

Synthesis and characterization of silver nanoparticles using the Shrub Solanum betaceum and evaluation of their anticancer activity

Aejitha Subbaian

Asian College of Engineering and Technology

Kaviya Babu

Asian College of Engineering and Technology

Apshana Mubarak Ali

Asian College of Engineering and Technology

Neelakandan Mani

Muthayammal College of Arts and Science (Autonomous)

Ramakrishnan Arumugam (✉ ramakrishnana1991@gmail.com)

Karpagam Academy of Higher Education

Gobinath Thilagar

Asian College of Engineering and Technology

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Abstract

The biological activity of *Solanum betaceum* leaves and its nanoparticles has been poorly studied in the field of anticancer science. In this research, the synthesis of silver nanoparticles was accomplished by the new effortless green synthesis process, using *S.betaceum* leaves extract as reducing and capping agent. The effects of various parameters i.e., pH, reactants ratio, and temperature on the synthesis of AgNPs were studied. The production of *S.betaceum* leaves extract AgNO₃ NPs was characterized by powder X-ray diffraction (XRD), UV-visible, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The *S.betaceum* leaves fabricated AgNO₃ NPs were tested for their cytotoxicity action against cancer cells (Leukaemia cell line (K562), Bone cancer - MG63, Breast cancer - MCF7). It was found that the formation of AgNPs enhanced with time at a higher temperature and alkaline pH. The UV visible absorbance spectrum of AgNO₃ NPs exhibited the absorbance band at 280 nm and SEM studied highlighted average crystalline sizes of nanoparticles of 0.2µm to 0.5µm. The in vitro cytotoxic effect of *.betaceum* leaves fabricated AgNO₃ NPs inhibited the proliferation of Leukaemia cell line (K562), Bone cancer - MG63, Breast cancer - MCF7 with IC50 concentration. Overall, based on our results, we strongly believe that the synthesis of AgNO₃ NPs using commonly used *S.betaceum* leaves can be promoted as a potential eco-friendly option to chemical methods currently used for nanoparticle synthesis of anticancer compounds.

Introduction

As of late, there is an advancement in research territory to expand the techniques to expect to create eco-friendly nanoparticles, which doesn't utilize more energy and harmful metals [1]. Green sources can act as both stabilizing and reducing agent for the synthesis of shape and size controlled metal and metal oxide nanoparticles [2]. Synthesis of nanoparticles using green plants, microorganisms and enzymes have been proposed as a sustainable and environmental friendly over regular chemical and physical methods, since they are expensive and hazardous to environment [3].

In this present study, plants are easy to obtain and renewable resources from the natural environment. Currently, several plants have an extensive range of industrial and pharmaceutical applications, due to the presence of several bioactive compounds of high interest for biomedical science and industries [4]. Recently, some of the studies showed that *Solanaceae* can be used for the green synthesis of silver nanoparticles [5]. However, there is limited research shedding light on the use of green plants for the nanoparticle synthesis.

Silver nanoparticles have drawn awareness of several researchers for their exclusive chemical and biological behaviors [6], which are strongly related to their size and morphology [7]. Within the large family of metal oxide nanoparticles, Silver nanoparticles have impressive properties, including optical, electrical, and thermal, high electrical conductivity [8]. Silver nanoparticles have been used to reduce pollutants such as pesticides, heavy metals, and microorganisms from drinking water [9]. Furthermore,

silver nanoparticles have high promising biological functions such as antimicrobial, anticancer, antioxidant, anti-inflammatory, wound healing, and antimalarial activities [10].

Knowledge of bone cancer and its management is timely importance to fight the cure of cancer disease, within the framework of Integrated Vector Management. Around the world, cancer is particularly dangerous, since the number of cancer cases enlarged in current years. Thus the utilize of biological tools as well as a green technology appeared as an alternative approach to reduce the employment of chemical compounds. Since the use of dangerous chemical compounds led to massive side effects and development of disorders. Modern studies reported that high anticancer activity of green synthesised silver nanoparticles using *Punica granatum*, *Anagallis tenella*, *Cocos nucifera*, *Ocimum sanctum*, *Chrysanthemum indicum*, *Dalbergia spinose*, and *Cinnamomum camphora* [11].

