Synthesis, Characteristics And Antimicrobial Activity Of Ag Nanoparticles

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Abstarct:** The biosynthesis of transition metal nanoparticles is gaining importance due to their biocompatibility, low toxicity and environmental friendly nature. Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology. In the present study, synthesis of silver nanoparticles (AgNPs) has been demonstrated using extracts of Ginger (Gingeber officinale) reducing aqueous silver nitrate. The reduction of silver ions occurred when silver nitrate solution was treated with aqueous extract of Gingeber officinale at 60 º C. Synthesised silver nanoparticles (AgNPs) were confirmed by analyzing the excitation of Surface Plasmon Resonance (SPR) using UV – Vis spectrophotometer at 425 nm. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face center cubic (fcc) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature with size ranging from 7-34 nm, which were characterised by Scanning Electron Microscope. FTIR spectroscopy reveals the functional group responsible for the reduction of Ag Nps. The structural morphology was confiremed by SEM analysis.The chemical composition of the nanoparticles is characterized by Energy Dispersive X-ray spectroscopy. Synthesized silver Nps show good antimicrobial effect against various pathogens which were analyzed by well diffusion method.

I. Introduction

The Field of nanotechnology is the most active area of research in modern materials science. In recent years, researchers in the field of nanotechnology are finding that metal nanoparticles have all kinds of previously unexpected benefits. They are mostly prepared from noble metals, that is, silver, gold, platinum and palladium, silver nanoparticles (AgNPs) being most exploited [1]. Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors. Additional applications include molecular diagnostics and photonic devices, which take advantage of the novel optical properties of these Nanomaterials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings. Many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria.

Different types of nanomaterials like Copper, Zinc, Titanium [2], Magnesium, Gold [3] 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 [4]. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, Catalytic and antibacterial activity [5].

Various protocols used for nanoparticles production often require the use of toxic chemical solvents/surfactants [6–8] and strong reducing agents (e.g., borohydride or hydrazine) [9–12], which typically generate large quantities of hazardous waste. Hence, nanoparticles synthesis procedures that eliminate the use of hazardous reagents [9, 13–15] and afford greener, more cost-effective alternatives are becoming more desirable as the number of nanoparticle applications increases. This is particularly true for biomedical research applications of metallic nanoparticles, which are rapidly growing due to their potential as therapeutic [16, 17] and contrasting agents [18]. Biological methods have emerged as an alternative to the conventional methods for synthesis of NPs. Synthesis of inorganic nanoparticles by biological systems makes nanoparticles more biocompatible and environmentally benign [19]. Moreover, the process is cost effective too [20]. Many bacterial as well as fungal species have been used for silver nanoparticles synthesis [21]. But most of them have been reported to produce AgNPs intracellular. This synthesis always takes longer reaction times and also demands subsequent extraction and recovery steps. On the contrary, in plant extract mediated synthesis, the reaction times have also been reported to be very short compared to that of microbial synthesis. Most importantly, the process can be suitably scaled up for large scale synthesis of NPs [22]. Many plants such as *Pelargonium graveolens* [23], *Medicago sativa* [24], *Azadirachta indica* [22], Lemongrass [25], *Aloe vera* [26], *Cinnamomum camphora* [27], *Emblica officinalis [28], Capsicum annuum [29], Diospyros kaki [30], Carica papaya* [31], *Coriandrum* sp. [32], *Boswellia ovalifoliolata* [33], *Tridax procumbens, Jatropha curcas, Solanum melongena*, *Datura metel*, *Citrus aurantium* [34], and many weeds [1, 35] have shown the potential of reducing silver nitrate to give formation of AgNPs.

We herein report the synthesis of silver nanoparticles by the reduction of aqueous Ag^+ and with the extract of ginger (*Zingiber officinale*) rhizome .Ginger is known for its medicinal values such as ginger has been used to treat skin diseases, colorectal cancer, arthritis, heart condition and also have been reported for its antibacterial properties [36,37,38]. Thus the approach is cost efficient and completely biogenic method for synthesisizing silver nanoparticles. An investigation detail of present work is as follows.

