

## Biogenic Synthesis of Silver Nanoparticles from *Alphonsea sclerocarpa* Leaf Extract and Their Anticancer Activity Against K562 Cells

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### Abstract

*Alphonsea sclerocarpa* Thwaites, a member of the family Annonaceae, is a small tree that grows up to 10–15 m in height, with simple and alternate leaves. Although known for its medicinal properties, the plant remains relatively underexplored. Therefore, the present research was undertaken to evaluate the anticancer potential of the ethanolic leaf extract of *A. sclerocarpa*. The extract was prepared using the Soxhlet extraction method and further analysed through TLC, HPLC, SEM, and TEM techniques. Cytotoxic activity was assessed using the MTT assay. Silver nanoparticles synthesized from the extract exhibited strong cytotoxic effects against malignant K562 cells, demonstrating significant growth inhibition, while showing minimal toxicity (less than 10% inhibition) in normal HEK293 cells.

**Keywords:** MTT assay; K562 cells; HEK293; TLC; HPLC; SEM

### 1. Introduction

Medicinal plants have played a vital role in the development of human culture. They have been a primary source of medicine and have remained at the forefront of healthcare across virtually all civilizations. These plants are considered valuable reservoirs of traditional remedies, and many modern medicines have been derived from them. *A. sclerocarpa*, a member of the Annonaceae family, is an endemic species found in South India and Sri Lanka, widely recognized for its medicinal potential. Its therapeutic properties are mainly attributed to the presence of diverse phytochemicals, particularly alkaloids. Previous studies have demonstrated the antimicrobial and antioxidant activities of crude leaf extracts of *A. sclerocarpa* (Suman Joshi *et al.*, 2017). Antioxidants are defined as “substances that delay or inhibit oxidative damage to a target molecule” (Halliwell *et al.*, 1990)

### 2. Materials and methods

#### 2.1. Collection of plant material

*A. sclerocarpa* leaves, a medicinal plant, were gathered from the Seshachalam forest area.

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## 2.2. Chemicals and Reagents

All chemicals, solvents, and reagents used in the study were of analytical grade and were procured from reputable suppliers, including Merck, Sigma, SD Fine, and SRL.

## 2.3. Soxhlet Extraction

The dried plant material (300 grams) was subjected to successive solvent extraction using a Soxhlet apparatus. The extraction was carried out sequentially with solvents of increasing polarity: hexane, chloroform, ethyl acetate, methanol, and water. For each solvent, the Soxhlet apparatus was operated at 45°C for 35 cycles. Freshly powdered plant material was used for each extraction to ensure a sufficient yield of the crude extract. After each extraction cycle, the remaining plant residue was dried and reloaded with the next solvent in the sequence. The resulting extracts were labelled according to the solvent used and stored for further analysis.

## 2.4. Thin Layer Chromatography (TLC)

Thin-layer chromatography was performed using silica gel 60 GF plates (Merck, Germany). Samples were applied as small spots using TLC-specific capillary tubes and allowed to air dry. The loaded plates were then placed in a TLC chamber saturated with the appropriate solvent system, sealed with a lid to prevent atmospheric interference, and allowed to develop until the solvent front reached approximately three-quarters of the plate length.

After development, the plates were examined under a UV light at 254 nm. For additional visualization, the plates were exposed to iodine vapor in an iodine chamber for 10–15 minutes to reveal compound spots. Fractions displaying similar banding patterns were pooled, concentrated using rotary evaporation, and subjected to further purification by High-Performance Liquid Chromatography (HPLC). TLC was used throughout the purification process to monitor and assess the purity of the collected fractions.

## 2.5. High-Performance Liquid Chromatography (HPLC)

The samples were analysed using a Shimadzu HPLC system (Kyoto, Japan) equipped with a dual-pump LC-20AD binary system and a photodiode array (PDA) detector (SPD-M20A). Separation was performed on a Merck C18 reverse-phase column (4.6 mm × 250 mm I.D.) using a linear binary gradient.

The mobile phases consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile). The gradient program was initiated at 10% B, increased to 70% over 25 minutes, then returned to 10% at 35 minutes, followed by a 40-minute column wash. The flow rate was maintained at 1.0 mL/min, with an injection volume of 1.0 mL. Detection was carried out at 254 nm using a PDA detector. This wavelength was selected due to the strong absorbance of aromatic compounds, particularly those containing benzene rings, and the high-intensity emission line of mercury lamps at this wavelength.

Fractions were separated and purified using the optimized binary gradient of acetonitrile and 0.1% formic acid in water. The isolated pure compounds were labelled as P1, P2, P3, P4, and P5 for further evaluation.

## 2.6. Structural Characterization

HPLC purification yielded five distinct fractions with varying degrees of purity, labelled as P1, P2, P3, P4, and P5. Among these, the compound designated as P5 was isolated with a recovery of 42 mg and a high purity of 97%. This chemical was structurally characterized and demonstrated significant anticancer action.

## 2.7. Cell Culture and Anticancer Activity

To evaluate the anticancer potential of the structurally characterized pure phytochemical, multiple cell lines were selected, including DU-145 (prostate cancer), HEK 293T (human embryonic kidney), and K562 (chronic myeloid leukemia). DU-145 and HEK 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), while K562 cells were maintained in Roswell Park Memorial Institute (RPMI-1640) medium. The RPMI-1640 medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cell viability was assessed using the trypan blue dye exclusion method. Cells were seeded at an approximate density of  $2 \times 10^3$  cells/mL and were subcultured twice weekly.

