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BIOSYNTHESIS AND CHARECTERIZATION OF GOLD NANOPARTICLES USING AQUEOUS LEAF EXTRACT OF CAJANUS CAJAN

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ABSTRACT

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology. Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The present study focused on the bio synthesis and characterization of Gold Nanoparticles using aqueous leaf extract of Cajanus cajan. A quantity of 10 ml plant extract was mixed with 90 ml of 10^{-3} M gold chloride for the synthesis of gold nanoparticles. The optical property of AuNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany). The morphological features of synthesized gold nanoparticles from Cajanus cajan plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). The phase evolution of calcined powder as well as that of sintered samples was studied by X - ray diffraction technique (Philips PAN analyticl, The Netherlands) using Cu radiation. The chemical composition of the synthesized Gold nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of gold nanoparticles. The particles distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs) to find out the particle size distribution.

KEYWORDS: Cajanus cajan, biosynthesis, Gold Nanoparticles, Nanotechnology.

INTRODUCTION

The Gold Nano particles have been synthesized using different techniques. Although chemical and physical methods have been reported in literature, most of the methods are extremely expensive and also toxic and potentially dangerous to the environment. The Biological methods of synthesis of nanoparticles such as microorganism, enzymes, fungus and plant or plant extracts have been suggested as possible eco-friendly alternative methods for chemical and physical methods. Sometime Nanoparticles synthesized from plant or plant parts can prove advantages over other biological methods (Panneerselvam C et al, 2012). Attention has been made towards the use of biological synthesis processes without use of toxic chemicals in the synthesis protocols to avoid adverse effects in biomedical applications for the synthesis of biocompatible metal. Metallic nanoparticles are presently applied in different fields such as electronics, biotechnology, chemical and biological sensors, DNA labelling, drug delivery, cosmetics, coatings and packaging (Kohler et al. 2001 and Schatz et al. 2000). There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies formulated to synthesize nanoparticles of particular size and also of shape depending on specific requirement. Plasmonics uses the unique optical properties of metallic nanomaterials to manipulate the transfer of light on the nanoscale and is a promising technology for integrating the large data-carrying capacity of optical interconnects with nanoscale electronic devices. Stabilization of gold nanoparticles using Phyto-synthesis and microwave heating techniques is an emerging area in the field of advanced nanoparticles synthesis. Several plants and plant products have been successfully used for efficient and rapid extracellular synthesis of silver and gold nanoparticles. The synthesis methods using organisms, both unicellular and multicellular like yeast, fungi and bacteria which were able to synthesize inorganic materials either extracellularly (Mann, 1996) or intracellularly (Ahmed John S and Koperuncholan M, 2012). Some plants can absorb and accumulate metals from water and soil in which they are grown. These are named as ‘hyper-accumulators’. In recent years, several plants have been successfully used and reported for efficient and rapid extracellular synthesis of silver, copper and gold nanoparticles. Green gold nanoparticles derived from phytochemicals can be show excellent biocompatibility, such biogenic gold nanoparticle with high biocompatibility may be clinically useful as contrast enhancement molecular imaging agents.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Cajanus cajan* was collected and washed thoroughly thrice with distilled water, shade-dried up to 5 days and prepared fine powder by grinding. The fine powder of the plant material was sterilized at 121°C for 15 min and weighed. Sterilized fine powder, 20 g each was taken, mixed with 200 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C for further studies to avoid microbial contamination.

Synthesis of gold nanoparticles

Biosynthesis of gold nanoparticles, gold chloride prepared at the concentration of 10^{-3} M with pre-sterilized Milli Q water. A quantity of 10 ml plant extract was mixed with 90 ml of 10^{-3} M gold chloride for the synthesis of Gold nanoparticles. Gold chloride has taken in similar quantities without adding plant extracts to main respective controls. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations were recorded.

UV-vis analysis

The optical property of AuNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany). After the addition of HAuCl4 to the plant extract, the spectrums were taken in different time intervals up to 24hrs between 450 nm to 540 nm. Then the spectrum was taken after 24hrs of HAuCl4 addition.

