Nanoparticle syntheses of biological application in orchid plant stem extract. An endemic flora in India

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Abstract: Nanoscience is improving of modern world young research in have fast current years in the field of bimolecule science. Nanosynthesis of properties is of basic importance in advanced plant biomolecule components search in the biomedical. Indian flora towards World long history of sidda medicine in kolli hills triple people. In the present investigation work designed to nanosynthesis of silver and gold nanoparticle has been done using a selected medicinal plant part Blbophyllum kaitense (Orchidaceae) stem though there are biochemical present in the plant. The synthesis of silver nitrate ($AgNO_3$) and Chloroauric acid ($HAuCL_4$) for the synthesis of silver and gold nanoprticles respectively with the plant stem extract. The plant stem extract is mixed with ($AgNO_3$) and ($HAuCL_4$) incubated furthermore studied synthesis of nanoparticle using UV Vis spectroscopy. The nanoparticle were molecule morphology characterization of FT-IR spectra, Scanning electron spectroscopy, Transmission electron spectroscopy equipped with XRD. The generally found to be spherical crustal shaped but it size range of 102 nm. Whereas the synthesized gold nanoparticle were found to be dispersed crystal nanoparticle in the size range of 108nm. The silver synthesis nanoparticle TEM analysis was employed to visualize was found to be spherical shaped in the size range of 98nm. Whereas the synthesized gold nanomolecules were spherical shaped in the range of 102nm. The work carried out showed the stem extract is excellent bio reductant. The antimicrobial activity of synthesis silver and gold nanomolecules active against human pathogenic organisms *Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi.*

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Keywords: Blbophyllum kaitense, $AgNO_3$, $HAuCL_4$, UV Vis spectroscopy, FT-IR spectra and TEM analysis.

1. Introduction

Bio-nanotechnology combines biological chemical principles with physical and chemical approaches to produce nano-sized particles with specific functions. It also represents an economic substitute for chemical and physical methods of nanoparticles formation. Nanoparticles exhibit completely. New or improved properties based on specific character such as size, distribution and morphology [1]. In Indian medical system (Ayurveda) gold is used as medicine in the preparation of nano level Swarna Bhasma [2].

Biological methods for the production of nanoparticles are considered as a safe and environment friendly and it is a cost effective method and toxic chemicals in completely eliminated [3]. Carried out that the plant extract can be used as an excellent source for synthesizing the nanoparticles as well [4]. Observed that the Artocarpus hircutus demonstrates strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions. This provides evidence for developing large scale commercial production of value added products for biomedical or nanotechnology [5].

Furthermore the color change from colorless to brown of a mixture containing only $AgNO_3$ solution and Achillea bibersteinii extract occurred within 180 min at 40°C. the highest color intensity was observed in a solution containing 10mL of silver nitrate 5mM and 0.8ml of plant extract [6]. Recently green synthesis of gold nanoparticles using Argemone Mexicana L. leaf extract a reddish brown colour solution was obtained [7]. Different researchers reported the silver nitrat, it started to change colour (within 30 minutes) from brown to blackish green in case of Bryophyllum. The colour change might be due to excitation of surface Plasmon vibration indicative of the formation of AgNP [8].

Bring out the use of toxic chemicals for the synthesis of gold and silver nanoparticles. So it can be used for biological applications. This synthesis approach of gold and silver nanoparticles is cost effective and can be widely researched because of their unique physical properties, chemical reactivity and potential applications in catalysis, biological labeling, biosensing, drugdelivery, antibacterial and antiviral activity, detection of genetic disorders, gene therapy and DNA sequencing [9]. Current report observed that the Bulbophyllum kaitense leaves, pseudobulb synthesis of silver and gold nanoparticles. The future using such plant extract to develop bio nanomedicine against various human pathogen and as well as food. Cosmetic with drinking water purified industries [10].

Bulbophyllum kaitense for nanoscience and nanomedical with cosmetic based industries [11]. Previously reported that the especially the medical properties of gold have been gold known for 2,000 years. Since the nineteenth century gold based compounds have been used in many antimicrobial applications. An alternate and feasible method to synthesis gold nanoparticles is to employ biological methods using many biological sources especially plants [12] Recent advances nanotechnology development of plant component increasing renewable in the medical field.

2. Plant Material and Methods

Habitat of epiphytes

Habitat of lithophytes

Figure.1 The plant Bulbophyllum kaitense reichb growing adaptation of morphology.

