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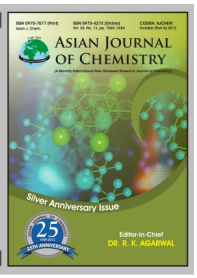


# Green Synthesis, Characterization and *in vitro* Antibacterial Studies of Gold Nanoparticles by Using *Senna siamea* Plant Seed Aqueous Extract at Ambient Conditions

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Biosynthesis of gold nanoparticles was achieved using chloroauric acid by aqueous extract of *Senna siamea* plant seed. Here, we reported the eco-friendly method for the preparation of gold nanoparticles in the presence of water, using aqueous extract of *Senna siamea* plant seed at room temperature. The aqueous extract of plant seed acts as a reducing and capping agents. The nanoparticle formation was confirmed by UV-visible spectroscopy and TEM images. The obtained gold nanoparticle was of triangular and hexagonal in shape. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the gold nanoparticles. The crystalline property was confirmed by X-Ray diffraction analysis. It is also verified that the efficacy of gold nanoparticles as a potential antibacterial agent and results obtained were well supported to our quest. Rapid and green synthesis methods using biological extracts have shown a great potential in nanoparticles synthesis protocols.

**Key Words: *Senna siamea*, Seed aqueous extract, Green synthesis, Gold nanoparticles, Antibacterial studies.**

## INTRODUCTION

Nanometer sized particles has the capability of exhibiting excellent catalytic activity and its abundance is preferable, though metal being a poor catalyst for bulk. Due to its relative high surface area to volume ratio, wider band gap between the valence and conduction band when being divided to near atomic size and their interface dimensional properties which significantly differ from those of the bulk material. Nanoparticles are particles with at least 1 dimension less than 100 nm in size[1]. Owing to its vast growth in engineering, biotechnology and agriculture fields, it is necessary to study its chemical and physical properties and characterize these nanomaterials according to them. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles. Metal nanoparticles have highly specific surface area because of their unique physico-chemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties. Synthesis of noble nanoparticles for the applications such as electronics, environmental and biotechnology is an area of constant interest[2].

There is growing need to develop environmentally benign metal nanoparticle synthesis process that do not use toxic chemicals in the synthesis protocols to avoids adverse effects in biological application. Compared with traditional chemical synthesis, biomolecule assisted synthesis of nobel metal nanomaterials have a number of advantages. Since biomolecule assisted synthesis carried out in a room temperature under aqueous conditions, energy input is reduced and the aqueous extracts used are nontoxic. These factors minimize environmental damage and enhance human health. In this method there is no need to use high pressure, energy, temperature and eliminates elaborate process of maintaining cell cultures. Surface complexation of nano gold with amino acids and proteins is an emerging field of research. A number of living organisms are already well-known to elaborate nano structured composites such as cyanobacteria, bacteria, fungi, actinomycetes, biomolecules and various plant materials such as *Cinnamomum camphora*[3], *Medicago sativa*[4-6], *Pelargonium graveolens*[7], *Avena sativa*[8], *Azardirachta indica*[9], *Tamarindus india*[10], *Emblica offcinalis*[11], *Aloe vera*[12], *Coriandrum sativum*[13], *Carica papaya*[14], *Parthenium hysterophorus*[15], *Tritium vulgare*[16], *Acanthella elongata*[17], *Sesuvivm potulacastrum*[18] and gold nanoparticles also synthesizedby biomolecules like honey[19]. Gold nanoparticles do not blink nor bleach and their non-toxicity and biocompatibility make this attractive for biological applications[20,21]. There has been a considerable effort to investigate their size and shape dependent optical properties, both experimentally[22,23] and theoretically[24,25]. Their scattering and absorption properties have been widely investigated and characterized, which has led to many

new applications[26]. In recent studies, the photoluminescence from single gold nanoparticles has proven to be a complementary property to absorption and scattering for imaging and sensing purposes[27-31]. This present investigation deals with aqueous extract of *Senna siamea* seed mediated synthesis, characterization and antibacterial study of gold nanoparticles.

## EXPERIMENTAL

All analytical reagents and media components were purchased from Hi-Media (Mumbai, India).

**Preparation of aqueous extract of *Senna siamea* seed:** Collected *Senna siamea* plant seeds shown in Fig. 1.were washed several times with deionized water. Finely crushed seeds (50 g) was boiled for 10 min at 70 ºC in 300 mL water and filtered. The filtrate is cooled to room temperature and used as reducing agent and stabilizer. The extract obtained was filtered using Whatman filter paper no.1 and maintained at 4 ºC for further studies.

