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Plant mediated synthesis of gold nanoparticles using fruit extracts of ananas comosus (L.) (pineapple) and evaluation of biological activities

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ABSTRACT

Plant mediated synthesis of metallic nanoparticles is an increasing commercial demand due to the wide applicability in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. In the present investigation, synthesis of gold nanoparticles is done by using fruit extracts of *Ananas comosus* (L.). Nanoparticles were characterized by using UV visible absorption spectra. Their morphology, elemental composition and crystalline phase were determined by scanning electron microscopy, energy dispersive X-ray spectroscopy and selected area electron diffraction. FT-IR analysis was used to confirm the presence of gold nanoparticles in the extracts. The synthesized gold nanoparticles were generally found to be effective as antimicrobial agents against some important human pathogens like *E.coli* and *Streptobacillus sp.* which are affecting and cause diseases like food poisoning and rat-bite fever to human beings respectively.

Keywords: *Ananas comosus* (L.)*;* gold nanoparticles; FT-IR; SEM-BSE.

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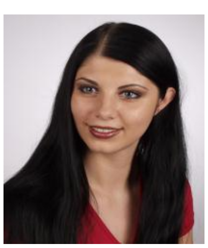

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Introduction

Nanobiotechnology is an upcoming branch of nanotechnology which has been playing an important role in the field of medical science, bioelectronics and biochemical applications and it often studies existing elements of living organisms and nature to fabricate new nano-devices. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research [**1**]. The biosynthetic method employing plant extracts has received attention as being simple, eco-friendly and economically viable compared to the microbial systems like bacteria and fungi because of their pathogenecity, and also the chemical and physical methods used for synthesis of metal nanoparticles [**2**].

Synthesis of nanoparticles using biological entities has great interest due to their unusual optical [**3**], chemical [**4**], photoelectro-chemical [**5**] and electronic properties [**6**]. The synthesis & assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable green chemistry procedure, probably involving organisms ranging from bacteria to fungi and even plants [**7,8**]. Hence, both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellulary [**9**].

Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods [**10**]. The use of plants for the preparation of nanoparticles could be more advantageous [**11**], because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures [**12**]. Several plants and their parts have been successfully used for the extracellular synthesis of metal nanoparticles [**12**].

In the present study, fruit extract of *Ananas comosus* (L.) was used to synthesize gold nanoparticles from $AuCl_4$. *Ananas comosus* (L.) comes under the Kingdom- *Plantae*, Family – Bromeliaceae, Genus- *Ananas*, and Species- *comosus.*

Pineapple is a perennial monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit and it is cultivated predominantly for its fruit that is consumed fresh or as canned fruit and juice and it is the only source of bromelain, a complex proteolytic enzyme used in the pharmaceutical market and as a meat-tenderising agent. Pineapple has been used as a medicinal plant in several native cultures and its major active principle, Bromelain, has been known chemically since 1876. The primary component of bromelain is a sulphydryl proteolytic fraction. It also contains peroxidase, acid phosphatase, several protease inhibitors and originally bound calcium. Pineapple has vitamin B_1, B_6 and its high content of vitamin C would also contribute to a little (over 30%) of its antioxidant potential [**13**]**.** It shows significant effects on hematological and biochemical parameters with Doliprane [**14**]**.** Ethanolic extract of Ananas comosus L. leaves has anti-diabetic, anti-dyslipidemic and anti-oxidative activities, which may be developed into a new plant medicine for treatment of diabetes and its complications [**15**]. No IgE-mediated reactions to pineapple have been described and no major allergens have yet been identified [**16**].

Experimental

Preparation of pinapple extract

Chloroauric acid ($HAuCl_4$) was purchased from Thomas Baker, Mumbai and was used without further purification. Fresh pineapple was purchased from the local market and authenticated by the Department of Bioscience, P.G. Centre, Hassan, University of Mysore. In typical preparation of pineapple extract, 20 g of peeled pineapple slices were ground in a blender, filtered through mesh and centrifuged twice at 10,000 rpm for 15 min at 4°C by REMI cooling centrifuge to remove cell-free debris. The resulting supernatant was then filtered through a 0.2 μm filter paper and employed for the synthesis of gold nanoparticles. Double distilled water was used to dilute the aqueous chloroauric acid stock solution and the original pineapple extract.

