



## ORIGINAL ARTICLE

# Biosynthesis of silver nanoparticle using flowers of *Calotropis gigantea* (L.) W.T. Aiton and activity against pathogenic bacteria



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## KEYWORDS

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**Abstract** Silver nanoparticles (AgNPs) from silver nitrate solution are carried out using the flower extract of *Calotropis gigantea*. Silver nanoparticles were characterized by UV–vis spectrophotometer, X-Ray diffractometer (XRD). Reduction of silver ions in the aqueous solution of silver during the reaction was observed by UV–vis spectroscopy. Crystalline nature of synthesized silver nanoparticles was studied by XRD pattern, refraction peak using the Scherrer's equation. Antibacterial activity of the silver nanoparticles was performed by disc diffusion method against *Bacillus subtilis*, *Pseudomonas putida* and *Escherichia coli*. The antibacterial activity of synthesized silver nanoparticles by flower extract of *C. gigantea* was found against *B. subtilis* (10 mm). Synthesised AgNPs has the efficient antibacterial activity against Gram positive bacteria.

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## 1. Introduction

The studies on silver nanoparticles (AgNPs) in the consumer products could be attributed to their potent antimicrobial activity against a wide range of pathogenic microorganisms and it is an exciting area of nanotechnology (Klaine et al., 2008; Ahmad et al., 2003; Kowshik et al., 2003; Sinha and Paul, 2015). Silver nanoparticles are known to possess anti-

microbial activities against different pathogens including viruses and provide an excellent opportunity to develop new antiviral compounds.

The production and release of the AgNPS are to be continually documented (Impellitteri et al., 2009; Maregesi et al., 2008; Vorobyova et al., 1999; Zaidan et al., 2005). The recent studies also revealed that the AgNPs application have the capacity to control several plant diseases (Park et al., 2006). Nanoparticles play a pivotal role in the drug delivery due to high surface-to-volume ratio and thus can efficiently interact with various pathogens compare other biomolecules. Recent researches focus on the biologically synthesized nanoparticles with a size range from 1 to 100 nm (Zhang et al., 2016; Logeswari et al., 2015). Because medicinal and aromatic plants produce many secondary metabolites in the life cycle. (Gupta and Chadha, 1995; Singh et al., 2015).

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Nanotechnology is an advanced technology, which deals with the synthesis of nano-particles, processing of the nanoparticles and their applications [Nazem and Mansoori, 2008; Lead et al., 2018]. The term nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. Physical Professor Richard. P. Feynman gave the concept of nanotechnology in his lecture [Murty et al., 2013]. The medicinal application of nanotechnology is known as nanomedicine [Panneerselvam et al., 2011; Bhakya et al., 2015]. Today, nanotechnology and nanoscience approaches to particle design and formulating are stacking to develop the market for many drugs and are forming the basis for a highly profitable niche within the industry [Bandyopadhyay, 2009; Chaloupka et al., 2010]. Biosynthetic methods using plant extracts [Chandran et al., 2006; Gardea-Torresdey et al., 2002] or bacteria [Joerger et al., 2000], fungus [Shankar et al., 2003], honey [Philip, 2010] is a simple and viable process which is an alternative to yield nanoparticles.

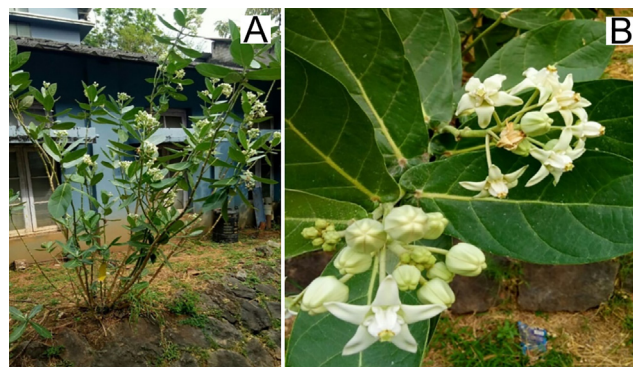
Nano-sized particles ranging below 10 nm are of great interest, because of the chemical and physical behaviour of the particles arising from the quantum size effect. The various types of nanoparticles are inorganic nanoparticles, polymeric nanoparticles, solid lipid nanoparticles, liposomes, nanocrystal, nanotube [Ismail et al., 2016]. Silver ions are released by the nanoparticles which will enhance the bacterial activity. Bactericidal effect of silver nanoparticles is size dependent because nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhances the reactivity of nanoparticles [Rishabh, 2019].

