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PHYTOSYNTHESIS OF GOLD NANOPARTICLES USING *CAESALPINIA PULCHERRIMA* (PEACOCK FLOWER) FLOWER EXTRACT AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

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Caesalpinia pulcherrima (Linn) popularly known as Peacock flower in India belongs to family Caesalpiniace. In this work, we describe environment friendly technique for green synthesis of gold nanoparticles from AuCl4 solution using the Caesalpinia pulcherrima (Peacock flower) flower extract as reducing agent. The gold nanoparticles were characterized using UV-visible spectroscopy and transmission electron microscopy (TEM). The UV- visible spectra indicate a strong Plasmon resonance that is located at ~450 nm. The TEM analysis shows that products have spherical morphology with size ranging between 10-50 nm. The study also indicates that gold nanoparticles show good antimicrobial activities when compared to the standard antibiotics.

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1. Introduction

Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm. A new branch of nanotechnology is nanobiotechnology. Bionanotechnology is the integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for the synthesis of nanomaterials [1-6]. Nano scale particles have emerged as novel antimicrobial agents owing to the high surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistances against metal ions, antibiotics and the development of resistant strains [7].

Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. These methods of synthesis can be divided on intra cellular and extracellular [8]. Silver nanoparticles synthesized by various techniques have received special attention because they have found potential application in many fields such as catalysis, sensors, drags delivery system. Additionally, silver nanoparticles possess an excellent biocompatibility and low toxicity [9-10].

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Nanoparticles can be broadly grouped into two: namely organic and inorganic nanoparticles, due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic nanoparticles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nanomaterials have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good biocompatibility, capability of targeted drug delivery and controlled release of drugs [11-14]. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo therapy of biological targets [15].

Herein we report the synthesis of gold nanoparticles by the reduction of aqueous $AuCl_4$ with *caesalpinia pulcherrima* (peacock flower) flower extract. The *caesalpinia pulcherrima* comes under the Kingdom- Plantae, Family – Fabaceae, Genus- *Caesalpinia*, Species- *pulcherrima.* In traditional Indian medicine *C. pulcherrima* is used in the treatment of tridosha, fever, ulcer, abortifacient, emmenagogue, asthma, tumors, vata and skin diseases. Flowers are known for its medicinal values such as cholera, malaria, bronchitis. *Caesalpinia pulcherrima* flower has been used as an analgesic and anti inflammatory [16], Antioxidant [17], anthelmintic [18].

Fig. 1 Caesalpinia pulcherrima flower plant

2. Materials and methods

Flower extraction

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

Synthesis of gold nanoparticles and purification:

In a typical experiment gold nanoparticles were synthesized by taking 5 ml suspension of flower extract was added into 45 ml solution of hydrogen tetra chloroaurate (0.002M) separately for reduction into Au^{3+}. $AuCl_4$ obtained from Loba Chemie Pvt. Ltd. Mumbai, incubated at room temperature for 3-4h. The primary detection of synthesized gold nanoparticles based on observing the color change taking within an hour, the media color changes from yellowish to dark brown. The gold nanoparticles solution thus obtained was purified and concentrated by repeated centrifugation at 15,000 rpm for 20 min. Supernatant is discarded and the pellet is 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

After the incubation the gold nanoparticles were isolated and concentrated by repeated centrifugation of the reaction mixture at 15,000 rpm for 20 minutes. The supernatant was replaced by distilled water each time and subjected for the optical measurements which were carried out using UV-Vis spectrophotometer. The gold nanoparticles were characterized by Elico SL 164 double beam UV-Visible Soectrophotometer [19] between 450 nm. 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.* collected from authenticated stock culture of our college itself.

Culturing of Potato Dextrose Media

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

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

3. Results and discussion

Gold nanoparticles were synthesized from Hydrogen tetra chloroaurate solution containing Au^+ ions by treating with the *Caesalpinia pulcherrima* 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 (Shown in Figure 2). They turned brown and the intensity of color was increased with the time of incubation.

