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Synthesis And Characterization Of Silver Nanoparticles Using *Hydnocarpus Alpina*, Its Application As A Potent Antimicrobial And Antioxidant Agent – A Novel Study

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Abstract: The biological synthesis of silver nanoparticles (AgNPs) was reported using *Hydnocarpus alpina* leaf extracts as reducing agent. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this research article, a simple and eco-friendly biosynthesis of AgNPs was prepared using *Hydnocarpus alpina* leaf extract as reducing agent. The AgNPs were characterized by UV-vis, FT-IR, XRD and SEM techniques. The UV-Vis spectrum of AgNPs in aqueous solution showed absorbance peak around 414 nm due to Surface Plasmon resonance. AgNPs were confirmed with XRD which exhibited intense peak. Besides the above, the FTIR analysis showed that C-O, -C-N-, -C=C-, -C□C-, N-H, O-H and C-H are the functional groups are present, which may involved in the AgNPs synthesis. Again, AgNPs size was confirmed by SEM analysis which showed that particles were in the range of 43 to 52 nm in size. Further, the biosynthesized AgNPs are to analyze antimicrobial activity and *in vitro* antioxidant activity has been evaluated.
Keywords: AgNPs, UV-vis, XRD, FTIR, SEM, *Hydnocarpus alpina*, antibacterial activity and *In vitro* antioxidant activity.

1. Introduction and Experimental

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level[1]. Different types of nanomaterials like copper, zinc, titanium[2], magnesium, gold[3], alginate[4] and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms[5]. Silver was known only as a metal until the recent advent of the nanotechnology era, when it became recognized that silver could be produced at the nanoscale[6,7]. AgNPs are the basic essential elements in the wall of nanotechnology and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent properties[8]. These unique features of nanoparticles may lead to play a crucial role in biomedicine, energy science, optics and other health care applications[9]. Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100 nm that can drastically modify their physico-chemical properties compared to the bulk material[10]. The chemical[11] and physical[12] processes are the classical general methods used for the fabrication of nanoparticles, but these methods are not environmentally benign[13] and due to the presence of some toxic metals in the synthesis process that may create some dicey effects in biomedical applications[14]. Biological methods of nanoparticles synthesis using microorganisms[15] and plant or plant extract offer numerous benefits over chemical and physical methods. It is cost effective, environmental friendly, easily scaled up for large scale synthesis. In this method there is no need to use high pressure, energy, temperature and toxic chemical that may have adverse effect in the medical application [16-18]. Moreover, these nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as mechanics, optics, biomedical sciences, chemical industry, electronics, space industries, drug gene delivery, energy science and catalysis[19,20].

The main objectives of this study were (i) to synthesize the AgNPs using aqueous Leaf extract of *Hydnocorpus alpina*, (ii) to characterize the AgNPs by using UV-vis Spectroscopy, X-ray diffract meter (XRD), FTIR, SEM, (iii) to analyze antimicrobial properties against pathogenic Gram-negative *Salmonella typhi* and *Salmonella marse* bacteria. (iv) to analyze an *in vitro* antioxidant activity. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co (St. Louis, MO, USA), silver nitrate, dimethyl sulfoxide (DMSO), trichloro acetic acid (TCA) were purchased from Himedia Chemicals Pvt. Lim. India and all other chemicals used including the solvents were of analytical grade. And also, good quality deionized water was used for the entire experimental work.

Figure A: *Hydnocorpus alpina*

The herbal plant *Hydnocarpus alpina* (fig A) were collected from Kolli Hills (Namakkal District Tamilnadu), it is a major mountain range and is an outline of the Eastern Ghats. Properly washed 50 g of fresh leaves was added in 250mL ultra-pure water in 500mL Erlenmeyer flask and boiled for 10–15 min. Whatman filter paper (No. 40) was used for the filtration of boiled material to prepare the leaf extract, which was used as such for metal nanoparticle synthesis.

1.1 Synthesis of Silver Nanoparticles

Aqueous solution of silver nitrate (AgNO3) at concentration of 1 mM/mL was prepared and used for the synthesis of AgNPs. Ten milliliter of *Hydnocarpus alpina* leaf extract was added into 90 mL of aqueous solution of 1mM/mL silver nitrate for reduction into Ag+ ions and exposed to bright sunlight at 50 °C; the change of color takes place in 15h from yellowish to reddish brown color (Fig 1(a)).

Figure 1(a): Synthesis of AgNPs from extract of *Hydnocorpus Alpina* leaf extract.