The *Bulbophyllam kaitense* (Tamil vernacular name: Oru ethal elai) Belongs to the family orchidaceae was first identified at Sethurpatti nadu urachi kolli hills of Namakkal District, Tamil Nadu, India. Herbarium specimens were Prepared and taxonomic identification of the plant *Bulbophyllam kaitense* was confirmed at the Rapinat Herbarium and Centre for Molecular Systematic, Tiruchirappalli, with the voucher number: RHT. 872. A voucher specimen of Plant was deposited to that the Rabinate Herbarium for future reference.

***BULBOPHYLLUM KAITENSE* REICHB**

Kingdom - Plantae
Unranked - Angiosperms
Unranked - Monocots
Order - Asperagales
Family - Orchedaceae
Genus - Bulbophyllum
Species - kaitens

3. Green Bio-synthesized Silver and Gold Nanoparticles

3.1 Chemical

Silver nitrate (AgNO3), Chloroauric acid (HAuCL4) and other components were purchased from Himedia, Mumbai, India.

3.2 Preparation of Plant Extract

The stems of B. kaitense were washed thoroughly thrice with distilled water and were shade dried for 10 days. The fine powder was obtained from the dried plant materials by using Kitchen blender. The plant powder was sterilized at 121 °C for 15 minutes. 50 g of powder was taken and mixed with 200 mL of Milli Q water and kept in boiling water bath at 60 °C for 10 minutes. The extracts were filtered with whatman filter paper No. 1. The filtered extract was stored in refrigerator at 4°C for further studies.

3.3. Biosynthesis of Silver and Gold nanoparticles

Plant stem cut piece for air dry

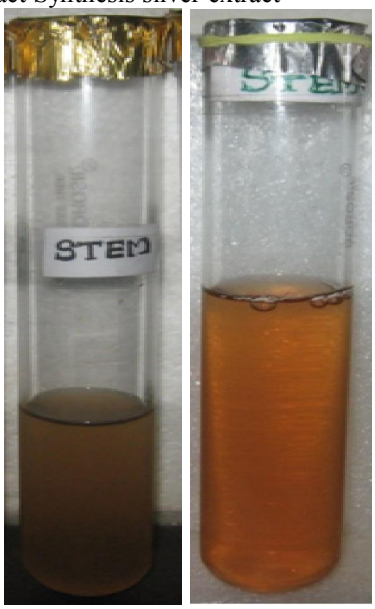
For the biosynthesis silver nanoparticles, 1.5 ml of plant extracts is mixed with 30 ml of AgNO3 solution (1 mM) and incubated at 28 °C for 24 hours. Small aliquot of solution is used for the UV–V is spectroscopy and FTIR is performed to the extract which was exposed before and after addition to the silver nitrate solution. The reactions mixture is

centrifuged at 6000 rpm for 10minutes and the pellet was re suspended in small amount of sterilized double distilled water and then small amount of suspension was sprayed on glass slideto make thin film. The thin film was kept in hot air oven to dry and then the thin film was used for the SEM and TEM analysis equipped with EDAX (Model JEOL, JSM-5610). The same procedure is followed for gold nanoparticles synthesis.

Prepare for plant stem powder

Figure.2 The plant powder preparation stem cut and stem powder

Plant extract Synthesis silver extract



Synthesis gold extract

Figure 3. Nanoparticle synthesized of silver and gold plant stem extract.

3.4. UV-VISIBLE Spectral Analysis of Bioreduction of Silver and Gold Synthesis Plant Extract

The bioreduction of Silver and Gold in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml of de-ionized water and subsequently measuring UV-visible spectra of the resulting diluents. UV-visible spectroscopy analyses of Silver and Gold nanoparticles produced were carried out as a function of time needed for bioreduction at room temperature on Thermo Heyios 2 model spectrophotometer at 190 – 1100 nm.

3.5. FTIR Analysis of Bio-synthesis for Silver and Gold Plant Extract

A pellet for infrared (IR) analysis was obtained by carefully grinding 2 mg of Silver and Gold bio-synthesis plant extract with 200 mg of dry potassium bromide, ground well in mortar under an IR lamp for 30 mines and then pressing in a mold. The IR spectrum of Silver and Gold nanoparticle plant extract from 400 to 4000 cm-1 was obtained using a Perkin-Elmer spectrum GX.

3.6. EDAX Measurements Analysis of Silver and Gold Nanoparticles

In order to carry out EDAX analysis, the extracts reduced Silver and Gold nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 NSEM instrument equipped with a thermo EDAX attachments. Energy dispersive X-ray spectrometers take advantage of the photon nature of light. In the X-ray range the energy of single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultralow noise preamplifier

connected to the low noise are a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses within a so called multi channel analyze a complete image of the X-ray spectrum is building up almost simultaneously. This digitalquantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the X-raystogether with processing electronics to analyses the spectrum.

