

Biosynthesis of silver nanoparticles using the extract of *Alternanthera sessilis*—antiproliferative effect against prostate cancer cells

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Abstract Green synthesis of silver nanoparticles was carried out using the aqueous extract of *Alternanthera sessilis* under various experimental conditions. The aqueous extract of *Alternanthera sessilis* showed significant potential for the quick reduction of silver ions. The synthesized silver nanoparticles were characterized with UV-visible absorption spectrophotometer, XRD, SEM, and FTIR analysis. The average crystallite size as calculated from x-ray diffraction studies and SEM analysis was found to be less than 100 nm. The cytotoxic activity of synthesized nanosilver was carried out against prostate cancer cells (PC3) by MTT assay and found to show significant activity. The present work of biosynthesis of silver nanoparticles using *Alternanthera sessilis* appears to be cost effective, eco-friendly, and an alternative to conventional method of synthesis.

Keywords *Alternanthera sessilis* · UV-visible spectroscopy · XRD · SEM · FTIR

1 Introduction

Uncontrolled growth and spread of abnormal cells lead to group of diseases and finally results in death, which is termed as cancer. It can be caused by both external factors (tobacco, chemicals, radiation, and viruses) and internal factors (hormones, immune conditions, and mutations) that may act together or in sequence to instigate or promote carcinogenesis (American Cancer Society 2011). The World

Health Organization reported that cancer is one of the leading diseases which will cause global death rates up to 15 million by 2020. In 2008, prostate cancer was the second most commonly diagnosed cancer in men. Recognizing the growing global cancer crisis, a smart vision is needed to implement the metal nanoparticles as a drug in the genomic era (World Statistical Information 2007).

Nanotechnology has gained attraction in the twenty-first century and grows rapidly due to the ability to manipulate and harness properties of assemblies that are at the nanosize scale of various biomolecules (Panneerselvam et al. 2011). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology (Linga Rao and Savithamma 2012). In recent times, the advances in the field of nanosciences and nanotechnology has brought to fore the nanosized inorganic and organic particles which are finding increasing applications in personal care products, industrial, medical instruments and therapeutics, synthetic textiles, and food packaging products (Ravishankar Rai and Jamuna Bai 2011).

Phytoconstituents like flavonoids, polyphenols possesses astonishing antitumor properties; but there is no proper utilization in terms of cancer drugs due to its solubility nature, less oral intake, and ineffective delivery. These can be overcome by the application of nanotechnology (Tabrez et al. 2013). Biosynthesis of nanoparticles using plant extracts is the favorite method of green, eco-friendly production of nanoparticles and exploited to a vast extent because the plants are widely distributed, easily available, safe to handle, and with a range of metabolites (Kulkarni et al. 2011). The successful use of silver nanoparticles (AgNPs) in diverse medical streams as antifungal, antibacterial (Panacek et al. 2009; Singh et al. 2008), and virucidal agents (Lara et al. 2010) has led to their applications in controlling phytopathogens.

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In recent years, the robust area of research focuses on the cytotoxicity study of silver nanoparticles. Several studies on the cytotoxicity of silver nanoparticles on different cell lines are reported. Antitumor activity of *Bacillus licheniformis*-mediated AgNPs against DLA cell line (Sriram et al. 2010) and bovine retinal endothelial cells (Sriram et al. 2012) in vitro and in vivo are reported. Sodium citrate-assisted silver nanoparticles were studied for its antiproliferation activity on human lung alveolar carcinoma epithelial cells (A549) (Zhou and Wang 2012). Silver and gold nanoparticles synthesized using guava and clove extracts showed anticancer efficacy against four different cancer cell lines viz. human colorectal adenocarcinoma, human chronic myelogenous leukemia, bone marrow, and human cervix (Raghunandan et al. 2011). Synthesized silver sulfide nanoworms showed good cytotoxicity against human cervical cancer cell line (HeLa) is reported by Xing et al. 2011.