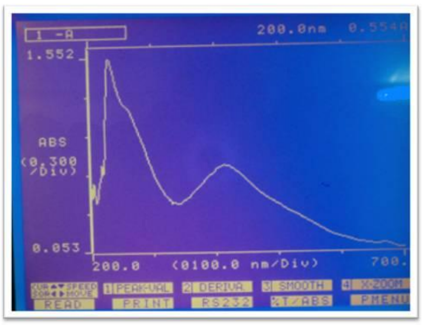

Figure 1(b): The UV- Visible Spectra of AgNPs synthesized using aqueous extract of *Hydnocorpus Alpina*

1.2 UV-Visible Spectroscopy.

Spectral analysis for the development of AgNPs at different reaction conditions were observed by using a Double Beam UV-Visible (UV-vis) spectrophotometer.

1.3 XRD- Study

A drop of synthesized AgNPs was coated on a glass plate and the coated film was characterized using X' Pert Powder PANALYTICAL X-Ray Diffraction Spectrometer. Debye-Scherrer's equation was used to determine the particles size of the silver nanoparticles from the 2θ values of the X-ray diffraction peaks.

Debye – Scherrer's Equation

$$D = K\lambda / \beta \cos\theta$$

Where K= constant, λ = wavelength of the X-rays, β = full width half maximum of the XRD peak (radians), θ = Bragg's angle of the XRD peak.

1.4 SEM and FTIR characterization of synthesized AgNPs

The synthesized AgNPs were fabricated on a glass plate. The morphology and size of the AgNPs was investigated by using VEGA3 TESCAN make Scanning Electron Microscopy provided with Vega TC Software, Further Secondary Electron Sputtering at an applied potential of 20 Kv was adopted prior to recording SEM. ART model of FTIR was recorded for the synthesized AgNPs in a Shimadzu Spectrophotometer.

1.5 Antimicrobial activity

Silver nanoparticles biosynthesized from *Hydnocorpus alpina* leaf extract were tested for antimicrobial activity by disc diffusion method against pathogenic Gram-negative *Salmonella typhi* and *Salmonella marse* bacteria. The pure bacteria cultures were sub cultured on nutrient agar media. Both strains were swabbed evenly onto the single plates using sterile glass rods. After incubation at 37±C for 24 hours, the levels of zone diameter inhibition of bacteria were measured.

1.6 Antioxidant property

Antioxidant property of the leaf extract was determined by DPPH assay, Hydrogen Peroxidase assay, Metal chelating assay and Reducing power assay[21, 22.]

2. Result and Discussion

2.1 UV-Visible (UV-vis) spectroscopy analysis

The color change from yellowish to reddish brown was noted in 15h this was confirmed the formation of AgNPs by recording UV spectra of the solution and observing the bands obtained at 480nm due to the Surface Plasmon Resonance (SPR). Fig 1(b) shows that the UV spectrum bands were formed in the range of 414-440nm. This confirms the formation of silver nanoparticles at room temperature. This study shows that 5ml of silver nitrate is easily reduced by 1ml of aqueous plant extract in 15 hours.

2.2 XRD analysis

The crystalline nature of the AgNPs synthesized from *Hydnocarpus alpina* has been studied by XRD analysis. The XRD spectrum of the AgNPs showed five diffraction peaks at 32.5º, 46.5º, 37.9º, 25.00 and 28.9º in the 2Ɵ range 20º to 80º (Figure 2(a)). Among these four peaks 37.9º is the obtained characteristic peak. Using X' Pert Powder PANALYTICAL X-Ray Diffraction Spectrometer, the diffraction peak values were matched and their corresponding values (122), (231), (111) and (210) were obtained for 32.5º, 46.5º, 37.9º and 28.9º respectively. So, it was confirmed that the AgNPs were crystalline in nature and were cubic face centered.

2.3 FTIR spectroscopy analysis

FTIR spectrum revealed that the absorption bands at above 3390 cm^{-1} are may be responsible for the presence of O-H and N-H stretching vibrations. A band at 2854 cm^{-1} is due to C-H stretching vibration. In fig 2(b) bands at 2284 cm^{-1} showed the presence of -C□C- groups. Bands at 1741 cm^{-1} and 1711 cm^{-1} are characteristic stretching vibration of carbonyl functional group in acid and aldehyde. A band at 1646 cm^{-1}

shows the presence of carbonyl functional group in amide. Bands 1628 cm^{-1}, 1618 cm^{-1} and 1614 cm^{-1} shows the presence of -C=C- group. N-H stretching vibration in the amide linkage is appeared at 1571 cm^{-1}. A band at 1459 cm^{-1} is may be assigned to symmetric stretching vibration of $–COO^-$ group and a peak at 1452 cm^{-1} showed the presence of aromatic C=C group. Germinal methyl group shows the absorption bands at 1410 cm^{-1}, 1406 cm^{-1} and 1384 cm^{-1}. C-H bending vibration is observed at 1320 cm^{-1}. 1282 cm^{-1} band showed the extract contains -C-N- groups. 1205 cm^{-1} band showed the extract that contains C-O groups. The FTIR analysis showed that O-H, N-H, C-H, -C□C- -C=C-, N-H, C=C, C-O, O-H and C-H are the functional groups present in the aqueous extract of *Hydnocarpus alpina* which may involved in the AgNPs synthesis.