3.7. SEM Analysis of Silver and Gold Nanoparticles

Scanning electron microscope was done in Hitachi S – 3500 N. By drop coating, Silver and gold nanoparticle were prepared for High-resolution scanning electron microscope analysis on to pure Titanium coated. The film on the SEM grids were allowed to stand for 2 min following which the extract solution was removed using a blotting paper and grid was allowed to dry, prior to the measurement. SEM measurement performed on a Hitachi S-3500 N use these conditions 20,000 X magnification, ~15 mm working distance. Instrument operated at an 25 KV accelerating voltage, objective aperture # 3and condenser lens strength set to 50.

3.8. TEM Analysis of Silver and Gold Nanoparticles

Transmission electron microscope was done in TANUVAS, Chennai. By drop coating, silver and gold nanoparticles were prepared for higher solution transmission electron microscope analysis on to carbon coated copper TEM grids. The film on the TEM grids were allowed to stand for 280 mines following which the extra solution was removed using a blotting paper and grid was allowed to dry, prior to the measurement. TEM measurements were performed on a JEOL 3010 instrument operated at an accelerating voltage of 300 KV.

4. Result

4.1. UV-VIS Spectra Analysis

As soon as, Bulbophyllum kaitens stem extract was mixed in aqueous solution of $AgNO_3$ and $HAuCL_4$. The bio synthesis reaction started within few minutes and colour reaction were observed in which clear $AgNO_3$ solution changed yellowish into orange coloure. Whereas plant extract pale yellowish $HAuCL_4$ nanoparticle solution turned to light brown coloured solution which indicates that the formation preliminary identify corresponding suggest synthesis nanoprticles. [Fig 1]. Experimental studies the UV-Vis spectra of silver and gold nanoparticles synthesized in plant extract are shown in [Fig 2]. UV-Vis spectra were recorded as reaction peak time and nm. Interestingly observed that the synthesis of silver nanoparticles the surface Plasmon resonance of silver occurrence of 432nm [Fig 2b]. An overview of the after addition plant gold synthesized extract. The colour of varied from pale yellow to light brown coloured. The evidence of synthesis gold nanoparticle is present in the plant extract. [Table 1], these data support a broad peak was observed 223nm synthesis gold nanoparticle.

4.2. FT IR spectra data analysis nanoparticle functioning group of characterizations is silver and gold

In our experience investigate to FT-IR spectra data analyses were find possible bio reducing molecules present in the extract. Spectra data of extracts were recorded before and after synthesis of nanoparticle. Plant extract [a], silver nitrate [b] and Chloro auric acide [C], [Fig 3a, b, c.] the synthesis nanoparticle molecule function group recorded in the table. 2.

Another interpretation of the infrared data usually have sharp feature of molecular characterization is specific groups vibration, making the data useful for sample identify the performed to biomolecules responsible for capping, reducing and stabilizing the silver and gold nsnoparticles present in the stem extract.

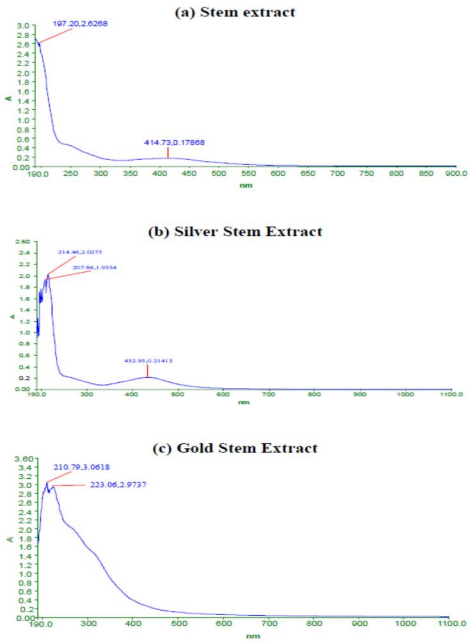


Figure.4 UV- Vis Spectroscopy analysis of stem extract after synthesized silver and gold nanoparticle

Carried out to FT-IR spectra of the plant extract, which carefully carried out find bands at 3429cm^{-1}, 3342cm^{-1} correspond to the O-H stretching of hydroxyl groups. Therefore the relatively strong absorption bands around 1638cm^{-1} indicated the

characteristics IR absorption of polysaccharides. Consistent with observation of band 2074cm^{-1}, 2077cm^{-1} from these individual lipid spectra it is clear that characteristic and distinct finger prints for triglycerides and phospholipids.

