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Green synthesis of gold nanoparticles using *camellia sinensis* and kinetics of the reaction

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ABSTRACT

In this paper we report the green synthesis of gold nanoparticles (AuNPs) by using a simple, faster, low cost, eco-friendly technique. The green synthesis of AuNPs was done by using fresh young leaves and leaf buds of tea (*Camellia Sinensis*). Reduction of $HAuCl_4$ by polyphenols present in young leaves and leaf buds of tea extract at room temperature provides AuNPs ($Au^{3+} \rightarrow Au^0$).The UV-Visible absorption spectrum of AuNPs in tea extract shows two bands at around 534 and 752 nm, which results from transverse and longitudinal surface plasmon resonance (SPR) respectively. In fluorescence spectroscopy study, AuNPs in tea extract shows fluorescence emission at 450 and 705 nm when excited at 350 nm. The kinetics of the reaction rate ($Au^{3+} \rightarrow Au^0$) with respect to time was studied with the help of UV-Visible and fluorescence spectroscopy. The reaction rate evaluated with the help of UV-Visible and fluorescence spectroscopy was found to be almost similar in results. The kinetics of the reaction also suggests that the reaction was fast and completed in 28 minutes. The amount of tea extract determines the core size of the AuNPs. The core size of the AuNPs decreases as the amount of tea extract increases and it causes the blue-shift of SPR band. The physical properties, particle size and morphology of AuNPs were characterized using X-ray diffractometer (XRD), field emission scanning electron microscope (FESEM) and high resolution-transmission electron microscopy (HR-TEM) techniques. The AuNPs size in the range ~2.94-45.58 nm with an average of 13.14 nm. Copyright © 2012 VBRI press.

Keywords: Gold nanoparticles; *Camellia Sinensis*; green synthesis; surface plasmon.

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Introduction

Currently, biosynthesis of AuNPs has gained much attention and emerged to be an active research area in the field of nanotechnology. Due to possessing some remarkable novel properties such as intense plasmon resonance, electrical, magnetic, thermal conductivity, chemical and biostability, catalytic activity, anti-bacterial activity, anti-HIV activity, anti-angiogenesis activity, anti-malarial agent and anti-arthritic activity gold and AuNPs have found tremendous interest over last few decades [**1, 2**]. Recent *in vitro* studies show that AuNPs donot cause cytotoxicity in human cell and therefore, AuNPs have received tremendous interest for modern biomedical sciences,including cancer photodiagnostics, photothermal therapy, biolabeling, nanodiagnostics, drug delivery, gene delivery, immunochromatographic identification of pathogens in clinical specimen [**2**]. In the field of nanotechnology the future application of AuNPs will open exciting possibility and they will be vital key materials in the 21st century **[3]**.

AuNps can be synthesized in a range of size and shape distributions via different techniques such as citrate reduction of $HAuCl_4$ in water [**4, 5**], seed-mediated growth method [**6**], metal vapour synthesis, electrochemical method through gold ionization and reduction **[7]**. However, most of these physical and chemical methods are still in the development stage and involve the use of toxic chemicals, high temperature and pressure [**1, 4**]. Consequently, the researchers in the field of nanoparticles have to investigate some alternative biosynthetic green approaches that utilize natural microorganisms and plant extracts for reduction of metal ions **[8]**.

Ionic forms of gold shown to have cytotoxicity on various cell types and adverse effects on red blood cells. Also it has been reported that AuNPs synthesized by physical and chemical methods aggregates in physiological conditions hindering its in *vivo* applications [**9, 10**]. Therefore, in this work we report a plant mediated green synthesis approach for the synthesis of AuNPs by using fresh young leaves and leaf buds of tea (*Camellia Sinensis*) extracts which can act as a reducing, stabilizing or capping agent. Moreover, through this work we have attempted to integrate green synthesis approach in the field of nano science. This green synthesis is a simple, low cost, stable for long time, green chemistry approach suitable for large scale commercial production and alternative to other biological, physical and chemical methods.

Camellia Sinensis, commonly known as tea is the species of plant whose leaves and leaf buds are used to production of Chinese tea. It has been used socially, habitually and medical drink by people for so long (since 3000 B.C.) **[11]**. Tea leaves contain many compounds such as polysaccarides, volatile oils, vitamins, minerals, purines, xanthine alkaloids (e.g. caffeine, theophylline, theobromine) and polyphenols of the flavonoid type such as theaflavins, catechins **[11]**. Tea catechin in green tea or theaflavins in black tea are known as stronger antioxidant compound **[11]**.

