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Original Research Article

Antibacterial Activity of Silver Nanoparticles Developed using *invitro* Leaf Extracts of *Dioscorea oppositifolia*.L

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A B S T R A C T

The aim of the study is to synthesize silver nanoparticles (AgNPs) using *in vitro* leaf extract of *Dioscorea oppositifolia* .L and to compare its antibacterial efficacy with *in vivo* fruit extract synthesized AgNPs of the same plant. The synthesized nanoparticles were characterized using UV–visible absorption spectrophotometer (UV-Vis), Fourier transform Infra-red Spectroscopy (FTIR) and Transmission electron microscopy (TEM). Disease free *In vitro* leaves were cultured and its extract was used to fabricate AgNPs observed at 423 nm with particle sizes between 8 nm and 11 nm. The antibacterial potency of *in vitro* leaf extract mediated nanoparticles compared to the *in vivo* fruit extract mediated nanoparticles was superior as denoted by the zone of inhibition (ZoI) when the nanoparticles were treated against four different Microbial Type Culture Collection (MTCC) strains.

Keywords

AgNPs,
TEM,
FE-SEM,
In vitro leaf extract

Introduction

Synthesis of nanostructures using living organisms is the common feature of nanobiotechnology (Kumar and Yadav**,** 2009). Among the use of living organisms, plants have found the top most place for application particularly in metal nanoparticles synthesis. The chemical methods are more hazardous where as biological methods are eco- friendly and provide a feasible approach for the synthesis of nanoparticles. There are few reports on *in vitro* development of callus and biosynthesis of AgNPs using the same which was reported by Nabikhan *et al.*,(2010).

Hence, plants are used as an alternative to generate nanomaterials by biomimetic synthesis process. Mude *et al.*,(2009). Biological means of synthesizing nanoparticles puts a high note over chemical means as it is cost effective and does not involve any toxic substance for synthesis. Antony *et al.*,(2011).

Microbial resistance towards the available antimicrobial agents has been a major factor for the development of novel microbe inhibitory agents. Silver possesses antimicrobial effects with unique properties

of conductivity and stability. Sadhasivam *et al.*,(2010).The synthesized particle dimensions were characterized with TEM, and DLS measurements. Ozay *et al.*,(2010). The antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens. Krishna raj *et al.*,(2010). Ag NPs could be attributed as therapeutic agent for biomedical and pharmaceutical applications to meet the demand of the growing population. Gopinath *et al.*,(2010). The size and shape of the Ag nanoparticles are dependent on the concentration of the $AgNO_3$ solution. Das *et al.*,(2011).The odds of microbes becoming resistant to AgNPs are slender because of the broad targeting range of nanoparticles on the microbes and is hence of advanced therapeutic value Kora et al.(2011).Compared to whole metal of silver, AgNPs possess efficient antibacterial properties because of high surface area to volume ratio along with high fraction of surface atoms, though the mode of activity is still unclear. Shahverdi et al.(2007).

This is the first ever report with regard to exploration of the biological reduction performed by *in vitro* tuber extract of *Dioscorea oppositifolia* L (TUNPs) and the comparison of antibacterial efficacy of the synthesized nanoparticles with its *in vivo* fruit extract derived AgNPs. *In vivo* antibacterial activity of fruit extract mediated AgNPs (FENPs) had been established by our previous report. Maheswari *et al.*,(2012).

Experimental Section

Plant Collection

Dioscorea oppositifolia L. were collected from the Kolli hills of Tamilnadu, India and the species was identified by comparing with the authenticated specimen deposited at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu.

Preparation of Hydrosol Containing AgNPs

Briefly, *in vivo* tuber and *in vitro* tuber powders (200 mg) were dissolved in 200 ml of distilled water and the extracts were filtered by using Whatmann No.1 filter paper and used for the bioreduction.

Synthesis of Silver Nanoparticles

The procedure followed by Song and Kim for the preparation of the nanoparticles was used (Song & Kim, 2009). 100 ml of the sample 0.1 mM aqueous Silver nitrate (Qualigens – 99.8%) solution was added and filtered. The mixture was gradually heated in a water bath at 65 $^{\circ}$C for 10 minutes after which a brownish colored hydrosol was formed. This was centrifuged at 10,000 rpm for 20 minutes at 4 $^{\circ}$C and the steps were repeated thrice. Final pellet was used for further study.

Characterization of AgNPs

UV–visible spectrometric measurements were carried out on Hitachi double beam equipment (Model Lambda 35), in the 200–600 nm range. AgNPs were analyzed by FTIR on a Spectrum RX 1 instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets and the spectra were recorded in the wavelength interval of 4000 and 400 nm^{-1}. TEM measurements were performed on a Tecnai 10 instrument operated at 120 keV. (Yu, 2007) The 3D structure of Silver nanoparticles has been elucidated using FSEM image.

