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Article

In Vitro, Characterization, and DNA-binding Studies of Synthesized Gold Nanoparticles using Orange (*Citrus sinensis*) Peels Extract

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ABSTRACT

Orange (*Citrus sinensis*) peel is one of the most underutilized biowastes. Most of the orange peels powders are the natural bleaching agent and it is the best source of Vitamin "C." The orange peel extracts acting as reducing and stabilizing agent for the metal nanoparticles, the orange fruit peels are enriched with different natural resources and it conveniently synthesizes the gold nanoparticles (AuNps) under microwave irradiation for 5 min (450 watts). The synthesized AuNps show strong UV–visible absorption at 534 nm, the green synthesized AuNps further optimized by Fourier-transform infrared spectroscopy, HR-scanning electronic spectroscopy, and HR-transmission electronic microscopy, X-ray diffractive spectroscopy), zeta potential, etc.

Key words: Green synthesis, Gold nanoparticles, Orange (*Citrus sinensis*) peel extract, DNA-binding studies.

1. INTRODUCTION

Nanotechnology is quite possibly the most trend setting innovations in every one of the spaces of science, because of the critical distinction in nature properties of nanoparticles and these properties (e.g. organic, reactant movement, mechanical properties, electrical conductivity optical absorption, thermal, and melting point) [1]. All these properties are depended on size, shape, and distribution of nanoparticles [2,3]. In addition, nanotechnology and science are a broad and interdisciplinary area for researcher and scientist, nanotechnology-based substances are now found with a wide range of household products and product intended for professional uses [4], including sports gear, cosmetic sunshine lotions, food packaging materials, clothing and paints, disinfectants, and medicine [5,6]. Among All the metal nanoparticles Gold Nanopaertricles (AuNps) are essential in various field. AuNps have more impact of researchers and scientific; the uses of AuNps are potential for drug delivery and medical field [7,8]. The orange (*Citrus sinensis*) peel extract acts as both reducing and stabilizing agents, hence, the orange peel extract can directly reduce $HAuCl_4$ to form AuNps without adding external reducers and stabilizing agent [9]. There are a few strategies incorporate obvious AuNps such as synthetic decrease, electrochemical, photochemical, and sonochemical and so forth be that as it may, these techniques are unsafe as they generally require the utilization of poisonous synthetic substances which lead to the natural poisonousness or organic perils [10,11]. To forestall the adverse consequences of the compound decrease techniques, specialists were intrigued to coordinate "green science" combination of nanomaterials using plant separates [12], biosurfactants, in watery medium and so on green amalgamation of AuNps has been accounted for utilizing an assortment of phytochemicals, including *Gymnema sylvestre* leaf extract, pomegranates, and *Syzygium* peel extract [13]. Here, green synthesis of AuNps is attempted using orange (*C. sinensis*) peels extract act as reducing and stabilizing agent. The orange peel extract is a naturally occurring phytochemical extracted from the plant (*C. sinensis*), a native tree of India [14]. This (*C. sinensis*) is utilized in customary Ayurvedic and Unani clinical arrangements for the treatment of mitigating, hepatodefensive, hypotensive infirmities, and as a cell reinforcement and is likewise utilized for the treatment of asthma and loose bowels [15]. The present study reports the synthesis of AuNPs with orange (*C. sinensis*) peel extract acting as the reducing and stabilization agent [16]. The synthesized nanoparticles were characterized by UV–visible (UV–Vis) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), HR-scanning electronic spectroscopy (SEM), and transmission electron microscopy (TEM) techniques. These AuNps were also studied for their applications of DNA-binding studies, average size calculation by XRD, and thermal stability counting using UV–Vis spectrometry.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

Chloroauric acid ($HAucl_4$) was purchased from Sigma-Aldrich for reliable grade of purity without further purification, nitric acid is purchased from Aura Chemicals Laboratories for the cleaning of glass wear. In this entire reaction, double-distilled water (DD H_2O) is used for purity without further purification and the orange (*C. sinensis*) peels extract. This consists higher medicinal values, orange fruit peels are collected from the local market of Osmania University, Hyderabad.