Experimental

Materials and instruments

UV-Visible and fluorescence spectroscopy measurements were carried out on Hitachi U-3210 double beam spectrophotometer and Hitachi F-2500 Fluorescence spectrophotometer respectively. The FT-IR spectra were recorded using IR Affinity-I Shimadzu spectrophotometer. The $HAuCl_4$ reduced tea extract were centrifuged at 16,000 rpm for 15 minutes individually. The deposited residue was dried and grinned with KBr to obtain pellet for FT-IR analysis. The morphology of the AuNPs was analyzed using FESEM and HR-TEM images.

Preparation of tea extract

Fresh young leaves and leaf buds of tea was collected from a local tea garden of Assam, India. 20 grams of fresh young leaves and leaf buds was washed several times with deionized water to remove dust particles and then leaf was cut and grounded with a mortar and a pastel. The finely grinded tea paste was transferred into a 100 mL round bottom flask and then stirred with 50 mL of deionized water at room temperature for 3 hours and then allowed to stay for 1 hour. The reddish brown color tea extract was decanted gently and filtered to remove the solid undissolved residues of tea leaves. This reddish brown color filtrate was used as reducing and stabilizing or capping agents for $HAuCl_4$.

Table 1. Addition of different amounts tea extract to 25.8 mM aqueous $HAuCl_4$ and corresponding SPR bands.

Sample	Volume of $HAuCl_4$ (mL)	Volume of tea extract (mL)	Volume of deionized water (mL)	λ_{max} = SPR band (nm)
S1	0.3	0.2	1.0	568
S2	0.3	0.6	0.6	538
S3	0.3	0.8	0.4	535
S4	0.3	1.2	0	534

Synthesis of AuNPs

Gold (III) chloride hydrate ($HAuCl_4.xH_2O$) 99.99% metal basis was obtained from Aldrich and used as such. 0.2 mL tea extract and 1.0 mL deionized water was added to 0.3 mL aqueous solution of $HAuCl_4$ (25.8mM) at room temperature and the mixture was shacked well to form a colloidal solution. Slow reduction takes place and completed in 28 minutes as shown by stable light purple or brilliant red color of solution which gives sample S1. To obtain sample S2, S3 and S4 the addition of the tea extract and deionized water was varied as shown in **Table 1**. It is evident from the experiment that the gradual increase of concentration of tea extract favours the reduction of Au^{3+} to Au. The polyphenolic compounds present in tea extract is the acting reducing agent which is responsible for this redox change.

Results and discussion

FESEM analysis

FESEM images were obtained by using FESEM ∑ (Sigma), Carl Zeiss. The FESEM image (**Fig. 1**) of the AuNPs in sample S4 confirmed that the particles are irregular spherical, hexagonal, triangular and elongated shapes.

HR-TEM analysis

HR-TEM images were obtained by using TEM, JEM-2100, 200kV, Jeol. The typical HR-TEM images obtained for AuNPs sample S4 under different magnifications are shown in the **Fig. 3(a), (b), (c), (d)**. The longitudinal surface plasmon resonance (LSPR) arising due to anisotropy of AuNPs is evident from the HR-TEM image

Fig. 3(c) of the AuNPs in sample S4. The HR-TEM images of AuNPs confirmed that particles are 74% irregular spherical, 10% hexagonal, 8% triangular and 8% elongated shapes with size range ~2.94-45.58 nm. The typical HR-TEM of a single nanocrystal (**Fig. 3(d)**) with clear lattice fringes having a spacing 23.36 nm reveals that the growth of AuNPs occurs preferentially on the (111) plane. The selected area electron diffraction (SAED) pattern of one of the spherical AuNPs in sample S4 is shown in **Fig. 3(e)**.

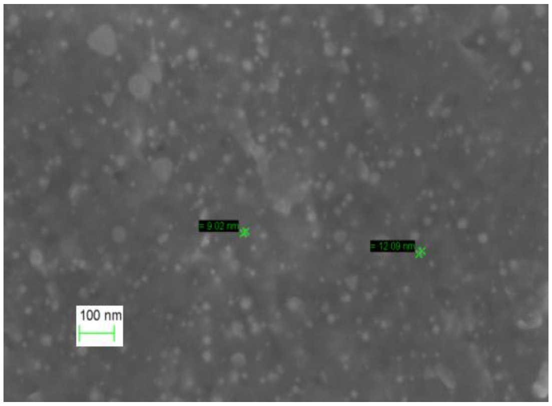

Fig. 1. FESEM image of AuNPs in sample S4.

