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Short Communication

Green Synthesis of Silver Nanoparticles using *Withania somnifera* Leaf Extract and its Antibacterial Effect

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***Key words*:** Antimicrobial activity, Silver nanoparticles, *Withania somnifera*, Leaf extract, Green synthesis

Nanotechnology, is a modern technology in science that deals with synthesis, design and application of nanomaterials by controlling their shape and size. Nanoparticles are defined as discrete objects having all of their three certain Cartesian dimension in the Nano range i.e., 1-100nm. The properties of these nanomaterials are different than those of their bulk materials due to their high surface to volume ratio. Hence, the nanomaterials are used in various application due to their improved properties. Nanotechnology finds its application in the fields of electronics food industry, packaging industry, bio medicals device, drug delivery system, medical diagnostic, biologicals tissue regeneration, space industry, optical devices, water purification, environmental monitoring, and many more [1].

Significant amount of research is being done on the various ways to synthesize silver nanoparticles, like physicals, chemicals and biologicals/ green method. Green method has gained more importance over the year as it is more eco- friendly than the other two method. It does not make use of toxic reducing agent that are employed in chemicals method and the energy input is comparatively much less than that used during physicals method of nanoparticles [2].

Withania somnifera commonly known as Ashwaghanda is a perennial plant belonging to order solanaceae. The plant has been found useful in the treatment of burn, wounds and dermatological disorder. *Withania somnifera* a traditional medicine plant of tropical country including India has the source of bioreducte and stabilizer. A group of Steroidal lactones. it leaves are use in Ayurveda and Unani system for the treatment of tumours and tubercular glands. The Biological activities of *Withania somnifera* are an anxiolytic –anti-depressive. The current study is expected to synthesis of silver nanoparticles using leaves extract of *Withania somnifera.*

Green synthesis of silver nanoparticles

Silver nanoparticles green synthesis was carried out by using *Withania somnifera* leaf extract as he reducing agent and silver nitrate as the metal salt precursor. For the preparation of aqueous leaf extract of *Withania somnifera*, fresh leaf of *Withania somnifera*. were collect from the Botanical Garden, Vinoba Bhave University, Hazaribagh, India, and washed thoroughly with tap water followed by distilled water to remove any grime material. These were cut into small pieces and air dried at room temperature (3-4). The extract was prepared by adding 10g of the 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 (5-6). The reaction solution was prepared by mixing an aqueous solution of 5mM Silver Nitrate ($AgNO_3$) to the leaf extract in 1:9 ratio and left undisrupted for 24 hours at room temperature. After 24 hours the solution was washed thrice by centrifugation at 10000 rpm for 15 minutes and the pellet containing the AgNP was dried in a hot air oven to obtain the AgNP powder (6-7).

Physiochemical characterization

The physiochemical characterization of AgNP were done using standard techniques like UV-Vis spectroscopy – Optical properties of synthesized AgNP was done through UV-Visible spectrophotometer (Carry 5000, Agilent, USA) by scrutinizing the spectral scan performed at the range of 200nm-800nm (8-9). Dynamic Light Scattering (DLS) – The size of the nanoparticles was determined by measuring the hydrodynamic diameter and zeta potential using Zetasizer (Malvern, UK).

Screening of antimicrobial properties in synthesized nanoparticles

The antimicrobial activity was analyzed with synthesized silver nanoparticles by well diffusion method against bacteria. Gram – negative bacteria (*Escherichia coli*, *Salmonella typhii*), Gram positive bacteria (*Staphylococcus aureus*, *Micrococcus lutes*). In brief, the dried silver nanoparticles were weighed and dissolved (5mg ml^{-1}) in sterile distilled water [10].

Well plate assay

All extract were subjected to agar well diffusion assay by measuring the diameter of Zone of inhibition (ZOI).

