ISSN 0975-6299 Vol 2/Issue 4/Oct – Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

NANOTECCHNOLOGY

BIOSYNTHESIS OF GOLD NANOPARTICLES OF *IXORA COCCINEA* FLOWER EXTRACT & THEIR ANTIMICROBIAL ACTIVITIES

NAGARAJ B[1]*, KRISHNAMURTHY NB[1], LINY P[1], DIVYA TK[1] AND DINESH R[2]

[1]Department of Biotechnology, Shridevi Institute of Engineering & Technology, Sira Road, Tumkur-572106, Karnataka, INDIA

[2] Centre for Nanomaterials, International Advanced Research Centre for Powder Metallurgy & New Materials (ARCI), Balapur PO, Hyderabad - 500-005, INDIA.

NAGARAJ B

Department of Biotechnology, Shridevi Institute of Engineering & Technology, Sira Road, Tumkur-572106, Karnataka, INDIA

*Corresponding author

ABSTRACT

The synthesis of eco-friendly nanoparticles is evergreen branch of nanoscience for biomedical application. Low cost of synthesis and non toxicity are the main features which make it more attractive potential option for biomedical field. Here, we report the synthesis of gold nanoparticles in aqueous medium using flower extracts of *Ixora coccinea* (Chetty flower) as reducing and stabilizing agent. On treating chloroauric acid solution with extract, rapid reduction of chloroaurate ions is observed leading to the formation of the highly stable gold nanoparticles in solution. The synthesized nanoparticles are confirmed by color changes and it has been characterized by UV-visible spectroscopy. The UV- visible spectra indicate a strong Plasmon resonance that is located at ~550 nm. Presence of this strong broad plasmon peak has been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm. The morphology and size of the biologically synthesized gold nanoparticles were determined using TEM. The images clearly show that the average size of the nanotriangles is about 200 nm, while, the spherical like particles show very small size about 5-10 nm. The study also shows that gold nanoparticles with antibiotic show more inhibitory zones than compared to the standard antibiotics.

KEY WORDS

Gold nanoparticles, Chloroauric acid, antimicrobial activity, UV-visible spectrophotometer.

INTRODUCTION

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology[1]. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease[2].

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and the potential use for human benefits[3]. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner[4].

Nowadays, the preparation of nanoscaled gold materials has become very important due to its unique properties, which are different from those of the bulk materials[5]. The properties of these particles in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanoparticles[6]. Production of noble metals such as gold, silver, and platinum nanoparticles are widely applied to human contacting areas as there is a growing need to develop environmentally friendly synthesis of nanoparticles that do not use toxic chemicals[7].

In the present study, Chetty flower extract has been used to synthesize gold nanoparticles. The Chetty flower comes under the Kingdom-Plantae, Family – Rubiaceae, Genus- *Ixora*, Species- *coccinea. Ixora coccinea* is said to be native to Asia and whose name is derived from an Indian deity (Fig 1). There are about 400 species spread from Africa to India to Southern Asia. They differ in leaf size, plant height, flower size and flower color. Growing wildly throughout Western ghats, also in gardens, this plant is spreading hard simple leaves, opposite elliptic, ovate, sessile and coriaceous. Flowers are scarlet red colored in dense corymbose cymes.

Some of the medicinal uses of the chetty plant parts are: to cure skin diseases, cure diarrhea, indigestion, ulcers, wounds, and it is also used as antiseptic. The flower of *Ixora* has anti inflammatory, aromatic, antipyretic properties and useful in extensive thirst and fatigue[8]. Fifty-four components have been identified from the flower extract of *Ixora coccinea*[9]. The root extract of *Ixora coccinea* shows good anthelmintic activity[10]. *Ixora coccinea* possess anti-inflammatory and also antitussive properties [11].

Figure 1
Chetty flower plant

MATERIALS AND METHODS

Flower extraction

The fresh flowers (20g) of chetty flower samples were collected from our college campus itself and authenticated by the department of Applied Botany, University of Mysore. Collected fresh flowers were washed, finely cut and soaked in 100ml boiling distilled water for 5-10 min and then it was filtered through Whatman filter paper no.1.

Synthesis of gold nanoparticles:

In a typical experiment, gold nanoparticles were synthesized by taking 5ml of flower extract and then it was added into 45ml 0.002M $AuCl_4$ obtained from Loba Chemie Pvt. Ltd. Mumbai and kept in dark for 3-4 hours. Within an hour cherry red solution was obtained. The gold nanoparticles solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant was discarded and the pellet was dissolved in deionized water. The bioreduction of Au^{3+} in aqueous solution was monitored by periodic sampling of aliquots of the suspension. The synthesized nanoparticles were screened for its antibacterial and antifungal activity by disc method.