Fig. 1. *Senna siamea* plant

**Biosynthesis of gold nanoparticles:** The stock solution ($2 \times 10^{-3}$ M) of chloroauric acid was prepared by using sterile deionized triple distilled water and the subsequent dilutions were made from this stock solution. A series of volume (2-10 mL) of plant seed extract was added to 5 mL of $2 \times 10^{-3}$ M aqueous $HAuCl_4$ solution and water being added to acquire a volume of 15 mL. After 3 h, the light yellow colour of $HAuCl_4$ solution turned to pink colour indicates the formation of gold nanoparticles, which remain stable for more than 2 months with out any changes in the absorption spectrum (Fig. 2).

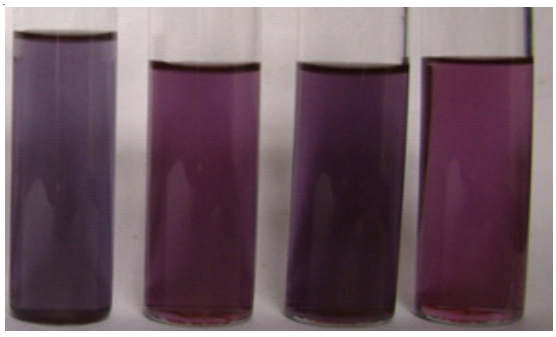

Fig. 2. Aqueous solution of 2X $10^{-3}$ M gold with aqueous extract of *Senna siamea* seed and different stages with colours of synthesized gold nanoparticles

**Antibacterial Studies (Agar well diffusion method):** The stock solutions of different concentrations were prepared. The plant seed extract and gold nanoparticles from the stock solution of concentrations 50, 100 and 200 µL were immediately dispensed into each agar wells of culture inoculated in muller hinton agar (MHA) plates using sterilized micropipette. Bacterial pathogens were obtained as follows: Two-gram positive (*Staphylcoccus aureus* and *Bacillus subtilis*) and three gram negative *(Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*) were used.

**Ultraviolet-visible Spectroscopy analysis:** UV-visible spectroscopy measurement was carried out on JASCO V 550 spectrophotometer operated at a resolution of 1 nm.

**Fourier transform-infrared spectra analysis:** Identification of possible biomolecules responsible for the reduction and stabilization of gold nanoparticles were recorded using ABB MB3000 FT-IR spectrophotometer.

**X-Ray Diffraction analysis:** XRD measurement was carried out on a Philips PW 1830 instrument operating at a voltage of 40 KV and at a current of 30 mA with $CuK_\alpha$ radiation.

**Transmission electron microscopy analysis:** A drop of the solution nanoparticles is placed on copper-grid pre-coated formvar film and the solvent was evaporated under vacuum. The Grids were observed under Transmission electron microscope.

## RESULTS AND DISCUSSION

UV-visible spectroscopy is one of the important techniques to ascertain the formation and stability of metal nanoparticles in aqueous solution[32]. The gold nanoparticles obtained at constant concentration of $HAuCl_4$ solutions with different amount of aqueous extracts of plant seed [2 mL, 4 mL, 6 mL, 8 mL, 10 mL] changes from pale yellow to violet colour. This change in colour represents the characteristic of surface plasmon resonance (SPR) of different size of gold nanoparticle in solution. Fig. 3 shows UV-visible spectrum of gold nanoparticle formation at constant concentration of $HAuCl_4$ solution with 6 mL of aqueous extract of *Senna siamea* seed. It is worth while mentioning that $HAuCl_4$ can be completely reduced to form gold nanoparticles. The colour changed and synthesized nanoparticles were confirmed by UV-visible spectroscopy at 543 nm. Nanoparticles were priced to form gold nanoparticles, which is confirmed by the fact that there is no further change of UV-visible spectrum after aqueous extract of *Senna siamea* seed is introduced into as-prepared gold solution.

There are three sets of spots could be identified from this diffraction pattern. The inner set with weak intensity was caused by reflections from the 1/3 {4 2 2} planes (triangle), the set with strong intensity could be indexed to the {2 0 0} planes (square) of fcc gold and the outer set with weak intensity is the reflection from {4 2 2} plane (hexagonal). These results are closer to the reported standard data (JCPDS File No. 893697).