2.2. Preparation of Orange (C. sinensis) Peels Extract

The orange (*C. sinensis*) peels are naturally available phytochemicals, bicompounds. This is basically cheap [17], easily available, and non-toxic and it has potential application in human disease therapy. Almost 15 g of orange (*C. sinensis*) peels are cut into small pieces and dried under hot air woven at 80°C for 30 min and powdered it [18]. From

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that powder, nearly 5 g of orange (*C. sinensis*) peels powder were taken in 250 ml of the Erlenmeyer flask containing DD H2O, and sonicated at 60°C for 15 min to facilitate the advancement of watery concentrate [19]. The shaped concentrate was separated by the syringe filter (pore size is 0.22 µm) to get clear arrangement. Likewise, the cleared orange (*C. sinensis*) peels extract solution removed and kept under cool conditions for additional of future reaction.

2.3. Synthesis of AuNps

A mixture containing 5 ml of $HAucl_4$ solutions and 50 mL of orange (*C. sinensis*) peels extract was heated in a microwave woven. The color of the reaction mixture changed slowly from pale yellow to red color, which indicates the formation of AuNps. The formation of AuNps is further confirmed by UV–Vis spectroscopy. The different concentration of AuNps was synthesized from reaction mixtures using different concentration of ($HAuCl_4$ from 0.1 to 0.5 mM), and each mixed with a single percentage of orange (*C. sinensis*) peels extract (0.5%) at different microwave irradiation times from 1 to 20 min.

2.4. Characterization

The green synthesized AuNps further optimized by FT-IR, HR-SEM, and HR-TEM, XRD spectroscopy, zeta potential, etc.

2.5. DNA-Binding Studies

Because of its well-known structure and specific recognition abilities, DNA is widely employed in bioanalysis, imaging, and drug delivery system; DNA (in any event ×10 concentrated against the final focus) was added to the suspension of AuNPs to a 10 µL last volume and a 0.5 µM last AuNP fixation and hatched for 30 min at 52°C. Before stacking onto the 0.6% agarose gel, 1 µL of half glycerol was added as a stacking arrangement [20]. Electrophoresis was completed for 30 min at 5 V/cm in 25 mM Tris, 250 mM glycine, pH 9.

3. RESULTS AND DISCUSSION

UV–Vis spectroscopy is a very powerful full technique to study the kinetic energy barrier of the formed AuNps. The surface plasmon resonance (SPR) UV–Vis spectroscopy shows the absorption wavelength at 535 nm. Moreover, the rest of the solution color modified from colorless to red color, it states conformation of synthesized AuNps [21]. The importance of orange peel (*C. sinensis*) extracts and concentration of $HAuCl_4$ solution observed on the synthesis of AuNps. Figure 1 shows the effect of orange peel (*C. sinensis*) extracts and different concentration of $HAuCl_4$, the synthesis of AuNps studied using domestic microwave [22], the different concentration of AuNps was synthesized from reaction mixtures using different concentration of ($HAuCl_4$ from 0.1 to 0.5 mM), and each mixed with a single percentage of orange (*C. sinensis*) peels extract (0.5%), microwave irradiation times is 5 min. Figure 1a demonstrates that formation of AuNps increments with concentration of $HAuCl_4$ (0.1–0.5 mM) at the consistent convergence of orange peel (*C. sinensis*) extracts (0.5%). On increasing the concentration of $HAuCl_4$, the absorption band intensity increased, it indicates the production of a greater number of nanoparticles [23]. Moreover, the different microwave time (1–20 min) can show the observable increment of synthesized AuNps (Figure 1b).

3.1. FTIR Analysis of AuNPs

The FTIR measurements of biologically green synthesized AuNps and orange peel (*C. sinensis*) extracts were studied out to identify, which functional groups are responsible for the reduction and stabilization of AuNps process [24]. Figure 2 shows that FTIR spectrum of orange peel (*C. sinensis*) extracts capped amps. The protuberant peaks present in the spectra are the following. The peak at 3402^{cm-1} is due

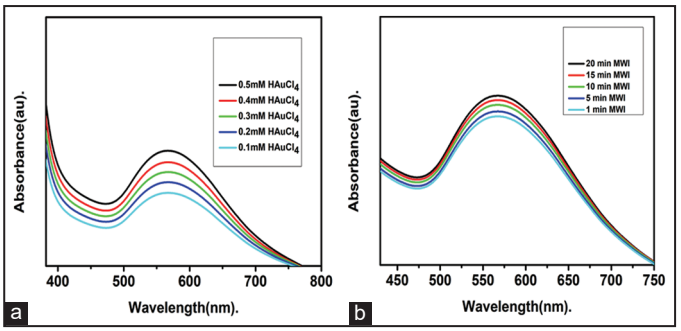

Figure 1: UV–visible spectra of synthesized gold nanoparticles (a) at different concentrations of chloroauric acid, (b) different microwave time irradiation.

