Volume: 5 Issue: 3 Pages: 49-53

Phytochemical Profiling, Green Synthesis and Physicochemical Characterization of Silver Nanoparticles using Methanolic Stem Extract of *Calotropis gigantea* and their Antimicrobial Evaluation

[*1] Deepika R and [2]K Chitra

[*1] M.sc. Biotechnology, Muthayammal College of Arts and Science Autonomous Rasipuram, Tamil Nadu, India.

[2] Assistant Professor, Department of Biotechnology, Muthayammal College of Arts and Science Autonomous Rasipuram, Tamil Nadu, India.

Article Info.
E-ISSN: 2583-6528
Impact Factor (QJIF): 8.4
Peer Reviewed Journal
Available online:
www.alladvancejournal.com

Received: 05/Feb/2026
Accepted: 10/March/2026

Abstract
The stem of *Calotropis gigantea* having several medicinal properties. It exists anti-inflammatory, analgesic, anti- bacterial, and anti- microbial activities due to the presence of bioactive compounds. The stem extract serves as many potential sources, so that the green synthesis of nanoparticles is used in the field of nanobiotechnology. This study focuses on phytochemical analysis confirmed the sample may have the presence of bioactive compounds of Nanoparticles. Uv-Vis Spectrophotometer used to characterize the peaks and the FTIR used to helps identify the functional groups and also Particle size analysis helps to determine the size and stability of the particles. The Anti- microbial activity is essential to inhibit the growth of pathogenic microorganisms. This study helpful to know the bioactive nanoparticles and potentials in biomedical and pharmaceutical fields.

***Corresponding Author**
Deepika R
M.sc. Biotechnology, Muthayammal College of Arts and Science Autonomous Rasipuram, Tamil Nadu, India.

Keywords: *Calotropis gigantea,* Green Synthesis, Silver Nanoparticles, Particle Size Analysis, Phytochemicals, Anti-microbial Activity.

1. Introduction

Calotropis gigantea, commonly known as the giant milkweed or crown flower, is a fast-growing, evergreen shrub belonging to the Apocynaceae family (Joseph., *et al* 2015). Calotropis gigantea is a plant found in several Asian countries, including India, Indonesia, Malaysia, the Philippines, Thailand, Sri Lanka, and China (Rates *et al*.,2001).

The stem of *Calotropis gigantea* is erect, cylindrical, and woody at the base. It has a thick, greyish-green bark that contains a milky latex, which is rich in alkaloids and glycosides. The latex is toxic in nature but has been traditionally used for medicinal purposes (Abeysinghe *et al*.,2018). The process of synthesis AgNPs Can be carried out one of the methods of green synthesis using *C. gigantea* stem extract. This research profiles the phytochemical composition and physicochemical properties via (UV, FTIR, Particle Size Analysis) and evaluates Anti- microbial Activity against some of the bacterial strains. Hence, this study aims to investigate the phytochemical constituents of Calotropis gigantea stem extract and evaluate its efficacy in the eco-friendly synthesis, characterization, and antimicrobial assessment of silver nanoparticles.

2. Materials and Methods

2.1 Sample Collection and Preparation

The plant stem (Calotropis gigantea) was collected from a kalleripatty village vazhappadi, Salem Dt, Tamil Nadu, India and in the month of December in 2024. The Various parts of the plant have been used for traditional medicine.

2.2 Preparation of Sample Extraction by Soxhlet Method

Stems of Calotropis gigantea were collected and washed under running tap water and once in distilled water. Those stems were shade dried and grind by mixer grinder to make as a powder form. The Soxhlet extraction method was used to prepare crude extract. Approximately 20 grams of powdered stem material was uniformly packed into a thimble and

extracted with 250 ml of methanol respectively. The extraction procedure must contain for 24 hours, or until the solvent in the extractors siphon tube turns colourless. The extract was placed in a beaker at 20℃ until all solvent had evaporated. The dried extract was stored, in the refrigerator at 4℃ for further usage.

