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Green Synthesis of Silver Nanoparticles by *Crescentia cujete* L. Leaves Extract and their Antibacterial Activity

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A B S T R A C T

Nanotechnology is the most promising technology, with wide range of applications in practically every aspect of life. Its applications rely totally on the type of the nanoparticles used. Metal nanoparticles are immensely valued. In spite of chemical synthesis being easier biological synthesis using plants, microorganisms, enzymes are preferred. The antibacterial property of silver has been recognized since ages. Silver has powerful inhibitory, bactericidal effect on broad spectrum of bacteria, fungi, virus. In this work, we have synthesized silver nanoparticles biologically using *Crescentia cujete* L. leaves extract and characterized by standard physical techniques. The antibacterial property of these nanoparticles were studied on gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*). The synthesized AgNP was found to be stable with a size of 52 nm and show SPR at 441 nm. The results clearly showed that the growth of Staphylococcus aureus as well as Escherichia coli were inhibited by the synthesized silver nanoparticles.

***Key words*:** Nanotechnology, Silver nanoparticles, *Crescentia cujete*, Biological synthesis, Antibacterial assay

Technology that is implemented at the nanoscale level, i.e., 1-100 nm is called Nanotechnology [15]. It is the most promising technology, with wide range of applications in practically every aspect of life, including defence, electronics, pharmaceuticals, sports, transportation, aesthetics and heat transfer [5-6], [8], [14], [20-21], [27]. The applications of nanotechnology rely totally on the type of the nanoparticles (NPs). Nanoparticle are submicron moieties ranging from 1 to 100 nm in diameters although there are examples of NPs several hundreds of nanometers in size [19]. Metal nanoparticles are immensely valued because of their extraordinary chemical and physical properties, like thermal conductivity and high surface-to-volume ratio [11]. Metal nanoparticles are implemented in almost every field like, nanomedicine, catalysis, photonics, drug delivery, sensors, electronics and biodiagnostics [11]. Synthesis of metal nanoparticles are achieved by different approaches including physical, chemical and biological method. Among these, chemical synthesis is the most common as its easier, efficient and cost-effective. In addition, of nanoparticle's size can be managed by changing various experimental factors. In spite of these benefits chemical synthesis are not ecofriendly as they use toxic chemicals. To solve these issues, biological synthesis using plants, microorganisms, enzymes are preferred. In this work, we have synthesized silver nanoparticles biologically using *Crescentia cujete* L. leaves extract.

The antibacterial property of silver has been recognized since ages [7], [22] and also when used in low concentrations it is non-toxic to humans [17]. Because of the rise in bacterial resistance to traditional antibiotics, researchers have been looking into the antibacterial efficacy of silver nanoparticles [18]. Silver and its derivatives have powerful inhibitory, bactericidal effect on broad spectrum of bacteria, fungi, virus [4], [13], [23]. In comparison to other metals silver shows increased toxicity to different microorganisms and decreased toxicity to mammalian cells [28]. Silver nanoparticles synthesized by different methods have shown to have effective antimicrobial property [1-4], [9], [12-13], [16], [23-24], [28]. Owing to these properties silver nanoparticles are used in a large number of products, like burn dressings, medical devices, water purification systems, scaffold [10], [25].

In this work, we have synthesized silver nanoparticles biologically using *Crescentia cujete* L. leaves extract. The synthesized silver nono particles were characterized by standard physical techniques to evaluate their physiochemical properties. The antibacterial property of these nanoparticles were studied on gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*).

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MATERIALS AND METHODS

Synthesis of silver nanoparticles (Ag NP)

Preparation of Crescentia cujete L. leaf extract

Fresh leaves of *Crescentia cujete* L. were collected from Botanical Garden, VBU Campus and washed thoroughly with tap water followed by distilled water to remove any grime material. These were cut into small pieces and dried at room temperature. The extract was prepared by adding 10 g of leaf to 100 ml distilled water and boiled for 20 minutes. The extract was then filtered twice using Whatman No. 1 filter paper to get the clear extract and stored in Erlenmeyer flask at 4C for preparation of nanoparticles.

Synthesis of silver nanoparticles (Ag NP)

Aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared. To this solution, leaf extract was added in 1:1 ratio and left undisturbed for 48 hours in dark. The colour change from pale yellow to brownish yellow was an indication of formation of nanoparticles. After 48 hours the solution was washed thrice by centrifuging at 5000 rpm for 20 minutes and the pellet containing AgNP was collected.

Physiochemical characterization

The physiochemical characterizations of silver nano particles were done using standard techniques. The optical properties were determined by UV-Vis spectroscopy. The scanning was performed using UV-visible spectrophotometer (Cary 5000, Agilent, Santa Clara, CA, USA) by scrutinizing the spectral scan at a range of 200–800 nm. The hydrodynamic size and zeta potential of the silver nanoparticle was measured by dynamic light scattering (DLS) using Zetasizer (Malvern, UK). The size of the silver was further determined by electron microscopy by using FE-SEM (Carl Zeiss, Jena, Germany). The samples were dried in the hot sun and were imaged at 20 KV.

