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Research Article

Green Reactants Act as a Natural Precursor for Facile Synthesis of Nanoparticles using *Withania somnifera*

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ABSTRACT

Nanotechnology includes physical and chemical approaches intended for their phytofabrication of metal nanoparticles. Most of the time, these methods are not as safe as these are associated with the utilization of extremely noxious and hazardous substances that produce biological problems. Due to their manifold applications, various biological methods are gaining admiration for the fabrication of silver nanoparticles (AgNPs). The utilization of vegetative parts in the biosynthesis of nanoparticles (NPs) emerges as a commercial and ecological approach. The spectroscopic profile confirms the occurrence of a functional peak at 360 nm. Fourier transform infrared spectroscopy (FTIR) studies indicate that phenol groups are responsible for phytofabrication of NPs. The results of microbicidal screening confirm that broad spectrum of inhibition was found to be observed in 400 μ L of biosynthesized AgNP against *E. coli* (23 mm) and *B. cereus* (22.3 mm). Therefore, the progress of simple photosynthesis methods avoid deleterious and harmful properties has fascinated major attention in this field.

INTRODUCTION

Nanotechnology is the science of manipulation of nanomaterials with various properties used to make novel devices.^[1] Conventional approaches in the synthesis of AgNPs include physical and chemical methods.^[2] Apart from these methods, lethal and hazardous substances implicated in the phytosynthesis acquire various biological risks and are accountable for enormous health problems. A variety of biological agents act as *in vitro* reducing and capping agents (plant and microbial derivatives) are responsible for bioreduction. These methods are own for the production of various nanomaterials like gold, silver, zinc, copper, iron, etc.^[3,4] In the field of medical microbiology, disease diagnosis^[5] is facilitated with the aid of gold nanomaterials. Nanoparticle comes in contact with membrane structure, damages the membrane

permeability by means of depolarization. Silver ions attach to proteins present in tissue and finally leads to disruption of cell integrity and death.^[6-8]

Superior cytotoxicity against bacteria is brought about by the amalgamation of diverse compounds such as antibiotics, AgNP complexes, with enhanced properties of their active surfaces.^[9] Nanomaterials are produced mechanically by electro-spinning process, which encourages biocidal properties and biocompatibility with human cells.^[10] The permeability in membrane allows small-sized AgNPs to accumulate in the internal membrane of the cell.^[11] NPs counter against the eukaryotic cells by means of phagocytosis and endocytosis.^[12] The mechanism depends on the prescribed quantity with cytotoxicity effects.^[11] AgNPs come in contact with the plasma membrane and discharge Ag^+ ions into cytoplasm, followed by the ion exchange mechanism,

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and blocks the synthesis of sulfur-containing proteins on ribosomes.^[13]

Withania somnifera L. is a medicinal plant with essential phytoconstituents belonging to the Solanaceae family, commonly known as red ginseng, *ashwagandha*, withanolides that contribute to most of the biological activity of *W. somnifera*.^[14] Leaves of the plant is rich in biochemical compounds such as alkaloids, steroidal lactones, tannin, etc.^[15] Vegetative parts of leaves are enriched with more than 12 alkaloids, 40 withanolides containing a glucose molecule at carbon. In leaves, the concentration of withanolides usually ranges from 0.001 to 0.5 % dry weight.^[16] The pharmacological properties of this plant are due to Anolides, a combination of steroidal lactones found in the leaves of this plant.^[17] The *Withania somnifera* extract, when administered orally, prevented the increase in LPO levels.^[18-20] These biosynthesized compounds are believed to manipulate the mechanism in the cortical and basal forebrain cholinergic-signal transduction cascade.^[19] Thus, *W. somnifera* contains bioreduction agents such as terpenoids, flavonoids, proteins, and alkaloids, which initiate the synthesis of nanoparticles.^[21]

In the present investigation, biofabrication of AgNPs using *Withania somnifera* was examined. The optimization of phytofabricated AgNP was done to indicate the presence of nanoparticles. Bioefficacy of AgNP was using microbicidal assay, and characterization was confirmed using FTIR, scanning electron microscopy (SEM), energy dispersive X-ray (EDX), and dynamic light scattering (DLS) analysis.

MATERIALS AND METHODS

Plant Materials

Leaf samples of *Withania somnifera* were selected for the study. The leaves were immersed in running water, desiccated under shade conditions, and homogenized using an electrical blender. The obtained leaf samples were stored and used for further studies.

