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Green Synthesis and Characterization of Silver Nanoparticles by using *Carthamus tinctorius* and its Antifungal Properties

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ABSTRACT

Development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. In this study, synthesis of stable silver nanoparticles (AgNP's) was done using *Carthamus tinctorius* leaf extract. UV-Vis spectrometer is used to monitor the reduction of Ag ions and formation of AgNP's in medium. Scanning electron microscope has been used to investigate the morphology of prepared AgNP's. A silver nanoparticle is characterized by FT-IR; to identify the functional groups of carbonyl, hydroxyl, amine and protein molecules which form a layer covering AgNP's and stabilizes the AgNP's in medium. Green synthesis of nanoparticles was found 153nm and it was determined by Zeta potential analyzer. Antifungal activity shown by synthesis of silver nanoparticles against pathogen such as *Aspergillus flavus, Aspergillus niger and Trichoderma viride* by agar well diffusion method. Fungal efficiency of synthesized silver nanoparticles was also analyzed by viable counts. The Haemolysis percentage was found to be 3.8 for green synthesized silver nanoparticles using Carthamus tinctorius extract which is relatively less toxic. Thus the AgNP's biosynthesis using *Carthamus tinctorius* would prove to be a novel tool in the study of nanotechnology.

Key words: *Carthamus tinctorius, FT-IR, Silver nanoparticles, UV-Vis spectrometer, Zeta potential antifungal activity.*

Research based on advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past decades for its physiochemical properties such as size, distribution and morphology. They have been studied for catalytic activity, optical properties, electronic properties, antifungal properties and magnetic properties [1-5] and its application. Green Synthesis and Characterization of Silver Nanoparticles using different plant extracts published in 159 Journals in different fields such as biomaterial production, biochemistry, medical and pharmaceutical products, toothpastes, optical receptors, biosensing, etc. [6-9]. Chemical, physical, and biological methods have been developed to synthesis nanoparticles but chemical and physical methods are involved in the production of toxic byproducts which are hazardous moreover the methods are very expensive [10, 11]. To synthesis stable metal nanoparticles with controlled size and shape, there has been search for inexpensive, safe, and the reliable is "green" approach. The novel methods so called green/biosynthesis have been recently developed by a variety of plant extract such as *Ocimum Sanctum* [12], *Petroselinum crispum* [13], *Murraya koenigii* [14], *Coriandrum Sativum* [15] for the synthesis of metal nanoparticles. Nature has devised various processes for the synthesis of nano and micro length scaled inorganic materials which have contributed in the development of relatively new and largely unexplored area of research based on the biosynthesis of the nanomaterials. Synthesis using bio-organisms is compatible with the green chemistry

principles. "Green synthesis" of nanoparticles makes use of environmental friendly, non-toxic and safe reagents. Nanoparticles synthesized using biological techniques or green technology have diverse nature, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents [12]. Among various plants, we have chosen *Carthamus tinctorius* leaves extract for the present study since it has several pharmacological effects such as anti-fertility, antidiabetic, antihyperlipidemic, antioxidant, and hypotensive activities [16, 17].

Phytochemical screening indicated the presence of chemicals such as quercetin 3-glucoronide, linalool, camphor, geranyl acetate, geraniol and coumarins. The major fatty acid was petroselinicacid followed by linoleic acid [18]. Sathyavathi et al. [15] also reported biosynthesis of silver nanoparticles using *Carthamus tinctorius* leaf extract and their application in nonlinear optics. For instance, the antifungal activity of different metal nanoparticles such as silver colloids is closely related to their size; that is, the smaller the silver nuclei, the higher antifungal activity. Moreover, the catalytic activity of these nanoparticles is also dependent on their size as well as their structure, shape, size distribution, and chemical–physical environment. Thus, control over the size and size distribution is an important task. Generally, specific control of shape, size, and size distribution is often achieved by varying the synthesis methods, reducing agents and stabilizers.

