



Green synthesis of silver nanoparticles from different parts of the plant *Macrotyloma uniflorum*

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ABSTRACT

The biological synthesis of silver nanoparticles is a revolutionary step in the field of nanotechnology. *Macrotyloma uniflorum* seeds are known for the removal of kidney stones though no scientific explanation is known regarding the mechanism responsible for its litholytic activity. Here we describe the synthesis of silver nanoparticles through aqueous extract of different parts of *M. uniflorum*. UV-visible spectroscopy studies were carried out to assess the formation of silver nanoparticles. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopic (SEM) were used to characterize the silver nanoparticles. The resulting images divulge that silver nanoparticles were quite poly-dispersed, the size ranging from 50 nm to 100 nm. Electron Dispersive X-Ray (EDXR) showed the composition of silver nanoparticles as silicon, carbon, zinc, calcium, potassium etc. The initiative of biological synthesis of silver nanoparticles using dried plants biomass appears to be cost effective, eco-friendly as compared to other conventional methods of nanoparticles synthesis. This study suggests that different parts of *M. uniflorum* have the potential to produce silver nanoparticles. Nanoparticles are intermittently able to react very quickly which makes them useful as catalysts to speed up reactions. Therefore, the biosynthesized silver nanoparticles might be useful for the development of newer and more potent drugs.

INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes and chemical composition (Elumalai et al., 2010). The use of these silver nanoparticles in medical industry and their potential use for human benefits are remarkable (Banerjee et al., 2011). Although chemical and physical methods may successfully produce pure, well-defined nanoparticles (Srivastava et al., 1976), compared to biological synthesis, these methods are quite expensive and dangerous to the environment (Herlekar et al., 2014). Use of biological methods, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly and cost effective manner (Prasad et al., 2014). In recent years, plant-mediated biological synthesis of nanoparticles is achieving significance due to its uniformity and eco friendliness. *Macrotyloma uniflorum* (commonly known as kulthi or horsegram or gahat) plays an important role in the inhibition/dissolution of calcium oxalate and gallbladder stone. Medicinal values and edibility of the pulse are already established (Pandey et al., 1996). Studies on the extraction of silver nanoparticles from aqueous extract of *M. uniflorum* seeds have already been done (Vidhu et al., 2011). However green synthesis of nanoparticles from powdered extract of different parts of the same plant has remained a relatively unexplored research area. Therefore, the present work has been undertaken to synthesize, characterize and compare silver nanoparticles from different parts of the plant i.e. leaves, roots, stems and seeds.

Many research papers reported the synthesis of silver nanoparticles using plant extracts such as *Helianthus annuus*, *Basella alba*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*. In 2013, Auxilia et al. did the phytochemical analysis of seeds extracts of *M. uniflorum* and reported that several phytochemicals were present including tannins, leucoanthocyanins, flavonoids and phlobatannins and saponins (Auxilia et al., 2013). Philip et al., (2011) used plant extract from *M. uniflorum* for green synthesis of noble metal nanoparticles. The plant extract was used as capping agent for synthesizing silver nanoparticles. The reduction of silver nanoparticles was found to be enhanced on using aqueous extract because of the presence of caffeic acid. It was concluded in his work that several factors influence the formation of silver nanoparticles such as plant source, organic compounds in plant extract. Organic compounds like alkaloids, polyphenols, proteins and even some natural pigments are present in plant extracts. Potential of other plant parts of *M. uniflorum* such as roots, stems and leaves as a capping and reducing agent is not tested and well defined. In the present study we found that other parts of the *M. uniflorum* plant are also a good source of silver nanoparticles. In 2012, Yi et al. developed chemical reduction method by seed mediated method in the presence of tri-sodium citrate and polyvinyl pyrrolidone (PVP) at room temperature. In this work PVP molecules were used as capping agent. In 2012, biosynthesis of silver nanoparticles using *Olea europaea* leaves extract was done by Awwad et al. It was found that the

