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Synthesis of silver nanoparticles using the seed extract of *Ensete superbum* and their antibacterial activity assessment

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Seeds of *Ensete superbum* have been used in Ayurvedic medicine to treat kidney stones and diabetes. The current work report the synthesis and characterization of silver nanoparticles (AgNPs) and their antibacterial property. The synthesis of AgNPs was done by mixing silver nitrate solution with aqueous *E. superbum* seed extract. The pale-yellow colour of the seed extract was changed to deep brown due to the reduction of silver ions to AgNPs, under ambient conditions. The characterization of AgNPs was carried out by UV–Visible spectroscopy, TEM, XRD, and FTIR. The peak absorbance of the UV–Vis spectra was at 420 nm confirming the formation of AgNPs. TEM showed the existence of spherical and hexagonal-shaped nanoparticles. XRD results show that the AgNPs were face-centered cubic (fcc) lattices. FTIR analysis established a link that the presence of different classes of compounds viz. flavonoids, alkaloids, saponins, and terpenoids in *E. superbum* seed extract is responsible for the reduction and stabilization of AgNPs. The current study aims to point out the application of AgNPs as an antibacterial agent against *S. typhi*, *P. aeruginosa*, *K. pneumoniae*, and *V. cholerae*, using the well diffusion method. The AgNPs effectively inhibited bacterial growth against *P. aeruginosa* and *S. typhi.*

Keywords: Antibacterial activity, *Ensete superbum*, Musaceae, Seed extract, Silver nanoparticles.

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Introduction

Nowadays, researchers show a great interest in synthesizing metal and metal oxide nanoparticles (NPs) using plant extract. They are environment-friendly, stable, clinically adaptable, biocompatible, and cost-effective. Among metal NPs[1], silver nanoparticles (AgNPs) are gaining enormous interest in the research community due to their wide scope of application in microbiology, pharmacology, and parasitology. Biogenic synthesis of AgNPs using plant extract as a reducing agent and their antibacterial activity is widely reported[2].

Plant seed extracts are well established for the biosynthesis of nanoparticles. To date, various seed extracts have been utilized for the biosynthesis AgNPs. Several plant seed extracts such as *Durio* zibethinus[3], *Tectona grandis*[4], *Persea americana*[5], and *Trigonella foenum*-graecum[6] produced AgNPs with high antimicrobial activities. Several plant seeds such as *Nigella arvensis*[7], Linseed[8], *Embelia ribes*[9], *Melissa officinalis*[10] are used for the synthesis of spherical shape AgNPs.

Ensete superbum (Roxb.) Cheesman. (Musaceae Family) is commonly known as wild banana. It is found in the Western Ghats of India . The plant is well documented for culinary (vegetables and fruits) and multiple medicinal requirements of the local people, traditional healers, and many communities of India and Ethiopia[11]. Seeds and pseudostem of *E. superbum* are reported to be used for the treatment of diabetes, debility, calculi, measles, leucorrhoea, stomachache, chickenpox, smallpox, and child delivery-related pain in Ayurveda[11]. Fruits are used for the treatment of burning sensation and kidney stones by the communities in the Western Ghats, Shimoga region, Karnataka[12]. People of coastal Karnataka utilize stems, fruits, and seeds for food purpose[13]. TLC study of the seeds and pseudostem of *E. superbum* has revealed the presence of carbohydrates, flavonoids, alkaloids, saponins, and terpenoids[14,15]. Chroman derivative ($C_{16}O_4H_{22}$) and tetrahydro-β-carboline were isolated from *E. superbum* seed and pseudostem[16,17]. Seeds contain fatty oil, proanthocyanidin, triterpenoid esters,

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pelargonidin, pro-pelargonidin glucosides, 4-hydroxy-3-methylhex-5-enyl, 2-hydroxy-9-phenylphenalenone, 2-hydroxy9-(4-hydroxyphenyl)-phenalen-1-one (hydroxylanigorufone), 8-hydroxy-7-methoxy-6-phenylphenalen-1-one, phenylphenalenones derivatives and fractions such as VIDR-2T, VIDR-2GC, and VIDR-2GD[11].

The objective of this study was the green synthesis of silver nanoparticles using aqueous seed extract of *E. superbum*, characterization of the synthesized nanoparticles, and evaluation of the antibacterial efficacy of the AgNPs in laboratory conditions.