2.3 Phytochemical Analysis of the Sample (Calotropis gigantea)

All of the Calotropis gigantea methanol extraction underwent preliminary phytochemical analysis using conventional techniques.

2.3.1 Test for Reducing Sugar

10 ml of Benedict's solution was added to 1 ml of solution of plant extract in a test tube and boiled for few minutes and then cooled simultaneously. A red colour precipitate of cuprous oxide should form in the presence of reducing sugar, which revealed the presence or absence of reducing sugar.

2.3.2 Test for Tannins

1ml of 10% potassium dichromate solution was added with 1ml solution of the extract in a test tube. A yellow precipitate was formed in the presence of tannins, which revealed the presence or absence of tannins.

2.3.3 Test for Flavonoids

In the alcoholic extract of plant material two to three drops of conc. HCl acid were added. Immediate development of a red colour indicates the presence of flavonoids, which revealed the presence or absence of flavonoids.

2.3.4 Test for Saponins

In a test tube add 1 ml solution of plant extract was diluted with 2 ml of distilled water. One centimetre layer of foam indicates the presence of saponins, which revealed the presence or absence of saponins.

2.3.5 Test for Carbohydrate

In a test tube 1 ml of plant extract add phenol and sulphuric acid a yellowish orange colour indicates the presence of Carbohydrate, which revealed the presence or absence of carbohydrates.

2.3.6 Test for Steroid

In a test tube 1ml of sulphuric acid was added to 1 ml solution of chloroform extract. Red colour indicates the presence of steroid, which revealed the presence or absence of steroid.

2.3.7 Test for Alkaloid

A few drops of dilute HCl are added to the plant extract, followed by the addition of Mayer's reagent. The formation of a cream precipitate indicates the presence of alkaloids, which revealed the presence or absence of alkaloid.

2.3.8 Test for Protein and Amino acid

1ml of extract was treated with few drops of million's reagents or ninhydrin's solution. Formation of purple or yellow colour indicates the presence of protein and amino acid, which revealed the presence or absence of protein and amino acid

2.3.9 Test for Terpenoids

1 ml of extract was treated with 2 ml of chloroform and 3ml of conc. Sulphuric acid. Reddish brown colour indicates the presence of terpenoids, which revealed the presence or absence of terpenoids.

2.4.0 Test for Starch

1 ml of plant extract treated with 2ml of 5% potassium hydroxide solution was added. Formation of canary yellow colour indicates the presence of starch, which revealed the presence or absence of starch.

2.4 Synthesis of Silver Nanoparticles

To produce green AgNPs, 5 ml of *Calotropis gigantea* leaf extract with 95 ml of a 1mM aqueous silver nitrate solution. The pH was carefully adjusted to 9.5 by adding 1 M NaOH solution drop by drop. This mixture was continuously stirred for 12 h and stored in dark area to prevent any photo activated effects. The change in solution colour, transitioning from red to dark brown, signified the successful formation of AgNPs.

2.5 Characterization of Silver Nanoparticles

The characterization of silver nanoparticles (AgNPs) content involves determining their physical, chemical, and biological properties. This is essential for applications in medicine and environmental science. The main techniques used include:

2.5.1 UV-VIS Analysis

Using a UV-VIS SPECTROPHOTOMETER, the optical properties of silver nanoparticles were ascertained. Following the creation of silver nanoparticles the spectra were obtained.

2.5.2 FTIR Spectroscopy Analysis

The Fourier Transform Infrared (FTIR) method was used to characterize the plant stem extract. FT-IR spectroscopy is a type of vibration spectroscopy in which an infrared source emits radiation into the sample; the radiation absorption causes quanta of energy to be deposited into vibration modes, there by stimulating vibration motions. The obtained spectra were analysed to identify the functional groups present in the plant stem.

