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Investigation of Antimicrobial activity and Mechanism of Silvernanoparticles Synthesized from Aerial roots of *Rhaphidophora Aura* leaf Extract

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Abstract: *In the present study, an eco-friendly and easy procedure of green synthesis of non-toxic silver nano particles through the leaf extract diospyros benumb. The synthesized silver nanoparticles have effectively inhibited the growth of bacteria while showing minimal toxicity. The antibacterial activities and acting mechanism of silver nanoparticles are examined using Escherichia coli, Streptococcus mutants, Bacillus subtitles and Pseudomonas aeroginosa respectively by analyzing the growth, permeability and morphology of the bacterial cells following treatment with AgNPs. The synthesized materials were characterized using X-ray diffraction, UV-Vis spectroscopy, and FESEM.*

Keywords: *Green synthesis, diospyros benumb leaf, Silver nano particles, Antibacterial*

I. Indroduction

Nanotechnology is anticipated to open novel opportunity to prevent and fight against the disease using atomic scale tailoring of materials. [1] In the field of nanotechnology the synthesis of nanoparticles of different sizes, chemical compositions, and controlled monodispersity is very essential because they exhibit unique properties which are not seen in bulk materials [2-3]. Metallic Nanoparticles (NPs) with antibacterial activity are most promising materials because of its large surface to volume ratios and it exhibit increased chemical activity. Nanoparticles play crucial role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering (4) and which has considerable attention because of their various applications. Recently, there is an urge to develop environmentally benign nanoparticles synthesis processes. In recent years, the plant mediated biosynthesis of NPs is beneficial over physical and chemical methods because it is eco-friendly, a cost-effective and replace the complex process of maintaining cell cultures and could be suitably scaled up for large-scale nanoparticles synthesis method and there is no need to use high pressure, energy, temperature, and toxic chemicals(5).

2. Materials Methods

2.1 Biosynthesis of Silver nanoparticles

The collected fresh diospyros benumb leaves were thoroughly washed thrice with tap water and then with double distilled water to remove dust particles. The leaves were then shade dried, cut and finally used for the biosynthesis of AgNPs. Plant leaf extracts were prepared by boiling 10g of dried leaves in 100 ml of demonized water in 300 mL Erlenmeyer flask for 30minutes at 60° C. This was filtered through a Whatmann no.1 filter paper and used within 1 hour or stored at -20° C till use. Different concentrations of diospyros benumb leaf extracts (10, 30 , 60 ,120, 180 Mins) was treated with 100ml of aqueous 1mM $AgNO_3$ solution with constant stirring on Erlenmeyer flask for reduction of Ag+ ions and then periodically color change of the solution was checked from yellow to brown solution.

2.2 ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

The antimicrobial activities of aqueous plant extract diospyrose benumb determined using disc diffusion method. The antibacterial activities of silver nanoparticles were studied against the gram-negative bacterial strains, Escherichia coli, Streptococcus mutants, Bacillus subtitles and Pseudomonas aeroginosa The bacterial strains grown in nutrient broth at 37°C with continuous shaking at 200 rpm for 24h.The nanoparticles were sub cultured on Muller Hinton agar medium (MHA). Incubation was kept for a period 48 hours at 50°C and stored in 5°C in refrigerator to maintain stock culture. The serial dilutions were conducted with different level of concentrations 100 µl, 200 µl, and 300 µl/L of silver nanoparticles. The zone growth of the plate incubation was 48 hours using a negative bacterium as a standard positive control. Growth kinetics was determined by measuring optical density at 560 nm at every 1h interval from the time of inoculation.

3. Results and Discussion

3.1 UV-Vis Spectra Analysis of AgNPs

Fig (1) show the UV-Visible spectrum of silver nanoparticles recorded from the reaction medium at the interval of (10, 30, 60, 120 and 180 Mins). The maximum absorption characteristic Surface Plasmon peak is located at 412 nm indicates the formation and presence of Ag nanoparticles. The color changes are due to excitation of surface Plasmon vibration in silver nanoparticles. AgNPs have free electrons, which have rise to Surface Plasmon Resonance (SPR), and absorption is due to the vibration of electrons of Ag nanoparticles in resonance with light energy. The maximum absorption spectra band are shifted to longer wavelength due to the increase in time of exposure on surface of Ag nano particles which indicates the formation of more or larger number Ag particles in different size and structure from 412 nm to 424 nm as shown in fig(1).

