Novel Green Synthesis of Silver Nanoparticles Using *Scolopia crenata* Stem Bark Extract: An Endemic Medicinal Plant of India for Biomedical Applications.

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A B S T R A C T

The green synthesis of nanoparticles has attracted significant interest owing to its eco-friendly and sustainable nature. This study utilized the stem bark extract from *Scolopia crenata* as a reducing and stabilizing agent to synthesize silver nanoparticles (AgNPs). The synthesized AgNPs were characterized by UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray spectroscopy (EDX), X-ray diffraction (XRD), zeta potential analysis, and Particle Size Analysis (PSA). The AgNPs exhibited a spherical shape and were well dispersed, with average sizes ranging from 66 to 100 nm. Biological evaluations revealed that AgNPs derived from *S. crenata* demonstrated superior antioxidant, anti-inflammatory, and anticancer activities compared to both the crude extracts and $AgNO_3$. This suggests that biomolecules surrounding the nanoparticles may enhance their biological efficacy. Our findings highlight the potential therapeutic applications of biogenic AgNPs isolated from *S. crenata* for therapeutic applications. Further research is warranted to isolate and identify specific biomolecules that contribute to the enhanced biological activity of these nanoparticles. Understanding the underlying mechanisms could pave the way for developing more effective and targeted pharmacological interventions.

Highlights

- Green synthesis of AgNPs from *Scolopia crenata* aqueous stem bark extracts.
- Characterization of AgNPs was done by UV-visible spectroscopy, SEM, EDX, FTIR, XRD, ZETA, and PSA
- The nanoparticles were stable in charge, distinct, and spherical in shape, with an average size of 69±2 nm in the colloidal form.
- AgNPs display potential antioxidant and anti-inflammatory activities, with a broad range of anticancer activities.

INTRODUCTION

Nanotechnology is experiencing rapid growth, with wide-ranging applications in electronics, biotechnology, and medicine [1]. Scientists can create innovative materials with distinctive characteristics by manipulating matter at the atomic scale, particularly when particles are less than 100 nm in size [2]. Nanoparticles (NPs) are especially significant in this field, demonstrating enhanced capabilities that are not found in their larger counterparts [3]. NPs can be categorized as organic or inorganic, with the latter group, including magnetic NPs, noble metals (such silver and gold), and semiconductors (such as titanium dioxide and zinc oxide), attracting considerable interest owing to their exceptional qualities and versatility, especially in medical imaging and targeted treatments [4, 5]. The dimensions, form, and surface properties of nanoparticles are crucial for determining their chemical, physical, and biological behaviors [6]. These attributes are often influenced by the synthesis methods employed, which can be broadly categorized as physical, chemical, biological, or hybrid [7]. Conventional synthesis techniques frequently utilize organic solvents and potentially harmful chemicals, raising environmental and safety concerns [8]. In contrast, green nanotechnology promotes sustainable practices by using natural materials such as plant extracts and microorganisms as reducing and capping agents in nanoparticle production [9]. This eco-friendly method has proven effective in synthesizing various metallic nanoparticles, including silver, gold, and zinc, offering advantages such as scalability, safety, and reduced environmental impact [10]. Silver nanoparticles (AgNPs) are particularly noteworthy for their biomedical applications, because of their antibacterial, antiviral, and anticancer properties [11]. Green synthesis of AgNPs is gaining popularity because of its reduced toxicity and the effectiveness ofvarious plant extracts in this process [12]. These extracts contain secondary metabolites that act as reducing and stabilizing agents, facilitating the production of well-defined nanoparticles while enhancing their biological activities [13]. As the quest for natural substances continues, it is essential to explore new treatment options that minimize side effects associated with synthetic chemicals [14, 15]. One promising candidate is *Scolopia crenata* (Wight &Arn) Clos, a native species in India belonging to the Flacourtiaceae family [16]. This evergreen tree, found
in the tropical forests of Andhra Pradesh, Karnataka, Tamil Nadu and Kerala, has traditionally been used for its therapeutic benefits, particularly in managing musculoskeletal pain. Despite its ethnomedicinal importance, research into the phytochemical composition and biological activities of *S. crenata* remains limited [16]. Various parts of the tree are used in folk medicine to treat diverse ailments, suggesting its potential for further investigation [17].

