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Gloriosa superba L. assisted green synthesis of nanoparticles and their bactericidal activity against pathogenic bacteria

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Green Synthesis of silver nanoparticles is receiving higher attention due to facile preparation, wide application in medical field and ecofriendly. In the present investigation, we are reporting crystallization of silver ions to nano sized particles by aqueous leaves extract of *Glroriosa superba* through bioreduction. Preliminary confirmation of formation silver nanoparticles was confirmed by UV-Vis Spectro photometer, where strong plasmon resonance of silver nanoparticles was observed around 435 nm. Fourier transform infrared spectroscopy [FTIR] and transmission electron microscope [TEM] were performed to examine the formation and size of silver nanoparticles [AgNPs]. Further these NPs were evaluated for antibacterial activity against four pathogenic bacteria *viz*., *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacter aerogenes*, and *Escherichia coli*. The field of plant disease biocontrol may benefit greatly from the use of green synthesised silver nanoparticles.

Keywords: Antibacterial and *Gloriosa superba* L., Nanoparticles, TEM

INTRODUCTION

Many approaches are available for the synthesis of silver nanoparticles viz., chemical and photochemical reactions in reverse micelles, thermal reduction of silver ions, through radiation, microwave-assisted process and also through green interaction route. The plant-mediated synthesis of silver nanoparticles is simple and easy process for large-scale production of nanoparticles. Over the past few years, synthesis and characterization of plant mediated nanoparticles has gained increasing momentum as they possess largesurface area to volume ratio therefore the NPs can exhibit newproperties than their macroscopic counterparts (Hano and Abbasi, 2021).

Nanomaterials have a long list of applicability in improving human life and itsenvironment. It is evident from, Indian System of Medicine;Ayurveda gives the detailed knowledge of nano-scale fabrication used for medicinalpurposes (Roopashree *et al*. 2021)and silver has been widely used for food preservation (Manna and Mondal, 2023), cosmetic industries and also in medicine. This makes silver an excellent choice for multiple roles in the medicalfield (Meher *et al*. 2024). Silver is generally used in the nitrate form to induce antimicrobial effect and when nanoparticles are used, there is a huge increase in the surface area to be incontact with microbial cells (Bruna *et al*. 2021).Silver nanoparticles (AgNPs) have gained significant attention due to their remarkable antimicrobial, antifungal, and anticancer properties. Their broad-spectrum antimicrobial activity has made them particularly valuable in the fields of medicine, agriculture, and environmental protection (Sofi *et al*. 2022). AgNPs can effectively target various pathogens, offering an alternative to traditional antibiotics and chemical agents (Sondi and Salopek-Sondi,

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2004). Given these properties, AgNPs are increasingly considered a key player in plant disease control, where they can help combat plant pathogens and improve crop health.

Gloriosa superba L., a medicinal plant, has shown potential for synthesizing various metal nanoparticles such as gold, cerium oxide, copper oxide (CuO), and zinc oxide (ZnO), which also exhibit significant bactericidal activities (Flores-Rábago*et al*. 2023). However, the focus on AgNPs in this study is motivated by their superior antimicrobial efficacy and widespread use in various applications, which makes them an ideal candidate for exploring plant disease management solutions. Additionally, the use of plant extracts, such as that of *Gloriosa superba* L., for the green synthesis of AgNPs offers an eco-friendly, cost-effective, and sustainable approach to nanoparticle production (Gopinath *et al*. 2016).

Biological systems have long been known toreduce metal ions into nano-sized particles (Manna and Mondal, 2023) and many researchers have recentlyreported the biogenic synthesis of silver nano-particles using a different biological resources like bacteria (Saravanan *et al*.2017), fungi (Ottoni*et al*. 2017) and plants (Bankalgi*et al*. 2016; Inamdar *et al*. 2018). Similarly plant mediated synthesis of nanoparticles have been reported from different authors from various plant parts like, seed (Sadeghi *et al*., 2015), leaf (Bankalgi*et al*. 2016), bark (Bharathi *et al*. 2018), stem (Shinde *et al*. 2018) and fruit (Shaikh*et al*., 2018). These AgNps have shown wide range of applications viz., anti-bacterial and anti- fungal (Parveen*et al*. 2012), antioxidant (Bhakya*et al*. 2016) and anti-cancer activity (Rajeshkumar*et al*. 2016).

