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Synthesis and Characterization of Biogenic Silver Nanoparticles and Its Antimicrobial Analysis

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ABSTRACT

The sustainable green chemistry biosynthesis process for the generation of toxic free, biocompatible and environment-friendly nanoparticles constitutes major role in the present nanotechnology research. The present study described the importance and significance of biosynthesis of AgNPs. The advantages of using a plant extract mediated synthesis of AgNPs include toxicity free and quick synthesis method, economic viability, cost effective and ease in handling large scale synthesis. Curcuma caesia Roxb. (Black turmeric) is a perennial herb having bluish black color rhizomes and it is having huge medicinal properties. The formation of brownish orange color indicated the synthesis of AgNPs by using rhizomes of Curcuma caesia dry powdered rhizomes with $AgNO_3$ solution and this was easily detected and characterized by UV-Visible Spectroscopy, Fourier Transform Infrared (FT-IR) Spectroscopy, XRD, TEM, EDS etc. The formed biogenic silver nanoparticles were optimized by pH, time, temperature, concentration of silver nitrate, concentration ratio of leaf extract and $AgNO_3$. AgNPs show effective antibacterial and antifungal activity. The synthesized biogenic AgNPs are more susceptible to Gram negative bacteria than Gram positive bacteria. They could thus be an efficient alternative to conventional antibiotics and may be used as an antibacterial agent.

Graphical Abstract

Photograph showing change in color before and after adding aqueous *Curcuma caesia* extract to $AgNO_3$

Keywords: Green synthesis**,** *Curcuma caesia*, UV-Visible spectroscopy, FTIR, XRD, TEM, Biogenic AgNPs.

INTRODUCTION

The nanotechnology field is a fast-growing research niche [1]. Nanoparticles are particles with at least one dimension less than 100 nm. Nanotechnology is a collective term that implies the capacity to work with materials at a nanometer scale [2]. In spite of their small size, nanoparticles have high potency, and are emerging with wide range of applications in different disciplines of knowledge, ultimately leading to industrial and technological growth. It is widely accepted that nanotechnology is emerging as a major factor for commercial success in the 21st century and is regarded as "the next industrial revolution".

Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others [3] nanoparticles possess different properties compared to the same material in its coarser or bulk form [4]. Once a material is reduced below 100 nm in size, its components demonstrate unusual features based on quantum mechanics, rather than macroscopic Newtonian mechanics, which influence a variety of material properties such as conductivity, heat transfer, melting temperature, optical properties, and magnetization [5].

As the particles are micronized, they tend to be affected by the behavior of atoms or the molecules themselves and to show different properties from those of the bulk solid of the same material. It is attributable to the change of the bonding state of the atoms or the molecules constructing the particles. The fraction of the atoms or the molecules located at the surface on the particles plays a great role, since they are more active than those inside the solid particles because they are free, which leads to easy bonding with the contacting materials and causes various changes in particle properties [6].

Due to wide range of possible applications, the development of metal [7, 8] and semiconductor-polymer nanocomposites [8, 9] presents new challenges and opportunities for future technologies. As an intermediate between molecular and bulk states, inorganic nanoparticles often exhibit unique properties (e.g., electrical, optical, magnetic, catalytic). Among metal nanoparticles, AgNPs are playing a major role in the field of nanotechnology and nanomedicine. In the last decades, nanostructured silver has been the subject of intensive research.

Besides its technological significance, silver is an important material from a scientific point of view since it can be considered as an ideal model system for studying the physical properties of nano-sized metal particles [10]. AgNPs have received considerable attention due to their attractive physical and chemical properties. Interdisciplinary research has widened the horizons of material research, drawing new inspirations from biological systems. The towering environmental concerns had triggered the researchers to device novel methods of synthesizing the nanomaterials in biological systems such as bacteria, fungi and plants, termed as "green chemistry" approaches. So, there is a growing interest in the use of environmentally safe 'green' reducing agents [11]. Biologically synthesized (biogenic) silver nanoparticles have been a very interesting area of research in the past few years due to their non-requirement of high pressure, energy, temperature and toxic chemicals [12].

