



## Green Synthesis and Characterization of Silver Nanoparticles Derived from *Bauhinia variegata*: Exploring Anticancer Activity against Breast Cancer

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(Received: 27 October 2023

Revised: 22 November

Accepted: 26 December)

### KEYWORDS

*Bauhinia variegata*,  
Nanoparticles,  
Silver nanoparticles,  
Characterisation,  
antimicrobial and  
anticancer activity.

### Abstract:

#### Objective

Cancer nanobiotechnology has the potential to redesign the techniques of cancer detection, diagnosis, and treatment. This study is aimed to synthesize stable silver nanoparticles using the bioreduction method. Using the biological materials to make metal nanoparticles has a lot of potential and is environmentally safe. Silver nanoparticles (SNPs) will be synthesised using *Bauhinia variegata* bark extract. It will be studied whether synthesized SNPs could be used as a natural medication.

#### Method

Silver nanoparticles are formed by incubating boiling bark extract of the plant *Bauhinia variegata* with the boiling solution of silver nitrate ( $\text{AgNO}_3$ ). The SNPs were characterised by UV-Visible spectrophotometer, SEM, TEM, XRD, and FTIR. The anti microbial activity was studied using Disc diffusion method. The free radical scavenging activity was examined using DPHH assay. In vitro cytotoxicity of the SNPs was evaluated against MCF7 liver cell line at different concentrations.

#### Results

A change in color from pale yellow to reddish-brown and a strong peak at 450 nm under UV spectral analysis confirmed the formation of SNPs. In this, the plant extracts act as the reducing agent for the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ . FTIR analysis indicated the functional groups responsible for the formation of SNPs. Results obtained from Transmission Electron Microscopic (TEM) studies indicated that the formed SNPs were of 20nm. The antibacterial activities of the formed SNPs proved to be more effective against *E.faecali*. The Free Radical Scavenging Activity and the preliminary anti-cancerous activity was checked and cell viability was found to be 10% at 500 $\mu\text{g}/\text{ml}$ . The viability of MCF7 cells was likewise seen to decrease as the sample concentration increased.

**Conclusion:** The SNPs showed potent cytotoxic activity against MCF7 cell line. The  $\text{IC}_{50}$  value was studied as 500 $\mu\text{g}/\text{ml}$  at which the cell viability was recorded as 10 %. It was also observed that with increasing concentration of the sample, the viability of MCF7 cells decreased. This study demonstrates that SNPs derived from *bauhinia variegata* bark extract can be a useful therapeutic herb in treating cancer.

### Introduction:



Cancer is a large group of diseases that can begin in almost any organ or tissue of the body and spread to other organs when abnormal cells grow uncontrollably, invade adjacent parts of the body, and/or spread to other organs. Tumors can be benign or malignant (1). The cancer cells can spread to other parts of the body through the blood and lymphatic system (2). Prevalent cancer treatment involves the surgical removal of the cancerous parts, the chemotherapeutic killing of the cancer cells, endocrine therapy, and the radioactive elimination of the deadliest cancer cells (3). Nanotechnologists take use of the cancer cells' diverse microenvironment to create potent nanodrugs that can be used to treat cancer cells selectively and accurately (4). Cancer nanobiotechnology has the potential to redesign the techniques of cancer detection, diagnosis, and treatment (5). The plant-based SNPs showed significant anticancer activity when administered at various concentrations to other human cancer cells such as lung cancer, liver cancer, human cervix, and carcinoma cells (6).

Plants are a rich source of secondary metabolites with a wide range of functions, and they play an important role in converting bulk metals to metal ions (6). Several research have been conducted to examine plants' ability to synthesis nanoparticles of various sizes and forms, as well as their role in a variety of biological domains. In terms of availability of biomass in large quantities, cost-effectiveness, ease of handling, and environmental friendliness, biological materials outperform conventional chemical and physical processes. Green synthesis is the process of making products that are less hazardous to the environment and humans by using natural components or ecofriendly chemicals that are safe to handle (7). Green nanostructure manufacturing not only reduces the use of hazardous industrial chemicals, but also allows for one-step nanoparticle creation (8). By altering the boiling point, melting point, permeability, solubility, and shelf-life of materials at the nanoscale, researchers have unprecedented freedom to achieve goals (9). Various biosynthesized SNP uses, such as larvicidal, anticancer, and antioxidant medicines, as well as photocatalysis, have been documented in recent decades (10–12). SNPs produced using *Piper nigrum* leaf extract were proven to be particularly potent against MCF-7 and Hela cancer cell lines (13). According to another research investigation SNPs produced with *Prosopis cineraria* and *Coriandrum*

*sativum* exhibited excellent anticancer potential against MCF-7 cancer cells by acting as reducing and capping agents (14).