II. **Experimental Details**.

2.1. Materials

Silver nitrate (AgNO3, 99.99%) was purchased from Sigma Aldrich chemicals. Fresh ginger is purchased from a local market.

2.2. Biosynthesis of Silver Nanoparticles using plant Extract

A five gm of fresh ginger rhizome is washed thoroughly. It is crushed using a mortar and 50 ml of water is added to it. It is boiled for 5 minutes and filtered. The extract was stored at 4° C for further experiments. This filtrate is used as a reducing and stabilizing agent for silver nanoparticles.

In the phyto -synthesis protocol, aqueous silver nitrate (90 ml of 1mM) was added to 10 ml of the plant extract and kept for stirring at 60° C for 4hrs. The bioreduction of silver ions was monitored soon after 5 minutes by the colour change of the mixture. The colour changed from dull white to brown and then deep brown. The observed colour change of the reaction mixture is shown in **Fig1a&1b**.

The colour change is due to the excitation of Surface plasmon Resonance which plays an important role in the confirmation of silver nanoparticles formation [42].The fully reduced solution was centrifuged at10000 rpm for 15 minutes. The supernatant liquid was removed and the pellet obtained was washed in distilled water. The pellets are then dried and stored for further characterization.

Fig. 1a. (Silver Nitrate with immediate addition of plant extract)

Fig. 1b (After reduction of Silver Nitrate)

III. Results and Discussion

3.1 UV – Vis spectrum analysis of Silver nano particles.

The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by Perkin Elmer Lambda UV – Vis double beam spectrophotometer, SAIF, SPU, AVADI. **Fig 2**. Exhibits the UV – Vis spectra ranges from 340 to 740 nm. The UV – Vis spectra showed maximum absorbance at 422 nm corresponding to the Surface Plasmon Resonance of Silver nanoparticles. It is reported earlier that absorbance

at around 430 nm for silver is a characteristic of these noble metal particles [43].

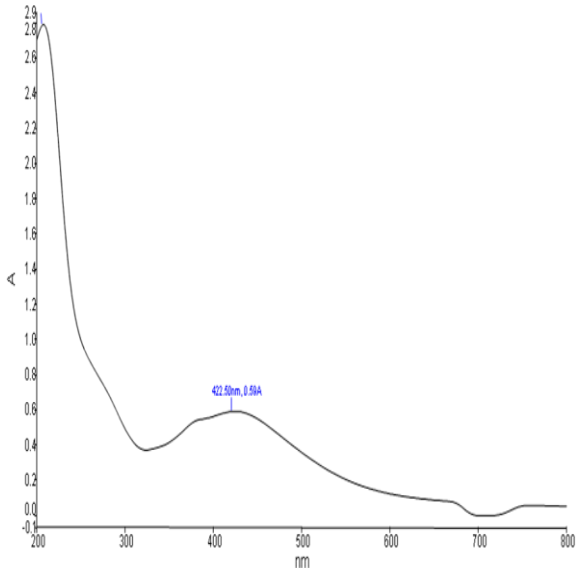

Fig. 2. UV spectrum of synthesised Ag nanoparticlesusing fresh ginger extract.

