

2.3 Characterization of AgNPs

2.3.1 UV-Vis Spectroscopy

The synthesized AgNPs was established by sampling the aqueous component behind the reaction and the absorption maxima was scanned by T80 UV-Vis spectrometer at the wavelength of 200–800 nm.

2.3.2 FTIR Spectroscopy

In the FTIR spectrum, the samples were determined using the model of Perklin Elmer spectrum 1FTIR spectroscopy, sample range 500–4000 cm^{-1}.

2.3.3 XRD Analysis

The particle size and nature of the AgNPs were determined using XRD. This was carried out using diffraction meter, brucker, Germany model D8 advance model by 30 kV and 30 mA with Cu ka radians at 2 θ angle.

2.3.4 HRTEM Analysis

HRTEM (Model-Tecnai, G2 20 twin) was utilized to study the surface morphology and size of the integrated nanoparticles. For HR-TEM investigation, the example was set up by dissolving 2 mg of the nanoparticles in 10 ml of methanol by sonication process. Two drops of this arrangement were put on the carbon-copper matrices and were permitted to evaporate the dissolvable.

2.3.5 EDS Analysis

The energy dispersive X-ray spectroscopy was performed by a Bruker EDX spectrometer to determine the elemental composition of the samples.

2.4 Antibacterial Activity

The well diffusion method (Anonymous, 1996) was utilized to show the antibacterial properties. Different concentration of the extract (100 μg/ml) was set up by AgNPs. The test microorganisms were seeded into particular medium by spread plate strategy 10 μl (10 cells/ml) with the 24 h societies of microbes development in supplement stock. After hardening the channel paper wells (5 mm in distance across) impregnated with the concentrates were put on test living being seeded plates. Chloramphenicol (10 μg) utilized as standard for antibacterial test. The antibacterial test plates were hatched at 37 °C for 24 h. The distances across of the restraint zones were estimated in mm.

2.5 Cytotoxicity Activity

2.5.1 Cell Line

The human lung cancer cell line (A549) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37 °C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cytotoxicity effect of the AgNPs was measured by the MTT assay [17, 18]. The cells were maintained in DMEM medium, added with 10% of bovine serum at 37 °C in humidity field atmosphere with 5% CO2%. The cells were plated in 96 well a flat bottom tissue culture plates at concentration of approximately 1 × 105 cell s/well and allowed to attach overnight at 37 °C. Then, the cells were incubated by different concentrations of the AgNPs bulb extract of *Ledebouria revoluta* for 48 h. After the incubation, the medium was redundant and 100 µl fresh medium was added with 5 µl of MTT assay (5 mg/ml). After 4 h, the medium added in 100 µl of DMSO to dissolve the Formosan crystals. Then, the absorbance was read at 570 nm in a microliter plate reader. The IC_{50} value was calculated.

2.5.2 Statistical Analysis

All the measurements were carried out in triplets and the results are expressed as mean ± SD using on way analysis (ANOVA) through SPSS software.

3 Results and Discussion

3.1 UV-Vis Spectroscopy

The aqueous of *L. revoluta* with watery arrangement of the nitrate (AgNo3) start to change the shading from yellow to darkish brown shading. It showed the development of AgNPs with decrease of silver particle. The assimilation groups were gotten at 472 nm (Fig. 1). The presence of the dark colored shading was because of excitation of the surface Plasmon vibrations. The arrangement of AgNPs was observed by UV-Visible spectroscopy in the 200–800 nm extend [15]. The result got for UV range for the AgNPs arranged from *P. maderaspatens* is root uncovered the most extreme retention was observed to be at 479 nm.

3.2 FTIR Spectroscopy

The FTIR spectroscopy estimation was concentrated to distinguish the conceivable biomolecules for the development and adjustment of AgNPs. The pinnacles showed up at 3209.55, 2926.01, 1622.13, 1527.62, 1217.08, 1014.56 cm^{-1} (Fig. 2). The solid assimilation tops at 3209.55 cm^{-1} uncovers the nearness of O-H extending liquor and phenol gathering. The pinnacle 2926.01 cm^{-1} maybe allotted to alkenes is clear from C-H extending the pinnacle seemed 1622.13 cm^{-1} was relegated to the extending C = O of gathering amide. The pinnacle acquired at 1527.62 cm^{-1} N-O extending in nitro gathering. The pinnacle got at 1217.08 cm^{-1} shows fragrant C-N. The pinnacle acquired at 1014.56 cm^{-1} compares to C-O extending ether gathering.