Table.1 FT-IR analysis of before and after synthesized nanoparticle were functioning groups

S.No	Groups	Plant stem extract [nm]	Silver nanoparticle [nm]	Gold nanoparticle [nm]
1	Chloroalkanes	686	685	660
2	CO	1638	1637	1637
3	C-H	2075	2074	2077
4	OH or N-H groups	3424	3429	3342

It frequency is found in the range between the weaker band at 1637cm^{-1} according to amide I, the exact position determined data of the pure lipid compounds are more complex. Tree distinct absorption bands are appetent of which the CH_3 and CH_2 group. Thus various outcomes seen in the present strong bands between 660 cm^{-1} and 685cm^{-1} related variation of carbohydrates, lipids and proteins. Proteins are the largest group and the repeat unit in proteins gives of the protein infrared spectra. Amide I is the most intense absorption band is proteins.

It is primarily governed by the stretching vibrations of the C=O and C-N groups. Strongly suggested that the FT-IR spectra find out the presence of carbohydrate, proteins, DNA and lipids varying composition and quantity as evidenced the appearance of difference in both molecule function group and absorption intensity of synthesized plant extract.

4.3. Energy Dispersive X-RAY Spectra

Activation of the through energy dispersive x-ray [EDAX] spectra confirmed the presence of silver and gold nanoparticle. The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in Kev. Identification lines for the major emission energies for silver [Ag] and gold [Au] are displayed and these correspond with peaks in the spectrum. Thus giving confidence that Ag and Au has been exactly [fig.5 a,b,c]. The presence of elemental signal was confidently correctly.

4.4 Scanning Electron Microscopy Morphology Analysis of Silver and Gold Synthesized Nanomolecules

Broadly to the scanning electron microscopic pocus of synthesis nanoparticle shape and sizes was observed that the stem extract nanoparticle are spherical crustal shape but it is size range of 102 nm the recorded fig 5 a, b. Above investigate determined to the gold synthesis nanoparticle indicating that them also were dispersed crystal shape nanoparticle in the size range of 108 nm. Our knowledge suggested that the silver and gold nanoparticle synthesis correctly size and shape.

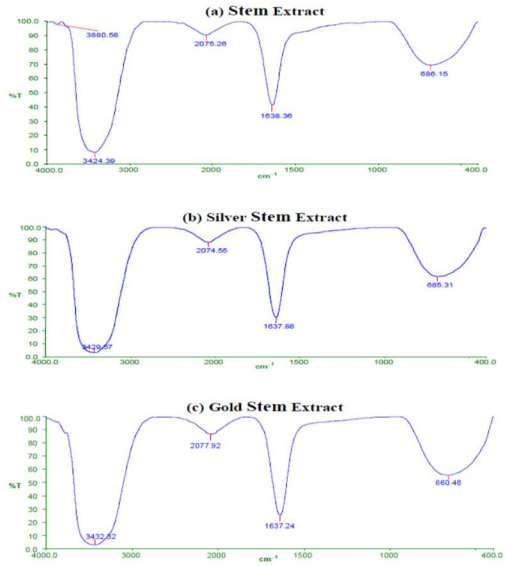


Figure.5 FT-IR Spectroscopy analysis of stem extract after synthesized silver and gold nanomolecules

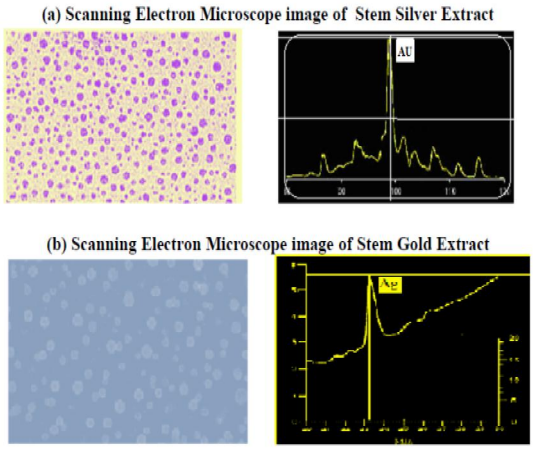


Figure.6 Scanning electron microscopy and energy dispersive x- ray fluorescent spectroscopy analysis of synthesized nanopartcle

4.5. Transmission Electron Microscopy

Advance discovery of transmission electron microscopic was found out to visualize were the

enlarged shown silver nanoparticle spherical shape but its range in 98nm. Other then gold nanoparticle closely shows spherical shape range of size in 102 nm. Strongly evidence of transmissions electron microscopic monograph. Fig. 6 c, d.

