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Green synthesis of silver nanoparticles using *Macrotyloma uniflorum* seed sprout and their Characterization

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Abstract
Development of reliable and eco-friendly green processes for synthesis of metallic nanoparticles is an important step in the field of nanotechnology, since the other techniques have several disadvantages. In the present study, we used a novel approach to synthesize stable silver (Ag) NPs using *Macrotyloma uniflorum,* seed sprout powder and observed a rapid reduction of silver ions leading to the formation of stable silver nanoparticles in solution when exposed to sun light compared to other modes of synthesis. The UV-Vis spectrum of Ag NP in aqueous solution shows an absorbance peak around 450 nm due to Surface plasmon resonance. Silver nanoparticles were confirmed with FTIR analysis showed the presence of difference functional groups involved in capping the silver nanoparticles again, silver nanoparticles size was confirmed by SEM analysis which shows that particles were in the range of 30 to 50 nm in size. This study, to the best of our knowledge, is the first attempt to perform eco-friendly synthesis of Ag NPs using seed sprouts of *Macrotyloma uniflorum,* which may benefit various industries with wide range of applications.

Keywords: Green synthesis, Ag NPs, UV-VIS Spectra**,** FTIR, Zeta, SEM

Introduction
In recent science nanotechnology, a new emerging, promising approaches for innovations and fascinating field of science and one of the most active areas of research in modern materials science and technology to fulfill the human needs (Siddiqui *et al*., 2015; Kala Rani *et al.*, 2018) [1,2]. Noble metal nanoparticles have been the subject of intense research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials.(Supraja and Arumugam, 2015) [3]. The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts. The silver particles of between 1 nm and 100 nm in size are considered as silver nanoparticles. (Sahayaraj, 2014) [4]. Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses (Forough and Farhadi, 2010) [5]. Research in the silver nanoparticles has become vital owing to their applications in various areas. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology and distribution (Awwad *et al*., 2013) [6]. Ag-NPs have attained special focus in view of their distinctive properties, like good electrical conductivity, chemical stability, catalytic and antibacterial activity (Sharma *et al*., 2009) [7]. Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior (Krutyakov *et al*., 2008) [8]. Silver nanoparticles are used in the development of new technologies in the areas of electronics, material sciences and medicine and because of their extensive applications in various areas more research is being conducted on the silver nanoparticles by the scientists throughout the world. (Safaepour *et al*., 2009) [9].
Nanoparticles have been synthesized by both chemical methods (Murray *et al*., 2002) [10] and physical methods (Ayyub *et al*., 2001) [11] but these routes for synthesis of particles/crystallites require tedious and environmentally challenging techniques. Application of green chemistry to the synthesis of nanomaterials has vital importance in medicinal and technological aspects (Mondal *et al*., 2011) [12]. The growing needs to develop clean, non-toxic and ecofriendly procedures for synthesis of nanoparticles have resulted in researchers seriously looking at biological systems for inspiration. Biological methods for the production of nanoparticles are

considered as a safe and environment friendly alternative to the conventional physical and chemical methods as it is a cost effective method and the usage of high pressure, energy, temperature and toxic chemicals is completely eliminated (Prashar *et al*., 2009) [13]. Therefore the synthesis of nanoparticles using biological method is preferred over the physical and chemical methods and the term "Green nano synthesis" has been proposed for the synthesis of nanoparticles through biological route (Banu and Rathod, 2011) [14]. Thus, there is an increasing demand for "green nanotechnology". (Garima Singhal *et al*., 2011) [15]. Using plants for the nanoparticle synthesis can be advantageous over the other biological methods as the process of maintaining cell cultures can be eliminated and nanoparticle synthesis using seed sprouts extracts can be more economical when compared to the whole plants because of the feasibility in the downstream processing steps.

Macrotyloma uniflorum (Horsegram) is predominantly a South Indian crop and termed as poor man's legume. It serves the farmer excellently under subsistence farming conditions and it is suited to marginally poor soils and those deficient in Nitrogen. The grain of *Macrotyloma uniflorum* are often used as a green vegetable in many parts of India and are very well known to rich in protein and possess medicinal properties. It contains 22% protein, 1.0% fat and 62.0% carbohydrate. It provides thiamine, riboflavin, niacin, vitamin B and ascorbic acid. Cooked seeds possess an earthy flavour and the soups are nutritious. Roasted grains are salted and consumed as confectionary items. It is used as animal feed particularly horse and cattle (boiled, salted and fed). The chemical composition is comparable with more commonly cultivated legumes. Like other legumes, these are deficient in methionine and tryptophan, though horse gram is an excellent source of iron and molybdenum (Marimuthu.and Krishnamoorthi, 2013) [16].