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1. LB agar was prepared by pouring 20ml each in sterile petri dishes and allowed to solidify.
2. Four well of 4mm were bored in each plate with an aseptic cork borer, when plates were solidified
3. The overnight broth culture (50µl) was used so as to ensure the concentration of these organism to contain 1×10^6 cfu/ml [11].
4. The culture was spread on the solidify agar surface using sterilized spreader.
5. Two different concentrations of extract (50%, 75%) were poured into the wells under sterilized condition.
6. The plates were then incubated at 37±2 °C in case of all culture but *M. luleus* were incubated at 30±2 °C.
7. The zone of inhibition (ZI) around the wells was measured in mm after 24 hr of incubation [12-13].

Green synthesis and characterization of AgNP

The synthesis of silver nanoparticles (AgNP) was carried out by using *Withania somnifera* leaf extract as shown in (Fig 1). The synthesized nanoparticles were dried and suspended in HF medium for characterization. The HF medium was selected for suspension preparation following the medium used for the used for the biological assays. The nanoparticles were characterized for their physicals and optical properties as shown in (Fig B). The UV- visible spectrum analysis exhibited a sharp surface Plasmon resonance (SPR) peak. The exhibition of SPR has been reported to be distinguished properties of nanoparticles to classify their optical properties [14]. Green synthesized AgNP showed (SPR) at 448nm. previous reports have also described the possession of SPR peak at a range of 300 – 450nm in the case of Ag nanoparticles synthesized by other procedure [6]. The stability of the nanoparticles in the HF medium was further checked using Dynamic light scattering by determining their zeta potential. As shown in (Fig 2B), the AgNP was found to have a zeta potential of -22±08mV in HF medium which indicate the stability of AgNP. The physiochemical characterization of the nanoparticles confirmed their Nano properties and successful synthesis of nanoparticles.

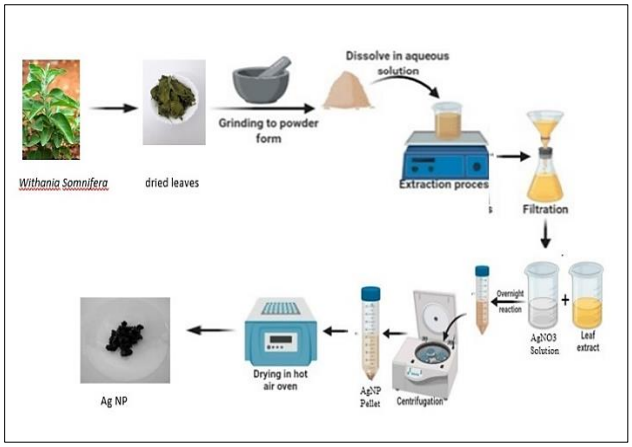
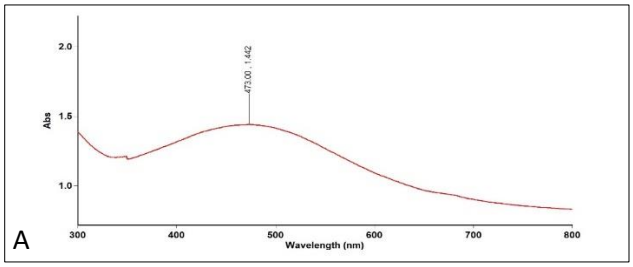
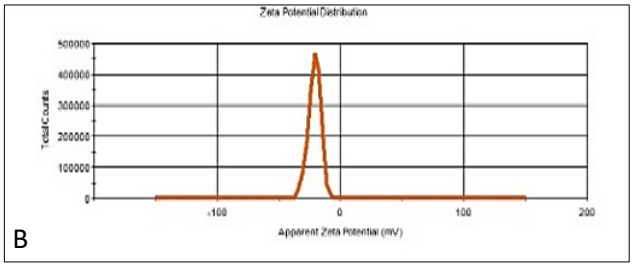
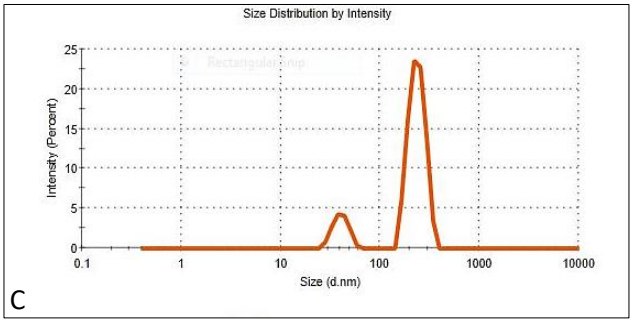
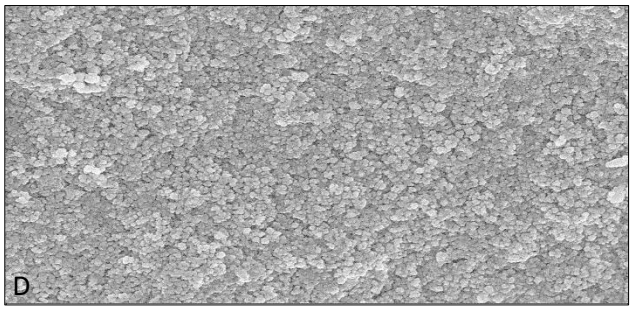


