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Green synthesis of gold nanoparticles from the leaf extract of *Nepenthes khasiana* and antimicrobial assay

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ABSTRACT

Synthesis of nanoparticles from various biological systems has been reported, but among all, biosynthesis of nanoparticles from plants is considered as the most suitable method. The use of plant material not only makes the process eco-friendly but also the abundance makes it more economical. The aim of this study was to investigate the ability of this plant to synthesis gold nanoparticles and study the properties of the nanoparticles thus produced. Antimicrobial activity and medicinal values of *Nepenthes khasiana* fascinated us to utilize it for biosynthesis of gold nanoparticles. The synthesized gold nanoparticles were characterized by UV-*vis* spectrophotometry, Scanning Electron Microscopy, X-ray Diffraction, Fourier Transform Infra-red Spectroscopy and Transmission Electron Microscopy. Different time intervals for the reaction with aqueous chloroauric acid solution increase in the absorbance with time and became constant giving a maximum absorbance at 599.78 nm at three hours of incubation. The results from XRD, TEM and SEM supports the biosynthesis of triangular and spherical shaped Gold nanoparticles between 50nm to 80 nm. In this study, the antimicrobial property of the AuNPS was exploited against human pathogenic micro-organisms. The results of TEM, SEM, FT-IR, UV-VIS and XRD confirm that the leaves extract of *N. Khasiana* can be used to produce Gold nanoparticles with significant amount of antimicrobial activity.

Keywords: Nanoaprticles, FTIR, AuNPs, *Nepenthes* khasiana, SEM, Antimicrobial.

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Introduction

The recent developments in nanotechnology have delivered a far-reaching research by intersecting with various other branches of science and forming impact on all forms of life. With the advancement of technologies and superior scientific understanding paved a way for research and development in the field of plant biology towards intersection of nanotechnology. One such interference is employing plants or plant parts in the synthesis of nanoparticles **[1-3]**. Nanoparticles are of numerous scientific interests as they are effectively a bridge between bulk materials and atomic or molecular structures. Chemical synthesis of metal nanoparticles leads to the production of toxic compounds, which remains adsorbed on the surface that have adverse effects on human health **[4]**. The usage of eco-friendly materials like plant extracts, microbes and enzymes not only eliminate the hazards of toxic material but also many constraints **[5-8]**. Utilization of plants becomes the most voted choice for generating metal nanoparticles for being easily available, environmentally benign, a hoard of metabolites, complex metabolic pathways, ability to tolerate heavy metals, cost effective and less tedious purification steps **[9-11]**. Plant system also requires small incubation period than microbial systems and can be easily scaled up for commercial production **[12]**. Gold nanoparticles so far have been synthesized from a wide array of plant systems **[13-18]**. The protective and reductive activity of plant biomolecules is accountable for the reduction of gold ions **[19]**. They have advantage over other metal nanoparticles for being biocompatible and non-toxic nature **[20-22]**. *Nepenthes khasiana* belongs to Nepenthaceae family is an endemic plant of India. The species has very localized distribution and endemic in nature. The plant is ethno medically important locally. Any process, which can gainfully utilize *N. khasiana*, has the great advantage that it would encourage the people to cultivate this endangered plant more thus leading to its conservation and adding more value to the plant. Moreover, biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single step green synthesis process.

To the best of our knowledge, gold nanoparticle synthesis from *N. khasiana* is reported for the first time by reducing a solution of Gold (III) chloride. In our study, we report a green method for the synthesis of gold nanoparticles at room temperature by using leaf extracts of *N. khasiana* as reducing/stabilizing agents and the probable mechanism for the formation of nanoparticles.

Experimental

Materials and methods

The synthesis of AuNPs was carried out from *Nepenthes khasiana* leaves, which were collected from Experimental Fields of CSIR-NEIST Jorhat and Gold (III) chloride [Sigma-Aldrich, USA; 99.99% pure]. Mature leaves (20 gram) of *Nepenthes khasiana* were weighed, cleaned and cut into small pieces. Leaves were then added to 200 ml autoclaved double distilled water and boiled for 30 minutes and filtered through 0.45 µm membrane filter (Millipore). The filtrate was used as reducing agent and stabilizer.

