International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2025; 7(2): 201-208
www.pharmacyjournal.org
Received: 12-06-2025
Accepted: 15-07-2025

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Biogenic synthesis of *Cleoma viscosa* ethanolic extract Silver Nanoparticles and its Antioxidant and *In vitro* anticancer activity

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DOI: https://doi.org/10.33545/26647222.2025.v7.i2c.211

Abstract
Biogeneic-derived silver nanoparticles offer eco-friendly synthesis with promising antioxidant and anticancer therapeutic applications. *Cleome viscosa*, was collected from Coimbatore and identified by distinct features like small yellow flowers and glandular hairy stems. 2.5 kg of leaves and roots were harvested from multiple sites to capture phytochemical variation. The plant material was washed, shade dried, and ground to powder. Soxhlet extraction with ethanol was conducted for 48–72 hours. Phytochemical profiling via LC-MS analysis. Silver nanoparticles were synthesized by mixing 1 mM silver nitrate with extract in a 1:5 ratio, confirmed by color change due to surface plasmon resonance. CvAgNPs Antioxidant activity studied by using DPPH assay. MTT assays and AO/EB analysis done in SK-MEL-3 cells, respectively. The collected Cleome viscosa (2.5 kg) was shade dried, powdered, and extracted with ethanol for 48–72 h, yielding crude extract stored at 4°C. LC-MS analysis identified quercetin (302.25 g/mol), naringenin (272.25 g/mol), dodecanoic acid (200.32 g/mol), tetradecanoic acid (226.42 g/mol), and propanamide (73.09 g/mol). Green synthesis of AgNPs showed a color shift to turbid green with an SPR peak at 300–450 nm. HR-TEM revealed spherical nanoparticles of 5–40 nm, while FT-IR indicated -OH, -NH, and -COOH groups. Antioxidant activity of CvAgNPs reached 71% inhibition at 100 µg/mL versus 97.22% for ascorbic acid, and MTT assay showed 72.92–78.14% cytotoxicity at 40 µg/mL. Cleome viscosa AgNPs exhibit strong antioxidant, cytotoxic, and apoptotic activity, highlighting therapeutic potential. Further *In vivo* study required for the human application.

Keywords: *Cleome viscosa*, Silver nanoparticles, Antioxidant activity, Cytotoxicity, Apoptosis

Introduction
Skin cancer complications include local tissue damage, infection of ulcerated lesions, and potential metastasis to lymph nodes and organs. Untreated cases can cause disfigurement, pain, and functional impairment. Recurrence, psychological distress, and side effects from treatments are also common, emphasizing the importance of early detection and management [1].

Cleome viscosa is a medicinal plant valued for its wide range of therapeutic properties, including antioxidant, antimicrobial, and wound healing effects [2]. Traditionally, it is used to treat ailments such as rheumatism, malaria, skin wounds, and digestive disorders. Rich in flavonoids, fatty acids, and vitamins, this plant plays a significant role in natural medicine by promoting health and aiding recovery, while its non-toxic nature makes it safe for various applications [3]. Its holistic benefits underscore its importance in both traditional and modern herbal practices.

Biogenic synthesis of nanoparticles [4] has emerged as a sustainable and innovative approach in nanotechnology, offering a green alternative to conventional physico-chemical methods that often involve hazardous chemicals and high energy consumption [5]. The use of plant extracts for nanoparticle synthesis has drawn significant attention due to their abundance of bioactive compounds capable of acting as reducing, stabilizing, and capping agents. *Cleome viscosa*, commonly known as Asian spiderflower, has been extensively utilized in traditional medicine and is currently gaining interest for its phytochemical richness [6]. The plant contains flavonoids, phenolic acids, alkaloids, and glycosides, all of which provide

antioxidant, antimicrobial, and anti-inflammatory properties while also participating in redox mechanisms necessary for metal ion reduction [7].

Silver nanoparticles are widely recognized for their unique physicochemical characteristics, including strong surface plasmon resonance, high surface-to-volume ratio, and significant reactivity that enhances their biological activities. Traditional chemical methods of synthesis, though efficient, raise concerns about cytotoxic chemical residues and environmental hazards, necessitating the development of eco-friendly alternatives. The present study focuses on the biogenic synthesis of silver nanoparticles using the ethanolic extract of *Cleome viscosa* and evaluates their antioxidant and *in vitro* anticancer activities.

Materials and Methods

Plant collection and identification

Cleome viscosa, commonly known as Asian spiderflower, was collected from the garden in Coimbatore and accurately identified for use in this research study. The plant was determined based on distinctive botanical phenotypes, including its small yellow flowers, sticky indumentum, and glandular hirsute stems and leaves, which are key characteristics for distinguishing *C. viscosa* from related species. Leaves and roots were harvested in substantial quantities, approximately 2.5 kg of plant material, ensuring a representative sample from diverse locations to account for potential variation in phytochemical content [8].

