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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *CENTELLA ASIATICA* (L.)URBAN.

A. K. Dixit and Naureen Shaba Khan*

Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495009 India.

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***Corresponding Author**
Naureen Shaba Khan
Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495009 India.

ABSTRACT

Nanotechnology is a blazing and burning area of present day research due to the fabulous, fiery and terrific distinguishing properties of nanoparticles from their bulk counter parts. Nanoparticles are the key to this technology. They are called as the building block as they exhibit large surface to volume ratio from their heavier source. Due to this they are in great demand and a compulsive requirement in every sector of society. Channeling of Bio-nanotechnology through this broad area has offered a new path of Nano-particle synthesis. Following this stream green chemistry approach has brought a drastic change in present scenario because it offers a comparatively safer and productive output in terms of environmental condition and suitability of method execution, it offers an ecofriendly and energy saving way to get the NPs. Keeping in view the benefits of verdure synthesis, present fraction of research work comprises the phytofabrication of Ag NPs using *Centella asiatica*'s leaf extract as reducing and capping agent. The synthesized Ag NPs were characterized by UV-Vis Spectroscopy that confirms the formation of metallic nanoparticles by conferring the peak at (λmax) ~426nm Further, Transmission Electron Microscopy (TEM) and Nanoparticle Tracking analysis (NTA) were conducted to speculate the shape and size of phytosynthesized Ag NPs. The particles were found to be polydispersed spherical in nature with size distribution around 40±19nm. Also, the stability of Ag NPs was determined by Zeta sizer analysis which reveals negative zeta potential denoting negative surface charge (- 14.2 mV). Nanoparticles were found to be highly stable over a considerable period of time, which can be attributed to capping layer derived from reducing agent used to synthesize them.

KEYWORDS: Green synthesis, Ag NPs, *Centella asiatica*, Characterization.

INTRODUCTION

Nanotechnology is the leading edge technology with tremendous possibilities in diversified sectors of society. The term Nanotechnology is a combination of two different terms, 'nanos' meaning "dwarf" and "technol" a branch deals with atomic and molecular mechanisms. It is flourishing rapidly due to the sharp and fiery properties of "Nanoparticles". Size, surface charge, morphology and the state of existence are the key that strongly governs the properties of these tiny tools. They offers large surface to volume ratio, and a comparatively larger and interactive spatial planes for the chemical reactions to take place. They exists in different forms like organic nanoparticles i.e. carbon containing (Carbon Nanotubes, fullerence, buckyball etc), inorganic metal nanoparticles (Au, Ag etc) and engineered nanoparticles (nanocomposites, dendrimers etc).

There are basically two approaches of Nanoparticle synthesis. First is 'Top down method' which involves the breakage of complex, bulky matters in their constituent fractions. And, second is the Bottom up method which consist of the union of tiny substances to reach the nano realm. Former is attained by physical method like ball milling, mechanical attrition etc. which consumes a large amount of energy and also leads to the generation of unintentional nanoparticles. Whereas, the latter comprises chemical synthesis and biological methods. In chemical method of nanoparticles synthesis toxic organic and inorganic solvents are used in addition to large amount of power consumption. Nanoparticles synthesized by physical and chemical methods have been proved to cause toxicity in water, soil, microbes plants and animals (Khan et al. 2016). In the biological method of nanoparticles synthesis a wide variety of organisms have been exploited till date. The small microscopic creatures, unicellular entities i.e. Bacteria i.e. *P. stutzeri* AG259 (Tanja et al 1999), *Enterobacter cloacae* (Minaeian et al 2008); cyanobacteria i.e. *Plectonema boryanum* (Lengke et al 2007), including fungi i.e. *A. fumigates* (Bhainsa et al 2006) and the producers i.e. green plants have been exploited for the synthesis of metal nanoparticles. It has been observed since a decade back that different varieties of plants have been used in nanoparticle synthesis. Every part of the plant has been used for the same. Aerial shoot portion along with the underground roots have been used as a bioreductant. Seed extract of *B. nigra* (Raksha pandit 2015), Root extract of *Morinda citrifolia* (Suman et al 2013), stem extract of *Breynia rhamnoides* (Gangula et al 2011) stem bark extract of *Boswellia ovalifoliata* (Ankanna et al. 2010), leaf extract of *Acalypha indica* (Krishnaraj et al 2010), *Ocimum sanctum* L. (Khan et al. 2015) and *Bacopa monnieri* L. (Khan et al. 2015) leaf bud extract of *Rhizophora mucronata* (Umashankari et al