The production and stabilization of the reduced AuNPs in the colloidal solution was monitored by UV-vis spectrophotometer analysis using Analytikjena Specord 200. The synthesis of gold nanoparticles was monitored at different time interval (0 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr and 5 hr) range. Plant extract (10 ml) was added to 90 ml of the Gold (III) chloride solution (0.001M) with continuous stirring. The spectrum was scanned from 200 to 900 nm wavelengths. For bulk production of AuNPs, 25 ml of the plant extract was slowly added to 225 ml of the Gold chloride (III) solution (0.001M) and was allowed to incubate for 3 hours with gentle stirring. After 3 hours, the mixture was centrifuged at 10,000 rpm for 20 mins at room temperature. The resulting pellet was washed with autoclaved double distilled water twice and allowed to air dry. The AuNPs thus obtained were used for further analysis. FTIR analysis was done KBr Press method. 1- 2 mg dried powdered sample were grinded with KBr in a motar and ~13 mm KBr disc were prepared in disc making accessory by using 10 ton pressure for 1 min. X-ray diffraction (XRD) measurement were carried out by Rigaku X-ray diffractometer (ULTIMA IV, Rigaku, Japan) with CuK X-ray source (= 1.54056Å) at a generator voltage 40 kV, a generator current 40 mA with the scanning rate 2° min^{-1}.

The samples were characterized morphologically by doing SEM. A pinch of dried AuNPs was coated on carbon with platinum in an auto fine coater and then material was subjected to analysis. TEM micrographs of AuNPs were obtained. The specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, one or two drops of the suspension deposited onto holey-carbon coated grids and dried under Infrared lamp. Antimicrobial activity of the AuNPs was screened by Kirby-Bauer disc diffusion method against two human pathogenic bacteria and two human pathogenic fungi. The sterile disc were dipped in AuNPS solution and placed in the agar plates and kept for incubation. Bacterial plates were incubated for 24 hours and fungal plates were incubated for 48 hours. Ciprofloxacin disc (5mcg/disc) [HiMedia, India] was used as standard. The inhibition zone was measured after respective incubation period.

Results and discussion

UV-vis and FT-IR spectra analysis

UV-*vis* spectra recorded at different time intervals for the reaction with aqueous chloroauric acid solution showed an initial increase in the absorbance, which later decreased with higher incubation period and became constant giving a maximum absorbance at 599.78 nm at three hours of incubation. The appearance of the lower color confirms the formation of gold nanoparticles in the reaction mixture and efficient reduction of the Au^{3+} to Au^{0} (**Fig**. **1**). Colored solution allowed measuring the absorbance against distinct wavelength to confirm the formation of AuNPs. UV-*vis* spectra recorded at different time intervals for the reaction with aqueous solution showed appearance of absorbance peak at 599.78 after 3 hours. In order to determine the rate of $AuNP_S$ formation the kinetics of the reaction with respect to time was studied with the help of UV-*vis* spectroscopy. The corresponding UV-vis spectra recorded

from $HAuCl_4$ plant extract reduction at various time intervals is shown in **Fig. 1**. On reduction of $HAuCl_4$ by leaf extract for various time intervals shows a decrease in the intensity of Au^+ at 400 nm bands and appearance of absorbance band at about 599 nm. A gradual increase in the intensity of absorbance band without any shift with increasing time from spectra indicates the slow reduction of Au^{3+} to Au^0. No significant change in the intensity from spectra also suggests that the reduction is going over upto 3 hrs.