In this manuscript, we attempted to corroborate the reduction of water soluble silver ion using *Carthamus tinctorius* leaf extract to silver nanoparticles by green synthesis method

MATERIALS AND METHODS

Nutrient broth (M002-100G), Silver nitrate of Himedia and all reagents of analytical grade.

Preparation of Plant Extract

Aqueous Extract of *Carthamus tinctorius* was prepared as described by Jenila Rani Duraraj with some modifications. Fresh *Carthamus tinctorius* was brought and the juice was extracted from the leaves and heated at 85 °C for 10 minutes. The mixture was filtered by using Whattman No.1 filter paper & the extract was stored at 4°C. [7].

Synthesis of Silver nanoparticles

0.1M silver nitrate solution was prepared in distilled water. 10ml, 15ml& 20 ml of the $AgNO_3$ solution was taken in a glass beaker and kept in magnetic stirrer for 15 minutes at 65°C. 1ml of plant extract was added drop wise in different volumes of AgNO3 solution with continuous stirring. The mixture was kept on magnetic stirrer for 15 minutes to observe colour change to reddish brown, change in colour indicates synthesis of silver nanoparticles. [8] .All experiments are carried out in triplicate.

Characterization of silver nanoparticles

The synthesized silver nanoparticles are characterized with the help of UV-Visible spectrophotometer. (Double beam ELICO BL198). To determine the functional groups of aqueous extract of *Carthamus tinctorius* and their possible involvement in the synthesis and stabilization of silver nanoparticles, Fourier Transform Infra Red (FTIR) analysis was carried out for the control samples (Plant extract) and the test sample (plant extract after reaction with silver nitrate). A particle size analyzer (zeta potential) was used to find out the size distribution of AgNP's. In order to find out the particle size of AgNP's, sample was prepared in distilled water. i.e. 1ml of

sample was diluted with 9ml of distilled water and proceed for analysis. The analysis was carried out in computer controlled particle size analyzer [(ZETA seizers Nanoseries (Malvern Instrument Nano ZS) [9].

Carthamus tinctorius

Carthamus extract

silver nitrate

silver nano particle

Carthamus tinctorius leaves

Silver nano particle in powder form

Antifungal activity of green synthesized AgNP's

Three fungal strains are acquisized from mysore university, mysore, *Viz*. *Aspergillus niger,Aspergillus flavus and Trichoderma viride,*. Determination of antifungal activity of green synthesized silver nanoparticles was done using two method viz., agar well diffusion method and colony forming unit as per the protocol elaborated by Mittal [10a].