silver nanoparticles showed the presence of proteins which coat the silver nanoparticles known as capping proteins. In 2013, Awwad et al. synthesized silver nanoparticles from carob leaves extract. Investigation suggested that the carboxyl, hydroxyl and amine group in carob leaf extract were mainly responsible for the reduction of Ag^+ ions to Ag^0 nanoparticles. Further study confirmed that carbonyl group of amino acid residues acted as capping agent to prevent agglomeration which suggested the formation of a layer covering silver nanoparticles and confirmed the presence of possible protein acting as reducing and stabilizing agent (Awwad et al., 2013). Geethalakshmi et al. (2010) synthesized silver nanoparticles using *Trianthema decandra* extract and in this study it was confirmed that bioreduction of Ag^+ ions to silver nanoparticles are due to reduction by capping material of plant extract. In 2009, Jain et al. synthesized silver nanoparticles from papaya fruit extract and they found out that polyols are mainly responsible for the reduction of silver nanoparticles. FTIR analysis confirmed that bioreduction of silver ions to silver nanoparticles are due to the reduction by capping material of plant extract. It was also discovered from synthesis of silver nanoparticles from leaf extract of *Cardiospermum halicacabum* L. that the carboxyl groups in aspartic and glutamine residues and the hydroxyl groups in tyrosine residues of the proteins are responsible for the Ag^+ ion reduction (Shekhawat et al., 2013). In 2013, Donda et al. synthesized silver nanoparticles using extracts of *Securinega leucopyrus*. The result indicated that the carboxyl, hydroxyl and amine groups of leaf extracts are mainly involved in synthesis of silver nanoparticles. The green synthesis of silver nanoparticles from *Cleome viscosa* was done and IR studies confirmed that the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal and hence acts as a capping agent (Lakshmi et al., 2011).

MATERIALS AND METHODS

M. uniflorum seeds were collected from local shop in Uttarakhand. The seeds, roots, stem and leaves were taken and dried for 3-4 hours at 60°C. These were ground to a fine powder.

Preparation of silver nitrate solution (1mM)

1mM silver nitrate was added to the 100ml of distilled water and the solution was stirred well continuously until the silver nitrate dissolved completely. This 1mM Silver nitrate solution is stored in brown bottle at 4°C for further use for the synthesis of Silver nanoparticles from *M. uniflorum* extract.

Synthesis of silver nanoparticles

1mM aqueous solution of silver nitrate was used for synthesis of silver nanoparticles. For the synthesis of AgNPs, 1mM silver nitrate was added to the plant extract to make up a final solution of 200 ml. These were incubated at room temperature for 30 minutes. The color change of the extracts from yellowish green to dark brown was checked

periodically. The reaction mixtures were centrifuged at 18,000 rpm for 25 minutes in order to obtain the pellet which was used for further study. Supernatant was discarded and the pellet was dissolved in deionised water. The pellets were stored at 4°C for further use.

Characterization of Silver nanoparticles:

UV Spectroscopic analysis of Silver nanoparticles

The UV-visible spectra were recorded using UV-visible spectrophotometer with samples in quartz cuvette. The reduction of pure Ag²⁺ ions was analyzed by measuring the UV-Vis Spectrum of the reaction medium after 1 hour incubation by diluting a small aliquot of the sample with distilled water (Dinesh et al., 2012).

SEM and EDX Analysis

Purified silver nanoparticles in suspension were characterized for their size and shape biosynthesized. EDXR (Energy Dispersive X-ray) analysis of purified nanoparticles was carried out using the same instrument for confirming the elemental composition of

the sample (Kathireswari et al., 2014).

TEM Analysis

The preparation of TEM specimens includes mechanical grinding, electro-polishing and ion beam thinning. As a result, artefacts can originate at different stages of sample preparation. In transmission electron microscopy (TEM), a high-energy electron beam interacts with an electron transparent specimen in order to investigate the structure and composition (Rao et al., 2011).

RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Song et al., 2009). As the seed extract was mixed in the aqueous solution of the silver ion complex, it begin to change the colour from light brownish colour to yellowish colour due to reduction of silver ion (Fig.1); which indicated formation of silver nanoparticles.