2.5.3 Particle Size Analysis

Particle Size Analysis (PSA) plays a significant role in the characterization of plant extracts. The efficiency of extraction, solubility, bioavailability, and stability of plant-based formulations are directly influenced by particle size distribution. Understanding the particle size helps in optimizing extraction methods and improving product quality in industries. Several studies indicate that smaller particle sizes enhance the extraction of bioactive compounds due to increased surface area. Particle size affects they drying process, storage stability, and dissolution rate in liquid and solid formulations. Methods such as laser diffraction, sieve analysis, and dynamic light scattering (DLS) are commonly used for PSA in plant extracts.

2.5.4 Antimicrobial Activity of Stem Extract

To perform an antimicrobial activity by using bacterial strains such as *pseudomonas, protease, klebsiella, staphylococcus* species.

Media Preparation

The Nutrient agar was prepared by the composition of peptone, beef extract, yeast extract, sodium chloride and the gelling agent agar and dissolved into the distilled water. The prepare media was autoclaved at 121℃ for 15 minutes. The sterilized media was poured into sterile Petri plate and allow for solidification. A standardized suspension of the test microorganism is then evenly spread over the agar surface using a sterile cotton swab. Wells are created in the agar using a sterile pipette tip, and the test sample, typically in a specific concentration, is introduced into the wells. The plates are incubated at 37℃ for 24 hours for bacterial strains. After incubation, the antimicrobial activity is evaluated by measuring the diameter of the clear inhibition zones around the wells, indicating microbial growth inhibition.

3. Result

3.1 Preliminary Phytochemical Analysis

The Phytochemical characteristics of *Calotropis gigantea* were summarized in the table. The result revealed the presence of secondary metabolite (Phytochemicals) such as Quinine, Tanin, Protein, Terpenoids and Steroids, etc. The results were shown in the (Figure 1)

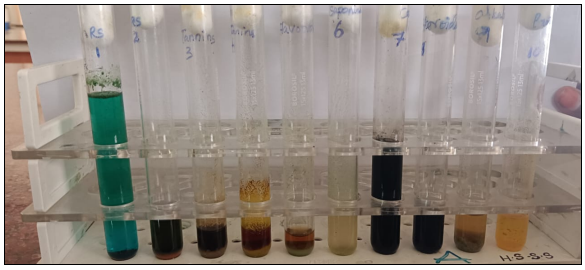

Fig 1: preliminary phytochemical analysis of stem extract

Table 1: Preliminary phytochemical analysis of stem extract

S. No	Phytochemical constituent	Observation
1	Reducing sugar	+
2	Tannins	+
3	Flavonoids	-
4	Saponins	+
5	Carbohydrate	-
6	Steroid	-
7	Alkaloid	-
8	Protein	+
9	Terpenoids	+
10	Starch	-

All of the bioactive substance were found in the plant stem extract using Phytochemical screening, with exception of Flavonoids, Carbohydrate, Steroid, Alkaloid, starch. Phytochemical have potential biological properties. Identification of bioactive chemicals helpful in the process of drug development and Pharmacological Formulation. The extract contains secondary metabolites that are significant bioactive agents which may play a role in its medicinal effects. Tannins play a major role in various Medicinal and Pharmacological properties including potential Antibacterial, Antifungal and Anti-inflammatory effects. The amount of Phytochemical substance varies considerably from species to species, depending on the age and various Ecological and Climate factors.

