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BIOGENIC SYNTHESIS OF GOLD NANOPARTICLES (AUNPS) USING *CROCUS SATIVUS* AND *BERBERIS LYCIUM* EXTRACTS AND THEIR CHARACTERIZATION

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ABSTRACT

Biogenic synthesis, so-called Green synthesis is a promising tool for medicines and nanotechnology. In Biogenic synthesis we used natural reducing agents to synthesize nanoparticles. In the present studies gold nanoparticles (AuNPs) were synthesized by reducing gold chloride (Chloroauric acid) with the help of leaf and flower extracts of *Berberis lycium* and *Crocus sativus* respectively. Both the plant's extracts were highly effective to reduce Chloroauric acid into gold nanoparticles. This approach is simple, economic, stable and is synthesized in an eco-friendly mode to obtain a self-assembly of AuNPs. The nanoparticles obtained are characterized by UV–vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD). Antibiotic resistance is the world's major public healthcare problem. AuNPs particles play a vital role in nanobiotechnology as biomedicine against Drug-resistant bacteria. Several classes of antimicrobial nanoparticles (NPs) and nanosized carriers for antibiotics delivery have proved their effectiveness as alternative agents to combat the antimicrobial resistance experienced with conventional drugs. The Gold nanoparticals have many applications in biology and medicine due to their unique optical and physical properties. These versatile reagents can be used for biosensor development, as cellular probes, as drug delivery vehicles, or as optical contrast agents among others. The present research opens a new avenue for the green synthesis of nanomaterials.

KEYWORDS: Biogenic synthesis, Green synthesis, nanotechnology, *Berberis lycium, Crocus sativus,* nanoparticles.

INTRODUCTION

The nanoparticles (NPs) have engrossed extensive attention for their unusual chemical and physical properties such that they show great potential applications in biotechnology, catalysis, medical imaging, novel electronics, and optics. Due to their unique and tunable surface Plasmon resonance and their applications in biomedical science including drug delivery, tissue/tumor imaging, photothermal therapy and immuno-chromatographic identification of pathogens in clinical specimens, gold nanoparticles have been considered as an important field of research.

Metal nanoparticles (NPs) have attracted great attention for their unusual chemical and physical properties such that they show great potential applications in biotechnology, catalysis, medical imaging, novel electronics, and optics. The term nanotechnology was invented by Professor Norio Taniguchi at the University of Tokyo in 1971. Gold nanoparticles have been considered as an important area of research due to their unique and tunable surface Plasmon resonance (SPR) and their applications in biomedical science including drug delivery, tissue/tumor imaging, photothermal therapy and immuno-chromatographic identification of pathogens in clinical specimens.). Many methods have been developed for synthesizing metal NPs, especially gold nanoparticles (AuNPs). Recently there is an ever-growing need to develop clean, non-toxic and eco-friendly synthetic procedures and researchers have been looking at biological systems for inspiration. From the green chemistry approach, it has been concluded that the biosynthesized gold nanoparticles are biocompatible, inert, bind readily to a large range of bio-molecules such as proteins/enzymes, DNA, aminoacids and expose large surface area for the immobilization ion of such bio molecules and environmentally friendly chemicals. There are several reports on biological methods of nanoparticles synthesis using microorganisms, which have been exploited for toxic metals recovery via reduction of the metal ions. There have been numerous reports on the synthesis of gold NPs using plant materials as environment-friendly substitutes to chemical and physical methods. The work reported on the microorganisms and plants in the synthesis of Au nanoparticles has been reviewed by Plants have been proven very efficient and alternative for the synthesis of nanoparticles in the past few decades. Metal nanoparticles are synthesized by phytochemicals present in plants, which work as reducing and capping agents. For the synthesis of gold, silver and gold-silver-copper alloy nanoparticles, plants have been exploited to a greater extent.