Phytochemical Analysis

Plant materials were assessed for the occurrence of various biologically active compounds like flavonoids, alkaloids, glycosides, steroids, phenols, saponins, and tannins due to the methods proposed by Harborne *et al.*^[22]

Characterization of Phytoassisted Silver Nanoparticles

The major shift in the optical color of the solution to dark brown accounts for the complete bioreduction of Ag⁺, overnight samples of synthesized AgNPs were measured using UV-2550 Shimadzu Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The rapidly formed

biosynthesized AgNPs were obtained by centrifugation at 10,000 rpm for 10 minutes in a centrifuge and carefully washed with sterile ddH₂O, and freeze-dried and stored at -80°C. Based on the fast reduction of AgNO₃ into AgNPs, the capable AgNPs sample prepared from 15 mL of fruit extract was used for further characterization using several methods, including UV-visible spectroscopy, FTIR, SEM, EDX spectroscopy, and diffraction light scattering analysis.

UV-vis Spectroscopy

Visual examination of the phytosynthesized AgNP has experimented for any color change, and 1 mL of the combined mixture was withdrawn successively at different time levels by diluting a small aliquot (100 µL) of the reactant sample 10-fold in deionized water for the study of surface plasmon resonance of silver nanoparticles. The reduction process of pure Ag⁺ ions was evaluated by using a UV-vis spectrophotometer (Shimadzu 1601 model, Japan) at the resolution of 1 nm in the range of 200–800 nm.

Fourier Transform Infrared Spectroscopy Analysis

The functional assignments of the NPs were qualitatively confirmed by using FTIR spectroscopy^[23] with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. Shimadzu FT-IR model number 8400 was used to perform FTIR analysis. A 3 mg of powdered leaf samples was mixed with 300 mg of dried KBr, and thin pellets were prepared for analysis of the sample. A similar process was carried out for phytosynthesized AgNPs using an extract. Scanning of samples was performed in a range of 400-4000 cm⁻¹.

Scanning Electron Microscopy (SEM)

The SEM with an ultra-high-resolution is used for detecting the arrangement and composition of purified AgNPs, analyzed by using a 10-kV. A mixture of purified silver nanomaterials was obtained after repetitive centrifugation, carbon-coated copper grids were used for sputter coating, and imagery descriptions of nanoparticles were analyzed by FEI QUANTA-200 SEM.

Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) was used in analyzing the size distribution pattern of the biofabricated AgNPs, Model DLS (Malvern, UK). In this experimental analysis, 0.15 M PBS (pH 7.4) was used to dilute the samples, 10-fold dilution was done and the dimensions were recorded in the range between 0.1 and 10,000 nm.

Microbicidal Screening of Phytosynthesized AgNPs

Antimicrobial activity of synthesized AgNPs was evaluated against the pathogen such as *Bacillus sp.*, *Escherichia coli*, *Mycobacterium mucilaginosus*, *Klebsiella terrigena*, *Pseudomonas aeruginosa*, *Shigella*, *Staphylococcus epidermis*, *Fusarium oxysporum*, *Penicillium*, *Aspergillus*



niger by Kirby Bauer method as experimented by Elbeshehy *et al.*^[24] Overnight culture was maintained in LB broth at 30°C (approximately $\sim 1 \times 10^8$ CFU/mL), lawn culture was done with 5 mL of LB agar medium on the LB agar medium. Incubation of fungal cultures was done at 25°C for 48 hours, inoculated into potato dextrose broth and was spread in potato dextrose agar medium. After drying of the agar medium, 40 μ L of the concentration of AgNPs were loaded at the equivalent space on agar well (6 mm) and grown for 24 hours at 30°C. A similar quantity of filter-sterilized leaf extract was used as a control. The microbicidal activity was determined by averaging the diameter of the inhibition zone. To determine the activity, the diameter of the inhibition zone was measured around the whole. Three repeated experiments has been done for antimicrobial evaluation.

