Research Article

Bryo-Synthesis of Gold Nanoparticles

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ABSTRACT

Nano technological revolution unfolded a new arena of interdisciplinary research involving biology, chemistry, pharmaceutical engineering and medicine. The secret gleaned from nature had led to the generation of biogenic machinery for the manufacture of nanomaterials. Present investigation disclosed the gold nanoparticles' biosynthesizing capability of the gametophyte of a bryophyte for the first time. Rapid, cost-effective, one-step process of synthesis had been achieved. Newly genre AuNPs were confirmed by a change in extract colour from light yellow to dark red and surface plasmon resonance spectra obtained in a range of approximately 540 nm. Nanoparticles with an average diameter of 70 ± 5 nm were generated. Different instrumental techniques, like TEM, XRD, EDAX, particle size analyzer, and FTIR, were used to characterize the synthesized AuNPs.

Keywords: Biological Synthesis, Bryophyte Gametophyte Extract, Gold Nanoparticles, *Taxithelium nepalense*.

INTRODUCTION

Colloidal gold had been used for medicinal purpose from time immemorial. In ayurvedic medicine *Swarna Bhasma* (gold ash) is used for rejuvenation and revitalization.[1] In Ayurved Prakasha and Rasa Chandanshu, it is mentioned that any disease not curable with all available medicines can be cured by use of gold.[2-3] Even modern science accepted its medicinal importance and used it in form of its salt, colloidal solution and nano-particles.[4-7] Gold nanoparticles due to its biocompatibility and strong interaction with soft bases played a major role in the treatment of cancer.[8] Recent investigations revealed that gold nanoparticles could inhibit ovarian cancer, metastasis,[9-10] function of VEGF,[11] chronic lymphatic leukemia,[12] rheumatoid artharitis,[13] inflammation[14-16] etc. It had shown a strong vermifugal activity[17] and antimicrobial potentiality.[18] Conventional synthetic methods of AuNPs had involved a number of chemical methods. With the rapid development of these chemical methods, concern for environmental contaminations was regularly heightened as the chemical procedures involved in the synthesis of nanomaterial generates a large amount of hazardous by-products. Thus, there was an urgent need for 'green chemistry' that includes clean, nontoxic and environment friendly methods of nanoparticle synthesis with precise control over the shape and size. In the recent years, 'green synthesis' of the nanoparticles had paid much more attention in the rapidly growing area of nanoscience and nanotechnology.[19] Furthermore, because of the increase demand of AuNPs in medicine and many industrial applications there was also a growing need for cost effective production system. Therefore, there had been tremendous excitement in the study of gold nanoparticle synthesis by using biological system. The biological method for the synthesis of gold nanoparticles utilized different biological agents like bacteria, fungi, actinomycetes, algae and higher plants.[20-22]

Working towards the goal to enlarge the scope of the biosynthesis of nano-materials, we explore the potential of a bryophyte, *Taxithelium nepalense* (Schwagr.) Broth., gametophyte extract for the first time to reduce tetrachloroauric acid to gold nanoparticles.

MATERIALS AND METHODS

Reagents and Chemicals

Tetrachloroauric acid ($HAuCl_4$, $6H_2O$) was obtained from Sigma Aldrich. Freshly prepared double distilled water was used throughout the experimental work.

Collection of specimen

The specimens were collected from Narendrapur Ramakrishna Mission Ashrama, Narendrapur, Kolkata, India. The specimen were brought to the laboratory and identified following standard literature.[23]

Biological synthesis of gold nanoparticles

The gametophyte of *Taxithelium nepalense* was thoroughly washed several times with Milli-Q water. 10 g of the plant bodies were quick frozen in liquid nitrogen. The frozen tissue was powdered in a mortar in liquid nitrogen and then homogenized in 10 ml of Milli-Q water using a polytron at full speed. The Milli-Q water was added further at a ratio of 1:10 of tissue (weight) to water (volume). It was then filtered through Whatman filter paper no. 1. The filtrate was used as reducing agent and stabilizer. Then in a typical experiment, 40 mL of that filtered broth was added to 60 mL aqueous chloroauric acid ($HAuCl_4$) solution (3 mM final concentration) and agitated for 12 hours at room temperature (37°C).

Simultaneously, only the filtered broth of *Taxithelium nepalense* and only chloroauric acid solution were maintained under same conditions. Within 12 hours dark red solution was obtained.

