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A New Route of Synthesis and Characterization of Biocompatible Gold Nanoparticles from Synthetic Japanese Natural Medicine

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Abstract: *Redox behavior of phenolic substrate is well established method for preparation of gold nanoparticles (GNPs) from gold salt. Natural medicine containing phenolic hydroxyl group was used as not only reducing agent but also as stabilizer. Newly synthesis of GNPs at ambient condition shows good stability over a period of time with mono-dispersed in nature. GNPs were characterized by ultraviolet visible spectroscopy, transmission electron microscopy, X-ray powder diffraction.*

Keywords- *ambient temperature, eco-friendly synthesis, gold nanoparticles, natural medicine, redox reaction.*

I. INTRODUCTION

Usefulness of biomaterials and metal catalyst in many reactions is an urgent need of the society to fulfill our requirements. As per the environmental issue it is our responsibility to avoid hazardous effect of metal. So choice of biocompatible and micro- to nano-gram scale catalyst instead of toxic and gram scale is critical. Recent development of nanometer scaled metal particles have attracted significantly with new area of research – *Nanomaterial Science and Technology* [1,2]. They are widely precious in the field of chemical synthesis to biomedicine [3-6].

Gold nanoparticles (GNPs) have properties that are different from the bulk metals due to their small size. They are useful in catalysis, magnetism, single electron tunneling devices, DNA sequencing, biomolecular recognition and many others [7,8]. The discovery of gold nanoparticles (GNPs) as catalyst for the reduction of nitroaromatic compounds has established new possibilities for the production of aniline derivatives [9-12]. This help scientists to probe new possibility for planning efficient and clean synthetic routes for the preparation of polyfunctional nitrogenated compounds of highly demanded chemicals [13]. A very recent report shows chemoselective reduction of substituted nitroaromatic compounds for preparation of polyfunctional nitrogenated compounds by means of cascade reactions [14]. Mirkin and co-workers reported a new method for colorimetric detection of targeted DNA sequences based on gold colloidal particles size controlled by oligonucleotide linkers of varying length [15]. Thus growing interest of GNPs in the field of catalysis to bioanalytical field provides a progressive movement to the improvement of their novel preparation methods [16].

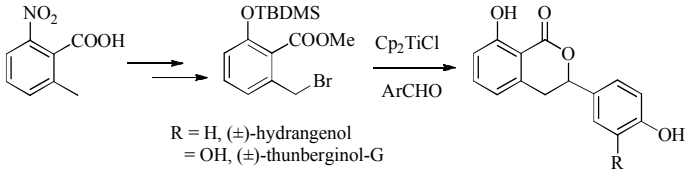
The conventional methods for preparation of GNPs describes reduction of tetrachloroauric acid ($HAuCl_4$) in aqueous medium. Most widely used reducing agents are sodium citrate, sodium borohydride, block copolymers or ascorbic acid [17]. In this process GNPs dispersed in solution due to ionic repulsion and absorbed on the surface and have the general tendency to aggregate due to shielding of charge. So choice of stabilizer is crucial to prevent aggregation which is soluble in the medium. Many have used water-soluble polymers such as starches, PEG, SDS, amine etc [18]. Organic metal nanocomposites have diverse advantages which lead the scientists to find out the proper materials [19]. Water-dispersible GNPs have been synthesized by redox technique at room temperature using poly(4-vinylphenol) (PVPh) as a simultaneous template, stabilizer and reducing agent [20]. Still alternative method is require for sensitive use of biomedicine as *PEG* compounds themselves show some evidence of genotoxicity [21]. Thus the hot spot of current research in nanomaterial science and technology is the synthesis of GNPs from biomaterial using plants or plant extracts [22,23], fungus [24-26], and bacteria [27,28]. Very recently synthesis of GNPs using chloroplasts as reductants and stabilizers have been reported and showed great potential for ultrasensitive detection of biomarkers in vitro and in vivo based on surface-enhanced Raman spectroscopy (SERS) [29]. Most of the method needs the complex biological components and costly biological resources.

