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Green Synthesis of Silver Nanoparticle Using Plant Root Extract of *Croton sparsiflorus* and their Antimicrobial Activity

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Abstract: ***Silver nanoparticles have a wide range of applications in the fields of catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals and in biomedicine. Plants possess a wide range of phytochemical constituents, which are responsible for their medicinal properties. These phytochemicals contribute to the capping and stabilization of the silver nanoparticles. The silver nanoparticles synthesized are found to have enhanced properties when compared to that of the extract. The reducing and stabilizing property of Croton sparsiflorussilver nanoparticles were studied and it was found that silver nanoparticles exhibited better toxic activity. Hence the silver nanoparticles obtained were characterized using UV-Vis analysis and SEM. The nano-aggregates that were synthesized by the treatment of root ethanol extract of C. sparsiflorus possessed the mean diameter of 36.51-42.49 nm, which is strong indication of formation silver nanoparticles. Since the diameter of silver nanoparticles revealed by scanning electron micrograph (36.51 - 42.49 nm) lie in the considerable range for nanoparticle (0-100 nm) . Applications of such eco-friendly nanoparticles in bactericidal and other medical applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials) .***

Keywords: Silver nanoparticles, green synthesis, toxic activity, UV-VIS and SEM

1. Introduction

Nanotechnology is emerging as a rapid growing field with its immense application in the field of science and technology for the purpose of manufacturing new materials measuring atnano scale level (Jahn.W et al., 1999) .Extensive research in the field of nanobiotechnology, especially in synthesis of nanomaterials from Copper, Zinc, Titanium, Gold and Silver and explore their therapeutic applications. Nanomaterials are employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms (Shankar SSet al., 2004) .Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics (S. Schultz *et al.*2000) , antimicrobials and therapeutics (M. Rai and A.Yadav 2009; Elechiguerra*et al.*2005) catalysis (Crooks 2007) and micro-electronics (Gittins*et al.*2000) . However, there is still need for economic, commercially viable as well environmentally cleansynthesis route to synthesize silver nanoparticles.

Many preparations of the building blocks of nanotechnology involve hazardous chemicals, low material conversions, high energy requirements, and difficult, wasteful purifications; thus, there are multiple opportunities to develop greener processes for the manufacture of these materials. Many of the green chemistry principles apply readily to the synthesis or production of nanoscale materials. A more efficient and less hazardous synthesis of metal nanoparticles has been developed, producing greater amounts of particles, in less time, under milder conditions, while using less hazardous reagents than the traditional preparation (Dahl JA *et al.*2007) .

Croton sparsiflorus (family: Euphorbiaceae) is a small annual herb, growing up to 1 feet tall. The Euphorbiaceae family presents about 7, 500 species distributed mainly in tropical areas with larger dispersion centres in America and Africa. It has great importance not only for the number of species but also for its economic implications related to medicinal and cosmetic industry as well as for its toxicological aspects (Sun YP et al., 2001) .

This plant contains an array of bioactive phytochemical constituents that includes glycosides, saponins, tannins, flavonoids, terpenoids, alkaloids, phenolics etc. Three proaporphine bases provisionally designated Crotsparine, N-methyl-crotsprine, and N, O- dimethyl crotsparine; two dihydroproaporphines, Crotsparinine and N-methyl crotsparinine and the known Aporphine alkaloid have been isolated from *Croton Sparsiflorus* (Erren TC et al., 2007) .The antimicrobial activities of these chemical constituents confirm the presence of broad spectrum antibiotic compound in this species. The alkaloid fraction of extract of *C. sparsiflorus* is reported to possess anti-inflammatory and antipyretic property (Geethalakshmi R et al., 2010)

In this present investigation, a new strategy is employed in synthesis of metal nanoparticles by using natural phytochemicals extracted from the root of *Croton sparsiflorus* plant, instead of using toxic chemical polymers in synthesis of nanoparticles. In continuation with our interest in metal nanoparticles that are extensively used in many electrochemical, electroanalytical and bioelectrochemical applications owing to their extraordinary electrocatalytic activity. Although metal is a poor catalyst in bulk form, nanometer sized particles can exhibit excellent catalytic activity due to their relative high surface area to volume ratio and their interface dominated properties. In this paper, we reported the synthesis of silver nanoparticles using the aqueous extract of *Croton Sparsiflorus*employing different experimental conditions and thereby enhancing the importance of plant sources and implementing green chemistry for future research.

All the nano-phytochemical composites were evaluated for their antibacterial activity. Based on results of antibacterial activity, biological and size analysis of silver nanoparticles was extrapolated.