Pineapple extract-mediated synthesis of gold nanoparticles

In this study, pineapple extract is used to obtain phytochemically-derived reducing agents for the production and stabilization of gold nanoparticles. The nanoparticles were examined for their consistency in Surface Plasmon Resonance (SPR) properties and reduction rate by varying the concentration of the pineapple extract. The same plasmon resonance band was observed at 600 nm at various concentrations indicating uniformity in the formation of gold nanoparticles. In a typical experiment, dark conditions and a preincubation at 90°C were applied separately to a 0.002M $AuCl_4$ aqueous solution and the pineapple extract to achieve temperature equilibrium and the final total reaction mixture volume was 20 mL. Biosynthesis of gold nanoparticles (Pineapple-AuNPs) was begun by adding pineapple extract at 50% (v/v) with a final concentration of 0.002M $AuCl_4$. The formation of nanoparticles was monitored by UV-Vis spectroscopy. The mixture was centrifuged at 10,000 rpm for 15 min at 4°C. The process of centrifugation and redispersion was repeated three times to remove unbound pineapple phytochemicals. Rapidly produced pineapple-AuNPs within 20 min were collected and purified by repeated centrifugation as described above, and used to determine physicochemical and biocompatible properties [**17**].

Characterization of gold nanostructures:UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + fruit extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently measuring UV-vis spectra of the solution. UV-vis spectra of these aliquots were monitored as a function of time of reaction on Elico UV-vis spectrophotometer (Model SL 164 double beam) operated at a resolution of 1 nm.

SEM analysis

A scanning electron microscopy (SEM) image was obtained using JEOL JSM 7500F Field Emission Scanning Electron Microscope with a back scattered electrons (BSE) detector (marked as COMPO). K575X Turbo Sputter Coater was used for coating the part of the sample with chromium (deposited film thickness – 20 nm). The microstructure of samples was supported by chemical analysis carried out using energy dispersive X-ray spectroscope (EDX) at 20.0 kV and 15.0 mA.

FT-IR analysis

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm^{-1}. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Uvasol® purchased from Merck, Germany. The number of scans 16 and the resolution of 4 cm^{-1} characterized these measurements.

Antifungal and antibacterial activity

Microbial cultures

Aspergillus niger, Aspergillus flavus, E. coli and *Streptobacillus sp***.** collected from authenticated stock culture of our college itself. *A. niger* and *A. flavus* were maintained in Potato dextrose broth (Hi-Media). *E. coli* and *Streptobacillus* were cultured in Nutrient broth (Hi-Media).

Microbial activity by agar well diffusion method

Antifungal and antibacterial activity was measured using well diffusion method. Wells were prepared in the medium using sterile gel puncture. Overnight Bacterial cultures were swabbed with a sterile cotton swab on plates containing Muller Hinton agar (MHA) medium (Hi-Media) and fungal species on Potato dextrose agar (PDA) medium (Hi-Media). Then 10 µl of antibiotic solution and 10 µl of gold nanoparticles were added to the wells. Wells with antibiotic solution alone were served as positive controls. The Petri plates were incubated at $37^{O}C$ for 24 hrs for bacteria and 48 hrs for fungi.

Results and discussion

The extracellular synthesis of gold nanoparticles occurred during the exposure of pineapple fruit extract to 0.002M $AuCl_4$ aqueous solution. The complete reduction of gold ions was observed after 3-4 hours. The colour change in reaction mixture was observed during the incubation period, because the formation of gold nanoparticles is able to produce the particular colour in the reaction mixtures due to their specific properties. The appearance of dark brown color is a clear indication of the formation of gold nanoparticles in the reaction mixture. The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an in-phase oscillation which is known as Surface Plasmon Resonance-SPR [**18**].