Chemically synthesized bioactive compound may be excellent alternative methods for synthesis of GNPs. This encouraged the current author to use chemically synthesized natural medicine such as (±)-hydrangenol and (±)-thunberginol-G as the potential biological template for preparation of GNPs. The hypothesis of the present work is based on the utilization of redox active phenolic hydroxyl group of the chemically synthesized natural compound as the potential source for reduction of gold salts into their corresponding nanoparticles. Although expected, author was fortunate enough that the template act as simultaneous stabilizer and reducing agent. Present work reports that (±)-hydrangenol and (±)-thunberginol-G are capable to reduce gold ion under aqueous condition in ethanol-water media to yield highly stable gold nanoparticle.

II. EXPERIMENTAL

2.1. Preparation of the template

Initially the chemical synthesis was carried out from commercially available 2-methyl-6-nitrobenzoic acid. This was converted to the radical precursor for Barbier type of reaction followed by radical addition to the suitably substituted aldehyde to result (±)-hydrangenol and (±)-thunberginol-G (**Scheme 1**) as depicted earlier by the same author [30].



Scheme 1. Chemical synthesis of the organic template

2.2. Synthesis of GNPs [31-33]

2.2.1. Method A

To a stirred solution of 2.5 mL 1mM ethanolic solution of (±)-hydrangenol 0.1 mL of 0.1M KOH solution was added. After dissolution, 1.0 mL of 1mM $HAuCl_4$ was added slowly to provide homogeneity at room temperature. The yellow color slowly changed to violet color within 6h indicates the formation of gold nanoparticle.

2.2.2. Method B

To a stirred solution of 5 mL 1mM ethanolic solution of (±)-hydrangenol or (±)-thunberginol-G 2.0 mL of 1mM $HAuCl_4$ was added slowly to provide homogeneity at room temperature. The yellow color slowly changed to violet color within 2h for (±)-hydrangenol and within a minute for (±)-thunberginol-G indicates the formation of gold nanoparticle.

2.3. Chemicals

Aqueous 1% $HAuCl_4 \cdot 3H_2O$ solution (Sigma, > 49% as gold) and Millipore Milli-Q system (18 mΩ) were used for the preparation of colloidal gold. Anhydrous ethanol was purchased from Aldrich chemical company.

2.4. Spectral data of (±)-hydrangenol and (±)-thunberginol-G [30]

2.4.1. (±)-hydrangenol: IR (KBr) 3354, 1658, 1460, 1230, 1028 cm^{-1}; ^1H NMR (300 MHz, d_6-DMSO): δ 3.10 (dd, *J* = 2.5, 16.4 Hz, 1H), 3.37 (dd, *J* = 12.0, 16.4 Hz, 1H), 5.63 (dd, *J* = 2.5, 12.0 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 2H), 6.83 – 6.89 (m, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.50 (t, *J* = 7.9 Hz, 1H), 9.62 (s, 1H, Phenolic OH), 10.92 (s, 1H, Phenolic OH); ^{13}C NMR (75 MHz, d_6-DMSO): δ 34.4, 81.4, 109.3, 116.1(2C), 116.3, 119.1(2C), 129.2, 129.3, 137.2, 141.5, 158.7, 161.8, 170.2; HRMS calcd for $C_{15}H_{12}O_4Na$ $[M+Na]^+$ 279.0633, found 279.0649.

2.4.2. (±)-thunberginol-G: IR (KBr): 3303, 1658, 1614, 1203 cm^{-1}. ^1H NMR (300MHz, d_6-DMSO): δ 3.12 (dd, *J* = 2.9, 16.5 Hz, 1H), 3.33 (dd, *J* = 11.8, 16.5 Hz, 1H), 5.59 (dd, *J* = 2.9, 11.8 Hz, 1H), 6.77 – 6.91 (m, 5H), 7.51 (dd, *J* = 7.8, 7.9 Hz, 1H), 9.07 (s, 1H), 9.10 (s, 1H), 10.95 (s, 1H). ^{13}C NMR (75MHz, d_6-DMSO): δ 33.1, 80.0, 108.0, 113.7, 114.9 (2C), 117.3, 117.9, 128.5, 135.8, 140.1, 144.7, 145.3, 160.4, 168.9. HRMS calcd. for $C_{15}H_{12}O_5Na$ $[M+Na]^+$ 295.0583, found 295.0584.