## 2.8. Cytotoxicity (MTT) Assay

The cytotoxic effects of the extracts on both normal (HEK 293T) and cancerous (DU-145 and K562) cell lines were evaluated using the MTT assay. Cells were seeded into 96-well plates at a density of  $5 \times 10^3$  cells per well and allowed to adhere for 24 hours in a final volume of 200  $\mu$ L, with or without varying concentrations of the extract. After incubation, 20  $\mu$ L of MTT solution (5 mg/mL in PBS) was added to each well, followed by an additional 3-hour incubation at 37°C. The resulting formazan crystals, formed by the reduction of MTT by mitochondrial dehydrogenases, were dissolved in 100  $\mu$ L of DMSO. Absorbance was measured at 570 nm using a multimode plate reader (BioTek Synergy Mx). The percentage of cell proliferation inhibition was calculated relative to untreated control cells.

## 2.9. Synthesis of Silver Nanoparticles

The aqueous fraction of *A. sclerocarpa* was utilized for the synthesis of silver nanoparticles (AgNPs), followed by structural characterization. The potential antibacterial and anticancer activities were evaluated, with K562 (chronic myeloid leukemia) blood cancer cells used as the cancer model. Normal, non-cancerous HEK293 (human embryonic kidney) cells served to assess the biocompatibility and biosafety of both the aggregate plant extract (PE) and the synthesized AgNPs.

## 2.10. Characterization of Silver Nanoparticles:

- **UV-Visible Spectroscopy:** The synthesized AgNPs were initially identified through visual observation of color changes. Subsequently, the reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  was confirmed using UV-Visible spectroscopy (Thermo Scientific) at a 1 nm resolution, with a scanning range of 200–800 nm.
- **FTIR Analysis:** The synthesized AgNPs were analysed using Fourier Transform Infrared (FTIR) spectroscopy, employing the KBr pellet method on a Shimadzu IR Affinity-1S (Japan) instrument, covering the spectral range of 4000–500  $\text{cm}^{-1}$ .
- **SEM with EDAX:** Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDAX) were employed to evaluate the synthesized AgNPs. For analysis, the AgNPs were mounted on a stub and gold-coated using a Quorum Q150 RES for three minutes at 10 mA, achieving a coating thickness of 2.3 nm and a density of 19.32  $\text{g/m}^3$ . SEM was used to determine the shape, size, and surface morphology, while EDAX was utilized to analyse the elemental composition.
- **TEM Analysis:** The morphological characterization of the composites was carried out using a FEI Tecnai transmission electron microscope. Samples for TEM imaging were prepared by placing a 10  $\mu$ L methanol-dispersed sample onto carbon-coated copper grids and allowing it to air-dry at room temperature for 24 hours. Imaging was performed at an acceleration voltage of 200 kV. To prevent thermal damage at this high voltage, phosphotungstic acid (PTA) was applied, forming a thin protective film on the sample surface.

## 2.11. Biological assays of silver nanoparticles aggregated with plant extract Anti-microbial activity

The antibacterial activity of the synthesized AgNPs was evaluated using the agar well diffusion method. The assay was performed against four Gram-positive bacterial strains (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Streptococcus mutans*) and four Gram-negative bacterial strains (*Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*). All bacterial cultures were procured from IMTECH, Chandigarh, and 24-hour-old cultures were used for the experiments.

For the assay, 1 mL of bacterial suspension was dispensed into sterile Petri plates, followed by the addition of molten nutrient agar medium, which was mixed thoroughly and allowed to solidify. Wells of 6 mm diameter were then created using a sterile cork borer, and each well was loaded with 100  $\mu$ L of synthesized AgNPs (50 mg dissolved in DMSO). Streptomycin (10  $\mu$ g/mL in DMSO) was used as a positive control. The plates were incubated at 37 °C for 24 hours, after which the antibacterial activity was assessed by measuring the diameter of the inhibition zones around the wells.

## 2.12. Cell inhibition assay (MTT assay)

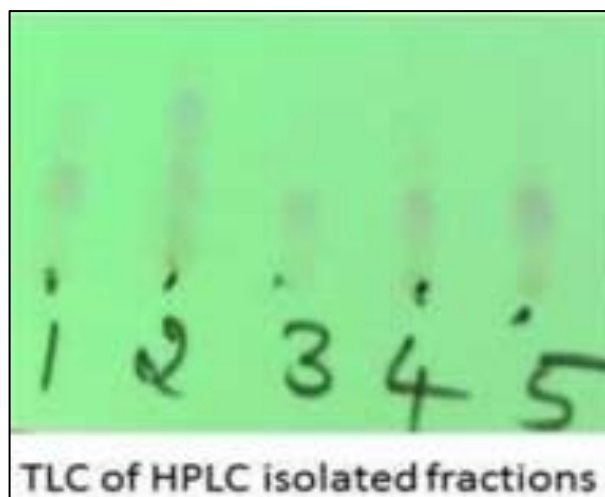
The anticancer activity or inhibitory potential of the synthesized [(*A. sclerocarpa*)-(AgNPs)] aggregate was evaluated using multiple cell lines. For the cell inhibition assay, K562 (leukemia blood) cancer cells were chosen, while HEK293 (human embryonic kidney) cells served as the non-cancerous control (Amgoth *et al.*, 2016). The experiment was conducted in two sets: one containing control and blank wells, and the other containing [(AgNPs)-(PE)]. In each well of a 96-well plate, approximately  $4 \times 10^5$  well-cultured cells were seeded. The prepared [(AgNPs)-(PE)] aggregate was introduced into wells containing approximately  $4 \times 10^5$  cells/well at six different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, and 3.5  $\mu$ g/mL). The plates were then incubated for 24 hours at 37 °C in a CO<sub>2</sub> incubator. Following incubation, the MTT reagent was added, and upon dissolution in 100  $\mu$ L of dimethyl sulfoxide (DMSO), the formation of purple formazan

crystals was observed. After the 24-hour treatment, the 96-well plates were removed from the incubator, and the cell inhibition assay was analysed using a BioTek Synergy H4 Multimode Reader (Jang, 2016).