Characterization

The gold nanoparticles were characterized by Elico SL 164 double beam UV-Visible Soectrophotometer[12]. The morphology of the samples was studied by high-resolution transmission electron microscopy (HRTEM; JEOL JEM-2010F).

Antifungal and antibacterial activity

Aspergillus niger, Aspergillus flavus, E.coli and *Streptobacillus sp.* were collected from authenticated stock culture in our college itself.

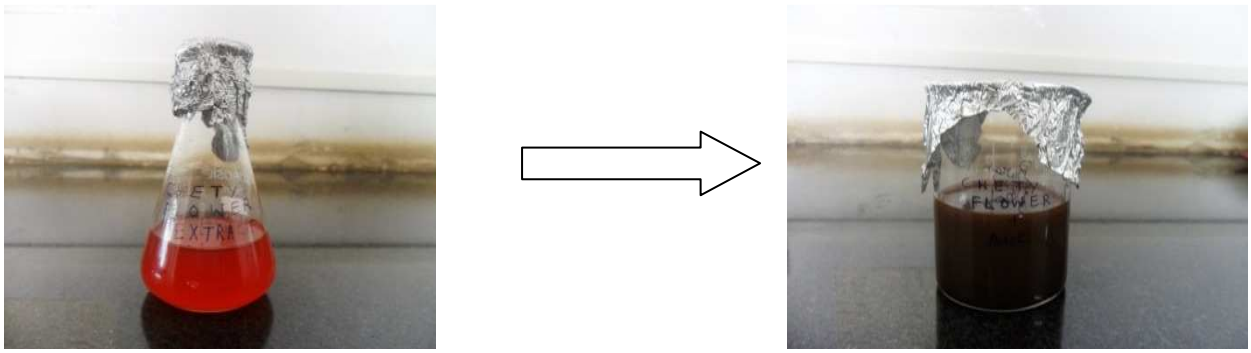
Culturing of Potato Dextrose Media

2.4g of potato dextrose broth and 2g of agar were dissolved in 100ml of distilled water. The contents were subjected to autoclaving at 121^{o} C for 20min at 15lbs pressure.

Potato dextrose agar media plate is prepared by pouring the nutrient agar media into the petriplates. The microbial suspension of *Aspergillus niger, Aspergillus flavus E.coli* and *Streptobacillus sp.* were spread over the media. The standard antibiotic disc was also placed in one side of the petriplates which was the control and the pretreated antibiotic discs with the synthesized nanoparticles in another side. The inoculated petriplates was covered and it was kept for incubation at room temperature.

RESULTS AND DISCUSSION

Gold nanoparticles were synthesized from Hydrogen tetra chloroaurate solution containing Au^+ ions by treating with the chetty flower extracts. The color of the solution changed to deep brownish color within 30 min of reaction with the Au^+ ions. The appearance of the deep brownish color indicated formation of gold nanoparticles (Fig 2).

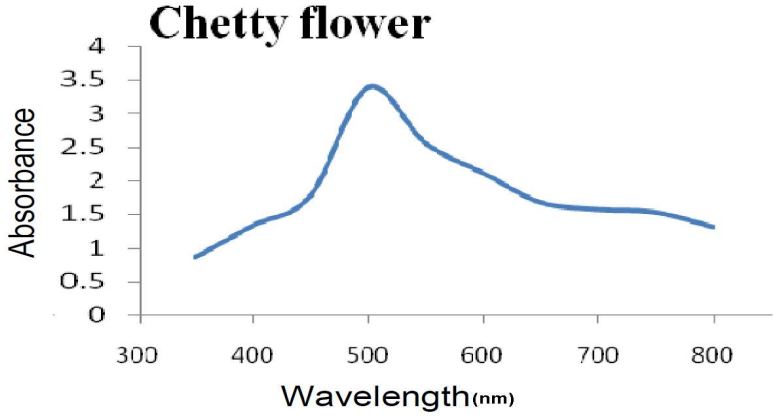


Before adding $AuCl_4$ After adding $AuCl_4$

Figure 2
Test for Chetty flower to show the synthesis of gold nanoparticles

The formation of gold nanoparticles was confirmed by color changes followed by UV-Visible spectrophotometer analysis. The UV-Visible spectrophotometer proved to be very useful technique for the analysis of some metal nanoparticles. The UV- visible spectra (shown in Graph 1) indicated a strong Plasmon resonance that was located at ~550 nm. Presence of this strong broad plasmon peak had been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm[13].