Preparation of the ethanolic extraction

Fresh, healthy plants are collected, washed thoroughly with sterile water to eliminate dust and impurities, and then shade dried to retain heat- or light-sensitive compounds. The dried material is powdered and subjected to Soxhlet extraction with ethanol for 48–72 hours, enabling efficient recovery of bioactive components. The obtained extract is filtered to remove plant residues and concentrated under reduced pressure using a rotary evaporator. Finally, the crude extract is stored at 4 °C to ensure stability and prevent degradation until further experimental use [10].

Examination of *Cleoma viscosa* ethanolic extract phytochemical analysis using LC-MS

Liquid chromatography–mass spectrometry (LC-MS) serves as an advanced analytical method for detailed characterization of phytoconstituents in plant extracts. For Cleome viscosa, LC-MS was applied to evaluate the ethanolic extract, enabling precise identification of diverse bioactive molecules with high accuracy and sensitivity. The system operated with a binary pump linked to a Mariner Biospectrometry platform and a quadrupole time-of-flight (Q-TOF) mass analyzer using electrospray ionization (ESI). Chromatographic separation was achieved on a Phenomenex 5 µ C8 column (150 × 2 mm). A mobile phase of methanol and 0.3% formic acid flowed isocratically at 0.1 mL/min, ensuring consistent detection of phytochemicals [11].

Synthesis and characterization of Silver Nanoparticles (Ag-NPs)

The green synthesis of silver nanoparticles (AgNPs) utilizing *Cleoma viscosa* ethanolic extracts represents an intriguing intersection of nanotechnology and natural resources, initiating with a precisely controlled 1 mM silver nitrate solution in double-distilled water to furnish silver ions for the experiment, where the combination of this solution with the *Cleoma viscosa* extract at a specific 1:5 ratio is pivotal, influencing the synthesis process and leading to a visual transformation from green to turbid green, indicative of silver nitrate reduction and nanoparticle formation, thus showcasing the potential of biogenic reduction through bioactive components in *Cleoma viscosa* acting as reducing agents to convert silver ions into metallic silver nanoparticles, a process confirmed by distinctive color changes attributed to surface plasmon resonance (SPR) [12].

Antioxidant activity of *Cleome Viscosa* Ethanolic Extract Silver Nanoparticles (CvAgNPs) and Ascorbic acid

The antioxidant activity of silver nanoparticles synthesized using *Cleome viscosa* ethanolic extract (CvAgNPs) was evaluated in comparison with ascorbic acid as a standard reference. Different concentrations ranging from 0.390625 to 100 µg/mL were prepared for both CvAgNPs and ascorbic acid. The percentage inhibition of free radicals was assessed through a standard radical scavenging assay, where higher inhibition indicated stronger antioxidant capacity [13].

MTT Assay of *Cleome viscosa* Ethanolic Extract (CvAgNPs).

Cleome viscosa silver nanoparticles (CvAgNPs) demonstrated dose-dependent anticancer activity against SK-MEL-3 melanoma cells, with efficacy quantified via IC_{50} values (concentration inhibiting 50% cell viability) expressed in µg/mL. The study evaluated CvAgNPs across concentrations (50–2.5 µg/mL), revealing potent cytotoxicity. IC_{50} reflects the nanoparticle concentration required to halt 50% of cancer cell proliferation, highlighting CvAgNPs' therapeutic potential against skin cancer [14].

Results and Discussion

Plant Collection, Identification and Extraction

Cleome viscosa, commonly referred to as Asian spiderflower, was successfully collected from the garden in Coimbatore and authenticated based on distinct morphological traits such as small yellow flowers, glandular hairy stems and leaves, and sticky indumentum, which ensured accurate identification for research purposes. Approximately 2.5 kg of plant material, including both roots and leaves, was gathered from diverse spots to minimize variability and provide a representative sample for analysis. The collected specimens were carefully washed with sterile water to remove adhering particles and subjected to shade drying in order to preserve light- and heat-sensitive phytochemicals. The dried material was ground into fine powder and extracted with ethanol using the Soxhlet method over 48–72 hours, facilitating maximum recovery of active constituents. Following extraction, the solvent was removed under reduced pressure with a rotary evaporator and the concentrated crude extract obtained was stored at 4 °C to retain its stability and prevent loss of bioactive compounds.