Green plants can be used for the effective synthesis of nanoparticles. The shrub, *S.betaceum* is a flowering plant widely present in worldwide. It contains fibers, vitamin C, potassium, polyphenols and carotenoids [12]. *S.betaceum* fruits contain a high amount of polyphenols compare than another tropical fruit. This family plant has been described for the effective and cheap synthesis of silver nanoparticles with several biological activities [5], therefore, we selected it for the synthesis of potentially eco-friendly silver nanoparticles. The synthesis of *S.betaceum* silver nanoparticles was characterized by X-ray diffraction (XRD), UV-spectroscopy, UV-visible and Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The *S.betaceum* silver nanoparticles were tested for their anticancer activity against cell line models. The impact of the synthesized nanoparticle on the cancer cell was studied with microscopy technique.

Materials and Methods

Solanum betaceum extract

Fresh *S.betaceum* mature leaves were collected from the Nilgiri district in Tamilnadu, India (11.4916° N, 76.7337° E). Leaves were rinsed completely with de-ionized water, cut into small pieces and dried at room temperature; 20gm of leaves powder were added into a conical flask containing 100ml of de-ionized water and heat for 20 minutes at 60⁰C. The leaves extract was filtered by Whatman No.1 filter paper and store in the refrigerator at 4°C for two weeks on further analysis.

Green synthesis of AgNO₃ nanoparticles

3.40 gm of silver nitrate dissolved in 200 ml of distilled water which is referred to as 0.1m of silver nitrate. The ratio of 8:2 silver nitrate and *S.betaceum* extract was added into a flask, the reaction mixture was kept at shaker for 12 h at room temperature using an even shaking at 90 rpm. Then, the reaction mixture was centrifuged at 4000 rpm for 10 minutes the product was collected and rinsed with de-ionized water.

Observation by varying physico-chemical parameters

To evaluate the effects of various physico-chemical parameters, the nanoparticle synthesis reaction was carried out under different reaction conditions. Effect of ratio was studied by varying the ratio of silver nitrate and *S. betaceum* extract solution. Ratio variation was observed from 2:8, 4:6, 5:5, 6:4, and 8:2. Effect of pH was studied by varying pH, 0.1 N KOH and 0.1 N HCL was added to adjust the pH of the solution. The pH variation was observed at pH 2, 4, 6, 8, and 10 with high accuracy. Effect of temperature was measured by varying the temperature at 27°C, 40°C, and 50°C. The reaction mixture was centrifuged at 4000 rpm for 10 minutes the product was collected and rinsed with de-ionized water and analyzed using UV-Vis spectrophotometer operating at a resolution of 1 nm among 200 to 400 nm to determine optimal condition for the maximal synthesis of AgNO₃ NPs.

Characterization of *S. betaceum* prepared AgNO₃ NPs

The *S. betaceum* prepared AgNO₃ NPs were characterized by several techniques like UV–vis spectroscopy analysis, XRD, FTIR, and SEM, which gives essential information for the understand the different physicochemical features of synthesised nanoparticles.

The UV spectral analysis *S. betaceum* prepared AgNO₃ NPs was performed by determining the optical density (OD) using UV-Vis spectrophotometer operating at a resolution of 1 nm among 200 to 400 nm. The XRD analysis was used for phase purity. Particle size of *S. betaceum* prepared AgNO₃ NPs and functional groups was observed by FTIR spectroscopy. The shape of the *S. betaceum* prepared AgNO₃ NPs were analysed by scanning electron microscopy (SEM).

Cytotoxic activity

The cytotoxic activity of *S. betaceum* prepared AgNO₃ NPs against Leukaemia cell line (K562), Bone cancer - MG63, Breast cancer - MCF7 was determined by the following previously described method. Cancer cells were seeded in a 96-well plate and incubated at 24 hrs at 37°C, 5% CO₂, 95% air and 100% relative humidity condition. The cells were treated with different concentration of AgNO₃ NPs and incubated for 24 hrs. The cells without drugs were treated with DMSO and used as control. After 24 hr of incubation, 25 µl of an MTT solution (5 mg/ml) was added to wells and plate was further incubated for 4 hr. The culture medium in each well was replaced with 100 µl of DMSO and plate was gently agitated for 15 min. Absorbance was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader. On comparing with untreated cells, the percentage of viable cells in treated culture was calculated and IC₅₀ was taken.

Statistical analysis

The One-way analysis tool of variance (ANOVA) was used to assess the statistical significance between different groups using Software Package for the Social Science (SPSS) version 15 followed by Duncan's multiple range test (DMRT). The results are expressed as Average ± SD. The significance was assessed at $p \leq 0.05$.

