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Green synthesis and characterization of gold nanoparticles using leaf extracts of *Withania somnifera* (Linn.) (Ashwagandha)

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Abstract: In this study, a novel technique for biosynthesizing of gold nanoparticles (GNPs) using *Withania somnifera* as reductants and stabilizers is reported. Biosynthesis of anisotropic gold nanoparticles using ethanolic extract of *Withania somnifera* leaf and their potential as IR blockers has been demonstrated. The phenolic groups (–OH) residue was identified as the active functional group for gold ion reduction. These gold nanoparticles were characterized by of UV–Vis spectrophotometer, FTIR, TEM. The presence of proteins was identified by FTIR, UV–Vis and fluorescence spectroscopy. The micrograph revealed the formation of anisotropic gold nanoaprticles. The characteristics of the nanoparticles formed suggest application of gold and gold nanoparticles as chemical sensors in the future. Given the simple and eco-friendly approach for synthesis, these nanoparticles could easily be commercialized for large scale production. The present research opens a new avenue for the green synthesis of nanomaterials.

Keywords: Green Synthesis, *Withania Somnifera*, Ethanolic Extract, Gold Nanoparticles

1. Introduction

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100nm. Tremendous growth in this emerging technology has opened novel fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reprography, single electron transistors, light emitters, nonlinear optical devices, and photo electrochemical applications [1-9]. Gold nanoparticles are of interest because of the unique properties (*e.g.,* size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibres, cryogenic superconducting materials, cosmetic products and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing gold nanoparticles [9-11]. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction and radiolysis are widely used for the synthesis of gold nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry).

Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. This article presents an overview of gold nanoparticle preparation by green synthesis approaches. Therefore, to reflect on the current state and future prospects, especially the potentials and limitations of the above mentioned techniques for industries [12].

Withania somnifera, is an erect, evergreen, perennial shrub which is a widely used medicinal plant considered as aphrodisiac and rejuvenating, anti-inflammatory and anti tumor agent [13]. It is widely used as an important drug in Ayurvedic formulations. The genus *Withania somnifera* belongs to the division Magnoliophyta, class Magnoliopsida, order Solanales and family Solanaceae [14-20]. It is best known classically for its rejuvenating properties and hence called “Indian Ginseng” [21-25]. Roots of *Withania somnifera* (Ashwagandha) reportedly exhibit antioxidant, immune modulatory and haematopoietic properties [26-30]. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans than those attributed to macronutrients and micronutrients [30-32].

In present study anisotropic gold nanoparticles were synthesized using *Withania somnifera* leaf ethanolic extract and demonstrated their potential in absorption of infrared rays. These green synthesized nanoparticles were examined by ultraviolet visible spectroscopy, transmission electron microscopy (TEM), energy dispersive X-ray analysis (EDAX), and Fourier transform infrared (FTIR) spectroscopy to determine their size and shape.

2. Materials and Methods

2.1. Plant Material Source

Withania somnifera leaf (Figure 1a) was procured from the local market and planted in earthen pots at Green house of Botanical Garden, Department of Botany, Berhampur University, Odisha, India.

2.2. Preparation of Crude Extracts

The air dried leaf and stem were milled to get a coarse powder. About 100g of dry powder was extracted with petroleum ether at room temperature using soxhlet apparatus for 8hrs or the extraction was continued until the liquid was clear (Figure 1b). The extracts were then filtered and concentrated to a dry mass under vacuum. The mark left after petroleum ether extraction was air dried and then extracted with solvents ethanol as done earlier and the extracts were similarly filtered and concentrated under vacuum.

2.3. Qualitative Chemical Evaluation

The different extracts thus obtained were qualitatively tested for the presence of various phytochemical constituents (Table 1) [26-30].