Evaluation of Antimicrobial Activity

Antibacterial analysis was performed using *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 1675), *Klebsiella pneumoniae*

(MTCC 7028), *Salmonella typhii* (MTCC733) and *Staphylococcus aureus* (MTCC 7505) obtained from MTCC, Indian Institute of Microbial Technology (IMTECH), Chandigarh, India. A disk diffusion method was implemented to assay the nanoparticles for bactericidal activity on Luria Bertani (LB) agar plates. A rapid and inexpensive method of determining the ZoI using a vernier caliper was used to determine the vulnerability of the microbes to the bactericidal agent Fayaz et al .(2010).Standard disks were purchased from HIMEDIA Laboratories, India. Each disk had a diameter of 6 mm. 50 µL of the test sample was loaded and air dried. Bacterial cultures about 10^6 CFU/mL were spread plated on LB agar, infused with the sample loaded disks and incubated at 37 ˚C for 18 hours. The diameters of ZoI were measured and the assays were performed in triplicate.

Results and Discussion

Characterization of LENPs

In vitro leaves filtrate after addition of aqueous $AgNO_3$ (0.1mM) was subjected to visual observation which indicated the transition of color to brown which indicates formation of AgNPs [Figure 1 A and 1 B, Fayaz et al .(2010). Due to resonance with light wave, AgNPs with free electrons give rise to specific resonance peak .The Surface Plasmon resonance peaks of the silver nanoparticles can be turned over a broad range from the visible to the near infrared. (Mulvaney ,1996) Optical measurement showed an absorbance peak at 423 nm (Figure 2). This peak is specific for AgNPs .Ghosh et al ,(2012)FTIR spectrum of the nanoparticles obtained in the present study is presented in Figure 3. Among them the absorption bands are observed in the range of 400- 4000 cm^{-1} are 695 cm^{-1}, 1636 cm^{-1}, 2068 cm^{-1}, 3431 cm^{-1} and 3979 cm^{-1}. The band at 1636 cm^{-1} can be correlated to primary amine peaks which may correlate to proteins. The band at 2068 cm^{-1} has been identified closer to COOH –acid group. The bands at 695, 3431 cm^{-1} can be correlated to alcohols and phenols corresponding to polyphenolic encapsulates. This corresponds to the fact that proteins and polyphenols are important for nanoparticle synthesis and encapsulation. TEM image revealed the size of the nanoparticle to be between 8 nm and 11 nm (Figure 4). The surface morphology and size of the bioreduced silver nanoparticles was determined using a transmission electron microscope (TEM, Tecnai 12 Cryo, FEI, Eindhoven, and The Netherlands). The morphology and size of the silver nanoparticles were also characterized by a higher resolution transmission electron microscope (HRTEM,JEOL-JEM-2100,Peabody,MA). Suriyakala et al (2013).The *in vitro* leaf nanoparticles was observed as spherical in nature.(Figure 5)

Antibacterial Activity of the AgNPs

After 18 h of incubation, leaf extract was efficient compared to LENPs. Fruit extract was efficient comparatively to FENPs against *E. faecalis* and *S. aureus*. FENPs were efficient compared to fruit extract against *S. typhi* and *K. pneumonia* (Figure 6 and Table 1).

To conclude, the antimicrobial activity of AgNPs fabricated using a biosynthetic method was evaluated. A comparison was made between the *in vivo* fruit synthesized AgNPs and *in vitro* leaf synthesized AgNPs of *Dioscorea oppositifolia* L. In this analysis, the AgNPs displayed antimicrobial activity against all four cultures tested. *In vitro* leaf synthesized AgNPs of *Dioscorea oppositifolia* L revealed higher microbicidal activity compared to *in vivo* fruit

synthesized AgNPs indicating that the aseptic plants are more suitable for AgNps synthesis than normal field plants. The aseptic plants produce very smaller size nanoparticles than the field plant. So, we suggest that aseptic plants can be considered better for nanoparticle production than the field plants.

Table.1 Zone of Inhibition of AgNPs and the Plant Extracts of *Dioscorea oppositifolia* L

S.No.	Organism studied	LE	LENPs	FE	FENPs
1.	*Salmonella typhi* (MTCC733)	11.0	12.5	10.1	7.3
2.	*Staphylococcus aureus* (MTCC7505)	7.8	8.2	3.2	5.1
3.	*Enterococcus feacalis* (MTCC459)	3.5	6.5	2.4	1.0
4.	*Klebsiela Pnuemonia* (MTCC7028)	5.0	6.0	7.0	4.5

LE- Leaf extract, LENPs- Leaf extract mediated AgNPs
FE- Fruit extract,FENPs- Fruit extract mediated AgNPs

Figure 1 A: In vitro developed leaf of *Dioscorea oppositifolia*.L.

