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Research Article

Synthesis of *Evolvulus alsinoides* derived gold nanoparticles for medical applications

R. Anbarasu*, G. Selvan, S. Baskar and V. Raja

P.G. and Research Department of Physics, Thanthai Hans Roever College, Perambalur – 621 212, Tamil Nadu, India

***Correspondence Info:**
R. Anbarasu
P.G. and Research Department of Physics,
Thanthai Hans Roever College, Perambalur – 621 212,
Tamil Nadu, India
E-mail: anbarasu.try102@gmail.com

Abstract

Plant mediated synthesis of metallic nanoparticles is an increasing commercial demand due to the wide applicability in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. This is greatly due to their special features, which include unusual optical and electronic properties, high stability and biological compatibility, controllable morphology and size dispersion, and easy surface functionalization. In the present investigation, synthesis of gold nanoparticle is done by using leaf extracts of *Evolvulus alsinoides*. Gold nanoparticles (AuNPs) were characterized by using UV visible absorption spectra. Their morphology, elemental composition and crystalline phase were determined by scanning electron microscopy, energy dispersive X-ray spectroscopy and FT-IR analysis was used to confirm the presence of gold nanoparticles in the extracts. The plant derived gold nanoparticles were also showing more inhibition activity in both bacterial and fungus strains. In bacteria, gram negative strains are highly affected by the test samples than gram positive. In fungal strains, the highest effect was noticed in *Trichophyton rubrum* while less effect was observed in *Candida albicans*.

Keywords: *Evolvulus alsinoides,* gold nanoparticles, Antimicrobial activity, metallic nanoparticles

1. Introduction

The terrestrial and watery ecosystem provide many bioactive compounds but which could be loss their potential against microorganisms due to the antibiotic resistance [1]. So, alternative antibiotic is an urgent need for pharmaceutical world. Metal nanoparticles are of great importance due to their high surface area and a high fraction of surface atoms and their pharmacological applications. Nanotechnology can be delineated as a researcher for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm [2][3]. Nano-biotechnology combines biological principles with physical and chemical processes to generate nano-sized particles with specific functions [4][5]. The biosynthetic method employing plant extracts has received attention as being simple, ecofriendly and economically viable compared to the microbial systems like bacteria and fungi because of their pathogenecity, and also the chemical and physical methods used for synthesis of metal nanoparticles [6]. It does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures [7]. Several plants and their parts have been successfully used for the extracellular synthesis of metal nanoparticles [8][9].

Koperuncholan and Ahmed John (2011a)[10] defined as gold nanoparticles have been used for more than 400 years for the treatment of certain illness, and the staining of glass enamels. But nowadays, the preparation of nano-scaled gold materials has become very important due to their unique properties of medicinal usages. Gold (Au) is known as a powerful disinfecting agent for killing unicellular microorganisms and has the strongest antimicrobial effects. Also, it is known to exhibit superb inhibitory effects of algal growth [11]. Presently available gold-based inorganic antimicrobial agents are produced in the forms of gold-supported inorganic powders, gold colloids, metal gold powders [12]. The gold-supported inorganic powders are the most used and thus are representative of a typical inorganic antimicrobial agent.

2. Materials and Methods

2.1 Plant Material

The plant materials (*E. alsinoides)* were collected from Tiruchirappalli district of Tamil Nadu in India during the period of October to November 2015.

2.2 Aqueous extraction

The plant leaves were collected individually and washed thoroughly thrice with distilled water. After that, it was 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. The 20 g of sterilized fine powder was taken and 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 No. 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C for further studies to avoid microbial contamination.

2.3 Biosynthesis of gold nanoparticles

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

2.4 Characterization of gold nanoparticles

The characterization of gold nanoparticles was done by the examining size, shape and quantity of particles. Number of technique is used for this purpose, including UV-visible spectroscopy, Scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Dynamic light scattering (DLS).