Antibacterial assay

Test organisms used were *Micrococcus luteus* ATCC4698, *E. coli* PTA8019, *Salmonella typhi* ATCC29630, *Staphylococcus aureus* BAA2686.

LB agar plates were prepared by pouring 20 ml LB agar in Petri dishes and allowed to solidify. LB broth culture of each bacterial strain (50 l) were used. Concentration of the microorganisms was 1 X 106cfu/ml. These bacterial cultures were spread on solidified agar plates using sterile spreader. Wells of 4mm were bored using sterile cork borer. Different concentration of nanoparticles were poured in each well. One well was set as control and it contained distilled water. These agar plates were then incubated overnight at 37±2 C. Zone of inhibition formed was determined.

RESULTS AND DISCUSSION

Green synthesis and characterization of silver nanoparticle

The green synthesis of silver nanoparticles (AgNP) was done by *Crescentia cujete* L. leaf extract as reported in the previous segment. The solution turned pale brown specifying the formation of AgNP (Fig 1). The synthesized nanoparticles were dried in oven followed by characterization. The UV-Vis spectrum analysis shows a sharp peak in (Fig 2A). The appearance of an absorbance peak has been described as one of the distinguishing aspects of nanoparticles that can be used to identify their optical qualities [26]. Green synthesized silver nanoparticle showed peak absorbance at 441 nm. The stability of Synthesized silver nanoparticles was checked by determining their zeta potential using dynamic light scattering. As shown in (Fig 2B), the silver nanoparticle was found to have a zeta potential of 30 ± 4 mV in HF medium, which indicated the stability of AgNP. The size of the AgNPs was further learned by FE-SEM as shown in (Fig 2C). The physicochemical characterization confirmed their nano properties and successful synthesis of nanoparticles.

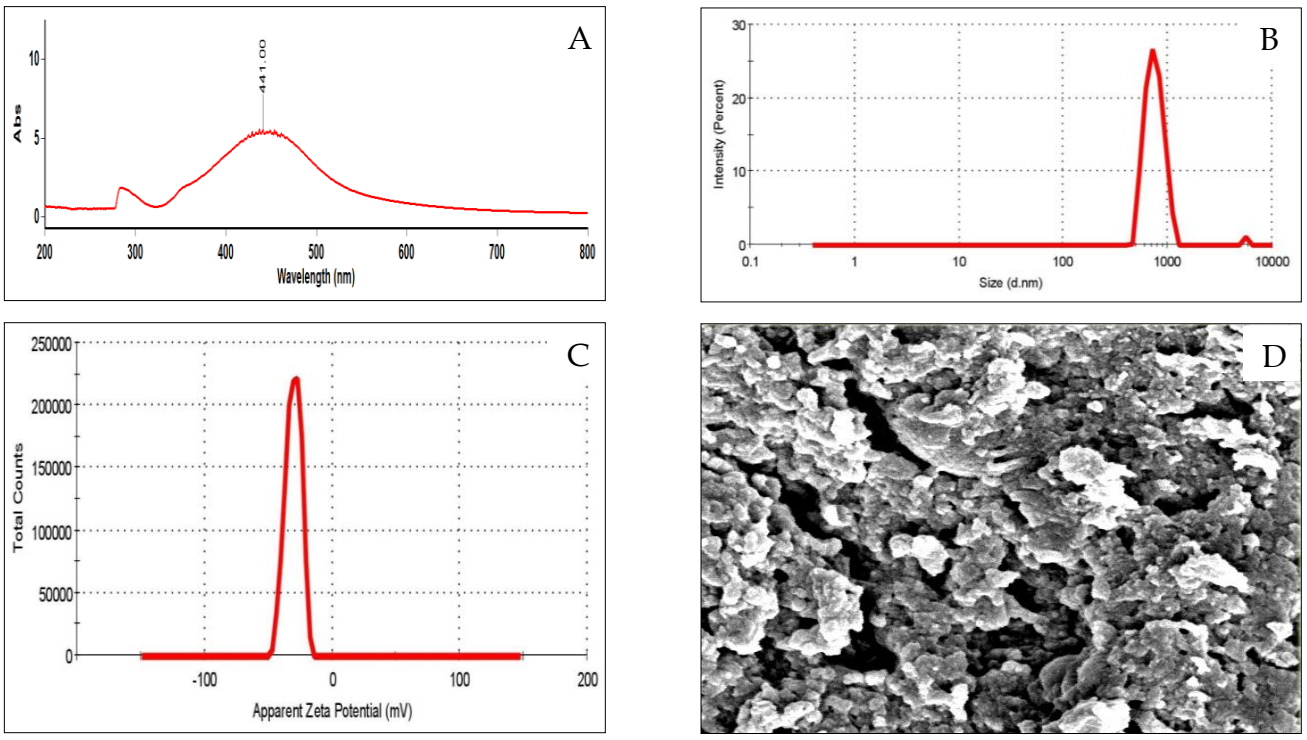

Fig 2 Characterization of biologically synthesized AgNP. (**A**) UV–Vis spectrum of AgNP; (**B**) hydrodynamic diameter of AgNP as dictated by dynamic light scattering; (**C**) zeta-potential of AgNP dictated by dynamic light scattering; (**D**) optical image of AgNP as determined by FE-SEM