MATERIALS AND METHODS

Curcuma caesia Roxb (Black turmeric) is a perennial herb plant having bluish black color rhizomes and it is having huge medicinal properties. It is considered as a medicinal plant to possess various properties such as anti-fungal activity reported by Banerjee and Nigam[13], smooth muscle relaxant and anti-asthmatic activity [14], broncho dilating activity [15], antioxidant activity [16], anxiolytic and central nervous system depressant activity, locomotor depressant, anti-convulsant[17], anthelmintic activity[18], antibacterial activity [19], antiulcer activity [20]. The phytochemical studies of *Curcuma caesia* revealed that the presence of multiple phytoconstituents like essential oils with camphor, arturmerone, (Z) ocemene, arcurcumene,1,8-cineole, elemene, borneol, boranyl acetate,

curcumene, etc [21]. The current research is designed to investigate the impacts of biologically synthesized biogenic AgNPs on bacterial pathogens.

Synthesis of biogenic silver nanoparticles using plant extract: In the last few years, synthesis of silver nanoparticles biologically using plant extracts have been gaining more attention due to the presence of active phytochemicals. It has been seen that AgNPs synthesized by plant extract are more stable than the ones synthesized using other organisms like bacteria and fungi [22].

Antibacterial and antifungal activities of biogenic silver nanoparticles: The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal and fungicidal effects of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution [23]. AgNPs are effective against *Candida* sp., Dermatophytes and a few phytopathogenic fungi [24]. AgNPs show powerful antimicrobial properties even in far lower concentration [25].

Cleaning and sterilization of glassware: Potassium dichromate (60 g) was dissolved in warm water (100 mL), cooled and concentrated H_2SO_4 (60 mL) was added slowly. It was mixed thoroughly and used for cleaning of glassware. The glassware was first soaked in chromic acid cleaning solution (10% potassium dichromate solution in 25% concentrated H_2SO_4) for 3h and then washed thoroughly in tap water. Then these/glassware were washed with a commercial detergent with tap water and was finally rinsed in distilled water and oven dried at 80°C. Dried glassware and media were sterilized in autoclave at 121°C, 15 lbs pressure for 20 min and then dried in a hot air oven.

Collection of rhizomes of *Curcuma caesia*: *Curcuma caesia* plants were obtained from Maredumilli, Rampachodavaram, Rajamahendravaram in Andhra Pradesh, India. Their rhizomes were then obtained, cleaned, dried and further used to prepare aqueous rhizome extract.

Preparation of aqueous Rhizome extract: The dried rhizomes of *Curcuma caesia* were powdered using a dry grinder. The aqueous plant extract was prepared by adding 1g of the rhizome powder to 100 mL of distilled water and heated at 80°C for 3 min. This extract was then filtered using Whatman 40 filter paper and the filtrate was refrigerated at 4°C for further use.

Synthesis of biogenic silver nanoparticles: 5 mL of the aqueous rhizome extract was added to 95 mL of 5 mM Silver nitrate (Sisco Research Laboratories) and incubated at room temperature for 30 min. The solution turned brownish orange in color.

Characterization of synthesized biogenic silver nanoparticles

UV-Visible Spectroscopic studies: The synthesized AgNPs were studied at regular intervals using Shimadzu 2900 UV-Visible Spectrophotometer between ranges of 300-800 nm and at a scanning speed of 600 nm min^{-1}.

Fourier Transform Infrared (FT-IR) Spectroscopic studies: The functional groups present in the rhizome of the plant and its role in the synthesis of AgNPs was determined by FT-IR studies. The dried rhizome powder and biogenic AgNPs were mixed with KBr to make pellet and the FT-IR analysis was carried out in transmittance mode by Shimadzu FT-IR 8300 in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}.

Powder X-Ray Diffraction (XRD) studies: The synthesized dry AgNPs powder was analysed with Cu Kα1 filtered radiation (λ = 1.540598 Å) at a voltage of 40 kV and a current of 30 mA using Seifert JSO Debye Flex XRD in a range of 10° to 70°. The obtained pattern was for FCC cubic crystal structure. The crystalline size was calculated using the Debye-Sherrer formula:

$$D = 0.89\ \lambda / \beta \cos \theta$$

Where, D is the mean grain size, λ is the X-ray wavelength for Cu target, β is the FWHM (full width at half maximum) of diffraction peak and θ is the diffraction angle.