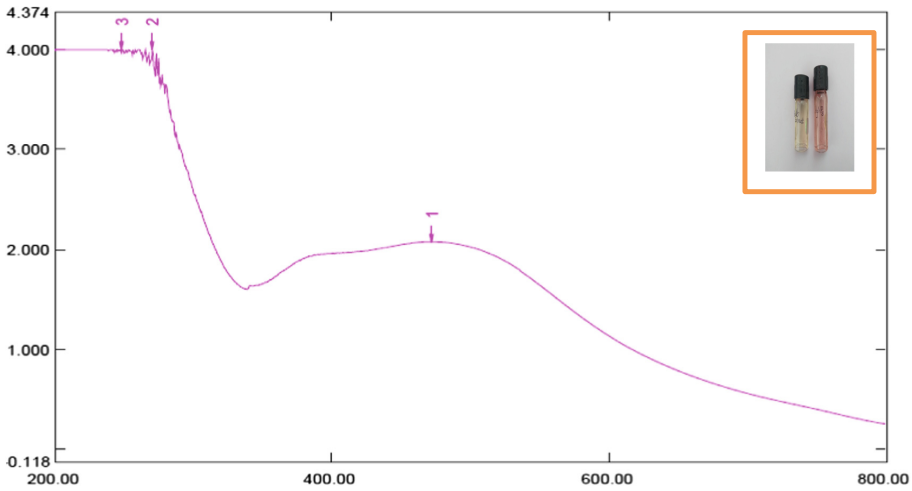

Fig. 1. UV-Vis absorption spectra of silver nanoparticle synthesized extract (AgNPs)

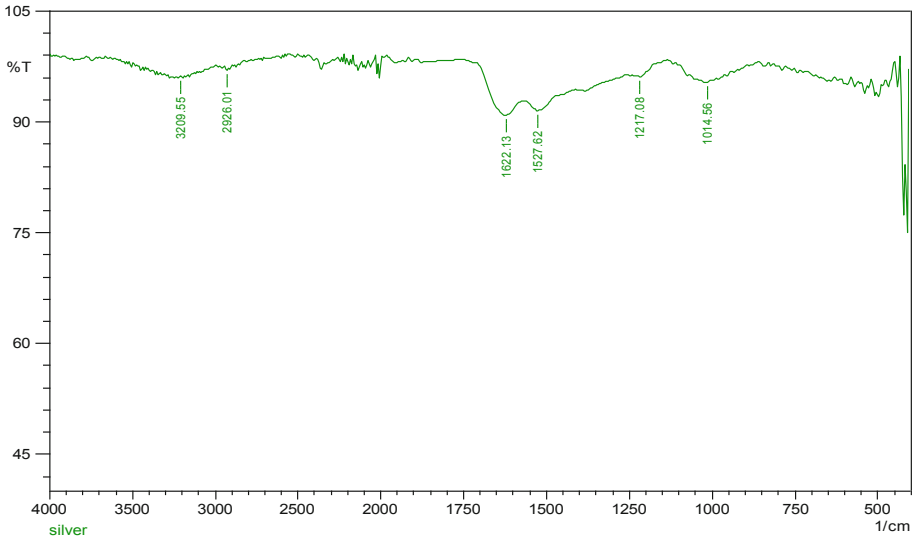

Fig. 2. FT-IR spectrum of silver nanoparticles synthesized by *Ledebouria revoluta* bulb part

3.3 XRD Analysis

The X-ray powder diffraction designs shows distinctive precious stone planes namely (210), (122), (111), (231), (142), (241), (220) and (311) of the nanometals, which relates to the crests with two theta esteems 27.91, 33.45, 46.56, 54.92, 57.32, 64.50, 77.32, same outcome uncovered (Fig. 3). Unidentified crystalline pinnacles (27.89, 32.30, 46.26, 54.79) are likewise clear in numerous works in which the XRD design incorporates the important 20 territory [16].

