RESEARCH ARTICLE

Synthesis and Characterization of Gold Nanoparticles from *Lobelia Nicotianifolia* Leaf Extract and its Biological Activities

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Biosynthesis of gold nanoparticles is one among the best and cheap economical viable process, which is environmental friendly. The purpose of this study is to synthesis the gold nanoparticles using *Lobelia nicotianifolia* leaf extract and to investigate its biological activities. The synthesized gold nanoparticles were characterized by UV-vis spectroscopy, TEM, SAED, FTIR and XRD, the nanoparticles produced at maximum absorbance 532 nm. The Characterization study proved that the size and shape of AuNPs were spherical in shape, with an average size of 80 nm. Synthesized AuNPs were evaluated for various in-vitro biological studies.

Introduction

Nanotechnology have attracted attention of researchers due to multifaceted application. Green synthesis of nanoparticles has numerous scientific interests, they are associated effectively with bulk materials and atomic or molecular structure [**1**]. The effectiveness of nanoparticles were mainly determined by the size [**2**] and unique shape. These nanoparticles were extensively used in chemical, energy, electronics and space industries [**3**] Plasmonics [**4**] biosensing [**5**] enzyme electrodes [**6**] super conductors [**7**] and in cancer therapy [**8**] Plant mediated synthesis is being widely explored and a number of plant parts such as leaves *Alternanthera sessilis* [**9**] *Ixora coccinea* flowers [**10**] *Ananas comosus* fruits [**11**] *Citrus unshiu* fruit peels [**12**] were successfully used to synthesize the silver and gold nanoparticles.

A simple biosynthetic method in which, leaf extract *Lobelia nicotianifolia* was used for the synthesis of AuNPs. The *Lobelia nicotianifolia* has numerous medicinal use including antibiotic, antiseptic, anti-inflammatory activities [**13**]. The synthesized gold nanoparticles of *Lobelia nicotianifolia* has medicinal properties, efforts were made to identify those medicinal values [**14**] of AuNPs.

Experimental

Chemical used for this research were analytical grade obtained from Thomas Baker. Leaf were collected from Western Ghats forests of Kodagu district of Karnataka.

Preparation of leaf extract

Collected leaf were shade dried and powdered using pestle and mortar, sieved through 20 mesh sieve 5gm of powder was added to 200ml of double distilled quartz water. The sample was boiled for 30 minutes, the solution was filtered through Whatman filter paper No:1 (pore size 25µm) [**15**] and cooled at room temperature for 30 minutes.10ml of extract was added to 90ml of 1mMl $AuCl_3$ solution and allowed for synthesis of AuNPs. The colour change indicate the synthesis of AuNPs [**16**]. The sample were subjected to UV spectral analysis for every 30 minutes for 6 hours confirmed that the AuNPs synthesis.

Characterization of synthesized Au nanoparticles

Purification of AuNPs

The synthesized AuNPs were centrifuged at 10,000 rpm for 20 minutes [**17**]. The residue was thoroughly washed with double distilled water and freeze dried using Modulyod lyophlizer.

UV-visible spectroscopy analysis

AuNPs bio reduction was monitored by periodic sampling of aliquots [**18**] 3ml was subjected to UV-vis–spectral analysis (Lab India UV 3000) for every 30 minutes between the ranges 450nm - 650nm. It is evident that the reduction reaction of AuNPs reaches saturation gradually with the increase in time period. [**19**].

Fourier transform infrared spectroscopic analysis

5µ gms of freeze dried AuNPs were pressed with 0.2000 gm of KBr pellets for IR spectrum, which were examined under FTIR spectrometer (JASCO) over the range 400 – 4000 cm^{-1} of wavelength.

Transmission electron microscopy measurements.

Morphology, size [**20**] and shape of AuNPs were determined by the Transmission electron microscopic studies [**21**]. AuNPs were placed on the carbon coated copper grid [**22**] and dried by removing extra solution with blotting paper and dried in the room temperature. FEI Tecnai G2 F20 STFE-TEM was operated at 200 kv, resolution of 0.24 nm and Cs of 1.2 mm [**23**].

X-ray diffraction analysis

X-ray diffraction diffractometer, PANalytical X'Pert MRD model at 30kv, 40mA with CuKα at 2θ angle [**24**]. Was used to investigate the crystal structure of the AuNPs. The finely powdered nanoparticles were loaded on to the sample holder and readings were taken. The size of AuNPs was calculated using Debye-Scherer's equation by determining the width of the (111) Bragg's reflection, $D = 0.94 \beta \cos \theta$ [**25**].