2.5. UV-Vis spectroscopy

UV-Vis spectroscopy was utilized to measure the presence of a Surface Plasmon Resonance (SPR) peak using U-2800 Spectrophotometer. UV-Vis spectroscopic measurements were operated at a resolution of 1 nm at a range of 200–800 nm with a scanning speed of 400 nm/min. The presence of surface plasmon band around 545 nm observed in UV-visible spectrum indicates the formation of gold nanoparticle.

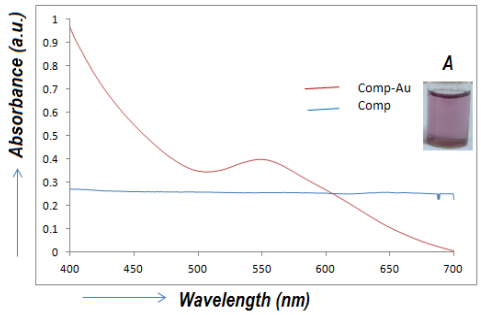
2.6. Transmission electron microscopy measurements and X-ray diffraction analysis

The diameter of the GNPs was determined by transmission electron microscopy (TEM) on JEOL JEM-2010 electron microscope using a 200 kV accelerating voltage. Sample of HRTEM were prepared by placing a drop of gold suspension on a clear, dry 300 mesh copper grid coated with carbon film and allowed to dry for few hour. The typical HR-TEM image of GNPs had clear lattice fringes.The X-ray diffraction pattern (XRD) of GNPs was confirmed by using the Rich-Seifert XRD 3000P. The scanning was done in the region of the 2θ angle from 20° to 80°.Typical X-ray diffraction pattern of the sample deposited on glass surface is in good agreement of the cubic phase gold nanocrystal. The diffraction peaks, which appeared at 38.2°, 44.4°,

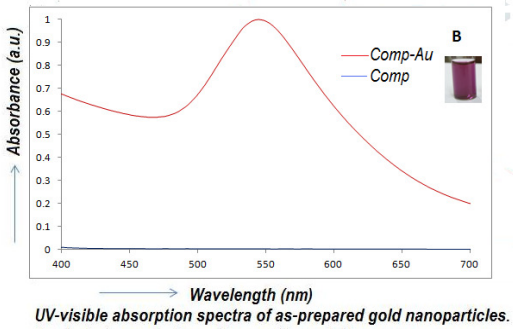
64.8°, and 78.8°, were recognized to the (111), (200), (220), and (311) planes, respectively. The distinctive selected areas electron diffraction (SAED) pattern with bright circular rings suggested that the GNPs were highly crystalline.

III. RESULTS AND DISCUSSION

A solution of compound in ethanol was prepared and KOH was added with stirring. Addition of yellow solution of gold-salt changes the color slowly which suggested the formation of GNPs. UV-Vis spectra of GNPs displayed surface plasmon resonance (SPR) band at about 547 nm (**Fig. A**) for (±)-hydrangenol according to the method A but a sharp peak was obtained for (±)-thunberginol-G without use of KOH solution (**Fig. B**) according to the method B. Bragg reflections of fcc gold (**Fig. 1**) are thus in agreement with the electron diffraction results (**Fig. 2**).



UV-visible absorption spectra of as-prepared gold nanoparticles. Inset photograph shows the reaction solution.



UV-visible absorption spectra of as-prepared gold nanoparticles. Inset photograph shows the reaction solution.