The lattice constant was calculated according to Bragg's law. The crystallinity index was calculated using the formula-

$$Icry = Dp\ (SEM, TEM)/Dcry\ (XRD)$$

Where, Icry-Crystallinity Index, Dp-The particle size obtained from either SEM or TEM morphological analysis, Dcry-The particle size calculated according to Debye-Sherrer formula in XRD.

Transmission Electron Microscopic (TEM) studies: A drop of solution containing biogenic AgNPs was placed on the carbon coated grids and kept under vacuo desiccation before analyzing with Hitachi H 7650 at 100 kV of acceleration.

Energy Dispersive Spectroscopic (EDS) studies: The dry AgNPs powder was used for this purpose using Hitachi S 3400 N operating at 30 kV of acceleration and at a magnification of 25k and the energy dispersive spectrum (EDS) was recorded.

High Resolution Transmission Electron Microscopic (HR-TEM) studies: The stable biogenic AgNPs were washed and diluted by distilled water to attain the absorbance range of 0.5. Then one drop of diluted AgNPs sample was placed on Copper grid with Ultrathin Copper on holey carbon disc and was allowed to dry in vacuo. After drying, the synthesized AgNPs were visualized using High Resolution Transmission Electron Microscope operating at 200 kV of acceleration. The SAED pattern was also obtained.

Optimization of synthesized biogenic silver nanoparticles

pH: The pH of this reaction was optimized at different pH, where the reaction pH was maintained at 3, 5, 7, 9 and 11. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH. The absorbance of the resulting solutions was measured spectrophotometrically.

Time: The time of this reaction was optimized at different time intervals, where the reaction time was monitored from 0 min to 6h. The absorbance of the resulting solutions was measured spectro-photometrically.

Temperature: The time of this reaction was optimized at different time intervals, where the reaction time was monitored from 10° to 80°C. The absorbance of the resulting solutions was measured spectrophotometrically.

Concentration of silver nitrate solution: In this reaction, the concentration of silver nitrate was optimized using different concentrations, where the reaction was maintained at 1 mM, 2 mM, 3 mM, 4 mM and 5 mM. The absorbance of the resulting solution was measured spectrophotometrically.

Concentration ratio of plant rhizome extract and silver nitrate: Similarly, the concentration ratio of plant rhizome extract and silver nitrate was optimized with the increasing concentration (1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 6 mL) of leaf extract in 5 mM silver nitrate solution. The absorbance of the resulting solutions was measured spectrophotometrically. Further, the optimized reaction solution was kept as such in dark at the room temperature and the stability of the synthesized particles was monitored up to 60 days by using Hitachi U 2900 UV-Visible Spectrophotometer (Krishnaraj *et al*., 2012).

Source of Bacterial sample: Pure cultures of bacteria *Escherichia coli* (CFR001), *Staphylococcus aureus* (CFR002), *Klebsiella pneumoniae* (CFR003) were obtained from Department of

microbiology, Andhra University. All the bacterial cultures were maintained in Nutrient Agar (HiMedia, Mumbai, India) slants at 5 ± 1°C.

RESULTS AND DISCUSSION

Biogenic synthesis of silver nanoparticles (AgNPs): In the present study, the formation of brownish orange color indicated the synthesis of AgNPs by using rhizomes of *Curcuma caesia* plant as shown (Figure 1). Extracts of dry powdered rhizomes were used to biologically synthesize AgNPs. The formation of biogenic AgNPs was seen by color change in $AgNO_3$ solution to brownish orange. The $AgNO_3$ solution without addition of extracts of dry powdered rhizomes can be treated as control. This bio reduced aqueous component was further used for characterization purpose.

Figure 1. Photograph showing change in color before and after adding aqueous *Curcuma caesia* extract to $AgNO_3$.