Biosynthesized AgNPs from leaf extract of *Vitex negundo* L. proved to be an antitumor agent against human colon cancer cell line HCT15 (Prabhu et al. 2013). In vitro cytotoxicity effect was analyzed by AgNPs synthesized using *Sesbania grandiflora* leaf extract against human breast cancer (MCF-7) (Jeyaraj et al. 2013). The potential silver nanoparticles synthesized from calli extract of *Citrullus colocynthis* was investigated on human epidermoid larynx carcinoma cell line (Satyavani et al. 2011). Govender et al. 2013, studied cytotoxic activity of *Albizia adianthifolia* (AA)-mediated silver nanoparticles and showed mechanistically the activation of AA AgNP in the intrinsic apoptotic pathway in A549 lung carcinoma cells.

Ethanol extract of *Dioscorea membranacea* roots showed highest cytotoxic activity than other five plants (*Bridelia ovata*, *Curcuma zedoaria*, *Derris scandens*, *Nardostachys jatamansi*, and *Rhinacanthus nasutus*) against prostate cancer cell lines (Saetung et al. 2005). Human prostate cell proliferation in vitro study was attenuated using the ethanol extract of *Punica granatum* L. var. *spinosa* which suppress the proliferation activity at an IC₅₀ value of 250.21 µg/mL (Sepehr et al. 2012). Acetone extract of *Tridax procumbens* showed 82 % cytotoxic activity compared to aqueous extract against prostate epithelial cancerous cells (PC3) by MTT assay (Vishnu Priya et al. 2011). *Glochidion zeylanicum* (Gaertn.) showed significant cytotoxicity activity on PC3 compared to HepG2 and HT29 cell lines was reported by Sharma et al. 2011. Root, stem, flower, and leaf acetone extract of *Lasienthera africanum* was tested for its anticancer activity on PC3 cell lines. Acetone extract of leaf showed significant anticancer properties compared to the other parts of this plant (Matheen et al. 2012).

Alternanthera sessilis is a weed and occurs in both wet lands and uplands and can grow on a variety of soil types. It is a weed of rice throughout tropical regions and of other cereal crops, sugarcane, and bananas, and has many utilities. In south-

east Asia, young shoots and leaves are ingested as vegetables. Previous phytochemical studies have reported the isolation of flavonols, triterpenoids, steroids and tannins; β-sitosterol, stigmasterol, campesterol, and lupeol being few of its important constituents. The herb has been reported to have antipyretic, hepatoprotective, antiulcer, antibacterial, hematinic, and diuretic activities (Sahithi et al. 2011). *Alternanthera sessilis*-assisted silver nanoparticles exhibited 100 % cell inhibition of breast cancer cells (MCF-7) at IC₅₀ value 25 µl/mL (Firdhouse and Lalitha 2013).

In the present work, we have explored the green synthesis of silver nanoparticles using aqueous extract of *Alternanthera sessilis* as an alternative to chemical methods of synthesis and studied its antiproliferative effect against prostate cancer cell line (PC3).

2 Experimental

2.1 Preparation of the extract

Fresh leaves of *Alternanthera sessilis* (20 g) were weighed and washed and boiled with 100 ml of Millipore water for 5 min. The extract was filtered using Whatman filter paper and refrigerated for further studies.

2.2 Synthesis of silver nanoparticles

The aqueous extract of *Alternanthera sessilis* was treated with 3 mM of silver nitrate solution under various conditions, i.e., room temperature (27–30 °C), higher temperature (75 °C), and sonication using ultrasonic bath (PCI Ultrasonics 1.5 L (H)). The reddish brown color silver solution was centrifuged (Spectrofuge 7 M) at 13,000 rpm for 15 min. The silver nanoparticles were redispersed in water, centrifuged again, and the supernatant solutions were analyzed.

2.3 Characterization of synthesized silver nanoparticles

The synthesized silver nanoparticles were characterized by UV-visible spectroscopy, x-ray diffraction (XRD), SEM, and Fourier transform infrared spectroscopy (FTIR) analysis.

2.3.1 UV-visible spectroscopy

The formation of nanosilver was confirmed by UV-visible absorption spectra using double-beam spectrophotometer 2202 (SYSTRONICS).