RESULTS AND DISCUSSION

Phytochemical Investigation of *Withania somnifera*

The results of bioactive constituents of plant samples were summarized in Table 1. The plant samples were subjected to phytochemical analysis by extracting with various solvents. Acetone and aqueous extract displayed positive results towards the majority of compounds except for saponin and terpenoid. Ethanol and methanol extract possess alkaloid, flavonoid, phenol, and glycosides extract, whereas hexane extract displayed negative results towards the majority of compounds except for steroids. The flavonoids and terpenoids are the biomolecules present in the leaves of *W. somnifera* act as a bioreductant which are responsible for conversion of silver nitrate into silver ions.^[25]

Spectroscopic Profile of Plant Assisted AgNPs

Optimization Factors in Photosynthesis of AgNPs

Spectroscopic profile of plant assisted AgNPs were represented in Fig. 1-4. Various biosynthesis of fabricated AgNPs have been documented using vegetative parts from, *Lippia nodiflora*.^[26] Diverse factors such as time, pH, temperature, and concentration of AgNO₃ were evaluated for the optimization of biosynthesis of AgNPs.

The visual changes experimented could be recognized due to the spectroscopic peaks.^[27] The range and shape of nanomaterials influence the biological properties.^[28] In the existing study, the biosynthesized AgNPs at 70°C, shows a broader peak at 360 nm. The amplification of nanoparticles is enhanced by the visual intensity of solution obtained by the bioreduction process.

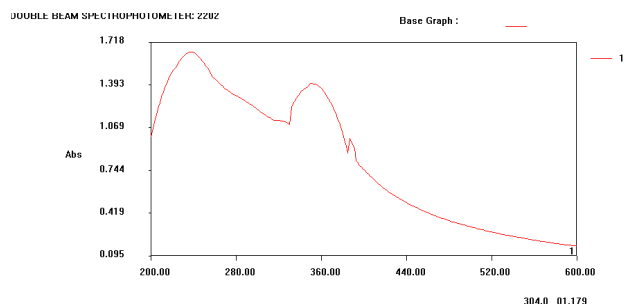


Fig. 1: Experimental analysis of biofabricated NPs at 70°C

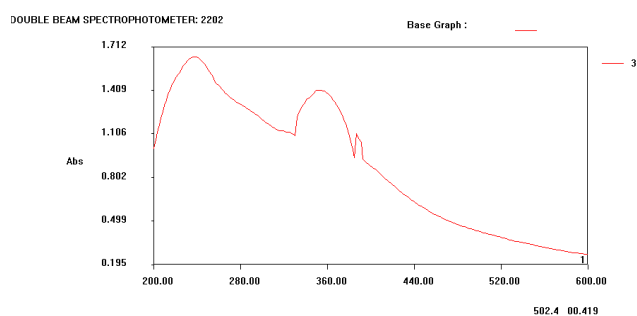


Fig. 2: Experimental analysis of biofabricated NPs at 2 hours

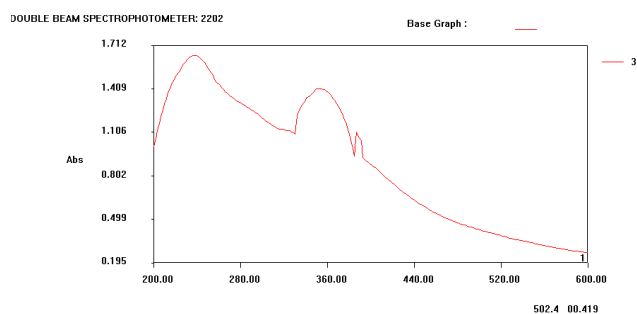


Fig. 3: Experimental analysis of biofabricated NPs at pH 4

Table 1: Bioactive constituents in *Withania somnifera*

Phytoconstituents	A	E	EA	M	PE	H	W
Alkaloid	+	+	-	+	+	-	+
Flavonoid	+	+	+	+	-	-	+
Saponin	-	-	-	+	-	-	+
Tannin	+	-	-	-	-	-	+
Phenol	+	+	+	+	+	-	+
Glycosides	+	+	+	+	-	-	+
Terpenoid	+	-	-	+	+	-	-
Steroid	+	-	-	-	-	+	+

A - Acetone; E - Ethanol; EA - Ethyl acetate; M - Methanol; P - Petroleum ether; H - Hexane; W - Water