Agar well diffusion method

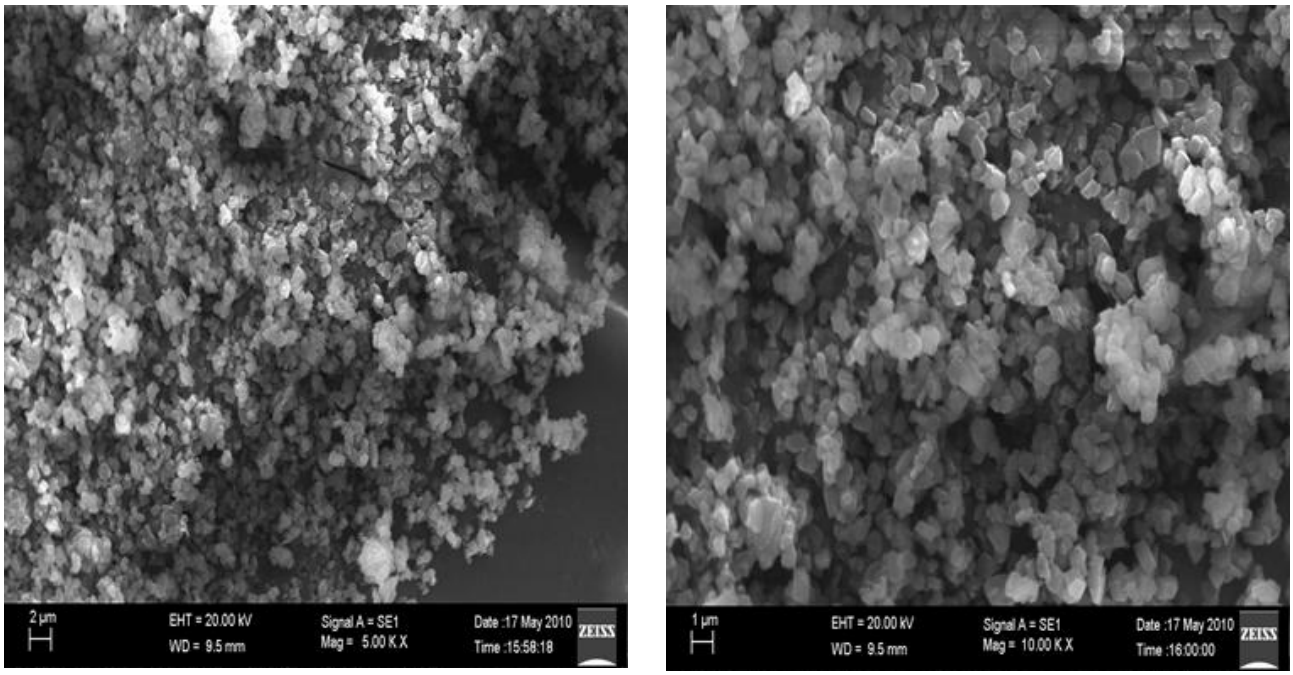
Aspergillus niger,Aspergillus flavus and Trichoderma viride, was inoculated in nutrient broth and incubated overnight at 37 °C. 0.1ml of culture was added in soft agar and mixed well. This was poured on basal agar plate. After 30 min. of incubation at 4 °C, plates were punched using 6mm cork borer. Control plate was prepared by adding 10µl of silver nitrate solution & 10µl of plant extract in wells. Variable volumes of different concentration synthesized AgNP's (10µl, 20µl, 30 µl) were added in respective wells. All plates were incubated at 37 °C for 48hrs. After incubation, zone of inhibition was measured.[10b] by using disc diffusion method. Different standard antibiotic discs (AX10, AK30, NX10, NF300 and streptomycin) are kept against given fugal strains as a positive control [7].

Colony forming unit method

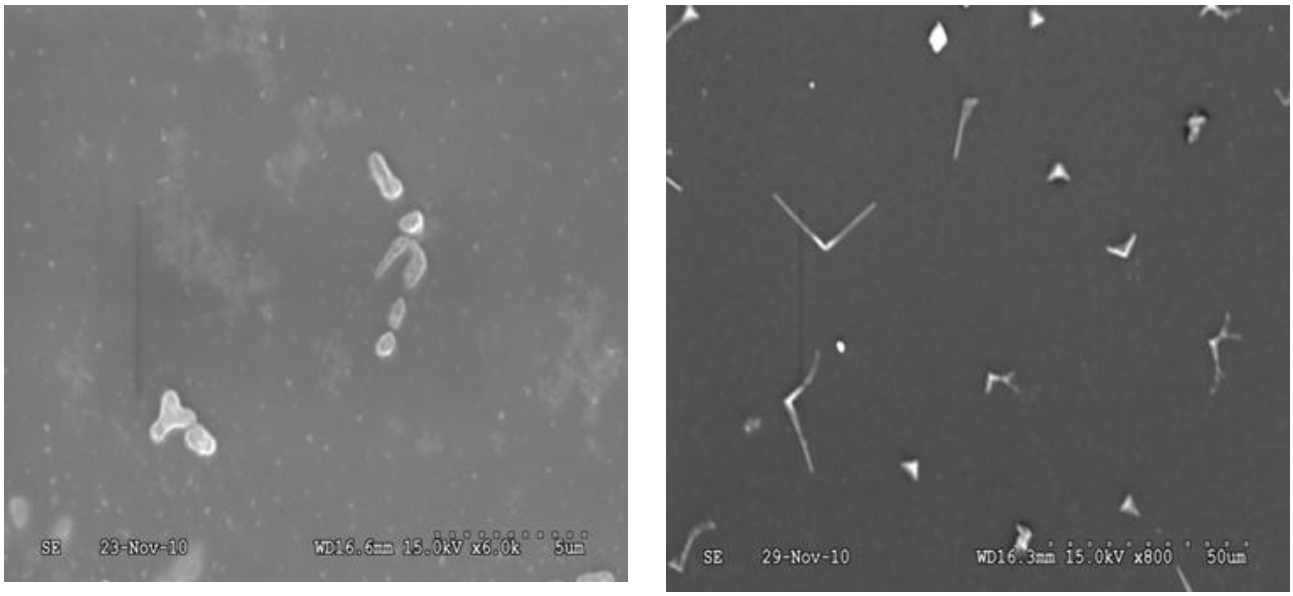
Same strains are inoculated in 5ml of nutrient broth containing various volumes (0.1ml, 0.2 ml, 0.3ml, 0.4ml, 0.5ml and 0.6ml) of Green synthesized silver nanoparticles. After overnight incubation, each tube was diluted up to 10-5 dilution using sterile saline (0.8% W/V), as described by Sondi and Sondi [11].