SEM analysis

The morphological features of synthesized gold nanoparticles from *A. vasica* plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs of the addition of HAuCl4 the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

X-ray diffraction method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherlands) using Cu radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. The Au sample was

scanned in the range 10.0000 - 90.0000o in continuous scan mode. The scan rate was 0.60/sec.

FTIR analysis

The chemical composition of the synthesized Gold nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75o C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

DLS & Zeta potential analysis

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of gold nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 250C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particles distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs).

RESULTS AND DISCUSSION

Biological reduction of gold salt

Reduction of gold salt into gold nanoparticles during exposure to plant extracts was observed as a result of the colour change. The colour change is due to the Surface Plasmon Resonance (SPR) phenomenon (plate 1).

UV-Vis spectrophotometer analysis

The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of gold nanoparticles were observed around 540 nm in case of *C. cajan*. From different literatures it was found that the gold nanoparticles show SPR peak at around 540 nm. From our studies we found the SPR peak for *C. cajan* at 540 nm. It is confirmed that *C. cajan* leaf extract has more potential to reduce Au ions into Au nanoparticles, which lead us for further research on synthesis of gold nanoparticles from *C. cajan* leaf extracts. The intensity of absorption peak increases with increasing time period. This characteristic colour variation is due to the excitation of the SPR in the metal nanoparticles. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Au+ ions is complete within

2 hrs after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticles solutions with time.

On the behalf of the UV-vis data it was cleared that reduces metal ions. So the further characterizations were carried out with *C. cajan* (Figure 1). The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions (Wiley et al. 2006). Huang et al. (2007) reported formation of gold nanoparticles when constant aqueous HAuCl4 at 50 ml, 1 mM with 0.1 g biomass produced gold nanoparticles as indicated by sharp absorbance at around 540 nm in *Cinnamomum camphora*.

SEM Analysis of AuNPs

SEM provided further insight into the morphology and size details of the gold nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes several nano meters i.e. between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles (Figure 2).

X-ray diffraction (XRD) analysis of AuNPs

The *C. cajan* leaf extract-mediated synthesized Au nanostructure was confirmed by the characteristic peaks observed in the XRD image which was shown in Figure 3. The XRD result shows four distinct diffraction peaks at 38.12°, 44.27°, 64.27° and 76.23°, which are indexed for the planes (111), (200), (220) and (311) respectively of the face centered cubic Au. Other peaks were also observed along with the main peaks. This may be due to the crude nature of the extracts containing other metabolites and salts.

FTIR analysis of AuNPs

FTIR measurement was carried out to study the interaction of the nanoparticles and to identify the possible biomolecules in *C. cajan* leaf extract responsible for capping leading to efficient stabilization of the NPs. The intense IR bands (Figure 4) are observed at 3436, 2367, 2075, 1635 and 684 cm-1. The bands observed at 3436 are assigned to the stretching vibrations of Secondary amine (N-H asymmetric stretching). The broad band at 2367 cm-1 is due to Tertiary amine salt (-NH+ stretching) modes. The absorption bands located at 2075, 1635 and 684 may be attributed to RCH=N=N, O-NO2 Stretching asymm and C-S stretching

modes, respectively. The bonds or functional group such indicates that's. Therefore, it may be assumed that water soluble compounds such as flavonoids, gold nanoparticles synthesized using the *C. cajan* leaf extract surrounded by some proteins and metabolites such as terpenoids, flavonoides which are capping ligands of the nanoparticles.

DLS-Size Distribution

The dynamic light scattering (DLS) is a technique widely used for determining the size of colloidal nanoparticles. Distributions of the hydrodynamic diameters of the GNPs were measured by two different light scattering-based techniques. DLS measurements were performed at 90° with three repetitions of AuNPs. The results are presented in Figure 5. The intensity size distribution reveals a broad peak, indicating a potential aggregation of gold nanoparticles. The particle size distribution (PSD) of synthesized gold nanoparticles, it was found that Au nanoparticles size were in the range of 50-100nm. The highest fraction of AuNPs present in the solution was of 95 nm is very appropriate since it gives lowest average size of nanoparticles.