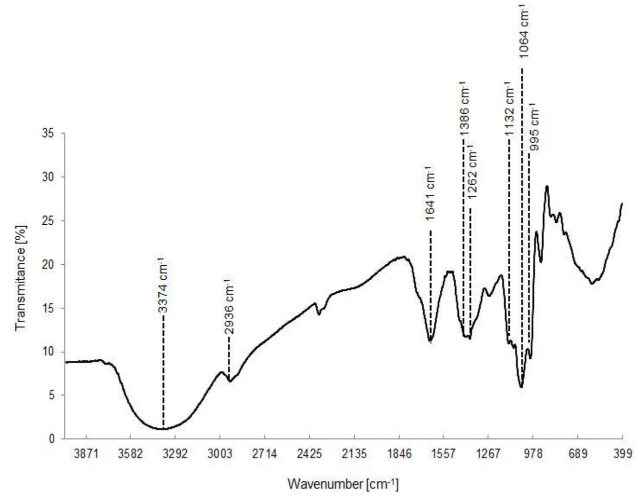

Fig. 1. FT-IR absorption spectrum obtained from gold nanoparticles biologically synthesized by reduction of $AuCl_4$- ions using fruit extract of *Ananas comosus.*

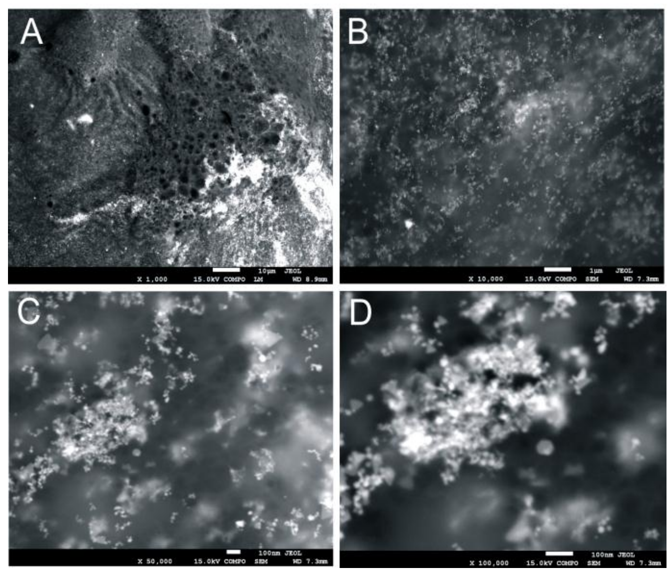

Fig. 2. SEM-BSE images (A, B, C and D) with different magnifications illustrating the formation of gold nanoparticles biologically synthesized by reduction of $AuCl_4$- ions using fruit extract of *Ananas comosus*.

UV-Vis spectroscopy analysis showed that the SPR absorbance band of gold nanoparticles synthesized using *Ananas comosus* fruit extract centered at 600 nm and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [**19**].

The interaction sites of fruit extracts and gold nanoparticles were characterized by FT-IR spectroscopy. Plant mediated synthesis caused that nanoparticles are surrounded by some proteins and metabolites identified in the spectra as follows. The band at 1064 cm^{-1} corresponds to the C – N stretching vibration of aliphatic amines or to alcohols/phenols. The weak bands that appeared at 995 and 1132 cm^{-1} can be ascribed to C – O – C or C – O stretching modes in phenolic compound or phenolic derivatives (**Fig. 1**). The absorption appearing at 1262 and 1641 cm^{-1} can be assigned to the amide III and amide I bands of proteins and the band at 1386 cm^{-1} corresponds to C – N stretching vibrations of aromatic amines. The band at 2936 cm^{-1} is characteristic of the secondary amines. The intense broad absorbance at 3374 cm^{-1} corresponds to the hydroxyl functional group in alcohols and phenolic compounds [**20-24**].

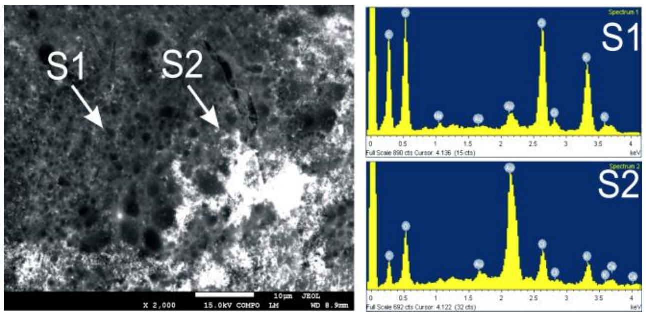

Fig. 3. EDX analysis illustrating the formation of gold nanoparticles biologically synthesized by reduction of $AuCl_4$- ions using fruit extract of *Ananas comosus*.