Recently, synthesis of nanoparticles using plants, particularly medicinal plant extracts, is gaining more attention due to the presence of active phytochemicals [**26-27**]. The color identification is a preliminary analysis to confirm the formation of AgNPs. The formation of brownish orange color indicates the synthesis of AgNPs. It may be due to the excitation of Surface Plasmon Resonance (SPR) effect and reduction of $AgNO_3$. The intensity of brown color increased in direct proportion to the incubation period. Ahmad *et al*. (2010) reported that *Ocimum sanctum* extracts have taken 1 h to synthesize the AgNPs. However, the reports of [**28**] shows that the AgNPs are formed in 10 min in the solution of *Azadirachta indica*. *Curcuma caesia* Rhizome extracts show the formation of brownish orange color in 5 min. This indicates that the AgNPs synthesis process has been started. The intensity of brown color increases with increase in the duration of incubation.

Ultraviolet-visible Spectroscopic studies: AgNPs were observed strongly in the range of 400-450 nm in visible region as shown in (Figure 2). In the present work, the biogenic AgNPs are rapidly formed (at pH 7) after the addition of *Curcuma caesia* extract, evident from the appearance of brownish orange color at 418 nm which is the characteristic wavelength of AgNPs with increase in absorbance at regular time intervals as depicted in figure 2. The formation of biogenic AgNPs was easily detected and characterized by UV-Visible Spectroscopy owing to the reduction of silver nitrate and due to the Surface Plasmon resonance (SPR), i.e., the interaction of electromagnetic radiation and the electrons in the conduction band around the nanoparticles [**29**]. AgNPs were observed strongly in the range of 400-450 nm in visible region evident from the appearance of brownish orange colour at 418 nm which is the characteristic wavelength of AgNPs with increase in absorbance at regular time intervals [**30**].

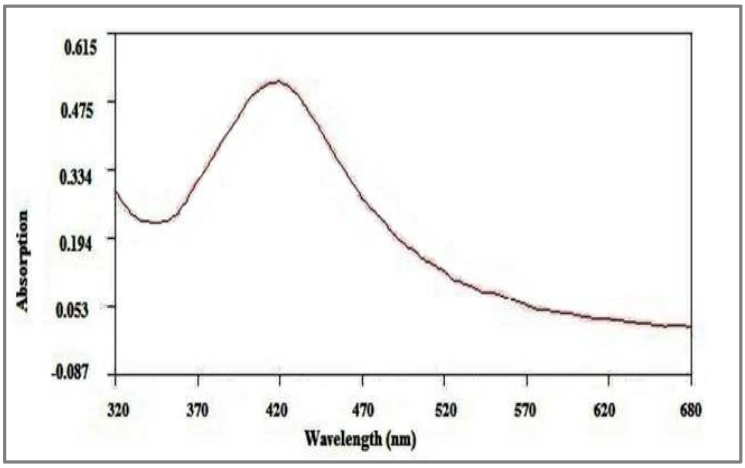

Figure 2. Confirmation of formation of AgNPs at 72 h via UV-Vis spectra at 418 nm wavelength.

Fourier Transform Infrared Spectroscopic studies: FT-IR spectrum of dry powder aqueous extract and synthesized AgNPs are shown in (Figure 3). The IR-spectrum of the AgNPs showed absorption bands at 1038, 1380, 1635 and 3430 cm^{-1}. The absorption bands at 1038 cm^{-1} correspond to C-N stretching vibrations of the amine. The absorption bands at 1635 cm^{-1} correspond to amide 1 band of proteins due to carbonyl stretch in proteins and absorption bands at 3430 cm^{-1} are due to the O-H stretching in alcoholic compounds. The sharp band at 1380 cm^{-1} is due to C-H stretching vibrations of aromatic and aliphatic amines.FT-IR spectroscopy studies were carried out to identify the biomolecules that not only capped, but also helped in reduction and stabilization of synthesized biogenic AgNPs. The absorption bands that appear in the FT-IR spectrum of the aqueous extract could also be seen in the IR spectra of Phyto capped synthesized AgNPs. This shows that the phytoconstituents present in the aqueous extract protect the AgNPs from aggregation.The IR spectrum of the AgNPs showed absorption bands at 1038, 1380, 1635 and 3430 cm^{-1}. The absorption bands at 1038 cm^{-1} correspond to C-N stretching vibrations of the amine. The absorption bands at 1635 cm^{-1} correspond to amide 1 band of proteins due to carbonyl stretch in proteins and absorption bands at 3430 cm^{-1} are due to the O-H stretching in alcoholic compounds [**31**]. The sharp band at 1380 cm^{-1} is due to C-H stretching vibrations of aromatic and aliphatic amines. IR spectroscopic study thus confirmed that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e., capping of AgNPs) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of AgNPs in the aqueous medium [**31**].