Results and Discussion

Effect of temperature on AgNO₃ NPs synthesis

Figure 1a shows the absorption spectra of AgNO₃ NPs at different temperatures. With the increase in temperature, the fabrication of nanoparticles is enhanced, as indicated by rapid change in the colour of the solution. The peak intensity was changed gradually, as temperature varies from 27°C to 50°C. The shift in the UV spectral absorption maximum is due to the localization of surface resonance of the AgNO₃ NPs. This indicates that the size of the fabricated nanoparticles decreases with increasing temperature (50°C), which may be due to the faster reaction rate at a higher temperature. At high temperature, the kinetic energy of the molecules increases and silver ions get consumed faster, thus leaving less possibility for particle size growth. Thus, smaller particles of uniform size distribution are formed at a higher temperature. The present study is in complete correlation with the work reported based on neem leaves, where the intensity of peak maximum increases with increasing temperature. Previous studies have been reported that increase in temperature leads to increase in the intensity of the bands as a result of peak shift resulting in a decrease in the diameter of fabricated AgNO₃ NPs (Bindhu and Umadevi, 2014). The present study is correlated with the work based on *Tithonia diversifolia* plant leaves extract (Dada *et al.*, 2018), where the intensity of peak increases with increasing temperature. However, excellent representation was obtained at room temperature because the *S.betaceum* leaves extract effectively reduced and stabilized NPs at room temperature. Stable *S.betaceum* leaves extract AgNO₃ NPs were formed at room temperature, justifying the green synthesis direction.

Effect of pH on AgNO₃ NPs synthesis

Figure 1b shows changes in peak absorption intensity on increasing pH of the solution. This indicates that pH 10 is the most favourable pH for the synthesis of AgNO₃ NPs using *S.betaceum* leaves extract. Further, it was observed that the pH enhances the rate of the reduction reaction, the colour changes were observed very fast when AgNO₃ mixed with *S.betaceum* leaves extract. The shift in the peak wavelength indicates that the size of the particles increases with increasing pH of the solution. As the diameter of the particles gets larger, the energy required for excitation of surface plasmon electrons decreases, as a result, the absorption maximum shifted towards the longer wavelength region. Moreover, it was observed that at acidic pH i.e. pH < 7, the formation of nanoparticles is suppressed. At high pH, the bioavailability of functional groups in Neem leaf extract promoted the synthesis of nanoparticles. Previous studies have suggested that increasing the pH of the solution is more favourable for the nanoparticle synthesis, reported that absorbance of the AgNO₃ NPs obtained from olive tree extract increases with the increasing pH of the solution from 2 to 8, further increasing resulted in the absorbance decrease. However, in the present study increasing pH from 2 to 20 the absorption increases gradually, indicating that alkaline pH is more favourable for the AgNO₃ NPs fabrication.

Effect of ratio on AgNO₃ NPs synthesis

Figure 1c shows the absorption spectra of AgNO₃ NPs obtained with changing the concentration of *S. betaceum* leaves extract and AgNO₃. When the concentration of *S. betaceum* leaves extract to silver salt is in the ratio of 8:2, a very weak absorption band is observed. With the increase in the concentration of reaction mixture (silver salt 8:2 *S. betaceum* leaves extract), the peak intensity increases dramatically, indicating an enhancement in the production of AgNO₃ NPs. The absorption intensity increases direct proportional with increasing concentration of silver salt. The absorption was lower at the higher volume of extract indicating a slow reduction of AgNO₃, as the volume of AgNO₃ solution increased the absorption peak become sharper with excellent heightening [13].

UV- visible absorption spectrum

The nanoparticle formation was visually monitored, the solution colour was altered from pale green to brown through the chemical reaction showing the formation of *S. betaceum* prepared AgNO₃ NPs (Fig. 2a). Silver nanoparticles (AgNO₃ NPs) received huge interest in the research community due to their good optical properties leading various industrial and pharmaceutical applications. *S. betaceum* prepared AgNO₃ NPs exhibited characteristic UV absorption spectrum with the absorption wavelength of 280 nm (Fig. 2b). An absorption band at 280 nm observed in UV-Visible absorption spectra is plausibly attributed to electronic excitation in tryptophan and tyrosine residues in proteins [14]. Apart from the 280 nm peak, AgNO₃ NPs also exhibited one prominent additional peak at 326 nm, which are belongs to the presence of phytomolecules bound to the surface of AgNO₃ NPs [15].