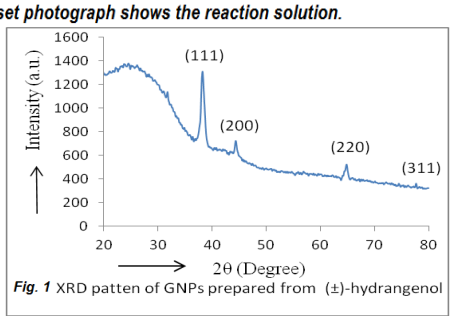


Fig. 1 XRD patten of GNPs prepared from (±)-hydrangenol

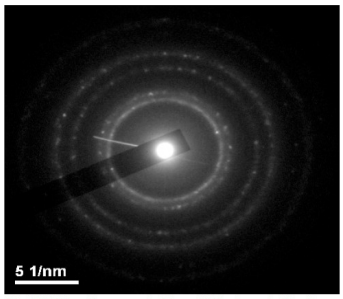
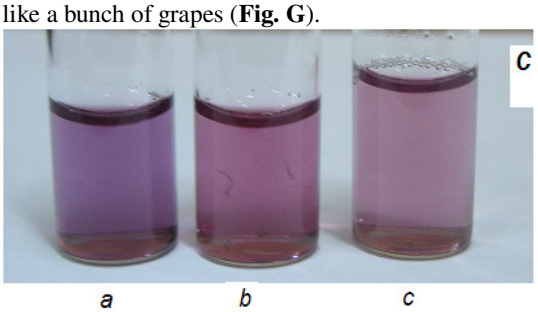
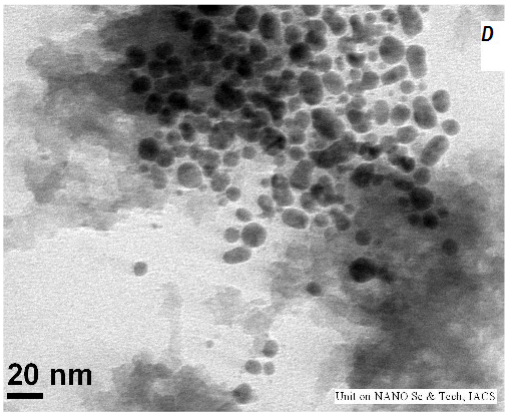


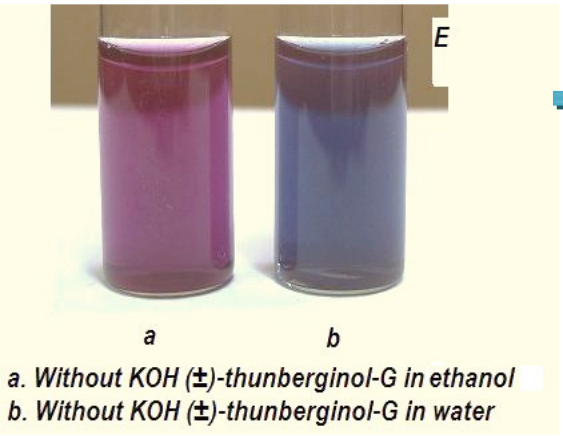
Fig. 2 SAED pattern recorded from GNPs shown in Fig. D

For (±)-hydrangenol the intensity of the SPR band does not increase to an expected extent but remains broad over time which means there may be an aggregation of the particles with distribution of broad rang particles size. The color of the reaction solution was bluish pink (**Fig. C, a**). This is a good agreement of polydispersed GNPs as observed by TEM image (**Fig. D**). So, it was considered by checking the stability of the GNPs by altering the using and not using of base which support the formation of GNPs rapidly including the solubility of the template. A comparison using with and without KOH solution showed that sharp SPR band was obtained without KOH solution from the pink color GNPs solution (**Fig. E, a**) of (±)-thunberginol-G. At high magnification, monodispersed GNPs was observed by TEM image (**Fig. F**) but in low magnification it shows like a bunch of grapes (**Fig. G**).