*The results are triplicates of the samples

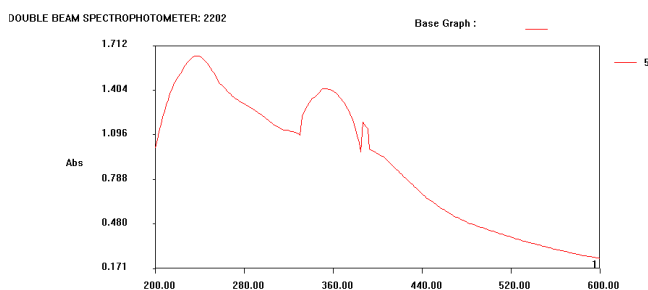


Fig. 4: Experimental analysis of biofabricated NPs at 2 mM

UV spectrum of plant synthesized AgNPs possess an elevated peak at 2 hours. Electrostatic interactions flanked by the Ag^+ ions and proteins in *W. somnifera* are responsible for the bioreduction of Ag. In the present study, the UV-Vis spectrum obtained elevated levels of peak at pH 4. Positively charged photosynthesized NPs interact with cell surface of bacteria, which is negatively charged, this reductant reaction occurs at a low pH level.^[29] Proteins are capable of reducing silver ion and play a vital role in the formation of silver nuclei. The produced silver nuclei are sequentially developed by the bioreduction of Ag ions and formation of nuclei, which is the primary factor in the formation of AgNPs.^[30] In the existing investigation, the AgNPs synthesized at 2 mM concentration was found to be effective in producing a broader spectrum. The increase in maximum absorbance at different concentrations is due to the particle density, which strongly depends on the amount of silver reduction at the surface of the medium.^[31]

The FTIR spectrum of biosynthesized AgNPs was depicted in Fig. 5. The absorption of various functional assignments like O-stretching of phenol and alcohol, the group of N-H, O-H, C=O, the phytol compounds of amino acids, and proteins are said to be implicated in the process of biofabricated NPs.^[32] The functional assignments at 3430 cm^{-1} correspond to O-H stretching vibration. Previous studies reported that O-H stretching vibrations of the phenol group are responsible for the reduction

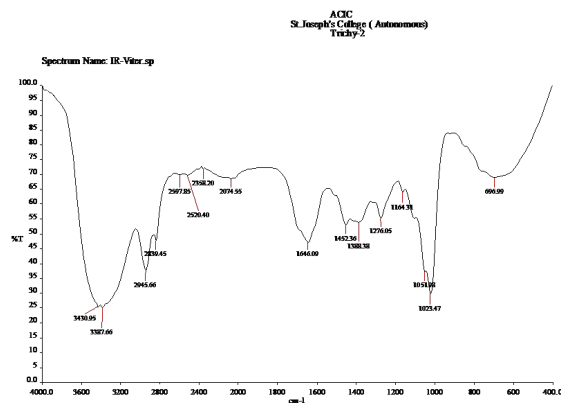


Fig. 5: Functional assignments of phytofabricated AgNPs

and capping of nanomaterials.^[33] The absorption peak at 2945 cm^{-1} belongs to CeH stretching, the peak at 1646 cm^{-1} is responsible for amide groups, and the results corroborated with previous reports. The absorption peaks at 3420 cm^{-1} was responsible for -OH group, followed by the region at 2920 cm^{-1} corresponds to CeH stretching. Absorbance peak at 1606 cm^{-1} indicates the presence of proteins by the amine or amide I group. Also, the peak at 1606 cm^{-1} was appended by AgNPs with the C-O functional groups.^[34] In the previous reports, the functional assignments at 2921 cm^{-1} confirm the existence of C-H group of an aromatic compound.^[35]

In the existing study, the wavenumber at 1452 cm^{-1} indicates the aromatic group, followed by the absorption spectrum at 1023.47 cm^{-1} belongs to the secondary AOH group, the FTIR spectrum was correlated with prior studies. The peak at 1408 cm^{-1} belongs to the OAH bend of polyphenol corresponds to an aromatic group, whereas the peaks at 1013 cm^{-1} indicate the occurrence of CAOAC and secondary AOH group^[36,37] of plant extract. The wavenumber at 696 cm^{-1} might be due to the presence of alkyl halides, confirmed by the preceding studies. The functional groups at 699 cm^{-1} and 504 cm^{-1} corresponds to alkyl halides stretching.^[38] The assignments at 2074.55 cm^{-1} indicate the occurrence of alkyne groups, and the band at 1646.09 cm^{-1} is responsible for C = O peak. The results correlated with the biosynthesis of NPs obtained from previous reports.^[39] The peaks at 1388.38 cm^{-1} correspond to C-N stretch vibrations and the amide I bands of proteins in the extract.^[40] The FTIR studies prove that the carbonyl groups of amino acids and peptides of proteins to attach to the surface of metal ions and play an essential role in forming a protective coat around the nanoparticles, which helps in stabilization of the medium.