METHODS

Collection of plant material

In March 2023, plant samples were collected from the vicinity of the Rainforest Office in the Gini-Gini Honnavalli Post, Sringeri Rural, located within the Balehonnuru Range of Sringeri Taluk, Chikkamagaluru District, Karnataka, India (577112). Adithya Rao G.S., a Deputy Range Forest Officer employed by the Government of Karnataka, conducted the plant identification. A specimen for herbarium purposes was placed in the Botany Department at the Sahyadri Science College (SSC/DB/2022/0049) in Shivamogga, Karnataka, India.

Preparation of plant extract

The extraction of 25 g of pulverized stem bark was carried out using a Soxhlet apparatus for 48 h, 250 mL of distilled water. Subsequently, the obtained aqueous extract was concentrated by rotary evaporation, followed by desiccator drying. The resulting product was stored in an airtight container at 4°C until further use. This water-based extract served a dual purpose in the production of silver nanoparticles (AgNPs), acting as both a reducing agent and a stabilizer.

Solvents and Reagents

All solvents and chemicals used in this study were of analytical grade and were sourced from Hi-media, India.

Phytochemical screening

Phytochemical screening was performed using various chemical tests as outlined in the literature [18–20].

Synthesis of silver nanoparticles.

An aqueous plant extract (1 mL) was added to a 10 mL solution of 10 mM $AgNO^3$. The reaction was carried out in dark at room temperature to prevent undesired photochemical effects. Following incubation, a significant color shift was observed, with the solution changing from clear to dark brown, suggesting the formation of nanoparticles. Subsequently, the nanoparticle-containing mixture was subjected to centrifugate at 10,000 rpm for 10 min. This process of centrifugation and resuspension in double-distilled water (DDW) was performed multiple times to eliminate contaminants. The nanoparticles were then dehydrated to, yield a powder form [21, 22].

Structural characterization of biosynthesized silver nanoparticles

Various analytical methods were used to verify the physicochemical characteristics of the AgNPs. A Shimadzu UV-1800 spectrophotometer was employed to record UV–vis absorption spectra across a wavelength range of 200–800 nm, using quartz cuvettes [23]. To identify the potential functional groups, Fourier-transform infrared (FTIR) spectroscopy was conducted on a Thermo Nicolet 6700 device, covering a range of 200–4000 cm^{-1} [24]. The crystalline nature of AgNPs was evaluated using X-ray diffraction (XRD) with a Shimadzu XRD-7000 model [25]. A JEOL JEM 2100 operating at 90 kV was used for scanning electron microscopy (SEM) to examine the surface morphology and particle size, complemented by high-resolution SEM analysis [26]. Energy-dispersive X-ray (EDX) analysis, performed with a JEOL EDX model JSM-5610 LV, confirmed the presence of Ag and other elements [27]. The surface charge of the synthesized AgNPs was determined using a zeta potential analyzer (HORIBA Nanoparticle Analyzer SZ100) [28].

Evaluation of *in vitro* antioxidant activity

DPPH free-radical-scavenging assay

The free radical scavenging activity of *S. crenata* stem bark extract and synthesized AgNPs was evaluated using the DPPH radical as a reagent [29]. Ascorbic acid was used as the standard for comparison. The DPPH scavenging activity of each sample was calculated using following equation

% Antioxidant activity = [(Ac − As)/Ac] × 100---(1)

Where, Ac and As are the absorbance values of the control and sample, respectively.

Hydrogen peroxide scavenging assay

The ability of *S. crenata* aqueous stem bark extract and synthesized AgNPs to scavenge hydrogen peroxide was evaluated [30]. A control was prepared using phosphate buffer without sample or standard. Each sample was tested in triplicate. The percentage inhibition was calculated using the appropriate formula.

$$\% \, Scavenged \, [H2O2] = [\frac{[(Ac - As)]}{Ac}] \times 100 \text{ ---(2)}$$

Where, Ac and As are the absorbance values of the control and sample, respectively.

Evaluation of *in vitro* anti-inflammatory activity

Anti-inflammatory effects of *S. crenata* aqueousstem bark extract and silver nanoparticles (AgNPs)were evaluated using the protein denaturation method, with slight modifications to previously described protocols. Aspirin was used as the standard pharmaceutical agent [19, 31]. After cooling, absorbance was measured at 660 nm, using double-distilled water as a reference solution. Each experiment was conducted in triplicate, and the percentage inhibition of protein denaturation was calculated using the formula.