3.2 XRD – analysis of synthesized silver nano particles.

Crystalline structure and size of the nanoparticles were carried out by XRD. It was carried out in Nuclear Physics Dept, University of Madras. A thin film of the Ag nanoparticles is prepared by dipping a glass plate in the solution to carry out the X- Ray diffraction studies. The diffraction pattern is recorded by Cu – Kα radiation with a wavelength of 1.78 A°, the scanning in the region from 20° to 90° for 2θ at 0.02°/min and a time constant with 2 s. The XRD pattern is as shown in **Fig 3**. The five distinct diffraction peaks at 27.57°, 31.96°, 33.26°, 46.05° and 64° corresponds to the (012),(104),(111), (200) (220) facets of the FCC crystal structure respectively. Thus the crystalline nature of the Silver nanoparticles is confirmed. The broadening of Bragg's peaks indicates the formation of nanoparticles. The mean size of the silvernanoparticles was calculated using the Scherer's equation $D = 0.94\lambda / \beta \cos\theta$ where D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X- Ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. It is found that the calculated average size is 33.4 nm from FWHM peaks. A few intense additional and yet unassigned peaks were also noticed in vicinity of the characteristic peaks of silver. These sharp Bragg peaks might have resulted from some bioorganic compounds/protein(s) present in the fresh ginger extract. The slight shift in the peak positions indicated strain in the crystal structure which is a characteristic of nanocrystals [44] The intensity of the Bragg reflections suggests strong X-ray scattering centers in the crystalline phase and pure crystalline silver structures have been obtained with average particle size of 7 nm to 34nm, and the shape was cubic in nature. It was reported that Ocimum sanctum leaf extract could bioreduce silver ions into crystalline silver nanoparticles (4-30nm) [45].

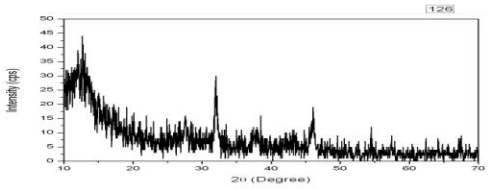

Fig. 3. XRD pattern of synthesized Ag nanoparticles using fresh ginger extract.

3.3 SEM analysis

Scanning electron microscopy analysis reveals the structural morphology of the synthesized silver nanoparticles. The SEM study has been carried out in SAIF, IIT, Chennai. The SEM image of Agnp is as shown in **Fig 4.** It reveals that the synthesized nanoparticles are predominantly spherical in shape. The SEM image shows the size of the nanoparticles ranging from 15 to 34 nm which is in good agreement with the size obtained by Scherer's formula. Similar result of the silver nanoparticles size was obtained for AgNps synthesized using Jartropha curcas and Henna leaf reported by Bar et al., and Kasthuri et al., 2009. [46, 47]

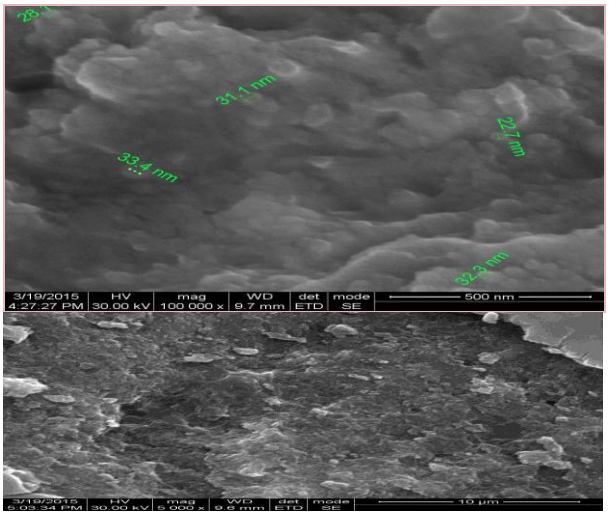

Figure 3. SEM images of Ag Nanoparticles

3.4 EDX analysis

Quantitative elemental analysis of sample was performed by EDX spectroscopic method. The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of silver nanoparticles. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 KeV due to surface Plasmon resonance. **Fig 4.** shows the EDX spectrum of spherical nanoparticles prepared with this bio reduction method. The strong peaks around 3 KeV correspond to the binding energies of Ag. Results obtained coincide with the peak value obtained for AgNp synthesized by using *Coleus aromaticus* [48]. Some other signals correspond to C and O was also observed which indicate the presence of plant phytochemical acting as capping elements. The results indicated that the reaction product was composed of high purity Ag nanoparticles.