Antibacterial activity

Gram positive bacteria like *Bacillus* and *Micrococcus luteus, E.coli* a gram-ve bacteria were tested against Au nanoparticles using nutrient agar diffusion method. The plates were smeared with respective organisms and 1:1 diluted 5µml of AuNPs discs. The inhibition zone was measured.

Anticancer activity

To study the cytotoxicity of synthesized AuNPs against human lung cancer cells A459, The cell viability test was conducted using MTT assay. The cells were seeded with 96 well plate for 24h.

Results and discussion

Characterization of gold nanoparticles can be easily attained by UV-spectrometer. The colour change in the reaction mixture, from red wine colour respectively indicated surface plasmon vibration of gold nanoparticles proving formation of nano particles in the range of 532 nm. The colour reaction was checked at every 30 mintues time interval for about 6 hours **Fig 1(a)**. The absorption peaks 532 nm not shifted with the increase of reduction time.

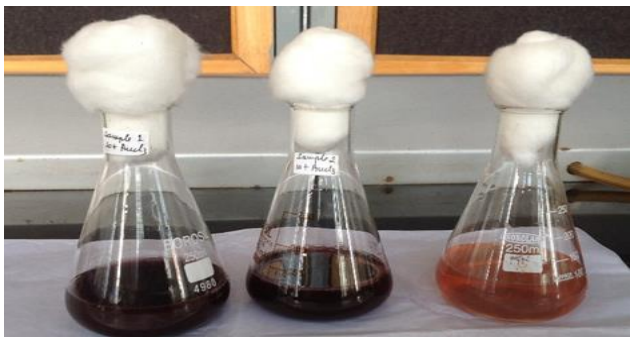


Fig. 1. (a) Optical photograph of colloidal solution.

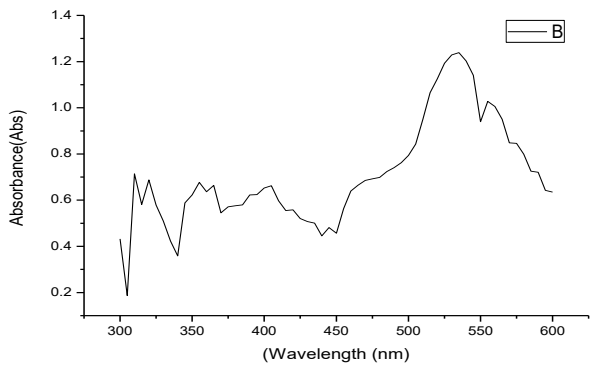


Fig. 1. (b) UV- vis absorption spectra.

Antimicrobial activity of Gram positive bacteria *Bacillus* and *Micrococcus luteus*, *E.coli* a gram –ve bacteria were tested against Au nanoparticles *Micrococcus luteus* showed clear inhibition zone **Fig. 2**.

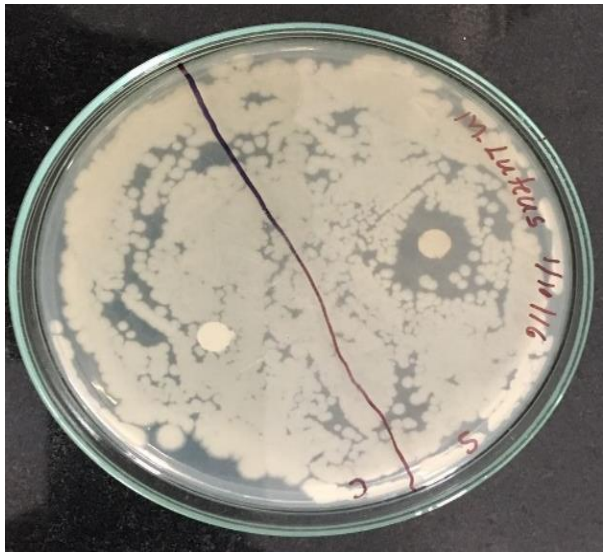


Fig. 2. Antimicrobial activity.

The TEM images of AuNPs showed that, they were hexagonal and trinangular in nature and very few of them dipicted spherical morphology [**26**] **Fig. 3a** and **Fig. 3b**. The tringale shaped AuNPs were nano dispersed with large surface area. The average edge length of gold nanotringales was 80nm, while hexagon were 50nm.