Scanning Images of Bio Fabricated AgNPs using FE-SEM

The SEM images of plant assisted AgNPs were represented in Fig. 6. The SEM determines distribution of biosynthesized AgNPs at the nanoscale, a surface analyzing technique, used to identify diverse particle shapes, morphology, range.^[39] In the existing study, images of biofabricated AgNPs was found to be spherical and uniformly distributed, biofabricated NPs that have carbonyl, primary amine group correlated with prior reports.

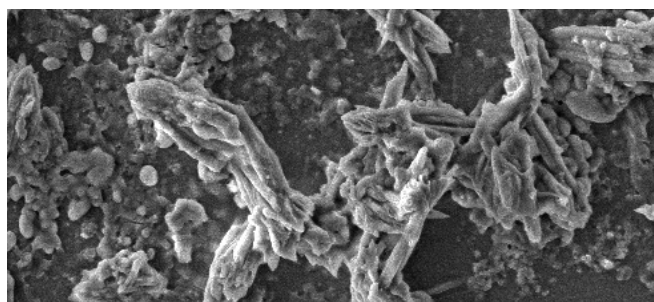


Fig. 6: FE-SEM micrograph of photosynthesized AgNPs



EDX Spectrum of Phytofabricated AgNPs

The EDS profile exhibited the presence of silver along with some peaks were depicted in Figs. 7 and 8. The peaks may be attributed due to the presence of bioactive constituents that are attached to the exterior surface of AgNPs, signifying the bioreduction process.

Dynamic Light Scattering Study

The DLS profile of phytofabricated AgNP was represented in Fig. 9. This is due to the experimentation of small particles^[41-43] as well as the existence of unreacted extract indicates the occurrence of the AgNPs. The hydrodynamic analysis of the distribution reveals that the size of AgNPs is 100 nm.

In vitro Microbicidal Assessment of *W. somnifera*

In vitro microbicidal assay of plant extract was tested against various pathogens using well diffusion technique (Table 2). Antibiotic resistance possesses serious hazard to human wellbeing. Therefore, there is an increase in the investigation of vegetative parts as a source for the management of the infectious disease.^[44,45] Among the extracts assayed methanol extract possess maximum inhibitory effect against *E. coli* (22.3 mm), *B. cereus* (21 mm), *S. aureus* (20.4 mm), followed by acetone extract (22, 20.5, and 19.6 mm). The inhibitory effect was found to be moderate in ethanol extract against *K. pneumoniae* (16.5 mm) and *M. mucilaginosus* (14.3 mm), followed by chloroform extract against *M. mucilaginosus* (13.4 mm). The level of inhibition was found to be minimum in

petroleum ether extract against *K. terrigena* (14 mm) and *M. mucilaginosus* (12.5 mm).

Methanol extract possess maximum fungicidal effect against *A. niger* (19.7 mm), followed by *Penicillium* (18.5 mm) and *F. oxysporum* (17.4 mm), followed by acetone extract (18.5, 17.3, and 16.3 mm). Chloroform