In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. (Panneerselvam *et al*., 2011) [17] Although biosynthesis of gold nanoparticles by plants such as alfalfa (Shetty *et al.*, 2006; Ahmed *et al*., 2002) [18, 19], *Aloe vera* (Gupta *et al.*, 2006) [20], *Cinnamomum camphora* (Singh *et a*l., 1996) [21], *Emblica officianalis* (Sood *et al.*, 2006) [22], lemongrass (Sharma *et al*., 2002) [23] and *Ocimum sanctum,* (Yogeswari Rout *et al*., 2012) [24] have been reported, the potential of the plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. In the present study green synthesis of silver nanoparticles were carried out using the pulses seed sprouts extract of *Macrotyloma uniflorum*.

Materials and methods

Sample collection

Seeds of *Macrotyloma uniflorum* were collected from ICAR-Krishi Vigyan Kendra and National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban, Tamil Nadu, India and the taxonomic identification made by Botanical Survey of India, Coimbatore.

Synthesis of silver nanoparticles

The *Macrotyloma uniflorum* seed was washed thoroughly with de-ionized water. The cleaned seed was sown in the glass bowl which was covered with lid. The seed sown glass bowl kept in laboratory under ambient temperature for allowing sprouts. *Macrotyloma uniflorum* seed sprouts were observed four days after sowing (Fig 1a &1b). All glasswares were thoroughly cleaned using aquaregia and rinsed with de-ionized water. Six grams of seed sprouts powder was dissolved in 100 ml of distilled water and boiled at 80°C for one hour. Then the extract was vacuum filtered and prepared 0.1mM $AgNO_3$. The extract obtained was filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use. The sprout filtrate was treated with aqueous 0. 1 mM AgNO3 solution at 1:2 ratio in a conical and incubated at room temperature for one hour. As a result, colour of the solution gradually changed from yellow to brown color. The colour changes indicated the formation of silver nanoparticles.

Fig (1a): Soaked horsegram seed

Fig (1b): Horsegram sprouts

Characterization of the Synthesized Silver nanoparticles

UV-VIS Spectra analysis

Synthesis of AgNPs solution with seed sprout extract may be easily observed by UV–vis spectroscopy. The bio-reduction of the Ag+ ions in solutions was monitored by periodic sampling of aliquots (1mL) of the aqueous component and measuring the UV– Vis spectra of the solution. UV– Vis spectra of these aliquots were monitored as a function of time of reaction in 400–600-nm range operated at a resolution of 1 nm. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. U-V Visible spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

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

FTIR analysis
For FTIR measurements, Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 minutes. the pellet was washed three times with 20 ml of de-ionized water. The samples were dried and analyzed on Spectrum 100 series of Perkinelmer operating at a resolution of 2cm $^{-1}$.

Particle Size analysis and Zeta potential
The mean particle size, polydispersity index (PDI) and zeta potential of developed nanoparticles were performed by DLS on Zetasizer HPPS-5001 (Malvern, UK) at 25^0C at a scattering angle of 90^0.

Results and discussion
In recent two decades, the synthesis of nanomaterial by means of green methods has increased the attention of researchers due to their application in various fields. Synthesis of silver nanoparticles was easily observed due to changes in the colour of the solution. The formation of silver nanoparticles was indicated by the appearance of signatory brown colour in the same procedure the colour of control solution remain unchanged during this period of the experiment
Formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV–vis spectral analysis. Extinctions spectra of silver hydrosol synthesized from different concentrations of AgNO3 are visible in the spectra (data not shown). Characteristic surface plasmon absorption bands are observed at 425nm for the reddish yellow colored silver nanoparticles synthesized AgNO3 and the fixed volume fraction of aqueous seed extract.
FTIR spectroscopy is used to probe the chemical composition of the surface of the silver nanoparticles and the local molecular environment of the capping agents on the nanoparticles. It has been reported that proteins can provide a good protecting environment for metal hydrosol during their growth processes. Details about wave number and bands assigned are shown in Table 1. Fig. 2 shows the presence of three bands 1644, 1402, and 1258 cm^{-1}. The strong absorption at 1644cm^{-1} is due carbonyl stretching vibration of the acid groups of different fatty acids present in the extract. The bands at 1650 and 1550cm^{-1} are characteristic of amide I and II band respectively. The amide band I is assigned to the stretch mode of the carbonyl group coupled to the amide linkage while the amide II band arises as a result of the N–H stretching modes of vibration in the amide linkage. The band at 1402 cm^{-1} is assigned to the methylene scissoring vibrations from the proteins. It is well known that proteins can bind to silver nanoparticle through either free amine groups or cysteine residues in the proteins and therefore stabilization of silver nanoparticles by the surface bound proteins is possible in the present green synthesis. The band at 1252 cm^{-1} can be assigned to poly phenols. The band at 1087 cm^{-1} corresponds to C–N stretching vibrations of aliphatic amines. IR spectroscopic study confirmed that the horse gram extract has the ability to perform dual functions of reduction and stabilization of silver nanoparticles.