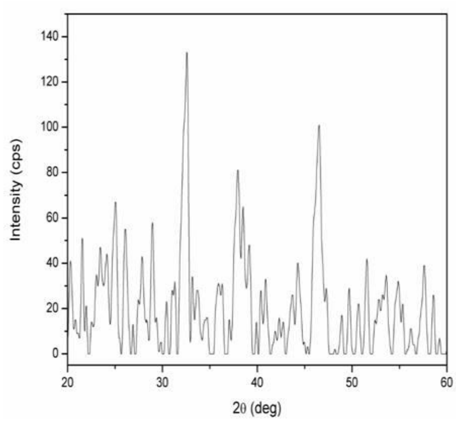

Figure 2(a): XRD Spectra of silver nanoparticles formed from *Hydnocorpus Alpina* extract

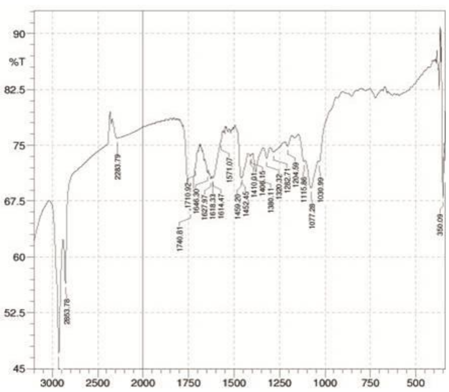

Figure 2(b): FTIR Spectra of AgNPs formed from aqueous *Hydnocorpus Alpina* extract

2.4 Scanning Electron Microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) experiments proved the formation of nanocrystalline silver particles, as shown in Fig 3(a). The nanoparticles predominately adopt a spherical morphology and are often agglomerated into small aggregates, comprising of 4–5 particles each, as Fig 3(a) illustrates. The obtained nanoparticles are quite uniform in size and up to 43 nm. In rare occasion, particles with higher sizes were also observed in the sample, but their population was rather low. The SEM images exposed that the small particle aggregates are coated with a thin organic layer, which acts as a capping organic agent. This also may well explain that fact that the nanoparticles showed a very good dispersion inside the bio-reduced aqueous solution, even in the macroscopic scale.

2.5 Antimicrobial activity of synthesized AgNPs

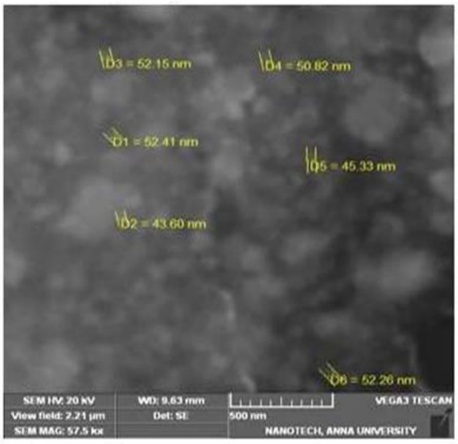

Figure3(a): Scanning electron micrographs of AgNPs synthesized with *Hydnocorpus Alpina* leaf extract

Figure3(b): Anti bacteriyal activity of AgNPs with *Hydnocorpus Alpina* leaf extract against *solmonalla typhi* and *solmonalla Marse*

For the antimicrobial evaluation of synthesized AgNPs, the disc diffusion method was used. In Disc diffusion study, the lowest concentration of AgNPs 20 µg/mL was found to be effective in killing the bacteria up to 1×10^7 CFU/mL concentrations, as there was no cell growth seen on plating after incubation at 37±C for 24 hours. The analysis of MIC/MBC study states that these ratios are responsible for inclusive killing of bacteria up to mentioned concentrations. Fig. 3(b) shows the inhibition of two challenge bacteria's by AgNPs extracted from *Hudnocorpus alpina* extract. It was evident that around the paper soaked in AgNPs with different concentrations has a significant inhibition zone against Gram-negative *Salmonella typhi* and *Salmonella marse* bacteria.