2.3.2 XRD analysis

A drop of synthesized silver nanoparticles coated on the glass substrate was examined by x-ray diffraction analysis

Table 1 Comparative experimental study on the biosynthesis of silver nanoparticles under different conditions

Aqueous extract of plant + silver nitrate solution (mL)	Time for the formation of silver nanoparticles (minutes)		
	Room temperature	Higher temperature (75 °C)	Sonication
1+6	180	90	30
1+7	220	80	25
1+8	260	60	20
1+9	340	40	15
1+10	360	30	10

(SHIMADZU Lab X XRD-6000) with a Cu K α radiation monochromatic filter in the range 10–80 °.

2.3.3 SEM analysis

Morphology and size of silver nanoparticles were investigated by scanning electron microscope using TESCAN instrument provided with Vega TC software for nanosilver coated on glass substrate.

2.3.4 FTIR spectroscopy

The functional groups present in the synthesized nanosilver were analyzed by FTIR spectroscopy- Tensor-27 (Bruker).

2.4 In vitro cytotoxicity assay of nanosilver

2.4.1 Preparation of cell culture

PC3 (human prostate cancer cell line) was obtained from NCCS Pune. It was maintained in Roswell Park Memorial Institute (RPMI) supplemented with 10 % fetal bovine serum (FBS), amphotericin (3 μ g/mL), gentamycin (400 μ g/mL), streptomycin (250 μ g/mL), and penicillin (250 units/mL) in a carbon dioxide incubator at 5 % CO₂.

2.4.2 Preparation of medium for cell culture

Roswell Park Memorial Institute medium The powdered media was dissolved in 900 ml of Millipore water in an autoclaved glass conical flask under sterile conditions. The antibiotics were added in the concentration as mentioned above and stirred well. Then, 3.7 g of sodium bicarbonate was added into the flask and 10 % FBS was added and mixed well. The liquid was slowly poured into the upper portion of a media sterilization unit (Corning) and filtered through a 0.2- μ filter under negative pressure. The medium was stored at 4 °C without delay.

Saline/trypsin/versene 10X saline A: 8-g NaCl, 0.4-g KCl, 1.0-g D-Glucose, and 0.35-g NaHCO₃ (tissue culture grade) were dissolved in 100-ml water and stored at 4 °C.

Versene: 1-g EDTA (tissue culture grade) was added into 90-ml distilled water. Then 5-N NaOH was added drop wise