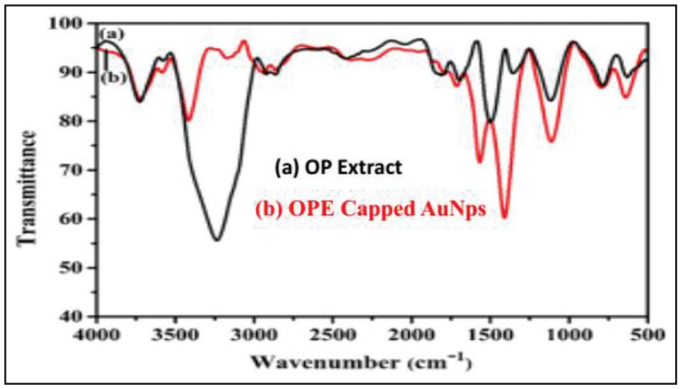

Figure 2: Fourier-transform infrared spectroscopy spectra of (a) orange (*Citrus sinensis*) peels extract and (b) orange (*C. sinensis*) peels extract capped gold nanoparticles.

to –OH is stretching, peak at 2963 compares to –CH extending, the pinnacle saw at 1608^{cm-1} in view of the C–C stretches of the fragrant gathering. The peak comparing two –OH twists of Polyphemus was seen at 1442^{cm-1} [25]. The pinnacle showed up at 1043^{cm-1} due to C-O-C-extending. Albeit, the FTIR spectra of the orange strip (*C. sinensis*) concentrates and AuNps are pretty much comparable, the different absorption tops in the range of the orange strip (*C. sinensis*) extricates are more exceptional than that of AuNps [26]. In addition, in the spectrum of nanoparticles, we can see some minor shifts in the position of various peaks compared with that of the orange peel (*C. sinensis*) extracts, hence indicating the involvement of the orange peel (*C. sinensis*) extracts in the synthesis and stabilization of AuNps.

3.2. Zeta Potential of Synthesized AuNps

The average hydrodynamic diameter of the AuNps was found around 15.380 nm with 0.423 PDI (Figure 3). The aqueous stability of AuNps was tested by zeta potential analysis [27]. Zeta potentials of the nanoparticles obtained by DLS measurements were −14.6.7 and thus the electrostatic interactions between electronegative AuNps.

3.3. XRD Results

The XRD investigation was done with air-dried AuNps nanoparticles. The XRD examination with orange strip (*C. sinensis*) extricates covered AuNps (Figure 4) has shown extraordinary diffraction peaks at 38.1°, 44.43°, and 64.3°, which listed the planes (111), (200), (220), and (311) Bragg's appearance and the examples noticed clearly revealed that the crystalline synthesized AuNps are a face-centered cubic phase. For these situations, the pinnacle relating to (111) plane is more serious than the pinnacle relating to (200), (220), and (311) planes, these outcomes are…

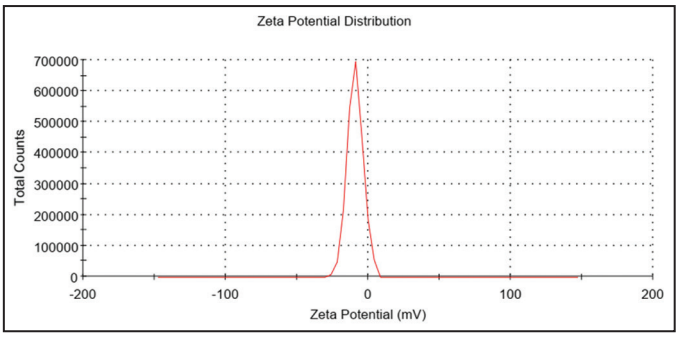

Figure 3: Zeta potential studies of orange (*Citrus sinensis*) peels extract capped gold nanoparticles.