Fig.1 Extracts after adding 1mM AgNO₃

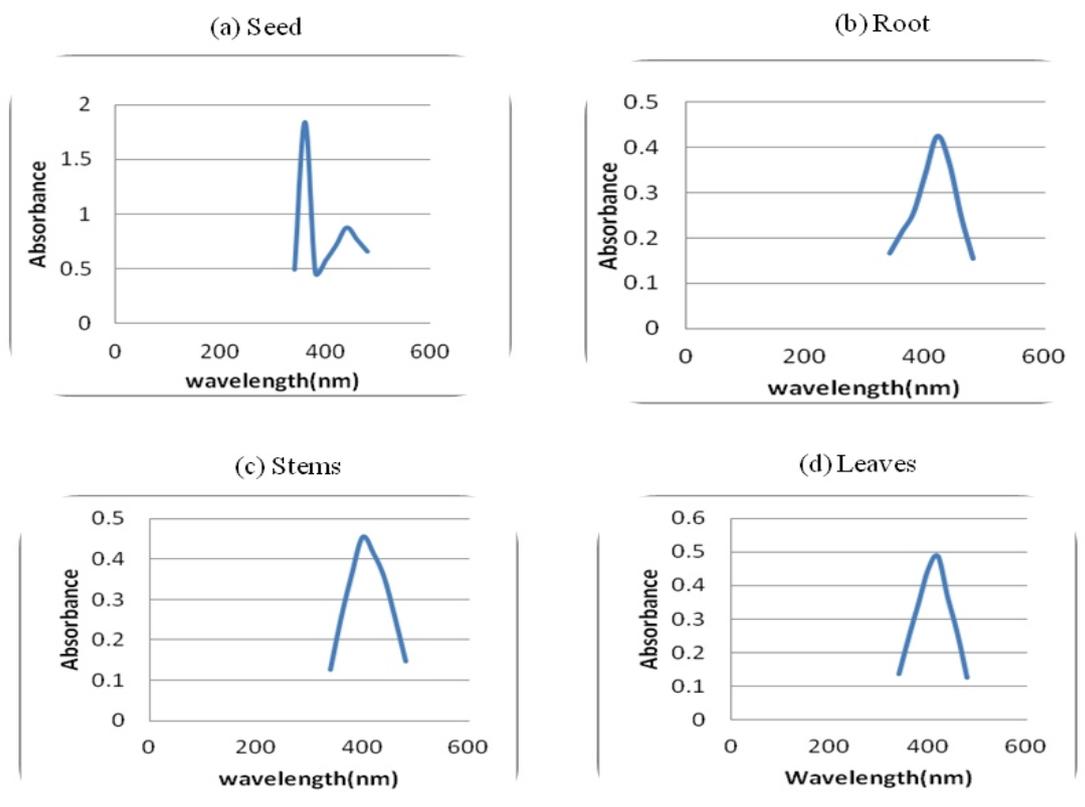


Fig 2: UV-Visible spectrophotometer analysis of different parts of *M. uniflorum*

UV-Vis spectrophotometry

It is usually recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. The reduction of pure Ag^{2+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium immediately after diluting a small aliquot of the sample into distilled water. The UV-visible absorption spectrum of the reaction solution taken after incubation period of 20 minutes showed the maximum absorbance at ~420nm in leaves, ~400nm in stems, ~420nm in roots and ~440nm in seeds (Fig. 2). Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 450 nm, broadening of peak implies that the particles are poly-dispersed.

SEM and TEM analysis

From SEM and TEM analysis, average particle size of the nanoparticles was found between 50nm- 100nm in leaves, roots and stems and 20nm-100nm in seeds. Very less amount of silver nanoparticles were formed in seeds as compared to leaves, roots and stems.

Electron Dispersive X-Ray

This graphs shows that there is very less amount of silver nanoparticles present in the seeds as compared to stems, roots and leaves. There are other chemical components present in the sample like carbon, oxygen, silicon, magnesium.