X-ray diffraction analysis shows that a number of prominent Bragg reflections were present which may be indexed on the basis of fcc structure of gold nanoparticles. Moreover, the

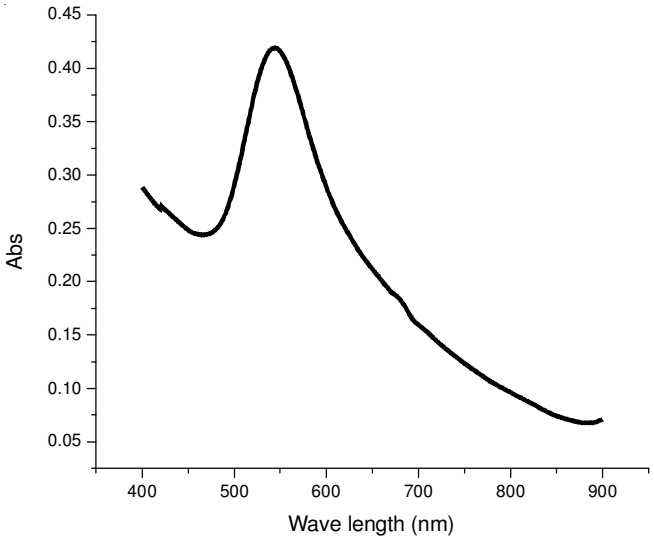


Fig. 3 UV Spectrum of Gold nanoparticles

intensities of the (1 1 1), (2 0 0) and (2 2 0) diffraction were much stronger than those of the (2 2 0) diffractions. The XRD pattern thus clearly shows that the gold nanoparticles were essentially crystalline and the results were consistent with previous results. Fig. 4 Represent the XRD pattern of gold nanoparticle, the additional peak indicates the biomass of protein present in aqueous extract of *Senna siamea* plant seed.

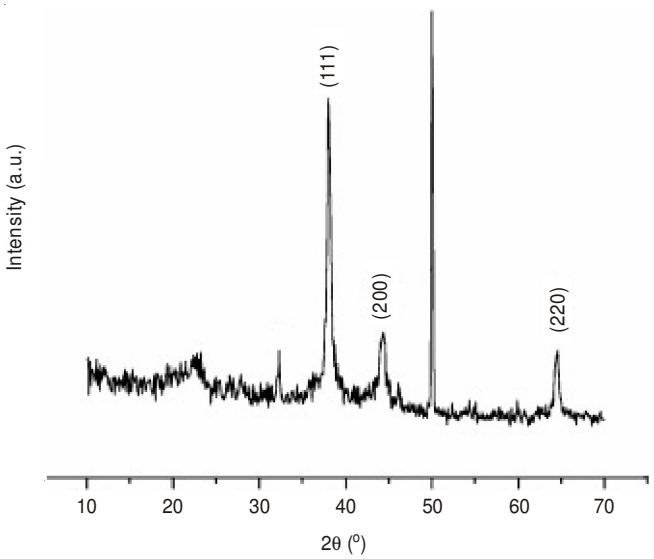


Fig. 4. X-ray diffraction pattern of gold nanoparticles

The morphology and size of as-formed gold nanoparticles were further determined by transmission electron microscopy analysis and the TEM image shows (Fig. 5a) that the nearly triangular shaped particles are more abundant than the hexagonal shapes. The resultant histogram represents that the size distribution of the particles sizes with almost 50 to 70 nm range and a very small percentage of nanoparticles exist in the long range of 100 nm. This may be due to growth at unusual facets which results in the formation of anisotropic (non-spherical) nanoparticles. Agglomeration in the nanoparticles can be seen due to the high temperature which results in the destruction of the stabilizing protein. Where as selected-area electron diffraction (SAED) pattern of one of the gold nanotriangle, which clearly shows (Fig. 5b) that it is single crystalline. The pattern was obtained by aligning the electron beam perpendicular to the triangular facet of the nanoplate. The hexagonal symmetry of the diffracted spots suggests that the single crystalline nature of gold nanotriangle lying flat on the tem grid[33].

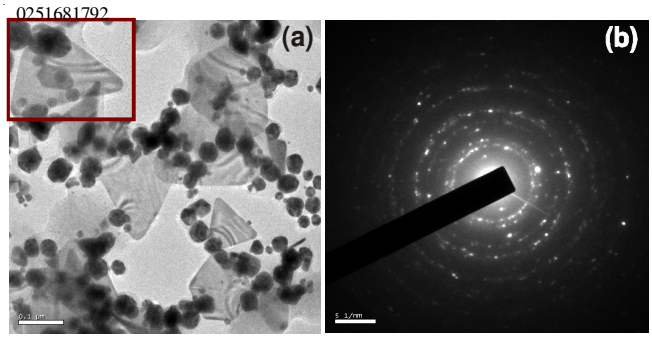


Fig. 5. (a) Transmission electron microscope images gold nanoparticles; (b) SAED pattern of gold nanoparticles