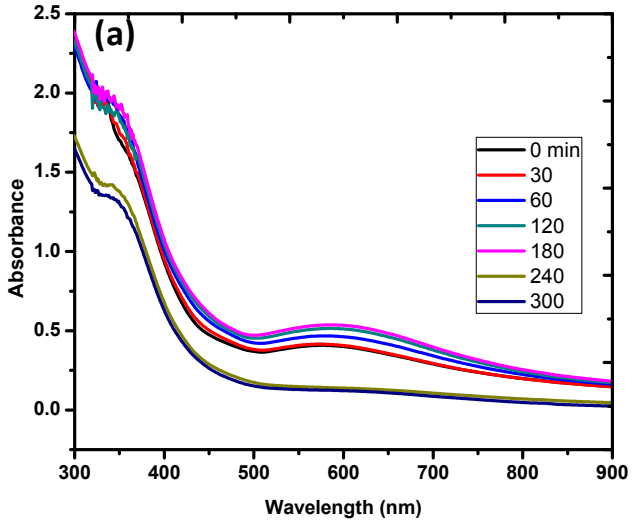

Fig. 1. UV–*vis* spectra of synthesized AuNPs.

The FTIR spectra of AuNPs with absorption peaks at 3432, 1631, 1599, 1384, 1351, 1030, and 760 were observed (**Fig. 2**). The spectra obtained to characterize the interaction between $HAuCl_4$ and plant extract has strong peak at 3432.0 shows the OH group (stretch H bonded, strong broad) along with the above mentioned peaks, some other peaks at 2959, 2925, 2804, 2718, and 2649 were also seen which corresponds to C-H group (stretch strong), C-H group (variable), C-O group (strong), =C- H group (strong), C=C group (variable). The highest absorption peak 3432 reflects that the OH group might be responsible for the reducing property of the extract.

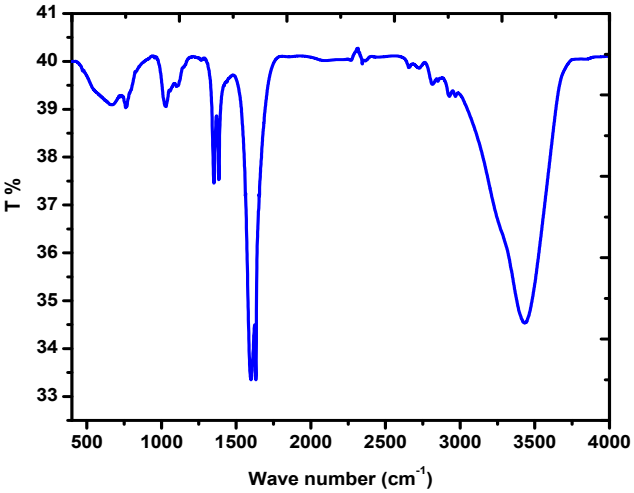

Fig. 2. FT-IR spectra of AuNPs.

SEM and TEM analysis

SEM images in (**Fig. 3a**) showed aggregation of AuNPs formed with diameter range from 50 nm to 80 nm. Analysis of the bio reduced green synthesized AuNPs s by TEM confirmed that they were in the nano range and of triangular and spherical shape (**Fig. 3b** and **b**'). The triangular shaped AuNPs formed were nano dispersed with large surface area. A large quantity of AuNPs was with thin smooth ends on the exterior of the nanoparticles with a range of 200 nm (Triangular shaped) in diameter at the highest resolution and the spherical shaped were of 50 nm.

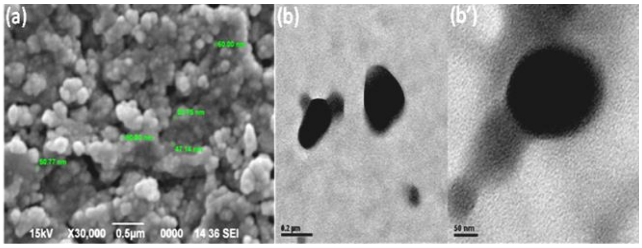

Fig. 3. (a) SEM and (b) TEM images of gold nanoparticles after bioreduction with *Nepenthes khasiana* leaf extract (b') at higher magnification.