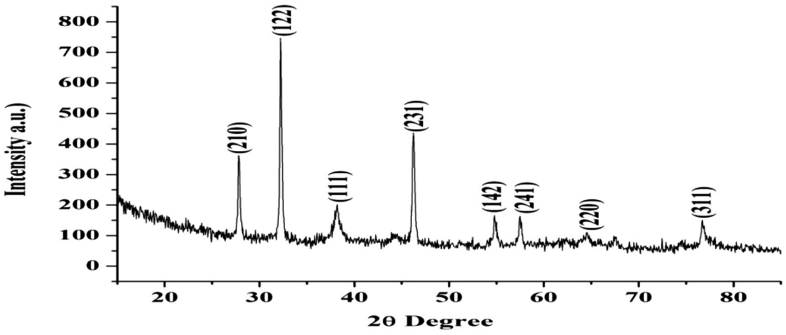

Fig. 3. X-ray diffraction patterns of synthesized AgNPs by *Ledebouria revoluta* bulb part

3.4 HRTEM and EDS

The morphology, measurement and circulation of the integrated nanoparticles were envisioned utilizing high goals transmission electron minuscule investigation. In the investigation dispersive spectroscopy (EDS) of the AgNPs the nearness of essential metal flag was affirmed (Fig. 5). HRTEM picture is the clear that the morphology of silver nanoparticles round shape (Fig. 4).The silver nanoparticles have circular geometry with a normal measurement of 18.05 ± 4.73 nm [17].

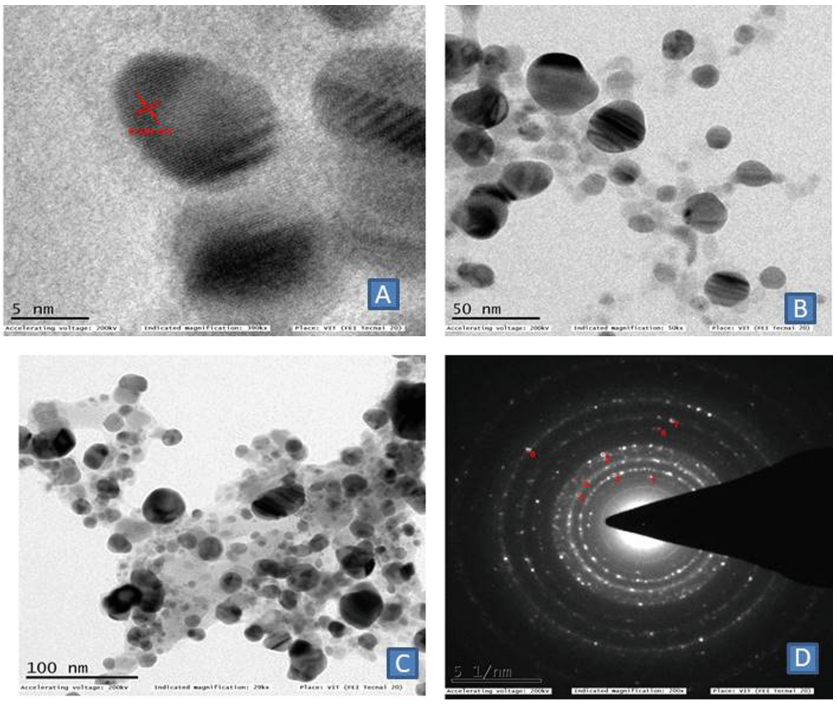

Fig. 4. High resolution transmission electron microscopic images of silver nanoparticles at different magnification. A-C size distribution histogram of TiO_2NPs and (D) SAED pattern.