The presence of circular lattice fringes in SEAD image reveals the single face-centered cubic (fcc) crystalline nature of the spherical AuNPs with (111), (200), (220) and (311) planes **[12]**. The particle size histogram distribution plot shown in **Fig. 3(f)** results from the counting of ~100 nanoparticles from HR-TEM image (**Fig. 3(c)**) and shows the particles in the size range of ~2.94-45.58 nm with an average of 13.14 nm (**Fig. 3(f)**).

XRD studies

XRD measurement of the $\mathrm{HAuCl_4}$.tea extract solution drop-coated onto glass substrates were done on a Philips X' PERT PRO instrument operating at a voltage of 40 KV and a current of 30 mA with CuKα radiation. Confirmation of formation of crystalline AuNPs was further confirmed by XRD analysis.

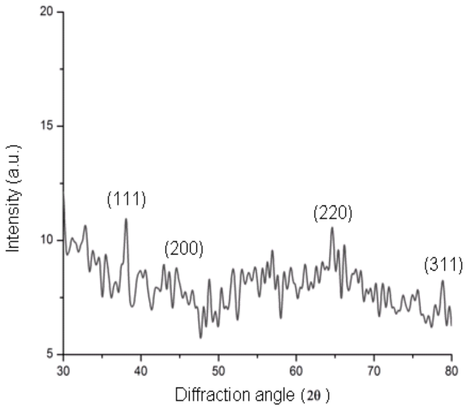

Fig. 2. XRD pattern of AuNPs obtained from sample S4

Fig. 2 shows XRD pattern with AuNPs sample S4 recorded from drop-coated films of the tea extract reduced AuNPs deposited on glass substrates. The presence of four peaks at 38.36°,44.51°,$64.87^{\circ}$and 78.85° in the 2θ range 30°-80° which can be indexed to the (111), (200), (220) and (311) Bragg reflections of fcc structure of metallic gold respectively [Joint Committee on Powder Diffraction Standards (JCPDS No.04-0784)], revealing that the synthesized AuNPs are composed of pure crystalline gold **[12]**.

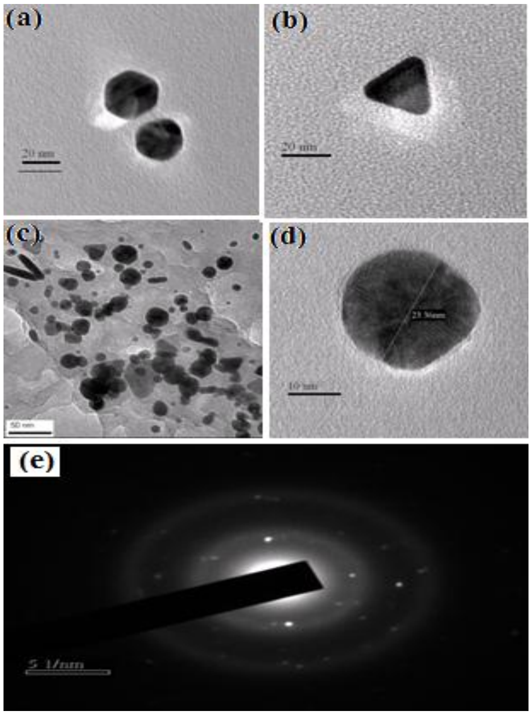

Fig 3. (a-d) HR-TEM images of AuNPs sample S4 under different magnification, **(a)** 20 nm (spherical and hexagonal shape), **(b)** 20 nm (triangle shape), **(c)** 50 nm, **(d)**10 nm single nanocrystal showing lattice fringes with spacing 23.36 nm, **(e)** SAED pattern of spherical AuNPs.