DLS- Zeta potential analysis of AuNPs

The Figure 6 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nanogold. The measurements render a sharp and clean peak, whereas the deviation between the peaks is relatively small, which indicates that the measurements are indeed repeatable and consistent. The overall absorbance of Zeta Potential revealed the energetically insufficient instability.

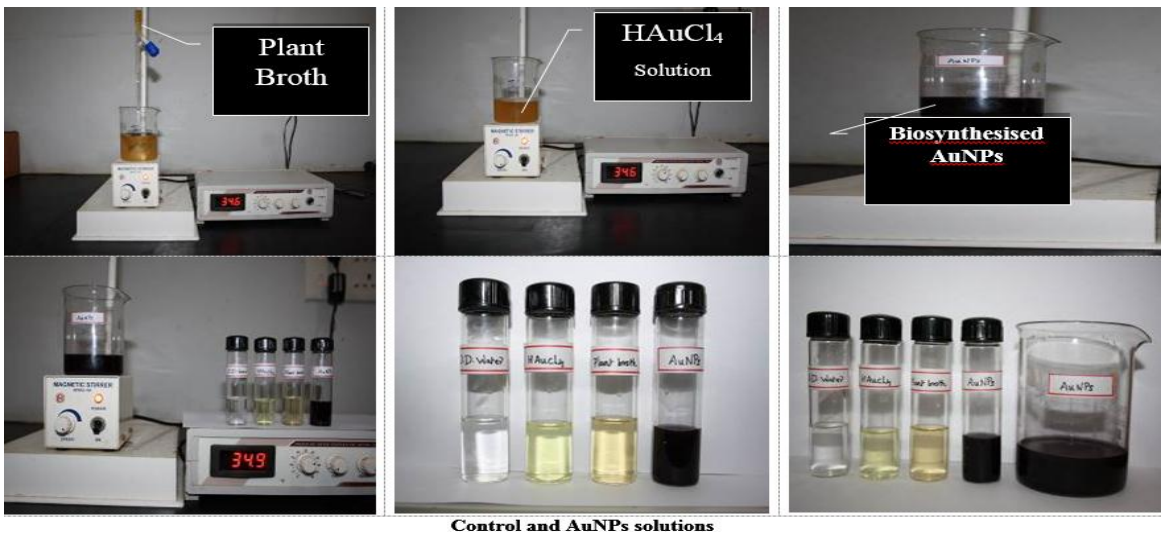

Plate 1: Step by step biosynthesis of AuNPs.

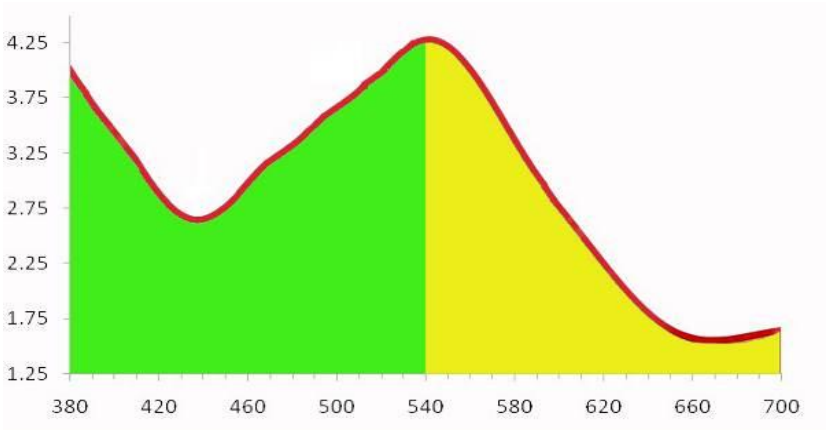

Figure 1. UV ananlysis of AuNPs.