2012), flower bud extract of *Couropita guinensis* (Nima and Ganesan 2015), flower extract of *Moringa oleifera* (Bindhu et al 2013), fruit extract of *Citrus limon* (Prathna et al 2011), rhizome extract of *Acorus calamus* (Nakkala et al 2014), tuber extract of *Curcuma longa* (Sathishkumar et al 2010), cone extract of *Pinus thunbergii* (Velmurugan et al 2013), peel extract of *Citrus sinensis* (Kaviya et al 2011), latex extract of *Jatropha curcas* (Bar et al 2009), callus extract of *Citrullus colocynthis* (satyavani et al 2011), clove extract of *Allium sativum* (Ahamed et al 2011), gum extract of *Boswellia serrata* (Kora et al 2012) and weeds like *parthenium hysterophorus* L. (Parashar et al 2009) are also used for this process. Use of green plants in metal nanoparticle synthesis has been strongly supported by various researchers a clear reflection of which is the gradual increase in the number of publication using the greens. This may be contributed to its feasibility, in expensive, less or no chance of contamination, no requirement of sophisticated laboratory, can be easily performed under normal temperature and pressure condition. And over all the green route of synthesis favors the environmental conditions as well. This makes it popular and a better alternative as compared to microbial assisted synthesis methods of nanoparticles synthesis. Another, strong point of discussion is the key responsible for the reduction phenomenon. The primary and secondary metabolites contained in the plant are the chief executor of the bio-chemical process and are the actual participants that switch on, accelerate and accomplish the phytonanosynthesis. The bioactive compounds of the green plants act as reducing, capping and stabilizing agents. And thus, they are assumed as the key to propel in the nanoworld.

The experimental plant *Centella asiatica L.* belong to family Apiaceae (Umbeliferae) is known for its wound healing and memory enhancing properties. It is commonly known as "Mandukparni". The medicinal value of the plant is reflected by its long back use in the ayurvedic and Chinese system of medicine (Meulenbeld and Wujastyk 2001). The active principles of this herb are 'asiatic acid, asiaticoside and asiaticoside A and B' (kartnig and Hoffmann-Bohm 1992) that offers tremendous possibilities of this herb in the sector of neurosciences and technology. The plant is well known for its splendid therapeutic and curative nature. It is effective in the treatment of stomach ulcers, mental fatigue, diarrhea, epilepsy, hepatitis, syphilis and asthma (Goldstein and Goldstein 2012). The present fraction of study was carried out to synthesize silver nanoparticle using the aqueous fresh leaves extract of *Centella asiatica* L. Fig.1 shows the biological properties of *Centella asiatica* (L.) Urban.