Powder X-Ray Diffraction studies: XRD studies were carried out to identify the crystalline nature of the synthesized biogenic AgNPs. Diffraction peaks were observed at 2θ values of 38.2°, 44.4° and 64.1° that can be indexed to (111), (200) and (220) reflection planes of face centered cubic (FCC) as shown (Figure 4). The mean size of the biosynthesized AgNPs was determined by Debye-Sharrer formula and found to be in the range of 2-10 nm. The lattice parameter was calculated according to Bragg's law and was found to be 4.077Å. The crystallinity index was calculated to be 1.3604.XRD studies were carried out to identify the crystalline nature of the synthesized biogenic AgNPs. Diffraction peaks were observed at 2θ values of 38.2°, 44.4° and 64.1° that can be indexed to (111), (200) and (220) reflection planes of face centered cubic (FCC). This study confirms that the resultant particles are (FCC) AgNPs [**32**]. The ratio between the intensities of diffraction peaks of (200) - (111) and (220) – (111) of the sample values were calculated to be 0.45 and 0.27 which were in agreement with the conventional values of JCPDS File No. 04-0783 (0.40 and 0.25) (Sun and Xia, 2002). The mean size of the biosynthesized AgNPs was determined by Debye–Sharrer formula [**33**] and found to be in the range of 2-10 nm. The lattice parameter was calculated according to Bragg's law and was

found to be 4.077Å which was also in agreement with the conventional value of JCPDS File No. 04-0783 (4.08Å) [34]. The crystallinity index was calculated to be 1.3604 and was confirmed that synthesized AgNPs were monocrystalline in nature and the FCC structure was well indexed [35].

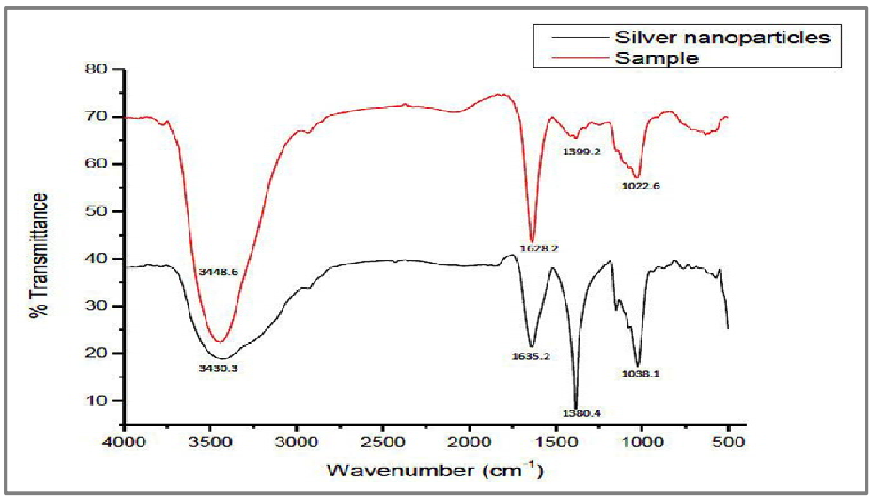

Figure 3. Fourier Transform Infrared spectrum of biogenic silver nanoparticles.