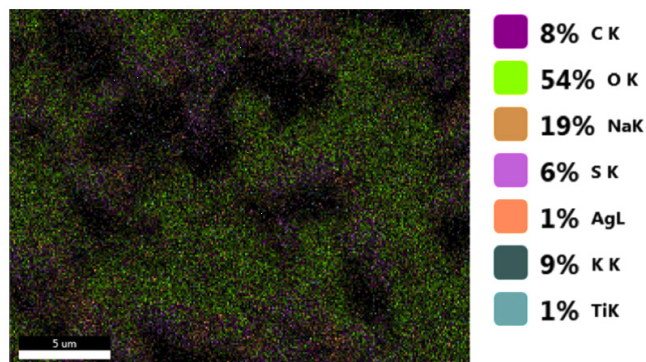


Fig. 8: Elemental overlay of synthesized AgNPs

Intensity Distribution

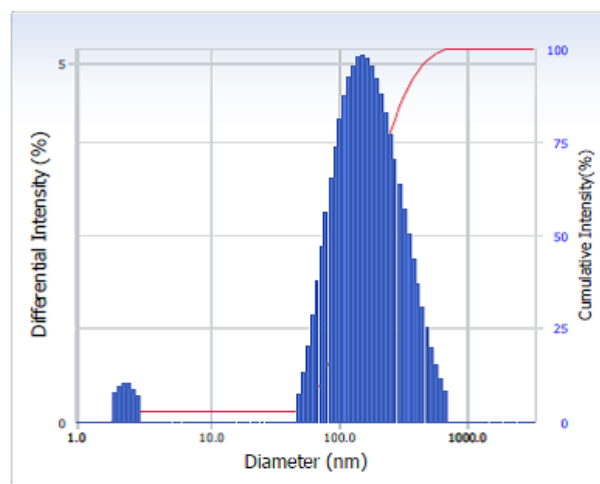


Fig. 9: DLS spectrum of biofabricated AgNPs

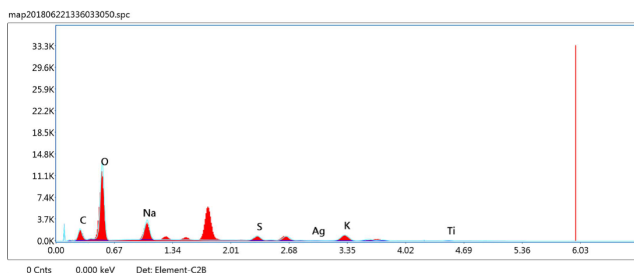


Fig. 7: Electrodiagnostic spectrum of phytofabricated AgNPs

Table 2: Microbicidal assessment in *Withania somnifera*

S. No.	Microorganism	<i>Withania somnifera</i>				
		Acetone	Chloroform	Ethanol	Methanol	Petroleum ether
1.	<i>Bacillus cereus</i>	20.5 ± 0	19.7 ± 0.1	19.4 ± 0.2	21 ± 0	18.7 ± 0.1
2.	<i>Klebsiella pneumonia</i>	17.7 ± 0.1	15.6 ± 0	16.5 ± 0.1	18.7 ± 0.1	14.6 ± 0.2
3.	<i>Pseudomonas aeruginosa</i>	18.1 ± 0	16.5 ± 0	17.5 ± 0	19.3 ± 0.2	16 ± 0
4.	<i>Staphylococcus aureus</i>	19.6 ± 0	18 ± 0	18.7 ± 0.1	20.4 ± 0.2	17.2 ± 0.2
5.	<i>Escherichia coli</i>	22 ± 0	20.6 ± 0.1	21.5 ± 0	22.3 ± 0.1	19.6 ± 0.1
6.	<i>Mycobacterium mucliaginosus</i>	15.3 ± 0.1	13.4 ± 0.2	14.3 ± 0.1	15.6 ± 0.1	12.5 ± 0
7.	<i>Klebsiella terrigena</i>	16.7 ± 0.1	14 ± 0	15 ± 0	16.4 ± 0.2	14 ± 0
8.	<i>Fusarium oxysporum</i>	16.3 ± 0.2	14.7 ± 0.1	16.5 ± 0	17.4 ± 0.1	12.6 ± 0.1
9.	<i>Penicillium</i>	17.3 ± 0.2	15 ± 0	17.7 ± 0.1	18.5 ± 0	13.6 ± 0.1
10.	<i>Aspergillus niger</i>	18.5 ± 0	17.4 ± 0.1	18.5 ± 0	19.7 ± 0.1	15 ± 0

*Values are mean of ± S.D, n=3

Table 3: Microbicidal screening of phytofabricated AgNPs using *Withania somnifera*