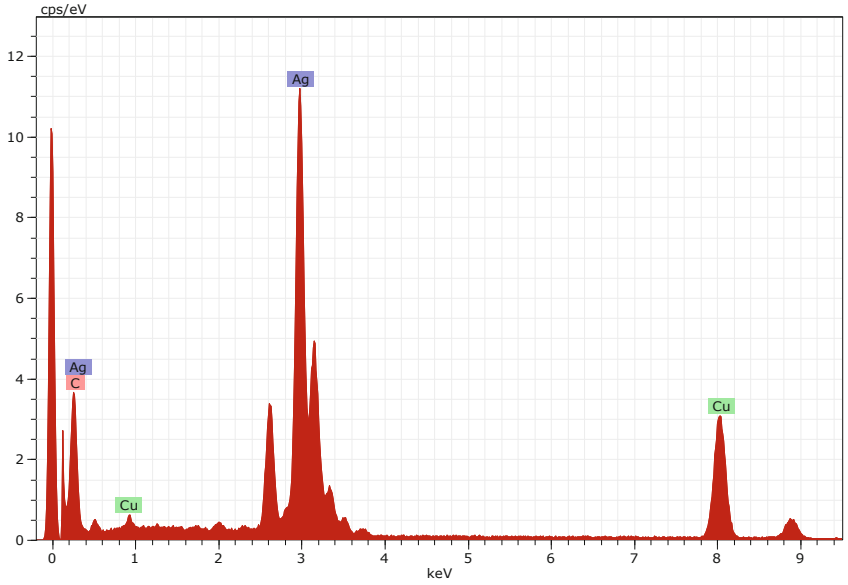

Fig. 5. EDS spectroscopy displays the purity and chemical composition of the AgNPs

3.5 Antibacterial Activity

The antibacterial activity test after effects of AgNo3 against various gram positive and negative microorganism were appeared Tables 1 and 2. Among utilizing three gram negative microbes the high restraint development in *Bacillus cerus* 5.66 ± 2.49 in 25hl (Fig. 6). In gram positive microorganisms the *Salmonella typhi* has been most astounding hindrance as 6 ± 2.44 in 25 µl. He revealed as the comparable outcome that the AgNPs has been great antibacterial movement [18].

Table 1. Gram positive bacterial activity of different concentration of Silver nanoparticles

S. no	Pathogenic bacteria	AgNPs extract Zone of inhibition (mm)				Standard (Chloramphenicol)
		10 µl	15 µl	20 µl	25 µl	
1	*Bacillus cerus*	0.33 ± 0.47	3.33 ± 1.24	3.66 ± 1.69	5.66 ± 2.49	3.66 ± 1.69
2	*Clostridium tetani*	2.33 ± 0.47	3.33 ± 0.94	3.33 ± 1.24	4.33 ± 1.88	3.66 ± 1.69
3	*Mycobacterium tuberculosis*	0.33 ± 0.47	1.66 ± 0.94	3 ± 1.41	4.33 ± 1.24	4.33 ± 2.05

Table 2. G negative bacterial activity of different concentration of Silver nanoparticles

S. no	Pathogenic bacteria	AgNPs extract Zone of inhibition (mm)				Standard (Chloramphenicol)
		10 µl	15 µl	20 µl	25 µl	
1	Vibrio cholera	1.33 ± 1.24	2 ± 1.63	4 ± 1.4	4 ± 1.69	4.33 ± 0.94
2	Salmonella typhi	0.33 ± 0.47	2.66 ± 0.47	2.33 ± 1.24	6 ± 2.44	4.66 ± 1.24
3	Klebsiella nemoniae	0.66 ± 0.47	0.33 ± 0.47	2.66 ± 0.94	3.33 ± 1.24	5.66 ± 1.24