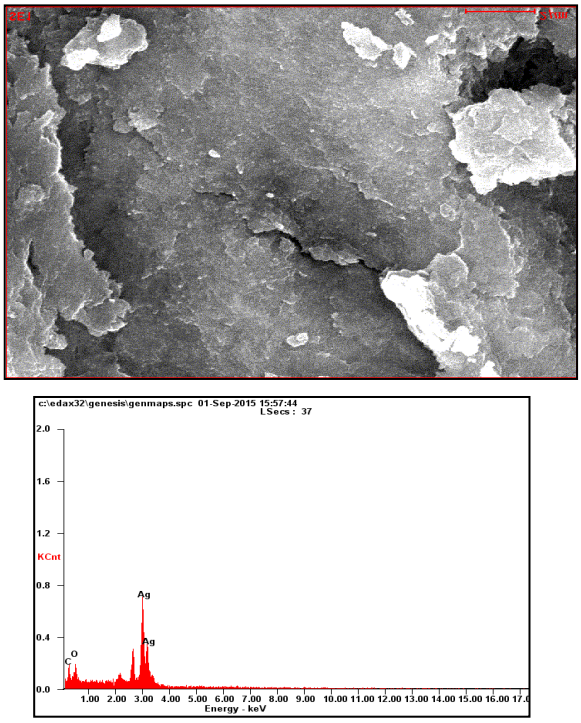

Figure 4. EDX Analysis of Ag Nanoparticles

3.5 FTIR- Analysis Of Ag Nano particles

FTIR analysis is carried out to identify the possible biomolecules responsible for reduction as well as efficient stabilization of the silver nanoparticles. FTIR analysis was carried out at SAIF, IIT, Chennai. **Fig 5** shows the typical spectrum obtained for Silver nano particles, (a), where the spectrum of fresh ^{ginger}, is also displayed for comparison. The spectrum of fresh ginger has several absorption peaks observed at 815 cm^{-1}, 1030 cm^{-1}, 1235 cm^{-1}, 1287 cm^{-1}, 1323 cm^{-1}, 1451 cm^{-1}, 1634 cm^{-1}, 2863 cm^{-1}, 2922 cm^{-1} and 3291 cm^{-1}. The bands at 2922 $cm^{-1}$and 2863 cm^{-1}could be due to alkenes C-H stretch which is associated with lipid molecules in the fresh ginger rhizome. The IR band at an intense broad line at 3291 cm^{-1} is characteristic of the hydroxyl functional group in alcohols and phenolic compounds. After reduction of silver nanoparticles the decrease in intensity from 3291to 3259 indicate the involvement of the OH group in the reduction process. The shift of the band from 1267 cm-1 to 1265 cm^{-1}, 1514 cm^{-1} to 1520 cm^{-1}, 1634 cm^{-1} to 1627 cm^{-1}, 2663 to 2667 cm^{-1} and 2922 to 2923 cm^{-1} were attributed to the binding of C-C, N-O, C=O,CH and alkenes CH group with the nanoparticles 29 (42). The bands at 1033 cm^{-1}, 1428 cm-1 , 2033 cm^{-1}, 2091 $cm^{-1}$and 2863 cm^{-1} are due to CO stretching in ether , ethylene, CH, CH2 The bands at 998 cm^{-1} , 1076cm^{-1} , 1110 cm^{-1} , 2111 cm^{-1} , and 2345 cm^{-1} are due to C-CH_3, C-H, C-O, Ctriplebond N, CO_2 reveals that these functional groups present in the fresh ginger extract could have acted as capping as well as stabilizing agent for the synthesized nanoparticles. The wave numbers at 1030 cm^{-1}, 1451 cm^{-1}, 2091 cm^{-1} and 2863 cm^{-1} C-O, CH_3, N-N, C-H-O respectively completely disappeared in the spectrum of Ag nanoparticles. Reduction of Ag1 to Ag^0 is mainly responsible for the total disappearance these bands after bio reduction.