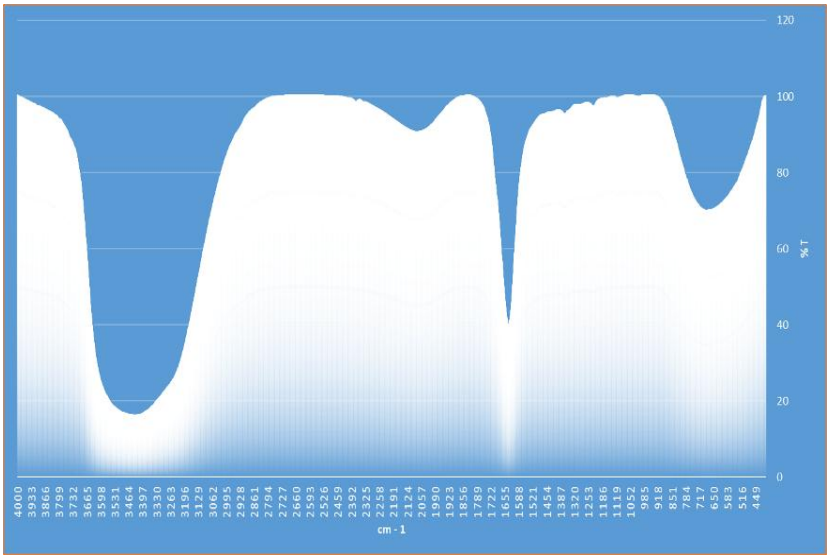

Figure 2. FTIR ananlysis of AuNPs.

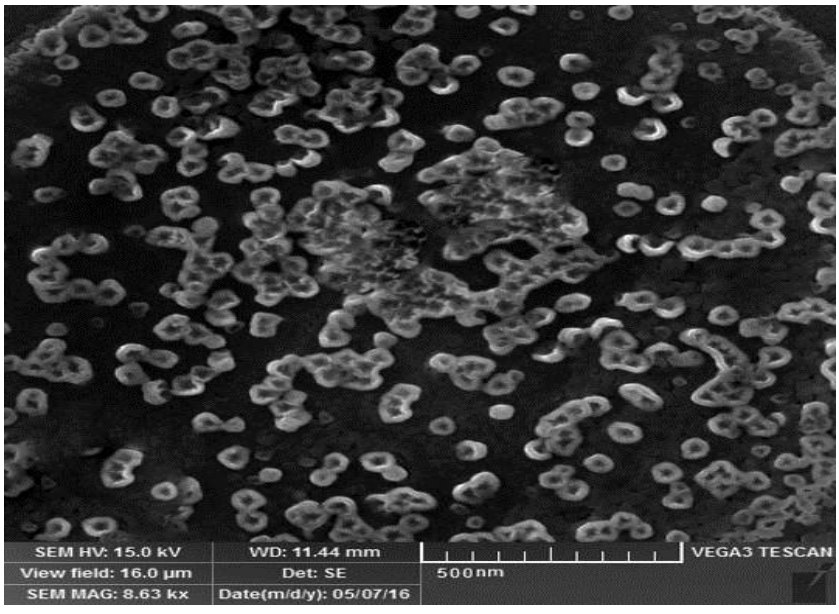

Figure 3 SEM Image of AuNPs.

