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Delonix Elata Leaf Extract Mediated Gold Nanoparticles and their Biological Applications

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Abstract : Biosynthesis of gold nanoparticleis a simple and eco-friendly method is used in various biomedical applications. Bio-reduction of chloroauric acid($HAuCl_4$) for the synthesis of Au NPs with the plant extract of Delonixelataleaf extract at room temperature. The properties of the synthesized nanoparticles were characterized by UV-Vis, XRD, TEM, DLS and Zeta potential. A UV-vis spectrum shows the SPR at 534nm. TEM study of the Au NPs is spherical in most cases and some other having intermittently triangular and nanorods were also observed and an average diameter of 24nm.Furthermore the biologically synthesized Au NPs were found to be a good antibacterial activity against gram positive *(Staphylococcus aureus, Streptococcus pyogenes)* and gram negative *(Pseudomonas aeruginosa* and *Proteus vulgaris*) bacterial pathogens.
Keywords : Biosynthesis, Delonixelata, AuNPs, XRD, TEM, DLS.

Introduction

Nowadays nanotechnology is a basis of many technological innovations,a major output of nanotechnology is to be on development of new materials in the nanometer scale, including nanoparticles. Au NPs have special optical and electronicproperties, such as unique and tunable surface plasmonresonance (SPR) and its having attracted considerable attention in catalysis, optics and biomedical applications[1]. For green chemistry way the plant based biological synthesis of Au NPs is gaining importance due to low cost, ecofriendliness, simple laboratory set-up required for the synthesis process is applied at room temperature[2,3]. The plant Delonixelata(Fig.1(a))possesses a broad range of pharmaceutical uses and is belonging to the family of Fabaceae. The leaf extracts are anti-inflammatory in nature and to get aid from rheumatic problems[4].The present study to describe for the first time Delonixelataleaf extract mediated biosynthesis of Au NPs. The synthesized nanoparticles were characterized by UV-vis, XRD, TEM, DLS and Zetapotential.

Experimental

Chloroauric acid ($HAuCl_4.3H_2O$) was obtained from Sigma-Aldrich, India. All other reagents used in the reaction were of analytical grade with maximum purity.

The freshly cleaned Delonixelataleaves were left to dry in a closed room for approximately 15 days. The dried leaves were pulverized with a sterile electric blender to obtain a powdered form. The plant leaf solution was prepared by taking 2g of finely powdered leaves in a 250ml Erlenmeyer flask with 100ml of deionized water and the mixture was boiled (70°C) for 10min before decanting.Then the extract (Fig.1(b)) obtained was filtered during a nylon mesh (0.2µm) and also by Whatmann.No.1 filter paper. The filtered extract was stored at 4°C for supplementary studies.

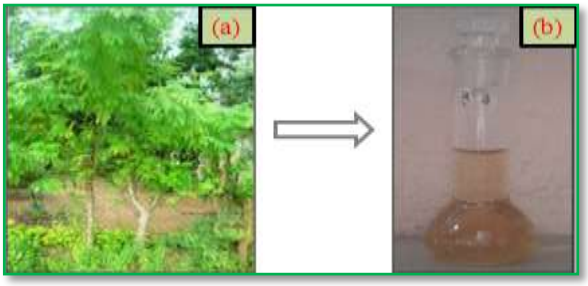

Fig.1:(a) Photograph of Delonixelata;(b) Delonixelata leaf extract

Synthesis of gold nanoparticles

In a sample vial a known amounts (0.2ml, 0.5ml, 1ml, 1.5ml and 2ml) of aqueous leaf extract were added dropwise to 10mL of 1mM aqueous$HAucl_4$solution and incubated at room temperature. After 30min the color of the solution changed from orange yellowto dark pink.This color change was due to excitation of surfaceplasmon resonance. This dark pink color is also a visual confirmation of the formation of Au NPs.