Berberis lycium is considered to be an important medicinal plant in practice of herbal medicine. The main therapeutic features described for this plant include, its roots that are used as remedy for swollen and sore eyes, healing of broken bones internal injuries, gonorrhea, curative piles, unhealthy ulcers, acute conjunctive and in chronic ophthalmic. It is also used as bitter tonic astringent, diaphoretic and febrifuge traditionally. *Berberis lycium* belongs to family *Berberidaceae*. It is a semi-deciduous shrub, 2 to 4 meter high, leaves are lanceolate or narrowly obovate-oblong, entire or with a few large spinous teeth, arranged alternately on the stem. Inflorescence a racemes, flowers yellow born in auxiliary clusters longer than the leaves. Fruit is black in color and called berries. It is found in Pakistan, India, Kashmir, Bhutan, China, Japan, Nepal, United Kingdom, Ireland, and Turkey and distributed in temperate and sub tropic parts of Asia, Europe and America.

Crocus sativus in continuation of the efforts for synthesizing gold nanoparticles by green route, if acile, rapid and single-potaqueous biosynthesis of gold nanoparticles using the extract of *Crocus sativus* (saffron) has been also reported here. Saffron contains more than 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components, many of which are carotenoids, including zeaxanthin, lycopene and various a- and b-carotenes.

In the present investigation, the synthesis of gold nanoparticles emphasizes the potentiality of *Crocus sativus* (saffron) and *Berberis lycium* in reducing and stabilizing gold nanoparticles.

MATERIALS AND METHODS

In the present study gold nanoparticles AuNPs were synthesized using *Crocus sativus* and *Berberis lycium* extracts. The present study was undertaken to elucidate; physiochemical composition of two medical herb extracts, synthesis of Gold nanoparticles and their characterization. The plant's *Crocus sativus* and *Berberis lycium* were separately collected from the local surroundings of Pulwama and Ganderbal district of Kashmir, during the month of November and June.

Synthesis

Chloroauric acid ($HAuCl_4$) was procured from Sigma-Aldrich Chemicals, India and used as received. All other reagents were of critical grade with maximum purity. Before using all glassware 's have been washed with distilled water and dried in an oven. The plant's *Crocus sativus* and *Berberis lycium* were separately collected from the local surroundings of Pulwama and Ganderbal district of Kashmir, during the month of November and June. For the synthesis of gold nanoparticles AuNPs aqueous extracts of Crocus sativus and *Berberis lycium* were prepared. 20 mg semi-solid extracts of both plants, already prepared, were separately dissolved in 100 ml of double distilled water in separate beakers and were boiled for complete dissolution. The aqueous extracts so obtained were filtered through Whatman filter paper No.1. The extracts were collected in separate flasks and stored at -4oC for further use. Chloroauric acid (HAuCl4) was procured from Sigma- Aldrich Chemicals, India and used as received. 0.5mM solution was prepared and was stored at the same temperature as mentioned above. Synthesis of gold nanoparticles AuNPs is quite a simple step in which 10 ml of aqueous plant extract was added to 90 ml of Chloroauric acid solution (0.5 mM) in a beaker. The beaker was kept on a magnetic stirrer. After a few minutes the color of the solution changed. Change in color preliminary confirmed the synthesis of gold nanoparticles AuNPs. In this study a simple and rapid green synthesis of AuNPs using an extract of *Crocus sativus* (saffron) and *Berberis lycium* has been reported. For the synthesis of gold nanoparticles, the effect of extract quantity and concentration of the metal ion solution were also evaluated to optimize the synthesis route producing the metal nanoparticles. The effect of extract concentration in the mixed solution on the biosynthesis of gold nanoparticles was first investigated. The different concentrations of extract in the mixed solution were obtained by changing the volume of the added extract solution. Different volumes (0.1–5 ml) of the extract solution were added to the $HAuCl_4$ solution of different concentrations. The concentration of extract and metal ion solution to be used was optimized to 4 ml and 0.5 mM, respectively. Gold nanoparticles can be prepared and the stability of particles was evaluated without any additional stabilizing chemicals/and or physical steps.