Haemolysis test

Silver nanoparticles cytotoxic effect was studied by performing haemolysis test, as per the producer given by Jenila Rani Durairaj [7] with slight modifications.



Nano particle analyser –[Malvern Instrument Nano ZS]



Scanning electron microscope analysis on silver nano-particle

RESULT AND DISCUSSION

Silver Nanoparticle synthesis

In present study, when we added the plant extract of *Carthamus tinctorius* to the 0.1M of silver nitrate aqueous solution , colour of reaction mixture rapidly change yellowish to reddish brown due to the reduction of silver ions Ag+ to silver nanoparticles and excitation of surface plasmon vibrations, which indicates the formation of silver nanoparticles (figure 1). Colour intensity was increased while increasing time incubation revealed the increased silver nanoparticle synthesis. Similar results were reported by Shankar SS, Ahmed A, Akkamwar B, Sastry M, Rai A, Singh A. [4b] using leaves of *Catharanthus*

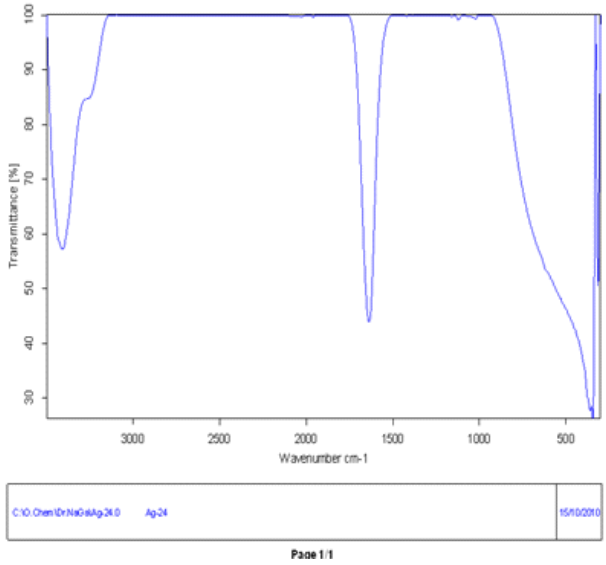
roseus. Here our plant extract of *Carthamus tinctorius* have wide capability to synthesized silver nanoparticles till reported.