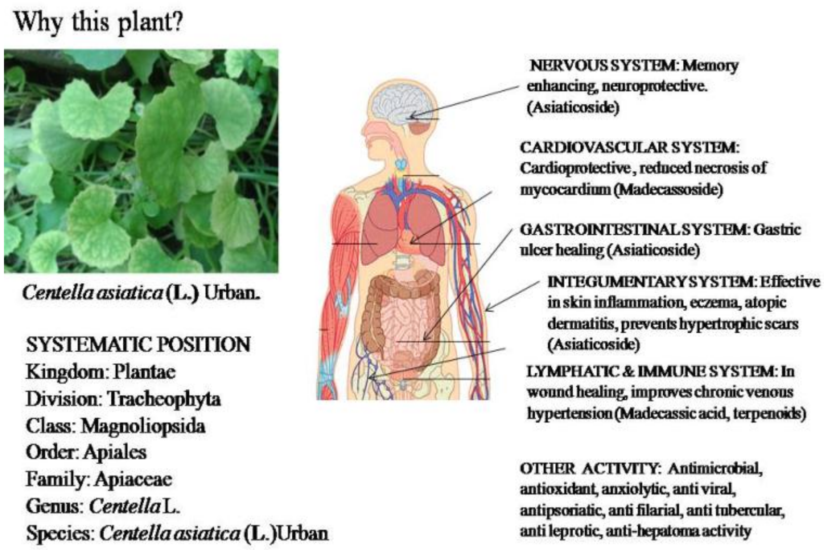

Fig.1.*BIOLOGICAL PROPERTIES EXHIBITED BY Centella asiatica* (L.) Urban ON HUMAN BODY modified after Chatterjee et al.1992, Chen et al. 2004, Chopra et al.1956, Shinomol et al. 2011, Shukla et al. 1999, Suguna et al. 1996, Viala et al. 1977.

MATERIALS AND METHODS: Fresh leaves of *Centella asiatica* L. were collected from the university campus, Guru Ghasidas Vishwavidyalaya, Bilaspur, India and were identified.

Preparation of leaf extract and synthesis of silver nanoparticles (Ag-NPs): Fresh leaves were cleaned with deionized water and crushed in mortal & pestle by adding deionized water dropwise. The paste is then mixed with 100 ml of deionized water and kept over the hot plate by adjusting its temperature at 60ºC for 5 minutes. It is set to gets cool down and then filtered by whatman no. 41. Then, the filtrate was treated with 1mM solution of freshly prepared silver nitrate.

Detection and characterization of Ag-NPs

Visual Inference**:** The preliminary screening of nanoparticle synthesis was done by observing the change in colour of the reaction mixture. The colour variation signifies nanoparticle synthesis.

UV-Vis Spectroscopy**:** The confirmation of silver nanoparticle synthesis was done by performing UV-Vis spectroscopy (Systronic -2203) of the test sample in the range of 350-550nm.

Nanoparticle tracking analysis **(*****NTA*****):** This system was used to find out the average size of the synthesized silver nanoparticles. Nanoparticle tracking analysis was performed through Nanosight LM 20 (UK) to estimate the size of the nanoparticles freely moving in liquid suspension. For this, 5 µl of the nanoparticle samples were diluted with 2 ml of nuclease free water and injected onto the sample chamber and observed through camera equipped with the instrument.

Zeta sizer Analysis: Zetasizer analysis was carried out to determine zeta potential of synthesized nanoparticles. The sample for zeta sizer analysis was done by diluting 60 µl of nanoparticle solution with 2 ml of nuclease free water. The prepared sample was taken into zeta dip cell and measured by Malvern Zetasizer 90 (ZS 90, USA).

Transmission Electron Microscopy (TEM)**:** TEM analysis of the sample was performed to speculate the shape and size of the synthesized silver nanoparticle. Aqueous sample was dropped (3 µl) over the carbon coated copper grid. Air dried grids were directly examined under transmission electron microscope (FEI Tecnai G2 Spirit Twin, Czech Republic) equipped with GATAN digital CCD camera. The diameter of nanosized particle was measured by using built-in gatan soft ware. Three grids of each sample were examined and the area that showed uniform size of particles was selected for photograph.