In vitro Antibacterial activity

The bacterial Pathogens**Gram positive***(Streptococcus pyogenes* MTCC442, *Staphylococcus aureus* MTCC90) and **Gram negative***(Pseudomonas aeruginosa* MTCC647, *Proteus vulgaris* MTCC426) used in the present study were procured from Institute of Microbial Technology (IMTech), Chandigarh.

In vitro Antibacterial assay

Antibacterial activity of biosynthesized Au NPswas carried out by utilizing agar well diffusion assay method. About 15-20ml of sterilized Muller-Hinton (MH) agar medium was transferred to sterile petriplates and allowed to solidify. After solidification, the test bacterial cell suspension (0.1%) was uniformly spread over the agar surface using sterile cotton swabs. After that 6 wells of 5mm in diameter were made using sterile cork borer and then the synthesized metallic nanoparticles dissolved in sterile distilled water with different concentration (20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml) were added separately into wells. Ciprofloxacin was used as a positive control and sterile distilled water used as negative control. Then the plates were incubated at 37°C for 24hrs. After incubation the diameter of the zone of inhibition was expressed in millimeter (mm).

Minimum Inhibitory Concentration (*MIC*)

The MIC determination used two fold serial dilution methods[5]with Muller – Hinton Broth. The biosynthesized Au NPs dissolved in sterile distilled water. From the stock solution 0.5ml was incorporated into 0.5ml of nutrient broth to get a concentration of 500µl/ml, and serially diluted by double dilution technique to achieve 500µg/ml, 250, 125, 62.5, 31.2, 15.6, 7.8 and 3.9µg/ml respectively, 50µl of standardized suspension of the test organism was transferred into each tube. The last tube of MHB with 50µl in inoculums serves as a positive control and MHB alone served as negative control. The whole setup in duplicates was incubated at 37ºC for 24 hours 27ºC. After the incubation the tubes were analyzed in spectrophotometer.

Results and Discussion

UV-Visible analysis

The leaf extract is an orange yellow liquid; there were no color changes upon mixing the$HAuCl_4$solution with the plant extract at room temperature, although the color gradually changed by time.The color of the mixture after 30min was faintly changed into pink and become progressively dark pink with time.Such a color change arises because of the coherent oscillation of electron gas on the surface of nanoparticles resulting in surface plasmon resonance (SPR), consequently the reduction of gold salt from three (Au^{3+}) to zero (Au^0) oxidation state might have been obsessed by some active components in plant extract[6,7].From the UV-visible spectrum (Fig.2), the observed nanogold occurs originally at 538nm at 0.2ml of leaf extract quantity. Even as an increasing leaf extract concentration from the SPR bandwidth decreases,

towards shift to shorter wavelength from 538-534nm[8], also decrease full width and half maxima supporting the reduction in particle size.

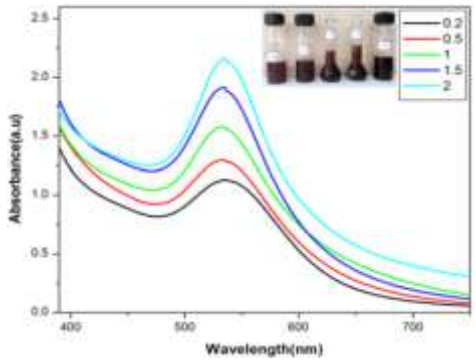

Fig.2: UV-Visible spectra of biosynthesized gold nanoparticles

XRD analysis

The experimentally obtained powder XRD pattern of biosynthesized Au NPs,shown in the Fig.3, reveals some characteristically well definedintense andbroader peaks at angles38.26°,44.37°, 64.68° and 77.75° corresponds to the diffraction plans (111), (200), (220) and (311) respectively.The value of the gold lattice parameter has been predictable to be a= 4.079Å, a value that is consistent with a=4.071Å reported by JCPDS ID: 89-3697.The average crystallites size according to the Debye–Scherrer equation is found to be 17.27nm.In addition, some unassigned peaks are also observed, suggesting that the crystallization of bio-organic phase occurs on the surface of the gold nanoparticles[9-12].

