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Green Synthesis of Glycyrrhizaglabra Silver Nano Particles and Confirmation of them through Microscopy and Spectrophotometric Techniques

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Abstract:*An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. This study reports the synthesis of silver Nanoparticle by usingGlycyrrhizaglabra leaf extract. Visually, the formation of silver nanoparticle was confirmed by observing the colour changes from pale yellow to dark brown colour and an intense peak was observed in the UV- spectrophotometer at 460 nm. The possible functional groups in the plant extracts were identified by FT-IR analysis.This novel green approach is a rapid, facile and used for large scale production of metallic nanoparticle.*

Keywords: *Nanoparticle, Glycyrrhizaglabra, UV –Vis, FT-IR and SEM.*

INTRODUCTION

Nanotechnology is a powerful new technology for taking apart and reconstructing nature at the atomic and molecular level. Nanotechnology embodies the dream that scientists can remake the world from the atom up, using atomic level manipulation to transform and construct a wide range of new materials, devices, living organisms and technological systems. Nanotechnology and nanoscience involve the study of phenomena and materials, and the manipulation of structures, devices and systems that exist at the nanoscale, <100 nanometers (nm) in size. To put 100nm in context: a strand of DNA is 2.5nm wide, a protein molecule is 5nm, a red blood cell 7,000 nm and a human hair is 80, 000 nm wide. The properties of nanoparticles are not governed by the same physical laws as larger particles, but by quantum mechanics.

"Nanotechnology is the creation of functional materials, devices and systems through control of matter on the nanometer length scale (1-100 nanometers), and exploitation of novel phenomena and properties (physical, chemical, biological, mechanical, electrical...) at that length scale."

Nanoparticles are small clusters of atoms about 1 to 100 nanometers long. 'Nano' derives from the Greek word "nanos", which means dwarf or extremely small. It can be used as a prefix for any unit like a second or a liter to mean a billionth of that unit. A nanosecond is a billionth of a second. A nanoliter is a billionth of a liter. And therefore a nanometer is a billionth of a meter or 10^{-9} m.

A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100nm, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures.

MATERIALS AND METHODS

Homogenate was prepared by weighing 20grams of fresh leaves of *Glycyrrhizaglabra*collected from Pattukkottai. Washed thoroughly (thrice) in distilled water and homogenized using a mortar and pestle. The homogenate was then filtered using a sterile gauze cloth. This homogenate extract prepared was then transferred to a sterile container. Simultaneously, the extract was evaporated to obtain dry powder. Both the extract and powder were used for the study of qualitative phytochemical analysis.

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Preparation of Silver Nitrate Solution

Commercially purchased silver nitrate (molecular weight-169.87) was used to prepare 1mM concentrations. Appropriate amount of silver nitrate was weighed and dissolved in distilled water.

Preparation of Silver

Nanoparticles

To 750 ml of each millimolar concentration of silver nitrate, 7.5 ml of the leaves of *Glycyrrhizaglabra* extract homogenate was added, respectively into a clean conical flask. The conical flasks were then exposed to the sunlight (while being continuously shaken) for the synthesis of the nanoparticles to begin. The colors of the mixture turns from green to brown when exposed to sunlight and once it turns to colorless the particles were settled at the bottom of the flasks. The particles were then centrifuged (high speed centrifuge) and the supernatant was removed. To the particles now settled at the bottom of the centrifuge tubes, about 1ml acetone was added for the removal of the moisture content from the nanoparticles. The nanoparticle suspension were transferred to a watch glass, air dried, collected, weighed and stored in a sterile container.

UV-VIS Spectra Analysis

The bioreduction of Ag^+ ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 800 nm ranges.

FT-IR Analysis

For FT-IR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu FT-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

FOURIER-TRANSFORM SPECTROMETERS

The Michelson Interferometer

Radiation leaves the source and is split. Half is reflected to a stationary mirror and then back to the splitter. This radiation has travelled a fixed distance. The other half of the radiation from the source passes through the splitter and is reflected back by a movable mirror. Therefore, the path length of this beam is variable. The two reflected beams recombine at the splitter, and they interfere (e.g. for any one wavelength, interference will be constructive if the difference in path lengths is an exact multiple of the wavelength. If the difference in path lengths is half the wavelength then destructive interference will result). If the movable mirror moves away from the beam splitter at a constant speed, radiation reaching the detector goes through a steady sequence of maxima and minima as the interference alternates between constructive and destructive phases.

If monochromatic IR radiation of frequency, $f\,(ir)$ enters the interferometer, then the output frequency, f_m can be found by;

$$f_m = \frac{v}{1.5 \times 10^{11}} \times f(ir)$$

Where v is the speed of mirror travel in mm/s

Because all wavelengths emitted by the source are present, the interferogram is extremely complicated. The moving mirror must travel smoothly; a frictionless bearing is used with electromagnetic drive. The position of the mirror is measured by a laser shining on a corner of the mirror. A simple sine wave interference pattern is produced. Each peak indicates mirror travel of one half the wavelength of the laser. The accuracy of this measurement system means that the IR frequency scale is accurate and precise.