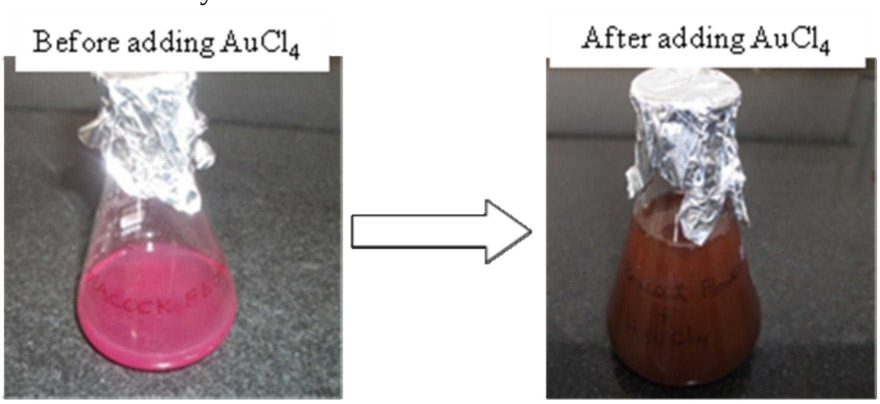
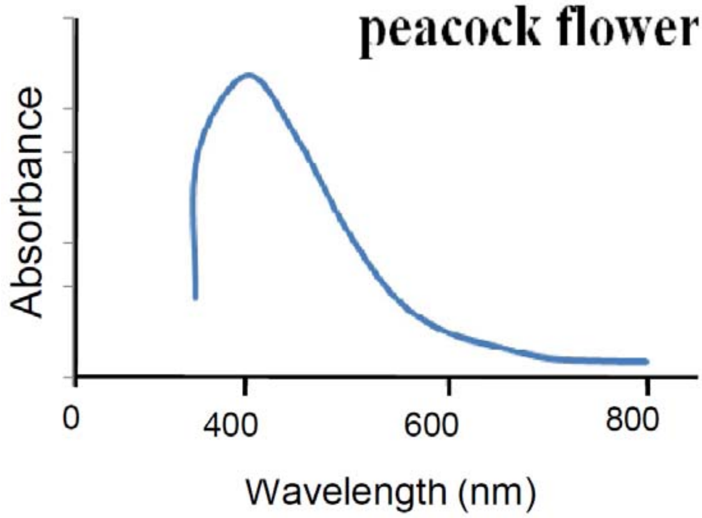


Fig. 2 Test for Peacock flower to show the synthesis of gold nanoparticles

The formation of gold nanoparticles was confirmed by color changes followed by UV-Visible spectrophotometer analysis. The UV- Visible spectrophotometer has proved to be very useful technique for the analysis of some metal nanoparticles. The UV- visible spectra (shown in Graph 1) indicate a strong plasmon resonance that is located at ~450 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 [20].



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

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 Peacock flower extract as reducing agent is shown in Figure 3. The micrograph shows formation of spherical nanoparticles. The sizes of the nanoparticles were in the range of 10-50 nm, showing a broad size distribution.