FT-IR measurements were carried out to identify the possible interactions between gold metal and the *Senna siamea* plant seed aqueous extract. The strong broad peak at 3445.7 $m^{-1}$ is characteristic of the O-H stretching vibrations[14]. The changes in the transmittances related to the bond with N atoms indicate that these atoms are the binding sites for the gold nanoparticles on the protein. The broad peaks show the hydrogen bonding but after the reaction, the nanoparticles bind to the N atoms, so the hydrogen bonding is absent. The most useful IR band for the direct measurement of secondary structure of protein is a broad band found 1639 $cm^{-1}$ [15]. This indicated that the secondary structure of the proteins is affected as a consequence of reaction with the binding with the nanoparticles. Similar changes are also observed in the case of gold nanoparticles indicating a strong bonding between them and the amide of the protein (Fig. 6). Further the nanoparticles synthesis by green route is found highly toxic against human pathogenic bacteria at different concentrations. This may be due to capping agent bio molecule which is present in the aqueous extract of *Senna siamea* plant seed.

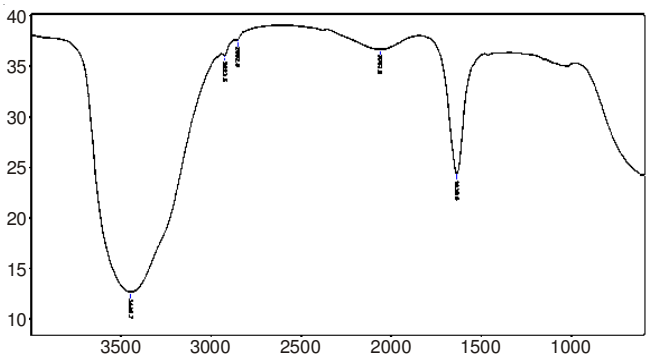

Fig. 6. FTIR spectrum of gold nanoparticles synthesized by using aqueous extract of *Senna siamea* seed

Optimal concentration that is a concentration which demonstrates a five log reduction in the test conditions were 50, 100 and 200 µL of gold nanoparticles for *Staphylococcus*

*aureus*, *Pseudomonas aeuroginosa* and *Bacillus subtilis* respectively. Among them, gram negative *Klebsiella pneumoniae* with 21 mm inhibition zone shows the highly sensitive to gold nano particles and subsequently *Bacillus subtilis* (18 mm), *Eschrichia coli* (15 mm), *Pseudomonas aeruginosa* (15 mm) and *Staphylococcus aureus* (13 mm) (Fig. 7). In the present study, although higher concentration (200 µL) of gold nanoparticles seems to decrease the number of *Bacillus subtilis*. It showed the slight negative effect on antibacterial activity of gold nanoparticles. Thus gold nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. For these gold nanoparticles, we also checked antifungal activity and obtained results were not encouraged. Further studies are required for gold nanoparticles in addition to use as antibacterial agent against drug resist organisms. Thus this study has added a new biological source for nanoparticles synthesis with clearly substantiated antibacterial nature.

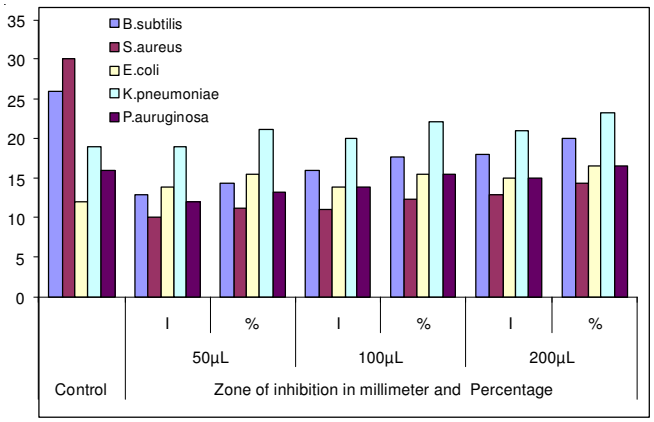


Fig. 7. *in vitro* antibacterial activity of gold nanoparticles against to human bacterial pathogens

## Conclusion

In this study, we have shown the feasibility of forming gold nanoparticles at the room temperature by using an *Senna siamea* plant seed aqueous extract, in which this plant seed aqueous extract simultaneously act as a reducing and capping agent for the formation of well dispersed nanoparticles. And the obtained nanoparticles were stable up to two months without any observable changes. Achievement of such a green synthesis of gold nanoparticles, contributes to a raise in the efficiency of synthetic procedures using environmentally benign natural resources. Furthermore the low cost of the method as well as its simplicity and efficiency offers an alternative to chemical synthetic methods of gold nanoparticles. Applications 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 (nanomaterials). Toxicity studies of *Senna siamea* mediated synthesized gold nanoparticles are under investigation.

## ACKNOWLEDGEMENTS

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