Table 1. Antimicrobial activity of the gold nanoparticles synthesized from Pineapple.

Antibiotics	*Aspergillus flavus* A	*Aspergillus flavus* A+P	*Aspergillus niger* A	*Aspergillus niger* A+P	*E.coli* A	*E.coli* A+P	*Streptobacillus* A	*Streptobacillus* A+P
Amphicilin	-	-	-	-	13.63±0 .45	18.05±0 .42	19.49± 0.38	20.33±0 .29
Penicillin	-	-	-	-	14.75±0 .66	17.55±0 .43	15.67± 0.40	19.52±0 .40
Bavistin	-	-	14.45±0 .48	15.67±0 .398	-	-	-	-

(Diameter of zone of inhibition (mm); Organisms)

The BSE-SEM images, elemental analysis with EDX confirmed the presence of gold nanoparticles in tested sample – bright points visible with help of Back Scattered Electrons (BSE) detector (**Fig. 2, 3**). As shown in **Fig. 2C**, the obtained nanoparticles have sphere shapes. During the separation the gold nanoparticles form aggregate in a large cluster shown in **Fig. 2C-D**. However, the distribution of agglomerates on the surface was almost uniform as shown in **Fig. 2B.**

The EDX quantitative analysis confirmed that the gold content has the highest elementary composition, while chlorine has a minor content together with only a trace of potassium and calcium (**Fig. 3 S2**).

Anti bacterial study indicated that antibiotics with gold nanoparticles extracted from Pineapple (A+P) exhibited more zone of inhibition compared to standard antibiotics (A) alone (**Table 1** and **Fig. 4**) and the effect of antibiotics were analysed based on the zone of inhibition around the microbial colonies. Ampicillin, Penicillin and Bavistin were used for anti bacterial studies against four organisms' viz. *Aspergillus niger, Aspergillus flavus, E.coli and Streptobacillus sps.* Ampicillin and Penicillin inhibited the growth of *E. coli* and *Streptobacillus* sp.

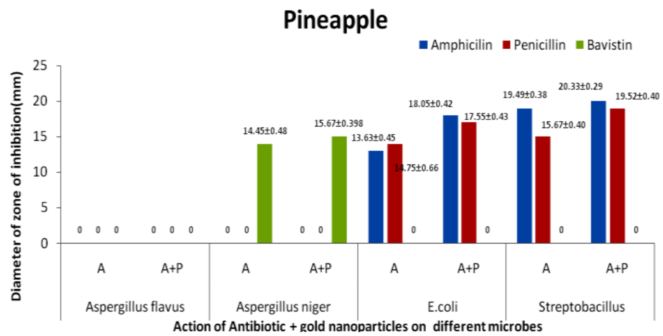

Fig. 4. Antimicrobial activity of gold nanoparticles synthesized from pineapple.

Pencillin's and Ampicillin's activity was increased highly due to gold nanoparticles against *E. coli* and *Streptobacillus* and the change was high for Ampicillin than Penicillin. Along with Gold nanoparticles, Ampicillin showed maximum inhibition against both bacteria than Penicillin. *Streptobacillus* was inhibited more by Ampicillin than *E. coli.* They did not show any effect individually against *Aspergillus niger* and *Aspergillus flavus* and also along with gold nanoparticles. Bavistin inhibited *Aspergillus niger* individually (14 mm) and with gold nanoparticles (15 mm). All three antibiotics did not show any effect on *Aspergillus flavus* indivdually and in coated form.

Reduction of gold ion into gold particles during exposure to the fruit extracts could be followed by color change. Gold nanoparticles exhibit dark brown color in aqueous solution due to the surface plasmon resonance phenomenon. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolved into an important branch of nanotechnology. The present study emphasizes the use of medicinal plants for the synthesis gold nanoparticles [**25**].