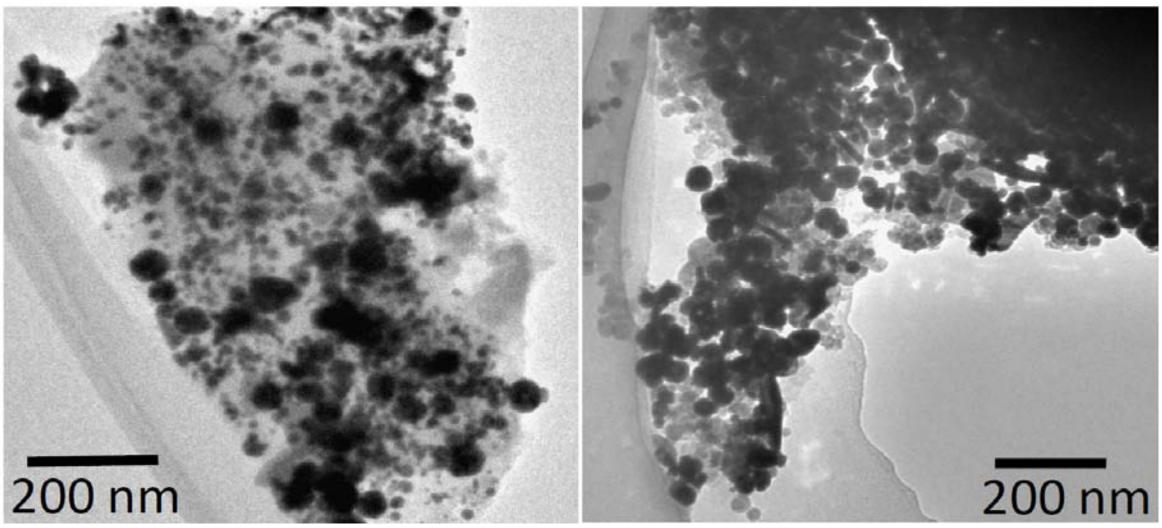
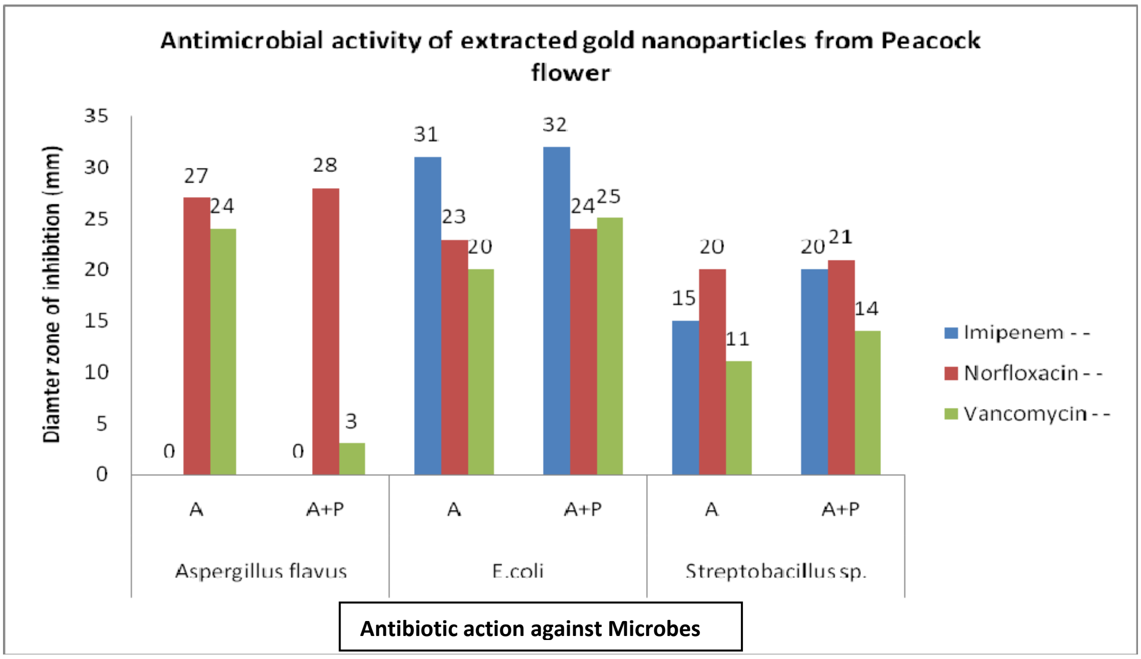


Fig. 3. TEM images of gold nanoparticles synthesized from Peacock flower.

Anti bacterial study indicates that antibiotic with gold nanoparticles extracted from the Peacock flower (A+P) exhibit more zone of inhibition compared to standard antibiotics (A) used (Table 1 and Graph 2). The zone of inhibition against *E.coli* is more when compared with the zone of inhibition exhibited on *Streptobacillus sp.* for gold nanoparticles with antibiotics like Imipenem, Norfloxacin and Vancomycin (Fig. 4). Vancomycin with gold nanoparticles has less zone of inhibition against *E.coli* and *Streptobacillus sp.* (25 mm and 14 mm, respectively), where as gold nanoparticles with Imipenem and Norfloxacin shows more inhibition against *E.coli* and *Streptobacillus sp.* (32 mm and 24 mm, respectively). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+P) shows more zone of inhibition against *E.coli* and *Streptobacillus sp.*

Table 1. Antibacterial activity of the gold nanoparticles synthesized from Peacock flower

Antibiotics	Diameter of zone of inhibition in mm							
	Organisms							
	Aspergillus niger		*Aspergillus flavus*		*E.coli*		*Streptobacillus sp.*	
	A	A+P	A	A+P	A	A+P	A	A+P
Imipenem	-	-	-	-	31	32	15	20
Norfloxacin	-	-	27	28	23	24	20	21
Vancomycin	-	-	24	3	20	25	11	14