The gold nanoparticles were separated out by centrifugation at 12000 g for 10 min, and the settled nanoparticles were washed with Milli-Q water (three times). The purified AuNPs were resuspended in Milli-Q water and ultrasonicated by Piezo-u-sonic ultrasonic cleaner (Pus-60w). Synthesis of AuNPs was repeated for three times (n=3) and subsequently utilized for characterization of the particles.

UV-visible spectroscopy

The absorption at 540 nm (maximum absorbance observed in the full range scan) was measured continuously to determine the stability of the solution. The UV-visible spectra of the solution were kinetically monitored in the range of 200–900 nm recorded in a Hitachi 330 spectrophotometer. Milli-Q water was used as blank.

Size measurement by dynamic light scattering (DLS) experiment

Particle size was measured by laser diffractometry using a nano size particle analyzer (Zen 1600 Malvern USA) in the range between 0.6 nm and 6.0 µm, under the following conditions: particle refractive index 1.590, particle absorption coefficient 0.01, water refractive index 1.33 and temperature 25°C.

X-ray diffraction (XRD) analysis

After bioreduction, the liquid reaction mixture was dried at 45°C in a vacuum drying oven. The vacuum dried mixture was then collected and used for powder XRD analysis. The spectra were recorded in a PW. 3040/60 PANalytical X-ray diffractometer (Cu Kα radiation, λ 1.54443) running at 45 kV and 30 mA. The diffracted intensities were recorded from 30° to 90° 2θ angles.

Energy dispersive X-ray (EDX) analysis

Energy dispersive X-ray (EDX) analysis was carried out by the Hitachi S 3400N instrument (Japan) and employed to know the elemental compositions of the particles. Samples were filtered and dried before measurements.

Fourier transforms infrared spectroscopic (FTIR) analysis

For FTIR analysis, the vacuum dried bio-reduced AuNPs were mixed separately with potassium bromide (KBr) at a ratio of 1:100 and the spectra were recorded with a Shimadzu 8400S Fourier transform infrared spectrophotometer using a diffuse reflectance accessory. The scanning data were obtained from the average of 50 scans in the range between 4000 and 400 cm^{-1}.

Transmission electron microscopic (TEM) observation of gold nanoparticles

TEM samples of the aqueous suspension of AuNPs were prepared by placing a drop of the suspension on carbon coated copper grids and allowing the water to evaporate. The micrographs were obtained by Tecnai G^2 spirit Biotwin (FP 5018/40) TEM, operated at 80 kV accelerating voltage.

RESULTS AND DISCUSSION

Characterization of the specimen

Plant body robust, yellow green, corticolous, dull to slightly glossy plants forming dense tufts. Main stem long, creeping, irregularly giving rise, leaves dense crectopatent (appressed to stem when dry), strongly concave, ovate with acute tips. Capsule inclined or horizontal. Curved constricted under mouth when dry, oval, 1.28 × 0.5 mm. Operculum conical, apiculate, calyptras cucullate. Peristome double, hypnoid, ~330 µ high, cilia single, basal membrane high. Spores 13 to 18 µ in diameter. The specimen was identified as *Taxithelium nepalense* (Schwagr.) Broth.[23]

Figure 1: Digital photograph of the gametophyte of *Taxithelium nepalense* used in the biosynthesis of gold nanoparticles.

Production and characterization of gold nanoparticles

It is well known that gold nanoparticles exhibit dark red colour in aqueous solution due to excitation of surface plasmon vibrations in gold nanoparticles.[21] Reduction of the gold ion to gold nanoparticles during exposure to the bryophyte gametophyte extract could be followed by colour change. Only the reaction mixture displayed a time dependent colour change, where as the broth and the chloroauric acid solution was observed to retain their original colour (Figure 2 inset). At the beginning, the reaction mixture was light yellow and became pinkish after one hour of incubation at room temperature (37°C); the pinkish colour changed to dark red colour gradually at 12 hour and then the red colour did not change with increasing incubation time. The appearance of the red colour indicated the occurrence of the reaction and the formation of AuNPs.