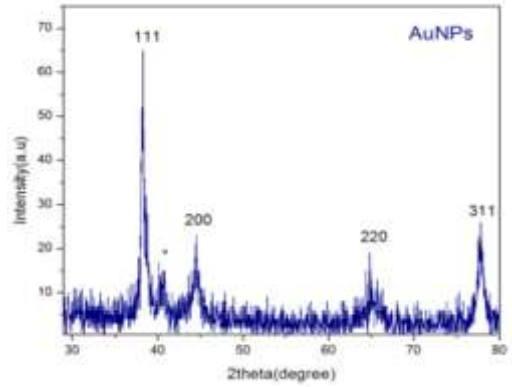

Fig.3: XRD pattern of biosynthesized gold nanoparticles

TEM analysis

The TEM images of the biosynthesized gold nanoparticles at different resolutions wereillustrated inFig.6 (a-c). The biosynthesized nanoparticles are spherical in most cases and some others having intermittently triangular, hexagonaland nanorods[13-15]. Significantly,a bunch of agglomerated into a small aggregation of two or more nanoparticles together, which in turn result due to the presence of excess amounts of reducing moieties and the interactions between the stabilizing molecules bound to the surface of particles and secondary reduction process on the surface of the performed nuclei. The prepared gold nanoparticles aggregates are coated among a thin organic layer, which acts as a capping bio-organic agent. The obtained nanoparticles are rather uniform in size and up to 24nm.The SAED pattern of the prepared gold nanoparticles is crystalline in nature and ordered lattice fringes of prepared gold nanoparticles are shown in Fig.5(d). For diffraction ring from inner to outer, which can be indexed as the "d" spacing values of 0.235, 0.203 ,0.143 and 0.122nm coincides with Bragg reflection planes (111), (200), (220) and (311) were obvious for the FCC phase of the metallic gold crystal.

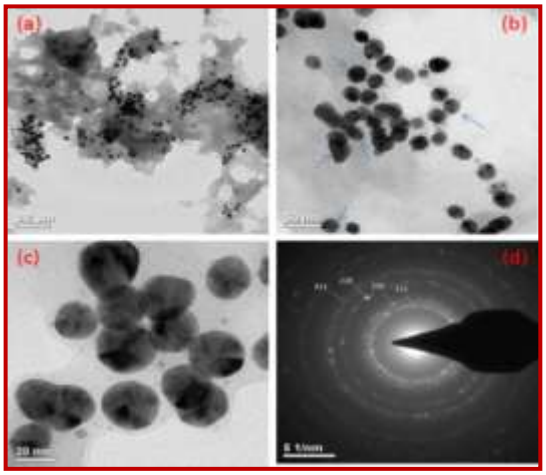

Fig.5: TEM and SAED images of biosynthesized gold nanoparticles

DLS analysis

The particle size determinations of the synthesized gold nanoparticles were shownby the intensity and laser diffraction revealed that particles obtained are polydisperse mixturesin Fig.8. The average diameter of the particles was found to be 81.27nm.In addition, several larger size particles appeared in DLS result, which is due to the agglomeration AuNPs in the solution.