### 3. Result

#### 3.1. TLC analysis

Based on chromatographic separation, five major fractions were obtained and designated as P1, P2, P3, P4, and P5. The TLC profile of these fractions is shown in Figure 10, where bands 1–5 correspond to P1–P5, respectively. Among them, P5 exhibited the highest purity and yield. The TLC plates were visualized under a UV transilluminator at 260 nm.



**Figure 1** TLC picture of preparative HPLC purified fractions (illuminated with UV light)

#### 3.2. HPLC analysis

To further confirm its purity, fraction P5 was subjected to High-Performance Liquid Chromatography (HPLC) using a Shimadzu system. A 100  $\mu$ L sample was injected, and the analysis was performed over 30 minutes. The chromatogram revealed a major peak eluting at 15 minutes, along with a minor contaminant peak at 16.3 minutes, confirming P5 as the predominant purified compound.

#### 3.3. Anticancer Activity of Crebanine:

The isolated and structurally characterized phytochemical Crebanine was evaluated for its anticancer potential against selected cell lines, including DU145 (prostate cancer) and K562 (myeloid leukemia). In addition, its effect on HEK 293T (human embryonic kidney) cells was examined to determine cytotoxicity toward normal cells.

The results of these evaluations are summarized in Tables 1, 2, and 3, with corresponding graphical representation in Graph 1. Specifically, Table 6 presents the anticancer activity against DU145 cells, Table 7 shows cytotoxicity toward HEK 293T cells, and Table 1 highlights the inhibitory effect on K562 cells. The percentage of inhibition was calculated and plotted, with compound concentration on the X-axis and inhibition percentage on the Y-axis.

**Table 1** Anticancer activity investigations of crebanine extracted from *A. sclerocarpa* against the DU145 cell line

|                     |         | Concentration of the Crebanine |         |         |           |            |             |
|---------------------|---------|--------------------------------|---------|---------|-----------|------------|-------------|
| Parameter           | Control | 1nM                            | 10nM    | 100nM   | 1 $\mu$ M | 10 $\mu$ M | 100 $\mu$ M |
| Average % of Growth | 100     | 193.473                        | 150.616 | 150.326 | 152.574   | 138.071    | 97.751      |
| % of Inhibition     | 0       | -93.473                        | -50.616 | -50.326 | -52.574   | -38.071    | 2.248       |

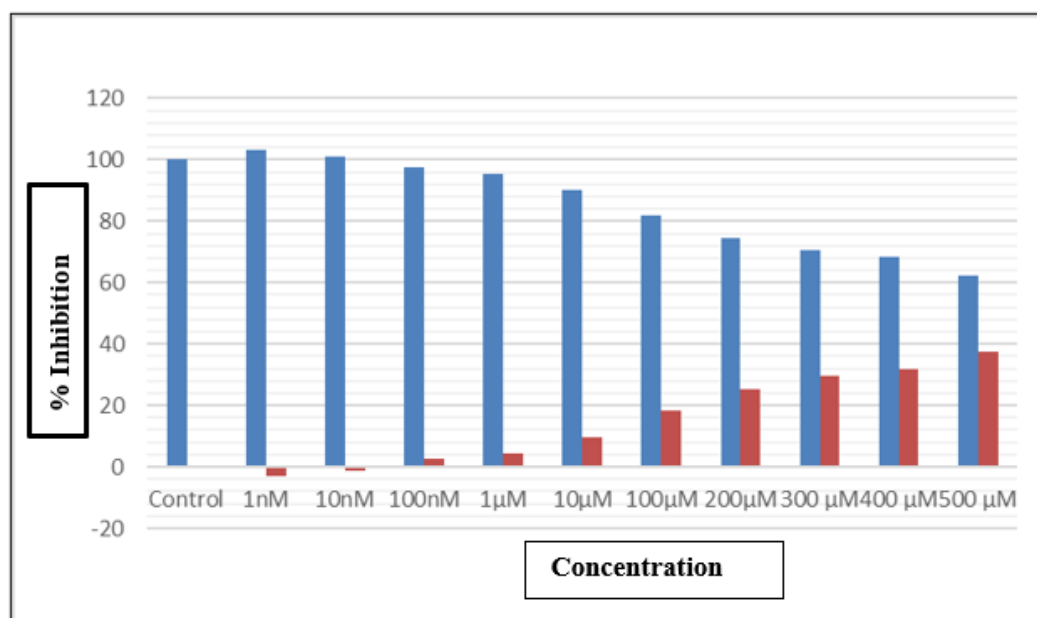
The cytotoxicity assay revealed that Crebanine exhibited a concentration-dependent inhibitory effect on K562 cells, with higher concentrations producing greater inhibition. In contrast, Crebanine showed no significant inhibitory effect on DU145 or HEK 293T cells. The  $IC_{50}$  value of Crebanine against K562 cells was determined to be 665  $\mu\text{g/mL}$ , indicating measurable but relatively weak anticancer activity in this leukemia model.