Materials and Methods

Materials and reagents

Silver nitrate AR grade ($AgNO_3$), nutrient agar, and nutrient broth were purchased from Sigma-Aldrich and ciprofloxacin was procured from a local chemist shop. The deionized water was used throughout the experimental procedure. All glassware were washed with dilute nitric acid HNO_3 and distilled water, then dried in a hot air oven.

Plant sample collection and identification

E. superbum seeds were collected in December 2019 from Anavatti, Shivamogga, Karnataka, India (Fig. 1). The plant sample was identified by Prof. V. Krishna, Professor, Department of Biotechnology, Kuvempu University and deposited at the Department of Biotechnology, Kuvempu University, Shankaraghatta (Voucher specimen number: KUBT2001).

Preparation of extract

1.0 g of dried seeds were coarsely crushed and mixed with 10 mL of deionized water. The mix was boiled in a water bath for 10 min and filtered with a Whatman No. 1 filter paper to get the extract. Then the extract was centrifuged at 5000 rpm for 10 min, and the supernatant was filtered in flasks and stored in a 4 ^{o}C refrigerator for further use.

Synthesis of silver nanoparticles (AgNPs)

The AgNPs were synthesized using 10 mL of 1 mM silver nitrate solution and 0.5 mL of aqueous seed extract of *E. superbum*. It was incubated at room temperature for 2 h. A colour change was observed from pale yellow to dark brown indicating that the reaction was terminated. In the meantime, the colour change of the mixture from pale yellow to dark brown was monitored by periodic sampling and scanning by UV-visible spectrophotometry[18]. The resultant mixture was centrifuged at 10,000 rpm for 10 min (REMI, India). The pellet obtained was washed 3 times with deionized water and finally with acetone. The purified pellet was dried and used for characterization and antibacterial studies.

Characterization of AgNPs

AgNPs were characterized by UV-Vis spectrophotometer (SHIMADZU UV-1800) in the wavelength range of 300–500 nm. The X-ray diffraction (XRD) patterns of AgNPs were obtained using in Rich Seifert X-ray diffractometer, operating at a voltage of 45 kV and a current of 40 mA with CuKα radiation between 2θ° angles ranging from 20 to 90°. Fourier transform infrared spectroscopy (FT-IR) study was carried out and the spectrum was recorded in the range of 4000-400 cm^{-1} using FT-IR (3000 Hyperion Microscope with Vertex 80 FTIR System) instrument (Bruker, Germany) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr

Fig. 1 — a) Fruits of *E. superbum*, b) seeds of *E. superbum*.

pellets. Transmission electron microscopy (TEM) images were taken using Philips CM 200 high resolution (2.4 Å) TEM operating at an accelerating voltage of 20-200 kV. The test sample was placed on the carbon-coated copper grid and dried before microscopy.

Antibacterial activity of AgNPs

Antibacterial activity of AgNps against clinical isolates of *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* was carried out by standard agar well diffusion method[9]. Bacterial strains were spread on the Petri dishes which contained autoclaved nutrient agar and then wells were made using a sterilized cork borer. The prepared wells were filled with an equal volume of AgNPs mixed in deionized water (50 µL) and standard drug (ciprofloxacin 125 µg/mL) separately. The plates were incubated at 37 °C. The zone of inhibition (ZI) was measured after 24 h. The same procedure was repeated for control (sterile water).

Results and Discussion

Synthesis of AgNPs and its UV–Vis characterization

The primary detection of the formation of AgNPs was done by visual observation. The change in colour of the reaction solution from pale yellow to a dark brown with the increase in time provides evidence of the formation of AgNPs (Fig. 2).

UV-visible spectroscopy is a widely used analytical technique to monitor the formation of AgNPs. Upon interaction with an electromagnetic field, the conducting electrons present in the outermost orbital of metal NPs collectively oscillate in resonance with certain wavelengths to exhibit a phenomenon called surface plasmon resonance (SPR). The excitation of SPR is responsible for the formation of colour and absorbance in a colloidal solution of AgNPs[2]. AgNPs are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm because of surface plasmon resonance[19].