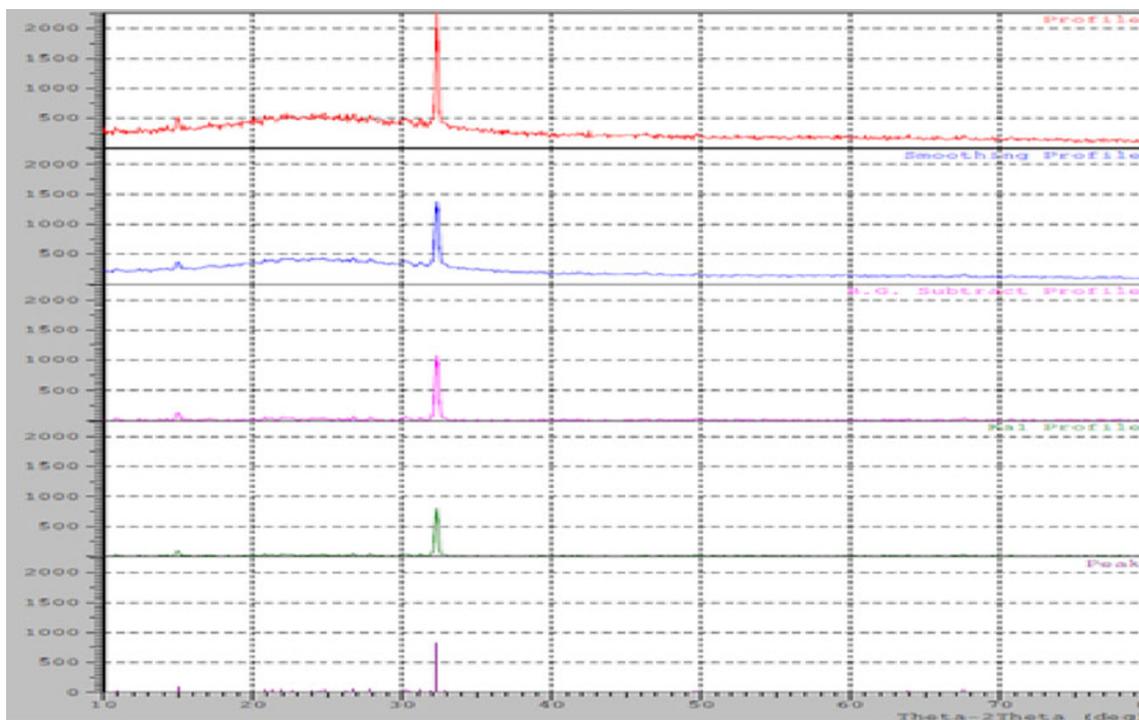


Fig. 1 XRD patterns of silver nanoparticles synthesized using *Alternanthera sessilis*

Table 2 Determination of crystalline size of AgNPs using Debye–Scherrer's equation

S.No	2θ	FWHM	$\beta = \pi * \text{FWHM} / 180$	θ	$\text{Cos}\theta$	$D = k \lambda / \beta \cdot \text{Cos}\theta$
1.	32.3005	0.25250	0.00440	16.15	0.96053	32.81

The crystalline size of silver nanoparticle is 32.81 nm

until it gets dissolved. The solution was filtered and stored at 4 °C. One hundred milliliters of saline/trypsin/versene (STV) was prepared by adding 25 mg of trypsin in a mixture of 10 ml of 10X saline A and 2.5 ml of versene. Double distilled water (100 mL) was added, sterile filtered, liquated, and frozen at -20 to -70 °C.

2.4.3 Treatment of cells

PC3 cells show a steady growth rate with a doubling time at approximately 33 h. The cells that reached confluency in 3 to 4 days were stored in liquid nitrogen and used for the experiments. The culture medium was removed from the T25 culture flask by decanting into a clean container inside the laminar airflow chamber. The cells were rinsed with medium to remove traces of serum, which may inhibit action of trypsin. STV solution (2 mL) was added to the flask containing cells and incubated at 37 °C for a few minutes. As soon as cells started dislocating from the surface, the flask was rinsed with 5 mL of serum-containing medium to arrest the trypsinization. The suspension of cells was collected in a sterile 15-mL centrifuge tube and the cells were pelleted at 1,500 rpm for 5 min. The cell pellet was resuspended in fresh medium with serum and a part of the cells were seeded back into the flask. The remaining cells were used for experiment and resuspended in cryopreservative medium (Synth-a-freeze) in a cryovial

and frozen at -70 °C for a day, then transferred to liquid nitrogen.

2.4.4 MTT assay

Cell lines were maintained in RPMI supplement with 10 % FBS, amphotericin (3 $\mu\text{g}/\text{mL}$), gentamycin (400 $\mu\text{g}/\text{mL}$), streptomycin (250 $\mu\text{g}/\text{mL}$), and penicillin (250 units/mL) in a carbon dioxide incubator at 5 % CO_2 . Approximately 1,000 cells/well were seeded in 96-well plate using culture medium, the viability was tested using trypan blue dye with the help of hemocytometer and 95 % of viability was confirmed. After 24 h, the different concentrations of silver nanoparticles (1.56, 3.12, 6.25, 12.5, 25 $\mu\text{l}/\text{mL}$) were added at respective wells and kept incubation for 48 h.

After 48 h of the drug treatment, the fresh medium was changed again for all groups and 10 μl of MTT (5 mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue crystals formed were dissolved with 50 μl of DMSO. The optical density was measured at 595 nm. The cell inhibition (in percentage) was determined using the following formula. Nonlinear regression graph was plotted between cell inhibition (in percentage) and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software.

$$\text{Cell Inhibition}(\%) = 1 - [\text{Abs}(\text{sample}) / \text{Abs}(\text{control})] \times 100.$$

Fig. 2 SEM image of silver nanoparticles synthesized using *Alternanthera sessilis*