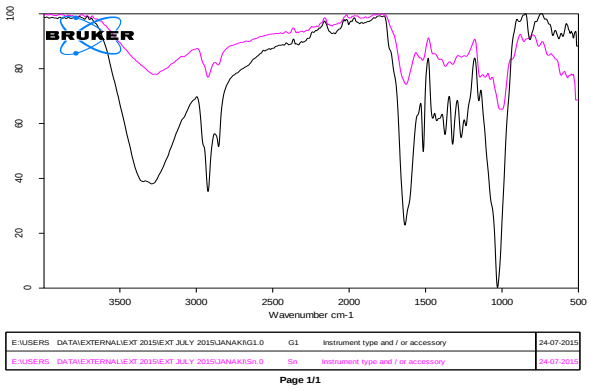

Figure 5. FTIR spectrum of Ginger and AgNps

IV. Anti microbial activity of Silver Nano particles

The Silver nanoparticles showed efficient antimicrobial activity compared to other salts due to their extremely large surface area, which provide better contact with microorganisms. The antimicrobial activity of silver nanoparticles was found to be size dependent, the nanoparticles of size 25 nm possessed highest antimicrobial activity. The mechanism of inhibitory action of silver nanoparticles on micro organisms is partially known. Silver nanoparticles have positive charge, it will attach with negative charged microorganisms by the electrostatic attraction in the cell wall membrane [49] and Silver nanoparticles are associated with the thiol groups of cell wall resulted in the generation of reactive oxygen species and

disrupting the cell [50]. The silver nanoparticles closely associated with cell wall of bacteria by forming 'pits' finally it affects the permeability and cause cell death [52]. The bactericidal and antifungal activity of silver nanoparticles was analyzed by Well diffusion method.

The zone of inhibition for the pathogens is as shown in **Fig 6**. The gram positive bacterium *Staphylococcus aureus , Bacillus subtilis* and *Salmonella tyhi and gram negative bacterium Ecceria coli and Klebsilla pneumonia* and the fungal strains Candida albicans, aspergillus flavus, were examined against silvernano particles. The zone of inhibition for these bacterium and fungi against silver nanoparticles is clearly tabulated in table1. The concentration of silver nanoparticles was varied with 25, 50,100 µg/ml. From the table it is observed that the first two concentrations were resistant to the bacterium and the fungi and the third concentration (100 µg/ml) showed good inhibition zone against them. Thus the silver nanoparticles synthesised using fresh ginger extract has Minimum Inhibition Concentration of 100 µg/ml. Our study coincided with the report of Mahitha et al (2011)[53]. They have examined the antibacterial activity of silver nanoparticles against the gram positive (*Staphylococcus aureus , Bacillus subtilis*) and gram negative(*Ecceria coli and Klebsilla pneumonia*) bacterium. Singh et al reported the Ag Nps using Ginger extract. But they reported that it took 11 days for Ag NO_3 to reduce to Ag Nps and they reported the inhibition zone of Ag Nps against only E.coli. In our present work, silver nanoparticles have been synthesized in a short period using ginger as we use magnetic stirrer and heater for the procedure. Further, we have reported that the Ag NPs have good antimicrobial effect against the bacteria causing food borne illness, [39], Urinary Tract Infections, blood stream infections[40] and fungi causing disseminated infection [41] that are very difficult to treat.

Table 1. Inhibition Zone of AgNp against microbes

Microorganism	**Inhibition zone for Silver nanoparticles conc (µg/ml) in mm**			**Inhibition zone for Standard in mm**
	25	50	100	
e.coli	R	R	10	10
klebsiella pnuemoniae	R	R	17	17
salmonella tyhi	R	R	20	18
staphylococcus aurus	R	R	14	17
bacillus subtlis	R	R	22	22
candida albicans	R	R	14	25
aspergillus flavus	R	R	9	17
aspergillus niger	R	R	R	17

Standard Antibiotics:
AMIKACIN for bacteria
KETOKONAZOLE – for fungi
C – Control - Water