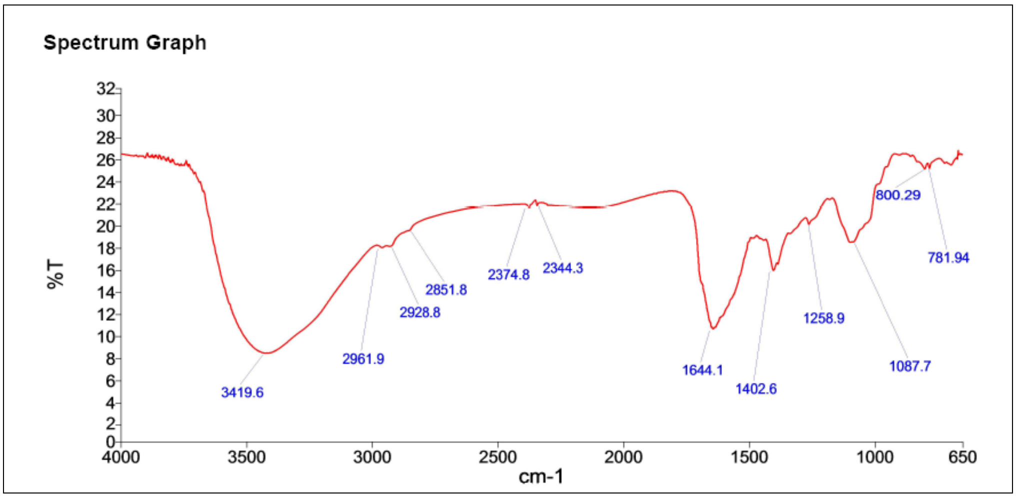

Fig 2: FTIR analysis of silver nanoparticles.

Table 1: Wave number and assignment of bonds for FTIR measurements of Nano particles

Wave number cm^{-1}	Assignment
Broad and Strong nature	
3400–3300	O–H stretching, N–H stretching (minor), hydrogen-bonded OH
1640–1600	aromatic C=C skeletal vibrations, C=O stretching of amide groups (amide I band),C=O of quinone and/or H-bonded conjugated ketones ; cyclic and acyclic compounds of aldehydes, keones and quinone
Medium and Weak nature	
2935–2925, 2850	asymmetric and symmetric C–H stretching of CH2 group
1460–1450	C–H asymmetric bending of CH3 groups
1420–1415	O–H deformation and C–O stretching of phenolic OH
1380	C–H bending of CH2 and CH3 groups, COO− anti-symmetric stretching
1270–1260	C–O stretching of aryl esters
1220	C–O stretching of aryl ethers and phenols
1080–1030	C-O-C stretching of carbohydrates
800–780	C-H deformation of substituted aromatic groups

Particle Size analysis data showed that green synthesized silver nanoparticles size distribution range between 340 to 370 nm. In the present study, the negative zeta potential was found at -19.2 to 20.1 mV (Fig.3a and 3 b). High absolute value of zeta potential specifies a high electrical charge on the surface of the nanoparticles, which can cause strong repellent force among the particles to prevent agglomeration. The stability of nanoparticles was determined by keeping the purified nanoparticles solution at room temperature for different day intervals.