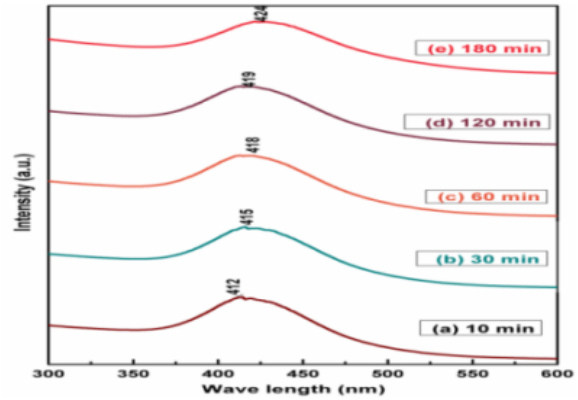


Fig:1 Shows the UV-Vis absorption spectra of reduction of silver ions to AgNPs synthesized by diospyros benumb leaves leaf extract with different time intervals (a) 10 min (b) 30 min (c) 60 min (d) 120 min and (e) 180 min

The formations of phase change and crystalline nature of Ag nanoparticles were confirmed by X-ray diffraction patterns of the biosynthesized silver nanostructure produced by the leaf extract in support with UV-Vis analysis.

3.2 XRD Analysis of Silver Nanoparticles

The structural confirmed peaks of Ag nanoparticles are located at 2θ = 38.0°, 44.2°, 64.4° and 77.3° for the indexing angles of reference planes at (111), (200), (220), and (240) which well matches with data base of Joint Committee on Diffraction standards (JCPDS) number 89-3722. Very high crystalline and nano level particles crystallographic planes of face centered cubic (FCC). The average crystalline size of the silver nanoparticles formed in the bio reduction process found to be 32 nm. The lattice constant calculated from this pattern was 4.0885 A°. The XRD data using full proof software and hexagonal structure as shown figure.

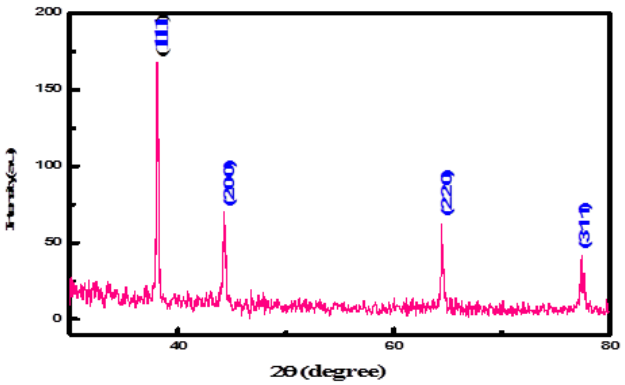


Fig2: XRD patterns of AgNPs by treating diospyros benumb leaves leaf extract with $AgNO_3$ solution.

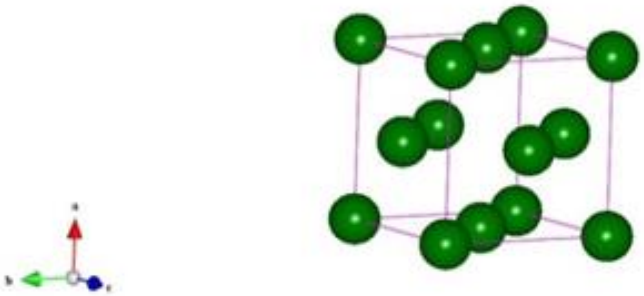


Fig 3: XRD patterns of AgNPs by Silver Nanoparticles Hexagonal structure using to full proof software.