S. No.	Microorganism	<i>Withania somnifera</i>				
		100 μ L	200 μ L	300 μ L	400 μ L	250 μ L AgNP
1.	<i>Bacillus cereus</i>	19.3 $\pm$ 0.1	20 $\pm$ 0	21.5 $\pm$ 0.1	22.3 $\pm$ 0.1	23.5 $\pm$ 0
2.	<i>Klebsiella pneumonia</i>	16.4 $\pm$ 0.1	16.5 $\pm$ 0.1	18 $\pm$ 0	19.7 $\pm$ 0.2	20.5 $\pm$ 0
3.	<i>Pseudomonas aeruginosa</i>	17.5 $\pm$ 0	18.4 $\pm$ 0.2	19.2 $\pm$ 0.1	20.7 $\pm$ 0.1	21 $\pm$ 0
4.	<i>Staphylococcus aureus</i>	18.3 $\pm$ 0.1	19.4 $\pm$ 0.1	20.5 $\pm$ 0	21.3 $\pm$ 0.2	22.3 $\pm$ 0.1
5.	<i>Escherichia coli</i>	20.5 $\pm$ 0	21.2 $\pm$ 0.2	22.3 $\pm$ 0.1	23 $\pm$ 0	24.3 $\pm$ 0.1
6.	<i>Mycobacterium mucilaginosus</i>	14.1 $\pm$ 0.1	15.5 $\pm$ 0	16.6 $\pm$ 0	17.2 $\pm$ 0.2	18 $\pm$ 0
7.	<i>Klebsiella terrigena</i>	15.5 $\pm$ 0	16 $\pm$ 0	17.5 $\pm$ 0	18.4 $\pm$ 0.1	19.5 $\pm$ 0
8.	<i>Fusarium oxysporum</i>	12.4 $\pm$ 0.1	14 $\pm$ 0	17.4 $\pm$ 0.2	17.5 $\pm$ 0	14 $\pm$ 0
9.	<i>Penicillium</i>	13 $\pm$ 0	16.6 $\pm$ 0.1	18.5 $\pm$ 0	19.6 $\pm$ 0.1	16.5 $\pm$ 0
10.	<i>Aspergillus niger</i>	15.3 $\pm$ 0.1	17 $\pm$ 0	19.2 $\pm$ 0.1	20.5 $\pm$ 0	17.4 $\pm$ 0.2

*Values are mean of $\pm$ S.D, n = 3

extract displayed moderate inhibition against *Penicillium* (15 mm) and *F. oxysporum* (14.7 mm). Petroleum ether extract remained sensitive to fungal species possessing minimum inhibition (13.6 mm, 12.6 mm) compared to other extracts.

Microbicidal Efficacy of Biofabricated AgNPs

In vitro antimicrobial assay of biosynthesized AgNPs using *W. somnifera* was represented in Table 3. Action of plant-mediated AgNPs leads to cell wall and membrane damage, leading to oxidative stress.^[46-48] 400 μ L of synthesized AgNP was found to be resistant against *E. coli* (23 mm), *B. cereus* (22.3 mm), and *S. aureus* (21.3 mm), followed by 300 μ L of synthesized AgNP (22.3, 21.5, and 20.5 mm). Das *et al.* reported the assay of AgNPs against multidrug-resistant *E. coli* and *S. aureus* and found that ROS generation initiates the bactericidal action.^[49,50] 200 μ L of AgNP possess moderate inhibition against *K. pneumoniae* (16.5 mm) and *K. terrigena* (16 mm). Anandalakshmi *et al.* documented the microbicidal action of phytofabricated NPs by *Pedaliu murex* plant against *E. coli* and *K. pneumoniae*.^[51] 100 μ L of AgNP displayed a minimum zone of inhibition against *K. terrigena* (15.5mm) and *M. mucilaginosus* (14.1 mm). Nanoparticles with larger surface areas provide higher interaction and ascendant intracellular penetration.^[52]

Fungicidal assessment of synthesized AgNP suggest that 400 μ L acquired maximum inhibition against *A. niger* (20.5 mm) and *Penicillium* (19.6 mm) followed by 300 μ L (19.2 mm, 18.5 mm). Significant fungicidal effect was observed against fluconazole resistant *Candida albicans*.^[53] 200 μ L of synthesized AgNP displayed moderate inhibitory effect against *A. niger* (17 mm), *Penicillium* (16.6 mm). The biological mechanisms of silver ions have the capacity to disrupt respiratory system.^[54-56] 100 μ L of phytosynthesised AgNP possess minimum level of inhibition against *Penicillium* (13 mm) and *F. oxysporum* (12.4 mm). The fungicidal assessment was observed against *Curvularia lunata*, *Bipolaris spicifera*, *Fusarium oxysporum*, and *Aspergillus niger*, is inhibited by the AgNPs.^[57]

CONCLUSION

Green reactants could accomplish this vital technique by biosynthesis of the AgNPs devoid of using any poisonous substance as bioreductant. Spectroscopic studies indicate the presence of bio-molecules obtained in plant extracts, act as reductant agents for biofabrication of AgNPs.

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