XRD

The XRD pattern revealed the formation of crystalline *S. betaceum* fabricated AgNO₃ NPs (Fig. 2c). The XRD spectrum showed strong diffraction peaks at (2 theta) 21.3°, 23.9°, 29.3°, 31.5°, 32.4°, 35.1°, 38.1°, 38.6°, 39.5°, 39.9°, 44.4°, 53.4°, 64.8°, 65.1°, 77.9° which are linked to 177, 103, 287, 72, 394, 98, 115, 247, 112, 71, 45, 1026, 128, 137, 61, 78 and 230 crystal planes respectively. No noise peaks were observed due to impurities and the peaks intensity profiles were a feature of the nanoparticle spherical structure. Debye Scherrer's formula was used to estimate the average crystalline size in the range of 27 nm. While comparing with earlier studies our results are quite comparable to [1], who fabricated silver nanoparticles using the *Ulva lactuca* seaweed extract, while [16] synthesized the nanoparticles using *Clitoria ternatea* and *Solanum nigrum* leaves extract.

FTIR

The FRIT spectrum of *S. betaceum* fabricated AgNO₃ NPs is shown in Fig. 2d. The absorption band showed at 3765.92 assigned to O-H stretch; 3695 .61 assigned to O-H stretch; 3417 .86 assigned to N-H stretch; 2924.09 assigned to C- H stretch; 2368.59 assigned to C = O = C stretch; 1805. 37 assigned to C = O stretch ; 1589.34 assigned to N-O stretch ; 1280.73 assigned to C-O stretch; 1041.56 assigned to CO- O-CO stretch ; 678.94 assigned to C-Br stretch ; .65 assigned to C- Br alkaline ; 570.07 assigned to C- I stretch as a result of this leaf sample. FTIR analysis confirmed that the bioreduction of Ag + ions to silver nanoparticles is due to the reduction by capping material of plant extract. Proteins present in the extract

can bind to silver nanoparticles through either the free amino or carboxyl groups in the proteins [17]. The present study indicated that the carboxyl ($-C=O$), hydroxyl ($-OH$), and amine ($-NH$) groups of leaf extracts are mainly involved in the fabrication of silver nanoparticles, these results were well correlated with work reported based on the *Clitoria ternatea* and *Solanum nigrum* leaves [16].

SEM

SEM of the *S.betaceum* fabricated $AgNO_3$ NPs (Fig. 3) displays that the sample is composed by a crystal like asymmetrically shaped nanoparticles. Most of the silver nanoparticles had a range of different sizes such as rectangles, squares, hexagons. Figure 2a and b show micrographs of the fabricated nanoparticles at different magnifications. The average size of the *S.betaceum* fabricated $AgNO_3$ NPs was 0.2 μm to 0.5 μm .

Cytotoxic activity

The cytotoxic activity of *S.betaceum* fabricated $AgNO_3$ NPs at different concentrations was evaluated using an MTT assay against the Leukaemia cell line (K562), Bone cancer - MG63, Breast cancer - MCF7 cells and the results indicated that the increasing nanoparticle concentration increase the cell death of cancer cells (Fig. 4d). About 150 $\mu g/ml$ of *S.betaceum* fabricated $AgNO_3$ NPs has inhibited the growth below 50% of K562, MG63, MCF7 cancer cells respectively. *S.betaceum* fabricated $AgNO_3$ NPs IC50 concentration for K562 cancer cells was found to be 32.22 $\mu g/ml$ (Fig. 4a). For MG63 140.13 $\mu g/ml$ were found to be an IC50 concentration (Fig. 4b). Meanwhile, IC50 concentration for MCF7 was found to be 75.73 $\mu g/ml$ (Fig. 4c).

It is well known that antioxidant activity is well correlated with the inhibition of cancer cells. Hence, it is expected that the *S.betaceum* fabricated $AgNO_3$ NPs with potent antioxidant activity would show considerable anticancer activity Fig. 5. Till now, several research studies proven that secondary metabolites such as polyphenols and flavonoids are toxic against cancerous cells and are harmless toward normal cells [18]. Likewise, it has been reported that *S.betaceum* fruit can kills cancer cells [18].

Abbreviations

$AgNO_3$ - Silver nitrate

NPs - Nanoparticles

UV - Ultra visible

FTIR - Fourier transform infrared

XRD - X-ray diffraction

SEM - Scanning Electron Microscopy

Declarations

Ethical Approval

Not Applicable

Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Ramakrishnan Arumugam and Gobinath Thilagar: Design and oversight of the research project, editing as part of manuscript preparation.

Aejitha Subbaia, Kaviya Babu and Apshana Mubarak Ali : conducted the current study project.