In this present study, interactions of biologically synthesized silver nanoparticles from flower extracts of *Calotropis gigantea* (L.) W.T. Aiton (Apocynaceae Juss.) and evaluated the antibacterial activity against *Bacillus subtilis*, *Pseudomonas putida* and *Escherichia coli*. The silver nanoparticles used in this study were synthesized by treating  $\text{AgNO}_3$  with flower extracts of *C. gigantea* reveals a source of antimicrobial effects. *C. gigantea* is a genus of plants that produce milky sap hence also commonly called milkweed [Kumar et al., 2010] and also known as Madar. It is considered as a common weed in some parts of the world mainly in Asian countries. The flowers are fragrant often used in making floral tassels in some mainland Southeast Asian cultures and their flowers are used for coughs, asthma, and loss of appetite and many other diseases. The use of silver nanoparticles from flowers of *Tagetes erecta* has been used against bacteria and showed an alternative to improve antimicrobial activity (Padalia et al. 2015). Here, we applied an eco-friendly route to produce silver nanoparticles from flowers of *C. gigantea*, combining natural products and metal nanoparticles, to obtain a therapy more efficient against bacteria.

## 2. Materials and methods

### 2.1. Collection and extraction of plant material

Fresh flowers of *Calotropis gigantea* L.W.T Aiton were collected from the campus of Vimala College (Autonomous) Thrissur, Kerala (Fig. 1) *C. gigantea* mediated synthesis of AgNPs was carried out based on green synthesis procedure with slight modifications (Babu and Prabu, 2011). *C. gigantea*

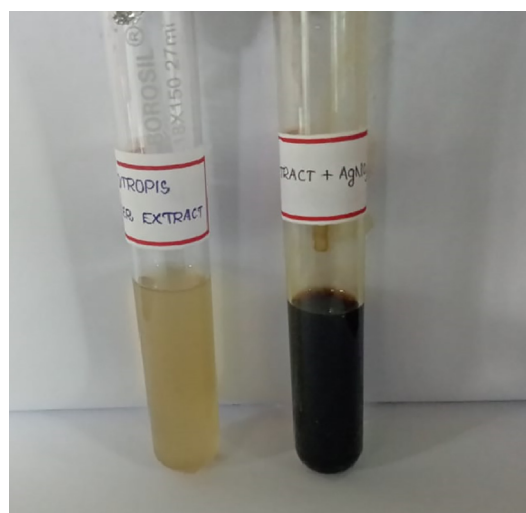


**Fig. 1** (A). Arbustive habit of a specimen of *Calotropis gigantea* collected in the Vimala college (Autonomous) Thrissur, Kerala. (B). Flowery branches showing different developmental stages of white flowers.

fresh flowers (20 g) were washed thoroughly with distilled water for 15 min and dried in shade condition. Flowers were finely crushed and stirred with 200 mL distilled water at room temperature for 15 min and filtered. This filter was used as green chemical reducing as well as stabilizing agent. Flower extract was stored at 4°C for further use.

### 2.2. Biosynthesis of silver nanoparticles

For the synthesis of AgNPs, 2 mL of flower extract and 15 mL of  $\text{AgNO}_3$  were used. The reaction mixture was prepared by adding 15 mL of  $\text{AgNO}_3$  (0.4 g  $\text{AgNO}_3$  in 250 mL water) and 2 mL of flower extract. This stoichiometric proportion was fixed based on initial testing with UV – Visible spectroscopy. The reaction mixture was kept at room temperature for 24 h in dark condition. Colour change pattern from yellow to dark brown indicates the formation of AgNPs (Fig. 2).



**Fig. 2** Solution of Silver Nitrate (1 mM) before (left) and after (right) exposure to the reaction (extract of *Calotropis gigantea* plus  $\text{AgNO}_3$ ) after 24 hrs incubation.

### 2.3. Characterization of silver nanoparticles

The colour change observed in the solution that showed the formation of silver nanoparticles. Synthesized silver nanoparticles were confirmed by sampling the reaction mixture at regular intervals and absorption maxima was scanned by UV–vis spectra, at the wave length 200–600 nm. Centrifugation of AgNP is widely used to get rid of unreacted materials and by products. Proper separation and purification are highly required for the characterization and applications of nanoparticles (Archana, 2016; Srikar et al., 2016). So, the reaction mixture was subjected to centrifugation at 1500 rpm for 10 min. Following which the pellet was re-dispersed in sterile distilled water was repeated thrice to ensure the better separation of free entities from the metal nanoparticles. The *C. gigantea* flower extract embedded with silver nanoparticles was freeze dried, powered and used for X-ray diffractometer (XRD) analysis. The diffracted intensities were recorded from 10 to 100 2 $\theta$  angles.