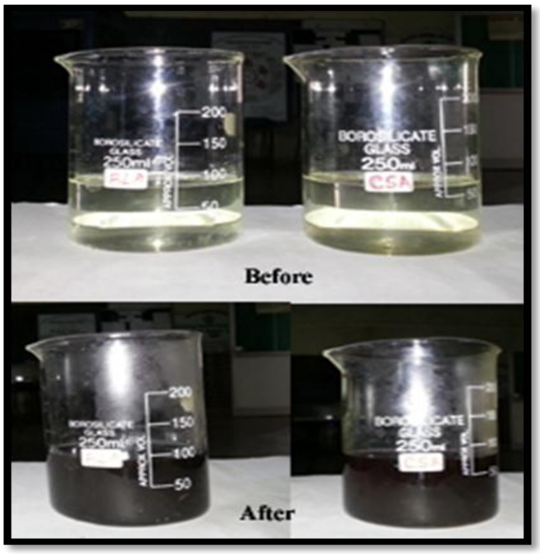


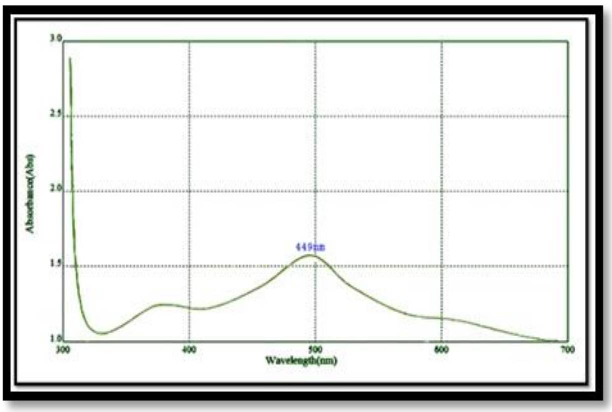
Fig. 1: Chloroauric acid before addition of Plant extract and after addition of plant extract.

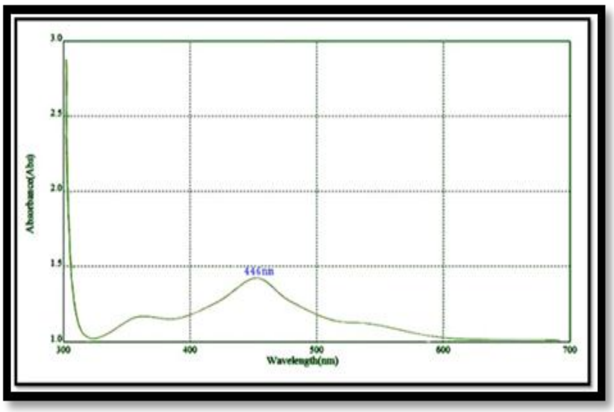
Characterization of Gold nanoparticles AuNPs

The optical property of AuNPs was determined by double beam UV-vis spectrophotometer (UV-vis Analyst- 0001, Electronic India). After the addition of $HAuCl_4.3H_2O$ to the respective plant extracts, the spectra were taken after 24 Hrs between 300 to 700 nm using double distilled water as blank and the final graph was obtained. The samples were prepared by dispersing the gold nanoparticles AuNPs of *Crocus sativus* and *Berberis lycium* uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard to analyze the samples and were characterized in the range 4000-400 cm-1 and the percentage transmittance was recorded. The spectrum obtained was compared with the standard infra red spectroscopy data. Important peak values determined the necessary functional group involved in the synthesis, capping, and stabilization of gold nanoparticles AuNPs. The gold nanoparticles AuNPs were studied by X-ray diffractometer (RIGAKU – JAPAN MINIFLEX) equipped with CuKα radiation (0.154 nm) operated at a voltage of 30 kV and 10 mA current. The scanning was done in the region of 20o to 80o for 2θ at 0.04o/min scanning speed. The diffraction patterns of synthesized gold nanoparticles AuNPs were obtained with the help of Integrated X-ray powder diffraction software (PDXL). The synthesized gold nanoparticles AuNPs were characterized for their morphological features, shape, and size with the help of scanning electron microscope.

