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Synthesis of Silver Nanoparticles via Capsicum annnuum L extract and their antibacterial studies

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ABSTRACT: Green synthesis method is considered to best alternate technique for synthesis of various nanoparticles as it is ecofriendly and safe. Also it is cost effective, less time consuming and simple method compared to chemical synthesis. The Silver nanoparticles have wide range of applications ranging from electrical to biological field due to their properties. It is considered toxic to the microorganisms and thus used for biological purposes. Here we report the synthesis of silver nanoparticle by using Capsicum annuum L as the reducing agent and also as capping agent. The results suggest the formation of silver nanorods of 50 nm length with good antibacterial properties.

Keywords: *Capsicum annuum; Silver nanoparticles; Green synthesis*;

1. INTRODUCTION

The development of green methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology, since these methods are considered to be safe and ecologically sound the nanomaterial's fabrication as an alternative to conventional methods was evolved [1]. Based on the various size and shape of the nanoparticles they exhibit unique optical, magnetic and electrical properties resulting in wide range of applications. Metallic nanoparticles are generally synthesized using wet chemical methods where the chemicals are often turned out to be toxic and flammable [2]. The synthesis of metallic nanoparticles using physical and chemical methods have helped us in yielding pure nanoparticles but still they are said to be quiet expensive, and potentially dangerous to the environment [3]. Thus synthesis of these nanoparticles using microorganism, plant extract is thought to be an alternative for the physical and chemical methods as they are considered ecofriendly [4]. The green synthesis technique are generally synthetic routes that utilize relatively nontoxic solvents such as water, biological extracts, biological systems and microwave assisted silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of application in the development of new techniques in the areas of electronics, medicine, materials sciences due to good conductivity and chemical stability, selective coatings of solar energy absorption, intercalation materials for electrical batteries, optical receptors, catalysts in chemical reactions, bio labeling, optoelectronics, medical devices, antibacterial and biomaterials production. Silver is said to be highly toxic to certain gram positive bacteria like S.aures and S.pneumonia and gram negative bacteria like E.coli and P.aeruginosa [6] that usually resist the conventional antibiotics used. A wide range of experiments are being conducted for a cost effective and ecofriendly synthesis of these silver nanoparticles.

Currently, many research works are available on the biosynthesis of silver nanoparticles using plant leaves extract, such as Ficus benghalensis [7], Rosa rugosa [8], Stevia rebaudiana [9], Chenopodium album [10], Nicotiana tobaccum [11] , Trianthema de candra [12], Polyalthia longifolia [13], Cycas [14], Pinus desiflora, Diopyros kaki, Ginko biloba, Magnolia kobus, and Pllatanus orientalis [15], Catharanthus roseus [16], Pungamia pinnata, Hemidesmus indicus, Syzygium cumini, Allium cepa, and Pandaanus odorifer [17], Sesuvium portulacastrum L [18] have been reported. Thus several plant biomass or plant extracts have been successfully used for extracellular biosynthesis of silver nanoparticles wherein the metal ions reduction occurs very rapidly and the reduction of Ag ions will be completed within hours. Also rapid synthesis and excellent yield of silver nanoparticles through the plant-mediated biosynthesis have a time-related (2 to 4 h) advantage in comparison with microbial synthesis (24 to 120 h) [19].

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Competing interests
The authors have declared that no competing interests exist.

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Here in this paper we report synthesis of silver nanoparticles using Capsicum annuum L which act both as a reducing agent and capping agent. C.annum L is taken at five different concentrations say in the range of 5gm to 25gm. A detailed report on the changes observed during the synthesis of silver for various concentrations are noted and reported here. Also their effects on gram negative bacteria E.coli and P.aeruginosa are discussed.

2. MATERIALS AND METHODS

Silver Nitrate ($AgNO_3$) was purchased from Sigma Aldrich and was used without any further purification. Capsicum annuum, nutrient agar medium (LB), nutrient broth and standard antibiotic Cefotxime Na was obtained from Centre for chemical science and technology, JNTU Hyderabad.

2.1 Synthesis Of Silver Nanoparticle

5, 10, 15, 20 & 25gm's of Capsicum annuum were weighed separately for five different concentrations. Then these were washed thoroughly using distilled water and dried. The dried chilies were crushed and 200ml of 50% ethanol was added to it. Beaker was placed in boiling steam bath for 15 to 20 minutes until the color changed to yellowish green. The extract was cooled to room temperature and was filtered. 40ml of distilled water was added to 40ml of the extract in order to dilute the solution. 20 ml 1M of silver nitrate was taken in a beaker and the diluted extract was added to it. The mixture was then boiled for 25min until a reddish brown color was observed. The solution was centrifuged at a high speed for about 40 min the pellet was collected and dried while the supernatant was kept separately in a conical flask for further settling down of the products. The pellet was incubated at 90° for 10 minutes and was stored for further characterizations.