In the present study AgNO3 was used for synthesis of NPs because silver is being used as bactericidal metal since many decades and is non-toxic to animal cells and highly toxic to bacteria (Marambio and Hoek, 2010; Nie*et al*. 2023) using aqueous leaf extract of *Gloriosa superba* L belonging family Colchicaceae. The plant is commonly called as flame lily, climbing lily. Every part of the plant is poisonous, especially the tuberous rhizomes. As with other members of the Colchicaceae, this plant contains high levels of colchicine, a toxic alkaloid.The alkaloid-rich plant has long been used as a traditional medicine in many cultures. It has been used in the treatment of gout, infertility, open wounds, snakebite, ulcers, arthritis, cholera, colic, kidney problems, typhus, itching, leprosy, bruises, sprains, hemorrhoids, cancer, impotence, nocturnal emission, smallpox, sexually transmitted diseases, and many types of internal parasites. By knowing the facts of medicinal properties of the plant and wider properties of $AgNO_3$, the present report was framed for fabrication of NPs.

MATERIALS AND METHODS

Collection of Plants and Extract Preparation

The healthy leaves of *Gloriosa superba* L. were collected from Department of Botany, Gulbarga University, Kalaburagi. 10 g of finely grinded leaves were boiled in 100 ml of distilled water on water bath at 60 ºC for 20 min. After cooling, the extracts were centrifuged and supernatant was used as reducing agent.

Synthesis of Silver Nanoparticles

For green synthesis of silver nanoparticles, 10 ml of filtered supernatant is dissolved $AgNO_3$ bearing the 1mM for reduction in silver ions and were incubated for 30 min under direct sunlight. Preliminary detection of Silver nanoparticles [AgNPs] was carried out by visual observation of color change(Fig. 1). Then samples were centrifuged at 6000 rpm for 30 min. The centrifugation was repeated three times from distilled water. Pellet was dried in watch glass and nanoparticles were weighed and stored at 4 ºC for further experimental studies (Vanlalveni *et al*. 2021).

Characterization of Silver Nanoparticles

The AgNPs were evaluated for the confirmation and characterization by different analytical techniques, Viz., optical and Spectral analysis were done by UV-VIS Spectrophotometer (Shimadzu UV-VISSpecrophotometer 2450), Fourier Transform Infrared Spectroscopy (Shimadzu FTIR Presiage 21) and morphological

and structural analysis of silver nanoparticles were carriedout by TEM equipment (JEOL model 1200 EX) by placing a drop of AgNP over carbon coated copper grids and allowing the solvent to evaporate.

Antimicrobial Activity
Bacterial Strains

Antibacterial activity *Gloriosa superba* leaf extract NPs were tested individually on different bacteria viz., *P. aeruginosa* (MTCC 2453), *Salmonella typhimurium*(MTCC 98), *Enterobacter aerogenes* (MTCC 111), and *Escherichia coli* (clinical strain). The above microorganisms were collected from Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India.

Antibacterial Testing by the Agar Diffusion Method

Primary evaluation of antibacterial activity of the NPs was done by agar well diffusion method by following the technique described by the Clinical and Laboratory Standards Institute (Wayne, 2009). Bacterial culture having 10^6 CFU/mL were inoculated onto petriplate containing nutrient agar medium using sterile swabs, where 6 mm wells were made and filled with 30 µL of 10µg concentrated silver nanoparticles, $AgNO_3$, *G. superba* leaf extract and standard antibiotic streptomycin (30 µg) used as positive control. Then the Petri dishes were incubated at 37 ± 1 ºC for 24 h. The antibacterial activity was done by measuring the zone of inhibition in diameters (mm).

RESULTS AND DISCUSSION

UV–VIS Spectrum of Silver Nanoparticles

Silver particles at nano-range exhibit an unusual optical phenomenon called Surface Plasmon Resonance (SPR), due to the cumulative oscillation of the conducting metal surface electrons in resonance with the non-particulate radiation. This property is largely governed and dependent upon the particle type, size, shape and the local chemical ambience. The characteristic fingerprint zone which exhibits this phenomenon (by the AgNPs) predominantly appears in the range of 400–500 nm respectively (Venugopal and Mitra, 2013; Anshiba *et al*. 2022). Similarly the *G. superba* assisted NPs have exhibited increase in the absorbance/ resonance between 380-450 nm from UV-Vis spectra(Fig. 2).

Transmission Electron Microscopy (TEM)

TEM analyse was employed to test the size and shape ofthe synthesized nanoparticles. From Fig. 3it can be concluded that AgNPs were mostly uniform and ovoid inshape with an average size of about 25-35 nm. These NPs were not in direct contact even in aggregate form and it is observed that, they were surrounded by a thin layer (to be characterized), indicating stabilization of the nanoparticles by a capping agent (Jagtap and Bapat, 2013).