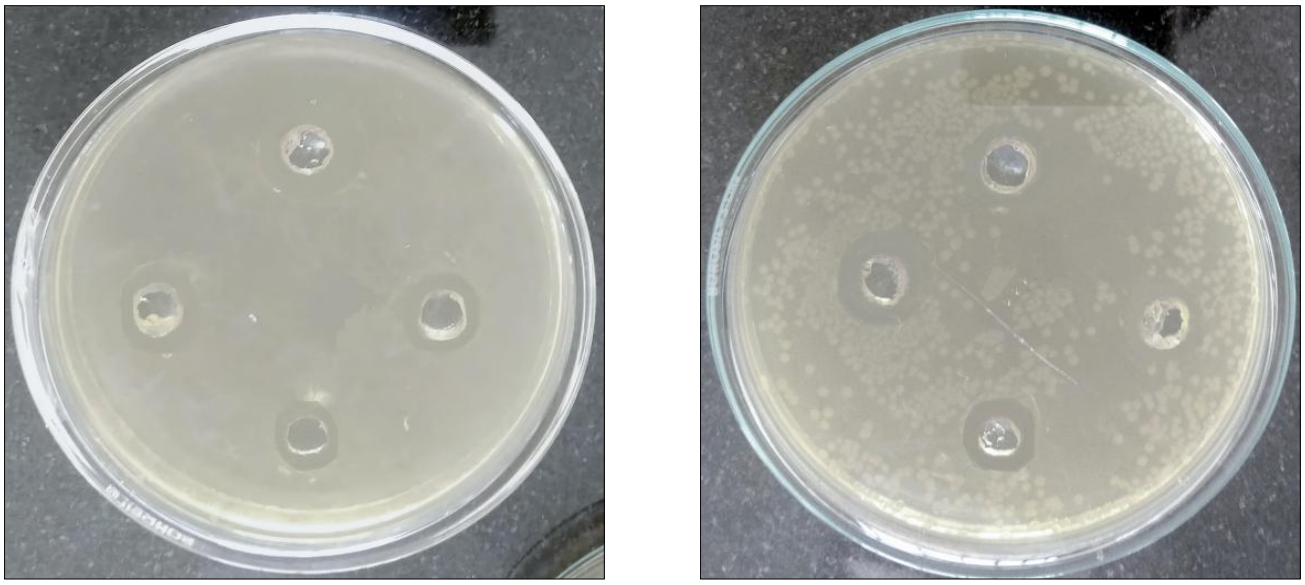

Fig 3 Antibacterial activity of biologically synthesized AgNP against (**A**) *Escherichia coli* and (**B**) *Staphylococcus aureus*

Table 1 Zone of inhibition formed (in mm) by biologically synthesized AgNP against (A) *Escherichia coli* and (B) *Staphylococcus aureus*

Concentration of silver nanoparticle	Zone of inhibition (in mm)	
	E. coli	Streptococcus aureus
50 µg/ml	22	18
100 µg/ml	24	20
150 µg/ml	25	21
200 µg/ml	26	22

Antibacterial activity of silver nanoparticles

The antibacterial activiy of silver nanoparticles synthesized biologically was checked against *Staphylococcus aureus* BAA2686 (gram positive) and *E. coli* PTA8019 (gram negative) strain and it inhibited both microorganisms. (Fig 2A-B) and (Table 1) shows the zone of inhibition formed by AgNP against both the bacterial strains. With increasing concentration of AgNP, the diameter of zone of inhibition also increased. The acting mechanism behind the formed zone of inhibition by the AgNPs is principally due to the disintegration of the plasma membrane, damage of the outer membrane and reduction of intracellular ATP.

CONCLUSION

The present study depicts the successful green synthesis and characterization of AgNP by an efficient, ecofriendly, cost-effective and economical method and gave useful insight into the creation of novel antimicrobial agents by enhancing the antibacterial mechanism against harmful microorganisms in a synergistic manner. The synthesized AgNP was found to be stable with a size of 52 nm. The 52 nm AgNP was characterized for their optical properties by showing SPR at 441 nm. The antibacterial property of the AgNP was measured by well diffusion method. The results clearly showed that the growth of Staphylococcus aureus as well as *Escherichia coli* were inhibited by the synthesized silver nanoparticles.

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