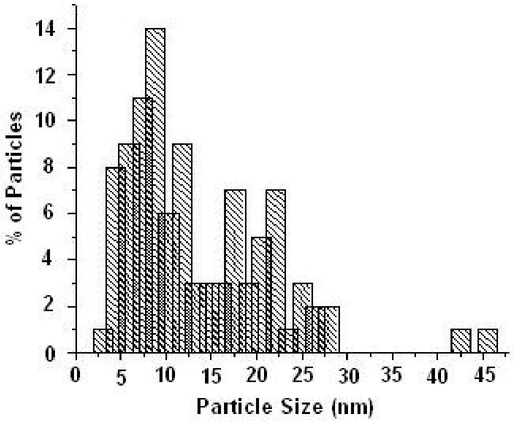

Fig. 3 (f) Histogram of the AuNPs size distribution analysis from corresponding HR-TEM image (c).

FT-IR studies

The presence of polyphenolic biomolecules in tea extract and their interaction with the surface of the AuNPs was confirmed by FT-IR spectra (**Fig. 4**). The IR bands (**Fig. 4(a)**) observed at 3395, 1654, 1639 and 1323 cm^{-1} in dried tea powder are characteristics of O-H, C=O and C-O stretching modes of the carboxylic acid group present in the arubigins and tannins. The bands at 1654 and 1639 cm^{-1} are assigned to the C=O stretch of the acid group present in the arubigins and tannic acid present in tea powder. Except for a slight shift in the C=O stretching bonds1654 to1648 cm^{-1} and 1639 to1631cm^{-1} rest of the IR bands remain unchanged in the spectra obtained from that AuNPs after the reaction with tea extract as shown in **Fig. 4(b)**. However, in the spectrum of AuNPs (**Fig. 4(b)),** the band due to C-O stretching at 1323 cm^{-1} is very weak. From these observations it is clear that the polyphenolic biomolecules present in tea extract are responsible for reduction and stabilization or capping cannot be ruled out.

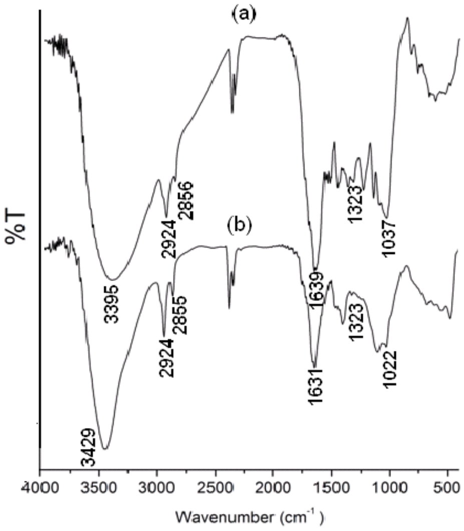

Fig. 4. FT-IR spectra of **(a)** dried tea powder, **(b)** dried tea powder of AuNPs.

UV-Visible and kinetic studies of AuNPs

The polyphenols present in the tea extract shows a band at 268 nm and before reduction $[AuCl_4]^-$ shows a band at 309 nm (**Fig. 5(a)**). After reduction, the formation of AuNPs was indicated by a visual color change to light purple or brilliant red color,disappearance of Au^{3+} band at 309 nm and appearance of two characteristics bands in the visible region at around 534 and 752 nm **[13]**. The band at 534 nm is attributed to TSPR and this band has some contributions from the spherical AuNPs present in the solution **[14]**. The band at 752 nm corresponds to the LSPR which is very sensitive to the aspect ratio **[6, 14]** .The SPR bands are due to the collective oscillations of the electron gas of the surface of AuNPs (6s electrons of the conduction band for AuNPs) that is correlated with the electromagnetic field of the incoming light, i.e.the excitation of the coherent oscillation of the conductive band **[3, 7]**. The SPR of the AuNPs consists of two components: scattering and absorption. The scattering component is known to be responsible for fluorescence enhancement and the absorption component for fluorescence quenching **[5, 6]**. The SPR spectrum depends on the nanoparticle size, shape of the material but also depends on some external properties of the nanoparticles environment such as medium dielectric constant, temperature, refractive index of the solvent **[15]**.