2.4. Synthesis of Gold Nanoparticles

100 ml of plant or leaf extract were collected in a 500 ml beaker which was kept on magnetic stirrer for stirring at 200 rpm at room temperature (Figure 1c), to this stirring solution 10 ml of 1mM auric acid solution was added drop wise. The ratio of extract and auric acid solution in above procedure is 10:1, similarly 5:1 and 1:1 ratios were followed for optimization of protocol [33] (Figure 1d).

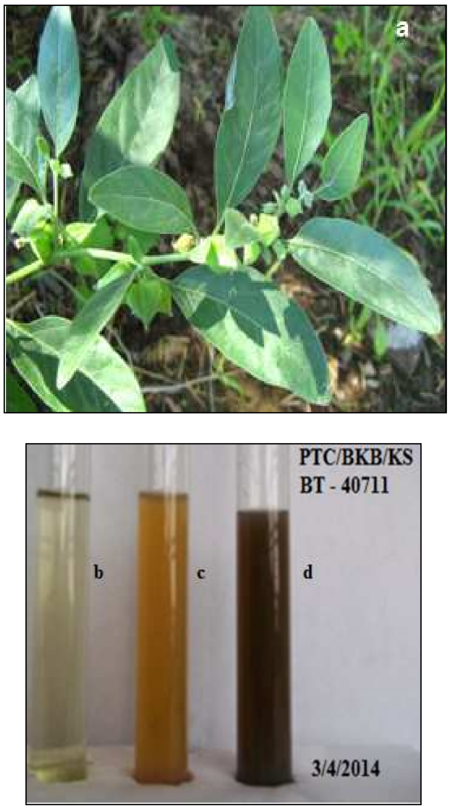

Figure 1. *Withania somnifera leaf (a), 1mM aqueous $HAuCl_4$ solution (b) leaf Extract (c) and AuNPs (d)*

Table 1. *Preliminary phytochemically analysis of various extract of Withania somnifera leaf extract.*

Sl. No.	Phytochemical Constituents	Ethanol Extraction
1	Flavonoids	+++
2	Alkaloids	+++
3	Steroids	++
4	Phenols	+++
5	Carboxylic Acids	+++
6	Xantho Proteins	++
7	Terpenoids	+++
8	Quinones	++

(++) =moderately present, (+++) =Appreciable amount

3. Measurements

3.1. UV-Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-1601 pc shimadzu spectrophotometer) or ultraviolet-visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region.

3.2. Fourier Transmission Infra Red Spectroscopy (FTIR)

The FTIR spectrum of *Withania somnifera* L. extract, gold nanoparticles and amine functionalized gold nanoparticles were recorded using were obtained using a BIORAD-FTS-7PC type FTIR spectrophotometer.

3.3. EDAX Spectroscopy

Energy-dispersive X-ray spectroscopes exploit the photon nature of light. In the X-ray range, the energy of a single photon is just sufficient to produce a measurable X-ray voltage pulse, and the output of an ultralow noise preamplifier connected to the low noise constitutes a statistical measure of the corresponding quantum energy. By digitally recording and counting a large number of such pulses within a so-called multichannel analyzer, a complete image of the X-ray spectrum is built up almost simultaneously.

3.4. Transmission Electron Microscope (TEM) Analysis

Transmission electron microscope (TEM) (Philips CM-10) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image was formed from the interaction of the electrons transmitted through the specimen; the image was magnified and focused onto an imaging device.

3.5. Statistical Analysis

All values are expressed as means ± standard deviation. The results were analyzed using one-way analysis of variance (ANOVA), and the differences among the formulation means were analysed using the Tukey–Kramer multiple-comparison test. The software Graph Pad in Stat was employed for the statistical analysis.