3.3 FESEM analysis

The formation of biosynthesized AgNPs was examined by FESEM s are the best analyzing tools for combined with shows the morphology of AgNPs investigated using FESEM and reveals that the green synthesized AgNPs particles are spherical in shape, with agglomerations .

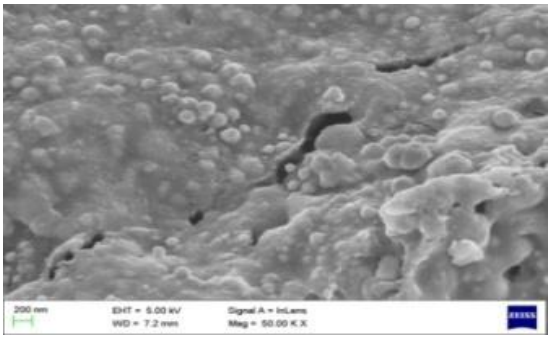
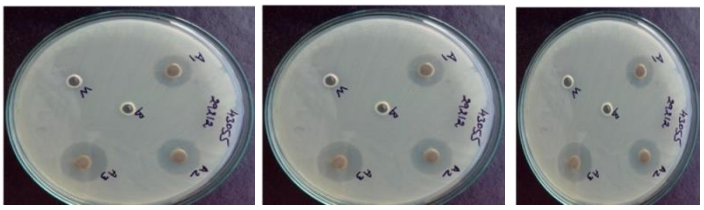


Fig 4: HRSEM image of AgNPs synthesized from diospyros benumb leaf extract

3.4 Antimicrobial Activity

The metallic nano particle naturally occurring element, is non-toxic, hypoallergenic, does not accumulate in the body to cause harm and considered safe for the environment. Bacteria depend on an enzyme to metabolize oxygen to live. Silver interferes with the effectiveness of the enzyme and disables the uptake of oxygen killing them. This process has the added benefit of not harming humans.The bacterial test were performed against two gram negative bacteria, E.coli and P. Aeruginosa on nutrient agar plates containing different concentration of Ag nanoparticles using the diameter of inhibition zone (DIZ) in a disk diffusion method.



a) E.coli b) S. mutants c) P. aeruginosa

Fig 5: Antibacterial activity of synthesized Silver nanoparticles(leaf extract) on a) Escherichia coli, b) S.Mutants c)P.aeruginosa, by the disk diffusion method.

The disks with AgNPs were surrounded by a clear and significantly larger DIZ for E. coli, S.mutants and P.aeruginosa. The average DIZ value for the Ag NP impregnated disks was almost 100% percentage observed. The Ag+ or Ag NP released from the surface area of aqueous extract to the surrounding medium, damaging proteins and genetic material in organisms leading to cell death or inhibited growth. Increasing the concentration of the nanoparticles and exposure time increases the growth delay of P. aeruginosa and E. coli, indicates that the concentration of the Ag nanoparticles is a prime parameter for the antibacterial activity. The DIZ was higher for higher concentration of the silver nanoparticles. A_1 (100 µl), A_2 (200 µl) and A_3 (300 µl) for E.coil, S. mutants and P. aeruginosa are clearly shown in the image below. The maximum antibacterial activity in 300µl/ml concentration of the synthesized AgNPswas 19 mm, 17mm and 16 mm for *E. aero genes*, P. aeruginosa and E. coli,, receptively.

4. Conclusion

We have described a simple and green synthesis of AgNPs by using reducing agents, which requires no special physical conditions and can potentially eliminate the problem of chemical agents that may have adverse effects, thus making nanoparticles more compatible with the eco-friendly approach. AgNPs have potential antibacterial activities against E. coli P. aeruginosa and E. coli cells. The results of experiments showed that the activity of respiratory chain dehydrogenases in E. coli might be inhibited by AgNPs with the higher concentration of AgNPs, the lower the activity of enzymes while making a break through the permeability of outer membrane firstly, resulting in the leakage of cellular materials. The pH, incubation condition and temperature conditions did not affect the growth of AgNPs treated cells.

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