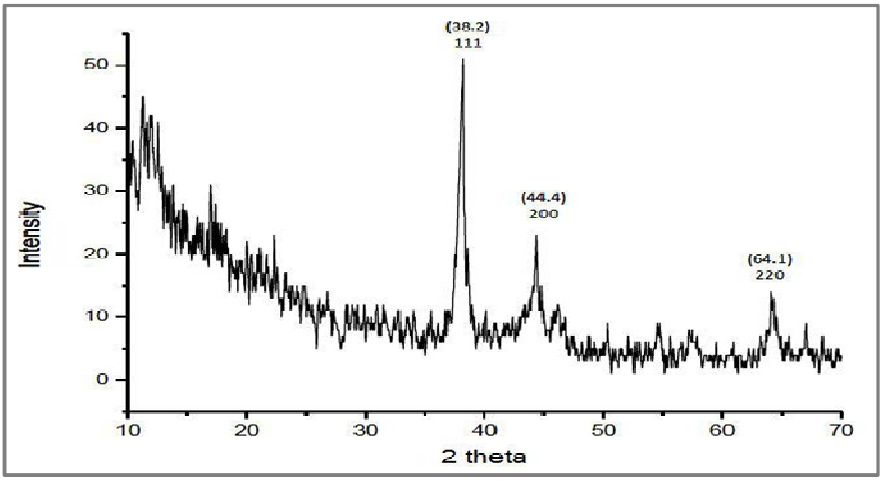

Figure 4. X-Ray Diffraction pattern of biogenic silver nanoparticles showing characteristics peaks centered indexed to the crystalline planes (111), (200) and (220) of face centered cubic silver.

Electron Microscopy/ Energy Dispersive Spectroscopic studies: Electron Microscopy studies were carried out to view the size of the synthesized biogenic nanoparticles. The HR-TEM image of synthesized AgNPs depicted in (Plate 3a) give clear indications regarding size, shape and size distribution of nanoparticles. The size of the synthesized biogenic AgNPs was found to be 2-20 nm. The SAED pattern of AgNPs reveals its crystalline nature. From TEM images, (Plate 4) it can be seen that the AgNPs are capped with phytoconstituents of rhizome of *Curcuma caesia* the result of EDS gives a clear idea about the elements present in the nanoparticles (Figure 5). The strong signal of the Ag atoms indicates the crystalline property.

To study the size of the biologically synthesized AgNPs, electron microscopy studies were carried out. The size and morphology were studied using HR-TEM giving clear indications regarding size,

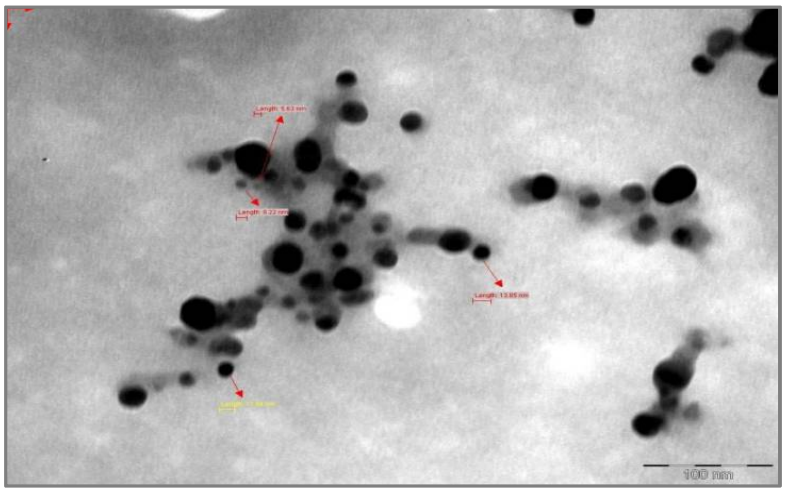

Figure 5. Transmission Electron Microscope image of biogenic silver nanoparticles capped with phytoconstituents of rhizomes of *Curcuma caesia.*

shape and size distribution of nanoparticles. The size of the synthesized biogenic AgNPs was found to be 2-20 nm compared to AgNPs synthesized by Krishnaraj *et al*. (2010) whose size ranged 20-30 nm. SAED pattern of AgNPs revealed its crystalline nature as reported them. From TEM images it was clear that the AgNPs were capped with phytoconstituents of rhizome of *Curcuma caesia* as shown in a similar work by Edison and Sethuraman [**36**] who used *Terminalia chebula* for instant green synthesis of AgNPs. In the EDS study, the strong signal of the Ag atoms indicates the crystalline property of the synthesized AgNPs. The carbon and oxygen peaks in the EDS analyses can be attributed to the surrounding residual material and/or the carbon tape used for SEM grid preparation [**37**].The concentration of silver in the synthesized biogenic AgNPs determined using Atomic Absorption Spectrophotometer was 24.89% of silver in 28 mg of synthesized silver nanopowder which was quite more than the silver found in AgNPs synthesized by Krishnaraj *et al*. (2012) and was recorded to be 25.4% of silver in 100 mg of synthesized silver nanopowder.