**Table 2** Cytotoxicity tests of Crebanine from *A. sclerocarpa* against the HEK cell line

| Parameter           | Control | Concentration of the Crebanine |         |         |                 |                  |                   |
|---------------------|---------|--------------------------------|---------|---------|-----------------|------------------|-------------------|
|                     |         | 1nM                            | 10nM    | 100nM   | 1 $\mu\text{M}$ | 10 $\mu\text{M}$ | 100 $\mu\text{M}$ |
| Average % of Growth | 100     | 142.323                        | 124.376 | 120.488 | 107.377         | 104.885          | 90.079            |
| % of Inhibition     | 0       | -42.323                        | -24.376 | -20.488 | -7.377          | -4.885           | 9.920             |

**Table 3** Anticancer activity of Crebanine extracted from *A. sclerocarpa* against the K562 cell line

| K562 cells (24h treated) | Control | 1nM     | 10nM    | 100nM  | 1 $\mu\text{M}$ | 10 $\mu\text{M}$ | 100 $\mu\text{M}$ | 200 $\mu\text{M}$ | 300 $\mu\text{M}$ | 400 $\mu\text{M}$ | 500 $\mu\text{M}$ |
|--------------------------|---------|---------|---------|--------|-----------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Average % of Growth      | 100     | 103.208 | 101.091 | 97.519 | 95.534          | 90.241           | 81.905            | 74.694            | 70.459            | 68.342            | 62.454            |
| % of Inhibition          | 0       | -3.208  | -1.091  | 2.480  | 4.465           | 9.758            | 18.094            | 25.305            | 29.540            | 31.657            | 37.545            |



**Graph 1** Crebanine anticancer efficacy against K562 cell lines

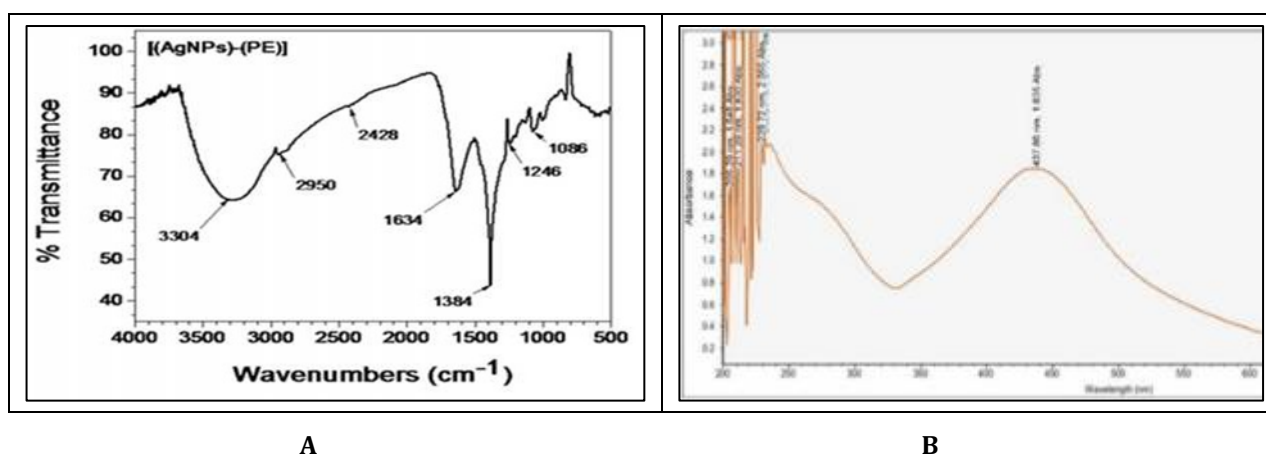
- **Silver Nanoparticles:** The biosynthesized silver nanoparticles were characterized through various analytical techniques. Spectroscopic methods such as UV-Visible and FT-IR spectroscopy were employed for conformational analysis, while SEM and TEM provided microscopic characterization. Additionally, XRD was used to examine the crystalline/amorphous nature of the AgNP aggregates, and EDAX analysis confirmed the elemental composition and concentration of the nanoparticles.
- **UV-Visible Spectroscopy of AgNPs:** UV-Visible spectrophotometric analysis confirmed the successful formation of silver nanoparticles. A broad absorption peak was observed at 437 nm, which is characteristic of AgNPs. Typically, silver nanoparticles exhibit a strong surface plasmon resonance (SPR) band between 400 and 500 nm (Sastry M. *et al.*, 1998). The UV-Visible spectrum is shown in Figure 17, where the wavelength is plotted along the X-axis and absorbance along the Y-axis.

The phytochemicals present in *A. sclerocarpa* extract facilitated the reduction of silver nitrate ( $\text{AgNO}_3$ ) into silver nanoparticles. Importantly, the nanoparticles were not separated from the plant extract, which simultaneously acted as both a reducing and stabilizing (capping) agent. Figure 18 illustrates the aggregated form of silver nanoparticles with the plant extract, represented as  $[(\text{AgNPs})-(\text{PE})]$ .

**FT-IR Analysis of  $[(\text{AgNPs})-(\text{PE})]$ :** The synthesized  $[(\text{AgNPs})-(\text{PE})]$  aggregates were further analyzed using Fourier Transform Infrared (FT-IR) spectroscopy to identify the functional groups involved in nanoparticle formation and stabilization. The obtained FT-IR spectrum (Figure 19) displays distinct peaks corresponding to various active functional moieties.