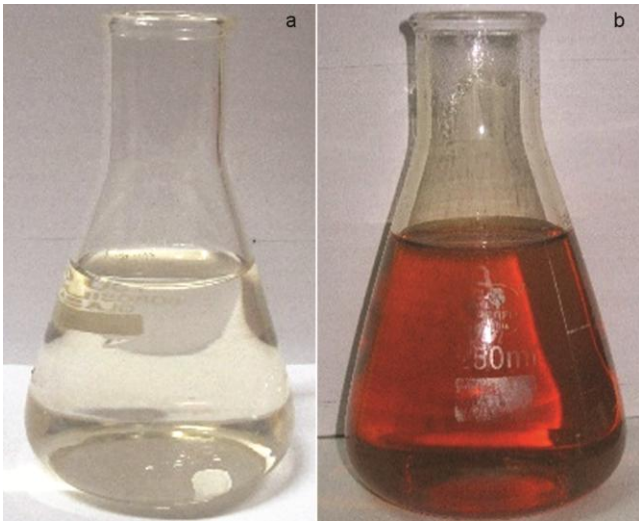

Fig. 2 — Aqueous seed extract of *E. superbum* with $AgNO_3$ solution, a) before incubation, b) after incubation.

The UV-Visible spectrum of the AgNPs synthesized using seed extract of *E. superbum* is shown in Fig. 3. The SPR of the nanoparticles produced a peak centred at 420 nm, indicating the reduction of silver nitrate into AgNPs.

TEM and selected-area diffraction (SAD) analysis

Transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs[20,21]. TEM images of the samples show nanoparticles predominate with spherical, triangle, and hexagonal shapes (Fig. 4a). The image also shows loosely bound particles were created due to the effect of sonication treatment. Most of the nanoparticles were roughly circular with smooth edges. Fig. 4b shows a SAD pattern of the silver nanoparticles. The AgNPs are crystalline and are evident from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate. SAD spots that corresponded to the different crystallographic planes of the face-centered cubic (fcc) structure of elemental silver are seen in Fig. 4b.

XRD study of the AgNPs

The XRD peaks at 2θ°degrees of 38.12, 44.30, 64.45, 77.41, and 81.55° correspond respectively to (111), (200), (220), (311), and (222) plans, confirming

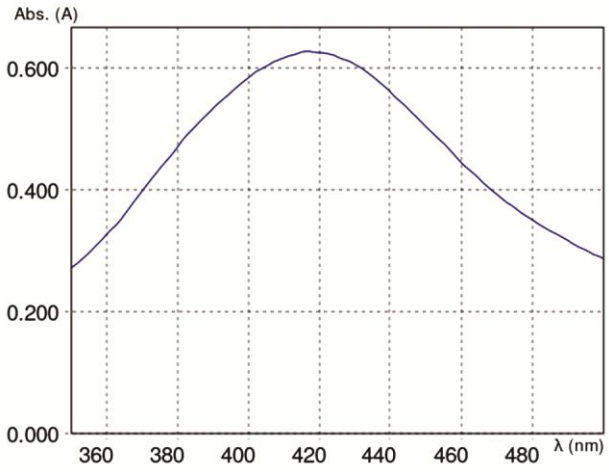

Fig. 3 — UV–Vis absorption spectrums of biosynthesized AgNPs.

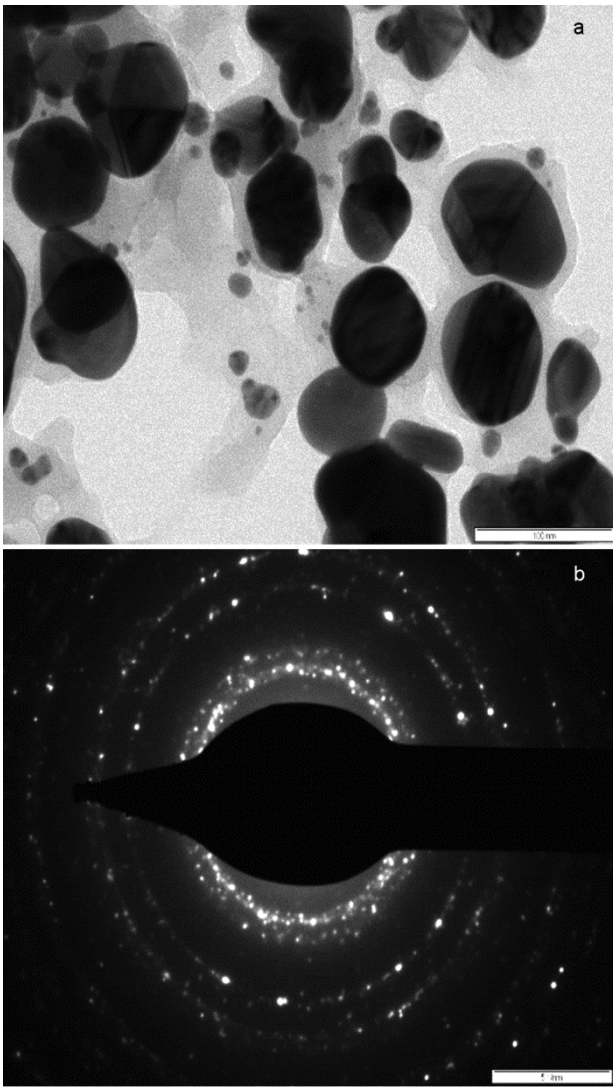

Fig. 4 — TEM images of biosynthesized AgNPs. a) Morphological feature of TEM image, b) Selected area electron diffraction pattern showing the characteristic crystal planes of elemental silver.