Fig 1 Schematic presentation of green synthesis $AgNO_3$ using *Withania somnifera* leaf extract. The extract was prepared from the freshly collected leaves. The synthesized AgNP was further characterized for its physiological properties









Physiochemical characterazation of silver nanopaticle

Fig 2 Physiochemical characterazation of green synthesized AgNP

(A) UV – Vis spectrum of AgNP (B) Zeta potential of AgNP determined by dynamic light scattering (C) Hydrodynamic diameter of AgNP as determined by dynamic light scattering (D) optical image of AgNP as determinend by FESEM

Fig 3 Antibactererial activity of AgNP against (*a*) *E. coli* (*b*) *Micrococcous lutes* (*c*) *Salmonella typhi* (*d*) *S. aureus*

Table 1 Antibacterial activity of synthesized AgNPs against pathogenic bacteria

Sample name	Zone of inhibition (mm)			
	25µl	50µl	75µl	100µl
Escherichia coli	2±1	4±2	6±1	9±2
Micrococcus luteus	6±2	8±1	10±2	13±1
Salmonella typhii	5±1	8±1	10±2	12±1
Staphylococcus aureus	4±1	8±1	12±1	12±1

SUMMARY

Biosynthesis of nanoparticles using the extract of medicinal plant in a non- hazardous mode has gained wide attention for various application in Nano medicine. The biologicals method of metal nanoparticles synthesis is more effective because of slow reduction rate of polydispersity of the final product. In our, study we have synthesized silver nanoparticles using leaves extract of *Withania somnifera* leaf extract. The synthesized $AgNO_3$ were found to have a size of 346±03nm and hydrodynamic diameter as determined by DLS. $AgNO_3$ was stable in disc diffusion medium with zeta potential of -23±01mV. The antibacterial studies showed significant activity as compared to their respective standard. From the results, *Withania Somnifera* Silver nanoparticles has attained the maximum antimicrobial against clinical pathogen and also very good stability of nanoparticles throughput processing. As we concluded, this type of naturally synthesized silver nanoparticles could be a better green resolution in medicinal chemistry. Silver nanoparticles were prepared by green method. The reduction of metal ions by leaf extract loading Ag Nano composites of quite distinct proportions. The synthesized AgNP were characterized by UV- Visible Spectrophotometer, DLS (Dynamic light scattering). Based on the current conclusion, it is accomplished that silver nanoparticles could be used as an antifungal agent in controlling the disease caused by fungi. The present study has opened up the possible way for synthesizing multidrug resistance antimicrobial AgNPs using natural biomolecules which could be employed in the pharmacy industry. By using such plant extract to develop Nano medicine along idea variety of human other veterinary pathogens.

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