% Protein denaturation activity = [(Ac − As)/Ac] × 100---(3)

Where Ac is the absorbance of the control and As is the absorbance in the presence of the extracts or standards sample.

Evaluation of Anticancer Activity of Silver Nanoparticles Using MTT Assay

The standard MTT assay was used to assess the impact of *S. crenata* and its synthesized AgNPs on the survival of non-cancerous fibroblast cells (L292) and their cancer-fighting potential against lung cancer (A549) and breast cancer (MDA-MB-231) cell lines. All cell lines were obtained from the National Center for Cell Science (NCCS) located in Pune, India. Dose-response curves were created for each cell line to calculate the percentage inhibition of cell growth (IC50 values) using the appropriate formula for determining growth inhibition percentage. Metabolically active cells reduce the yellow tetrazolium compound MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), primarily through the action of dehydrogenase enzymes, resulting in the production of reducing equivalents such as NADH and NADPH [32].

% Inhibition = OD of Test sample ÷ OD of control × 100---(4)

Where, Abs sample is the absorbance of a test sample and Abs control is the absorbance of the control reaction (including all reagents except the test sample). All the experiments were carried out in triplicates.

Statistical Analysis

All experiments were carried out in triplicates (n = 3) and were statistically assessed and are presented as mean ± SE.

RESULTS AND DISUSSION

Nanoparticles (NPs), defined by their size of less than 100 nm, are crucial elements in nanotechnology [33]. A key characteristic of NPs is their heightened biological and chemical reactivity [34]. Multiple methods, including physical, chemical, biological, and hybrid approaches, have been used to create various NP types. Green synthesis techniques have become popular for producing metal nanoparticles like silver, gold, iron, copper, and zinc [35]. Plant extract-based phytochemical synthesis has gained traction because of its eco-friendly nature, offering a safer method to create metal nanoparticles with desired structural features [36]. The high oxidizing capacity of phenolic compounds, particularly in alkaline environments, aids in initiating nucleation, facilitating the conversion of metal ions into nanoparticles [37]. In certain instances, oxidized phenolics attach to metal nanoparticle surfaces, thereby enhancing their stability. Natural phenolic substances, with their hydroxyl and carboxyl groups, exhibit proton-donating and adsorptive qualities, that are thought to be essential for nanoparticle formation. This research aimed to create silver nanoparticles (AgNPs) using *Scolopia crenata* stem bark aqueous extract for potential biomedical uses [38]. Initial phytochemical screening of plant extracts involves the detection of secondary metabolites. Plant-based systems have been selected as bio-reductants and stabilizers because of their bioactive compound content [39]. Various phytochemicals, such as alkaloids, flavonoids, tannins, phenols, and saponins, are known to act as reducing agents, enabling the biosynthesis of metal nanoparticles with diverse biological properties [40]. Phenolic compounds, recognized for their non-enzymatic nature and significant antioxidant activity, have recently attracted attention [41]. While phenolics are broadly linked to antioxidantproperties, specific groups such as flavonoids and tannins have been extensively researched for their biological effects [42].

In this study, phytochemical analysis of the *Scolopia crenata* stem bark aqueous extract revealed the presence of flavonoids, phenolics, terpenoids, tannins, and saponins [43]. These bioactive substances have been widely reported to possess anti-inflammatory, antibacterial, antioxidant, and anticancer properties in both traditional medicine and pharmaceutical studies [44].

Tests	Aqueous extract (AE)
Alkaloids	+ve
Flavonoids	+ve
Glycosides	-ve
Phenols	+ve
Saponins	+ve
Tannins	+ve
Terpenoids	+ve
Steroids	-ve

+ ve: presence; - ve: absence

Table 1: Phytochemical analysis of aqueous extracts of *S. crenata.*

Nanotechnology encompasses a broad range of scientific fields, including engineering, physics, biology, and medicine. Although the production of nanoparticles (NPs) from biological sources such as fungi and bacteria are effective, it can be costly and time-intensive. In comparison, the phytosynthesis of NPs using plant-based systems offers reduced health risks and notable benefits [45]. One major advantage of plant-mediated synthesis is its simplicity and cost-efficiency, as it eliminates the need for complex and expensive processes associated with microbial-based NP production. Recent research has emphasized the potential of cancer-fighting phytochemicals extracted from medicinal plants as a promising resource for creating biocompatible NPs, that are minimally harmful to health and can be manufactured on a large scale in a short period [46].