UV-Visible spectroscopic analysis

The characteristic red colour of the reaction solution was due to the excitation of the surface plasmon vibration of

Au^0 particles and provided a convenient spectroscopic signature of their formation. Both the control set showed no significant colour change in the same experimental conditions. The reduction of chloroauric acid was subjected to spectral analysis by using the UV-visible spectrophotometer. This showed an absorbance peak at 540 nm, which was specific for gold nanoparticles.[24]

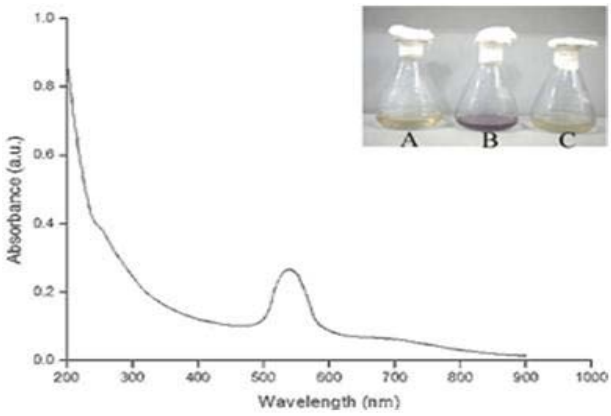

Figure 2: UV–visible spectra recorded as a function of reaction time of an aqueous solution of 3 mM $HAuCl_4$ with the bryophyte gametophyte extract. Inset: Represents three flasks containing only chloroauric acid solution **(a)**, reaction mixture of the filtered gametophyte extract with chloroauric acid **(b)** and only the filtered gametophyte extract of *Taxithelium nepalense* **(c)** The appearance of red colour only in the flask (b) due to the addition of filtered gametophyte extract of *Taxithelium nepalense* to 3×10^{-3} M aqueous $HAuCl_4$ solution after 12 h incubation period.

Particle size measurement

Particle size was determined by dynamic light scattering measurement. Laser diffraction revealed that particle size obtained in the range of 42 to 145 nm with an average diameter of 70 ± 5 nm.

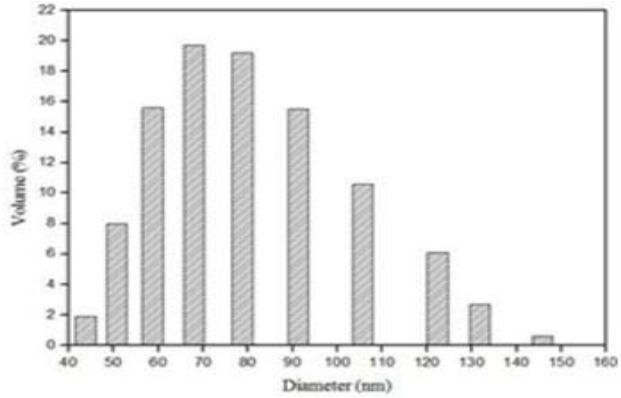

Figure 3: Histogram of particle size distribution as obtained from light scattering of the gold nanoparticles produced by the gametophyte extract of *Taxithelium nepalense*.

XRD analysis of the gold nanoparticles

In order to confirm the mono-crystalline nature of the AuNPs, XRD analysis was performed. Figure 4 showed the X-ray diffraction pattern obtained from the AuNPs. The Bragg reflections obtained from the AuNPs clearly correspond to the fcc crystalline structure of gold. The XRD pattern exhibited four identical diffraction peaks corresponding to the [111], [200], [220] and [311] appearing at 2θ = 38.2°, 44.5°, 65.6° and 78.6° of metal gold, respectively (International Centre for Diffraction Data, ICDD No. 04–0783), indicating that the precipitate was composed of pure crystalline gold.[24]

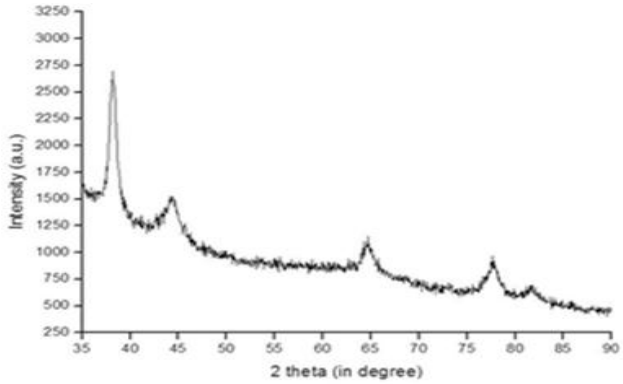

Figure 4: Representative XRD patterns of gold nanoparticles synthesized by the reaction of 3 mM aqueous $HAuCl_4$ solution with gametophyte extract of *Taxithelium nepalense*.