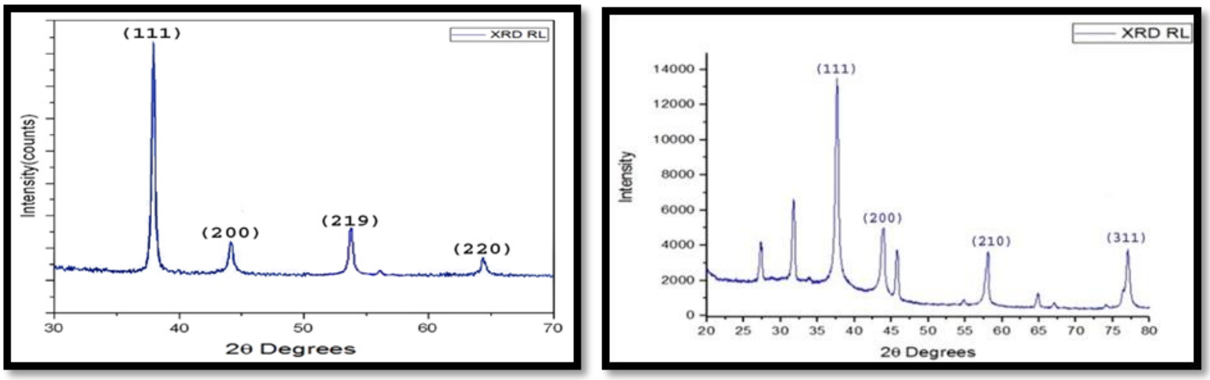
RESULTS AND DISCUSSION

In this paper, we report on the use of *Crocus sativus* and *Berberis lycium* extract for the synthesis of gold nanoparticles (AuNPs) from aqueous $HAuCl_4$ solution. The primary color of the solution mixture of extract and aqueous $HAuCl_4$ was colorless. The intensity of the color of reaction mixture changes to dark purple and dark brown respectively. This shows that the reaction medium behaves with time kinetics; the same is shown in Fig. 1. The color change in the solution indicates the formation of AuNPs. This observation shows that the reduction of Au ions takes place in the solution due to the addition of extracts. Synthesized gold nanoparticles were then confirmed by UV-Vis spectroscopy. The gold nanoparticles show characteristic absorption peak between 400 nm to 600 nm depending upon the size, shape, and morphology of gold nanoparticles. The synthesized AuNPs using *Crocus sativus* extract, showed surface plasmon peak value at 549 nm (Fig. 3) while that of *Berberis lyceum* extract AuNPs, when subjected to spectroscopic analysis, showed surface plasmon resonance value at 436 nm (Fig. 4). From UV-vis spectroscopy, the formation of gold nanoparticles was confirmed. The infra red analysis of *Crocus sativus* AuNPs revealed two important peaks of 2918.4 Cm-1 and 1339.61 Cm-1 which corresponded to Alkyl C-H stretch and Aromatic C=C Bending respectively. The gold nanoparticle solution of *Berberis lyceum* showed a wide number of peak values, each corresponds to a particular type of functional group and stretch or bend, which is linked to the type of compound present. A peak of 2956.1 Cm-1 indicated C-H stretch of alkanes. Results also revealed 2919.36 Cm-1 peaks indicated carboxylic acid OH stretch. A 1518.03 Cm-1 and 721.4 Cm-1 peaks indicated aromatic C=C bending and aromatic C–H bending respectively. The X-ray diffraction pattern of our samples was indexed with standard XRD spectrum of Joint Committee on Powder Diffraction Standards (JCPDS Card No. 53-61385) and it confirmed the formation of gold nanocrystals. The intense diffraction peaks due to AuNPs are clearly observed at (111), (200), (219) and (220) reflections of the centered cubic lattice structure of metallic gold. Similarly, four important 2ϴ angle peaks were observed for Berberis lyceum AuNPs, corresponding to (111), (200), (210) and (311), which correspond to face-centered cubic lattice of the gold nanocrystals.