*Bauhinia variegata* is a well-known medicinal plant that has been utilised for a variety of reasons by numerous ethnic groups for many years, which is why it is known as kanchan (gold) in Vedic literature (15). It is a species of flowering plant in the family *Fabaceae*, native to South Asia and Southeast Asia, Southern China, Burma, India, Nepal, Pakistan, and Sri Lanka. In numerous countries, *Bauhinia variegata* is titled as "Camel's foot tree" or "Orchid tree." It is a small to medium-sized tree growing to 10–12 meters (33–39 ft) tall, deciduous in the dry season. The leaves are 10–20 centimeters (3.9–7.9 in) long and broad, rounded, and bilobed at the base and apex. The flowers are conspicuous, bright pink or white, 8–12 centimeters (3.1–4.7 in) diameter, with five petals. The fruit is a pod 15–30 centimeters (5.9–11.8 in) long, containing several seeds. *Bauhinia variegata* stem bark contains variety of phytochemical classes like sterols, glycosides, flavonone glycoside, phenanthraquinone, reducing sugars and nitrogenous substances. Raj Kapoor et al., studied the anti-tumor activity of *Bauhinia variegata* in Swiss albino mice models against Dalton's ascitic lymphoma (DAL). It was observed that ethanol extract of the plant significantly enhanced the mean survival time of the models administered with the extract (16). Another study suggested that hydromethanolic extract of *Bauhinia variegata* was effective against melanoma tumor by B16F10 cell line in C57BL/6 mice (17). In vitro study showed that alcoholic extract of *Bauhinia variegata* was effective against *Bacillus subtilis* (ATCC 6635), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* (ATCC 29213) and *Vibrio cholera*. The extract showed more potent activity against gram positive bacteria (18). Similarly its other properties like anti-inflammatory (19), anti-diabetic (19), hepatoprotective (20), Antigoitrogenic (21), Nephroprotective (22), anti-ulcer (23) have been studied widely.

## Materials and methods

### Collection of experimental part of the tree:

*Bauhinia Variegata* bark were collected from Thiruvannamiyur, Chennai and were botanically confirmed by Dept of CAS Botany, University of Madras, Guindy campus, Chennai- 600025.



### Preparation of bark extract:

The stem bark were separated from wood and used for extraction. The collected material were washed thoroughly in water, chopped, air dried for a week at 35-40°C and pulverized in electric grinder. 20 gram of powered bark was weighed, boiled with 100ml distilled water and filter the extract using muslin cloth.

### Preparation of silver nitrate solution:

Different concentrations of silver nitrate solution were prepared (1mM,3mM,5mM) using the formula.

$$\text{Amount of silver nitrate} = \frac{\text{equivalent weight of silver nitrate} \times \text{Molarity} \times \text{Required Volume}}{1000}$$

### Synthesis of silver nanoparticles:

Three different conical flasks were taken and added with 9ml of silver nitrate and 1ml of bark extract solution in three different concentration 1mM, 3mM and 5mM respectively. The 1 ml bark extract solution was added drop by drop. These mixtures were kept in three different exposures- Sunlight, Room temperature, Microwave oven (at 35 °C) at different time intervals (5, 10, 15 mins). Reddish brown color was formed, which shows the reduction of silver from silver nitrate forming SNPs. The solutions were incubated for overnight at room temperature for complete settling of SNPs. The mixtures were centrifuged at 10,000 rpm for 10 to 20 mins. The supernatant was discarded; pellets were added with acetone and mixed using vortex mixture. The mixtures were poured in petriplates and kept for drying. 1mM solution which was kept in sunlight yielded more amounts of SNPs. Bulk synthesis of SNPs with 1mM solution using sunlight exposure was standardized.

### Bulk preparation of silver nanoparticles:

Silver nitrate (1mM), 90ml was taken in a conical flask. To that 10 ml of bark extract was added drop by drop. This mixture was kept at sunlight for different time intervals (1, 2,3,4,5 hours). Reddish brown color was formed and the absorbance of mixture was measured in UV visible spectrophotometer in the visible range (400-700 nm). This solution was incubated for overnight at room temperature for complete settling of SNPs. The mixture was centrifuged at 10,000 rpm for 10 to 20 mins. The supernatant was discarded and pellet was added with acetone and mixed using vortex mixture. The mixture was poured in petriplate and kept for drying. The colour change was noted by visual observation from pale yellow to reddish brown colour. The colour change indicates the formation of SNPs in

the solution. This result is similar to SNPs exhibiting a reddish brown colour in aqueous solution because of the excitation of surface Plasmon vibrations in SNPs.