A broad peak observed at  $\sim 3304 \text{ cm}^{-1}$  ( $\pm 10$ ) is attributed to the amide ( $-\text{NH}$ ) stretching vibration of the primary aromatic amine (Sastry M. *et al.*, 1998). The absorption band at  $\sim 2950 \text{ cm}^{-1}$  ( $\pm 10$ ) corresponds to the symmetric stretching of  $-\text{CH}$  groups in methyl moieties with  $\text{sp}^3$  hybridization. Additionally, the peak around  $\sim 2428 \text{ cm}^{-1}$  ( $\pm 10$ ) indicates asymmetric stretching of  $-\text{CH}$  groups, while the band at  $\sim 1634 \text{ cm}^{-1}$  ( $\pm 10$ ) represents  $-\text{NH}$  stretching vibrations associated with methyl functional groups (Sastry *et al.*, 1997; Biswajit Dey *et al.*, 2013).

Further characteristic peaks at 1384, 1246, and  $1086 \text{ cm}^{-1}$  suggest the presence of phytochemical functional groups derived from the plant extract, which may play a role in the capping and stabilization of AgNPs. The FT-IR spectrum was plotted using Origin Pro software (version 8.0), with % transmittance on the Y-axis and wavenumber ( $\text{cm}^{-1}$ ) on the X-axis.

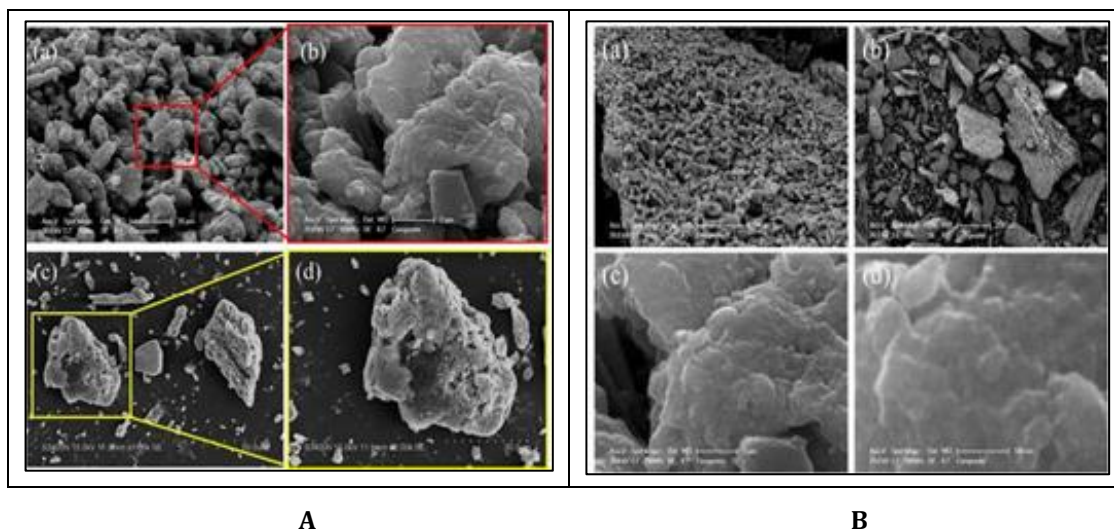


**Figure 2** A. The FT-IR spectra of  $[(\text{AgNPs})-(\text{PE})]$  B. UV-Visible spectroscopy of AgNPs

**Morphological Analysis of  $[(\text{AgNPs})-(\text{PE})]$ :** The morphology of the synthesized  $[(\text{AgNPs})-(\text{PE})]$  was examined using Scanning Electron Microscopy (SEM). Representative SEM micrographs are shown in Figures 3 and 4. Figure 20 (a–d) illustrates the surface morphology of  $[(\text{AgNPs})-(\text{PE})]$ , while Figure 21 provides a lower magnification image, confirming the irregular distribution of silver nanoparticles.

The SEM images reveal that the plant extract-assisted AgNPs tend to form irregular aggregates. This aggregation is attributed to the interaction of plant extract biomolecules encircling the silver nanoparticles, leading to clumping. Such aggregation is likely influenced by the inherent positive charge ( $\text{Ag}^+$ ) of the nanoparticles. In practical applications, sonication can be employed to reduce or prevent nanoparticle agglomeration.

To enhance conductivity during SEM imaging, the non-conductive AgNP–plant extract composites were sputter-coated with a thin conductive gold (Au) layer.

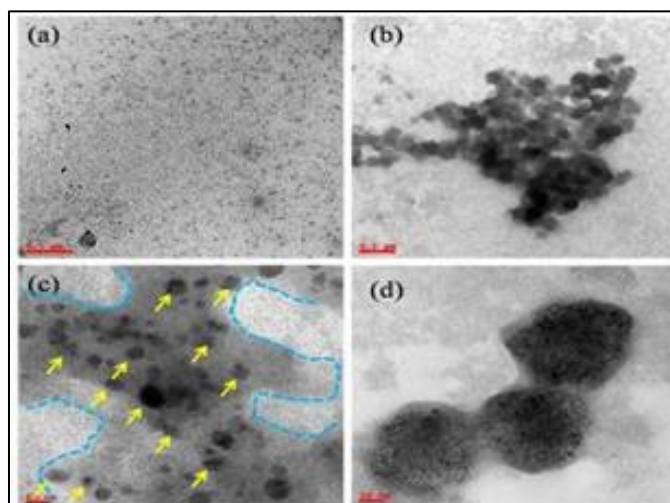


**Figure 3 A.** SEM pictures of AgNP aggregate covered with *A. sclerocarpa* extract [(AgNPs) (PE)]. At lower magnification, images (a, c) are obtained, while at higher magnification, images (b, d) are obtained

**Figure 3 B.** SEM pictures of plant extract from *A. sclerocarpa* embedded with AgNPs. A lower magnification was used to obtain images (a, c), whereas a higher magnification was used to acquire images (b, d).