Examination of *Cleoma viscosa* ethanolic extract phytochemical analysis using LC-MS

The LC-MS analysis of the ethanolic leaf extract of *Cleoma viscosa* revealed a diverse array of phytochemicals, each with significant mass values indicative of their potential health benefits. Among the key constituents identified was quercetin, with a mass value of approximately 302.25 g/mol,

renowned for its antioxidant and anti-inflammatory properties. Naringenin, another important flavonoid, was detected at about 272.25 g/mol, recognized for its various health benefits, particularly its antioxidant effects. The analysis also identified dodecanoic and tetradecanoic acid, with mass values of roughly 200.32 g/mol and 226.42 g/mol, respectively; both compounds are noted for their antibacterial and antimicrobial activities. 2,3-hydroxypropyl ester was found at approximately 132.12 g/mol, contributing to the extract's bioactivity, while 1,3-dictation, known for its emulsifying properties, had a mass value of about 370.56 g/mol. Furthermore, N-methoxy-N-methyl-3,4-dihydro-2H-thiopyran-6-carboxamide was identified at around 229.30 g/mol, indicating potential pharmacological effects. Lastly, propanamide was detected with a mass value of approximately 73.09 g/mol, suggesting various biological activities. These findings underscore the therapeutic potential of *Cleoma viscosa* jobi in traditional and modern medicine.

Green synthesis of silver nanoparticles assisted by *Cleoma viscosa* ethanolic extract and their characterization: The green synthesis of silver nanoparticles (AgNPs) utilizing *Cleoma viscosa* ethanolic extracts represents an intriguing intersection of nanotechnology and natural resources, initiating with a precisely controlled 1 mM silver nitrate solution in double-distilled water to furnish silver ions for the experiment, where the combination of this solution with the *Cleoma viscosa* extract at a specific 1:5 ratio is pivotal, influencing the synthesis process and leading to a visual transformation from green to turbid green, indicative of silver nitrate reduction and nanoparticle formation.

UV-Vis spectroscopy analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.
The UV-Vis spectroscopy analysis of silver nanoparticles synthesized using *Cleome viscosa* ethanolic extract confirms the successful formation of stable, small-sized, and well-dispersed AgNPs (Figure 1). The distinct SPR peak near 300 and 450 nm highlights the unique optical properties influenced by nanoparticle size and the phytochemical environment. This analysis validates the efficacy of the green synthesis method and provides a foundation for further characterization and application of these biogenic silver nanoparticles in antimicrobial, antioxidant, and other biomedical fields.

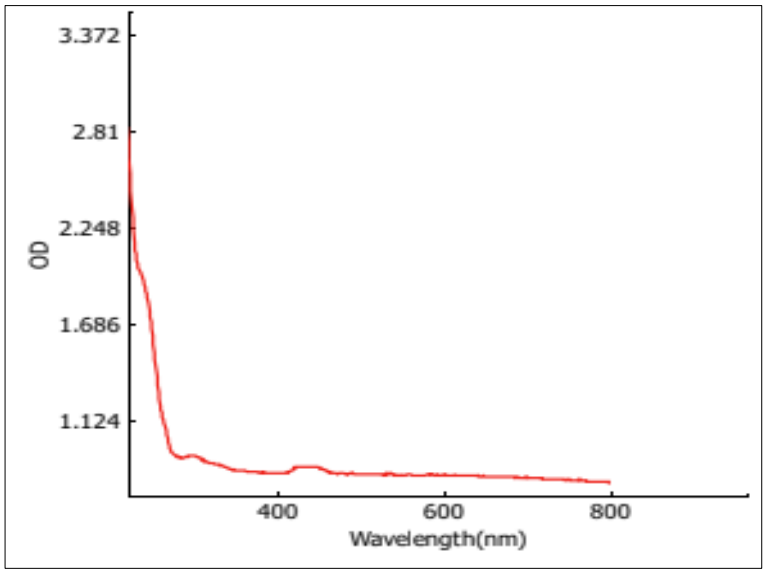

Fig 1: UV-Vis spectroscopy analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

FT-IR spectroscopy analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract
Fourier Transform Infrared (FT-IR) spectroscopy is pivotal in understanding chemical composition, surface functional groups, and potential applications of *Cleoma viscosa* ethanolic silver nanoparticles, offering insights into vibrational modes of molecular bonds and detailed chemical information, especially regarding functional groups on the nanoparticle surface, possibly revealing organic molecules from the extract acting as stabilizing or capping agents, indicating involvement of -OH, -NH, or -COOH groups in synthesis and stabilization, with potential to identify biomolecules from the extract interacting with silver ions during synthesis, such as proteins or amino acids (Figure 2).