Optimization of synthesized biogenic silver nanoparticles: Different parameters were optimized for synthesizing AgNPs including pH, time, temperature, concentration of silver nitrate, concentration ratio of leaf extract and $AgNO_3$. There is no particles formation at acidic pH 3 and 5. Color formation was rapid at alkaline pH 9 but peak was shifted towards 500 nm. Agglomeration was observed at pH 11 immediately after adding the $AgNO_3$ into the reaction mixture. At neutral pH 7, the reaction was started as soon as the $AgNO_3$ was added into the reaction medium and the formation was observed within 30 min of incubation. The colorless solution was turned to brownish color which indicates the formation of AgNPs. The characteristic absorption peak at 418 nm in UV-Vis spectrum further confirmed the formation of AgNPs. Temperature effect was also studied and found that the peak was found to be stable till 50° C. Thus, AgNPs were synthesized at room temperature.

Different concentration of silver nitrate was optimized for the maximum synthesis of AgNPs. Interestingly, 5 mM concentration of $AgNO_3$ supported rapid formation whereas the peak got reduced at 4 mM and 3 mM concentrations. Similarly, different concentration ratios of leaf extracts and $AgNO_3$ solution were also optimized for maximum production of AgNPs. Interestingly 50 mL reaction medium containing 5 mL of plant extract and 5 mM concentration of silver nitrate solution turned to brownish orange color with in 30 min of incubation period, indicating rapid formation of AgNPs. Thus, the optimized medium supported the maximum formation of AgNPs and the reaction occurred very rapidly. To access the stability of AgNPs formed in the reaction solution at pH 7, UV–vis analysis study was carried out. This study clearly showed no alteration in the peak at 418 nm even after 2 months of incubation period, indicating strong stability of biosynthesized AgNPs. Therefore, it is clear that the optimization process played a pivotal role in the particle's stability and aggregation.

Parameters like pH, time, temperature, concentration of silver nitrate, concentration ratio of leaf extract and $AgNO_3$ were optimized for synthesizing AgNPs as done by Krishnaraj *et al.* (2010)[24]. pH is one of the key factors playing a major role in nanoparticles synthesis. At neutral pH (7), the reaction was started as soon as the $AgNO_3$ was added into the reaction medium and the formation was observed within 30 min of incubation that turned to brownish orange color indicating the formation of AgNPs. The characteristic absorption peak at 418 nm in UV–vis spectrum further confirmed the formation of AgNPs which corresponds to SPR of AgNPs established previously [24]. Interestingly SPR occurred at 418 nm in the beginning of the reaction and it was stabilized in the same wavelength even after the completion of the reaction. Temperature effect was also studied and found that the peak was found to be stable till 50°C. Different concentration of silver nitrate was optimized for the maximum synthesis of AgNPs and 5 mM concentration of $AgNO_3$ supported rapid.

These results are in good agreement with the earlier investigations done by Krishnaraj *et al.* (2010) [24, 38] and nanoparticle formation is visually appreciable after 4 min of the beginning of the reaction. Different concentration ratios of leaf extracts and $AgNO_3$ solution were optimized and interestingly 50 mL reaction medium containing 5 mL of plant extract and 5 mM concentration of silver nitrate solution turned to brownish orange color with in 30 min of incubation period, indicating rapid formation of AgNPs. UV–Vis analysis study was carried out to access the stability of AgNPs formed in the reaction solution at pH 7 and showed no alteration in the peak at 418 nm even after 2 months of incubation period, indicating strong stability of biosynthesized AgNPs. Similar work was carried out by Krishnaraj*et al.* (2010) [24] with similar results. Therefore, it is clear that the optimization process played a pivotal role in the particle's stability and aggregation. Recently, there is an enormous interest in understanding the possible reaction mechanism for the plant mediated nanoparticles synthesis. Some studies have indicated that biomolecules present in the plant extract play a crucial role in reducing the ions to the nanosize. Although the reduction of Ag^+ ions is environmentally benign, it is chemically a complex phenomenon involving an array of biomolecules such as enzymes/proteins, flavonoids, phenols, vitamin, organic acids such as citrates, amino acids, and polysaccharides [24]. Rhizomes of *Curcuma caesia* have been reported to contain phenols, flavonoids, glycosides, tannins and alkaloids [39]. Phytochemicals would have played an important role in the reduction of respective salts to nanoparticles.