2.4.1 UV-vis spectroscopy

Absorbance spectroscopy is used to determine the optical properties of a solution. A Light is send through the sample solution and the amount of absorbed light is measured. The wavelength is varied and the absorbance is measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The examination of nanoparticles, the optical properties are much more complicated. For instance, the measured absorbance spectrum does not necessarily show the actual absorbance but the extinction of the light is both the absorbed and the scattered light from the particles. These wave lengths arise due to the surface Plasmon resonance of the particle. The gold nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the Perkin Elmer spectrophotometer at a resolution of 1 nm (from 300 to 700 nm) in 2 ml quartz cuvette with 1 cm path length.

2.4.2 Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDS)

SEM analysis use to employ for characterization of size, shape and morphologies of formed nanoparticle. SEM gives high-resolution images of the surface of a sample is desired. The scanning electron microscope works as same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon. Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than the one of photons. At the same time, it is possible to achieve high resolution pictures of the surface, making the instrument very useful in determining the size distribution of nanoparticles. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The Morphological characterization of the samples was done using JEOLJSM 5800 for SEM and EDS analysis.

2.4.3 Dynamic light scattering (DLS)

The DLS technique uses light to determine the size of particles in a solution. Light at a given frequency is sent through the solution from a laser. When the light interacts with the moving particles in the solution and is scattered, the frequency of the light is also changed. This change of light frequency is directly related to the size of the particles in the solution. The DLS is capable of measuring particles in the size range from a few nano-meters to a few micrometers. It is therefore applicable for determining the size of nanoparticles.

2.4.4 Fourier transmission infrared spectroscopy (FTIR)

FTIR is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It used to analysis of possible bio molecule and also bonding interaction between themselves. The FTIR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. When an infrared light interacts with matter, chemical bonds will show stretch, contract and bend form. These chemical functional group tends to adsorb infrared radiation in a specific wave number range of the structure of the rest of the molecule. The gold nanoparticle synthesis, FTIR data measures interaction between gold salts and proteins molecules, which accurate for the reduction of gold ions and stabilization of AuNPs. The characterization of functional groups on the surface of AuNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}. The

samples were prepared by dispersing the AuNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc.

2.4.5 X-ray diffraction (XRD)

The XRD is a technique used to study phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern, that diffraction gives the information about the atomic arrangement within the crystals. In gold nanoparticle, XRD gives phase structure and purity of the particle. XRD measurements of the reduced AuNPs were recorded on X-ray diffractometer (x'pert pananalytical) instrument operating at a voltage of 40 kV and current of 30 mA with Cu K (α) radiation. This method is also determine the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis.

2.5 Antimicrobial activity

The test sample was challenged against certain microbial strains (procured from MTCC and NCIM, India) for antimicrobial sensitivity using the disc diffusion method [13][14][15]. The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), Salmonella *typhimurium* NCIM 2501 (B5), *Vibrio cholarae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1) and *Cryptococcus* sp. MTCC 7076 (F2). *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4), A sterile cotton swab was used to inoculate the bacterial and fungal suspension on surface of MHA and PDA agar plates [16][17]. The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used [18]. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus) [19]. After incubation, the zone of inhibition was measured with ruler. All the trial was performed thrice and mean values were presented.

3. Results and Discussion

3.1 Green synthesis of AuNPs

The plant aqueous solution and Gold chloride solutions were prepared separately. A quantity of 1.5 ml of plant extract was mixed with 30 ml of 10-3 M of Gold chloride for the synthesis of gold nano particles. During gold nanoparticles synthesis, the change of color from pale green to dark pink colour suggested the formation of gold nanoparticles.