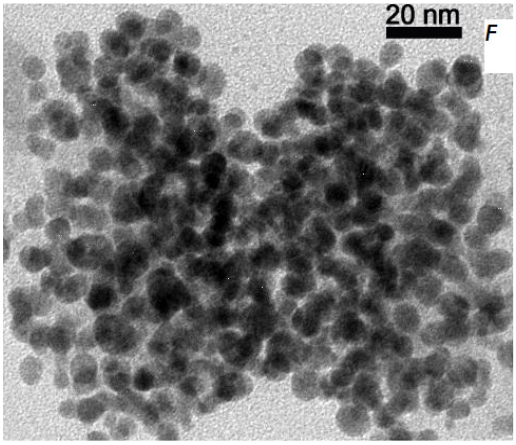


a. With KOH (±)-hydrangenol in ethanol
b. without KOH (±)-thunberginol-G in ethanol
c. Without KOH (±)-hydrangenol in ethanol



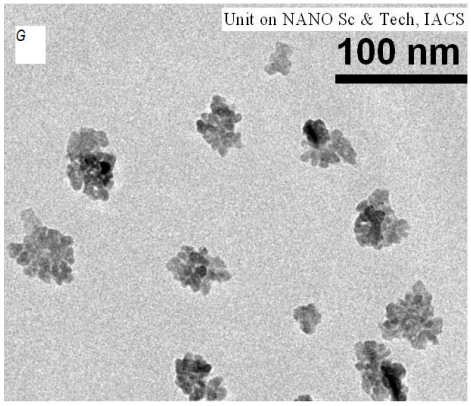


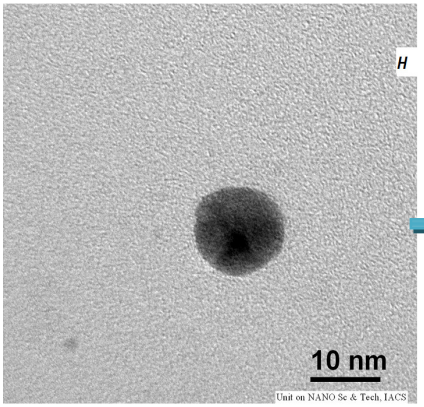
Solutions of GNPs synthesized/stabilized by (±)-hydrangenol or (±)-thunberginol-G (**Fig. C and E**)

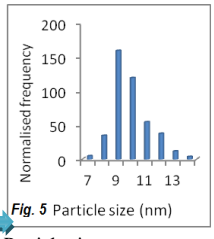


GNPs synthesized/stabilized by (±)-hydrangenol (**Fig. D**) and (±)-thunberginol-G (**Fig. F**)

UV-vis spectra show a shift of λ_{max} value from 548 nm to 535 nm within 50 minutes (**Fig. 3&4**) with increasing intensity of absorbance. This solution remains stable for a period of month as no change in λ_{max} value was observed. TEM image (**Fig. H&I**) of well separated GNPs was taken from low dense area which show average particle size is around 9-10 nm (**Fig. 5**). However the use of water as only solvent shows the opposite trend in UV-vis spectra (**Fig. 6&7**) that is a shift of λ_{max} value towards higher wavelength with broadening. The intensity gradually falls with longer time and the SPR band becomes broad which suggests aggregation of the metal particles. Polydispersed GNPs was observed by TEM image (**Fig. J**) suggesting the distribution in good agreement with the spectra. So, use of ethanol is crucial to prevent the aggregation of GNPs (**Fig. E**). It is expected as the template is partially water soluble. This aggregation is also a common when it is carried out beyond the room temperature. Biomolecules on the surface of GNPs cannot be observed under TEM image but visible in FE-SEM. This data was recorded after a month of preparation of the solution of **Fig. A** just to know the morphology of the template and the particles (**Fig. K**).







Particle size distribution histogram of more than 100 particles shown in images similar to Fig. G

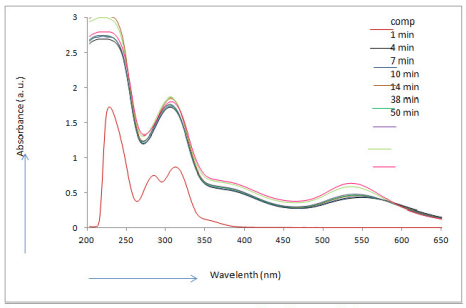
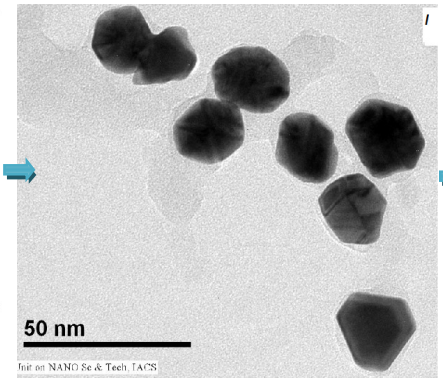