EDX observation of gold nanoparticles

An elemental composition analysis employing EDX showed the presence of a strong signal from gold atoms. Moreover, the presence of sharp optical absorption peak in the range of 2 to 3 keV which is typical for the absorption of metallic gold nano-crystallites.[25] This analysis indicated that the nanostructures were composed solely of gold.

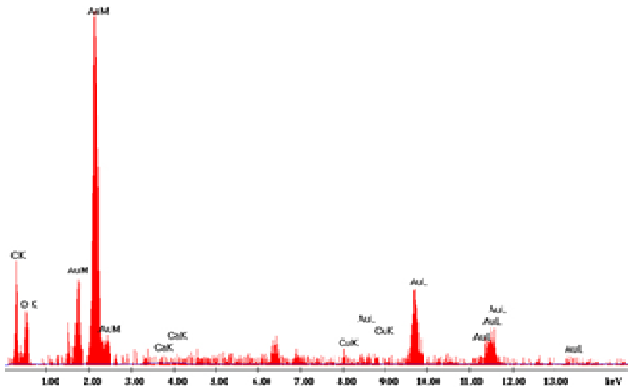

Figure 5: Recorded EDX spectrum showing sharp peak in between 2 and 3 keV confirming the presence of gold.

FTIR analysis of gold nanoparticles

FTIR absorption spectra of biosynthesized vacuum dried gold nanoparticles were shown in the figure 6. The spectra showed the presence of bonds due to O-H stretching (around ~3430 cm^{-1}) and aldehydic C-H stretching (around ~2920 cm^{-1}).[26] These peaks indicated the presence of proteins and other organic residues, which might have produced extracellularly by *Taxithelium*

nepalense. FTIR spectrum of AuNPs showed absorption band at 1632 cm^{-1}, corresponding to the amide I of polypeptides.[26] The peak at 1404.8 cm^{-1} may be assigned to the symmetric stretching of the carboxyl side groups in the amino acid residues of the protein molecules.[27] The FTIR peak at around 1240-1260 cm^{-1} signified amide III band of the random coil of protein.[28] Band at around 1052 cm^{-1} indicated C-O stretching.[29] These findings supported the results of Gole et al., who reported that proteins can bind to nanoparticles either through amino groups or cysteine residue or through electrostatic attraction.[30] Furthermore, Ahmad et al. and Xie et al. reported that protein can interact with AuNPs either through free amine, carboxyl or phosphate groups to stabilize them.[31-32] Thus, it can be concluded that the AuNPs are stabilized by surface bound protein molecules that also prevent aggregation.

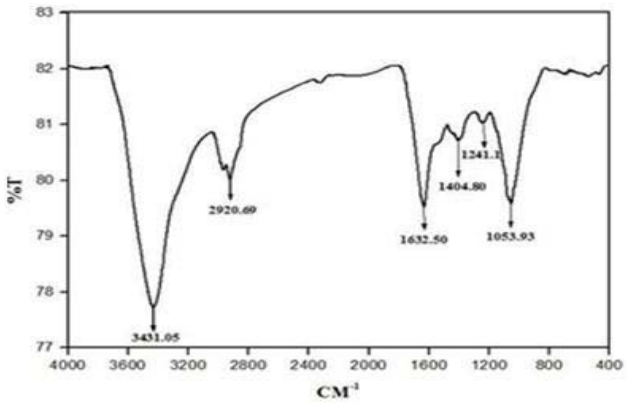

Figure 6: FTIR absorption spectra of bio-reduced chloroauric acid.

TEM image of gold nanoparticles

Transmission electron microscopic (TEM) image recorded different sizes of AuNPs which arose from the bioreduction of chloroauric acid by bryophyte gametophyte extract at room temperature (37°C) for 12 h. These observations revealed that spherical, triangular as well as hexagonal structures of the AuNPs were formed in the reaction solution. The diameters of these AuNPs were measured and the size was in the range of 42 to 145 nm. The average diameter of these AuNPs was of 70 ± 5 nm.