XRD Analysis

The XRD pattern shows the production of AuNPs by the reduction of Au^{3+} to Au^0 using *N. Khasiana*. The diffracted intensities have been recorded from 20° to 80° at 2 theta angles. The diffracted pattern in **Fig. 4** significantly corresponds to pure AuNPs. In the spectra clear peaks are not observed it indicates that the nanoparticles had a spherical structure. Broadening peak and noise were probably related to the effect of the nano particles as supported by SEM and the presence of various crystalline biological molecules in the plants extracts.

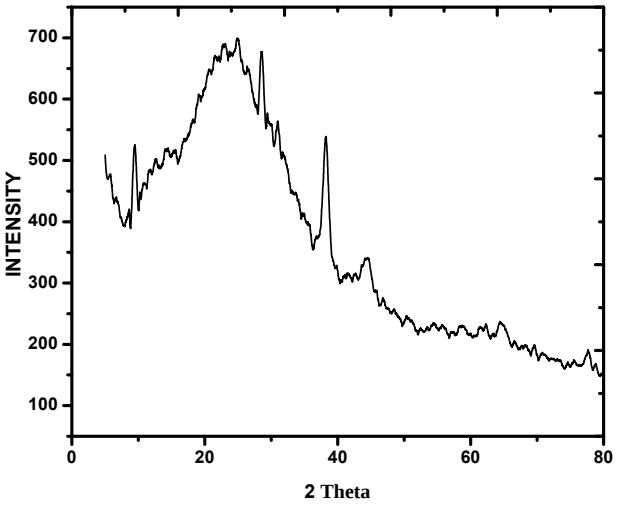

Fig. 4. XRD pattern of AuNPs synthesized from the leaf extract of *Nepenthes Khasiana*.

Antimicrobial activity

AuNPs showed antibacterial and antifungal activity against *E.coli*, *Bacillus* species (BH_2) and *Aspergillus niger*, *Candida albicans*. The antimicrobial activity of AuNPs was checked against the standard i.e. an antibiotic (Ciproflaxin).

AuNPs was less resistant against bacterial species; on the other hand, AuNPs was more resistant against fungal species, which can be precisely seen in the **Fig. 5**. The antimicrobial activity has been calculated by measuring the inhibition zone and mentioned in **Table 1**.

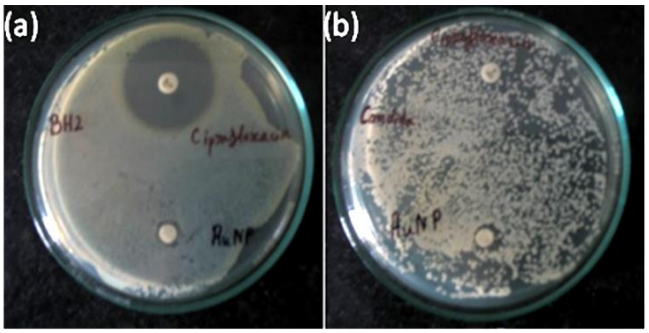

Fig. 5. Antimicrobial activity shown by AuNPs against Bacterial and Fungal species.

Table 1. Antimicrobial activity of AuNPs from the leaf extract of *Nepenthes khasiana*.

S. No.	Bacterial species	Inhibition zone (mm)	
		Standard	AuNPs
1	Bacillus sps. (BH_2)	30	10
2.	Escherichia coli	40	08
	Fungal Species		
3.	Candida albicans	NIL	10
4.	Aspergillus niger	NIL	16

Conclusion

In conclusion, we developed a eco-friendly, simple and efficient method for the synthesis of gold nanoparticles using leaf extract of *Nepenthes Khasiana*. The shape and size of the AuNPs were confirmed by SEM and TEM with triangular and spherical shape nanoparticle with an average size of 50 nm and 100 nm. The outcome of the experiments was positive concluding that the AuNPs synthesized shows good antimicrobial properties. The rate of reduction of metal ions using plant agents is found to be much faster and also at ambient temperature and pressure conditions. Future work should implement systematic experiments, which include development of gold nanoparticles of well-defined shape and size. Better understanding of the mechanism of gold nanoparticle biosynthesis will enable us to achieve better control over the size, shape and monodispersity which will lead to the development of high precision production and application of them for commercial use.

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