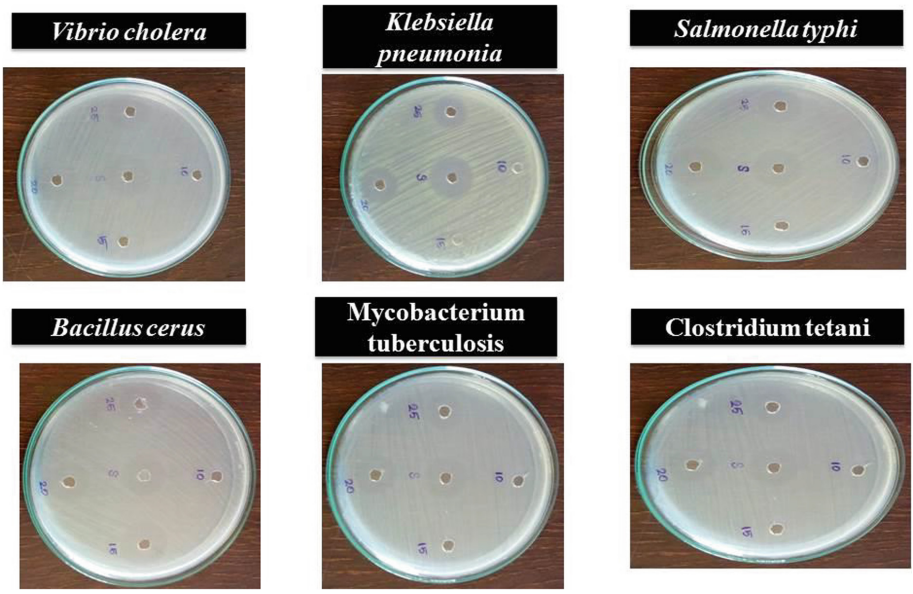

Fig. 6. Zone of inhibition of gram-negative bacterial strains using silver nanoparticles of different concentrations

3.6 Cytotoxicity Analysis of A549 Cell Line

The cytotoxicity of the AgNPs separate was against A549 cell line by MTT measure Fig. 7 demonstrate the cytotoxicity of AgNPs against A549 cell line. The cytotoxicity sway on cell development was inspected at various fixation (6.25, 12.5, 25, 50 and 100 µg) appeared in Table 3. The level of disease cell development restraint was observed to be high with the expanding the centralization of AgNPs the lC_{50} for *L. revoluta* was recorded at 72.65 µg/ml against A549 cells at 100 µg focus (Fig. 7). Detailed the AgNPs remove A549 cell line utilizing *S. aromatium* AgNPS extract [19].

Table 3. AgNPs treated A549 lung cancer cells

Conc	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	Cont
ABS	0.038	0.068	0.141	0.193	0.245	0.425
	0.034	0.065	0.143	0.192	0.247	0.422
	0.033	0.066	0.143	0.192	0.243	0.425
Avg	**0.035**	**0.066333**	**0.142333**	**0.192333**	**0.245**	**0.424**
					IC_{50} value	**72.65 µg/ml**

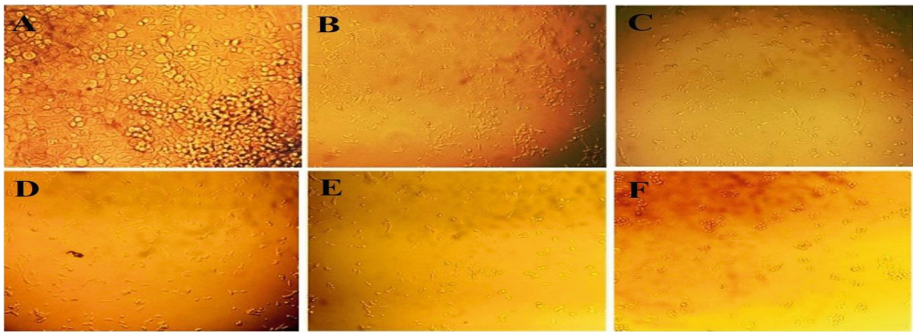

Fig. 7. Morphology of control and AgNPs treated A549 lung cancer cells. A. Control, B. IC_{50} Concentration (6.5 µg/ml), C. IC_{50} Concentration (12.5 µg/ml), D. IC_{50} Concentration (25 µg/ml), E. IC_{50} Concentration (50 µg/ml), F. IC_{50} Concentration (100 µg/ml).

4 Conclusion

Plant extract assisted green synthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized viz., Uv-Vis, XRD, FTIR, TEM and EDS. Their biological significance was proven by efficient in bacterial activity and A549 cell line.

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