3.2 Green synthesis of Silver Nanoparticles

Reduction of Ag ions into Ag particles exposure to the stem extract was obtained because of the colour change. Solution exhibits green to brown or yellow colour due to the surfaces. The results were shown in

Fig 2: Control

Fig 3: Synthesized nanoparticles of AgNO3

3.3 UV-Visible Spectrophotometer

The optical properties of silver nitrate nanoparticles were characterized based on UV-Visible Spectrophotometer. The reduction of pure silver nitrate nanoparticles was monitored by measuring the UV-Visible Spectrophotometer. UV-Visible Spectrophotometer of silver nitrate nanoparticles was recorded, by taking 3ml of sample as a function of time of reaction using a Cary 60 UV-Vis Spectrophotometer in the wavelength region 800 nm to 200 nm operated at a resolution of 5nm. The UV-Vis spectrum suggests the presence of conjugated organic molecules with aromatic rings and carbonyl groups. The strong absorption at 235 nm indicates a highly conjugated π-system, which could be due to polycyclic aromatic hydrocarbons, ketones, or other chromophores.

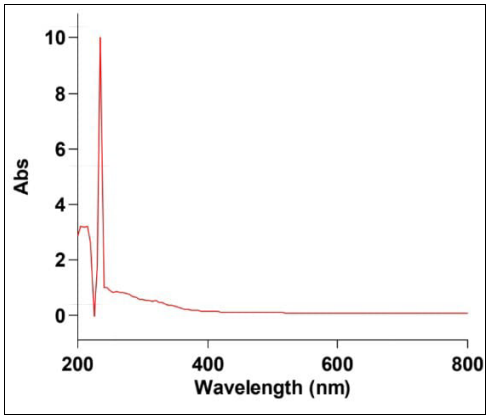

Fig 4: Characterization of UV-Visible Spectrophotometer

3.4 Fourier Transform Infra-Red Test

The FTIR analysis was conducted using an IR Spirit spectrometer with a resolution of 2 cm^{-1} and 45 scans. The spectrum was recorded in the range of 340 cm^{-1} to 4700 cm^{-1}, employing Happ-Genzel apodization. The peak table identifies key absorption bands at 616.19 cm^{-1}, 834.43 cm^{-1}, 1711.64 cm^{-1}, and 2929.05 cm^{-1}, with varying intensities. The peak at 2929.05 cm^{-1} exhibited the highest corrected intensity (103.36), suggesting significant vibrational modes. The data provides insight into the molecular composition and functional groups present in the sample, supporting further material characterization.

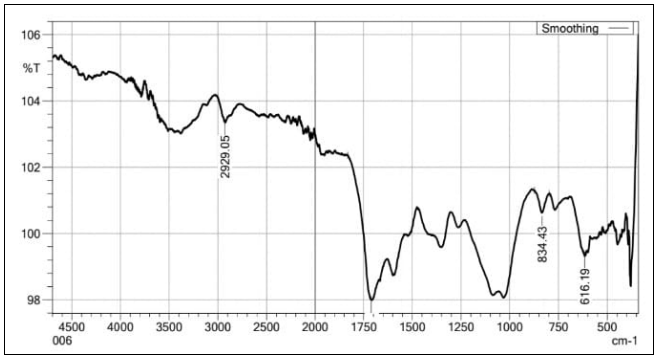

Fig 5: FT-IR Analysis of Calotropis gigantea stem extract

Table 2: FT-IR Analysis of silver nanoparticles synthesized by plant extract

S. No.	Peak Range	Intensity	Functional Group	Compounds
1	616.19	99.35	C-Br	Alkyl halides
2	834.43	100.62	C-H	Aromatic
3	1711.64	98.00	C=O	Ketone, ester
4	2929.05	103.36	C-H	Methylene, alkanes

3.5 Particle Size Analysis

The particle diameter is 166.7 nm, indicating the average size of the particles in the sample. 0.183, which suggests a narrow size distribution, indicating that the particles are relatively uniform in size. First peak: 208.3 nm (major peak) with a standard deviation of 101.8 nm. Other peaks (second, third, etc.) are 0.0 nm, meaning there are no significant additional peaks, which supports the uniformity of the sample. D (10) = 94.4 nm (10% of particles are smaller than this size). D (50) = 178.2 nm (median size, meaning 50% of the particles are smaller and 50% are larger). D (90) = 340.1 nm (90% of the particles are smaller than this size). These values suggest a moderate range of particle sizes but still relatively consistent. 2.951×10^{-8} cm²/sec, indicating how fast the particles diffuse in the medium. The results indicate that the particles in the sample are in the nano range (mostly between 100-300 nm). The low PDI (0.183) suggests that the sample has a relatively uniform particle size distribution, which is beneficial for applications requiring consistent particle behaviour. The D (50) value (178.2 nm) confirms that the majority of the particles are within the nanoscale range. Nano-sized particles with a uniform distribution can be used in catalysts.