The microstructures and size of the biosynthesized gold nanoparticles were studied by TEM (Transmission Electron Microscopy) analysis. The typical TEM images of the gold nanoparticles synthesized by chetty flower extract as reducing agent is shown in Fig. 3. The micrograph shows formation of nanotriangles and spherical like morphology. The size of the nanotriangles is about 200 nm, while, the spherical like particles show very small size about 5-10 nm. Recently, some researchers have obtained gold nanotriangles by using Tamarind leaf[14] and *Aloe vera* plant extracts[15]. Gold nanoparticles have been obtained also by using *A. indica*[16].



Graph 1
UV-Visible spectrum of gold nanoparticles synthesized by Chetty flower extract.

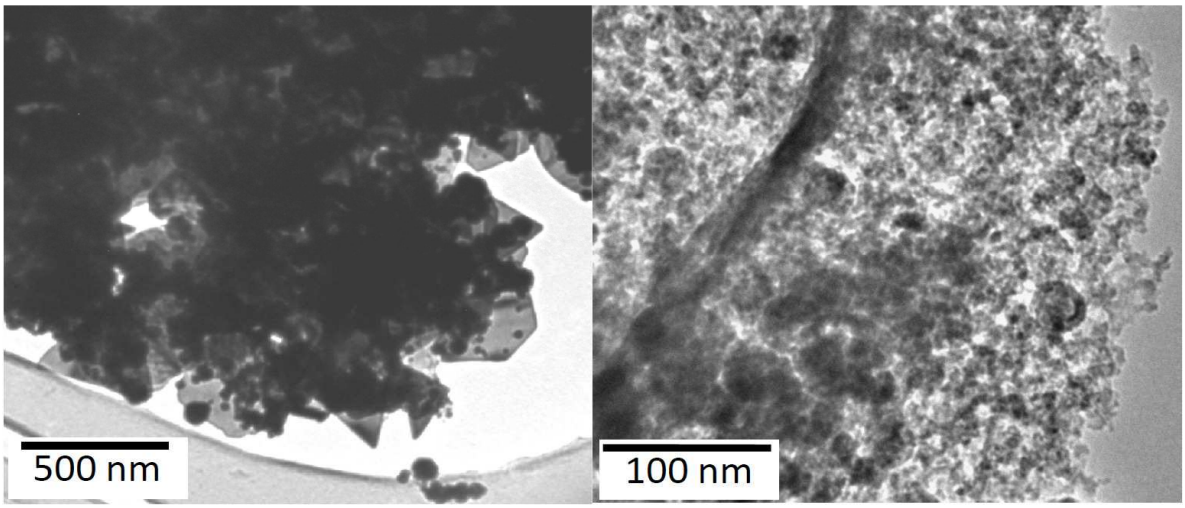


Figure 3
TEM images of gold nanoparticles synthesized from Chetty flower with triangle (left) and spherical like (right) nanoparticles.

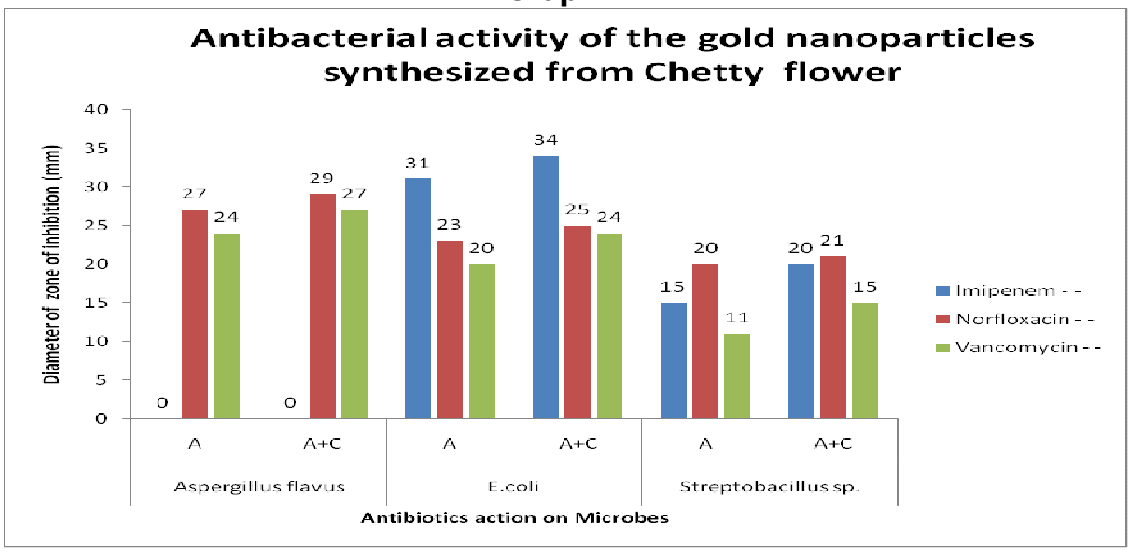
Anti bacterial study indicates that antibiotic + gold nanoparticles could be extracted from chetty flower (A+C) which exhibit more zone of inhibition when compared to standard antibiotics (A) used (Table 1 and Graph 2). The zone of inhibition on *E.coli* and *Streptobacillus sp*. is more when compared to the zone of inhibition exhibited by *Aspergillus flavus.* The extracted gold nanoparticles with the antibiotic Imipenem, Norfloxacin and Vancomycin (A+C) [34mm,25mm,24mm] exhibit high zone of inhibition when compared to the standard antibiotics (A). Imipenem [31mm], Norfloxacin [23mm] and Vancomycin [20mm] for the *E.coli*.(Fig 6). For the *Streptobacillus sp*.,extracted gold nanoparticles with the antibiotic Imipenem, Norfloxacin and Vancomycin (A+C) [20mm,21mm,15mm] exhibit high zone of inhibition when compared to the standard antibiotics (A) Imipenem [15mm], Norfloxacin[20mm] and Vancomycin [11mm] (Fig 7).