3.2 UV- vis spectrum analysis

Green synthesized AuNPs were confirmed by analysing the excitation due to the applied electromagnetic field of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 540 nm and the peak was observed between 535-550 nm. Figure 1 shows the UV absorption peaks of *E. alsinoides*. It clearly indicating the formation of spherical AuNPs through the plant extract. The change in colour is due to the excitation of surface plasmon vibration, which is indicated by the formation of gold nanoparticles at different time intervals. During each time interval, the peak became distinct and rising. This peak rising clearly denoted the increasing nanoparticles synthesis as the time increases. Similarly, the color also became intensified as the time increases.

3.3 Scanning electron microscopy

The SEM absorption of the product was recorded as synthesis of nanoparticles spherical in structure of about 80 nm in diameter (Figure 2). The SEM image showing gold nanoparticles synthesized using *E. alsinoides* extract confirmed the development of gold nanostructures

3.4 Energy dispersive spectroscopy

The EDS revealed the presence of pure gold (Figure 3) nanoparticles in higher percentages. Gold peak is higher than other peak. The EDX reading proved that the required phase of gold (Au) is present in the sample. This is probably due to the presence of substrate over which the NP sample was held during SEM microscopy.

3.5 Dynamic light scattering of particle size and zeta potential analyses

Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly disparity index of particles in a colloidal suspension. The Figure 4 shows the particle size of the synthesised nanoparticle sample. After analysing data, it was found that Au nanoparticles size were in the range of 50-100nm. The highest fraction of Au-NP present in the solution was of 80.29nm. From the plot it was evident that the solution was consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis.

Zeta potential measures the potential stability of the particles in the colloidal suspension. Gold nanoparticles generally carry a negative charge. The synthesized gold nanoparticles from the plant showed negative charge and were stable at room temperature. DLS-zeta potential showed negative charge (-28.7) which indicated that the sample is moderately stable at room temperature (Figure 5).

3.6 Fourier transform infra-red spectroscopy

FTIR gives the information about functional groups present in the synthesised gold nanoparticles for understanding their transformation from simple inorganic $HAuCl_4$ to elemental gold by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the bio-fabrication of gold nanoparticles mediated by the plant extracts (Figure 6). The *E. alsinoides* petal extract, in $HAuCl_4$ peaks were observed at recorded in the region between 4000 and 400 cm^{-1}. They include 3434 cm^{-1}, 2363 and 2079.16 cm^{-1}, 1637 cm^{-1} which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the bio-fabrication of the nanoparticles by the action of the protein or phytochemicals. The Figure 6 clearly illustrates the bio-fabrication of the AuNPs by the action of the phytochemicals such as phenols, flavonoids and alkaloids in *E. alsinoides*.

3.7 X-ray diffraction

The XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesised nanoparticles particles. The XRD pattern of synthesized particles were analysed and found peak profile of relevant particles. In this result, peaks were observed at 2θ of 38, 44, 65 and 77 are corresponding to the Bragg's reflections such as (111), (200), (220) and (311). Other peaks were also observed along with the main peaks. This may be due to the crude nature of the extracts which containing other metabolites and salts. These components would have reacted with the ionic gold during the synthesis reaction. These compounds might be reason for the formation of other peaks (Figure 7).

3.8 Antimicrobial studies

The antimicrobial activity assay is AuNPs sample was challenged against various NCIM and MTCC microbes using the disc diffusion method. The test concentrations (15 and 30 µL/disc) produce zone on MHA and PDA plates for bacteria and fungi, respectively. the sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4) in the bacterial division. But in fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Candida albicans* MTCC 1637 (F1). The higher (30µL/disc) concentration got larger zone effect than the small (15µL/disc) concentration against certain microorganisms (Table 1). All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. Koperuncholan (2015a)[20] reported the best antimicrobial activity of *piper nigrum*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes*. Several phytoconstituents such as terpenoids [21], flavonoids and tannins [22], are effective against certain microorganisms. The results of the present investigation clearly demonstrate the antibacterial and antifungal activities of the aqueous extracts of the leaves.