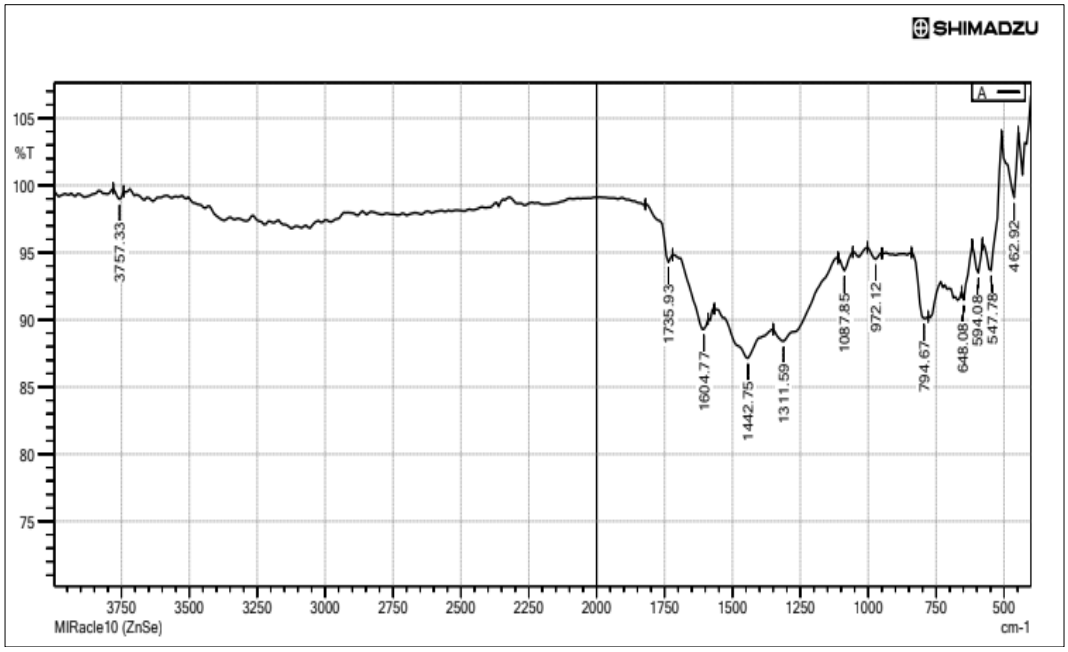

Fig 2: FT-IR analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

X-ray Diffraction (XRD) analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

X-ray Diffraction (XRD) is essential for characterizing crystal structure, phase composition, and physical properties of materials, spanning across materials science, geology, metallurgy, and pharmaceuticals, providing fundamental information about atomic arrangement within a crystal lattice, vital for understanding material behaviour and guiding new material development, while also being instrumental in identifying different phases present in a material, distinguishing crystalline components, and analyzing residual stresses through shifts in diffraction peaks, valuable for assessing structural integrity and optimizing manufacturing processes (Figure 3).

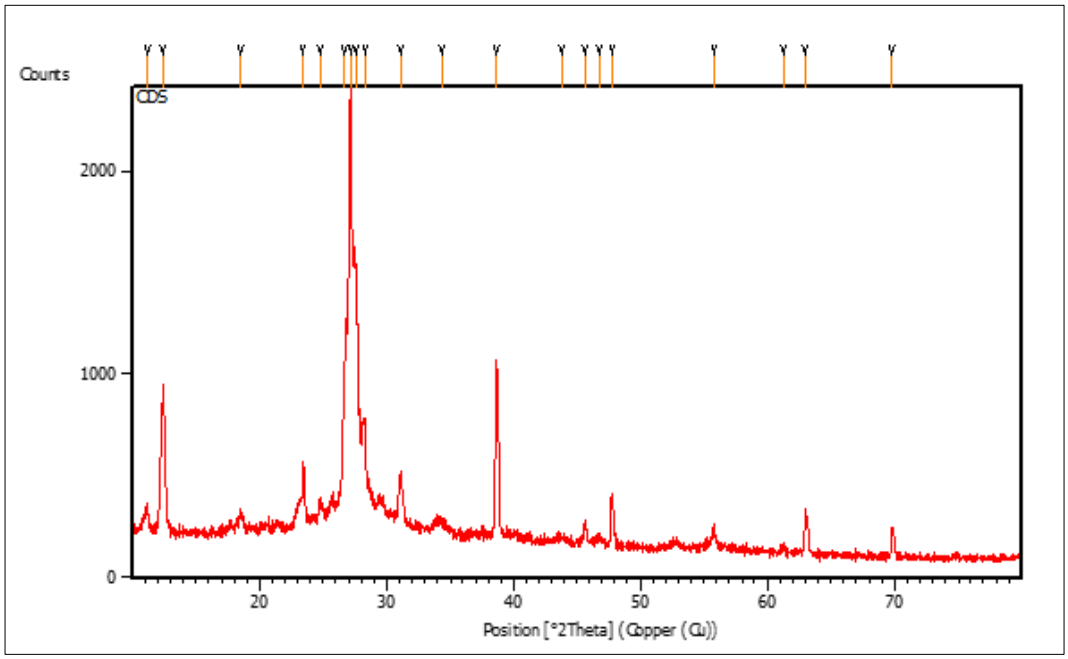

Fig 3: X-ray Diffraction (XRD) analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