Fig 6a.Inhibition zone of Ag Np against *S.aureus*

Fig 6b.Inhibition zone of Ag Np against as*pergillus flavus*

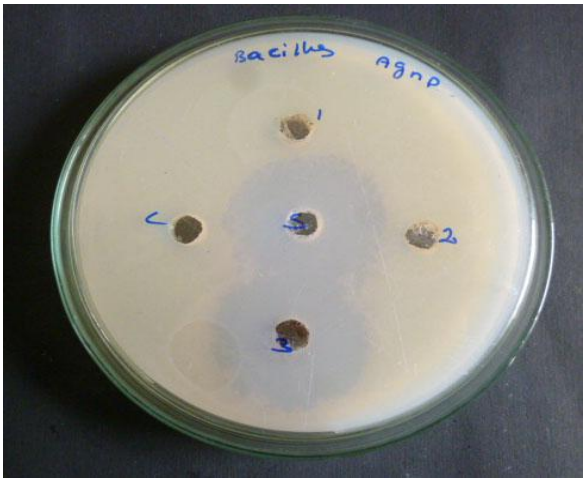

Fig 6c.Inhibition zone of Ag Np against *b. subtlis*

Fig 6d.Inhibition zone of Ag Np against *c albica*

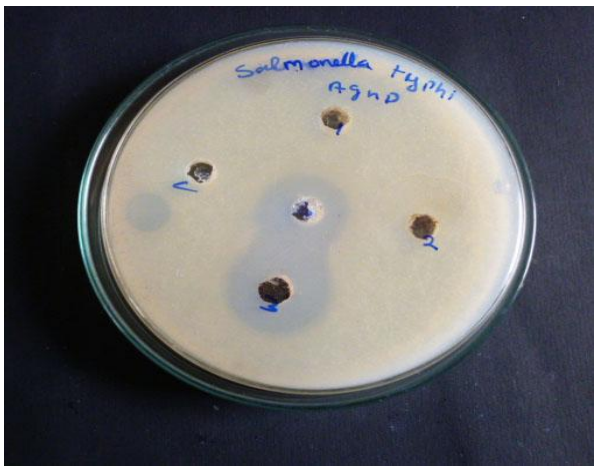

Fig 6f.Inhibition zone of Ag Np against E.coli

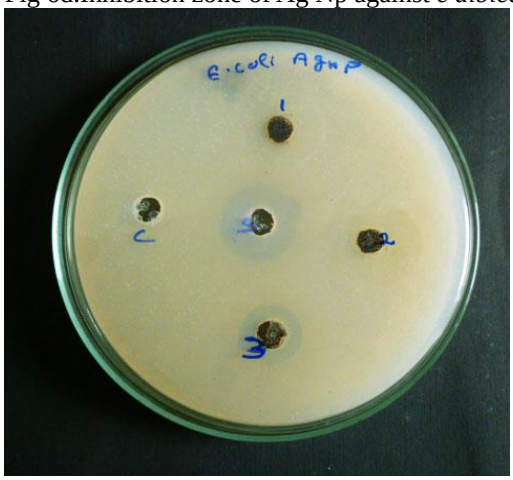

Fig 6e, Inhibition zone of Ag Np against *s. tyhi*

V. Conclusion

The silver nanoparticles in the range from 7-34 nm were synthesised in a rapid and ecofriendly manner. These particles have been characterised by UV absorption spectra with the absorption peak at 422 nm. The synthesize nanoparticles were confirmed by XRD and average size was found by using Scherer's formula as 20 nm which was established by SEM. The SEM analysis reveals the structural morphology which was spherical in shape. The EDAX confirms the significance presence of Silver nano particles. Capping and stabilizing agent plays an important role in the synthesis of nanoparticles which were characterised by FTIR. Finally the antimicrobial activity of these nanoparticles against various pathogens was successfully confirmed by well diffusion method with inhibition zone on the agar plate. Thus the biologically synthesised silvernanoparticles could be of immense use in medical field.

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