Neelakandan Mani: Preparation of the manuscript and edited as well and nanoparticle analysis work.

Findings

No findings

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Figures

Fig.1

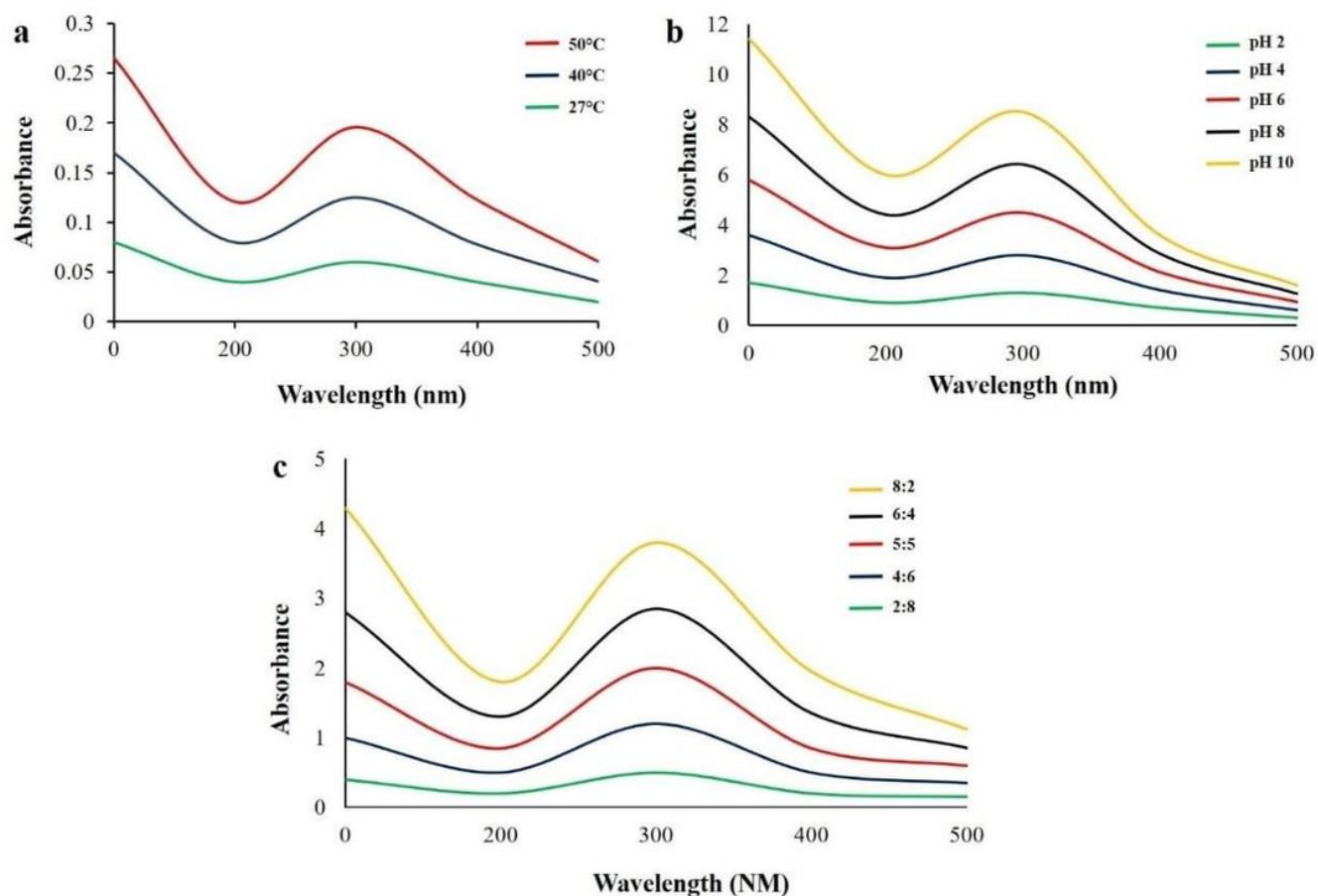


Figure 1

(a) Absorption spectra of AgNO₃ NPs obtained at different reaction temperature of 27°C, 40°C and 50°C, (b) Absorption spectra of AgNO₃ NPs at different pH values (pH 2, 4, 6, 8, 10) of the reaction mixture, (c) Absorption spectra of AgNO₃ NPs at various concentrations of *S. betaceum* leaves extract and AgNO₃ (2:8, 4:6, 5:5, 6:4, and 8:2).