### Characterization of biologically synthesized silver nanoparticles:

Biologically synthesized SNPs were characterized by UV-visible spectrometry, X-ray diffraction (XRD), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transmission infra red spectroscopy (FTIR).

The aliquot (2ml) of the reaction solution was taken and subjected to UV-Visible spectroscopic measurements. The UV-visible spectroscopic measurements were performed using  $\lambda$ -Helios SP Pye-Unicam spectrophotometer.

### X-Ray Diffraction (XRD):

X-Ray Diffraction used to determine the crystal structure of solid, including lattice constant and geometry, identification of unknown materials, orientation of single crystals, defects, etc. In this method, a monochromatic X-ray directed onto a sample and the interaction between these planes of atoms and X-ray lead to diffracted rays being emitted. The characterization of the purified SNPs was conducted with an XRD-6000 X-ray diffractometer (Shimadzu, Japan) operated at a voltage of 40 kV and a current of 30mA.

### Scanning Electron Microscope (SEM):

Scanning electron microscopy has provided further insight into the morphology and size details of the synthesized nanoparticles. Morphological characterization of the sample was done using SEM (JFC-1600) 5 $\mu$ l of the biologically SNPs were placed onto the clean aluminium foil sheet, which was air dried and sputter coated with gold particles for 15 seconds and then subjected for SEM analysis.

### Transmission Electron Microscope (TEM):

Size and shape of the SNPs can be identified using TEM analysis. Characterization of the sample was done using TEM (JEOL-2010) 200kV. Thin film of the sample were prepared on a carbon coated copper grid by just dropping a small amount of the sample on the grid, extra solution is removed using blotting paper and the film on the grid were allowed to dry by putting it under a mercury lamp for 5 minutes.

### Fourier Transmission Infra Red Spectroscopy (FTIR):



The formed SNPs from the bark extract were centrifuged at 20,000rpm for 20 minutes and then the pellet obtained is washed thrice with deionised water. Then it is dried in an oven at 60°C in an oven for an entire day. This powdered form is used for the further analysis of the nanoparticles. A Fourier Transform Infra Red Spectrometer is used to obtain the infra red spectra of absorption and emission of the formed SNPs. The advantage of using an FTIR is this simultaneously collects spectral data in a wide spectral range. 10mg of the powdered sample of the formed SNPs from the bark extract was subjected to FTIR analysis using a Nicolet 6700 spectrophotometer in the diffuse reflectance mode at a resolution of 4cm<sup>-1</sup> in Potassium Bromide pellets.

#### Anti-microbial activity:

Antimicrobial activity of biosynthesized SNPs from the bark extract of *Bauhinia variegata* by Kirby-Bauer disc diffusion method was carried out on four human pathogens *K.pneumonia*, *P.aeruginosa*, *P.vulgaris*, *E.faecalis*.

**Preparation of medium:** Nutrient agar medium (peptone-5g, yeast extract-3g, sodium chloride-5g, agar-20g, distilled water-1000g, pH 7.0). Then the medium was sterilized by autoclaving at 121°C for 15 mins at 15 psi pressure and used to determine the antibacterial activity of synthesized nanoparticle.

#### Disc diffusion method:

Biosynthesized SNPs from the bark extract of *bauhinia variegata* was subjected for antimicrobial activity by using gram positive and gram negative bacteria by disc diffusion method. Now sterile agar was poured aseptically into sterile petri plates and plates were allowed to solidify at room temperature in sterile condition. Spread the overnight culture of bacteria over the surface of the agar plate using a sterile glass spreader and incubated at 37 °C for 30 mins. Add 10 µl of each oil on sterile 6mm blank antimicrobial susceptibility disc; keep the oil impregnated discs onto the inoculated surface of the agar plate. The agar plate incubated overnight at 37 °C the zone of bacterial inhibition was recorded.