3. RESULTS AND DISCUSSION:

The synthesized nanoparticles were characterized using Ultraviolet and visible spectroscopy (UV), X-ray diffraction system (XRD), Dynamic light scattering system (DLS), Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS) and the results are discussed as follows

3.1 UV Absorbance

The UV characterization was carried out using a double beam spectrophotometer JASCO-V-7100. The absorption peaks for all the samples are in the range of 429 to 456 nm. It can be observed that the UV absorption of samples has a red shift from 429 to 456 nm when the concentration of the extract increased from 5g to 25g. Another observation that can be made is the flattening and broadening of the peaks with increasing concentration of silver. This hints towards an observation that the particles being formed have absorptions similar to silver nanoparticles, more silver is formed with lower concentration of extract used and the increase in the extract concentration is also increasing the particle size of the silver being formed.

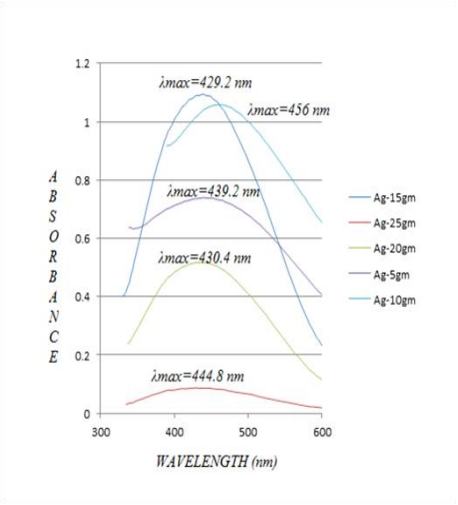

Fig.1: UV absorbance of silver nanoparticles obtained from 5 different concentration of papaya extract

3.2 *XRD Analysis*

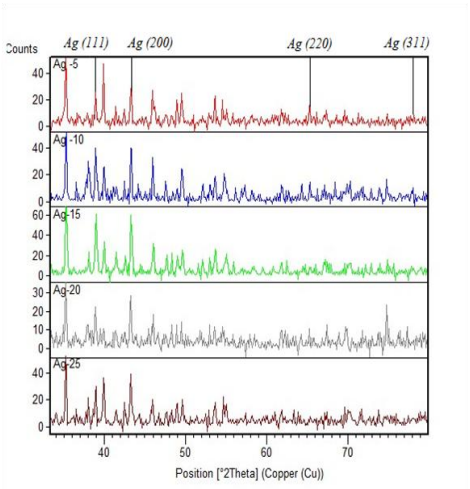

Fig.2: XRD diffraction of Ag with different concentration of reducing agent

XRD peaks of silver from 5 different concentration of the reducing agent are seen in figure 2 using a Bruker AXS-D8-Advance machine. The peaks match with silver (JCPDS#870717) having hkl values (111) (200) (220) & (311). The most interesting observation that can be made from the XRD patterns is that the silver nitrate used in the experiment does not seem to wash away even after multiple centrifugations. The patterns for silver nitrate are clearly visible in all of the samples patterns with some of them dwarfing the silver peaks. The most silver content is seen in the samples with extract 5g and 10g. Over this extract concentration, the particles form silver oxide suggesting towards the oxidative property of the extract being used. The results clearly show the formation of silver oxide in the case of samples 15g, 20g and 25g. The crystallite sizes of the prepared nanoparticles were calculated using the Debye Scherrer's formula. The calculated sizes seem to increase with increasing extract concentration which also match well

with the UV data presented earlier where the overall particle sizes had increased with increasing extract concentration.

3.3 *Particle Size Analyzer*

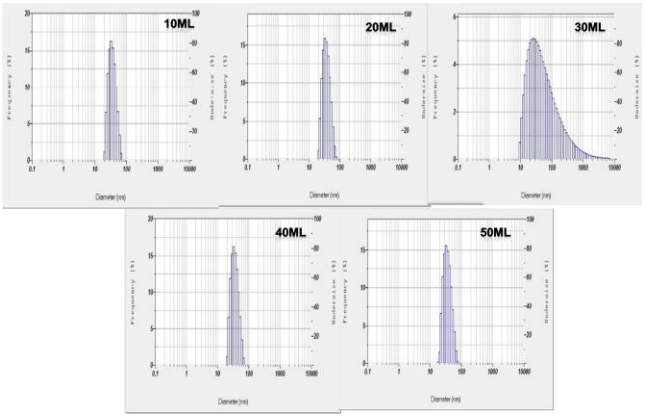

Fig.3: DLS Spectra of all silver nanoparticle samples.