**Transmission Electron Microscopy (TEM) Analysis of [(AgNPs)–(PE)]:** The morphology and size of the synthesized [(AgNPs)–(PE)] were further investigated using Transmission Electron Microscopy (TEM). TEM micrographs (Figure 5) confirm that the AgNPs are surrounded by *A. sclerocarpa* extract, resulting in aggregated nanoparticle formations.

Figures 5a and 5b (low magnification) depict the distribution of AgNPs coated with plant extract, showing nanoscale particles dispersed in the organic matrix. Particle size estimation, carried out using the free-hand polygon method, revealed that the PE-coated AgNPs ranged from ~20 to 40 nm in diameter, with an average size of approximately 33 nm. The nanoparticles exhibited irregular and uneven morphologies, with their size and shape largely influenced by the functional groups present in the phytochemicals of the extract.

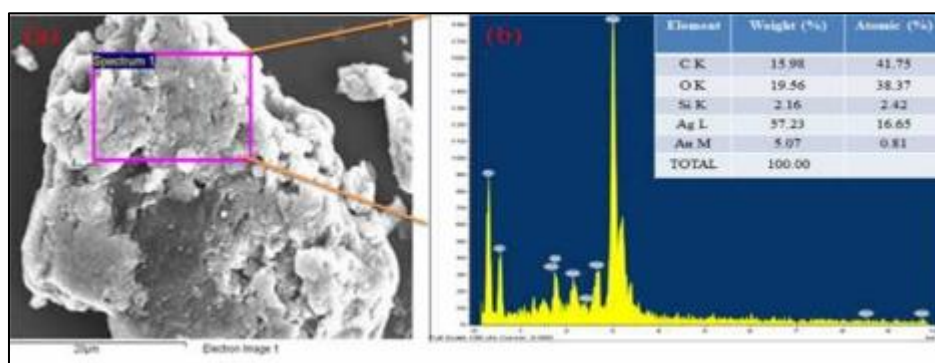


**Figure 4** TEM pictures of an AgNP aggregate coated with extract from *A. sclerocarpa* [(AgNPs)-(PE)]. Lower to higher magnifications are used to acquire the images from (a-d). The distribution of AgNPs coated with PE is depicted by yellow arrows, while the dispersion of *A. sclerocarpa* extract is shown by dotted blue lines

At higher magnifications (Figures 5c and 5d), distinct aggregates of AgNPs embedded in *A. sclerocarpa* extract are visible. Yellow arrows in Figure 5c highlight dispersed silver nanoparticles stabilized within the plant extract matrix.

To minimize charging and beam damage caused by the high accelerating voltage (200 kV) used during imaging, phosphotungstic acid (PTA) was applied to the samples. PTA provided a protective thin coating, thereby reducing the burn/charge effect commonly observed in extract-based nanostructures (Protima *et al.*, 2015).

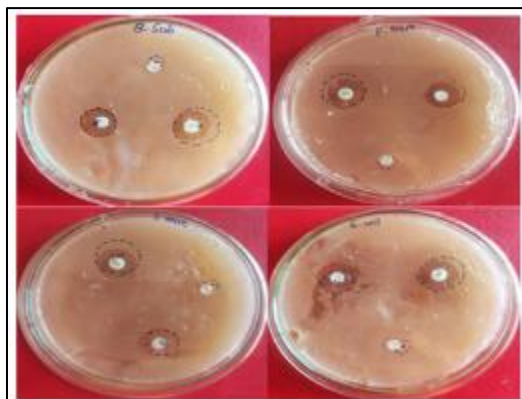
**EDAX analysis:** The elemental composition of the produced [(AgNPs)-(PE)] has also been examined using EDAX (Energy Dispersive X-ray Spectroscopy). The weight and atomic percentage of the elements in the synthesized [(AgNPs)-(PE)] aggregate are shown by the EDAX results in Figure 6 (a, b). While the principal peak is for source silver nanoparticles (AgNPs), secondary source elements C (carbon) and O (oxygen) are obtained from the plant extract. However, components like silicon (Si) and gold (Au) appear in the sample as a result of contaminants and impurities from sputter coating materials. The inset table in Figure 6 b shows that each element is present in the aggregate of [(AgNPs)-(PE)] that is formed. 16.65% atomic (%) and 57.23 weight (%) of Ag metal have been confirmed by the EDAX. The EDAX analysis demonstrated and validated the aggregated morphology of [(AgNPs)-(PE)] shown in TEM images (Figure 5).



**Figure 5** EDAX pictures of AgNP aggregates coated with extract from *A. sclerocarpa* [(AgNPs)-(PE)]. The presence of elements is confirmed by the inset table in Figure (b), which includes the elements' weight and atomic fraction

**Antibacterial Activity of AgNPs:** The synthesized silver nanoparticles were evaluated for their antibacterial potential in comparison to the crude extracts of *A. sclerocarpa*, which had already demonstrated strong inhibitory effects against bacterial strains. Antibacterial activity was assessed using both the broth serial dilution method and the agar well diffusion assay.