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy analysis was used to characterize and identify the biomolecules that were bound specifically on the synthesized AgNPs. FTIR spectrum measurements of the AgNPs showed the presence of eight intense bands at 3370, 1772, 1639, 1512, 1374, 1219,1110 and 1061cm^{-1}(Fig. 4). The spectra exhibited a broad peak located at 3370 cm^{-1}, which can be assigned as stretching vibrationsindicating the presence of hydroxyl groups. Less intense peak at 2918 and 2853 cm^{-1} could be assigned to presence of secondary amines and C–H stretching vibrations. The strong band at 1639 and 1374 cm-1 was mainly attributed to the amide bands, –C–N stretching vibrations of proteins. The peak located at 1110 and 1061 cm^{-1} can be assigned as the absorption peak of O–H (primary alcohol) and C–O stretching respectively (Ahluwalia *et al*. 2014). The spectrum supports the presence of alcohol, phenols, carbonyl and amines (both aromatic and aliphatic) and amide functional groups in the synthesis of silver nanoparticles (Jayaseelan *et al*., 2013).

Antibacterial activity

Microorganism inhibitory mechanism of Ag^+ ions is yet partially known. As per the evidences and previous reports states that the positive charge

Table: 1. Antibacterial activity of silver nanoparticles derived from *G. superba* L. against pathogenic bacteria

Test organisms	$AgNO_3$ (mm)	AgNPs (mm)	Streptomycin (mm)
S. typhimurium	03.21±0.18	06.18±0.28	12.06±0.33
P. aeruginosa	19.18±0.33	22.07±0.21	27.18±0.67
E. coli	12.27±0.20	16.17±0.27	22.03±0.89
E. aerogenes	09.12±0.21	15.21±1.20	21.33±0.67

on the Ag ion is crucial for its antimicrobial activity *i.e.*it may be through the electrostatic attractions between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles (Hamouda *et al*. 2001; Dibrov*et al*. 2002). According to Franci *et al*. (2015)AgNPs have less effect on the growth of Gram +ve bacteria compared to Gram –ve bacteria as both gram's bacteria have structural difference and different cell wall composition. In the present study we are reporting antibacterial efficacy of *G. superba* derived AgNPs against four gram negative pathogenic bacteria *viz.*, *S. typhimurium*, *E. aerogenes*, *P. aeruginosa* and *E. coli*. The attribution of inhibitory effect of NPs is exertion of mechanisms of toxicity are viz., movement of free ions into the cell followed by distraction of ATP production and DNA replication (Lok *et al*.2006) and accumulation of surplus levels of Reactive Oxygen Species [ROS] (Park and Park, 2009) this results in directive damage to cell membranes (Raffi *et al*.2008). These *G. superba* derived NPs have shown maximum zone of inhibition against *P. aeruginosa* (22.07±0.21) followed by *E. coli*(16.17±0.27),*E. aerogenes* (15.21±1.20) and *S. typhimurium* (06.18±0.28) (Fig. 5). Our results (Table 1) are in agreement withthe results ofBankalgi*et al*. (2016). Recent scientific reports clearly states that inorganic molecules like Ag, Zn, Cu etc., based nanoparticles have significant activity as bactericidal and many are in search of potent inorganic nanoparticles as effective antibacterial agents (Crabtree *et al*. 2003; Abuskhuna*et al*. 2004) because these have unique advantages over standard drug which are available in the market because many bacteria have developed resistance towards many conventional antibacterial agents and also developed multidrug resistance. Till date chemical based antimicrobial compounds are limited to use in medical devices and prophylaxis in antimicrobial facilities. Therefore, it is very necessary prepare cost effective bactericidal and find alternative way to overcome the multidrug resistance of microorganisms (Parveen *et al*., 2012; Parmanik *et al*., 2022).

CONCLUSION

In this report, *G. superba* L. leaf extract was used as a reducing agent for the synthesis of AgNPs. Facile method was used for green, and cost effective method to synthesize AgNPs at room temperature without using any harmful chemicals. The successful synthesis was confirmed by UV–VIS, FTIR and TEM analysis. From this study, AgNPs were observed to have strong andalmost equal antimicrobial activity when compared with the standard drugsterptomycin. This eco-friendly, cost effective method of AgNPs synthesis willopen a new field where the medicinal plants are become useful to generate thepharmaceutical products. Further scope is provided to identify the capping molecules which are narrowly responsible for having such activities.