In this study, introducing aqueous stem bark extract of *Scolopia crenata* to the reaction mixture led to a significant color shift from white to brown after 6 hours, signaling the conversion of silver ions (Ag^+) from $AgNO_3$ into silver nanoparticles (AgNPs). The reaction was completed within an hour, as indicated by the emergence of a brown hue (Figure 1). UV-Vis spectral analysis confirmed the formation of AgNPs by measuring the colloidal solution across a wavelength range of 100-700 nm. The analysis revealed a peak absorption at 350 nm, further substantiating the successful synthesis of AgNPs (Figure 2).

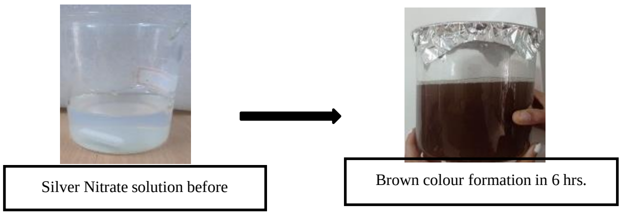

Figure 1. Synthesis of AgNPs using aqueous stem bark extract of *Scolopia crenata* with 10 mM $AgNO_3$.

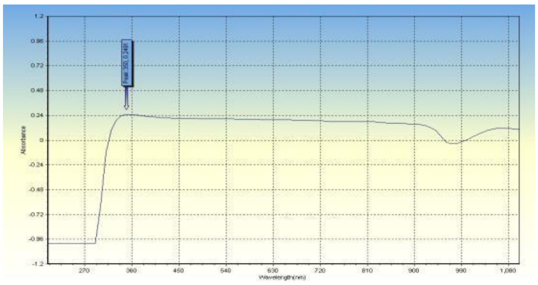

Figure 2. UV-VIS spectral spectroscopy of silver nanoparticles

Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDX) spectroscopy was employed to examine the structural and elemental characteristics of the AgNPs produced. The nanoparticles were determined to have dimensions of 69-71 nm. SEM analysis, used to evaluate the surface morphology of AgNPs revealed distinct spherical particles with a mean size of 69 ± 2 nm (Figure 3A). The elemental makeup of the AgNPs was verified through EDX analysis, which exhibited a notable signal in the metallic silver region between 2-4 KeV, indicating successful nanoparticle formation using the aqueous stem bark extract of *Scolopia crenata*. Additional elements, such as carbon, oxygen, and sodium, were also detected in the EDX spectrum, with silver being the primary component, constituting 40.43% of the total composition (Figure 3B).

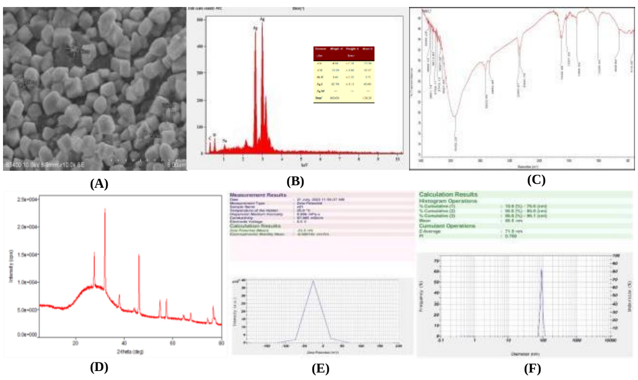

Figure 3: (A) SEM images illustrating the size and shape of AgNPs. (B) EDX analysis displaying the elemental composition of AgNPs. (C) FTIR spectra showing the characteristic peaks of AgNPs synthesized from *Scolopia crenata*. (D) XRD analysis of *S. crenata* AgNPs. (E) Zeta potential distribution of *S. crenata* AgNPs. (F) Particle size analysis of *S. crenata* AgNPs.