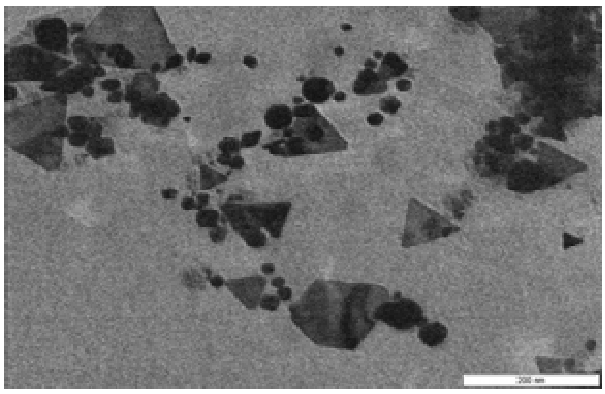

Figure 7: Transmission electron micrograph of gold nanoparticles after bioreduction of chloroauric acid.

CONCLUSION

Nanoparticles synthesis from biological route served as an important alternative in the development of clean, nontoxic, economical and environmentally friendly procedures. Here, we proposed a simple eco-friendly method for gold nanoparticles synthesis using bryophyte gametophyte extract. The bioreduction of chloroauric acid by *Taxithelium nepalense* yielded AuNPs in the range of 42 to 145 nm. The spectroscopic characterizations using various analytical instruments were useful in proving the composition of the nanoparticles and also in confirming their size and shape. FTIR evidenced the formation and stability of the bio-synthesized AuNPs which can be studied further to understand the chemical and molecular interactions responsible for the synthesis of the nanoparticles. Potential benefits of gold nanotechnology in medicine were inimitable owing to refined, highly targeted drug delivery and imaging platforms and unique transfection, as well as analytical and tissue engineering approaches. Thus, this simple and rapid, green synthesis of AuNPs can further be applied in various biomedical and biotechnological fields and their properties and applications can further be explored.

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REFERENCES

1. Hillyer JF, Albrecht RM, Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles, Journal of Pharmaceutical Sciences, 90, 2001, 1927–1936.
2. Mishra GS, Ayurved Prakash: Arthavidyotini and Arthaprakashini Sanskrit and Hindi Commentaries, Reprint edi., Chaukhamba Bharati Academy, Varanasi, 3/35, 1999, 352.
3. Savrekar SS, Rasa Chandanshu, C.C.R.A.S., New Delhi, Purva Khanda, 2011, 81.
4. Brown CL, Bushell G, Whitehouse MW, Agarwal DS, Tupe SG, Paknikar KM, Tiekink ERT, Nanogold Pharmaceutics (1) The use of gold to treat experimentally induced arthritis in rat models (2) Characterization of the gold in Swarna Bhasma: A microparticulate used in traditional Indian medicine, Gold Bulletin, 40, 2007, 3.
5. Boisselier E, Astruc G, Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity, Chemical Society Reviews, 38, 2009, 1759-1782.
6. Chottopadhyay A, Sarkar M, Biswas NM, Effect of gold on stimulation of reproductive function in immature female albino rats, Indian Journal of Experimental Biology, 44, 2006, 971-975.
7. Biswas NM, Chottopadhyay A, Sarkar M, Effect of gold on testicular steroidogenic and gametogenic functions in

immature female albino rats, Life Science, 76(6), 2004, 629-636.