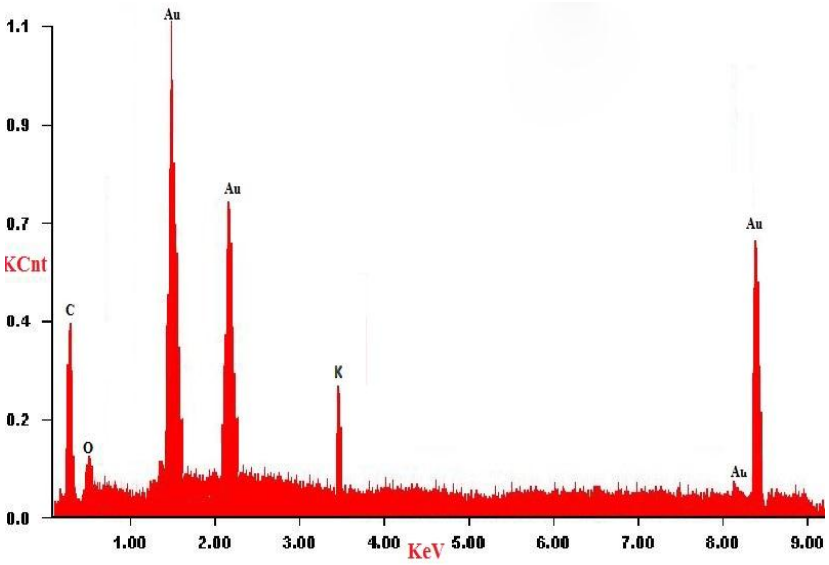

Figure 4: Au-NPs – EDS Analysis.

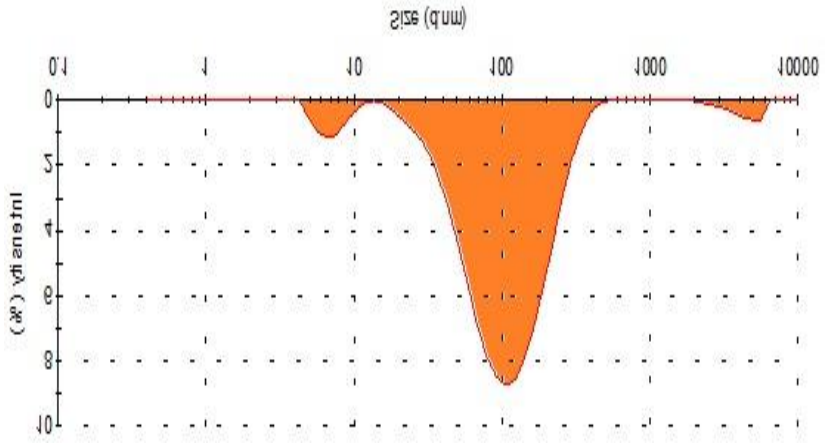

Figure 5: DLS-Size distribution of AuNPs.

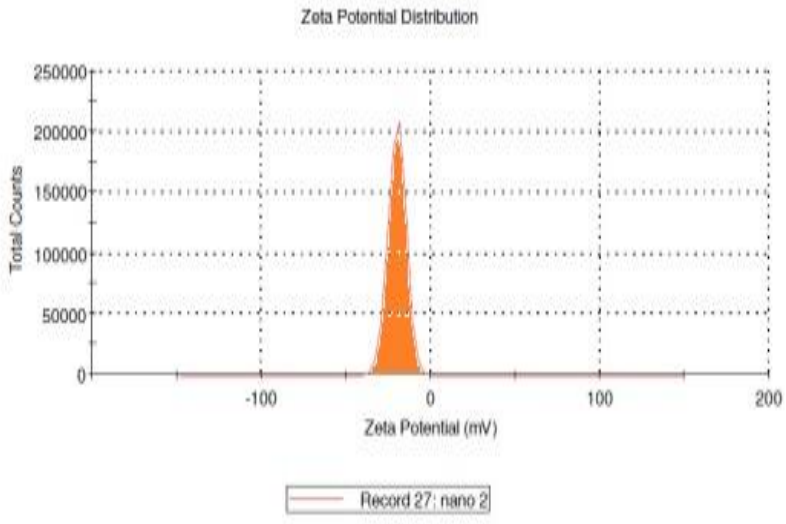

Figure 6: DLS Zeta potential of AuNPs.

CONCLUTION

The biological reduction of aqueous gold ions by *C. cajan* has been confirmed. In the present study, it was disclosed that the aqueous leaf extract of Cajanus cajan can be converted into AuNPs by green synthesis and the resulting Cajanus cajan - AuNPs will show potential antimicrobial and antifouling efficacy against microorganisms. This green approach towards the synthesis of gold nanoparticles has many advantages such as ease with which the process can be scaled up, economic viability, etc. This eco-friendly process of biological gold nanoparticles can potentially be applied in various products and applications.

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