#### Free radical scavenging activity by DPPH assay:

The ability of the SNPs to annihilate the DPPH radical (1, 1-diphenyl-2-picrylhydrazyl) was investigated. Stock solution of sample was prepared to the concentration of 1mg/ml. 20,40,60,80,100,120,140,160,180 g of each sample were added to DPPH (0.1%). The reaction mixture was

incubated for 30 min at room temperature and the absorbance (A) was recorded at 517 nm. The experiment was repeated for three times. BHT (Butylated Hydroxy Toluene) was used as standard control. The annihilation activity of free radicals was calculated as % inhibition according to the following formula,

$$\% \text{ Radical scavenging activity}(\% \text{RSA}) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

#### Cytotoxicity assay on cancer cell lines:

Chemicals and reagents:

**MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay.**

- ❖ Acridine orange.
- ❖ RPMI medium for culturing of cells
- ❖ Fetal Bovine Serum
- ❖ DMSO

#### Cell culture:

MCF 7/ HepG 2 cells were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 µg/ml), gentamycin (100µg/ml) and amphotericin B (1mg/ml). All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Cells were allowed to grow to confluence over 24 h before use.

#### Cell growth inhibition studies by MTT assay:

Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, MCF7cells were seeded at a density of 5×10<sup>3</sup> cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (1–100µg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10-5mg/ml) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments (12).

$$\text{Cell viability}(\%) = \frac{\text{Mean OD}}{\text{Control OD}} \times 100$$

## Results

### Synthesis of silver nanoparticles from the bark extract of *Bauhinia Variegata*:

*Bauhinia variegata* bark nanoparticles was synthesized using 1mM concentration. **Fig 1(a)** shows synthesized



SNPs in petriplate; **Fig (1b)** result shows dried form of SNPs.

#### **Characterization of silver nanoparticles synthesized from the bark extract of *Bauhinia Variegata*:**

##### **UV-Vis Spectral analysis**

Synthesized SNPs were subjected to UV-Visible spectroscopy which showed the absorbance peak at 450 nm (**Fig.2**). The maximum absorption of the SNPs from *bauhinia variegata* at 450nm indicates that the formation of the particles. The peak increased from the 0hr to a maximum of 450nm and thereby declined. This indicates that the silver nitrates were completely formed at 450nm.

##### **SEM Analysis:**

The analysis was carried out to identify the synthesized SNPs morphology, size and shape. **Fig.3** shows that synthesized SNPs from the bark extract of *B.Variegata*.

##### **TEM Analysis:**

TEM analysis of the biologically synthesized SNPs from the bark extract of *B.Variegata* from **Fig.4** showed the average size and shape of the particles, revealing that the size of nanoparticles were found to be 20 nm and nearly spherical in shape.

##### **XRD Analysis:**

**Fig.5** shows Three intense peaks in the whole spectrum of  $2^\theta$  values ranging from  $0^\circ$  to  $70^\circ$ . The formation of nanocrystal are evidenced by the peaks at  $2^\theta$  values of  $38.45^\circ$ ,  $44.48^\circ$ ,  $64.69^\circ$  corresponding to (111), (200) and (220) planes for silver, respectively. The unassigned peaks could be due to the crystallization of bioorganic phase that occurs on the surface of the nanoparticle. The peak broadening at half maximum intensity of the X-ray diffraction lines is due to a reduction in crystallite size, flattening and micro-strains within the diffracting domains.

##### **FTIR Analysis:**

FTIR measurements were carried out as in **Fig (6)**, it indicates the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized. The peaks at 608-629, ~816, 897-1162, 1325-1354, 1657-1682 and 1514-1556  $\text{cm}^{-1}$  corresponds to C-H bending in alkynes, acidic pectins, carbohydrates, C=O stretching and contribution from C-N stretching or C=C groups/aromatic rings, amide III, OH stretching alcohol/phenols or carbohydrates, C-H stretching in saponins, C-H bend/COO- symmetric stretching due to the acidic group of polygalacturonic

acid and OH deformation vibrations in the aromatic ring/phenol respectively.

#### **Antimicrobial activity of biologically synthesized silver nanoparticles from the bark extract of *Bauhinia Variegata*:**

20mg of bark extract of *Bauhinia variegata* was dispersed in 500  $\mu\text{l}$  of distilled water. From this 50  $\mu\text{l}$  and 100  $\mu\text{l}$  volume was impregnated on the disc. From the **Fig. 7(a)** synthesized SNPs showed clear zone of inhibition. The zone of inhibition values are mentioned in **Tab.1**. The antibacterial activity of the formed SNPs proved to be more effective against *E.faecali*.

##### **Free radical scavenging activity:**

Radical scavenging activity (RSA) of SNPs synthesized from the bark extract of *Bauhinia Variegata* was found to vary between 10% and 80%. The data was considered significant when compared with the standard used (BHT). The  $\text{IC}_{50}$  value was calculated as  $220\mu\text{g/ml}$ . The RSA values are shown in **Fig 8**.