Table.1 Antimicrobial activity of the different solvent extracts of *Evolvulus alsinoides* leaves

S. No	Test Microorganisms		AuNPs µL/disc		PC	Diseases	Route of Transmission
	Bacteria		15	30	10 mcg		
1.	*Aeromonas liquefaciens*	B1	10	13	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis*	B2	14	17	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae*	B3	14	16	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus*	B4	13	18	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium*	B5	12	14	0	Typhoid	Water / Food
6.	*Vibrio cholarae*	B6	12	15	16	Cholera	Water / Food
	Fungi						
7.	*Candida albicans*	F1	11	13	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp.	F2	13	17	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis*	F3	12	13	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum*	F4	13	16	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC- Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc) Samples – 15, 30 mg/ml (well)

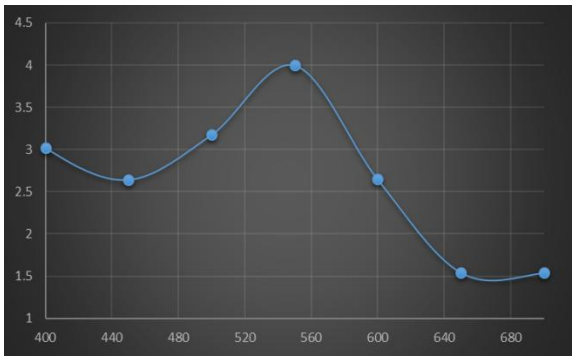

Figure 1: UV characterization of AuNPs

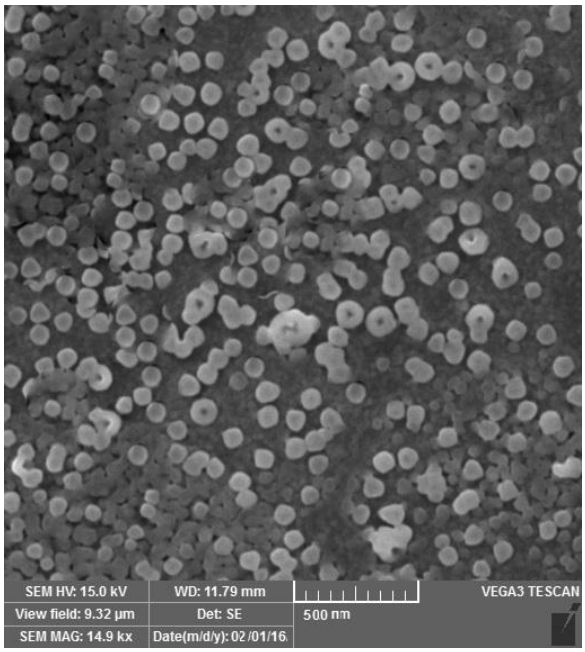

Figure 2: SEM characterization of AuNPs

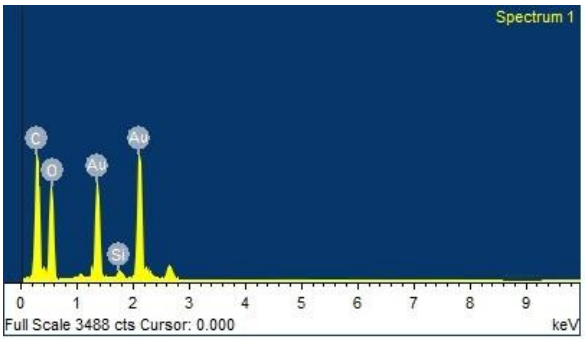

Figure 3: EDAX characterization of AuNPs