The amount of tea extract determines the size of the AuNPs. **Fig. 5** shows the UV-Visible absorption spectra of the AuNPs (sample S1-S4) obtained after 1 hour of the reaction. The SPR band becomes narrower from the sample S1 to S4 (**Fig. 5(b-e)**) with shifting towards the blue wavelength as the amount of tea extract is increased. The fairly sharp SPR band observed for sample S4 (**Fig. 5(e)**) at 534 nm indicating formation of most spherical AuNPs. The spherical AuNPs act as nuclei growth centers upon which the deposition of gold in the form of triangle or hexagonal occurred with the shape directing agent present in the solution **[16]**. Thus,the TSPR band at 534 nm is for spherical shape AuNPs and the LSPR band at 752 nm is for planer triangular, hexagonal or elongated shaped AuNPs. When the high amount tea extract was used to reduce the aqueous $HAuCl_4$, the polyphenolic biomolecules present in tea extract acting as capping agents strongly shaped spherical particles rather than nanotriangles, hexagonal or elongated nanoparticles though the reductive polyphenolic biomolecules were enhanced. Although less amount of tea extract reduces the Au^{3+}, but they failed to protect most of the quasi-spherical AuNPs from aggregating because of the deficiency of polyphenolic biomolecules to act as protecting agents. Sintering of AuNPs and their adherence to the nanotriangle is evident from the **Fig. 1** and **Fig. 2**. The addition of high amount of tea extract causes strong interaction between protective polyphenolic molecules and surface of nanoparticles preventing nascent nanocrystals from sintering, leading to size reduction of spherical nanoparticles **[16]**. As the particle size increases, the wavelength of SPR related absorption shifts to the longer wavelength.

In order to determine the rate of AuNPs formation the kinetics of the reaction with respect to time was studied with the help of UV-Visible spectroscopy by monitoring the absorption intensity of SPR band at 534 nm. The corresponding UV-Visible spectra recorded from $HAuCl_4$ (5.16mM)-tea extract (1.5mL) reduction at various time intervals of 3 minutes for 28 minutes is shown in the **Fig. 6**. On reduction of $HAuCl_4$ by tea extract for various time intervals shows a decrease in the intensity of Au^{3+} at 309 nm bands and appearance of TSPR band at around 534 nm. A gradual increase in the intensity of TSPR band without any shift with increasing time from spectra 1-15 indicates the slow reduction of Au^{3+} to Au^0. No significant change in the intensity from spectra 1-15 also suggest that the reduction completed in 28 minutes. As shown inset in **Fig. 6** pseudo- first order rate constant, k_{obs}, 8.09x10^{-4} min^{-1} was obtained from the slope of the nonlinear fits of A_t vs. time t according to the equation $A_t=A_\alpha+(A_0-A_\alpha)\exp(-k_{obs}t)$, where A_0 and A_α are the initial and final absorbance, respectively **[17]**.

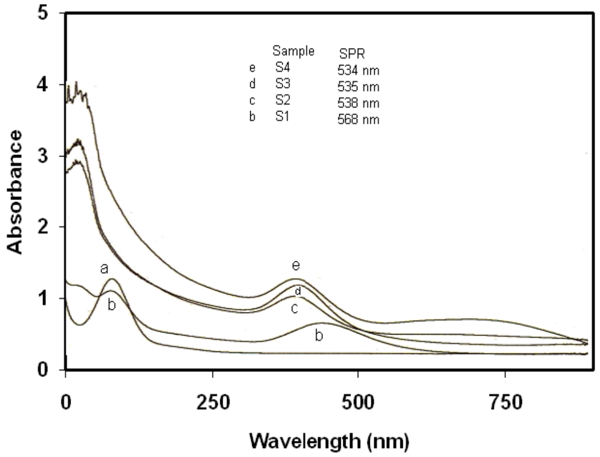

Fig. 5. UV-Visible spectra of **(a)** aqueous $HAuCl_4$, **(b-e)** AuNPs solutions for sample S1-S 4

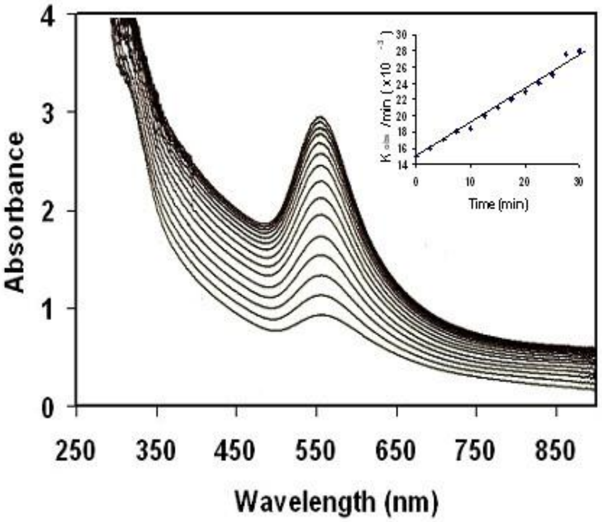

Fig. 6. UV-Visible spectra of AuNPs formation with $HAuCl_4$ (5.16mM) tea extract (1.5 mL) reduction at 3 minutes time interval