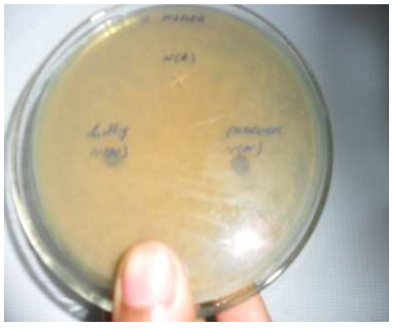
Table 1
Antibacterial activity of the gold nanoparticles synthesized from Chetty flower

Antibiotics	Diameter of zone of inhibition in mm							
	Organisms							
	Aspergillus niger		*Aspergillus flavus*		*E.coli*		*Streptobacillus sp.*	
	A	A+C	A	A+C	A	A+C	A	A+C
Imipenem	-	-	-	-	31	34	15	20
Norfloxacin	-	-	27	29	23	25	20	21
Vancomycin	-	-	24	27	20	24	11	15

On *Aspergillus flavus,* the zone of inhibition exhibited by the extracted gold nanoparticlesis with the antibiotic (A+C) Norfloxacin and Vancomycin [29mm,27mm] is more than the zone of inhibition exhibited by the standard antibiotic (A) Norfloxacin [27mm] and Vancomycin [24mm]. The pure antibiotic (A) Imipeneum and the extracted gold nanoparticles with the antibiotic(A+C) Imipeneum do not indicate any zone of inhibition (Fig 5). Equaling the standard antibiotics (A) Imipenem, Norfloxacin and Vancomycin and extracted gold nanoparticles with the antibiotic (A+C) do not show any zone of inhibition against the *Aspergillus niger* (Fig. 3).

Graph 2





Norfloxacin

Vancomycin

Imipenem

Figure 4
Antimicrobial activity of the gold nanoparticles synthesized from Chetty flower against Aspergillus niger

Norfloxacin *Vancomycin* *Imipenem*

Figure 5

Antimicrobial activity of the gold nanoparticles synthesized from Chetty flower against Aspergillus flavus

Norfloxacin *Vancomycin* *Imipenem*

Figure 6

Antimicrobial activity of the gold nanoparticles synthesized from Chetty flower against E.coli

Norfloxacin *Vancomycin* *Imipenem*

Figure 7

Antimicrobial activity of the gold nanoparticles synthesized from Chetty flower Against Streptobacillus sp.

Similar results were observed in many reports from flower extracts[17] (Padma and Dhara, 2010) but our report shows good results for antimicrobial activities of goldnanoparticles extracted from *Ixora coccinea* flower. Some authors reported the use of *Ixora coccinea* flower extract as an acid base indicator in different types of acid base titrations[18]. Some authours found that the active fraction from Ixora coccinea flowers prevented a decrease in body weight[19]. The alcoholic extract of the flowers of *Ixora coccinea* was studied for its effect on wound healing, using a dead space wound model in rats[20].