However, due to the limited diffusion and penetration of AgNPs into agar wells, the broth serial dilution method was considered more reliable. This approach allowed for the accurate determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, thereby validating the antibacterial efficacy of the synthesized AgNPs.



**Figure 6** The antibacterial (inhibition) activity of AgNPs-PE aggregates on a variety of bacterial species, including (a) *Streptococcus mutans*, (b) *Escherichia coli*, (c) *Pseudomonas aeruginosa*, and (d) *Staphylococcus aureus*

**Minimum Inhibitory Concentration (MIC):** The antibacterial efficacy of *A. sclerocarpa*-derived AgNPs was assessed by determining the Minimum Inhibitory Concentration (MIC) values against different bacterial strains. The MIC values

ranged from  $8.66 \pm 1.15$  to  $44.33 \pm 0.57$  mg/100  $\mu$ L, as summarized in Table 9. Among the tested organisms, *Streptococcus mutans* exhibited the lowest MIC value, indicating higher sensitivity, whereas *Pseudomonas aeruginosa* showed the highest MIC, reflecting greater resistance.

The observed variation in MIC values can be attributed to structural differences between the cell walls of Gram-positive and Gram-negative bacteria. The antibacterial action of AgNPs is primarily linked to the release of silver ions, which can penetrate bacterial cells and intercalate between purine and pyrimidine bases, leading to DNA denaturation (Ahmed *et al.*, 2016). Furthermore, AgNPs interfere with bacterial signalling by altering peptide substrates and inducing dephosphorylation of tyrosine residues in proteins. This disruption in phosphorylation, a key regulator of bacterial signal transduction, inhibits essential cellular processes and ultimately results in cell death (Shrivastava *et al.*, 2007; Nagababu *et al.*, 2017).

**Table 4** Minimum inhibitory concentration (MIC) (mg/100 $\mu$   $\mu$ L) values for antibacterial activity against several bacterial species treated with an aggregate of AgNPs coated with *A. sclerocarpa* extract

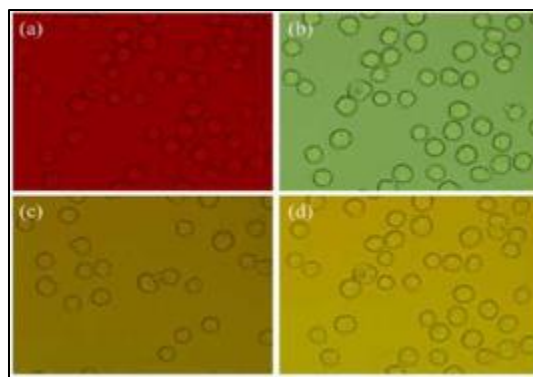
| Sl. No | Name of the Organism          | [(AgNPs)-(PE)]<br>(MIC Values) | Streptomycin<br>(MIC Values) |
|--------|-------------------------------|--------------------------------|------------------------------|
| 1      | <i>Escherichia coli</i>       | $30.0 \pm 01.73$               | $1.66 \pm 0.28$              |
| 2      | <i>Pseudomonas aeruginosa</i> | $44.33 \pm 0.57$               | $2.33 \pm 0.28$              |
| 3      | <i>Staphylococcus aureus</i>  | $14.66 \pm 1.15$               | $1.66 \pm 0.28$              |
| 4      | <i>Streptococcus mutans</i>   | $8.66 \pm 1.15$                | $1.66 \pm 0.28$              |

**Anticancer properties of AgNPs:** To evaluate the potential anticancer activity of *A. sclerocarpa*, the K562 cell line was cultured in the laboratory using standard procedures and methodologies, as described in the Materials and Methods section. The cancer cells were successfully cultured, and their growth was visualized using an optical microscope. Figure 8 (a-d) shows a picture of the cell.

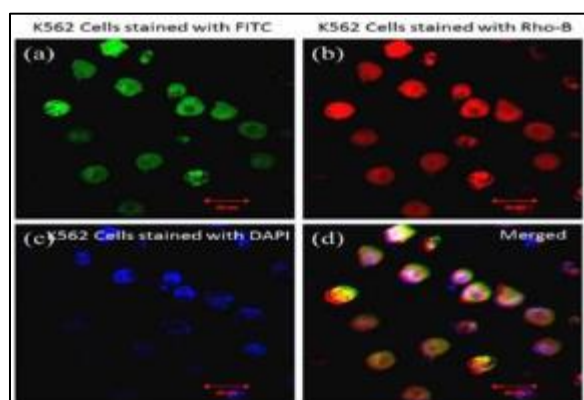
**Cell Inhibition Assay (MTT Assay):** The cytotoxic potential of [(AgNPs)-(PE)] aggregates was evaluated using the MTT assay. The bar graph (Graph 6) illustrates a dose-dependent increase in cell inhibition with rising concentrations of [(AgNPs)-(PE)]. More than 50% inhibition was observed at 1.0  $\mu$ g ( $IC_{50}$ ), while inhibition exceeded 75% at the highest tested concentration (3.5  $\mu$ g). Blank wells, containing no nanoparticles, served as negative controls and represented 0% inhibition.