The functional groups responsible for converting Ag^+ ions to Ag^0 nanoparticles were identified by Fourier Transform Infrared (FTIR) spectroscopy. Examination of the infrared spectra for both the plant extract and produced AgNPs indicated the existence of diverse functional groups, such as alkanes, nitro compounds, aromatics, alkyl halides, aliphatic amines, nitrogen compounds, ethers, carboxylic acids, alkynes,alcohols, esters, and primary and secondary amines (Figure 3C). The FTIR analysis of the AgNPs exhibited notable peaks at 3890.23 cm^{-1}, 3868.10 cm^{-1}, 3851.73 cm^{-1}, 3813.85 cm^{-1}, 3768.17 cm^{-1}, 3743.13 cm^{-1}, 3687.06 cm^{-1}, 3647.85 cm^{-1}, 3432.25 cm^{-1}, 2922.86 cm^{-1}, 2854.95 cm^{-1}, 2380.67 cm^{-1}, 2340.75 cm^{-1}, 1634.28 cm^{-1}, 1557.33 cm^{-1}, 1384.52 cm^{-1}, 1020.60 cm^{-1}, 668.60 cm^{-1}, and 419.45 cm^{-1}. These functional groups are believed to play a role in the reduction, capping, and stabilization processes of the nanoparticles, thus enabling the biosynthesis of AgNPs from the plant extract.

X-ray diffraction (XRD) analysis revealed information regarding the spatial arrangement and atomic spacing within the nanoparticles. The findings validated that the crystal planes showed peaks typical of monoclinic and cubic nanoparticle formations, as demonstrated by the XRD patterns. Figure 3 displays four distinct diffraction peaks associated with silver at 2θ values of 27.70°, 32.16°, 38.02°, 44.24°, 46.13°, 54.71°, 57.35°, 64.35°, and 67.33°. These angles indicate the crystalline structure of the produced AgNPs (Figure 3, D). Furthermore, Zeta potential analysis was conducted to assess the AgNPs' stability, yielding a high negative Zeta potential of -23.5 mV, which suggests robust stability (Figure 3, E). The distribution of particle sizes was determined, with a mean size of 71 nm (Figure 3, F), which is notable for potential therapeutic uses.

The widespread adoption of nanoparticles across various industries can be attributed to their distinctive qualities, such as expansive surface area, minimal toxicity, and simple separation processes. Silver nanoparticles (AgNPs) with dimensions less than 20 nm, including maghemite and magnetite, exhibit specific attributes that render them particularly suitable for biomedical applications. These applications include the separation of cell populations, purification processes, diagnostic magnetic resonance imaging (MRI), precise drug delivery, and cell biology research [12, 47].

Highly reactive and unstable molecules known as reactive oxygen species (ROS) contain unpaired electrons that produce free radicals. These free radicals are associated with various human ailments, including atherosclerosis, cancer, neurodegenerative conditions, and impaired physiological functions. ROS-induced oxidative stress plays a crucial role in degenerative aging and has been connected to the pathophysiology of cardiovascular, oncological, neurological, and respiratory disorders [48, 49].

This study evaluated the in vitro antioxidant properties of aqueous stem bark extract from *Scolopia crenata* and its synthesized AgNPs using DPPH and H_2O_2 assays. The DPPH assay, commonly employed to evaluate the antioxidant activity of plant-derived extracts and phytochemicals, measures the reduction of the methanolic solution of colored free radical DPPH by free radical scavengers. The absorbance at 517 nm correlates directly with the concentration of the free radical scavenger, and a decrease in absorbance indicates the reducing potential of the test extract [50, 51].

The antioxidant efficacy of the synthesized AgNPs was evaluated against that of a standard antioxidant. Although the AgNPs showed lower antioxidant activity than, the aqueous stem bark extract of*Scolopia crenata* demonstrated higher inhibition, similar to that of standard Vitamin C (Figure 4).

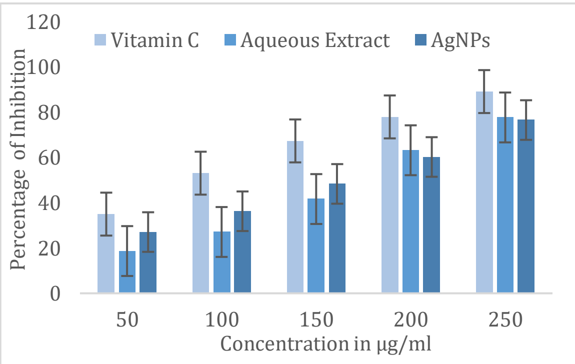

Figure 4. DPPH assay of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles.