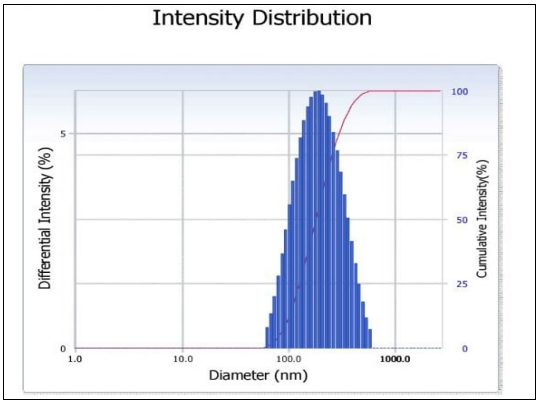

Fig 6: Intensity distribution of the particle size analysis by plant extract

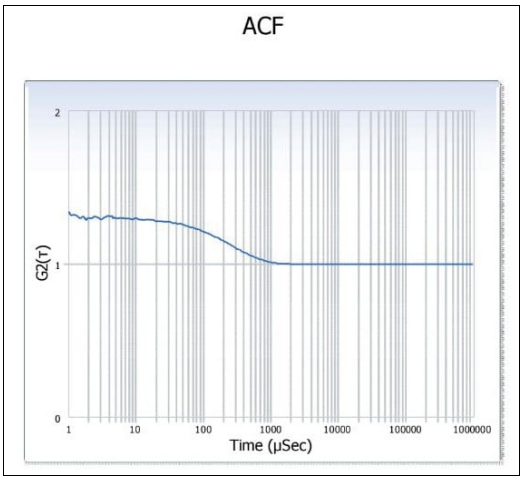

Fig 7: Auto-Correlation Function of Particle Size Analysis by plant extract

3.6 Antimicrobial Activity

The methanolic extract exhibited higher antimicrobial activities at all concentration compared to the aqueous extract. The extract showed varying degrees of inhibition on the tested microorganisms at a concentration of 25, 50, 75, 100 µl/ml. The *Calotropis gigantea* extract showed greater activity against *proteus mirabilis* (Zone of inhibition 14mm), *Staphylococcus aureus* (Zone of inhibition 14mm), *pseudomonas aeruginosa* (Zone of inhibition 15 mm).

The microbes against which the *Calotropis gigantea* leaf extracts were effective are pathogen already implicated in the etiology and severity of human diseases. Thus, the plant extract may be useful in pharmaceutical and medical formulations.

Table 3: Zone of inhibition presents in antimicrobial activity by plant extract

Name of the Organisms	Zone of Inhibition [mm]			
	Methanol [µl]			
	25	50	75	100
Proteus mirabilis	11	12	`13	14
Pseudomonas aeruginosa	10	11	14	15
Staphylococcus aureus	10	11	13	14