##### **Cytotoxicity of AgNPs on MCF7 cell lines:**

The in vitro cytotoxicity of the AgNPs was evaluated against MCF7 liver cell line at different concentrations. Cytotoxicity analysis of the sample shows a direct dose relationship; cytotoxicity increased at higher concentrations. The sample demonstrated a considerable cytotoxicity against MCF-7 cell lines. The result showed that MCF-7 cells proliferation was significantly inhibited by AgNPs with an  $\text{IC}_{50}$  value  $119.51\mu\text{g/ml}$  of the concentration. Cyclophosphamide is used as standard control. The SNPs showed potent cytotoxic activity against MCF7 cell line(**Fig.9**). The  $\text{IC}_{50}$  value was studied as  $500\mu\text{g/ml}$  at which the cell viability was recorded as 10% (**Tab.2**). It was also observed that with increasing concentration of the sample, the viability of MCF7 cells decrease.

##### **Discussion**

The cancer burden continues to grow globally, exerting a tremendous physical, emotional, and financial strain on individuals, families, communities, and health systems. The development of customised therapy and healthcare procedures for the benefit of the human population will be aided by a mechanistic knowledge of SNPs therapeutic effects. Green synthesis of silver nanoparticles (SNPs) by harnessing the natural abilities of plant secondary metabolites has advantages over routine physical and chemical synthetic approaches due to their one-step experimental setup to reduce and



stabilize the bulk silver into SNPs, biocompatible nature, and therapeutic significance(24). The SNPs prepared by different plant extracts from *Plumeria alba*, *Erythrina indica*, *Glycyrrhizauralensis*, *Indigofera tinctoria*, and *Mukia maderaspatana* show anticancer potential(25–27). *Bauhinia variegata* is commonly used in folk system by many cultures and tribes for treating several diseases. Each part of *Bauhinia variegata* is medically important due to the presence of variety of phytochemical constituents in them like alkaloids, oil, fat glycoside, carbohydrates, phenolics, tannins, lignin, saponins, flavonoids and terpenoids(28). Its bark extract is utilised to synthesize SNPs because it has received comparatively little investigation compared to its other sections. A change in color from pale yellow to reddish-brown and a strong peak at 450 nm under UV spectral analysis confirmed the formation of SNPs. The absorbance peak at 450 nm, was comparable with the reddish-brown color solution giving the surface Plasmon resonance absorption band due to the combined vibration of electrons of synthesized gold nanoparticles in resonance with light energy(29), suggesting that the reduction of silver caused the creation of SNPs. The plant extracts act as the reducing agent for the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ . The SEM data revealed that the produced nanoparticles were equally dispersed, due to Surface Plasmon phenomena. This could be attributed to the variety of capping agents found in the bark extracts, as well as the smooth production of fine SNPs. The SEM investigation, showed that the SNPs were spherical(30). From the TEM it was discovered that the manufactured SNPs were spherical in shape, with the majority of the particles falling between 11nm and 15nm in diameter(30). The crystalline stability of the SNPs was comparable to the XRD pattern of *Ananas comosus*, which shows the corresponding 2 position for the peaks of SNPs were  $38.45^\circ$ ,  $44.48^\circ$ ,  $64.69^\circ$ , and  $77.62^\circ$ , indicating that the silver particles are nanosize(31). FTIR analysis indicated the functional groups responsible for the formation of SNPs(29). SNPs antibacterial action was linked to the creation of "pits" in bacteria's cell walls, which resulted in enhanced membrane permeability and cell death(32). A study also indicated that the bactericidal actions of the silver ion are caused primarily by its interaction with the cytoplasm in the interior of the cell(33). The silver ion appears to penetrate through ion channels without

causing damage to the cell membranes; it denatures the ribosome and suppresses the expression of enzymes and proteins essential for ATP production, which causes the disruption of the cell. Recently it has been reported that plant-mediated SNPs display their potential against various pathogens through several mechanisms, but mostly SNPs kill the microorganisms by triggering the production of reactive oxygen species (ROS) in the cells(34). The antibacterial activity of the formed SNPs was proved. It was found to be more effective against *E. faecali*. When cells are exposed to plant-based SNPs, oxidative damage and caspase-3 activation are the main causes of cell death(35). Some researchers revealed that SNPs from *Mentha arvensis* induce toxicity against MCF-7 cells and Hep2 cells by mediating through the caspase-9-dependent apoptosis(36). The % toxicity increases with an increase in the concentration of the SNPs suggests that biosynthesized SNPs could be of immense use in the medical field to a certain extent as an anticancer agent.