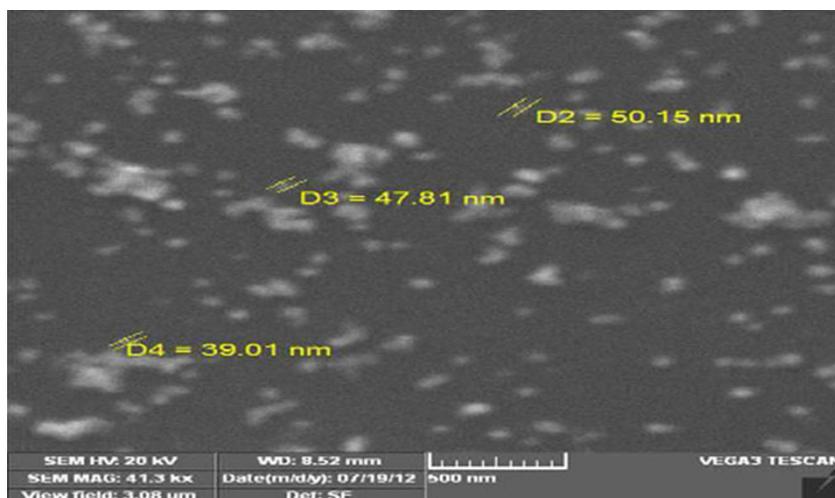
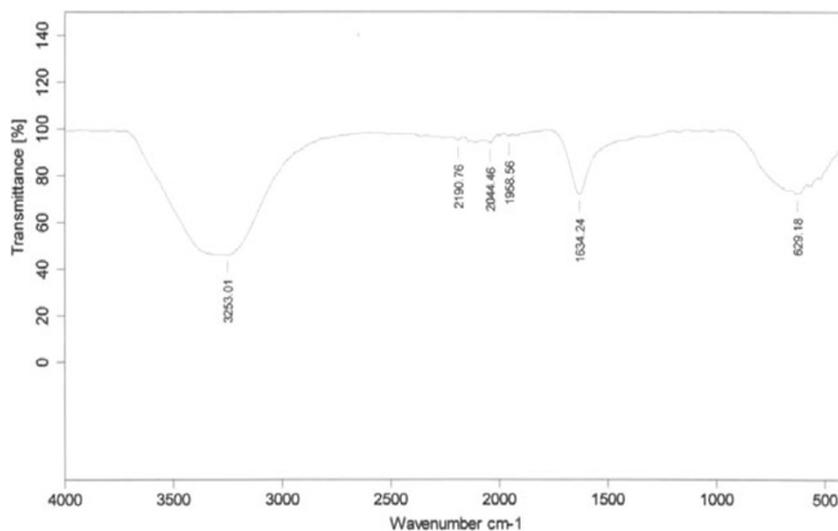


Fig. 3 FTIR spectra of silver nanoparticles synthesized using *Alternanthera sessilis*