RESULTS AND DISCUSSION

Aqueous leaf extract of *C. asiatica* was used to fabricate silver nanoparticles. Change in colour of mixture solution indicated that nanoparticle has been synthesized (Figure.2) The change in colour of mixture solution is mainly attributed to the bioreduction phenomena. It is assumed that silver ions (Ag+) donates one free electron present in their outer most shell and acquire a ground state configuration(Ag^0). The colour changes from yellow to dark brown signifies the formation of silver nanoparticles. The intensity of the colour reflects the amount of nanoparticles. These findings match with the results obtained by the researchers who have worked on phytofabrication of silver nanoparticles (Gupta et al 2014; Krishnaraj et al 2010).
UV-Vis spectroscopy is a useful technique which gives an idea about the synthesis of nanoparticles by showing peak at a range of 400 - 480nm (in case of silver nanoparticles) (Nazeruddin et al 2014). The Surface Plasmon resonance phenomenon was observed at 426nm which denotes maximum absorption takes place at that particular wavelength. (Figure.3) This confirms the successful synthesis and stabilization of silver nanoparticles. Further, our findings were compared with the previously reported work (Nazeruddin et al 2014; Okafor et al 2013) and strongly supported the observed inference who have reported the synthesis of silver nanoparticles from *coriandrum sativum* seed extract and various plant's leaf extract).

Aqueous leaf extract of *C.asiatica* L.

Leaf extract after treatment with 1 mM $AgNO_3$ (30minute incubation)

Fig.2:Synthesis of Silver Nanoparticles

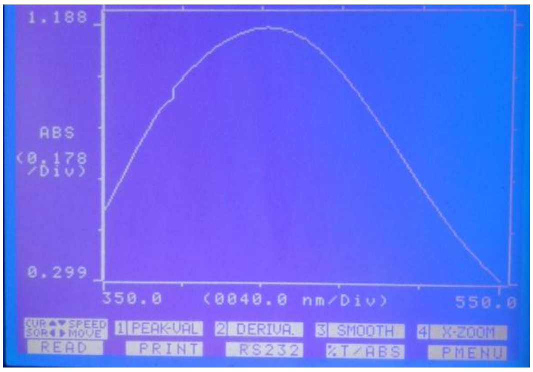

Fig.3: UV-Vis spectra of silver nanoparticles synthesized by using leaf extract of *C. asiatica* showing absorbance at 426nm.

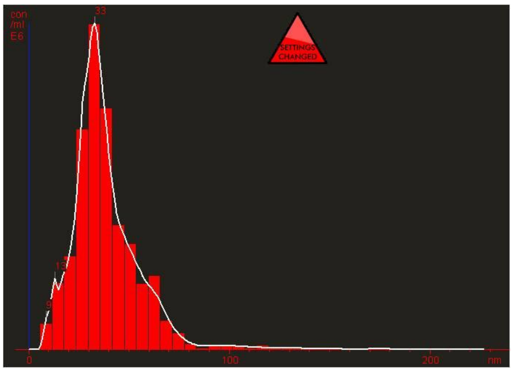

Fig.4: Nanoparticle tracking analysis (NTA) histogram showing particle size distribution and the average size of nanoparticles (40±19nm)