## CONCLUSION

The nanoparticles synthesised from the bark extracts of *Bauhinia variegata* showed potential for use in herbal medicine after being characterized by UV-Visible spectrophotometer, SEM, TEM, XRD, and FTIR. They also demonstrated antibacterial, free radical scavenging activity, and preliminary Cytotoxicity.

## Abbreviations

$\text{AgNO}_3$  : Silver nitrate

BHT : Butylated Hydroxy Toluene

DMSO: Dimethyl sulfoxide

DPPH : 2,2-diphenyl-1-picryl-hydrazyl-hydrate

*E. faecalis* : Enterococcus faecalis

FTIR : Fourier-transform infrared spectroscopy

IC<sub>50</sub> : Half-maximal inhibitory concentration

MCF-7 : Michigan Cancer Foundation-7

MTT : (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide)

*P. aeruginosa* : Pseudomonas aeruginosa

*P. vulgaris* : Proteus vulgaris

*K. pneumonia* : Klebsiella pneumoniae

ROS : Reactive oxygen species

SEM : Scanning electron microscope

SNPs : Silver nanoparticles

TEM : Transmission electron microscope

XRD : X-ray diffraction

## Acknowledgements



We'd like to thank the reviewers for their valuable input on this paper.

#### **Funding**

No funding was received for this study.

#### **Availability of data and materials**

Not applicable

#### **Ethics approval and consent to participate**

Not applicable

#### **Competing interests**

The authors declare that they have no competing interests.

#### **Consent for publication**

Not applicable

#### **Authors' contributions**

YS contributed to study design; PS contributed to manuscript editing; HG contributed to experimental studies; HG and PS contributed to data analysis. All authors read and approved the final manuscript.

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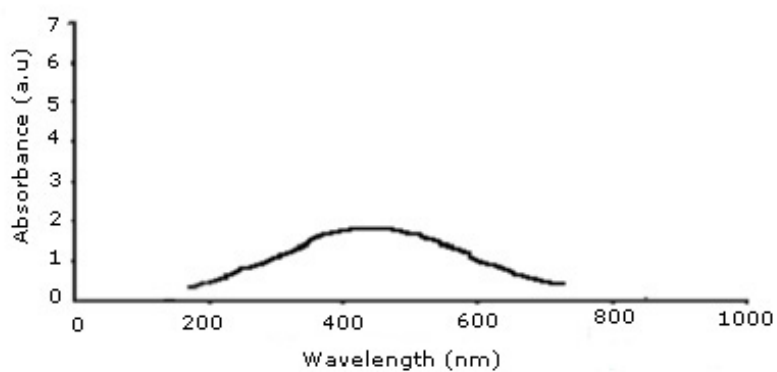
**Fig. 1(a) Silver Nanoparticles in Petriplate**



**Fig. 1(b) Silver Nanoparticles in dried form**

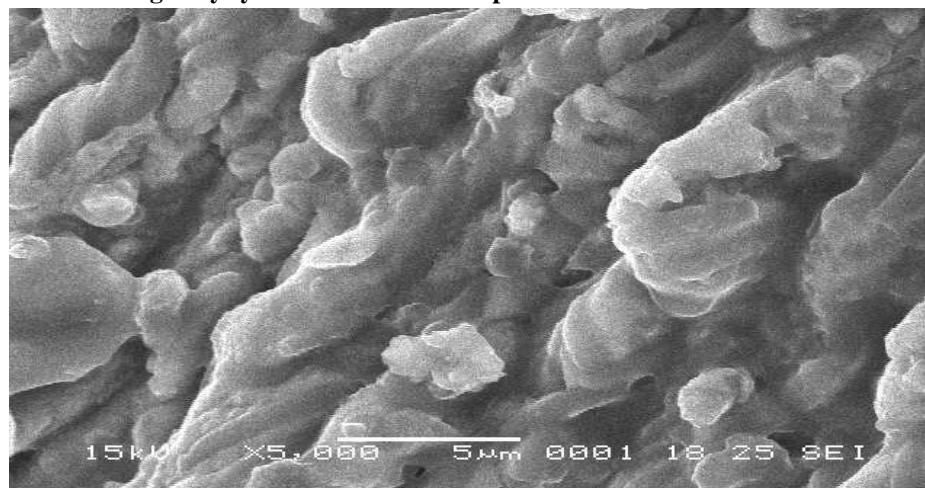


**Fig 2. UV-Visible spectroscopy absorbance peak of *Bauhinia Variegata***





**Fig.3 SEM result of the biologically synthesized silver nanoparticles from the bark extract of *B.Variegata***



**Fig.4 TEM result of the biologically synthesized silver nanoparticles from the bark extract of *B.Variegata***



**Fig. 5 X-ray diffraction (XRD) patterns of dried silver nanoparticles synthesized using bark extract at room temperature.**