Fig.2

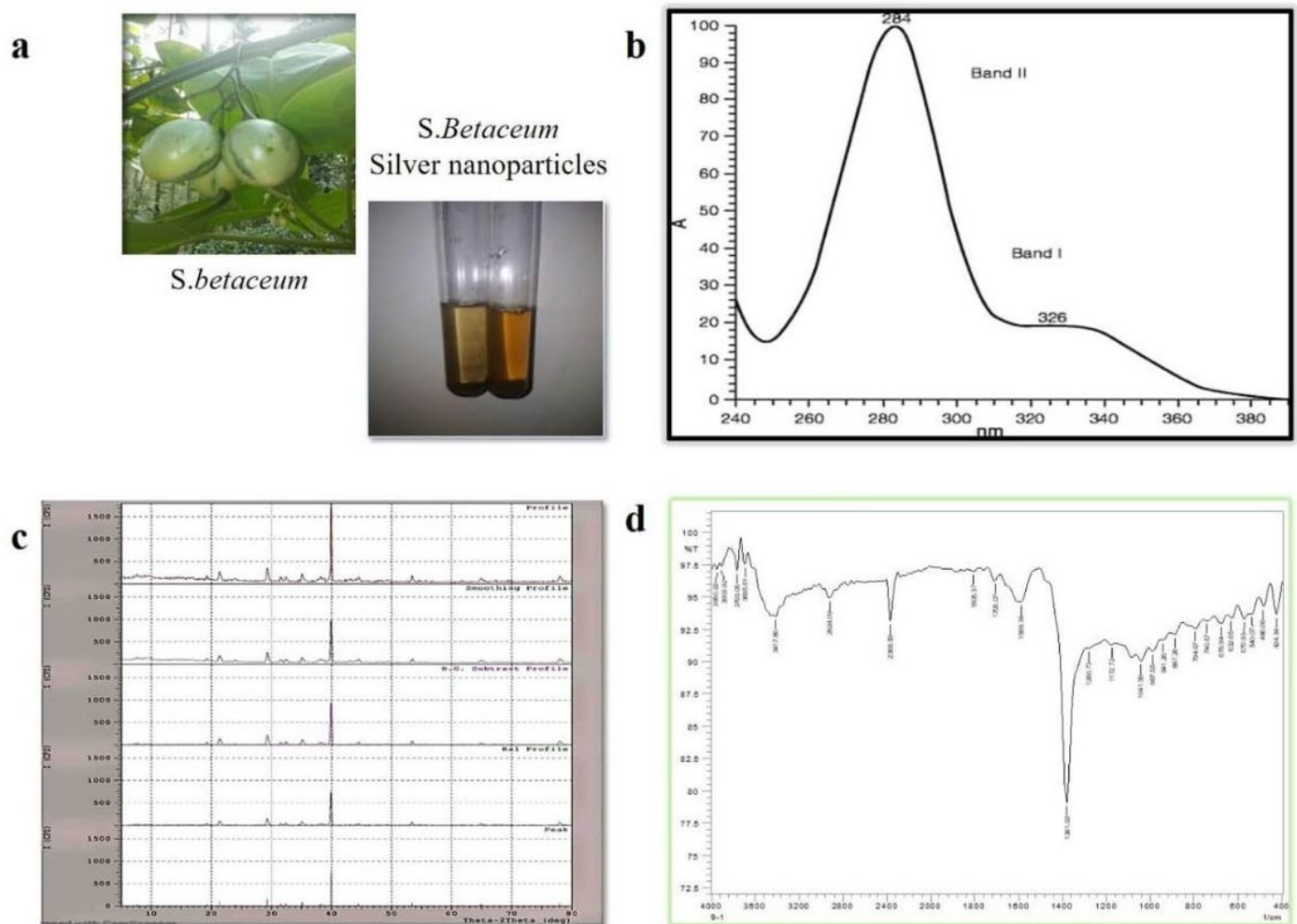


Figure 2

The *S. betaceum* fresh leaves (left) and (right) the synthesis of *S. betaceum* AgNO₃ NPs (a) UV- visible absorption spectrum of *S. betaceum* prepared AgNO₃ NPs showing a characteristic absorption peak at 284 nm (b) XRD pattern of *S. betaceum* prepared AgNO₃ NPs (c), FTIR spectrum of *S. betaceum* prepared AgNO₃ NPs recorded in the range of 500–4000 cm⁻¹ (d).