FE-SEM analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

FE-SEM analysis of silver nanoparticles synthesized using *Cleome viscosa* ethanolic extract revealed detailed insights into their structural and morphological features. The high-resolution imaging demonstrated that the nanoparticles were predominantly spherical, with smooth surfaces and uniform distribution, confirming homogeneity within the sample. Particle size was observed in the nanometer range, with no significant aggregation, indicating stability of the synthesized nanoparticles (Figure 4). The extended depth of field provided three-dimensional clarity, enabling comprehensive evaluation of topography and texture. Coupled with EDS, elemental analysis confirmed the presence of silver, validating successful biosynthesis. These results highlight uniform morphology and stable characteristics of the CvAgNPs.

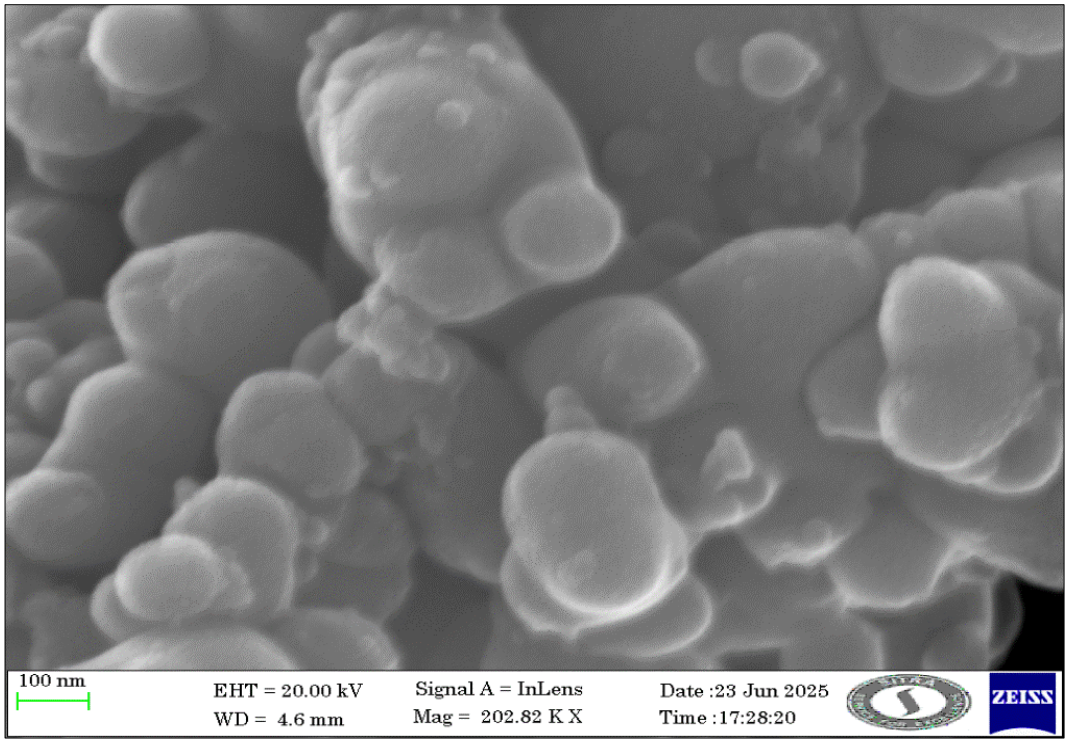

Fig 4: FE-SEM analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

HR-TEM analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

The size distribution of the AgNPs, as determined by HR-TEM, generally falls within the nanoscale range of 5–40 nm, with an average particle size often reported around 17–40 nm depending on synthesis conditions and extract concentration (Figure 5). This narrow size distribution is crucial, as it influences the nanoparticles' surface area-to-volume ratio, reactivity, and biological interactions. The HR-TEM micrographs allow for the direct measurement of individual nanoparticles, and the images often show clear, well-defined lattice fringes, a hallmark of high crystallinity.