The nanoparticles were primarily characterized by UV-Vis spectroscopy, which was proved to be a very useful technique for the analysis of nanoparticles. Reduction of Au ions in the aqueous solution of gold complex during the reaction with the ingredients present in the plant fruit extracts observed by the UV-Vis spectroscopy revealed that gold nanoparticles in the solution may be correlated with the UV-Vis spectra. As the fruit extracts were mixed with the aqueous solution of the gold ion complex, it was changed into dark brown color due to excitation of surface plasmon vibrations, which indicated that the formation of gold nanoparticles [**26**]. UV-Vis spectrograph of the colloid of gold nanoparticles has been recorded as a function of time by using a quartz cuvette with chloro auric acid as the reference. In the UV-Vis spectrum, the broadening of peak indicated that the particles are poly dispersed. The reduction of gold ions and the formation of stable nanoparticles occurred rapidly within 2-3 hours of reaction making it one of the fastest bioreducing methods to produce gold nanoparticles. The surface plasmon band in the gold nanoparticles solution remains close to 600 nm throughout the reaction period indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation. The UV-Vis spectrum of pineapple juice alone shows surface plasmon band at 430 nm indicates the synthesis of gold nanoparticles from the band at 630 nm. The gold nanoparticles formed were predominantly cubic with sphere shape. It is known that the shape of metal nanoparticles can considerably change their optical and electronic properties [**27**]. The SEM image showed relatively sphere shape nanoparticles formed with diameter in the range of 10 ± 5 nm. Energy dispersive spectrometry (EDS) micro-analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen. EDX spectra were recorded from the gold nanoparticles. From EDX spectra, it is clear that gold nanoparticles reduced by *Ananus comosus.*

FT-IR peaks in the extract ranges from 4000-400cm^{-1} which confirmed the presence of main groups occurred in natural plant extract from *Ananus comosus* (Pineapple). The absorption appearing at 1262 and 1641 cm^{-1} can be assigned to the amide III and amide I bands of proteins and the band at 1386 cm^{-1} corresponds to C – N stretching vibrations of aromatic amines. The band at 2936 cm^{-1} is characteristic of the secondary amines. The intense broad absorbance at 3374 cm^{-1} corresponds to the hydroxyl functional group in alcohols and phenolic compounds. Food poisoning and rat bite fever are the major problems around the world. Antibiotics is the only choice of treatment for these diseases, the reason for this is that these infections do not elicit pronounced immune response hence effective vaccination may not be possible. Meanwhile the traditional medicines have been used to treat these diseases for thousands of years. The plant is commonly used in traditional medicine, fruit extract of *Ananus comosus* is very good bioreductant for the synthesis of gold nanoparticles and synthesized nanoparticles are active against clinically isolated human pathogens *E. coli*, and *Streptobacillus.* In addition to antimicrobial activity, the phenolic extract of *Ananus comosus* shows significant antioxidant [**28**], antidiabetic [**29**], hypolipidemic [**30**], hypoglycemic [**31**] and also diabetic-dyslipidemic [**32**]. Bromelain is the mixture of enzyme which digests protein exhibits a potent antifungal activity against phytopathogens and suggests its potential use as an effective agent for crop protection [**33**] and the leaf extract of *Ananus comosus* shows significant antibacterial activity [**34**] but this is the only report to investigate antimicrobial activity of gold nanoparticles synthesized from fruit extract of *Ananus comosus.*

Conclusion

In conclusion, our study can be considered as the first report for the synthesis of gold nanoparticles using extracts of *Ananus comosus* fruit extracts. Gold nanoparticles were confirmed by color changes and were characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 600 nm for gold nanoparticles. The BSE-SEM images shows formation of spherical shape gold nanoparticles. The sizes of the nanoparticles were in the range of 10 ±5 nm, showing a broad size distribution**.** FT-IR peaks were in the extract ranging from 4000-400cm^{-1} which confirmed the presence of gold nanoparticles. *Ananus comosus* appears to have significant antimicrobial capacity resembling a broad spectrum antibiotic against *Streptobacillus Sp* and the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and is relatively resistant towards synthetic drugs.

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