2.6 *In vitro* Antioxidant activity of synthesized AgNPs from *Hydnocorpus alpine*

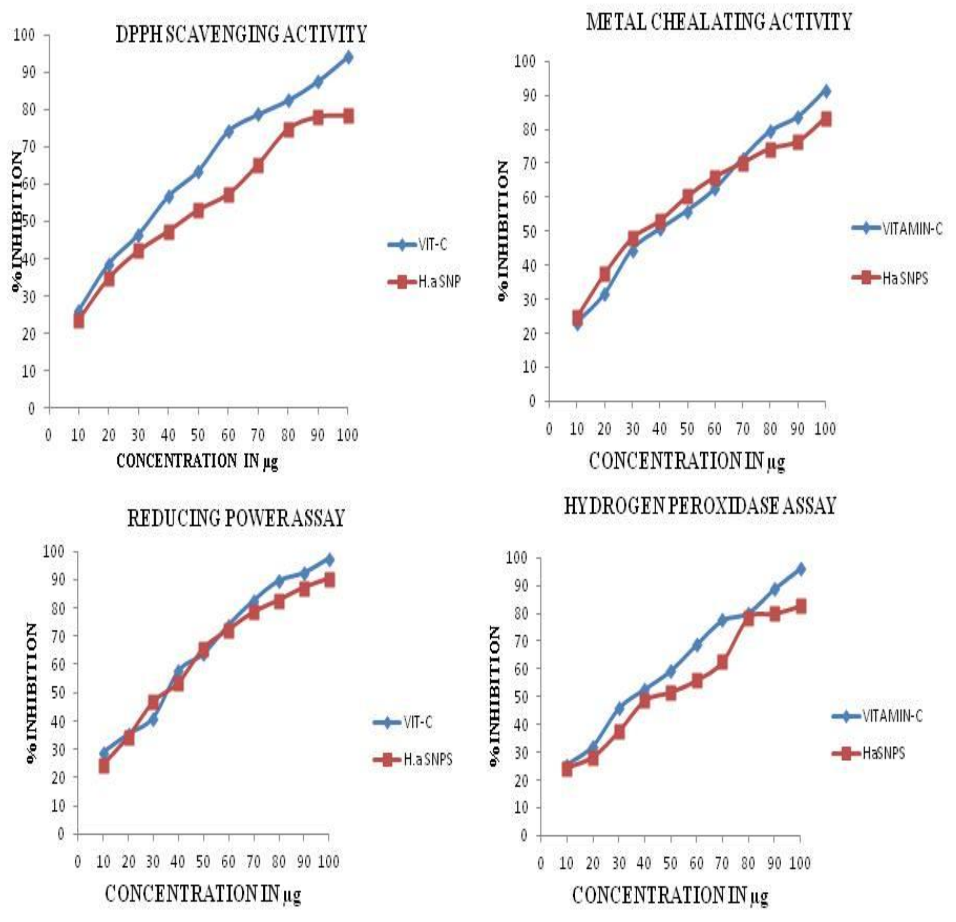

Figure 4: *In-vitro* Antioxidant activity of synthesized AgNPs from *Hydnocorpus Alpina*

We are reporting for the first time antioxidant activity of AgNPs capped with plant extract possessing free radical scavenging activity. Antioxidant activity of AgNPs is shown in terms scavenging capacity and % anti-oxidant activity in Fig 4. Capped AgNPs were found to be potent free radical scavenger when compared to standard. Free radical scavenging activity of AgNPs at maximum concentration. The table given below confirmed the free radical scavenging capacity of our synthesized AgNPs.

S. No	Parameter	Inhibition concentration 50% (IC ^{50} value)	
		Standard (µg)	SNPs (µg)
1	DPPH	34	46
2	Metal chelating activity	40	34
3	Reducing power assay	38	36
4	H2O2 scavenging activity	36	40

2.7 Conclusion

The rapid biological synthesis of AgNPs using leaf of *Hydnocorpus alpina* provides an environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The size of the AgNPs was between 42 and 52 nm. The bioreduced AgNPs were characterized using UV Spec, FTIR, XRD and SEM techniques. From a technological point of view, these obtained AgNPs have potential applications in the biomedical field and this simple procedure has several advantages such as cost effectiveness, Drug delivery, compatibility for medical and pharmaceutical applications, as well as large scale commercial production. The antimicrobial activities were carried out against Gram-Negative *Salmonella typhi* and *Salmonella marse* strains by using disc diffusion method. The synthesized nanoparticles have antioxidant activity due to capping can be used against deleterious effects of free radicals.

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