Figure 3 shows the DLS spectra (model HORIBA- SZ 100) of the samples synthesized. Dynamic light scattering helps in analyzing the average particles size along with the poly dispersivity of the silver formed. Which confirms the mean particle size of the samples indicating that the particles size increases as the volume of the extract added to the silver nitrate solution increases. It correlates well with XRD and UV data as mentioned before.

3.4 *Surface Morphology*

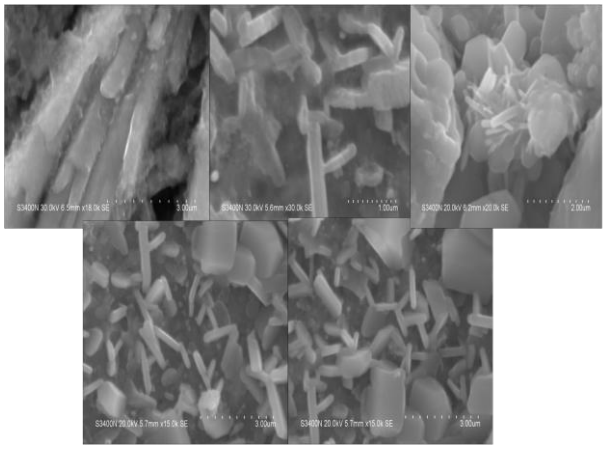

Fig.4: SEM Images of all silver nanoparticle samples.

Figure 4 shows the SEM analysis carried out using HITACHI S3400N model. The imaging was done at a voltage of 20kv and 30kv and was examined at magnifications of ×15,000, ×20,000 & ×30,000. The SEM images of the all the samples shows the rod shape of the nanoparticles formed by the reduction of silver nitrate solution for different concentration of the extract and the EDS confirm the presence of silver.

3.5 *Antimicrobial activity*

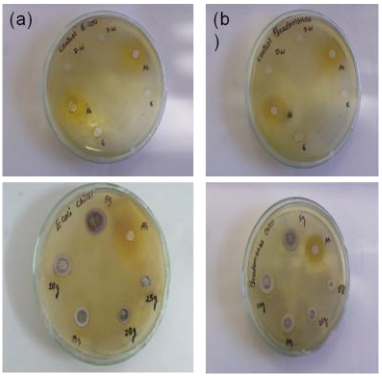

Fig.5: Antimicrobial activity of the silver nanoparticles on E.coli and P. aeruginosa

Table 1: Zone of inhibition for the formed nanoparticles compared to the standard antibiotic

S.N.O	SAMPLES	ZONE OF INHIBITION (mm)	
		E.coli	p.aeruginosa
1	Ag-10	18	15
2	Ag-20	17	14
3	Ag-30	16	13
4	Ag-40	14	11
5	Ag-50	13	10
Antibiotic	Cefotaxime Na	22	20
Control	Distilled water	-	-
Control	Empty (without any sample)	-	-

8gm Nutrient Agar was dissolved in 200ml of dis.H_2O, mixed thoroughly, autoclaved at 15psi pressure at 121°C for 1hr. 1.25gm Nutrient Broth was dissolved in 50ml of dis.H_2O,mixed thoroughly, autoclaved at 15psi pressure at 121°C for 1hr. 2 pathogenic strains *E.coli* and *P.aeruginosa* were taken and spread over 3 agar plates evenly LB agar plates. 6 Wells of 3 mm diameter were casted onto each of the agar plates using porers (3mm diameter). The samples (50 mg/lit AgNPs) along with standard antibiotic (cefotaxime Na) were loaded (30µl) on to the plates. A Control plate was filled with distilled water (negative control) and standard antibiotic (positive control). The square plates were incubated at room temp for 19-24hrs.

The antimicrobial activity of the silver nanoparticles was observed and the same was tabulated in table 1. The zone of inhibition in the case of E.coli and P.aeruginosa shows a linear pattern. Zone of inhibition pattern for the 5gm concentration of the chili extract was higher than the other concentrations for both the strains indicating the higher concentration of the silver.

4. CONCLUSION

The paper concludes that a capsicum extract can be used as an alternative medium for the preparation of silver nanoparticles. The formation of crystalline silver was concluded from the XRD results and the presence was confirmed by EDS data. The particle sizes were estimated to be in the size range of 50- 100 nm with a rod shape indicating that capsicum includes some kind of surfactant that helps silver morphologically change into rod shapes. The paper also concludes that the formed particles are not purely silver but also contains remains of silver nitrate precursor and silver oxide at higher concentration of capsicum extract. Hence it can be concluded that at concentration of 15g, silver nanorods of 50 nm length can be made using capsicum extract.

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