Hydrogen peroxide (H_2O_2) assay is a widely used technique for assessing the antioxidant potential of plant-derived extracts. Although H_2O_2 is relatively stable, it can produce highly reactive hydroxyl radicals that inflict cellular harm and toxicity. Various antioxidants, including phenols, polyphenols, and flavonoids, can neutralize H_2O_2, thus safeguarding mammalian cells from oxidative stress caused by hydrogen peroxide [52].

In this study evaluated the H_2O_2 scavenging capabilities of aqueous stem bark extract from *Scolopia crenata* and its synthesized silver nanoparticles (AgNPs) were evaluated using the H_2O_2 assay, with ascorbic acid as the reference standard. The findings revealed that the AgNPs demonstrated greater scavenging activity than the plant extract, achieving an inhibition percentage of 84.55 ± 0.14%. The plant extract exhibited a marginally lower inhibition percentage of 82.09 ± 0.14%, while ascorbic acid showed the highest antioxidant activity with an inhibition percentage of 92.89 ± 0.06% (Figure 5). These results underscore the robust antioxidant properties of both AgNPs and the plant extract, with the synthesized nanoparticles displaying enhanced activity compared to the plant extract, although both were surpassed by the standard ascorbic acid.

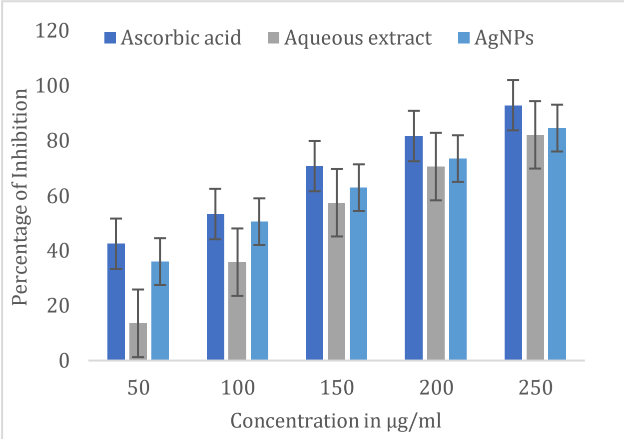

Figure 5. H_2O_2 assay of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles.

Inflammation is the body's immune response to harmful stimuli whereas infection refers to pathogen invasion. Inflammation, typically a localized reaction, is the body's defense mechanism against injury, illness, or stress. Although steroidal and nonsteroidal anti-inflammatory medications are commonly used to treat inflammation, they can cause undesirable side effects. To address these issues, researchers have developed nano-based herbal formulations as alternative anti-inflammatory agents. Multiple studies have shown that metallic nanoparticles (NPs) synthesized from plant extracts possess anti-inflammatory properties [53]. In this study, we conducted an *in vitro* protein denaturation assay to assess the anti-inflammatory effects of the aqueous stem bark extract of *Scolopia crenata* and the synthesized AgNPs. Known concentrations of the samples, were tested using aspirin as the standard reference drug. The results revealed a significant variation in protein denaturation among the groups. AgNPs exhibited strong anti-inflammatory activity, with an inhibition percentage of 78.84%, compared to 67.65% for the aqueous stem bark extract of *Scolopia crenata* and 89.64% for aspirin. These results indicate that the synthesized AgNPs have potent anti-inflammatory potential, similar to conventional anti-inflammatory drugs.

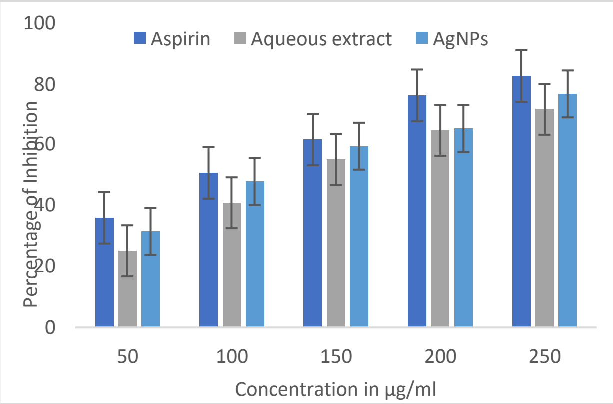

Figure 6. *In vitro* anti-inflammatory effect of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles.