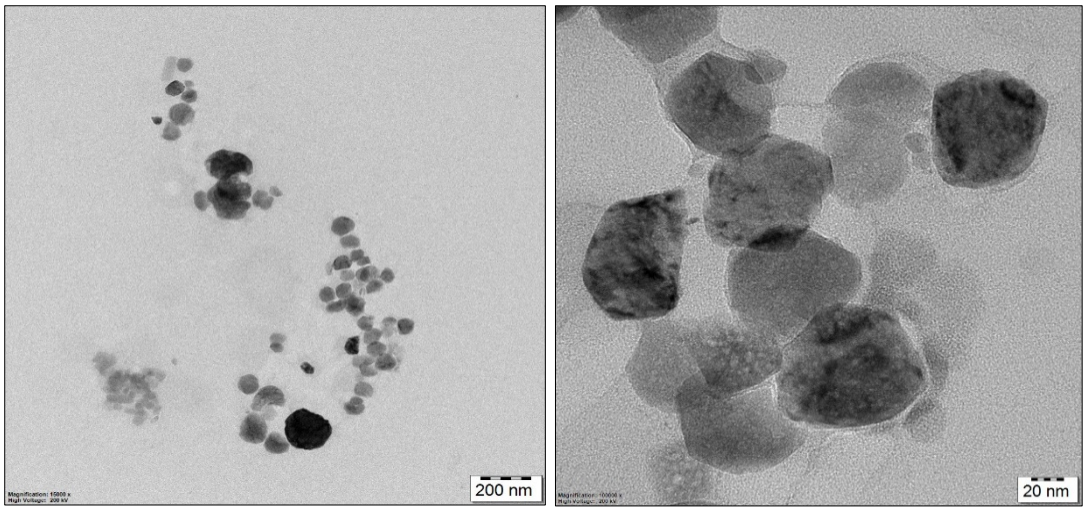

Fig 5: HR-TEM analysis of Silver Nanoparticles Synthesized using *Cleome viscosa* Ethanolic Extract.

Antioxidant activity of CvAgNPs.

The antioxidant activity of silver nanoparticles synthesized using *Cleome viscosa* ethanolic extract is a testament to the power of green nanotechnology in creating multifunctional materials with significant biomedical potential. The synergy between silver and plant-derived phytochemicals results in nanoparticles with robust free radical scavenging and reducing properties, making them valuable for the prevention and treatment of oxidative stress-related diseases. The eco-friendly synthesis process ensures the preservation of bioactive compounds, enhances the safety profile of the nanoparticles, and opens new avenues for their application in medicine, food, and cosmetics. As research progresses, CvAgNPs are poised to become an important tool in the fight against oxidative damage and its associated health challenges (Table 1 and Figure 6).

Table 1: Antioxidant activity of Silver Nanoparticles Synthesized Using *Cleome viscosa* Ethanolic Extract (CvAgNPs), and Ascorbic acid.

Concentration (µg)	Ascorbic acid (% of Inhibition)	CvAgNPs
100	97.22	71
50	92.54	64.65
25	85.27	62.53
12.5	78.73	54.12
6.25	72.84	43.32
3.125	64.58	30.39
1.5625	57.22	24.75
0.78125	24.02	9.13
0.390625	13.67	4.32

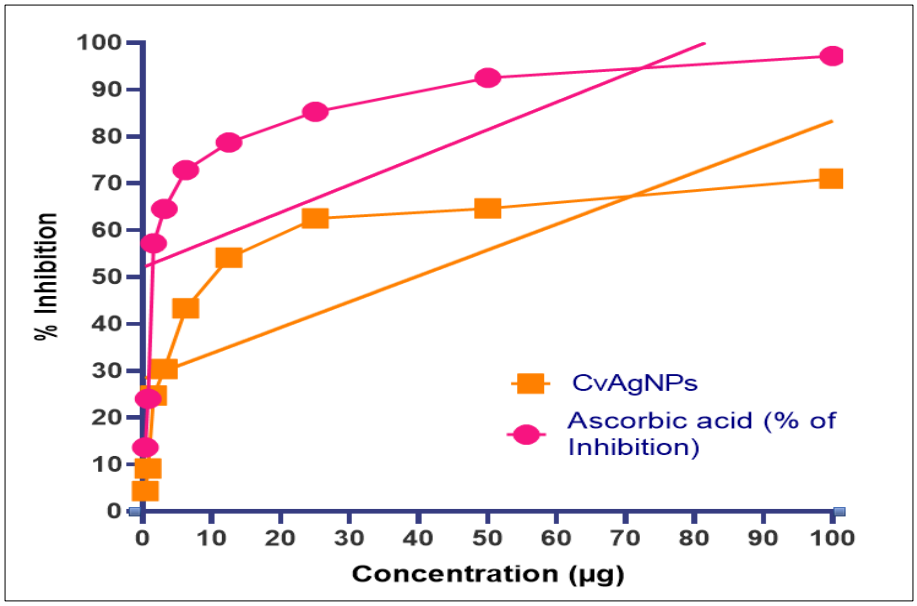

Fig 6: Antioxidant activity of CvAgNPs.

MTT Assay of *Cleome viscosa* Ethanolic Extract Silver Nanopartilces (CvAgNPs).