4. Results and Discussions

4.1. UV–Vis Spectrophotometry

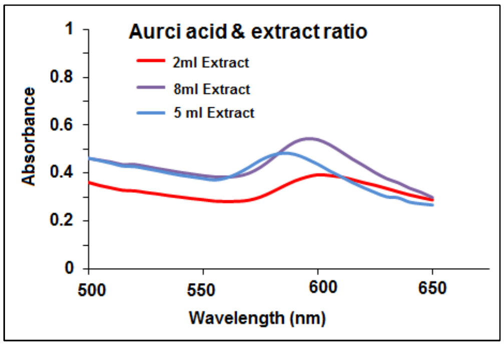

Figure 2. *UV-Visible absorption spectrum of gold nanoparticles of Withania somnifera L. leaf extract.*

Gold nanoparticles were characterized by UV visible spectrophotometer. Figure 2 shows the GNP formation using a constant $HAuCl_4$ concentration (1.3 X 10^4 M) with different concentrations of the extract. Photographs of figure-1 shows the color change of the gold nanoparticles with a changing *Withania somnifera* leaf extract concentration. The violet, pink to dark pink color observed is characteristic for the Surface Plasmon Resonance (SPR) of different sizes of gold nanoparticles [34]. The leaf extract quantities were varied from 2ml to 10ml, particularly in the range of low amounts of the leaf extract (2–5 ml in 10 ml metal ion solution), the absorption spectra exhibit a gradual increase of the absorbance accompanied with a shift in the k_{max} from 545 to 530 nm.

A strong correlation between the particle size and the maximum absorption peak has been previously observed [35]. They observed that as the particle size increases, the maximum absorption is red shifted. Accordingly, it can be concluded that the size of the particles decreases upon increasing the extract concentration from 0.2 to 2 ml as the absorption spectra are blue shifted [33]. The slight red shift in λ_{max} accompanied with a slight increase in the absorbance values at longer wavelengths (600nm) with an increase in the extract concentration from 3 to 6 ml is consistent with previously reported results for surfactant [34].

4.2. FTIR

The nature of amino acid residue in the plant protein responsible for gold ion reduction revealed by FTIR and fluorescence analysis of protein structure before and after reaction with metal ions is shown in Figure 3. The FTIR spectrum (Figure 3a) showed the bands at the interaction of nanoparticles with phytochemicals of *Withania somnifera* showed intense peaks at 2883, 1602, 1504, 1383, 1071, and 1333cm^{-1} relative shift in position and intensity distribution were confirmed with FTIR. The FTIR recorded (Figure 3b) for the dry *Withania somnifera* powder showed strong bands at 2831, 1733, 1611, and 1402 cm^{-1}. Comparing the FTIR spectra, it can be seen that for changes in the –COOH group for –OH, *i.e.*, the hydroxyl group, the peak appeared at 3314cm^{-1} in the raw material, but after encapsulation of nanoparticles, the peak was narrower and shifted to 3633cm^{-1} and the peak intensity reduced also for the –C– of the carboxylic group after encapsulation of nanoparticles. H bonds can be formed between the amide groups. This implies that the –COOH group in the compound was attached to the gold nanoparticles and there is a clear change in the spectra. After encapsulation, the 1732cm^{-1} in –C– bond stretching becomes masked or disappears [35, 36].

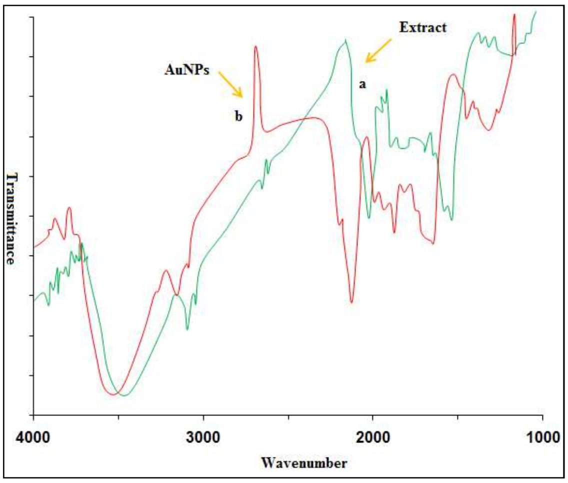

Figure 3. *FTIR spectrum of plant protein from the plant extract (a) and reaction with gold ions (b).*