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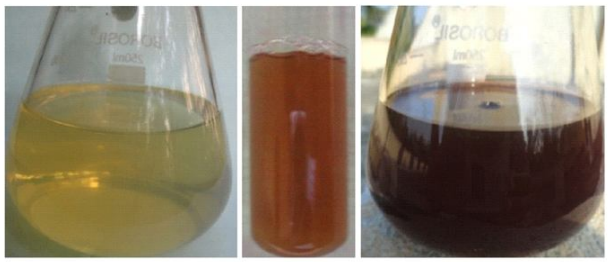

Fig. 1. Changes in the colouration indicating preliminary confirmation of reduction of Ag^{+} ions.

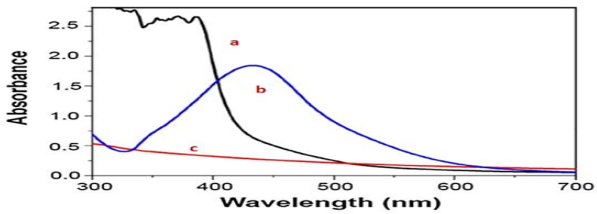

Fig. 2 : UV-VIS spectra of AgNPs displaying a strong peak at 435 nm

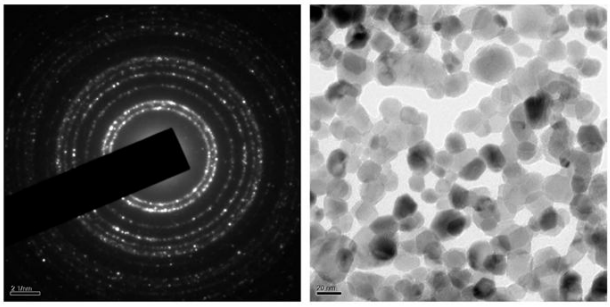

Fig. 3: TEM images showing the crystallite size of the material which were found to be around ~ 25 nm

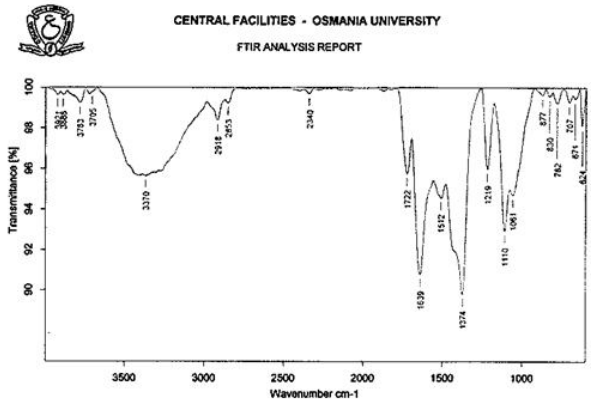

Fig. 4. FTIR spectra of AgNp derived from *G. superba* L.

bacterial cultures and UV–VIS spectra. They also extend their appreciation to Mr. A. Manjuantha of the Department of Physics, Gulbarga University, Kalaburagi, for his assistance in interpreting the

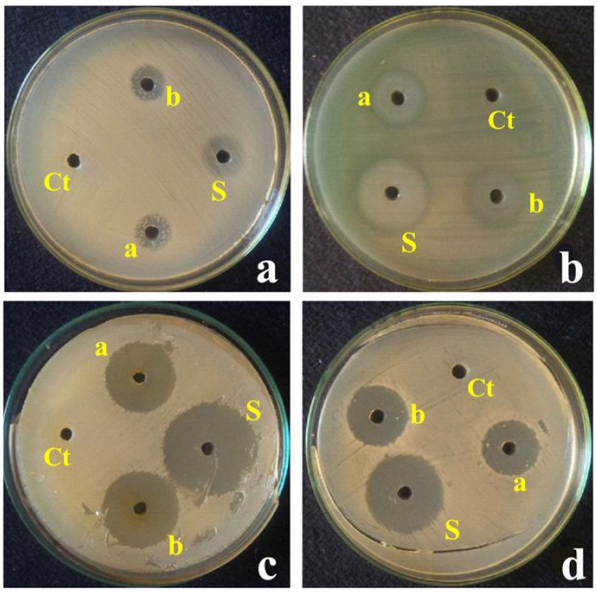

Fig. 5 : Antibacterial activity of *G. Superba* derived NPs, $AgNO_3$ and Standard (Streptomycin) **a.***S. typhimurium***, b.***P. aeruginosa* **c.** *E. coli***d.** *E. aerogenes***;**Ct= Control, S= Standard, a= $AgNO_3$, b= AgNPs

FTIR data. Additionally, the authors acknowledge the support of the Central Facilities for Research & Development at Osmania University, Hyderabad, Telangana, for performing the TEM analysis of the samples.

DECLARATION

Conflict of interest. The authors declare no conflicts of interest related to this work.

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