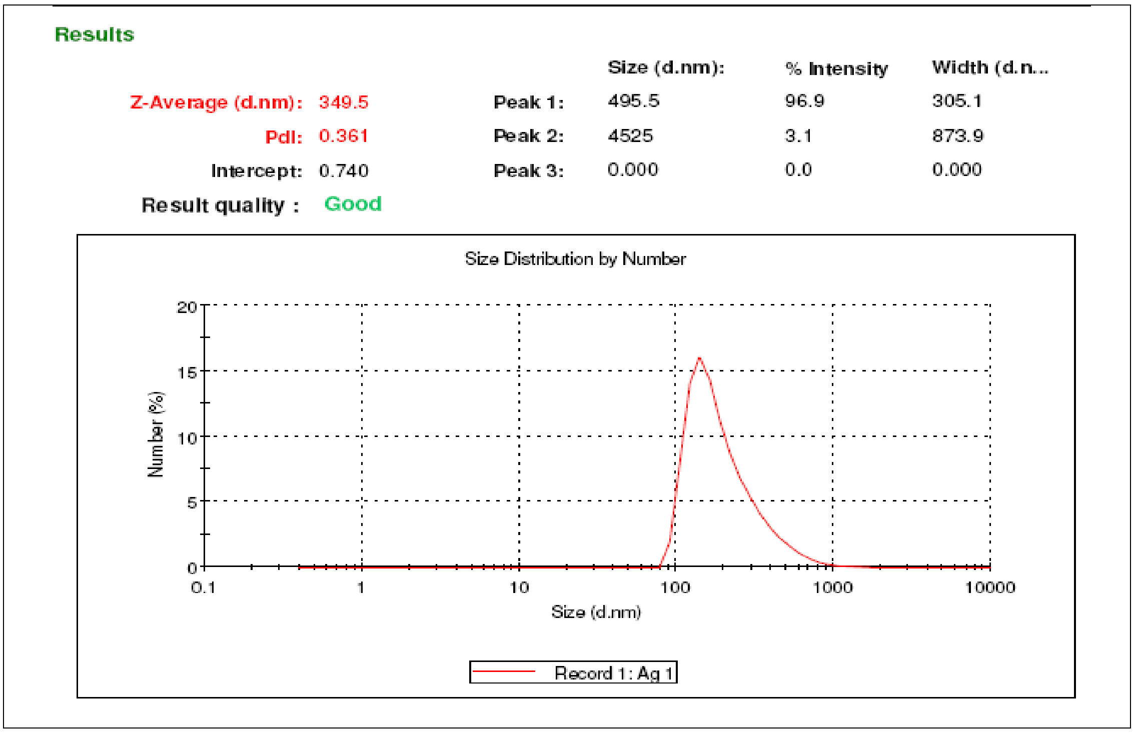

Fig 3(a): Particle size distribution

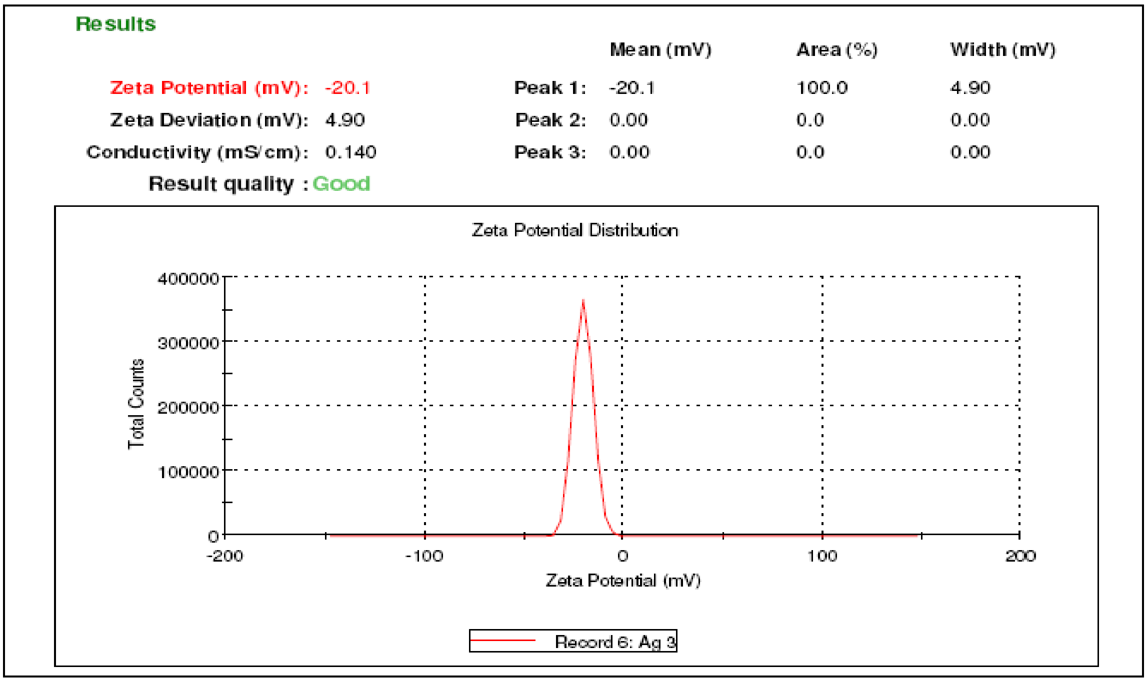

Fig 3(b): Zeta potential measurement analysis of silver nanoparticles.

Surface morphology of the *Macrotyloma uniflorum* mediated synthesized nanoparticles by SEM images (Fig. 4) show the particles are uniformly spherical in shaped. The average sizes of the particles were around 30 to 50 nm for plant extract mediated synthesized silver nanoparticles and it can also be observed that larger particles of AgNPs are formed due to aggregation of nanoparticles during sample preparation. Further study is necessary to explain this observation. The rough surface of the spherical silver nanoparticles was clearly elucidated by the SEM images.

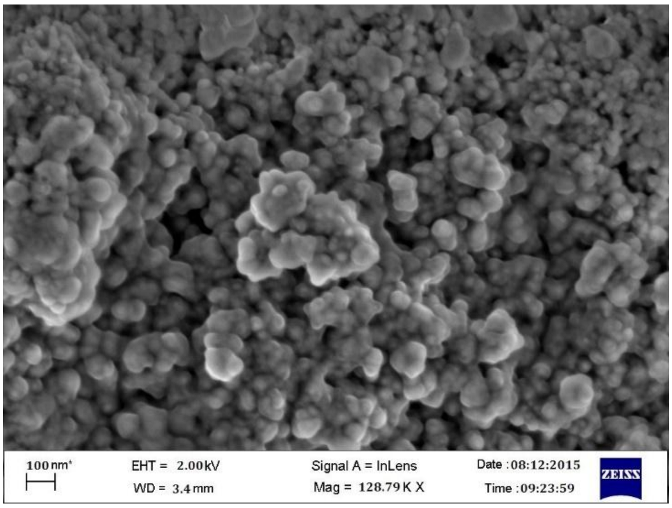

Fig 4: Scanning electron microscopic analysis of silver nitrate nanoparticles

Several factors influence the formation of silver nanoparticles such as plant source, organic compound in plant/seed extract. Organic compounds like alkaloids, polyphenols, proteins and even some pigments are present in plant extracts (Kumar *et al*., 2014) [25]. Earlier studies of horse gram extract on phytochemical analysis indicated, the presence of phenolic acids like p-hydroxy benzoic acid, 3,4-dihydroxy benzoic acid, pcoumaric acid, caffeic acid, ferulic acid, vanilic acid, syringic acid and sinapic acid. The phenolic acids are reported to be powerful antioxidants. Phenolic acids are large family of secondary metabolites having hydroxyl benzoic or hydroxyl cinnamic structures. It has been reported that they posses hydroxyl and carbonyl groups which are able to bind to metals. Phenolic compounds may inactivate ions by chelating. According to Morgan *et al*. (1997) [26] this chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than to specify chelating groups within the molecule.

Conclusion

In the present study, we focused on green synthesis of silver nanoparticles using seed sprout of *Macrotyloma uniflorum*. The physical property of synthesized nanoparticle was characterized using relevant techniques. Results showed that this plant and their extracts can be efficiently used in the synthesis of silver nanoparticles (AgNPs) as a greener route. Such nanoparticles fabricated from plants is the elixir of life and have been used in various applications for human benefit. This study, to the best of our knowledge, is the first attempt to perform eco-friendly synthesis of Ag NPs using *Macrotyloma uniflorum*, and sun light and produced the maximum yield which may benefit various industries with wide range of applications, including renewable clean energy, clean water, medical advances, cosmetics, food and food packaging, paints, coatings, information, technologies and aerospace developments.

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