Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **1 (6):** 1-6 (2013)

Research Article

International Journal of Pure & Applied Bioscience

Green synthesis of Silver Nanoparticle using *Terminalia chebula* and Assessment of its Antimicrobial Activity

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ABSTRACT

Nanobiotechnology gives emphasis for the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. Among the various agents used for nanoparticle synthesis, plants have found important application. The biomolecules found in plants induce the reduction of Ag^+ ions from silver nitrate to silver nanoparticles (AgNPs). The aqueous leaves extract of Terminalia chebula was used as reducing and stabilizing agent for the synthesis of silver nanoparticle. Synthesized nanoparticle is confirmed by the change of color from transparent yellow to dark brown indicates the formation of silver nanoparticles. UV-Vis absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The UV visible spectrum of colloidal solutions of SNPs was 199.00nm. The antimicrobial assays were done on human pathogens like Escherichia coli, Staphylococcus aureus, Salmonella typhi and Klebsiella pneumonia. The zone of inhibition of synthesized silver nanoparticles were found to be 18 mm for Salmonella typhi, 16 mm for S. aureus, 15 mm for E. coli and 20 mm for K. pneumoniae. The plant based route could be considered to be an environmental friendly, safe and economic biological method for the silver nanoparticles production.

Keywords: *Silver nanoparticles, Medicinal plants, Green synthesis, Ecofriendly.*

INTRODUCTION

In the present scenario, nanotechnology is an important enabling active area of research in modern material sciences. Nanoparticles deals with the synthesis and control of matter in scales less than 1µm, normally from 1 to 100 nanometers (nm)[1]. Nanoparticles show completely new or improved properties and have wide scope for their diversified application based on specific characteristics such as size, distribution and morphology.

Silver nanoparticles have found various and important applications for their bactericidal and fungicidal activity[2]. Antimicrobial effect is due to blockage of respiratory enzyme pathways, alterations of microbial DNA and the cell wall[3]. Historically, the synthesis of metallic nanoparticles utilized chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride to create uniform suspensions[4]. But chemical method is harmful in some way as the chemicals used are toxic, flammable, low synthesis rate etc. In current phase, green synthesis of nanoparticles is exploited to improve and also to protect the environment by the use of chemicals. Raveendran *et al*. 2003, suggested three important factors which should be considered for the synthesis of nanoparticles: solvent choice, the use of reducing agent and the use of non-toxic material for nanoparticle stablisation[5]. Recently, biological entities serving as both reducing and stabilizing agents for green synthesis of metallic nanoparticles[6]. Utilizing biological organisms such as microorganisms[7], enzymes[8] and plant extract or plant biomass could be an excellent alternative to chemical and physical methods for the production of nanoparticles in a cheap and eco-friendly manner compared to physical and chemical methods.

Synthesis of nanoparticles using plants can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture[9]. The microbial enzymes and secondary metabolites with an anti oxidant or reducing properties are usually reducing metal compounds into their respective nanoparticles. Plants have been reported to be used for synthesis of metal nanoparticles of gold and silver and of a gold-silver-copper alloy [10-14]. Colloidal silver is of particular interest because of its distinctive properties such as good conductivity, chemical stability, and catalytic and antibacterial activity[15-16]. In Chhattisgarh region during present study, we found that plant extracts prepared from *Terminalia chebula* can be used for synthesis of silver NPs under bright conditions. The objective of the present study was the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of medicinal plants and evaluation of synthesized silver nanoparticles against human pathogens.

MATERIAL AND METHODS

***Medicinal Plant Extract Preparation*:** The fresh and healthy leaves (20g) of *Terminalia chebula* sample (Table. 1) was collected from our University campus. Collected fresh leaves were washed, cleaned, finely chopped and soaked in 100ml milli-Q water. Further they were boiled in conical flask for 15-20 min. Obtained leaf extract was filtered through Whatmann filter paper no.1 and stored in refrigerator at 4ºC for further experiment.

***Synthesis of silver nanoparticles*:** Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich and 1 mM AgNO3 solution was prepared and stored in amber colour bottle and used in future experiment work. 10 ml plant leaf broth was added to 90ml 1 mM aqueous silver nitrate with constant stirring and allowed to react at ambient conditions for reduction into Ag^+ ions. The observed color change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles from leaves. The content was centrifuged at 20,000 rpm for 20 minutes. The supernatant obtained was used for the analysis like antimicrobial activity. Further the reduction of the Ag^+ ions was monitored over time by UV-visible spectral analysis.