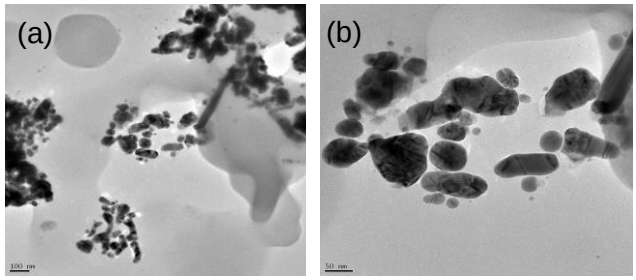
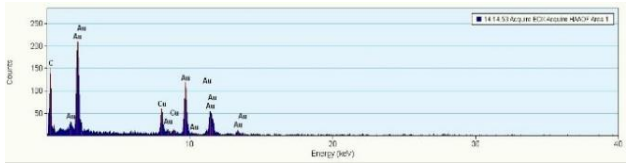


Fig. 3. (a & b) TEM images of AuNPs.



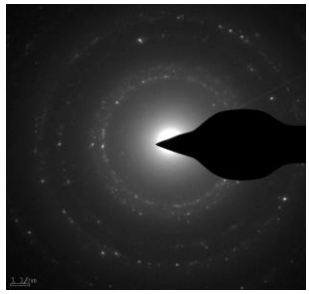


Fig. 3. (c & d) TEM images of AuNPs.

The TEM images at two different magnification, shows distribution of poly dispersed and agglomerated particles SAED confirms the *fcc* structure in corroboration with XRD pattern. EDX (counts vs. Energy in KeV) analysis shows, the presence of only Au metal was confirmed and no other elements were present.

FTIR studies were conducted to identify the absorption peaks, in which the reduction of gold salts into its respective nanoparticles **Fig 4**. The strong peak 3380 shows that O-H streching along with the following peaks 2928, 1380, 715 shows C-H groups 1696, shows C=O and 1280 and 1180 C-N streching and 1450 shows C=C aromatic ring streching. The heighest peaks 3380 reflect that the OH groups is responsible for the reducing property of the extract.

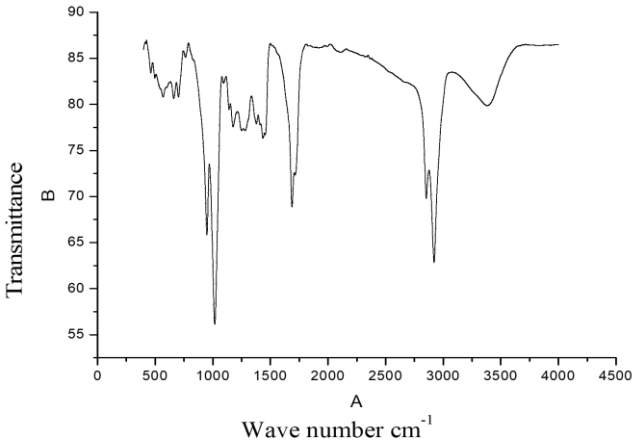


Fig. 4. FTIR analysis.

The XRD pattern of AuNPs (**Fig. 5**) shows 3 diffraction peaks (111), (200), (220), of the 2θ values of 38.2º and 64.6º can be assigned to (111), (200) and (220) planes respectively indicating that the AuNPs are fcc structured (JCPDS89-3722) [**27**]. The crystaline mean size of AuNPs was found to be 84 nm.

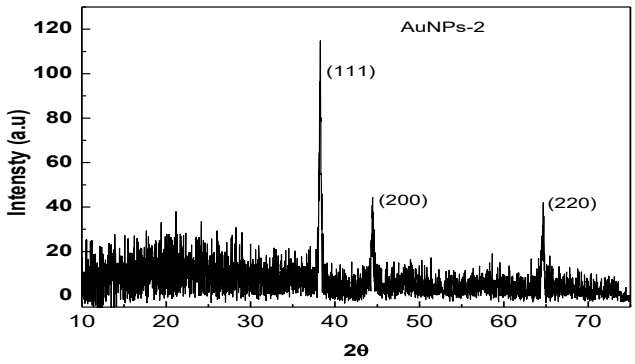


Fig. 5. XRD Pattern.

Debye-scherrer's equation, d = Kγ/βcosθ, where K-shape factor between 0.0 and 1.1, K- incident X-ray wave length (CuKα = 0.1542 nm), β-full width half maximum in radius of promoneent line (111) and θ-position of that line in the pattern, never the less, the overall XRD pattern indicates that the amorphous nature of the particle.

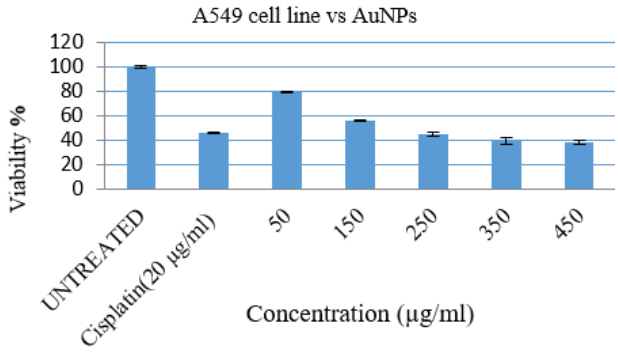


Fig. 6. (a) MTT assay.