The cytotoxic effects of the tested *Z. acanthopodium* fruit extracts were evaluated at concentrations of 5, 10, 20, 30, and 40 µg/mL, compared to the untreated control group. The percentage inhibition of cell growth showed a clear dose-dependent increase across all treated groups. At 5 µg/mL, the mean growth inhibition values ranged from 25.66% to 29.50%, while 10 µg/mL concentrations elicited inhibition rates between 37.48% and 42.01%. Increasing the dose to 20 µg/mL resulted in more pronounced cytotoxicity, with inhibition percentages spanning from 50.67% to 55.89% (Figure 7). At 30 µg/mL, cell growth was further inhibited, recording values between 63.37% and 69.77%. The highest concentration, 40 µg/mL, led to substantial inhibition, with rates varying from 72.92% to 78.14%. These data collectively demonstrate a marked concentration-dependent cytotoxic response, supporting the potent anticancer activity of *Z. acanthopodium* extracts (Figure 8).

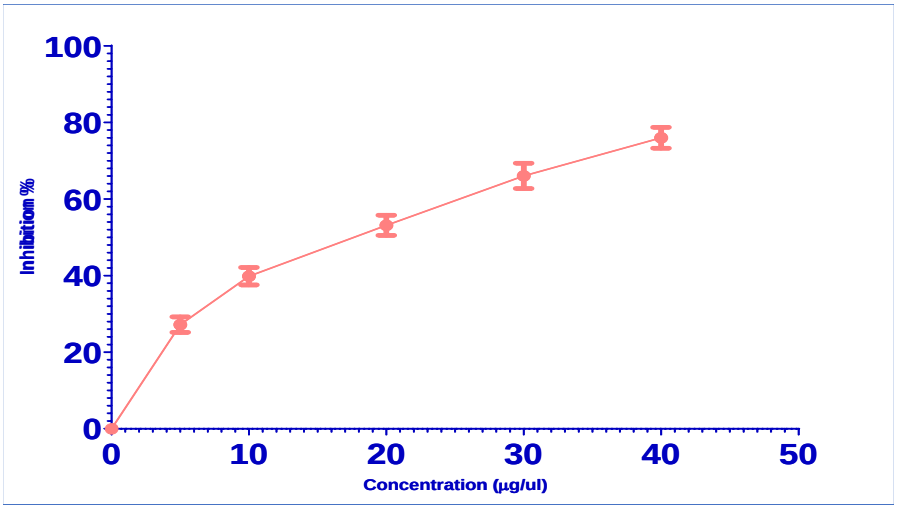

Fig 7: MTT Assay of *Cleome viscosa* Cv-Ag-NPs.

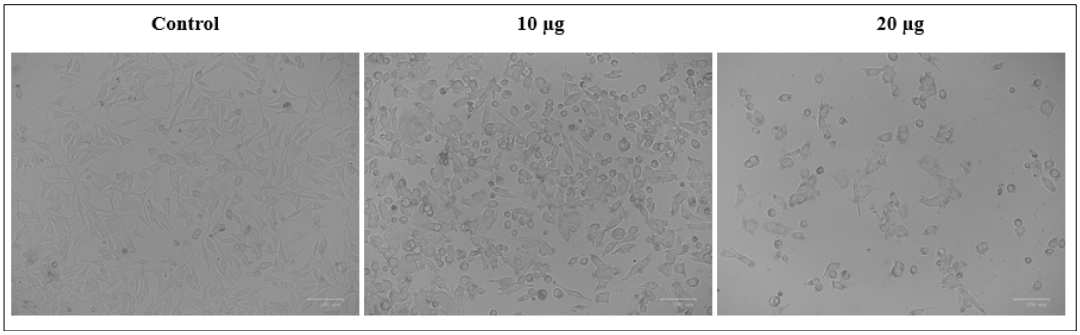

Fig 8: Microscopic observation of the CvAgNPs SKMEL-3 cells.

Apoptosis analysis of CvAgNPs treated SK-MEL-3

SKMEL3 cancer cells treated with CvAgNPs (10 and 20 µg/mL) for 24 hours were stained with dual acridine orange/ethidium bromide (AO/EB) and analyzed by fluorescence microscopy. Live cells exhibited green fluorescence with intact nuclei, while early apoptotic cells showed yellow fluorescence due to chromatin condensation and fragmented nuclei (Figure 9). Late apoptotic cells displayed orange fluorescence with condensed or fragmented chromatin, indicating advanced apoptosis stages. This staining method effectively distinguished cell viability and apoptotic phases, confirming CvAgNPs ability to induce apoptosis in SK-MEL-3 cells, suggesting its potential as a therapeutic agent for targeting melanoma by promoting programmed cell death through apoptotic pathways.