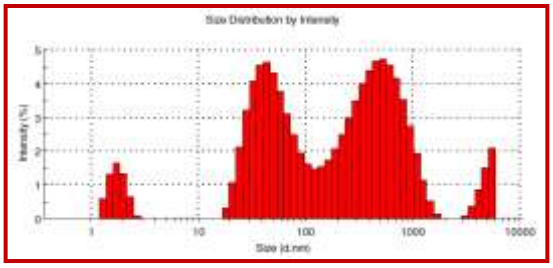

Fig.8: Particle size distribution curve for biosynthesized gold nanoparticles

Zeta potential

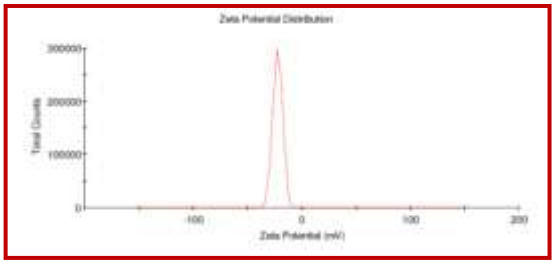

Fig.9: Zeta potential analysis of gold nanoparticles

Antibacterial activity and MIC of gold nanoparticles against bacterial pathogens

The antibacterial activity of gold nanoparticles was displayed in Fig.10 and inhibition values are given in Table.1.The mean zone of inhibition ranged from 11mm to 17mm at 80µg/ml concentration. The zone of inhibition varied significantly according to the concentration used[16,17]. Of all tested bacterial strains, *Streptococcus pyogenes* was highly inhibited and recorded wider zone of inhibition (17±0.54mm) at 80µg by gold nanoparticles. Similar resultswere observed in gold nanoparticles were more active against gram negative bacteria than a gram positive bacteria and this was attributed to change in the cell wall composition of bacteria[18].

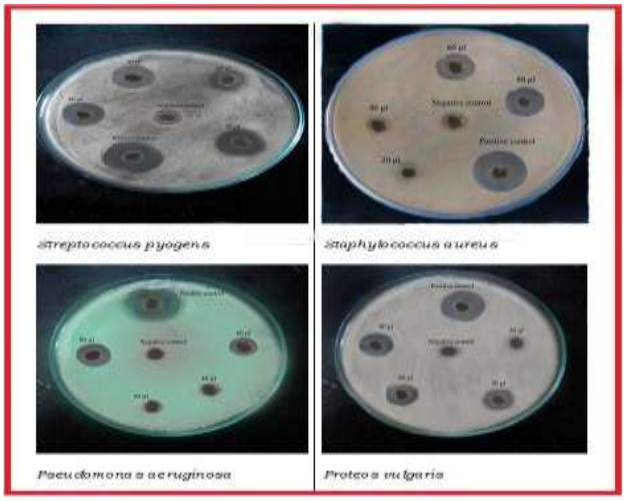

Fig.10:Antibacterial activity of gold nanoparticles

Table.1: Antibacterial activity and MIC of gold nanoparticles against bacterial pathogens

Bacterial pathogens	Zone of inhibition (mm)				MIC (µg/ml)	Positive control (Ciprofloxacin) (5 µg/disc)
	20µg/ml	40µg/ml	60µg/ml	80µg/ml		
Gram positive						
Streptococcus pyogenes	8±0.8	11±0.9	14± 0.7	17±0.54*	3.9	30.8±0.3
Staphylococcus aureus	NZ	NZ	10±0.09	12±0.32	31.2	25.6±0.5
Gram negative						
Pseudomonas aeruginosa	NZ	NZ	8 ± 0.3	11±0.21	31.2	28.4±0.4
Proteus vulgaris	NZ	8±0.1	11±0.6	13±0.22	15.6	27.4±0.5

At the meantime, all the concentrations showed a weak antibacterial activity compared with the standard antibiotic Ciprofloxacin, the recorded zone of inhibition ranged from 25 to 30mm at 5µg concentration. The MIC of gold nanoparticles was also analyzed in the present study. The MIC values ranged between 3.9 to 31.2µg/ml that was shown in Table.1.

Conclusion

In conclusion, the biosynthesis of gold nanoparticles have been synthesized using the bio-reducing agent of Delonixelataleaf broth and prepared nanoparticles successfully identified the visually inspection and UV-visspectrum. Further the synthesized nanoparticleshave been spherical morphology it has been confirmed by TEM analysis,particle size in the range of 24nm. Still our varied concentrations of biosynthesized Au nanoparticles are shown the proper response for all the test bacterial strains and finally plant biomassof flavonoids, responsible for the reduction of gold salt to gold nanoparticles.

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