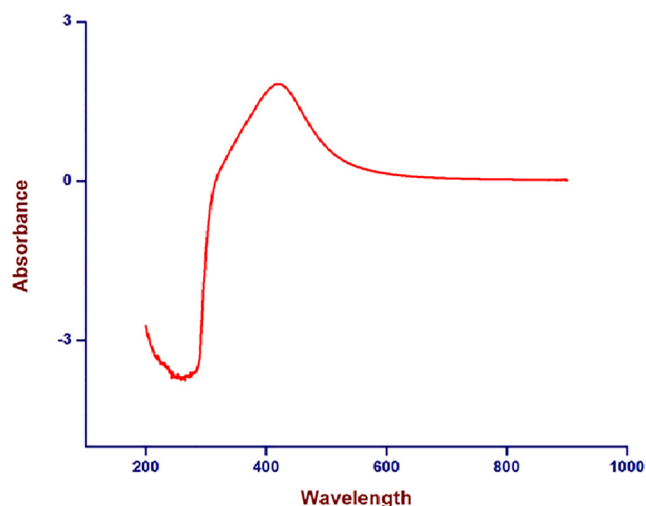
### 2.4. Invitro inhibitory activity of silver nanoparticles against bacteria

The antibacterial activity of *C. gigantea* and green synthesized silver nanoparticles were tested by the disc diffusion method for the determination of antibacterial activity against *Pseudomonas putida*, *Bacillus subtilis* and *Escherichia coli*. A modified agar disc diffusion method was adopted to determine antibacterial activity (Bhakya et al., 2016; Rajkuberan et al., 2015). The bacterial suspension,  $1 \times 10^6$  CFU mL<sup>-3</sup>. These three bacteria were inoculated on Muller-Hinton agar plates. 100  $\mu$ l samples of synthesized AgNPs solution were added to individual discs in separate plates. After incubation at 37 °C for 24 h, the zone of inhibition was measured.

## 3. Results and discussion

Plants from Angiospermae bloom periodically, what produce natural mass in a renewable way, sustainable. Reports have been showed flowers from different species in synthesis of variety types of metallic nanoparticles and applied as antimicrobial and antioxidant (Eswaraiah et al., 2020; Dahibhate et al., 2020; Tan et al., 2002). This is the first assay using flowers of *C. gigantea* to synthesis of AgNPs.

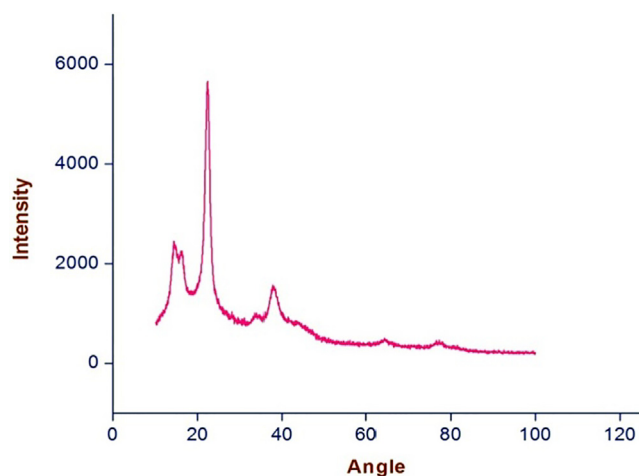
Reduction of silver ions in the aqueous solution of silver during the reaction with the ingredients present in the *C. gigantea* flower extracts was observed by UV–visible spectroscopy (Fig. 3). The change in colour was noted by visual observation in the flower extracts when it was incubated with silver nitrate solution. The colour of the flower extract changed to light brown within an hour and then later changed to dark brown during the 2 h incubation period (Fig. 2). The brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles. Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the flower of *C. gigantea* extracts observed by the UV–vis spectroscopy revealed the presence of silver nanoparticles may be correlated with UV–vis spectra. These changes were attributed to the excitation of Surface Plasmon Resonance (SPR) in the metal nanoparticles [Prabhuram et al., 2003]. Besides, the plasmon bands are



**Fig. 3** UV visible spectrum of *C. gigantea* flowers as a function of time. The absorbance peak corresponds to the plasmon resonance of silver nanoparticles at 422 nm.

broadened with an absorption tail in the longer wavelengths which may be due to the size distribution of the particles.