Significantly, cytotoxicity was predominantly observed in malignant K562 cells, where inhibition was substantial, whereas normal HEK293 cells exhibited less than 10% inhibition, indicating low cytotoxicity toward healthy cells. These findings suggest that [(AgNPs)-(PE)] aggregates possess selective anticancer activity and may serve as promising therapeutic candidates.

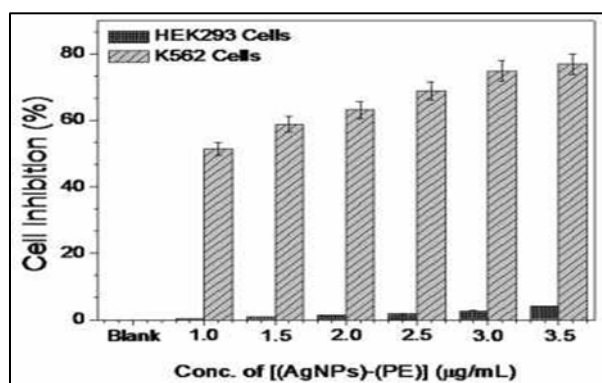
To further visualize the effects, K562 cells subjected to treatment were stained with various fluorescent dyes for morphological imaging. FITC (green) stained cellular components, Rhodamine-B (red) stained the cytoplasm, and DAPI (blue) targeted A-T-rich regions of nuclear DNA/RNA. Merged images revealed pronounced cellular damage, nuclear condensation, and morphological aberrations compared to untreated controls. These fluorescent observations corroborate the MTT assay results, confirming that [(AgNPs)-(PE)] induces cellular rupture and apoptosis in malignant cells.



**Figure 7** Optical microscope pictures depict the morphology of K562 (blood cancer cells from chronic myeloid leukemia) either as cultured or before therapy



**Figure 8** AgNPs coated with *A. sclerocarpa* extract [(AgNPs)-(PE)] were used to treat K562 cells for 24 hours. Images were obtained using laser scanning confocal microscopy



**Graph 2** Demonstrate the cell inhibition experiment using cell lines. Inhibition using AgNPs coated with *A. sclerocarpa* extract [(AgNPs)-(PE)]. Inhibition is stronger on malignant K562 cells, but negligible on non-cancerous HEK293 cells

#### 4. Discussion

The present investigation of the *A. sclerocarpa* plant extract was carried out using various analytical techniques, including TLC and HPLC. In Thin Layer Chromatography (TLC), which operates on the principle of adsorption, the components of the mixture are separated based on their affinity for the solid stationary phase. This analysis revealed five major fractions (P1, P2, P3, P4, and P5), among which the fractions exhibited the highest level of purity. High-Performance Liquid Chromatography (HPLC) was also employed to analyse the extract further. In HPLC, separation occurs due to the differential interactions of the mixture's components with the stationary phase inside the column, while the mobile phase is forced through under high pressure. This technique effectively identified the presence of bioactive compounds within the extract.

Silver nanoparticles were characterized using a range of techniques, including UV-Visible spectroscopy, FTIR, SEM, and TEM. UV-Visible spectroscopy confirmed the successful synthesis of silver nanoparticles. FTIR analysis was employed to identify the functional groups, showing characteristic peaks at 1384, 1246, and 1086  $\text{cm}^{-1}$ . SEM analysis revealed that the plant extract-assisted AgNPs formed irregular aggregates. TEM imaging further confirmed that the nanoparticles were surrounded by *A. sclerocarpa* extract, resulting in the formation of aggregated nanoparticles. Cell inhibition assay using the MTT method revealed that silver nanoparticles exhibited a strong cytotoxic effect against malignant K562 cells, showing significant growth inhibition. In contrast, normal HEK293 cells demonstrated less than 10% inhibition, suggesting minimal toxicity toward healthy cells.

## 5. Conclusion

In this study, crude extracts of *A. sclerocarpa* were prepared through Soxhlet extraction using a range of solvents from nonpolar to polar, and the extracts demonstrated notable antimicrobial, antibacterial, and antioxidant properties. Considering the urgent need for novel therapeutic agents to counter multidrug-resistant pathogens, the research further investigated the ability of *A. sclerocarpa* to synthesize silver nanoparticles (AgNPs) through the reduction of silver nitrate.

The structural and functional properties of the synthesized AgNPs were analysed using multiple microscopic and spectroscopic techniques. An aqueous extract of *A. sclerocarpa* leaves was employed for nanoparticle biosynthesis, and UV-visible spectroscopy confirmed their formation by displaying a distinct absorption peak at 437 nm, indicative of surface plasmon resonance. Morphological studies using SEM and TEM showed that the AgNPs were highly aggregated and measured approximately 33 nm in size. Elemental composition was validated by EDX, which confirmed a silver content of 16.65%. FT-IR analysis revealed strong absorption at 3304  $\text{cm}^{-1}$ , corresponding to OH and NH stretching vibrations, indicating that phytochemicals from the extract were attached to the nanoparticle surface, serving as capping and stabilizing agents.

In addition to structural characterization, the biological activity of the biosynthesized AgNPs was evaluated. Cytotoxicity assays revealed that the synthesized [(AgNPs)-(PE)] aggregates were biocompatible and non-toxic to normal HEK293 (human embryonic kidney) cells. In contrast, they exhibited significant anticancer effects against K562 leukemia cells, showing more than 75% inhibition after 24 hours of exposure. These findings suggest that *A. sclerocarpa*-mediated AgNPs hold strong promise as a natural, biocompatible agent with both antimicrobial and anticancer potential.

## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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