Catharanthus roseus

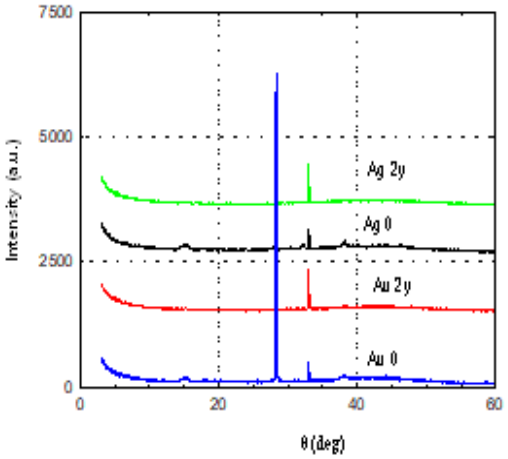
0.1M of silver nitrate

Reduction of silver ions Ag^{+} to silver nanoparticles

The synthesized nanoparticles are primarily characterized by UV-Vis spectroscopy for the analysis of nanoparticles. In our work, no absorbance peak was observed in control and synthesized AgNPs shows highest absorption peak at 420nm. The resullts are in ageement with Jenila Rani Durairaj [7], who observed the absorbance peak at 410 nm. In FT-IR analysis, the strong peak was observed at 3271.91 showed C-H stretching, indicates the presence of alkynes group and peak at 1633.49 showed N-H bonds stretching indicated the presence of primary amines group (figure 2). The presence of this functional group is responsible for the stabilization of synthesized silver nanoparticles and also acts as reducing agent. Exactly similar results reported by Firdhouse [12] that the peak at 3371cm^{-1} and 1635cm^{-1} by using pomegranate fruit peel extract.



Fourier Transform Infra Red (FTIR) analysis



Zeta analysis graph

Fig.2: FT-IR spectra and zeta analysis graph of green synthesized silver nanoparticles from aqueous extract of *Carthamus tinctorius*

Efficiency of nanoparticle depends on their size, smaller the size of nanoparticles higher the efficiency will be. In present study we got nano particle size of 194.2 d nm wich is determined by zeta potential(figure 2). Whereas Kirthika P. and Satoshi

kokura, et al, [9] reported the size of created nanoparticles 581nm & 316nm respectively, from five different herbal plants such as *Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus*. Hence *Carthamus tinctorius* proved to be the best reducing agent for green synthesis of silver nanoparticles.

Antifungal activity determination of biosynthesized silver nanoparticles

The silver nanoparticles through medicinal plants mediated synthesized showed good inhibitory activity against *Aspergillus niger,Aspergillus flavus and Trichoderma viride*. In this study the silver nanoparticle synthesized using aqueous extract of *Carthamus tinctorius* as reducing agent has exhibited a fairly significant antifungal activity against *Aspergillus niger,Aspergillus flavus and Trichoderma viride* with distinct zone of inhibition as per table no.1. The zone of inhibition was found to be increased with increased in concentration of Green synthesized silver nanoparticles from 10ul to 30ul. It was also observed that there was no zone of inhibition in the control (*Carthamus tinctorius* plant extract and silver nitrate solution) figure no. (3-8). Fungicidal efficiency of silver nanoparticles also analyzed by viable counts determined for the control of *Aspergillus niger,Aspergillus flavus and Trichoderma viride* and the silver nanocompsite containing samples (as shown in figure 9-12), was found to be more efficient fungicidal agent, as the concentration increases efficiency found to be increased. Similar results were reported by Mittal [10c].The Haemolysis percentage was found to be 3.8 by green synthesized silver nanoparticles which were prepared by using *Carthamus tinctorius* extract and it was relatively less toxic.

Table1. Zone of inhibition (in diameter in mm) obtained by silver nanoparticles produced by *Carthamus tinctorius* extract.

S. No.	Name of micro-organism(culture No)	Zone of inhibition in mm			NX	NF	AX
		10ul	20ul	30ul	10mcg	300mcg	10mcg
1	*Aspergillus flavus* (2514)	5	11	11	11		-
2	*Aspergillus niger* (2078)	10	11	11	-		10
3	*Trichoderma viride* (1692)	13	13	15	14		-

CONCLUSION

Silver nanoparticles were synthesized by using *Carthamus tinctorius*. Characterization of synthesized nanoparticles was studied by using UV visible spectra, FT-IR and Zeta potential. By the FT-IR,the functional group was found to be amide and maximum wavelength was at 1633.49. The maximum absorbance of silver nanoparticles by UV visible spectra was found to be at 420nm and the size was found to be 194 dnm by zeta potential. Synthesized AgNPs shows antifungal activity against fungus

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