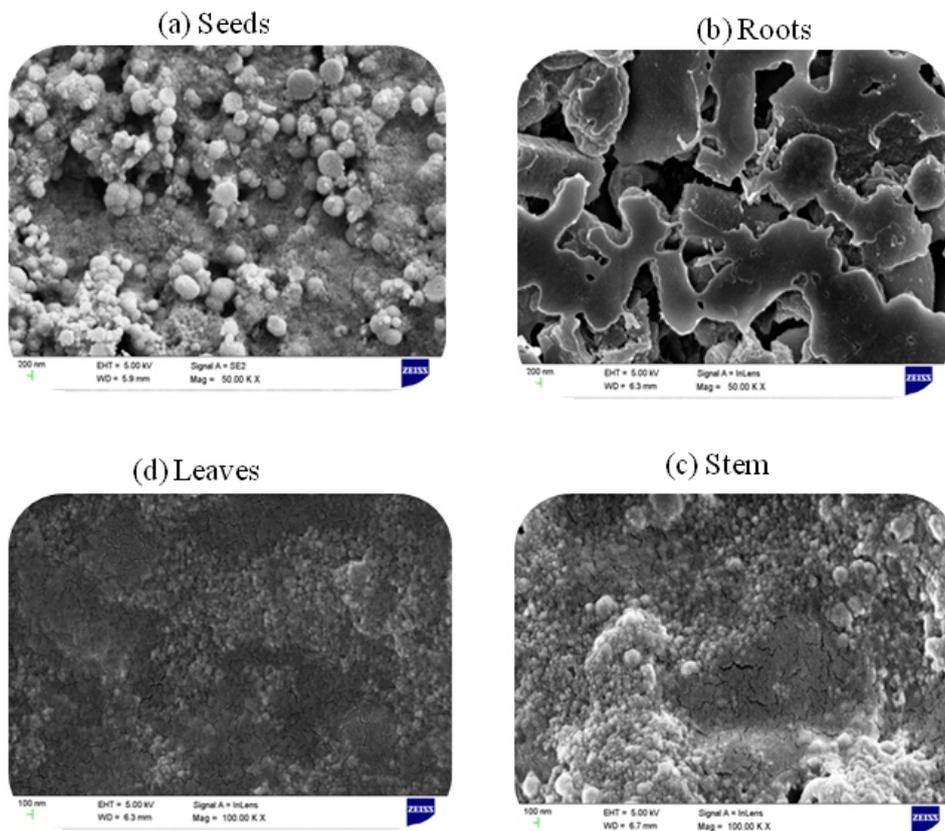


Fig 3: SEM analysis of different parts of *M. uniflorum*

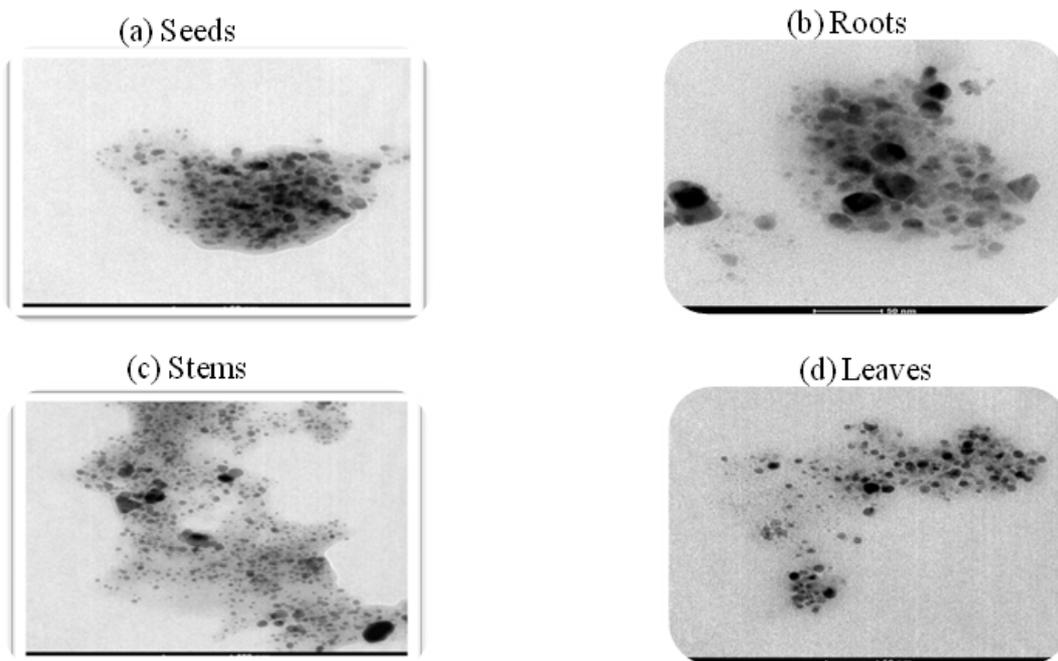


Fig 4: TEM analysis of different parts of *M. uniflorum*

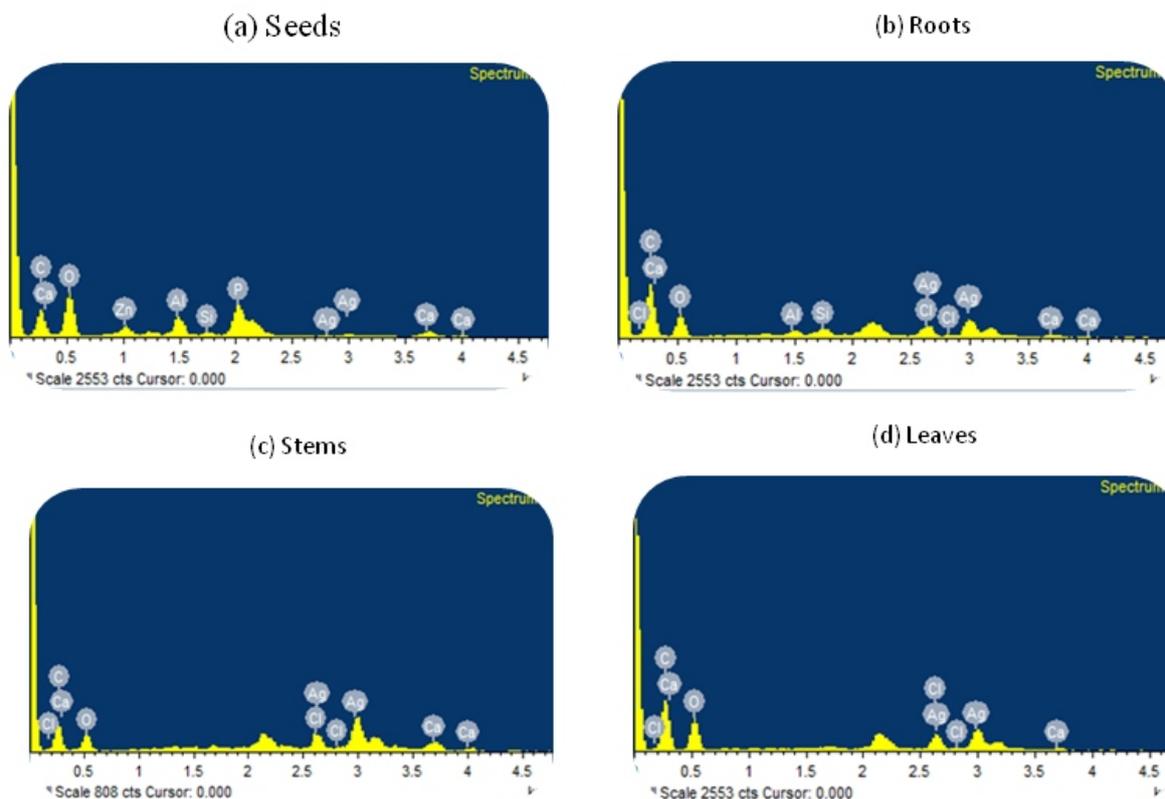


Fig 5: Electron Dispersive X-Ray analysis of different parts of *M. uniflorum*

CONCLUSION

In this investigation, the bio-reduction of aqueous Ag^{2+} ions by the leaf, stem, roots and seeds extract of the *M. uniflorum* plant has been demonstrated. The reduction of the metal ions through different extracts leads to the formation of silver nanoparticles of fairly well-defined dimensions. This is a simple, efficient and clean method to synthesize silver nanoparticles. The synthesized nanoparticles have been characterized by UV-visible, TEM, SEM and EDXR measurements. In the present study we found that leaves, roots, stems and seeds can be a good source for synthesis of silver nanoparticles. The colloid obtained by rapid reduction is found to consist of well dispersed nearly spherical particles having size around 50nm -100 nm which can be further used in medical industry.

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