Figure.1 B: Colour Change Profile

Figure.2 UV- visible Spectra

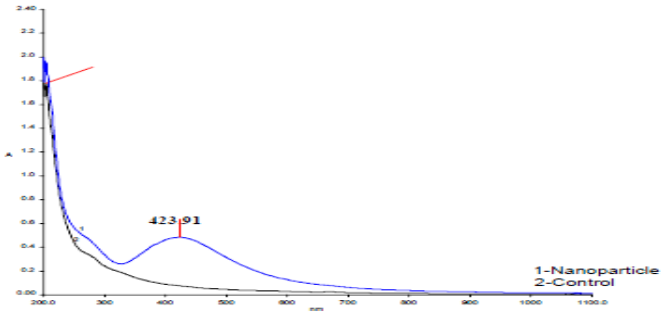


Figure.3 FTIR Spectrum

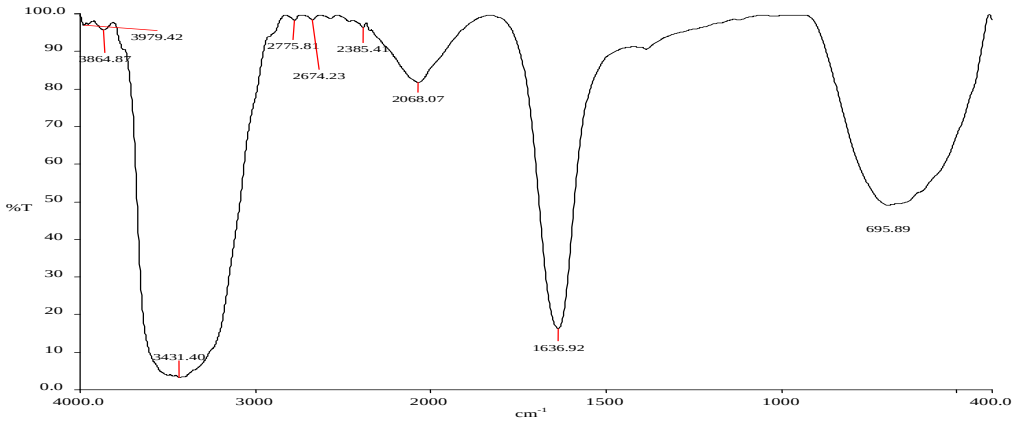


Figure.4 TEM Observation at 200 nm scale. TEM Image of Silvernanoparticles Synthesized from *In vitro* Leaf- Size of the Nanoparticle -17.65nm. And 33.22nm

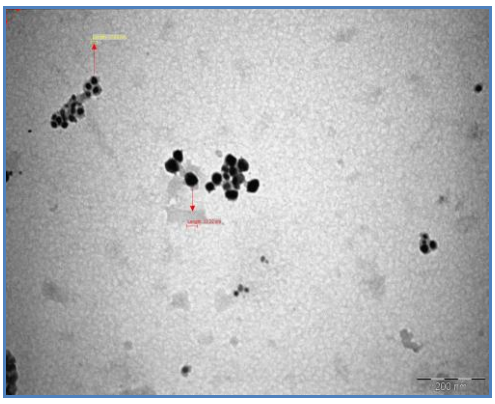


Figure.5SEM Image of Silver Nanoparticles Produced from *Invitro* Leaf of *Dioscorea oppositifolia* L.

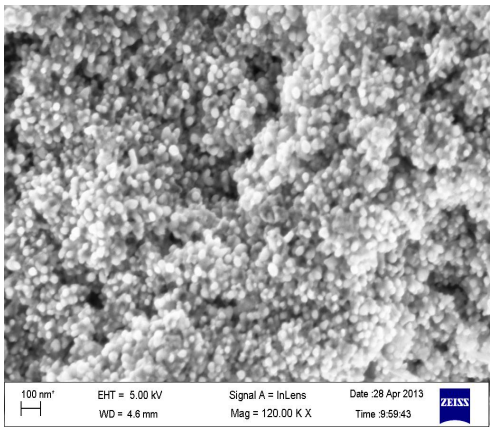
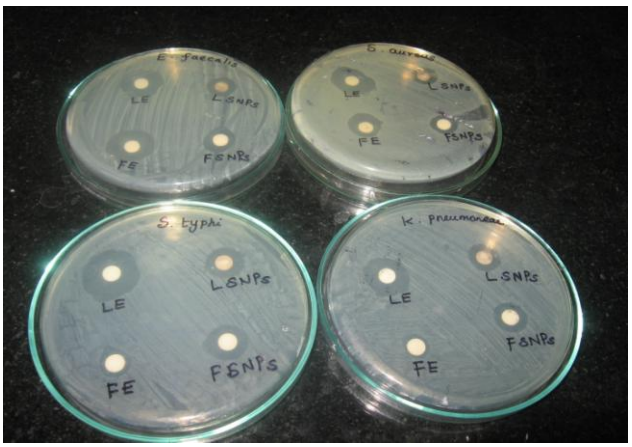


Figure.6 Antibacterial Activity of AgNPs



LE- Leaf extract; LENPs- Leaf extract derived AgNPs; FE- Fruit extract; FENPs- Fruit extract derived AgNPs

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