Graph 2: Antimicrobial activity of gold nanoparticles synthesised from Peacock flower extract

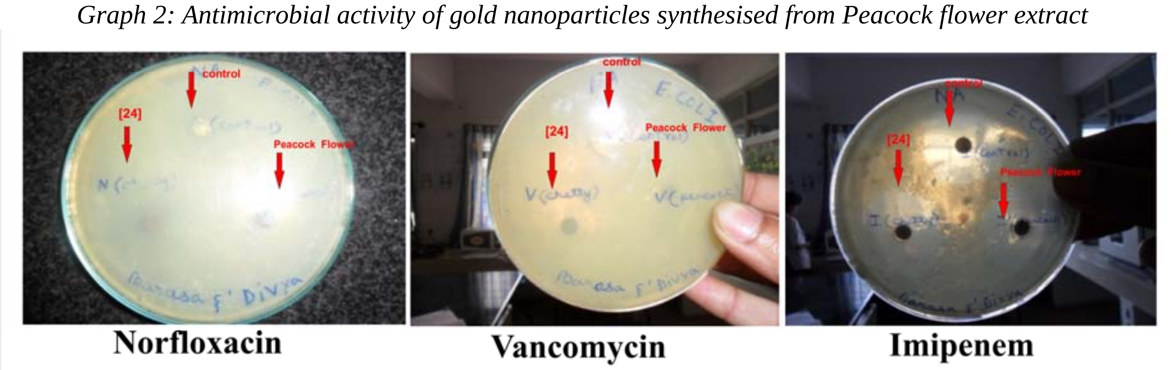


Fig. 4 Antimicrobial activity of the gold nanoparticles synthesized from Peacock flower against E.coli.

The antibacterials activity, compare to *Aspergillus niger*, *Aspergillus flavus* shows more zone of inhibition for the antibiotics Norfloxacin and Vancomycin in both antibiotics (A) and antibiotics with peacock flower extract (A+P). Both Norfloxacin and Vancomycin with gold nanoparticles does not show any reaction against *Aspergillus niger* but against *Aspergillus flavus* shows more inhibition (3 mm and 26 mm respectively). Compare to antibiotics (A), antibiotics with gold nanoparticles (A+P) shows more zone of inhibition against *Aspergillus flavus*. Sudhakar *et al*. studied the ethanolic extracts of the dry fruits of Caesalpinia pulcherrima [21], aerial parts of Euphorbia hirta and flowers of Asystasia gangeticum for antimicrobial activity. The three plants exhibited a broad spectrum of antimicrobial activity, particularly against Escherichia coli (enteropathogen), Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus [22]. Similarly, Chanda et al demonstrated that methanol extract of seeds and fruit rind of *C. pulcherrima* possesses antimicrobial and antioxidant properties which can be used to discover bioactive natural products [23]. Similar results were found in the case of gold nanoparticles obtained from Chetty flower [24].

4. Conclusion

The gold nanoparticles synthesized using extracts of Peacock flower samples was confirmed by color changes and was characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 450 nm for gold nanoparticles. The micrograph shows formation of spherical nanoparticles. The sizes of the nanoparticles were in the range of 10-50 nm, showing a broad size distribution. 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 can conclude that the synthesized nanoparticles are more efficient in the drug delivery process.