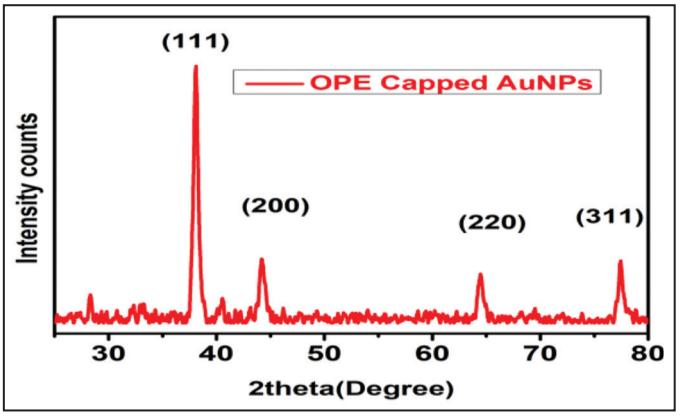

Figure 4: Powder X-ray powder diffraction pattern of orange (*Citrus sinensis*) peels extract capped gold nanoparticles.

3.4. Crystallites (Grain) Size From XRD Data Using Scherrer Equation

Scherer's condition is composed as-

Debye-Scherrer recipe:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

D = mean measurement of nanoparticles
β = the full width at half-most extreme estimation of XRD diffraction line
λ = the frequency of X-beam radiation source 0.15405 nm
θ = the half diffraction point Bragg point
K = the Scherrer consistent with the worth 0.9.

3.5. Tem Analysis

The TEM was used to determine the size, shape, and distribution of biosynthesized AuNps. Figure 5a shows that the prepared AuNps are mainly spherical in shape. The round nanoparticles were framed with diameters going from 5 nm to 20 nm and are of profoundly mono-scattered in nature. The resultant amps show that orange stripe (*C. sinensis*) extricates can shield Au nanoparticles from conglomeration. The histogram (Figure 5b) was designed by considering 120 AuNps and obviously indicated that the average size, dispersal was 15 ± 2 nm size.

3.6. Stability of AuNPs at Room Temperature

The colloidal strength of the AuNps particles got in these investigations was tried by the 3-months develop testing at 27°C and 52 h hatching

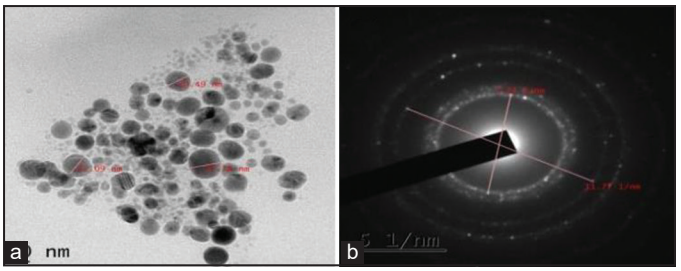

Figure 5: (a) Transmission electron microscopy image of orange (*Citrus sinensis*) peels extract capped gold nanoparticles and (b) corresponding SAED pattern.

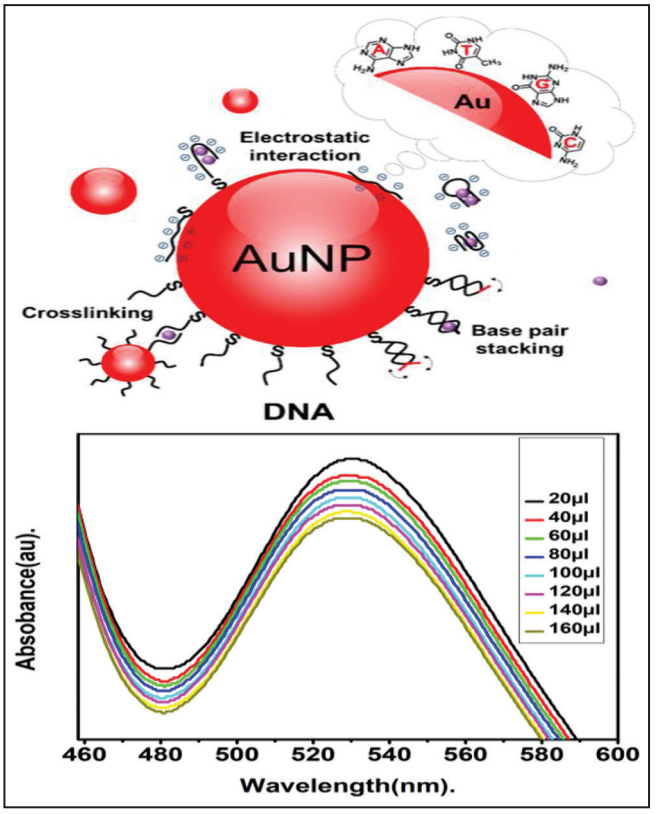

Figure 6: UV–visible spectra of synthesized gold nanoparticles with DNA-binding studies.

in 1 mM sodium chloride (natural saline) at room temperature. Their attributes were inspected by UV–Vis spectroscopy (SPR). It very well may be seen that all the incorporated AuNPs showed stay same outcome as previous UV absorbance from two distinctive scattering dependability tests (1.60 ± 0.69 and 1.55 ± 0.66 nm), with no accumulation. TEM results showed that the blended AuNps in these examinations are steady at 27°C and natural saline, which implies that they will stay stable when utilized for organic and clinical applications.