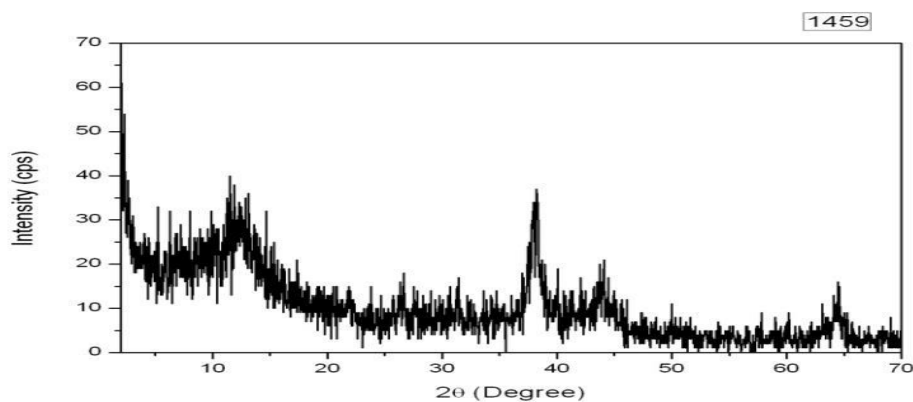


Fig. 6 FTIR results of the biologically synthesized silver nanoparticles from the bark extract of *B. Variegata*

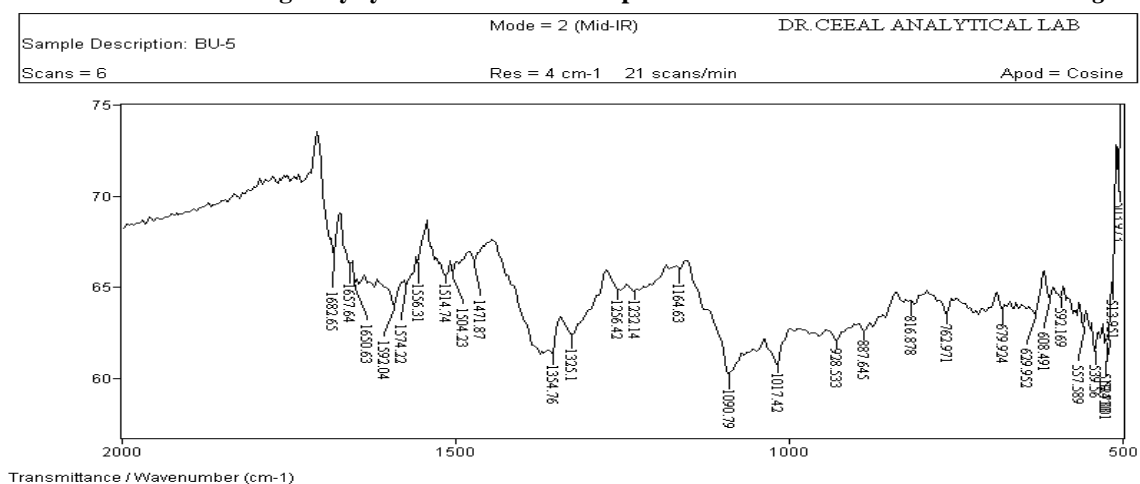


Fig 7 Zone of clearance showed by synthesized silver nanoparticles from bark extract of *Bauhinia variegata*

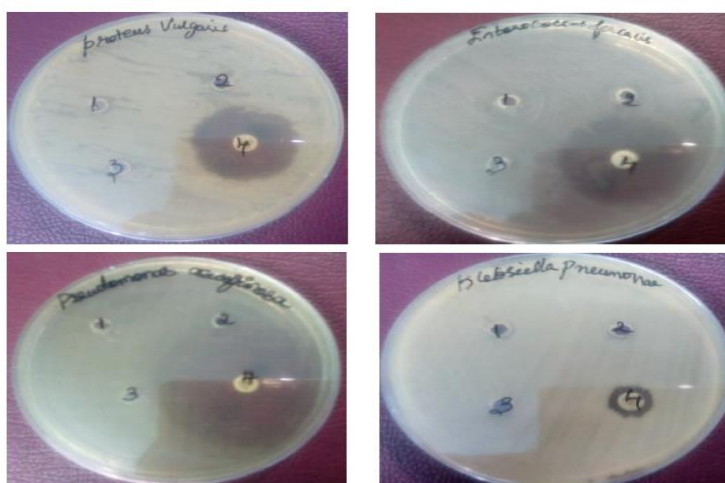


Fig. 8 Radical scavenging activity (RSA) of silver nanoparticles synthesized from the bark extract of *Bauhinia Variegata*