Fig.3

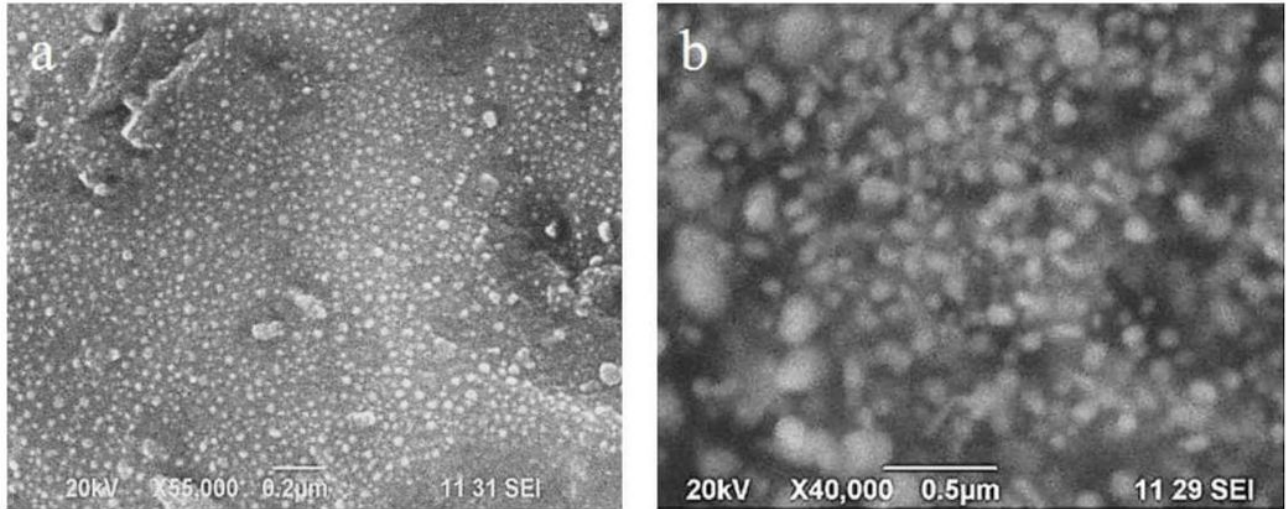


Figure 3

SEM images of *S.betaceum* fabricated AgNO₃ NPs at different magnifications at 20kV (a) X55, 000 and (b) X40, 000.