In the FT-IR instrument, the sample is placed between the output of the interferometer and the detector. The sample absorbs radiation of particular wavelengths. Therefore, the interferogram contains the spectrum of the source minus the spectrum of the sample. An interferogram of a reference (sample cell and solvent) is needed to obtain the spectrum of the sample.

After an interferogram has been collected, a computer performs a Fast Fourier Transform, which results in a frequency domain trace (i.e intensity vs. wavenumber). The detector used in an FT-IR instrument must respond quickly because intensity changes are rapid (the moving mirror moves quickly). Pyroelectric detectors or liquid nitrogen cooled photon detectors must be used. Thermal detectors are too slow. To achieve a good signal to noise ratio, many interferograms are obtained and then averaged. This can be done in less time than it would take a dipersive instrument to record one sca

Scanning Electron Microscopy (SEM)

The supernatant from the maximum time-point of production (of silver nanoparticles) was air-dried. The synthesized silver nanoparticles were fabricated on a glass substrates were done for the determination of the formation of silver nanoparticles. The morphology and size of silver nanoparticles were investigated using Scanning Electron Microscope (VEGA 3 TESCAN). The micrograph were recorded by focusing on clusters of particles.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical Investigation of *GlycyrrhizaGlabra* Leaf Extract

S.No	Phytochemical compound	Results of qualitative tests
1	Sugars	-
2	Terpenoids	+
3	Alkaolids	+
4	Phenolic compounds	+
5	Tannins	+
6	Flavonoids	+
7	Glycosides	+
8	Quinones	+
9	Steroids	+
10	Coumarins	+

Table 2: Indication of Color Change in the Synthesis of Silver Nano Particle(SNPs)

S.No	Plant leaf extract+AgNO3	Color change		pH change		Color intensity	Time	Result
	Scientific name	Before	After	Before	After			
1	*Glycyrrhizaglabra*	Light yellow	Brown	4.0	4.60	+++	20 min	Positive