2. Materials and Methods

2.1 Plant material and preparation of the Extract

C. sparsiflorus plant material was collected along with the roots. Roots were cut out and separated from the plant. Roots were washed thoroughly in running tap water, followed by distilled water, and then shade dried for 30 days. The dried root material of *C. sparsiflorous* was porously powdered using mechanical blender. The porously powdered root material was subjected for soxhlet extraction.

The dried, powdered roots of *C. sparsiflorus* (500 g) were extracted using petroleum ether, ethyl acetate, ethanol and double distilled water in soxhlet apparatus for 72 hrs each. The extractions were carried out until the solvent become colourless in the timble tube of soxhlet. These extracts were carried out by the above mentioned procedure for further analysis. All the four extracts were evaporated using rotatory flash evaporator and vaccum dried using desicator. Dried extracts were used for evaluation of biological activities and nanoparticle synthesis.

2.2 Synthesis of Silver Nanoparticles

The synthesis of metal nanoparticles in which 1 M aqueous solution of the following metal salts $CuSO_4$, $AgNO_3$, Zn-dust, $FeSO_4$, $MgSO_4$, $MnSO_4$, Pb $(CH_3COO)_4$ and $CoCl_2$ was prepared in separate tubes. The different solvents extracts of *C. spariflorus* root, prepared in their respective solvent were used for the synthesis of metal nanoparticles. This experimental setup was incubated at room temperature for 5 hrs in dark without disturbance. This eventually led to formation of nanoparticles, which was indicated by formation of precipitation with polydispersion and aggregation.

2.3 UV-Vis Spectra analysis

The reduction of pure metal salts, as an indicator of nanoparticle synthesis was monitored by studying UV-Visible absorption spectrum of the reaction medium at 5 hours after the initiation of metal nanoparticle synthesis by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Agilent) .

2.4 Drugs formulation

10 mg of Ciprofloxacin (400 mg) drug was dissolved in 0.5% of Dimethyl sulfoxide DMSO (0.5 % DMSO in 10 ml of double distilled water) . Whereas, the newly synthesized metal nanoparticles, *C. sparsiflorus*root extracts were also prepared in 0.5 % DMSO in 10 ml of double distilled water. The formulated drug, nanoparticles and the plant extracts were used for evaluation of bactericidal activity.

2.5 Source of multi-drug resistant pathogen bactericidal strains

The test bacterial strains *viz., Staphylococcus aureus*, *Klebsiellapneumoniae* (MTCC-3040) , *Pseudomonas aureginosa* (MTCC-3542) and *Escherichia coli* (ATCC11-273) were used for evaluation of antibacterial property of newly synthesized nanoparticle *viz.,* Cobalt, Copper, Zinc, Iron, Silver, Lead, Manganese, Magnesium nanoparticles against the plant extracts as negative control and standard drug ciprofloxacin as positive control.