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References

[1] K. Sobha, K. Surendranath, V. Meena, Biotechnology and Molecular Biology Reviews **5** **(**1), (2010).
[2] S.L. Iconaru, E. Andronescu, C.S. Ciobanu, A.M. Prodan,P. Le Coustumer, D. Predoi, Digest Journal of Nanomaterials and Biostructures **7**(1), 399 (2012).
[3] C. S. Ciobanu, S. L. Iconaru, E. Gyorgy, M. Radu, M. Costache, A. Dinischiotu, P. Le Coustumer, K. Lafdi, D. Predoi, Chemistryl Central journal, **6**, 17 (2012) doi:10.1186/1752-153X-6-17.
[4] C. S. Ciobanu, E. Andronescu, A. Stoicu, O. Florea, P. Le Coustumer, S. Galaup, A.Djouadi, J.Y. Mevellec, I. Musa, F. Massuyeau, A. M. Prodan, K. Lafdi, R. Trusca, I. Pasuk, D. Predoi, Digest Journal of Nanomaterials and Biostructures 6 (2), 609 (2011).
[5] D. Predoi, S. Derible, H. Duflo, J. Optoelectron. Adv. Mater. **11** (6), 852 (2009).
[6] F. Frumosu, S.L. Iconaru, D. Predoi, Digest Journal of Nanomaterials and Biostructures, **6**(4), 1859 (2011).
[7] H. Chan, M.H. Tsai, Rev.Adv.Mater. Sci.**18**, 734 (2008).
[8] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, M. Sastry. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids Surfaces, B: Biointerfaces **27**, 313-318 (2003).
[9] Zygmunt Sadowski, Biosynthesis and application of silver and gold Nanoparticles. Wroclaw University of Technology, Poland.
[10] C. S. Ciobanu, F. Massuyeau, L. V. Constantin, D. Predoi,Nanoscale Research Letters 6, 613 (2011).
[11] Z.P. Xu, Q.H. Zeng, G.Q. Lu, A.B. Yu. Inorganic nanoparticles as carriers for efficient cellular delivery. Chemical Engineering Science **61.** 1027- 1040 (2006).

[12] S. L. Iconaru, F. Ungureanu, A. Costescu, M. Costache, A. Dinischiotu, D. Predoi, Journal of Nanomaterials, 1, article number: 291512 (2011).
[13] C.S. Ciobanu, L. Pall, E. Andronescu, S.L. Iconaru, E. Gyorgy, D. Predoi, Revista de chimie **61**, 12 (2010).
[14] D. Predoi, Digest Journal Of Nanomaterials And Biostructures **2**(1), 169 (2007).
[15] J. Cheon, G. Horace, Inorganic nanoparticles for biological sensing, imaging and therapeutics. J. Mater. Chem. **19,** 6249- 6250 (2009).
[16] S.S. Patel, N.K. Verma, C. Chatterjee, K. Gauthaman**,** Screening of *Caesalpinia pulcherrima* Linn. Flowers for Analgesic and Anti-inflammatory Activities**,** International Journal of Applied Research in Natural Products **3**(3) 1-5 (2010).
[17] C. R. Pawar, R.E. Mutha, A. D. Landge, R. B. Jadhav, S. J. Surana. Antioxidant and cytotoxic activities of Caesalpinia pulcherrima wood. Indian journal of biochemistry biophysics **46**(2), 198-200 (2009).
[18] P.S. Dhaked, R.N. Panigrahy, S.N. Kshirsagar, *In vitro* evaluation of anthelmintic activity of *Caesalpinia pulcherrima (linn)* flower extracts in Indian earthworm, International Journal of Pharmaceutical Sciences Review and Research **7** (1), 89-91(2011).
[19] S. V. Padma, B. Dhara, Facility for ecological and Analytical Testing, Indian Journal of Biochemistry and Biophysics **47,** 157-160 (2010).
[20] A. Henglein, 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).
[21] K. V. N. S. Srinivas, Y. K. Rao, I. Mahender, B. Das, K. V. S. R. Krishna, K. H. Kishore, Flavanoids from *Caesalpinia pulcherrima* Linn. Phytochemistry **63(**7), 789-793(2003).
[22] M. Sudhakar, C.V. Rao, P.M. Rao, D.B. Raju, Y. Venkateswarlu, Antimicrobial activity of Caesalpinia pulcherrima, Euphorbia hirta and Asystasia gangeticum**,** Fitoterapia **77**(5) 378-80 (2006).
[23] S. Chanda, J. Parekh, Y. Baravalia, S. Parekh, Antimicrobial and antioxidant efficacy of various solvent extracts of seed and fruit rind of Caesalpinia pulcherrima, Archives of clinical microbiology **4** (2010).
[24] B. Nagaraj, N. Krishnamurthy, P. Liny, TK. Divya, R. Dinesh , Biosynthesis Of Gold Nanoparticles Of Ixora Coccinea Flower Extract & Their Antimicrobial Activities, International Journal of Pharma and Bio Sciences, **2**(4) 557-575 (2011).