To confirm the crystalline nature of the particles studies were carried out using X-ray diffraction. The XRD pattern obtained was shown in Fig. 4. It is important to know the exact nature of the silver particles formed and this can be deduced from the XRD spectrum of the sample. XRD spectra of pure crystalline silver structures have been published by the Joint Committee on Powder Diffraction Standards. The XRD pattern shows that the intense peaks in the whole spectrum of 2 $\theta$  values ranging from 10 to 100. The phytosynthesized silver nanostructures were characterized with powder XRD technique. The peaks at 37.8°, 44.0°, 64.2° and 77.0° in the XRD pattern corresponds to diffraction from (111), (200), (220) and (311) planes of Ag, respectively. In addition to this, some extra peaks (14.3°, 16.1°, 22.3°, 34.0°) were also observed in



**Fig. 4** XRD spectrum of silvernanoparticles formed after 24 h of incubation of the *C. gigantea* flowers filtrate treated with silver nitrate solution.

the XRD pattern. This could be due to certain photochemical compounds of flower extract present in the sample (see Fig. 6).

Antibacterial activity was evaluated for the synthesized silver nanoparticles against *E. coli*, *B. subtilis*, *P. putida* by using the disc diffusion method. The results showed that the biosynthesized silver particles from *C. gigantea* flower extracts have resistance against Gram positive bacteria like *B. subtilis* (10 mm) and at the same time gram negative bacteria like *E. coli* (9.5 mm) showed high resistance against the

nanoparticles (Table 1 & Fig. 4). The results were observed in terms of IZ around the disc caused by diffusion of antibacterial properties from the plant-based silver nanoparticles impregnated disc into the surrounding medium (Fig. 5).

The antimicrobial properties of silver nanoparticles depend on size and environmental conditions (size, pH, ionic strength and capping agent). The exact mechanisms of antimicrobial or toxicity activities by silver nanoparticles are still in investigation and a well debated topic. The positive charge on the Ag ions is suggested vital for antimicrobial activities. In order for silver to have any antimicrobial properties, it must be in its ionized form. In its ionized form, silver is inert but on coming in contact with moisture it releases silver ions [Klueh et al., 2000, Mushir et al., 2016, Reddy et al., 2014]. Several phyto constituents have been isolated and identified from different parts of the *C. gigantea* belonging to the category of alkaloids,

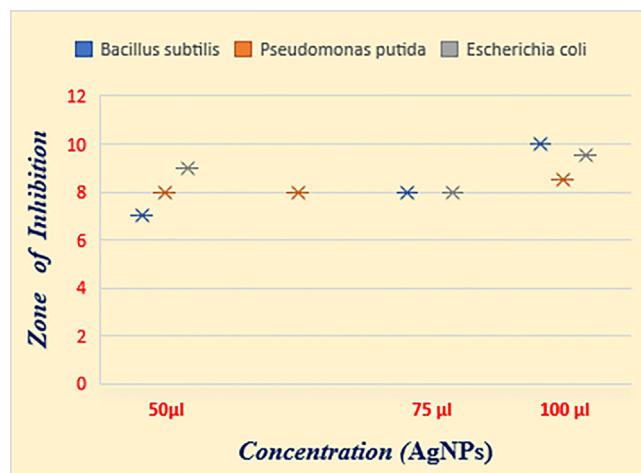


Fig. 5 Inhibition zone by Bacteria.

**Table 1** *In vitro* inhibitory activity of Silver Nanoparticles against bacteria.

Bacteria	Silver nanoparticle from <i>Calotropis gigantea</i>		
	Diameter of Zone of Inhibition(mm) of aqueous concentration of NPs		
	50 µl	75 µl	100 µl
<i>Bacillus subtilis</i>	7 ± 0.2	8 ± 0.3	10 ± 0.3
<i>Pseudomonas putida</i>	8 ± 0.1	8 ± 0.1	8.5 ± 0.1
<i>Escherichia coli</i>	9 ± 0.3	8 ± 0.3	9.5 ± 0.1