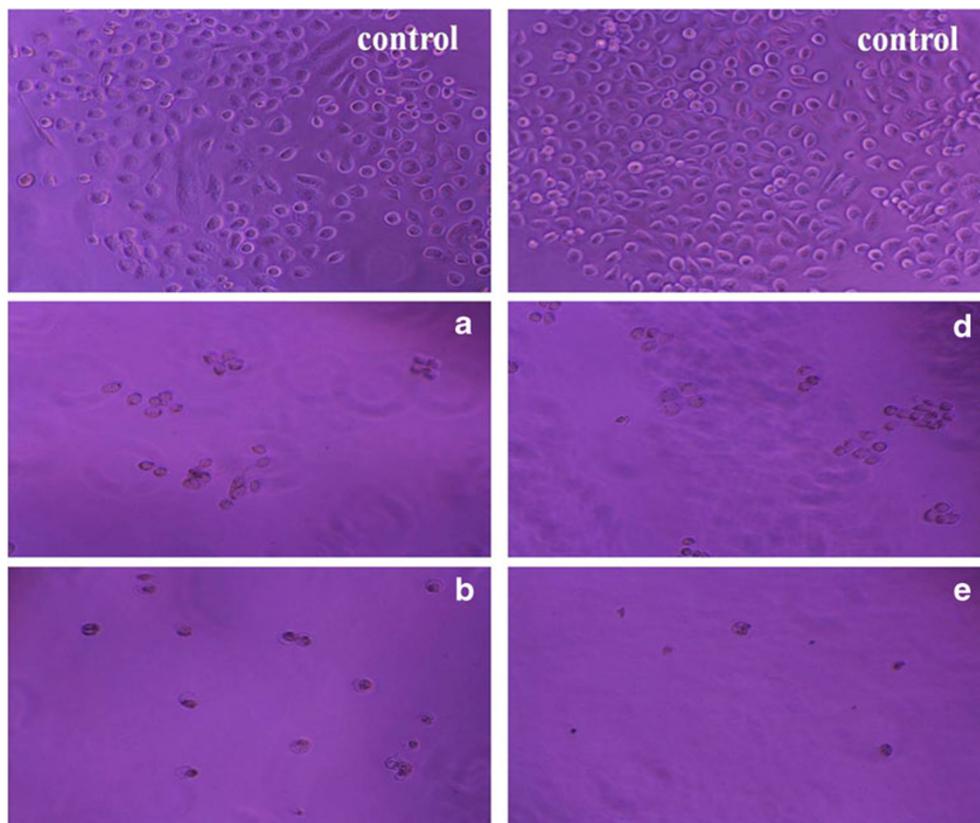
3 Results and discussion

The aqueous silver ions were reduced to silver nanoparticles when aqueous extract of *Alternanthera sessilis* was added. After 6 h, the yellow-colored solution changed to reddish brown color which indicates the formation of silver nanoparticles in room temperature. The formation of the silver nanoparticles was monitored by UV-visible spectrophotometric

analysis. The UV-visible spectra showed the maximum absorbance at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. A comparative study on various experimental conditions was carried out to identify the effect of aqueous extract of *Alternanthera sessilis* and silver nitrate solution on the rate of bioreduction of silver ions.

The results of formation of silver nanoparticles at different concentrations of silver nitrate (6, 7, 8, 9, and 10 ml) under

Fig. 4 Cytomorphological changes and growth inhibition of silver (a–b) and silver nanoparticles synthesized using *Alternanthera sessilis* (d–e) on PC 3 cell line at 12.5 and 25 $\mu\text{l/ml}$ concentrations, respectively



various conditions are given in Table 1. The sonication method results in easy and rapid synthesis of silver nanoparticles within 40 min as compared to other methods which may be due to the effect of ultrasound, which has the ability to create clean, highly reactive surfaces on metals and thereby enhancing the rate of reactions.

Figure 1 shows the XRD patterns of drop-coated silver nanoparticles synthesized using aqueous extract of *Alternanthera sessilis*. The XRD pattern shows one intense peak of Bragg's reflection with 2θ values of 32.30° which may be indexed to the (101) based on the face centered cubic structure of silver nanoparticles. The particle size of the silver nanoparticles was calculated using Scherrer's equation, given in Table 2.

Debye–Scherrer's equation

The Debye–Scherrer's equation is commonly used to determine the crystalline size of nanoparticles.

$$D = k \lambda / \beta \cdot \cos \theta$$

where,

- D Average crystalline size (in nanometer)
- k Dimensionless shape factor (0.9)
- λ X-ray wavelength (0.1541 nm)
- β Angular/line broadening at FWHM of the XRD peak at the diffraction angle
- θ Diffraction angle

Figure 2 represents the SEM image recorded from drop-coated film of the silver nanoparticles synthesized using aqueous extract of *Alternanthera sessilis*. The SEM image showed spherical shape of silver nanoparticles formation with diameter range 30–50 nm.

Figure 3 shows the FTIR spectra of silver nanoparticles synthesized using aqueous extract of *Alternanthera sessilis*. The peaks located at $3,253$ and $1,634 \text{ cm}^{-1}$ may be due to the presence of $-\text{NH}$ or $-\text{OH}$ group and carbonyl stretching in proteins. The other peaks at $2,190$ and $2,040 \text{ cm}^{-1}$ are assigned to CC or CN triple bond, respectively.