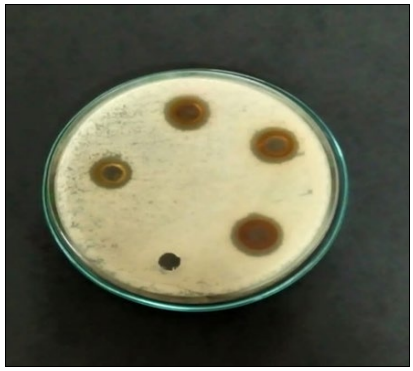

Fig 1: Proteus mirabilis

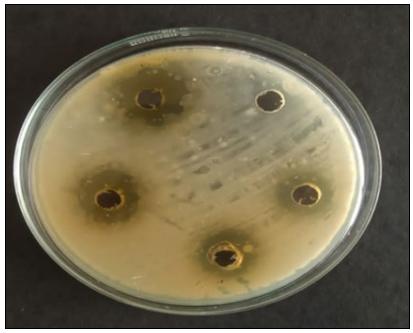

Fig 9: Staphylococcus aureus

Fig 10: Pseudomonas aeruginosa

Discussion

The preliminary phytochemical screening of *Calotropis gigantea* stem extract confirmed the presence of the bioactive compounds. Previous studies on *C. gigantea* have reported the presence of tannins, flavonoids, alkaloids, terpenoids, and saponins in different plant parts, particularly leaves and latex, the variations may be attributed because plant part used, extraction solvent, geographical location, and environmental conditions. The green synthesis of silver nanoparticles was

confirmed by the reduction of Ag^{+} ions to Ag^{0} upon exposure to the stem extract. This reduction was visually indicated by a colour change of the reaction mixture from green to brownish-yellow of the sample. The reduction of silver nitrate to silver nanoparticles was monitored by measuring absorbance as a function of reaction time. Approximately 3 mL of sample was scanned in the wavelength range of 200–800 nm at a resolution of 5 nm. The FTIR results confirm the involvement of various functional groups like alkyl halides, ketones, methylene etc., nanoparticle formation and stabilization. The Particle Size Analysis results indicate that the synthesized particles fall within the nanoscale range (100–300 nm). The major peak was observed at 208.3 nm with a standard deviation of 101.8 nm. No significant secondary peaks were detected, confirming sample uniformity. In the antimicrobial activity test, *Pseudomonas aeruginosa* showed the highest sensitivity and it exhibited the largest zone of inhibition indicating that the extract was most effective against the pathogens. The findings are consistent with previously reported literature on plant-mediated silver nanoparticle synthesis and antimicrobial evaluation, further supporting the potential of *C. gigantea* as a sustainable and eco-friendly source for biomedical nanoparticle production.

Reference

1. Alafnan A, Sridharagatta S, Saleem H. "Evaluation of the Phytochemical, Antioxidant, Enzyme Inhibition, and Wound Healing Potential of Calotropis gigantea (L.) Dryand. A Source of a Bioactive Medicinal Product." Front Pharmacol. 2021; 12:701369.
2. Deshmukh PT, Fernandes J, Atul A and Toppo E, *et al*. "Wound Healing Activity of Calotropis gigantea Root Bark in Rats." J Ethnopharmacol. 2009; 125:178-181.
3. De Pasquale A, Pharmacognosy: the oldest modern science, Journal of Ethnopharmacology. 1984; 11:1-16.
4. Gaurav Kumar, Karthik L, Bhaskara Rao KV, *in vitro* anti-Candida activity of Calotropis gigantea against clinical isolates of Candida, *Journal of Pharmacy Research*. 2010; 3:539-542.
5. Misra MK, Mohanty MK, Das PK. Studies on the method-ethnobotany of Calotropis gigantea and C. procera. Ancient science of life. 1993; (1-2):40-56.
6. Patel HV, Patel JD, Patel B. "Comparative Efficacy of Phytochemical Analysis and Antioxidant Activity of Methanolic Extract of Calotropis gigantea and Calotropis procera." *Int J Biol Pharm Res.* 2014; 5:107-103.
7. Rates SMK, Plants as source of drugs, Toxicon, 39,2001, 603-613
8. Sharma N, Shankar R, Gupta N, Prakash P. A preliminary phyto-pharmacognostical evaluation of *Calotropis gigantea* (L.) R. Br. (Alarka or Mandara) Root. *International Journal of Ayurvedic Medicine.* 2016; 7(1):44-48.