CONCLUSION

The gold nanoparticles synthesized using extracts of chetty flower samples was confirmed by color changes and was characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 500nm for gold nanoparticles. The TEM analysis shows large nanotriangle shape particle with 200 nm size and a small spherical like 5-10 nm size particles. This technique has proved to be very useful for the analysis of nanoparticles. Antimicrobial activity for the nanoparticles was carried out using standard antibiotics. Nanoparticles with antibiotic show more inhibitory zones than compared to the standard antibiotics. Hence, we conclude that the synthesized nanoparticles are more efficient in the drug delivery process.

ACKNOWLEDGEMENTS

We thank **Dr. M R Hulinaykar**, Managing Trustee, Sri Shridevi Charitable Trust (R.) and **Dr. M A Venkatesh**, Principal, SIET, Tumkur, India, for their encouragement to complete this investigations.

REFERENCES

1) Wim H De Jong and Paul JA Borm, Drug delivery and nanoparticles: applications and hazards. Int. J. Nanomedicine, 3(2): 133-149, (2011).
2) Patil M, Mehta DS and Guvya S, Future impact of nanotechnology on medicine and dentistry. J. Indian Soc. Periodontol, 12(2): 34-40, (2008).
3) Elumalai, EK, Prasad TNVKV, Hemachandran J, Viviyan S, Therasa, Thirumalai T and David E, Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. J. Pharm. Sci. & Res., 2 (9): 549-554, (2010).
4) Sastry M, Ahmad A, Khan MI and Kumar R, Niemeyer CM and Mirkin CA. Microbial nanoparticle production in Nanobiotechnology, ed. by Wiley-VCH, Weinheim, 126-127 (2004).
5) Armendariz, V, Size controlled gold nanoparticle formation by *Avena sativa* biomass. Journal of Nanoparticle Research, 6: 377-382, (2004).
6) Haverkamp RG, Pick your carats: nanoparticles of gold-silver-copper alloy produced *in vivo.* Journal of Nanoparticle Research, 9: 697-700, (2007).
7) Song JY, Kim BS, Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng., 32(1): 79-84, (2009).
8) Allimuthu M, Pharmacological evaluation of jungle flame (Ixora coccinea, Rubiaceae), OpenMED@NIC. 1129, (2005).
9) Gloria UO and Gibson UN, chemical composition of essential oil of ixora coccinea flower from Port Harcourt. Nigeria International journal of academic research, 3(2) Part II, 381-384, (2011).

10) Surana AR, Aher AN, Pal SC and Deore UV, Evaluation of anthelmintic activity of Ixora coccinea, Int. J. of Pharm. & Life Sci., 2(6): 813-814, (2011).
11) Missebukpo A, Metowogo K, Agbonon A, Eklu-Gadegbeku K, Aklikokou K and Gbeassor M, Evaluation of Anti-asthmatic Activities of *Ixora coccinea* Linn (Rubiaceae). Journal of Pharmacology and Toxicology, 6: 559-570, (2011).
12) Padma SV and Dhara B, Facility for ecological and Analytical Testing. Indian Journal of Biochemistry and Biophysics, 47: 157-160, (2010).
13) Henglein A, Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. J. Phys. Chem., 97 (21): 5457–5471, (1993).
14) Ankamwar B, Chaudhary M, and Sastry M, Gold Nanotriangles biologically Synthesized using Tamarind leaf extract and Potential application in vapor sensing. Synthesis and Reactivity in inorganic, metal-organic, and Nano-Metal Chemistry, 35: 19-26. (2005).
15) Chandran SP, Chaudhary M, Pasricha R, Ahmad A and Sastry M, Synthesis of Gold Nanotriangles and Silver Nanoparticles using *Aloe vera* plant extract. Biotechnology Progress, 22(2): 577-583, (2006).
16) Thirumurugan A, Jiflin GJ, Rajagomathi G, Neethu AT, Ramachandran S and Jaiganesh, R, Biotechnological synthesis of gold nanoparticles of Azardirachta indica leaf extract. International Journal of Biological Technology, 1(1): 75-77, (2010).
17) Padma SV and Dhara B, Facility for ecological and Analytical Testing. Indian Journal of Biochemistry and Biophysics, 47: 157-160, (2010).
18) Anant D, Dhanraj J, Shashikant D, Ramling P and Ajay G, Flower Extract of Ixora coccinea as a Natural Indicator in Acid Base Titration. Journal of Pharmacy Research, 3(10): 2512-2513, (2010).
19) Latha PG and Panikkar KR, Chemoprotective effect of Ixora coccinea L. flowers on cisplatin induced toxicity in mice. Phytother Res., 15(4): 364-366, (2001).
20) Nayak BS, Udupa AL and Udupa SL, Effect of *Ixora coccinea* flowers on dead space wound healing in rats. Fitoterapia, 70(3): 233-236, (1999).