that the AgNPs have a face centre cubic crystalline structure[22,23]. The data matched with the standard International Centre for Diffraction Data (ICDD), Card 010870717, confirming a face-centred cubic structure for the silver nanoparticles (Fig. 5). The absence of any peaks resembling metal or metal oxide other than pure silver in the diffraction data confirmed the purity of the AgNPs. Broadening of the peak indicated a smaller particle size[24]. The size of the AgNPs synthesized was calculated using the Scherrer equation:

$$D = (0.9\,\lambda)/(\beta \cos \theta)$$

where D is the average crystal size, 0.9 is the shape factor, generally taken for the cubic system, λ is the x-ray wavelength, typically 1.54 Å, β is the full width

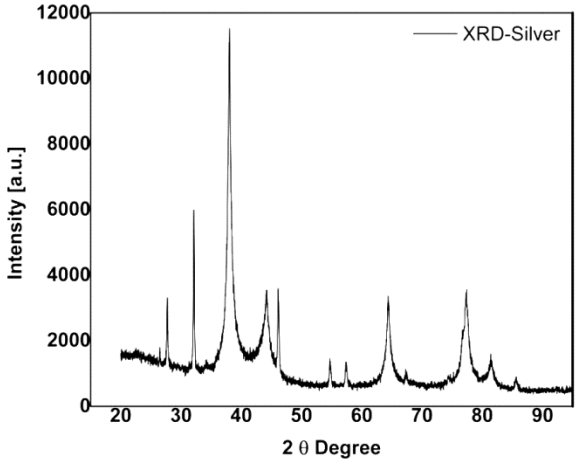

Fig. 5 — XRD spectrum of AgNPs.

at half maximum intensity in radians, and θ is the Bragg's angle. Using the above formula, the calculated crystallite size was approximately 5.8 nm.

FT-IR analysis of AgNPs

FTIR spectrum of the AgNPs is shown in (Fig. 6). FTIR analysis was performed to identify the possible biomolecules responsible for capping and efficient stabilization of the Ag nanoparticles[25].

The peak IR bands observed at 3858, 3740, and 3427 cm^{-1} are due to the presence of the OH group stretching vibration of phenolic compounds. The band appeared at 2920 cm^{-1} is due to aliphatic C-H stretching. The small peak at 1720 cm^{-1} corresponds to C=O aliphatic aldehyde or esters. The absorption peak seen at 1610 cm^{-1} is due to -C=C- stretching in the aromatic ring. The absorption bands seen at 1441 and 1370 cm^{-1} are due to C=C bending vibrations in the alkene molecules. C-C single bond was observed at 1152 cm^{-1} in the bending region. The absorption bands at 1075 cm^{-1} correspond to C-O bond bending. The band at 1018 cm^{-1} is attributed to C-Br bending.

The FTIR spectrum revealed that *E. superbum* seed extract is rich in phenolic compounds and other secondary metabolites. It is also reported that *E. superbum* extracts are rich in anthocyanins, flavonols, flavone, biflavonoid, and phenolic acid derivatives[26]. These biomolecules could be responsible for capping and efficient stabilization of the synthesized AgNPs.

A wide range of molecules, ranging from proteins to various low molecular weight compounds such as terpenoids, alkaloids, amino acids, alcoholic compounds, polyphenols, glutathiones, polysaccharides, antioxidants, organic acids, quinones, etc., have been reported to play a role in the green synthesis of NPs. The participation of sugars, terpenoids, polyphenols,