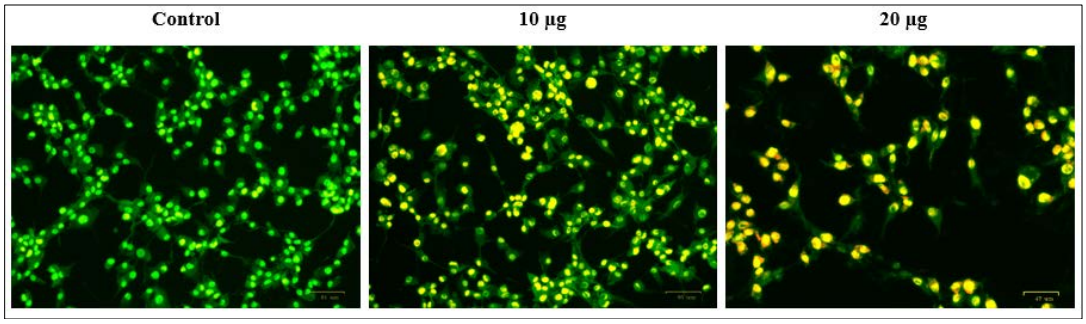

Fig 9: Apoptosis analysis of CvAgNPs treated SK-MEL-3

Discussion

The study centered on *Cleome viscosa*, a plant collected and authenticated from Coimbatore, demonstrating meticulous preparation to preserve its bioactive profile through ethanol extraction via Soxhlet method. The LC-MS analysis revealed a variety of phytochemicals including quercetin, naringenin, and several fatty acids, well-known for their antioxidant, antimicrobial, and potential pharmacological activities. This phytochemical richness aligns with previous studies attributing significant therapeutic properties to *Cleome viscosa* extracts, especially antioxidant and anticancer activities. The green synthesis of silver nanoparticles (CvAgNPs) using this extract illustrated an effective biogenic method highlighted by UV-Vis spectroscopy confirming stable nanoparticle formation, complemented by FT-IR, XRD, FE-SEM, and HR-TEM analyses that collectively validated the nanoparticles' crystalline structure, morphology, size range, and functional group involvement. These findings parallel earlier nanotechnology studies where plant-mediated synthesis produced nanoparticles with potent bioactivities.

Functionally, the CvAgNPs exhibited marked antioxidant capacity in free radical scavenging assays, though somewhat lower than ascorbic acid, demonstrating their biomedical relevance. Cytotoxicity assessed by MTT assay showed a strong dose-dependent inhibition of cancer cell proliferation, resonating with prior data on *Zanthoxylum acanthopodium* extracts, which similarly demonstrated concentration-dependent anticancer effects on MCF-7 and K562 cell lines. The growth inhibition pattern in both cases underscores the efficacy of plant-derived bioactive compounds and their nanoparticle formulations in targeting cancer cells.

Further, apoptosis assessment using AO/EB dual staining of SKMEL3 melanoma cells treated with CvAgNPs revealed progressive nuclear changes from viable (green) through early (yellow) to late apoptotic (orange) stages. This confirms the nanoparticles' ability to induce programmed cell death, consistent with literature describing apoptotic induction by phytochemical-enriched nanoparticles. The morphological evidence supports mechanistic pathways involving chromatin condensation and nuclear fragmentation, aligning with the pro-apoptotic roles of quercetin and naringenin identified in the extracts.

Comparing these results to previous studies on *Cleome viscosa* and related species highlights a convergent trend: plant extracts rich in flavonoids and fatty acids consistently show promising anticancer activity both in free extract and nanoparticle forms. The green synthesis approach not only enhances bioavailability and stability but also offers a sustainable, eco-friendly route for developing advanced therapeutic agents. Overall, this study integrates phytochemical profiling, nanotechnology characterization, and biological evaluation, reinforcing the therapeutic potential of *Cleome viscosa*-derived silver nanoparticles against cancer through antioxidant and apoptotic mechanisms, corroborating and extending previous scientific findings in the field.

Conclusion

The study successfully demonstrated the biogenic synthesis of silver nanoparticles using Cleome viscosa ethanolic extract, preserving its rich phytochemical profile, including quercetin and naringenin. Characterization confirmed stable, crystalline nanoparticles with nanoscale morphology. The CvAgNPs exhibited potent antioxidant activity and dose-dependent cytotoxic effects against cancer cells, inducing apoptosis through nuclear changes. These results highlight the therapeutic potential of *Cleome viscosa*-derived nanoparticles as effective, eco-friendly agents for antioxidant and anticancer applications, supporting further development in nanomedicine.

Conflict of interest: Nil

Funding: Nil

Acknowledgement: Authors would like to thanks JJTU university to provide necessary facilities for conducting the research.

Data Availability: Data generated while conducting the research are available to the corresponding author upon reasonable request

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