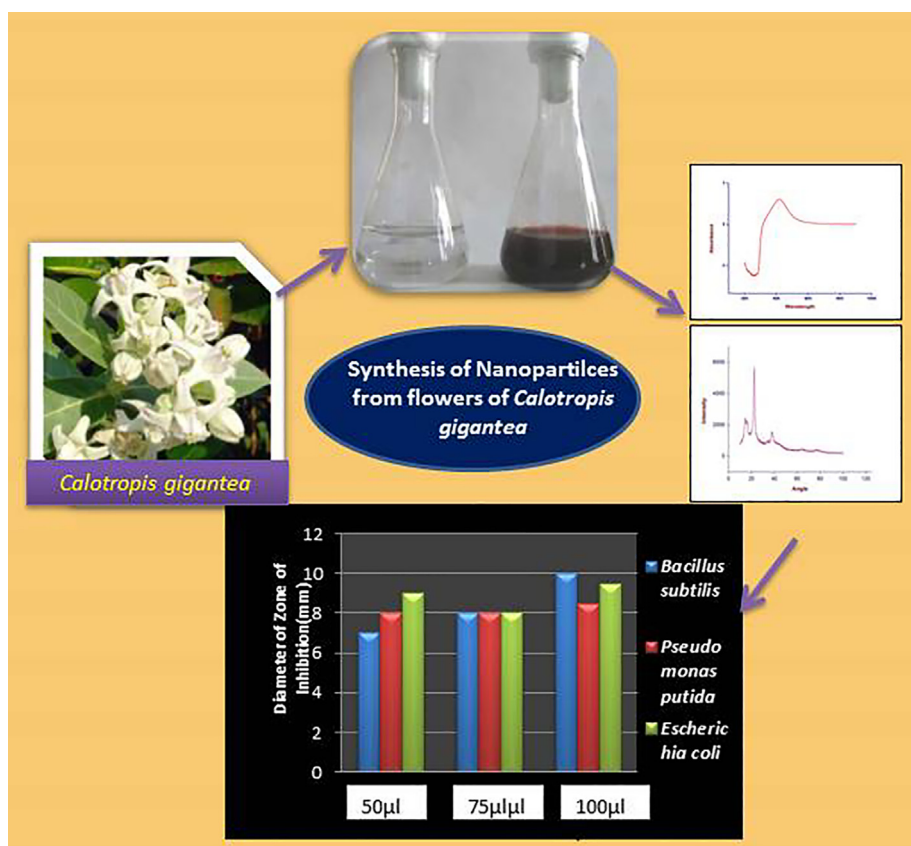


Fig. 6 Schematic representation of the production of silver nanoparticles from *Calotropis gigantea*.

glycosides, flavanols, tannins, saponins, sterols and triterpenoids and these phytochemicals are the responsive group for the synthesis of silver nanoparticles [Joshi et al., 2011].

Ag<sup>+</sup> ions are able to form complexes with nucleic acids and preferentially interact with the nucleosides rather than with the phosphate groups of nucleic acids. Thus, all forms of silver or silver containing compounds with observed antimicrobial properties are in one way or another sources of silver ions (Ag<sup>+</sup>); these silver ions may be incorporated into the substance and released slowly with time as with silver sulfadiazine, or the silver ions can come from ionizing the surface of a solid piece of silver as with silver nanoparticles [Sondi and Salopek-Sondi, 2004, Roucoux et al., 2003].

The results from this study reveal that Gram negative bacteria are resistant to the action of tested extracts and the same have been reported by several researchers for other plant based extracts. (Ncube et al., 2008). Cos et al., 2006 evaluated that Gram negative bacteria are generally more resistant when compared to the gram positive ones. This is because of Gram negative bacteria strains have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents (Nikaido and Vaara, 1985). The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherer and Gerhardt, 1971). Antibacterial study reveals that silver nanoparticles possess mild potential antibacterial activity against *B. subtilis*. Maximum zone of inhibition was observed in *Bacillus*.

#### 4. Conclusion

From the results obtained, the biosynthesized nanoparticles from *C.gigantea* flower extracts showed that the environmentally benign and renewable source of *C.gigantea* can be used as an effective reducing as well as stabilizing agent for the synthesis of AgNPs. The formation of AgNPs was observed by visible colour change from yellow to dark brown and confirmed by UV vis analysis. These nanoparticles showed characteristic absorption peak 422 nm in UV spectra. Crystalline nature of silver nanoparticles was studied using X-ray diffraction. Antibacterial activity evaluated for synthesized AgNPs showed that biosynthesized AgNPs from the flower extract have resistance against Gram positive bacteria like *B. subtilis*. This type of nanoparticle biosynthesis is an environmentally acceptable, nontoxic approach.

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