The synthesized nanosilver using *Alternanthera sessilis* was studied for its cytotoxic activity against prostate cancer cells (PC3) in vitro by MTT assay at different concentrations (1.56, 3.12, 6.25, 12.5, 25 $\mu\text{L}/\text{mL}$). The antiproliferation activity increases as the concentration of the nanosilver increases. It is quite obvious that the number of cancer cells decreases for the nanoparticles compared to that of silver ions. Figure 7a, b clearly shows the morphological changes such as cancer cell membrane lyses, coiling with the addition of silver and nanosilver synthesized using *Alternanthera sessilis* in Fig. 4d, e after 48 h. The reduction in the number of PC3 cancer cells is evidently observed at the highest concentrations (12.5 and 25 $\mu\text{L}/\text{mL}$) of PGAG-AgNPs compared to that of control.

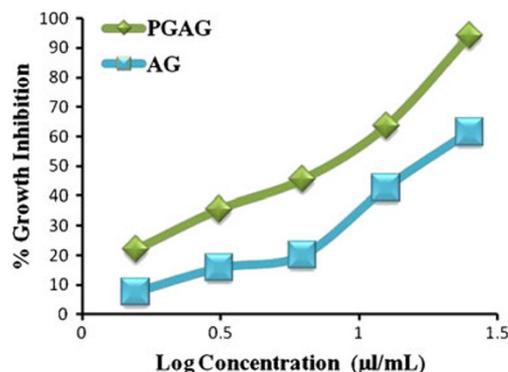


Fig. 5 Antiproliferative effect of silver (AG) and silver nanoparticles synthesized using *Alternanthera sessilis* (PGAG) on prostate cancer (PC3) cell line

The IC_{50} value of AgNPs was observed at $6.85 \mu\text{g}/\text{mL}$ compared to silver ions ($14.62 \mu\text{g}/\text{mL}$). The percentage growth inhibition of PC3 cell lines at different concentrations of silver (AG) and nanosilver (PGAG) is depicted in Fig. 5 and Table 3. The apoptosis rate was more in nanosilver compared to that of silver ions as shown in Fig. 4.

The results suggested that the *Alternanthera sessilis*-assisted nanosilver exerts its cytotoxic effect on prostate cancer cells possibly via an apoptosis-dependent pathway. Intracellular suicides program possessing morphological changes like cell shrinkage, oxidative stress, coiling, and biochemical response lead to apoptosis. The reason may be due to the interaction of silver nanoparticles with the functioning of cellular proteins which lead to the consequent changes in the cells. Otherwise, the deionization of silver ions may take place before entering the tumor cells due to the low stability and high reactive nature of Ag^+ ions. The complete apoptosis (95 %) was observed at 25 $\mu\text{L}/\text{mL}$ for prostate cancer cell (PC3); whereas 100 % growth inhibition was obtained for breast cancer cells (MCF-7). The results demonstrated that the antiproliferative effect of PGAG-AgNPs mainly depends on the time of exposure and its concentration. Thus, *Alternanthera sessilis*-mediated silver nanoparticles may provide as promising drug for chemotherapeutic treatment.

Table 3 Results of cytotoxic studies with silver and nanosilver synthesized using the extract of *Alternanthera sessilis*

Concentration ($\mu\text{L}/\text{mL}$)	Cell inhibition (%)	
	PGAG	AG
1.56	22.05	7.69
3.125	35.66	15.81
6.25	45.58	20.08
12.5	63.6	42.73
25	94.11	61.53

4 Conclusion

The biosynthesis of silver nanoparticles using the extract of *Alternanthera sessilis* was economical, non-toxic, and environmentally benign. The formation of silver nanoparticles was characterized by UV-visible spectrophotometer. The synthesized silver nanoparticles were stable due to the reducing and capping nature of phytoconstituents present in the aqueous extract of *Alternanthera sessilis* analyzed by FTIR spectra. The particle size of the synthesized silver nanoparticles is less than 50 nm which was confirmed by XRD and SEM analysis. Nanosilver shows good cytotoxic activity against prostate cancer cells and may serve as a potential anticancer drug for cancer therapy.

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