Fig.4

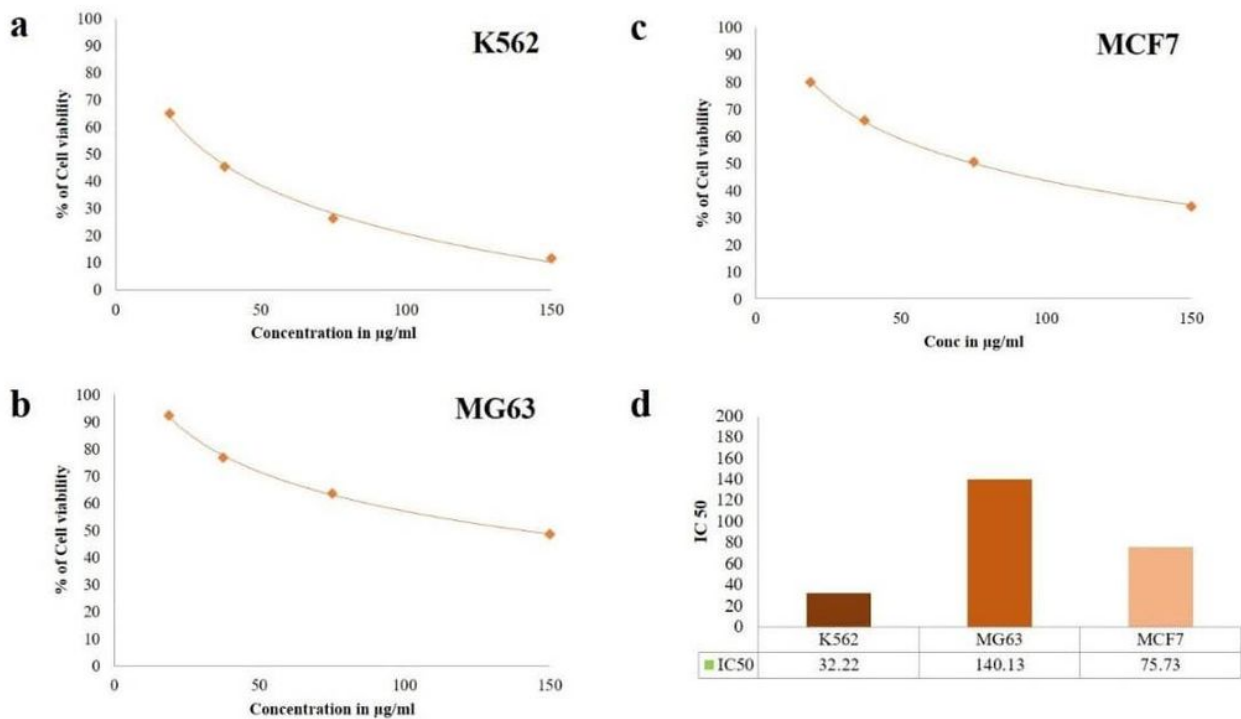


Figure 4

Cytotoxic activity of different concentrations of (18.75,37.5,75,150 µg/ml) of *S. betaceum* fabricated AgNO₃ NPs against K562 Leukaemia cell line (a), MG63- Bone cancer (b), MCF7- Breast cancer cells (c). Comparative IC₅₀ values of *S. betaceum* fabricated AgNO₃ NPs vs K562, MG63 & MCF7 cancer cells (d).

Fig.5

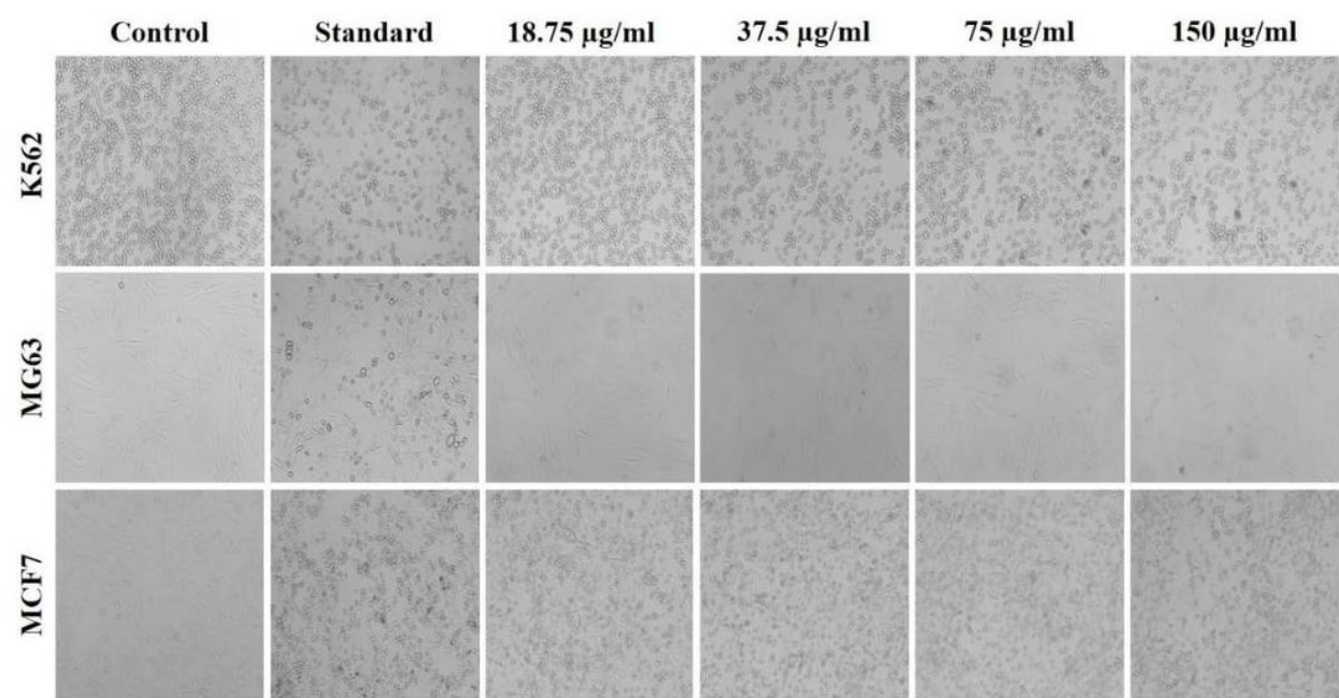


Figure 5

Cell viability in the cultured cells and pictures were taken using a cell imaging station after standard and *S.betaceum* fabricated AgNO₃ NPs respectively.

Supplementary Files

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