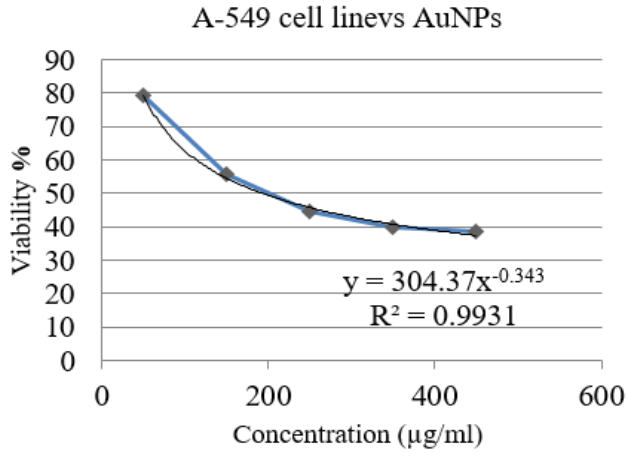


Fig. 6. (b) MTT assay.

Anticancer activity

Anticancer activity of AuNPs increases with the increase in the concentration of AuNPs, (**Fig. 6a** , **Fig. 6b & Fig 7**) shows the maximum cytotoxicity at 450µg of concentration efficiently facilitated into the human lung cancer cell line A-549 to supress cell prolifreration. AuNPs enters into the cells causing oxidative stress. This would reduces the cell viability by increasing DNA damage. AuNPs interacts with interacellular functional groups of nitrogen base and phosphate groups of DNA, causing DNA damage [**28**] and also suppress the signaling of protein [**29**].

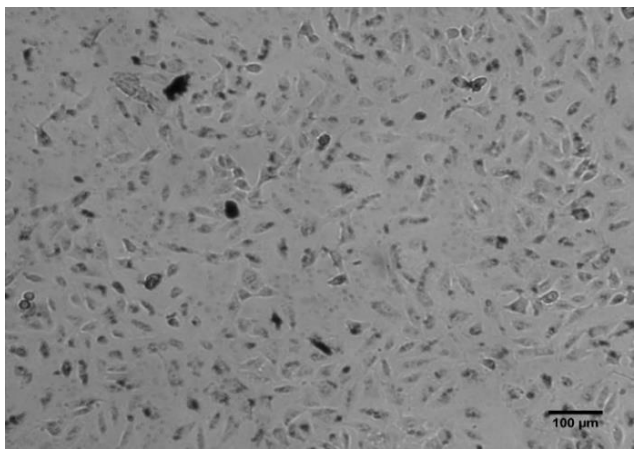


Fig. 7. MTT assay image.

Antidiabetic activities (**Fig. 8**) of biosynthesised nanoparticles was tested with α-amylase activity with IC_{50} value. The test samples showed mild α-amylase inhibitory activitiey as compared to standard Metformin.

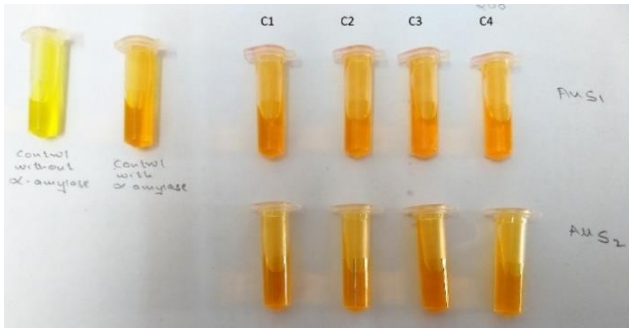


Fig. 8. Images of Anti diabetic assay.

Conclusion

Synthesis of AuNPs using leaf extract of *Lobila nicotianifolia*, demonstrated the role of reducing agents in forming AuNPs. The AuNPs produced by this method is simple,and abundantly available in the nature. The TEM, XRD, FTIR, proved that the shape and size, the synthesised particles are tirangular, spherical,with 50nm to100nm. The biological activities like anti- microbial, anticancer and antidiabetic proved that synthesised gold nanoparticles have greater impact on human health, medicine and pharma. Further deep study into their biological activity is a great boon for the field of medicine.

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Keywords

Gold nanoparticles, *lobelia nicotianifolia*, FTIR, TEM, XRD, antimicrobial.

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