3.7. DNA-Binding Studies

Although the negatively charged AuNPs capped by citrate are repulsive to the polyanionic DNA backbones, attraction interactions exist and enable the adsorption of unmodified DNA oligo nucleotides onto negatively charged AuNPs. In these cycle, adenine shows exceptionally restricting studies to AuNps, it ties with the outside of AuNps by means of the exocyclic amine bunch and the N7 atom. Auxiliary to adenine, guanine, and cytosine communicate with AuNps through the keto bunch

AQ8 **Table 1:** Average size calculation of synthesized AuNps by XRD pattern [37,38].

S. No.	Peak position 2 (theta values)	FWHM	Crystalline size D (nm)	Average D (nm)
1.	38.08576	0.55245	15.21262091	15.38058378
2.	44.2386	0.6369	13.46431842	
3.	64.46891	0.58867	14.56745614	
4.	77.45107	0.55712	18.27793965	

AuNps: Gold nanoparticles, XRD: X-ray powder diffraction

and connecting nitrogen. Thymine shows the most weak adsorption to the conflictingly blamed AuNps for possible association site through keto oxygen molecule so to speak. Till named DNA can attach with AuNps covalently through the advancement of Au-S bond this innovative game plan of DNA AuNps. Hence, in these study addition of buffer dissolved DNA to the plant extract capped AuNps volumetrically, Figure 6 shows that optimized (0.5 mM concentrated $HAucl_4$) AuNps to that buffer dissolved DNA were added (20, 40, 60, 80, 100, 120, 140, and 160 µL of DNA solution); the optimized UV–Vis peaks are gradually decreased (intercalation reaction). It proved that the surface of AuNps capped with DNA, amino acids, and the activity of AuNps are inactivate.

4. CONCLUSIONS

In this synthesis, the biological method with orange peel (*C. sinensis*) extracts was used to synthesize amps. The results indicated that the Au NPs, with better morphological features than those produced from fruit juices/extracts, could be synthesized from fruit waste orange peel (citrus sinensis) extracts ultra-small AuNPs (1.75 ± 0.86 nm) werefirst synthesized by bio waste orange peel (citrus sinensis) extracts, which is smaller than those (2.6 ± 1.1 nm) obtained from fruit extract (M. acuminate) in our previous experiment. More importantly, the major functional groups that are responsible for the production of the Au NPs, as well as the possible mechanism of the synthesis and stabilization, were investigated by FTIR spectra. The XRD investigation was done with air-dried AuNPs nanoparticles. [28,29] The XRD examination with orange strip (citrus Sinensis) extricates covered AuNPs (Fig. 9b) has shown extraordinary diffraction peaks at 38.1°, 44.43° and 64.3°, which listed the planes of (111), (200), (220) and (311) Bragg's appearance and the examples noticed clearly revealed the crystalline AuNPs is face centered cubic phase.[30,31] For these situation, the pinnacle relating to (111) plane is more serious than the pinnacle relating to (200), (220), and (311) planes, these outcomes are like the prior reports [32,33,34]. Most importantly, carboxyl, hydroxyl, and amide bunches in orange strip (*C. sinensis*) concentrates may take an interest during the time spent nanoparticle amalgamation, and carbonyl gatherings assisted with balancing out the size of AuNPs. Carboxylic corrosive and ester in the orange strip (*C. sinensis*) concentrates could shield AuNPs from accumulation. Finally, the synthesized AuNps average size is calculated as 15.380 nm (from XRD data). According to the TEM studies, it morphological size are demonstrated as 15 ± 2 nm. Moreover, most of the particles are spherical in nature and it is studied by 120 particles. Here, in these syntheses, we demonstrated the DNA-binding studies with better intercalation process, and we have reported the dispersion stability of the particles of AuNps tested by 5-month aging testing at room temperature and 72 h incubation method, it resulted that the formed AuNps nanoparticles are stable after 5 months.

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****Bibliographical Sketch***

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