MTT cytotoxicity assessment of the aqueous stem bark extract from *Scolopia crenata* and synthesized silver nanoparticles (AgNPs) using the MTT cell viability assay on non-cancerous L929 fibroblast cells. This assay evaluates compound toxicity by measuring its impact on cell viability. The study's findings demonstrated a concentration-dependent cytotoxic effect in both tested samples. The *S. crenata* aqueous stem bark extract showed a more pronounced decrease in cell viability than AgNPs. When exposed to a 250-µg concentration, cells treated with the plant extract exhibited a viability of 45.94 ± 0.23%, while those treated with AgNPs showed a viability of 34.96 ± 0.18% (Table 2, Figures 7 and 8). These results indicate that both the plant extract and AgNPs displayed cytotoxic properties, with the former demonstrating higher toxicity at the concentrations tested.

Table 2. Cytotoxicity studies of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles against L929 cell line.

Sample	Concentration in µg	Percentage of Cell Viability µg/mL
Aqueous stem bark extract of *S. crenata*	50	92.11±0.45
	100	87.52±0.43
	150	75.90±0.37
	200	62.36±0.31
	250	45.94±0.23
AgNPs	50	84.64±0.41
	100	72.28±0.36
	150	58.31±0.29
	200	46.90±0.24
	250	34.96±0.18

Results are expressed as mean ± SD (*n* =3)

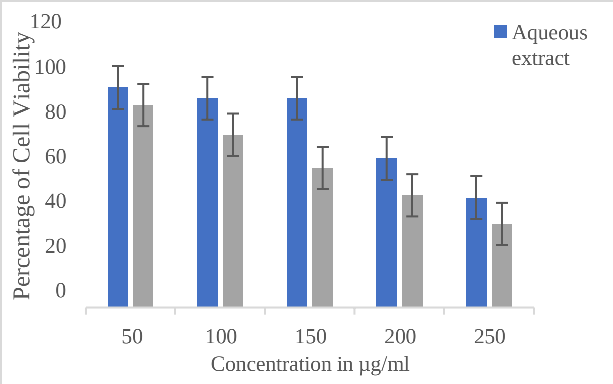

Figure 7. Cytotoxicity studies of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles against L929 cell line.

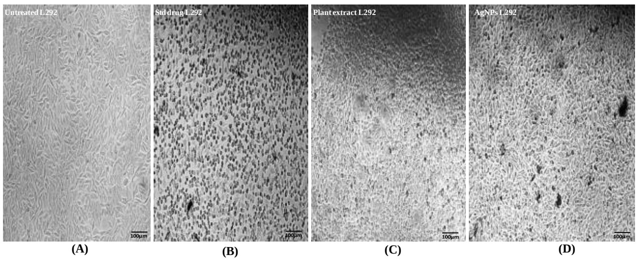

Figure 8. Morphological study of the effect of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles against L929 cell line. A: Untreated L929, B: Standard drug treated L929, C: Plant extract treated L929, D: AgNPs treated L929.

The survival and multiplication of cancer cells are characterized by disruptions in cell cycle control, with various signaling pathways being altered during the development of cancer. The suppression of natural cell death contributes to cancer progression and resistance to traditional treatments such as radiation and chemotherapy [54]. Cancer is a non-communicable disease, characterized by rapid and uncontrolled cell division. The high expenses associated with cancer care are often linked to difficulties in early detection and the intricacies of treatment, referred to as cancer theragnostic [55].

Lung, breast, and colorectal cancers, are among the most frequently diagnosed with lung cancer being particularly aggressive and lethal. Cancer treatment typically involves surgery, chemotherapy, radiation therapy, immunotherapy, and hormonal therapy [56]. However, these conventional treatments often result in severe side effects, ranging from mild discomfort to significant systemic toxicity. Oral cancer, the most prevalent form of head and neck cancer, commonly affects individuals ≥60 years of age. It is ranked among the top ten cancers in terms of occurrence, with risk factors including tobacco use, excessive alcohol consumption, and human papillomavirus (HPV) infection [57]. Despite advancements in research and treatment, survival rates for oral cancer have not significantly improved. Symptoms include persistent sores, lumps, or red and white patches in the mouth. The treatment options include surgery, chemotherapy, and radiation therapy [58].