Antibacterial and Antifungal Activities of Biogenic Silver Nanoparticles

Zone of inhibition in bacteria: The zone of inhibition in bacterial growth by the biogenic AgNPs and $AgNO_3$ shows that this study is dependent on the concentration in medium as shown in table 1 and table 2. The biologically synthesized AgNPs exhibit excellent antibacterial activity against both Gram positive and Gram negative bacterial pathogens.

Table 1. Zones of inhibition in bacterial growth by various concentrations of biogenic silver nanoparticles

Bacteria	Control (mm)	5µg mL^{-1} (mm)	10 µg mL^{-1} (mm)	15 µg mL^{-1} (mm)	20 µg mL^{-1} (mm)
Staphylococcus aureus	0	8.0±0.3	9.5±0.4	10.3±0.2	14.5±0.1
Klebsiella pneumonia	0	9.5±0.2	11.1±0.1	14.0±0.3	17.5±0.2
Escherichia coli	0	10.0±0.2	11.0±0.1	13.4±0.2	15.0±0.3

Table 2. Zones of inhibition in bacterial growth by various concentrations of silver nitrate

Bacteria	Control (mm)	5µg mL^{-1} (mm)	10 µg mL^{-1} (mm)	15 µg mL^{-1} (mm)	20 µg mL^{-1} (mm)
Staphylococcus aureus	0	9.4±0.1	11.8±0.2	13.1±0.2	16.3±0.2
Klebsiella pneumonia	0	10.2±0.2	12.4±0.1	16.3±0.2	20.1±0.1
Escherichia coli	0	12.0±0.1	13.4±0.3	14.8±0.3	18.1±0.2

The biologically synthesized AgNPs exhibit excellent antibacterial activity against bacterial pathogens [**40**] and attributed it to difference in cell wall structure between Gram negative and Gram-positive microorganisms [**41**]. Silver ions released by the AgNPs may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [**42**]. The antimicrobial activities of colloidal silver particles are influenced by the dimensions of the particles. The smaller particles lead to the greater antimicrobial effects [**43**]. It is necessary to emphasize that the tested AgNPs have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria. Experiments conducted using the scanning tunneling electron microscopy (STEM) and X-ray energy dispersive spectrometer (EDS) showed that AgNPs not only at the surface of cell membrane, but also inside the bacteria [**44**]. This suggests the possibility that the AgNPs may also penetrate inside the bacteria and cause damage by interacting with phosphorus and sulfur containing compounds such as DNA [**45**].

APPLICATION

The advantages of using a plant extract mediated synthesis of AgNPs include toxicity free and quick synthesis method, economic viability, cost effective and ease in handling large scale synthesis. AgNPs show effective antibacterial activity. The synthesized biogenic AgNPs are more susceptible to Gram negative bacteria than Gram positive bacteria. They could thus be an efficient alternative to conventional antibiotics and may be used as an antibacterial agent.

CONCLUSION

The sustainable green chemistry biosynthesis process for the generation of toxic free, biocompatible and environment friendly nanoparticles constitutes important role in current nanotechnology research. The studies described here throw light on the importance and significance of biosynthesis of AgNPs. The advantages of using a plant extract mediated synthesis of AgNPs include toxicity free and quick synthesis method, economic viability, cost effective and ease in handling large scale synthesis. AgNPs show effective antibacterial activity. The synthesized biogenic AgNPs are more susceptible to Gram negative bacteria than Gram positive bacteria. They could thus be an efficient alternative to conventional antibiotics and may be used as an antibacterial agent.

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