Fig. 3 UV-vis Spectra of (±)-thunberginol-G in ethanol-water with time



min	Abs.	λmax
0	0:02	~545
1	0.429	548
4	0.442	546
7	0.46	544
10	0.464	542
14	0.481	537
38	0.588	535
50	0.642	535

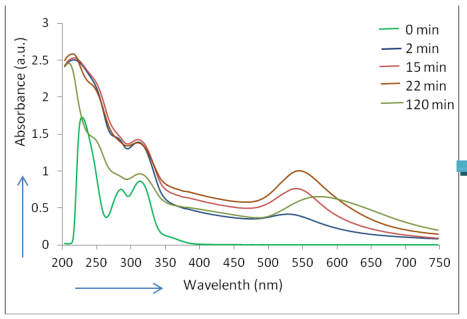
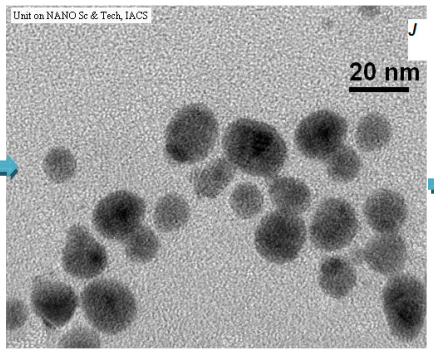


Fig. 6 UV-vis specta of (±)-thunberginol-G in water with time



min	Abs.	λmax
0	0:02	~545
2	0.418	529
15	0.761	541
22	1.001	545
120	0.656	575

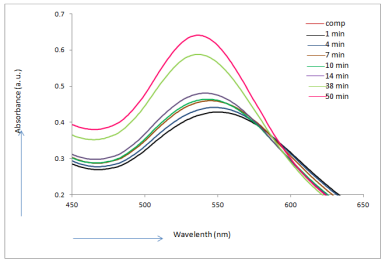


Fig 4. UV-vis Spectra of (±)-thunberginol-G in ethanol-water with time (Expanded)

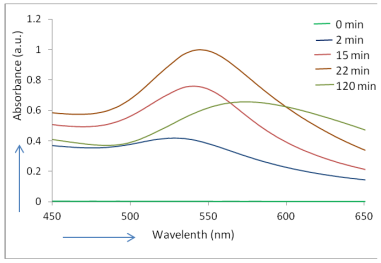


Fig. 7 UV-vis specta of (±)-thunberginol-G in water with time (Expanded)

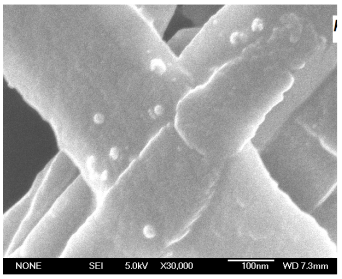


Fig. 3, 4, 5, 6 represents UV-Vis spectra of GNPs synthesized/stabilized by (±)-thunberginol-G
Fig. G, H, I, J represents GNPs synthesized/stabilized by (±)-thunberginol-G.

IV. CONCLUSION

In conclusion, author is successful to report that (±)-hydrangenol and (±)-thunberginol-G are capable to reduce gold ion to yield highly stable gold nanoparticle even after several days under aqueous condition in ethanol-water media and don't show any particles growth or loss of solubility in room temperature. It is expected that the newly developed method may bring opportunity to be useful in the field of biomedicine, biomarker, biosensor, bioseparations, bioimaging, biotherapy including catalysts, optical probes. At present due to limitation of the infrastructure it is unfortunate to show significant application in the above mentioned field but surely there is a scope in near future and will be published elsewhere.

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