Lung cancer is highly aggressive form of cancer that results in substantial mortality in both males and females. It is the third most deadly cancer globally, with common symptoms including weight loss, fatigue, hemoptysis (coughing up blood), and dysphagia (difficulty swallowing) [59]. The treatment approaches for lung cancer include chemotherapy, radiation therapy, laser therapy, immunotherapy, and surgery. However, these methods frequently cause significant side effects, such as nerve damage, toxicity, hair loss, and fatigue, as well as gastrointestinal issues such as diarrhoea and mouth sores [60]. Consequently, researchers are exploring novel therapeutic compounds to improve treatment outcomes. In this study, the aqueous stem bark extract of *Scolopia crenata* and its synthesized AgNPs were evaluated for their cytotoxic effects on lung cancer (A549) and breast cancer (MDA-MB-231) cell lines. The results showed that AgNPs exhibited significant cytotoxic activity, leading to a high percentage of cell death and reduced cell viability. Among the tested cancer cell lines, AgNPs demonstrated the greatest efficacy on breast cancer, followed by lung cancer. In contrast, the MTT cell viability assay results for the plant extracts showed moderate cytotoxic activity compared to AgNPs (Table 3, Figures 9 and 10). These findings indicate that the synthesized AgNPs show considerable promise as anticancer agents, particularly for targeting breast and lung cancer.

Table 3. Percentage of cell viability for the aqueous stem bark extract of *Scolopia crenata* and its synthesized silver nanoparticles against various cancer cell lines.

Samples	Concentration in µg	Percentage of Cell Viability for A549 µg/mL	Percentage of Cell Viability for MDAMB-231 µg/mL
Aqueous stem bark extract of *S. crenata*	50	78.93±0.25	71.13±0.27
	100	59.41±0.19	59.53±0.23
	150	48.11±0.16	42.54±0.17
	200	35.95±0.12	30.8±0.12
	250	25.68±0.09	18.64±0.08
AgNPs	50	71.78±0.23	55.69±0.22
	100	60.89±0.20	45.81±0.18
	150	47.85±0.16	34.29±0.14
	200	34.32±0.12	27.98±0.12
	250	20.62±0.08	17.55±0.08

Results are expressed as mean ± SE (*n* =3)

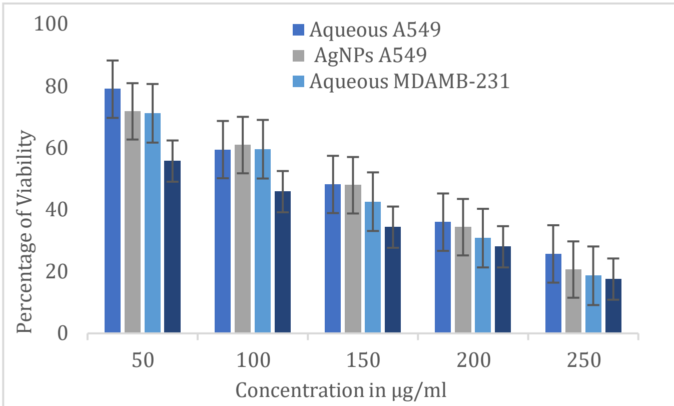

Figure 9. Percentage of cell viability for aqueous stem bark extract of *S. crenata* and its synthesized iron nanoparticles against different cancer cell lines.

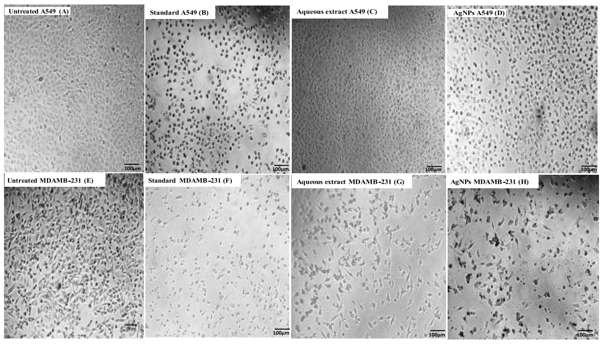

Figure 10. Morphological study of the effect of aqueous stem bark extract of *S. crenata* and its synthesized silver nanoparticles against A549 and MDAMB-231 cell line. A: Untreated A549, B: Standard drug treated A549, C: Aqueous extract treated A549, D: AgNPs treated A549, E: Untreated MDAMB-231, F: Standard drug treated MDAMB-231, G: Aqueous treated MDAMB-231, H: AgNPs treated MDAMB-231 cell line.

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