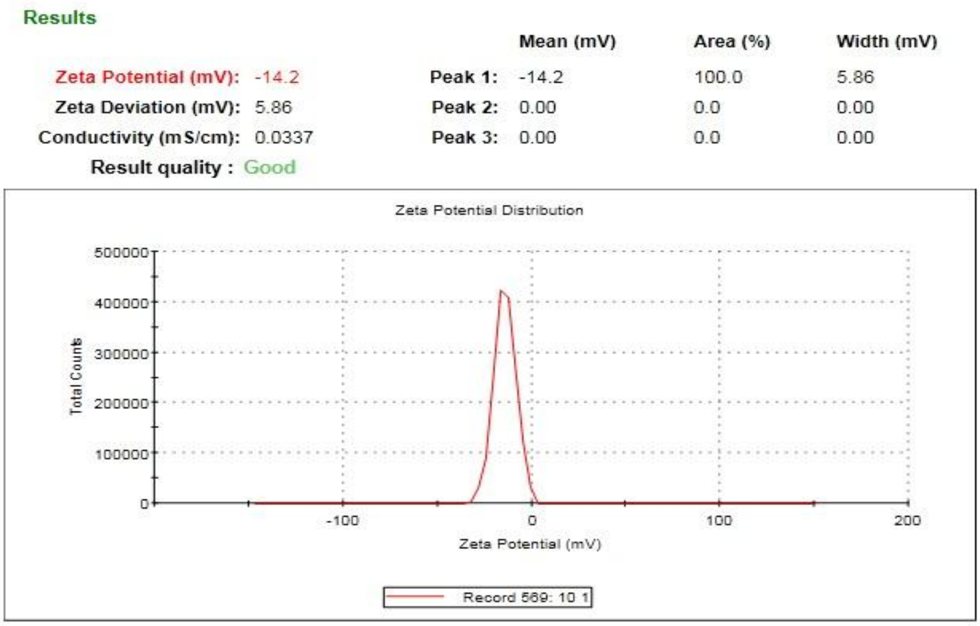

Fig.5: Zeta potential of silver nanoparticles (-14.2mV)

The nanoparticle tracking analysis was performed to determine the average number of nanoparticles dispersed in the reaction mixture. The mode of dispersion strongly depends upon the charge, size and shape of the dispersed media. These are the key factors that govern the aggregation/agglomeration of the synthesized nanoparticles. Based on the scattering phenomena the average particle size was found to be 40±19nm. (Figure.4) These findings were correlated with the observations of (Raksha Pandit 2015) for determining the size of silver nanoparticles by using *B. nigra* seed extract. In order to speculate the stability of the synthesized silver nanoparticle zeta sizer analysis was conducted. The stability of nanoparticles strongly depends upon their relative charge. (Figure.5). The synthesized silver nanoparticles possess a negative charge with zeta potential at (-14.2mV).

Transmission electron microscopy was performed to determine the shape of the silver nanoparticles and to further confirm the size range as depicted by the earlier reported analyzing tools. (Figure.6) The phytofabricated silver nanoparticles were found to be polydispersed with spherical geometry. Our findings was compared with the results of (Khalil et al 2014) who have corroborated silver nanoparticle synthesis by using olive leaf extract. Silver nanoparticles are used since ancient times as a strong antiseptic agent. Silver nanoparticles are prominently used in water purifiers, targeted drug delivery, cancer therapy, biosensor, cosmetics and as catalyst.

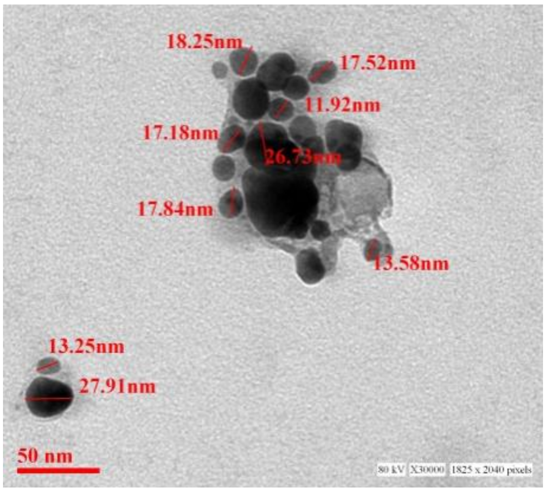

Fig.6: TEM micrographs (SAED pattern) of silver nanoparticles synthesized by using *C. asiatica* leaf extract

In conclusion, the present fraction of research work aimed to synthesized silver nanoparticle by using the leaf extract of medicinal herb *C. asiatica*. The nanoparticles formed are found to be stable and polydispersed in nature. (Gogoi et al. 2013) reported synthesis of silver nanoparticle from the same plant species. The cost effective as well as eco friendly method of nanoparticle synthesis i.e. the green synthesis method, offer a compatible and better alternative as compared to the typical methods. Use of aqueous solvent also reduces or completely ends up the chances of toxicity. The fate of nanoparticles in the environment is yet to be explored. However, their life cycle is more likely to be affected by the route of synthesis and the constituents of the process.

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