Note: **+++: Dark brown**

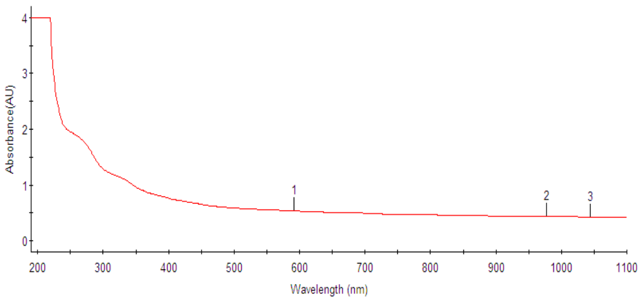


Figure 1: UV-VIS analysis of Glycyrrhizaglabra leaf extract

Spectrum Name:*Glycyrrhizaglabra*

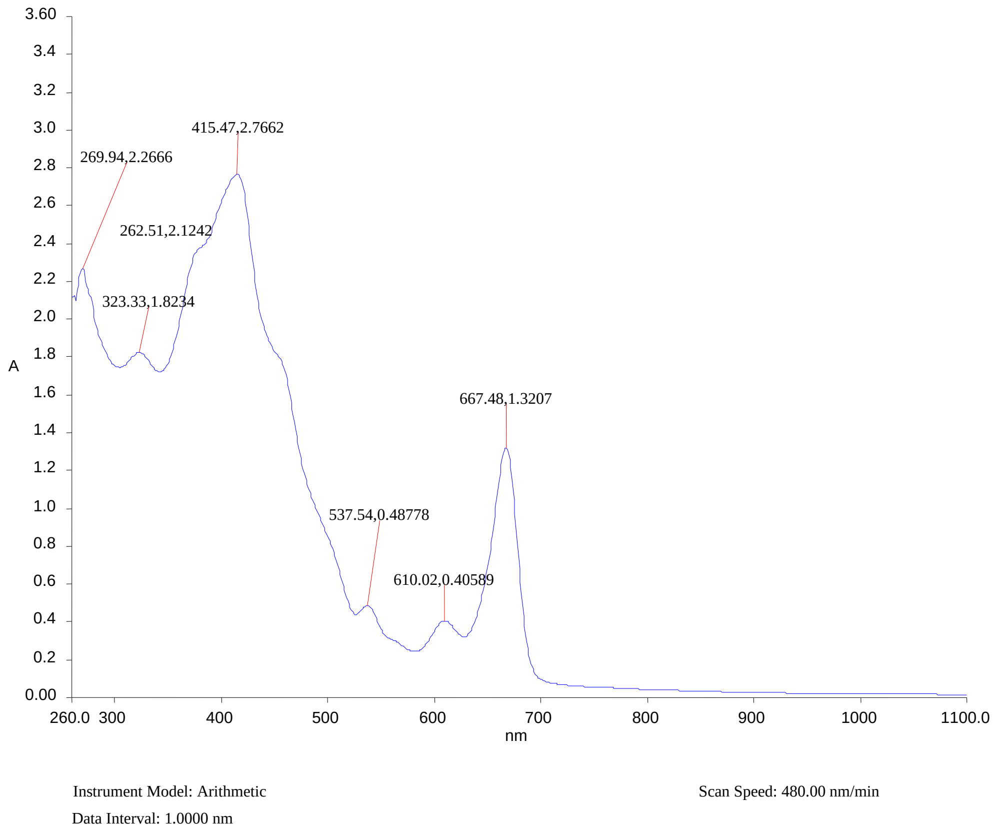


Figure 2: FT -IR analysis of Glycyrrhizaglabraleaf extract

Phytochemical Analysis

Plate: 1 showing the plant *Glycyrrhizaglabra* and the dry powder of leaves

The phytochemical analysis of *Glycyrrhizaglabra* leaf extract showed the presence of alkaloids, tannins, flavonoid, quinines, steroids, coumarins and phenolic compound. Sugar alone was absent in the leaf extract (Table1).

Various herbs and spices have been reported to exhibit antioxidant activity and antimicrobial properties. A majority of the antioxidant antimicrobial properties is attributed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer **(Khalaf*et al.*, 2007).**

Cancer chemoprevention by phytochemicals may be one of the most feasible approaches for cancer control. Phytochemicals obtained from vegetables, fruits, spices, teas, herbs and medicinal plants, such as alkaloids, terpenoids and other phenolic compounds, have been proven to suppress experimental carcinogenesis in various organs in pre-clinical models. Recent studies have indicated that mechanisms underlying chemopreventive potential may be a combination of antioxidant, anti-inflammatory, immune-enhancing and hormone modulation effects, with modification of drug metabolizing enzymes, influence on cell cycle and cell differentiation, induction of apoptosis, suppression of proliferation and angiogenesis, playing roles in the initiation and secondary modification stages of neoplastic development **(Rabi and Gupta, 2008).** Our plant also contains alkaloids, terpenoids and other phenolic compounds.

SNPs Synthesis

In the current study, Silver Nano Particle (SNPs) was synthesized. Syntheisis of SNPs were indicated by the Color Change from yellow to brown and the pH was changed from 4.0 to 4.60. Table -2 & Plate:2 shows the colour change during the synthesis of silver nano particles.

The synthesis and application of nanomaterial is in the limelight in modern nanotechnology. Plants including herbs, lower plants, higher plants, weeds etc. contain an array of secondary metabolites such as phenolic compound, terpenoids, essential oils, and flavonoids, which helps in the reduction of metal ion and formation of nanoparticles (**Haverkamp and Marshall 2009**). The present investigation demonstrated the formation of silver nano particles by the reduction of aqueous silver metal ions by plant extracts prepared using *Glycyrrhizaglabra.*

In the present study SNPs were synthesized by using flower extract of *Glycyrrhizaglabra* rapidly within 20 min of incubation period and yellowish brown colour was developed by addition of Silver Nitrate. The time duration of change in colour and thickness of the colour varies from plant to plant. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H+ ions to reduce the silver. It is well known that SNPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol.

Detection and Characterization of Phyto Silver Nanoparticles

Visual Observation: After treatment of flower extract of *Glycyrrhizaglabra*with $AgNO_3$, the colour change of the reaction mixture was visually observed. The time taken for the reaction mixture to change colour was noted.

The reduction of silver ions into silver particles during exposure to the plant extract was followed by colour change from colorless or pale yellow to brown. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nano particles (**Thirumurugan *et al.,* 2010).**

UV- Vis- Spectroscopy

The reduction of silver metal ions to silver nanoparticles was preliminarily analysed using UV-Vis Spectrophotometer between 300-700nm and depicted in Fig: 1. This analysis showed an absorbance peak at 420 nm which was specific for Ag nanoparticles. UV–visible spectroscopy is an important technique to determine the formation and stability of metal. Nanoparticle in aqueous solution. The reaction mixture changes the colour by adding various concentrations of metal ions. These color changes arise because of the excitation of surface plasmon vibrations in the silver Nanoparticle **(Thakkar*et al*., 2008)**. It shows yellowish to dark brown in colour. The dark brown colour of silver colloid is accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromagnetic field.