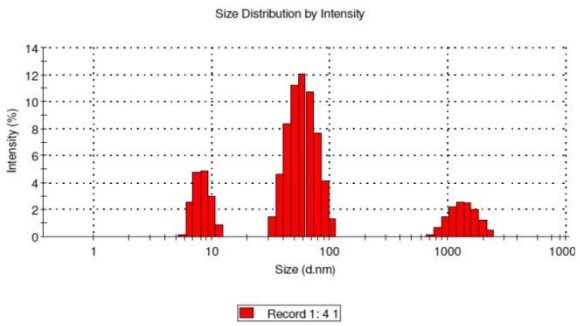

Figure.4 DLS Size distribution characterization of AuNPs

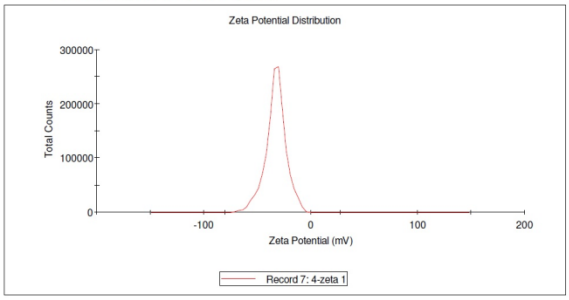

Figure 5: DLS Zeta potential characterization of AuNPs

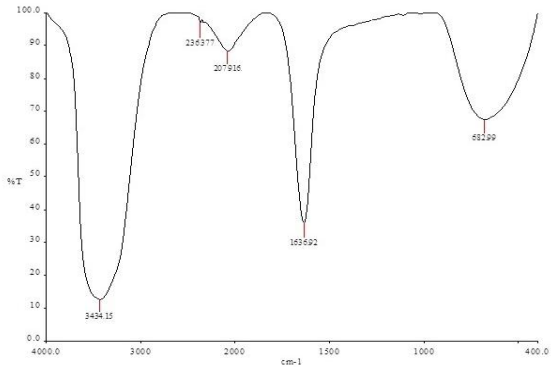

Figure 6: FTIR characterization of plant broth and AuNPs

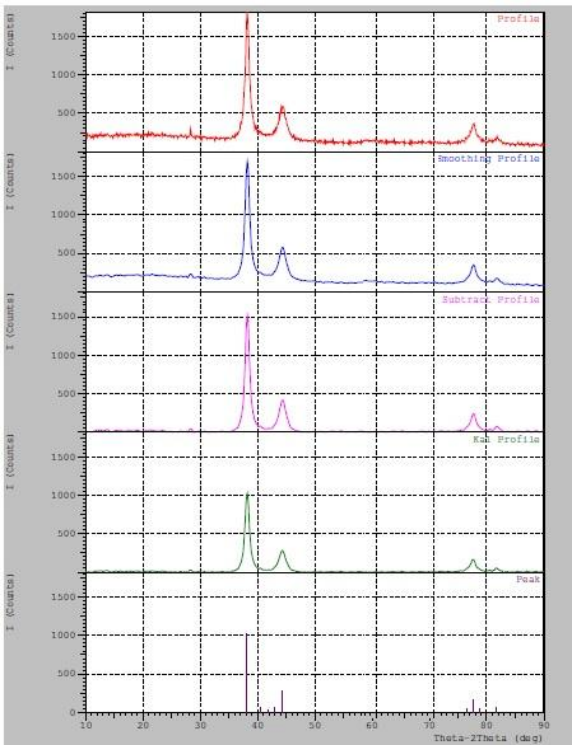

Figure 7: XRD characterization of AuNPs

4. Conclusion

We developed an eco-friendly, simple and efficient method for the synthesis of gold nanoparticles using leaf extract of *E. alsinoides*. The AuNPs are usually produced by the addition of a reducing agent to a solution of chloroaurate ions ($AuCl_4^-$), causing reduction of the gold ions and

aggregation of the Au atoms into AuNPs. Different organic compounds are usually added to form a protective layer on the surface of the AuNPs, thus preventing their aggregation into larger particles. The present study describes a "green chemistry" synthesis of AuNPs using environment-friendly reagents. The AuNPs showed good biocompatibility and good stability for over 4 weeks. Therefore, they can be used for imaging and drug-delivery applications in the human body.

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