Evaluation of bactericidal activity against MDR bacterial strains

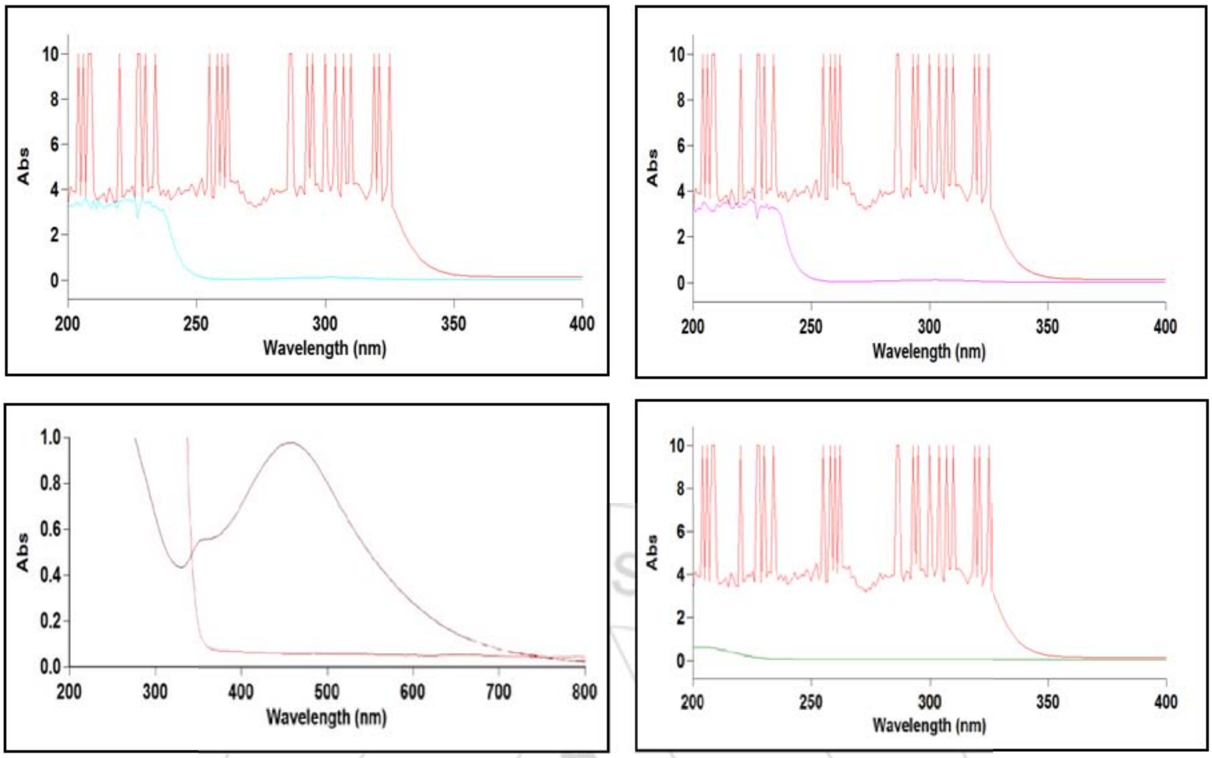
Antibacterial activities of the newly synthesized metal nanoparticles *viz.,* Cobalt, Copper, Zinc, Iron, Silver, Lead, Manganese, Magnesium nanoparticles against the plant extracts as negative control and standard drug ciprofloxacin as positive control was carried out by agar well diffusion method (46) . Test cultures of the bacterial pathogens were prepared by transferring a loop full of bacteria from nutrient agar slants into Nutrient broth and incubated at 37±1°C. Lawn cultures of the test pathogens were prepared by swabbing over sterile Nutrient agar plates with 24 hrs old multi-drug resistant pathogenic bacterial broth. Wells were punched with a sterile cork borer and 100 µl of the test metal nanoparticles/plant extract/standard drug ciprofloxacin was added to each well. Controls were maintained with respective solvents. Ciprofloxacin (1 mg/ml) were used as standard antibiotics. Following incubation at 37°C for 24 hrs, diameters of the zones of inhibition was measured in millimeter and documented.

2.6 SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of thesample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on thegrid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry byputting it under a mercury lamp for 5 min.

3. Results and Discussion

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation ofsurface plasmon vibrations in silver nanoparticles (Shankar *et al.*2004) . As the extract was mixed in the aqueoussolution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction ofsilver ion which indicated formation of silver nanoparticles. It is generally recognized that UV–Vis spectroscopycould be used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al*. 2006) .Figure 1 shows the UV-Vis spectra recorded from the reaction medium after 4 hours. Absorption spectra of silvernanoparticles formed in the reaction media has absorbance peak at 450 nm, broadening of peak indicated that theparticles are polydispersed.This distinct characterstic peak pattern that was observed for silver complex encouraged us to work on Scanning electron microscopic studies of $AgNO_3$ metal complex.The SEM image showing the high density silver nanoparticles synthesized by the extract*C. sparsiflorus* further confirmed thedevelopment of silver nanostructures.



UV-visible absorption spectras showing $AgNO_3$ metal salt reduction by the treatment of root extracts of *Croton sparsiflorus* against normal $AgNO_3$.
A. Petroleum ether extract induced $AgNO_3$ reduction spectra.
B. Ethyl acetate extract induced $AgNO_3$ reduction spectra.
C. Ethanol extract induced $AgNO_3$ reduction spectra.
D. Aqueous extract induced $AgNO_3$ reduction spectra