8. Bhattacharya R, Mukherjee P, Biological properties of naked nanoparticles. Advanced Drug Delivery Reviews, 60, 2008, 1289–1306.
9. Chen PC, Mwakwari SC, Oyelere AK, Gold nanoparticles: from nanomedicine to nanosensing, Nanotechnology, Science and Applications, 1, 2008, 45–66.
10. Edelman ER, Seifert P, Groothuis A, Morss A, Bornstein D, Rogers C, Gold-coated NIR stents in porcine coronary arteries. Circulation, 103, 2001, 429–434.
11. Bhattacharya R, Patra CR, Verma R, Griepp PR, Mukherjee P, Gold nanoparticles inhibit the proliferation of multiple myeloma cells, Advanced Materials, 19, 2007, 711–716.
12. Zent CS, Call TG, Hogan WJ, Shanafelt TD, Kay NE, Uptake on risk-stratified management for chronic lymphocytic leukemia, Leukemia and Lymphoma, 47, 2006, 1738–1746.
13. Nam J, Won N, Jin H, Chung H, Kim S, pH-induced aggregation of gold nanoparticles for photothermal cancer therapy, Journal of American Chemical Society, 131, 2009, 13639–13645.
14. Norton S, A brief history of potable gold, Molecular Interventions, 8(3), 2008; 120-125.
15. Jeon KI, Byun MS, Jue DM, Gold compound auranofin inhibits Ikappa B kinase (IKK) by modifying Cys-179 of IKKbeta subunit, Experimental and Molecular Medicine, 35, 2003, 61-66.
16. Kim NH, Lee MY, Park SJ, Choi JS, Oh MK, Kim IS, Auranofin blocks interleukin-6 signalling by inhibiting phosphorylation of JAK1 and STAT3, Immunology, 122, 2007, 607-614.
17. Kar PK, Murmu S, Saha S, Tandon V, Acharya K, Anthelmintic Efficacy of Gold Nanoparticles Derived from a Phytopathogenic Fungus, *Nigrospora oryzae*. PLoS One, 9(1), 2014, 1-9.
18. Mollick MMR, Bhowmick B, Mondal D, Maity D, Rana D, Dash SK, Chattopadhyay S, Roy S, Sarkar J, Acharya K, Chakraborty M, Chattopadhyay D, Anticancer (in vitro) and antimicrobial effect of gold nanoparticles synthesized using *Abelmoschus esculentus* (L.) pulp extract via a green route. RSC Advances, 2014, (PUBLISHED ONLINE) DOI: 10.1039/c4ra07285e.
19. Tikariha S, Singh S, Banerjee S, Vidyarthi AS, Biosynthesis of gold nanoparticles, scope and application: a review, International Journal of Pharmaceutical Sciences and Research, 3(6), 2012, 1603-1615.
20. Acharya K, Sarkar J, Deo SS, Mycosynthesis of nanoparticles. In: Bhowmik PK, Basu SK, Goyal A (eds) Advances in biotechnology, Bentham Science Publishers Ltd, 2009, 204-215.
21. Sarkar J, Ray S, Chattopadhyay D, Laskar A, Acharya K, Mycogenesis of gold nanoparticles using a phytopathogen *Alternaria alternate*, Bioprocess and Biosystems Engineering, 35, 2012, 637-643.
22. Sarkar J, Ray SK, Laskar A, Chattopadhyay D, Acharya K, Bioreduction of chloroaurate ions to gold nanoparticles by culture filtrate of *Pleurotus sapidus*, Materials Letters, 92, 2013, 313–316.
23. Gangulee HC, Hand book on Indian Mosses, Amarind Publication, New Delhi, 1985.
24. Verma VC, Singh SK, Solanki R, Prakash S, Biofabrication of anisotropic gold nanotriangles using extract of endophytic *Aspergillus clavatus* as a dual functional reductant and stabilizer, Nanoscale Research Letters, 6, 2011, 16-23.
25. Song JY, Jang H-K, Kim BS, Biological synthesis of gold nanoparticles using Magnolia kobus and Diopyros kaki leaf extracts, Process Biochemistry, 44, 2009, 1133-1138.
26. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN, Biosynthesis of silver nanoparticles using Coriandrum Sativum leaf extract and their application in nonlinear optics, Advanced Science Letters, 3, 2010, 01-06.
27. Das SK, Das AR, Guha AK, Gold nanoparticles: microbial synthesis and application in water hygiene management, Langmuir, 25, 2009, 8192-8199.
28. Cai S, Singh BR, A distinct utility of the amide III infrared band for secondary structure estimation of aqueous protein solutions using partial least squares methods, Biochemistry, 43, 2004, 2541-2549.
29. Renuga Devi TS, Gayathri S, FTIR and FT-Raman spectral analysis of paclitaxel drugs, International Journal Pharmaceutical Science Review and Research, 2, 2010, 106-110.
30. Gole A, Dash C, Ramakrishnan V, Sainkar SR, Mandle AB, Rao M, Pepsin-gold colloid conjugates: preparation, characterization, and enzymatic, Langmuir, 17, 2001, 1674-1679.
31. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M, Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, Thermomonospora sp. Langmuir, 19, 2003, 3550-3553.
32. Xie J, Lee JY, Wang DIC, Ting YP, High-yield synthesis of complex gold nanostructures in a fungal system, The Journal of Physical Chemistry C, 111, 2007, 16858-16865.

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