Characterization: UV–Vis Spectra analysis was carried out to confirm the silver nanoparticles formation in Shimadzu 1800 UV spectrophotometer (Kyoto, Japan). Silver (Ag) exhibit unique optical properties on account of their Surface Plasmon Resonance (SPR). The periodic scans of the optical absorbance between 190 and 1100nm at a resolution of 1nm were performed to investigate the reduction rate of silver ions by plant leaf extract. The progress of the reaction between metal ions and the leaf extracts were monitored after diluting a small aliquot of 100 μL of the plant extract sample with 1 ml deionized water and used for UV-Visible analysis.

Antibacterial Activity:

The silver nanoparticles synthesized using *Terminalia chebula* was tested for antibacterial activity by standard agar well-diffusion method[17] against pathogenic bacteria which includes *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia.* The pure cultures of bacterial pathogens were sub cultured on nutrient agar. 100μl of fresh overnight grown cultures of the respective bacteria were spread on Nutrient Agar containing Petri plates. Each strain was swabbed uniformly using sterile cotton swabs. Wells of 10 mm diameter were made on nutrient agar using gel puncture and 100 μL of AgNP solution was loaded into the wells. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured.

RESULTS AND DISCUSSION

***Synthesis and UV-Vis Spectra Analysis*:** The green synthesis of silver nanoparticles through herbal extracts was carried using aqueous silver nitrate solution. Medicinal plant including *Terminalia chebula was* used for the synthesis of silver nanoparticle. The method utilizes a non-toxic, agent which functions as both reducing and stabilizing agent during synthesis. The mechanism of the reaction involves the reduction of aqueous metal ion with plant leaves extract. Plant extracts color changes after the completion of the reaction. And it is well known that silver nanoparticles exhibit yellowish brown or dark brown based on their size[18]. Addition of the aqueous herbal extract to the 1mM aqueous $AgNO_3$ solution resulted

in change of color within 5 – 10 minutes which can be varied according to plant species chosen (Figure 1). The reason could be that the quantitative variation in the formation of SNPs (or) availability of H^+ ions to reduce the silver.

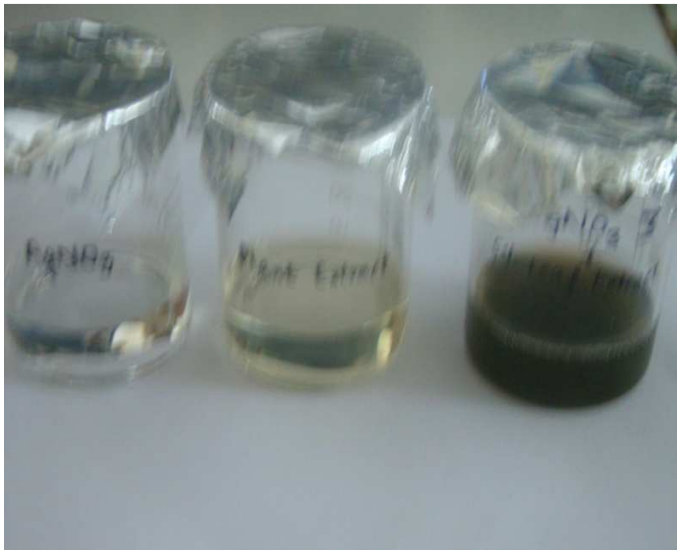
The change in color was obtained which resulted due to the excitation of the Surface Plasmon Resonance (SPR) vibrations of the silver nanoparticles formed[17]. The aqueous silver ions (Ag^+) when reacted to herbal extracts were reduced in solution thus resulting to the formation of silver hydrosol. The reaction could simply be tracked due to change in color and reconfirmed by UV-Vis spectroscopy. The UV visible spectrum of colloidal solutions of SNPs was 199.00nm (Fig. 2). And the broadening of absorption peak in the spectrum indicated that the particles are poly-dispersed[18] and the weak absorption peak at shorter wavelengths owing to the presence of numerous organic compounds which are known to intermingle with silver ions. The green leaves are the site of photosynthesis and accessibility of more H^+ ions to reduce the silver nitrate into silver nanoparticles.

The molecular origin for the formation of these silver crystals is speculated that the organic matrix contain silver binding proteins that provide amino acid moieties that serve as the nucleation sites[21]. Several plants have been successfully used for efficient and rapid extracellular synthesis of silver nano particles. Leaf extracts of geranium[19-21], lemongrass[22-23], cinnamon[24] have shown potential in reducing Au(III) ions to form gold nanoparticles Au(0) and silver nitrate to form silver nanoparticles Ag(0).

Antibacterial activity: The antimicrobial assays were done on human pathogens like *Escherichia coli*, *Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia.* After overnight incubation, zone of inhibitions formed in the plates which were measured using Zonal ruler. Antimicrobial activities of the synthesized silver nanoparticle are represented in (Table 2). The zone of inhibition of synthesized silver nanoparticles were found to be 18 mm for *Salmonella typhi,* 16 mm *for S. aureus*, 15 mm for *E. coli* and 20mm for *K. pneumoniae*. The antibacterial activity is probably derived, through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged Nanoparticles[25].