The phytosynthesis of silver nanoparticles was confirmed firstly by visual observation: the yellowish colour of petal extracts turned to brown after addition of $AgNO_3$ 10-3 M solution due to excitation of surface plasmon vibrations indicating the formation of silver nanostructures (**Priya*et al*., 2011).**

FT-IR Analysis

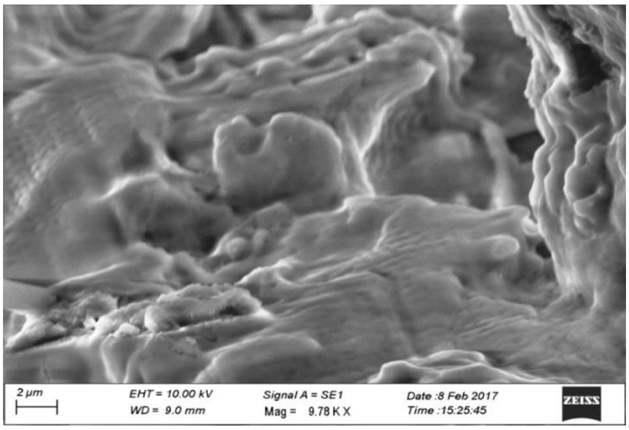
FT-IR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Ag Nanoparticle synthesized using *Glycyrrhizaglabra*leaf extract. This spectrum shows lot of absorption bands indicated the presence of active functional groups in the synthesized silver Nanoparticles. The intensity peaks are slightly increased for the period of silver nanoparticle synthesis like 3470, 2832, 2719, 1630 cm^{-1} Fig: 2 shows the band at 3470 corresponds to O-H and H Stretching vibrations of alkaline phenol. The peak at 2832 indicates to OH stretching vibrations to carboxylic acid. The peak at 2719 represents to C (trible bond) in plane bend to alkenes. The peak at 1630 corresponds to N-H, C-Br stretch in vibrations to primary amines. The weak band at 1362 indicates C-H stretching vibrations and it corresponds to the presence of aromatic acids in the flower extract of silver nano particles. The presence of active functional groups in seed extract results in the swift reduction of silver ions to silver Nanoparticle. To obtain good signal to noise ratio of silver nanoparticle were taken in the range 669–3400 cm^{-1}. (Fig: 2).

FT-IR spectra showing the presence of IR peaks assigned to polyphenols and also the existence of IR bands characteristic of amide I and amide II groups specific for proteins/enzymes suggest that flavonoids and proteins present in aqueous petal extracts of ornamental plants could be responsible for the reduction of silver ions and for the stabilization of the phytosynthesized noble metal nanoparticles **(Mallikarjuna *et al*., 2008).**

SEM Analysis

The SEM image showing the high intensity of silver nanoparticles synthesized by Glycyrrhizaglabraextract further confirmed the development of silver nanostructures.SEM provided further insight into the morphology and size details of the silver nanoparticles. SEM analysis showed the particle size of about 100 nm as well the crystal structure of the nanoparticles. The silver nanoparticles synthesized via green route are highly toxic to Multi Drug Resistant (MDR) bacteria hence has a great potential in Biomedical applications. The present study showed a simple, rapid, economical route to synthesize silver nanoparticles. Application of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications makes this method potentially exciting for the large scale synthesis of other inorganic materials (nano-materials) also. Plate 3 represents the SEM Analysis of AgNPs formed from Glycyrrhizaglabra leaf extract.

Plate: 3 Sem Analysis Of Agnps Formed From GlycyrrhizaGlabra Leaf Extract



SUMMARY AND CONCLUSION

In the preset investigation silver nanoparticles were synthesized from*Glycyrrhizaglabra*leaf extract and the synthesis of nano particles were confirmed by both visually and by spectrophotometrically. In visual observation the colour change of the solution, confirms the synthesis of AgNPs. UV-VIS, FT-IR and SEM analysis confirmed the presence of AgNPs by their respective peaks and images. The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AgNPs of *Glycyrrhizaglabra* leaf extract. The capping around each particle provides regular chemical environment formed by the bio-organic compound present in *Glycyrrhizaglabra* leaf extract, which may be chiefly responsible for the particles to become stabilized. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. The phytosynthesis of silver nanoparticles was demonstrated by visual inspection and by performing some spectral techniques (UV-VIS absorption, FT-IR spectroscopy and SEM analysis).

FT-IR results proved that bioactive compounds responsible for silver bioreduction could be proteins and flavonoids present in the aqueous extracts of *Glycyrrhizaglabra* presumed to act as reducing and capping agents for the silver nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles. From the nanotechnology point of view, this is a noteworthy development for synthesizing AgNPs economically. In conclusion, this green chemistry approach toward the synthesis of AgNPs possesses several advantages *viz*, easy process by which this may be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially stimulating for the large-scale synthesis of other inorganic materials, like nanomaterials. The present study included the bio-reduction of silver ions through medicinal plants extracts.

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