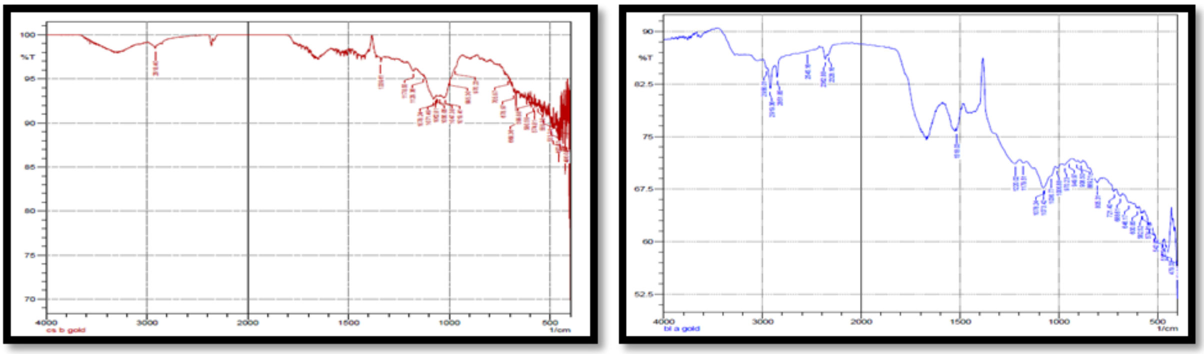




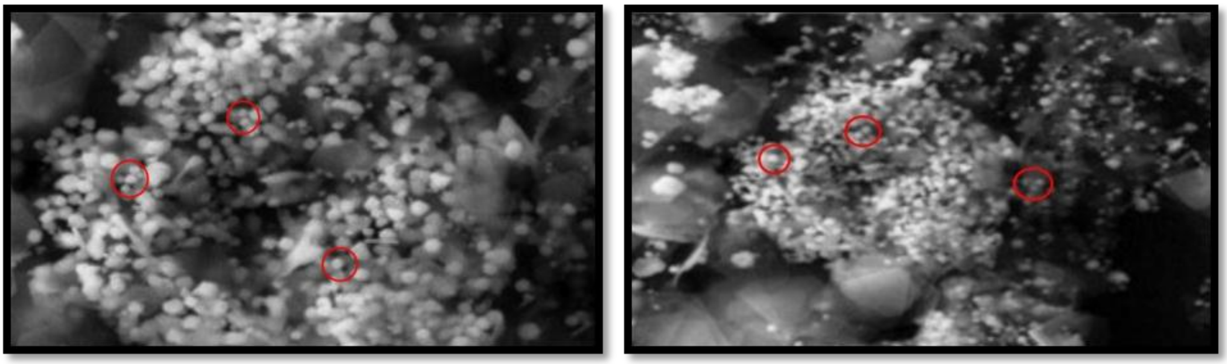
UV-vis spectrophotometeric analysis of (a) Crocus sativus and (b) Berberis lycium gold nanoparticle.



X-ray diffraction pattern of (a) Crocus sativus and (b) Berberis lycium gold nanoparticle.



Infra red spectroscopic analysis of (a) Crocus sativus and (b) Berberis lycium gold nanoparticle.



SEM analysis of Crocus sativus and Berberis lycium gold nanoparticles showing spherical shape of AuNPs.

CONCLUSION

The biosynthesis of gold nanoparticles using *Crocus sativus* and *Berberis lycium* has been reported in this study. It has been demonstrated that the *Crocus sativus* and *Berberis lycium* extracts are capable of producing fairly mono-dispersed spherical and triangular gold nanoparticles with well-defined dimensions with the size of 11–20 nm. Only 24 h of reaction time is required for conversion of almost all gold ions to gold nanoparticles at the room temperature. Using the clean-green chemistry involved in this way, we can think of the industrial scaling of nanoparticles. This field of gold nanoparticles biosynthesis is leading to new avenues in material science, chemistry, nanobiotechnology and biotechnology. This simple procedure for the biosynthesis of gold nanoparticles has several advantages such as cost-effectiveness, compatibility for biomedical and pharmaceutical applications as well as for large-scale commercial production. In future, it would be significant to select such plants, to understand the clear mechanism of biosynthesis and to technologically engineer the nanoparticles in order to achieve better control over size, shape and absolute monodispersivity to utilize the potential of herbal medicine in nanoscience for anti-tumor or biomedical applications.

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