Fluorescence and kinetic studies of AuNPs

In fluorescence spectroscopy study, AuNPs in tea extract shows fluorescence emission at 448-450 and 705 nm when excited at 350 nm. These emission bands are due to the local field enhancement via coupling to the TSPR and LSPR [**13, 18**]. The local field enhancement arises due to the resonant photons inducing coherent surface plasmon oscillations of their conduction band electrons [**2, 3, 6, 7**]. From the **Fig. 7** it shows that the fluorescence emission intensity of TSPR band at 448 nm and LSPR band at 705 nm decreases with increasing the tea extract amount (sample S1-S4). This indicates that the number of AuNPs increases in the colloidal solution with the high amount tea extract. As in UV-Visible kinetic method, similar kinetic reactions with respect to time were carried out to determine the rate of AuNPs formation.

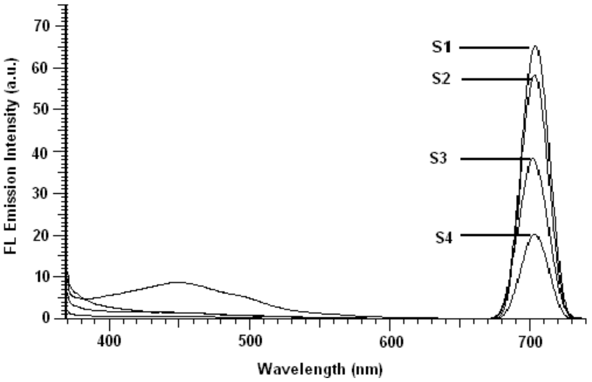

Fig. 7. Fluorescence emission spectra of AuNPs solutions for sample S1-S4.

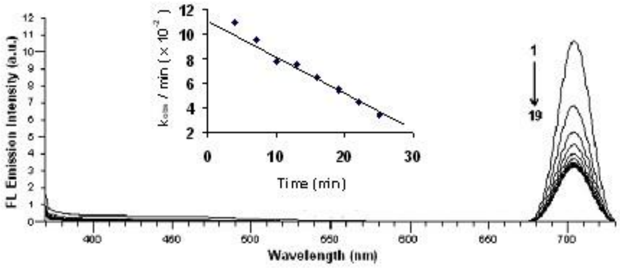

Fig. 8. Fluorescence emission spectra of AuNPs formation with $HAuCl_4$ (5.16mM)- tea extract(1.5mL) reduction at 3 minutes time intervals. Inset: Plot of k_{obs} vs. time (min).

Fluorescence kinetic study shows the fluorescence emission intensity at 450 and 705 nm gradually decreases with increasing reaction time from spectra 1-19 (**Fig. 8**) which indicates the slow progress of Au^{3+} to Au^0 reaction and no significant change in the emission intensity from spectra 1-19 was observed suggesting the completion of reduction of Au^{3+}. As shown inset in **Fig. 8** pseudo-first order rate constant, k_{obs}, 4.28×10^{-3} min^{-1} was obtained from the slope of the nonlinear fits of E_t versus time t according to the equation $E_t = E_\alpha + (E_0 - E_\alpha) \exp(-k_{obs}\, t)$, where E_0 and E_α are initial and final fluorescence emission respectively **[17]**. The reaction rates were evaluated with the help of uv-visible and fluorescence spectroscopy. The results were in close agreement with each other and suggest a fast reaction rate (i.e. completed within 28 minutes).

Conclusion

We have reported a plant mediated green synthesis approach for the synthesis of gold nanoparticles by using fresh young leaves and leaf buds (*Camellia Sinensis)* of tea extract as reducing, stabilizing or capping agent. The advantages of this synthesis are it is a simple, low cost, stable for long time, eco-friendly, green chemistry approach, alternative to other biological, physical and chemical methods. Tea extract mediated synthesized AuNPs of size range ~2.94-45.58 nm with an average 13.14 nm. In conclusion, we believe that this research work will therefore lead to further development of a low cost preparation of gold nanoparticles.

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