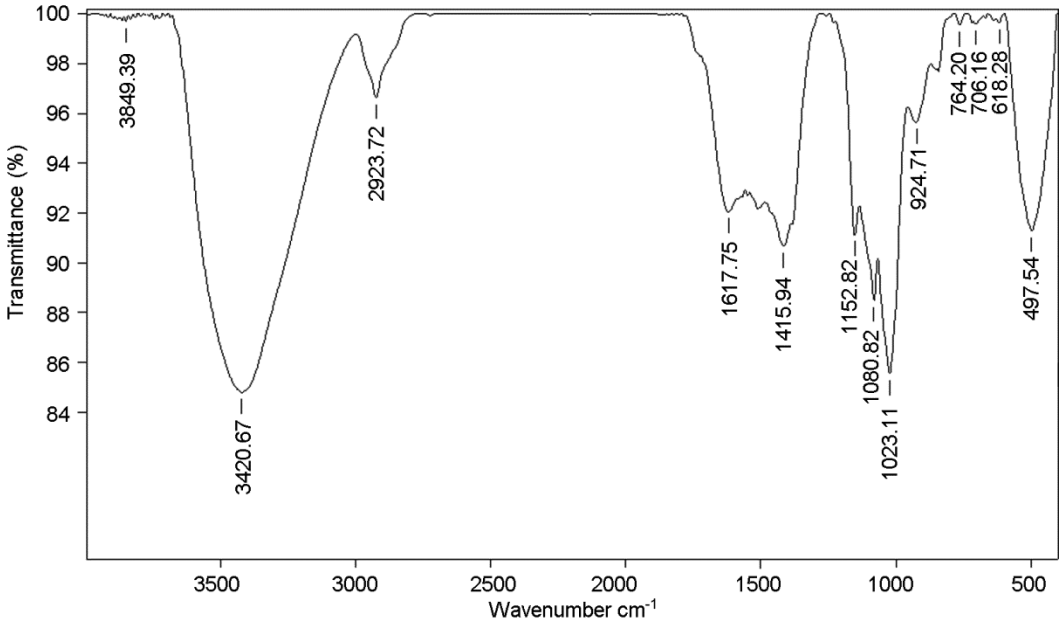

Fig. 6 — FTIR spectrum of AgNPs.

Table 1 — Antibacterial activity of biosynthesized silver nanoparticles

Name of Organisms	Zone of inhibition in mm	
	AgNPs (Mean±SEM)	Ciprofloxacin (Mean±SEM)
Pseudomonas aeruginosa	19.67±0.88	22.00±1.00
Salmonella typhi	18.33±0.67	22.67±0.88
Escherichia coli	16.00±1.00	19.67±0.33
Klebsiella pneumonia	15.33±0.88	23.00±1.15
Vibrio cholerae	12.00±0.58	19.67±0.88

alkaloids, phenolic acids, and proteins in the reduction of metal ions into NPs and in supporting their subsequent stability has also been postulated[27].

Antibacterial activity

Antibacterial activity of AgNPs was carried out against 05 bacterial clinical isolates, such as *S. typhi, P. aeruginosa, E. coli, K. pneumoniae, and V. cholera*. The diameter of the inhibition zone (mm) around each well with AgNPs is given in Table 1. The control (deionized sterile water) exhibited zero zones of inhibition (ZI). The highest antibacterial activity was observed against *P. aeruginosa* (19.67±0.88 mm) and the lowest bactericidal action was recorded against *V. cholera* (12.00±0.58 mm). When the ZI was compared with the standard drug (ciprofloxacin), it was found that the antibacterial activity of AgNPs was close to the standard drug. A similar type of work was carried out using AgNPs[28], and CuNPs[29] synthesized from *Terminalia arjuna* bark extract. *Cestrum diurnum*[30], *Clitoria ternatea* and *Solanum nigrum*[31], honey[32] were also reported to synthesize AgNPs and evaluated for their antibacterial activity.

Several studies have proposed the mechanism of the bactericidal action of AgNps. Kvitek *et al.*[33] suggested that AgNPs may attach to the surface of the bacterial cell membrane via interacting with sulfur-containing proteins[34], troubling permeability and respiration functions of the cell resulting in cell death[35].

Conclusion

This study reports the synthesis and characterization of silver nanoparticles using an aqueous seed extract of *E. superbum*. The antibacterial susceptibility of the synthesized AgNPs against several pathogenic microbes is highlighted in the present study. These AgNPs showed promising antibacterial activity against *P. aeruginosa* and *S. typhi*. Although the rapid and green synthetic methods using plant extracts have shown great potential in AgNPs, understanding the mechanism by which phytochemicals of *E. superbum* are involved in the synthesis and the mode of antimicrobial inhibition is still not fully understood.

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Conflict of interest

The authors declare no conflict of interest.

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