The present study suggests a simple and economical route to the synthesis of silver nanoparticles and their capability of rendering the antimicrobial efficacy. Also the synthesized SNPs improve the therapeutic efficacy and strengthen the medicinal values of these plants.

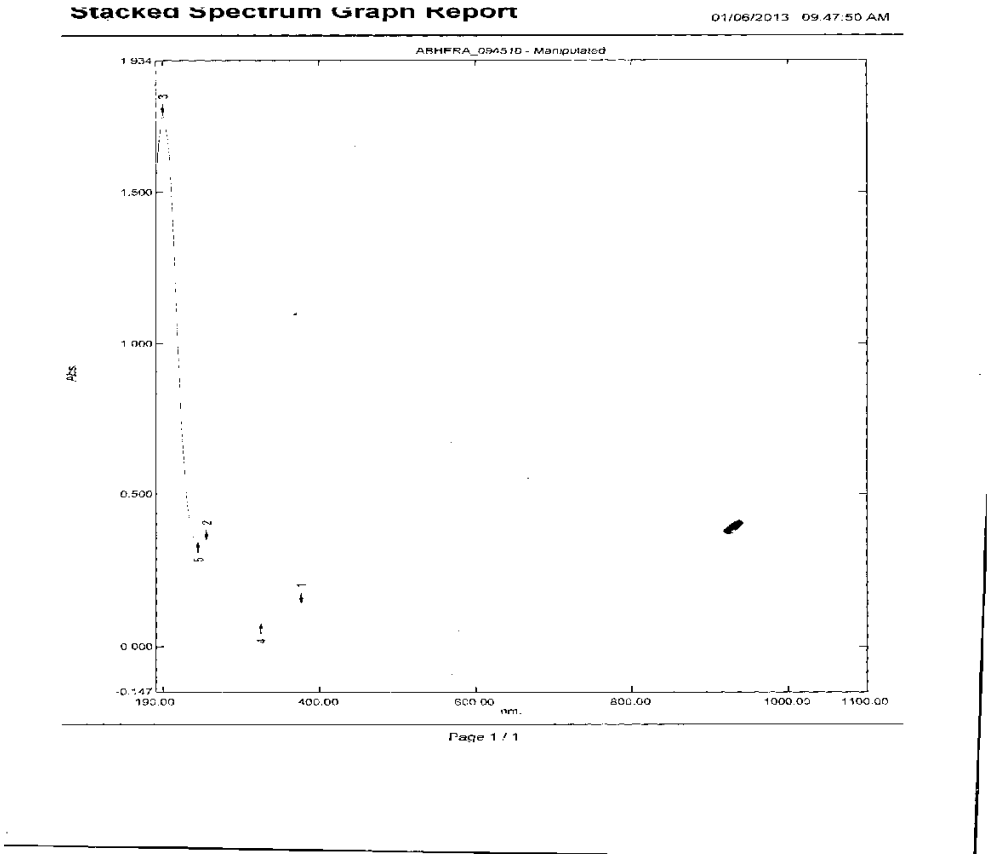
Fig. 1 Synthesis of silver nanoparticle indicated by change in colour



Terminalia chebula

(A) $AgNO_3$, (B) Plant Extract, (C) $AgNO_3$ + Leaf extract

Fig. 2 ***Terminalia chebula***



No.	P/V	Wavelength (nm.)	Abs.
1	↑	**199.00**	**1.760**

Table- 1: Medicinal Plant collected for the synthesis of silver nanoparticles

S. No.	Scientific name	Family	Common name	Medicinal uses
1.	*Terminalia chebula*	*Combretaceae*	Harad	for curing and cleansing ulcers and wounds, treating asthma and curing hiccups, anti-inflammatory and astringent

Table-2: Antimicrobial activity of leaf extract of *Terminalia chebula*

S. No.	Bacterial species	Inhibition zone (mm)		
		Control (Plant extracts)	SNPs	Silver nitrate
1.	*Escherichia coli*	7	15	9
2.	*Staphylococcus aureus*	9	16	11
3.	*Salmonella typhi*	10	18	12
4.	*Klebsiella pneumonia*	6	20	10

CONCLUSION

This work indicates that important herbal extract could be used as an efficient and potential green material for the reliable synthesis of silver nanoparticles. The synthesized phytonanoparticles have exhibited a wide range of activities to the bacteria strains and reveals high efficacy of silver Nanoparticle as a strong antibacterial agent. Thus, this phytonanoparticles has the potential for the development of drugs for various diseases and also useful in biomedical application.

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