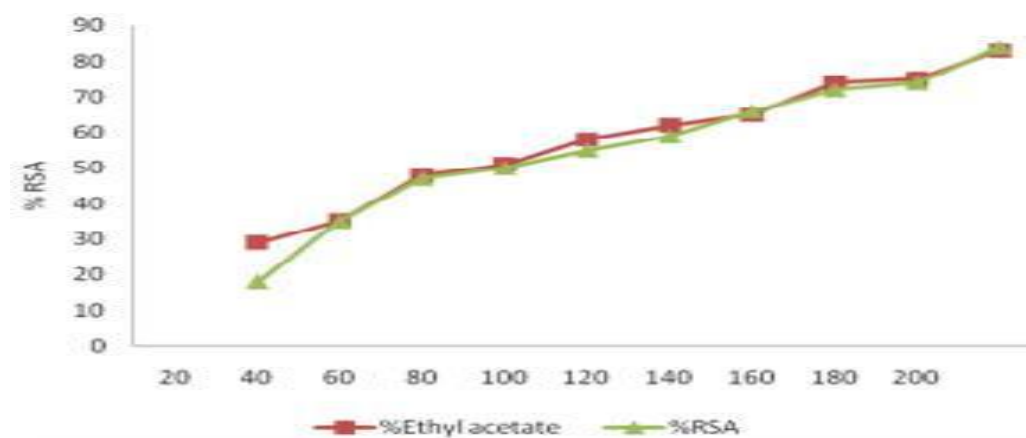


Fig.9 Cytotoxicity analysis of *Bauhinia Variegata* against MCF-7 cell lines

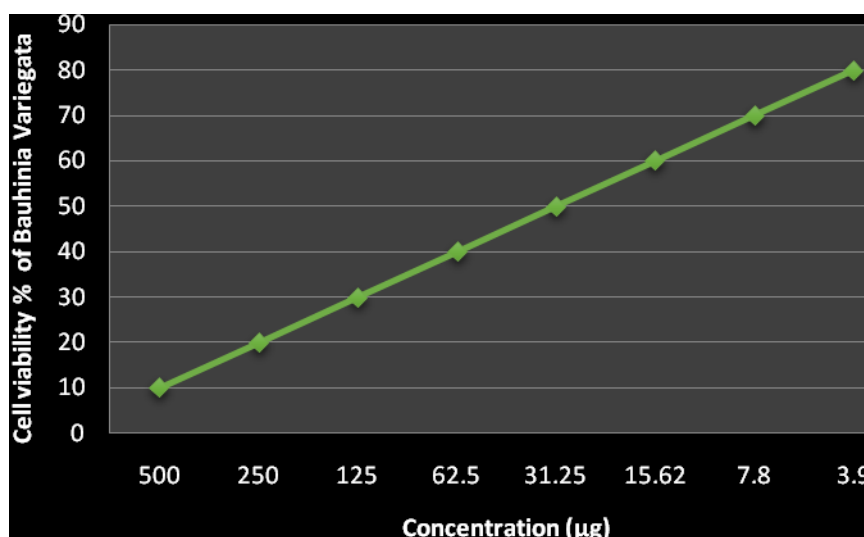


Table 1. Zone of inhibition values showed by synthesized silver nanoparticles from bark extract of *Bauhinia variegata*

S.No	Organism name	Zone of inhibition (50 μl/ml)	Zone of inhibition (100 μl/ml)
1	<i>K.pneumonia</i>	11 mm	13 mm
2	<i>P.aeruginosa</i>	10 mm	12 mm
3	<i>P.vulgaris</i>	12 mm	14mm
4	<i>E.faecalis</i>	14mm	15mm

Table 2. Cytotoxicity analysis values of *Bauhinia Variegata* against MCF-7 cell lines

S.No.	Concentration (μg)	Cell viability % of <i>Bauhinia Variegata</i>
1.	500	10
2.	250	20



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3.	125	30
4.	62.5	40
5.	31.25	50
6.	15.62	60
7.	7.8	70
8.	3.9	80