4.3. EDAX

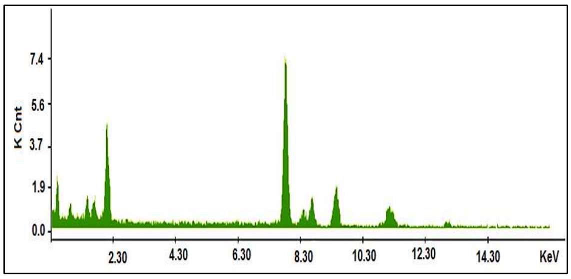

Figure 4. *EDAX profile of synthesized Gold nanoparticles.*

The green synthesized gold nanoparticles were investigated further using EDAX spectrometry, which confirmed the presence of gold with no contaminants. The optical adsorption peak was observed at approximately 2.30 keV (Figure 4), which is typical of adsorption of gold nanocrystallites due to surface plasmon resonance. The EDAX profile for gold nanoparticles synthesized using *Withania somnifera* showed strong gold atom signals at around 2.30, 8.10, 9.40, and 11.30 keV. EDAX for the gold nanoparticles showed strong signals for gold atoms and weaker signals for carbon, oxygen, and chloride, which were provenient from biomolecules of *Withania somnifera*, and the strong signals obtained at around 2.30 keV and 9 keV were similar to those in our earlier studies [37, 38].

4.4. TEM

This result was confirmed by TEM measurements at pH 3.5 and 9.6 (Figure 5). The sizes of the particles at pH3.5 and pH9.6 were larger while forming hexagonal structures. Furthermore, the particles formed in acidic medium were unstable and precipitated within 12h [39-46].

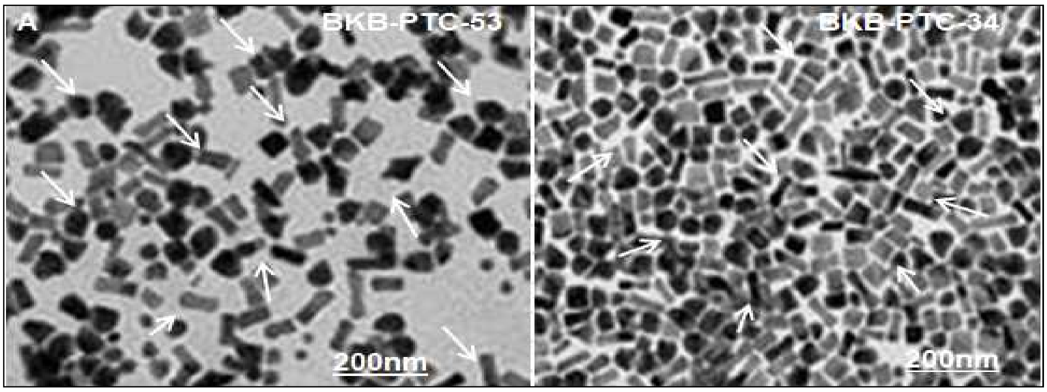

Figure 5. *TEM images of AuNPs at (A) pH 3.5 and (B) pH 9.6.*

5. Conclusion

The present study demonstrates bioreductive synthesis of nanosized gold particles using *Withania somnifera* ethanol leaf extract appears to be environmentally friendly, so that this protocol could be used for rapid production of gold nanoparticles. The size of the nanoparticles can be easily adjusted by using different amounts of leaf extract. The high phenolic content of the hot water extract of olive leaves having strong anti-oxidant properties helped in the reduction of gold cations to AuNPs. The characterization of AuNPs revealed that the morphology of the AuNPs depends on the extract concentration and pH of the used medium. At higher concentration of the extract and basic pH, the pseudo-spherical particles are capped by phytochemicals. In the future, selection of such plants may create a new platform for realizing the potential of herbal medicines in nanoscience for drug delivery and biomedical application.

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