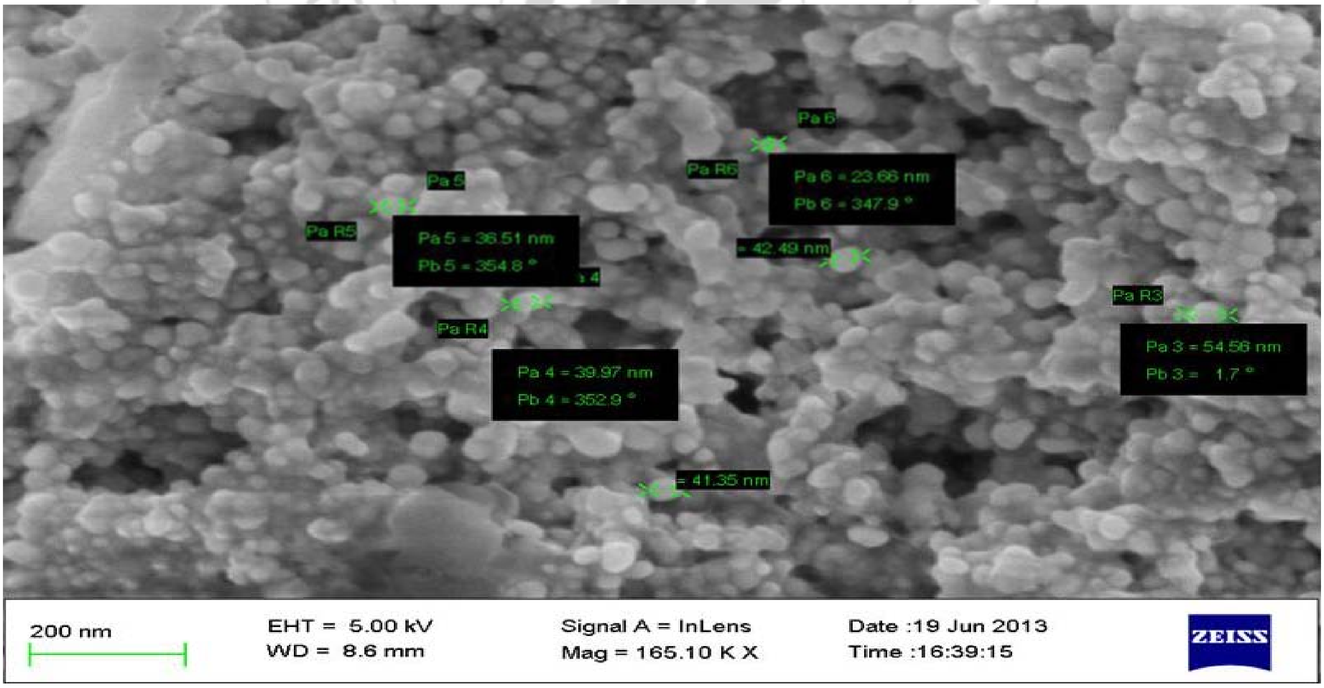


Figure 2: Scanning electron microscopic (SEM) studies of silver nanoparticles

3.1 Antibacterial screening of metal nanoparticles against multi-drug resistant bacterial pathogens.

The antibacterial activity of crude root extracts and also metal nanoparticle put a strong basis for this novel strategy of identifying and metal complexed plant based drugs against multidrug resistant bacterial pathogens. This study revealed that among the various root extracts viz., petroleum ether, ethyl acetate, ethanol and aqueous extracts, aqueous extract showed significant inhibition of *Staphylococcus aureus* and *Klebsiella pneumonia* with zone of inhibition of 19.62 mm and 10.14 mm respectively. Whereas, Petroleum ether extract proved to be potent against *Pseudomonas aureginosa* (9.12 mm) . However all the root extracts of *C. sparsiflorus* were less affected than the standard drug ciprofloxacin.

Among the various metal salt solution *viz.,* CoCl2, MgSO4, MnSO4, FeSO4, AgNO3, CuSO4, Pb (CH3COO) 4, Zn-dust. It was revealed that CoCl2 showed maximum inhibition against *S.aureus*. However, CuSO4 showed maximum inhibition against *K. pneumonia* and interestingly CoCl2 showed maximum inhibition zone against *P. aureginosa*.

4. Conclusions

In conclusion, the bio-reduction of aqueous Ag+ ions by the root extract of the*C. sparsiflorus* plant has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc.Reduction of silver ion into silver particles during exposure to the plant extracts could be followed by color change. Silver nanoparticle exhibit dark yellowish – brown color in aqueous solution due to the surface Plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential weeds for synthesizing the silver nanoparticles. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak, broadening of peak indicated that the particles are polydispersed. The SEM image showed relatively spherical shape nanoparticle formed with diameter range 30-50 nm. The nano-aggregates that were synthesized by the treatment of root ethanol extract of *C. Sparsiflorus* possessed the mean diameter of 36.51-42.49 nm, which is strong indication of formation silver snanoparticles. Since the diameter of silver nanoparticles revealed by scanning electron micrograph (36.51 - 42.49 nm) lie in the considerable range for nanoparticle (0-100 nm) .

Further the nanoparticle synthesis by green route was found highly toxic against species *viz., Staphylococcus aureus, Klebsiellapneumoniae* (MTCC-3040) and *Pseudomonas aureginosa* (MTCC-3542) which were used for evaluation of antibacterial property of newly synthesized nanoparticle *viz.,* Cobalt, Copper, Zinc, Iron, Silver, Lead, Manganese, Magnesium nanoparticles against the plant extracts as negative control and standard drug ciprofloxacin as positive control.

The reducing and stabilizing property of *Croton sparsiflorus* silver nanoparticles were studied and it was found that silver nanoparticles exhibited better toxic activity.

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