5. Antimicrobial Activity Essay in the Synthesis Nanoparticle Plant Extract

In contrast antimicrobial activity of nanoparticle synthesized silver and gold extract were against human pathogenic. Microbial growth *Pseudomonas aeruginosa, salmonella typhi* is highest zone of that against *Escherichia coli, Candida albicanse* synthesized gold extract. The minimum zone of inhibition *Escherichia coli* in both samples. The table shows 3 result obtain whereas in plant extract maximum zone of inhibition *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* for both saples. The synthesized silver nanoparticle highest zone of inhibition in *Candida albicans* were against all samples. Our decidedly clearly good antimicrobial activity of the plant synthesized *Bulbophyllum kaitense* stem extracts.

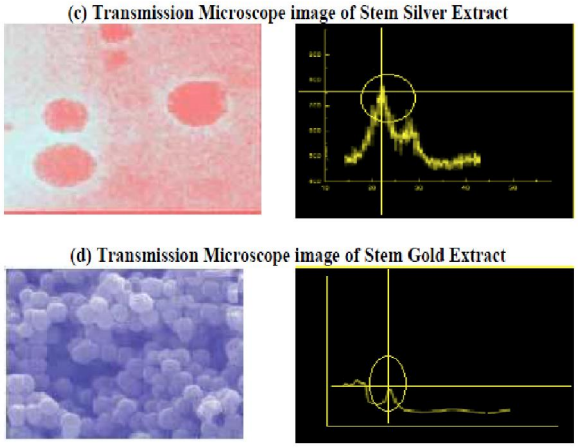


Figure.7 Transmission electron microscope and energy dispersive x-ray analysis of synthesized nanoparticle

Table.2 Antimicrobial activity of synthesized silver and gold nanoprticle in Bulphyllum kaitense stem extracts

S.No	Micro Organisms	Plant extract and synthesized nanoparticle		
		Plant Extract	Silver nano particle	Gold nano particle
1	Escherichia coli	11cm	9cm	10cm
2	Pseudomonas aeruginosa	11cm	10cm	12cm
3	Salmonella typhi	12cm	10cm	12cm
4	Candida albicans	Nil	12cm	Nil

6. Discussion

Binding to the extract was to $AgNO_3$ and $HAuCL_4$ biosynthesis reaction started with in few minutes and the color $AgNO_3$ solution changed into brown color, whereas pale yellowish $HAuCL_4$ solution which indicates that formation corresponding nanoprticle [13]. Similar report for the nanoporticle synthesis reaction was started after the leaf extract of *Erithrina indica* was introduced into aqueous silver nitrate solution [14]. Additional report evidence of the silver nanoparticles exhibit reddish pink color in aqueous solution due to excitation of surface Plasmon vibration in silver nanoprticles [15].

This another report the Fourier transform infrared spectroscopy showed the amines and secondary metabolites exciting in the pharmaceutical plant *Thymus vulgaris* leaf extract were responsible in the bioreduction and stabilization of silver nanopartcles [16]. Overall data function group analysis of biosynthesized nanoparticles peak at 3902,3888, 3853,3766, 3647 and 3473cm^{-1} was assigned as –OH stretching in phenolic compounds, peak at 2927cm^{-1} it represent C-H and also peak at 1646cm^{-1} represents C=O [17].

However the absorption peak at 3329, 1620, 1395, 1319 and 1049 and 1049cm^{-1}. The absorption peak 3329cm^{-1} is attributed to the O-H stretching vibrations of alkaloids or steroids. The absorption peaks at 1620 and 1395cm^{-1} indicates the C=O stretching vibration of fatty acids and carboxylic O-H bending fatty acid respectively [18, 19,]. It is also have been proposed that the analysis through energy dispersive x-ray spectrometers identification lines for the major emission energies for Au and Ag are displayed and this corresponding with peaks in the silver and gold has been correctly [10, 11, 7] Moreover the morphology of GNPs was studied using field emission gun scanning electron microscope the GNPs with unique morphological features were observed [20].

Overlook find the bio green synthesized AuNPs by TEM confirmed that they were in the nano range triangular and spherical shape [21,22,23]. Obviously the nanoparticles show the antibacterial activity against both gram positive and negative bacteria comparing the zone of inhibition it can be concluded that the silver nanoprticles have greatest antibacterial against *Salmonella* and least against *E.coli* [24,25,26,].

[27] The antimicrobial activity of aqueous *M. umbellatum.*

7. Conclusion

Highly decidedly demonstrates biosynthesis of silver and gold nanomolecules usin Bulbophyllum kaitense stem extract of a well-known medicinal plant. Nanomolecule were synthesized in molecule morphology and characterization was totally outlook by UV-Vis spectra, FT-IR, SEM and TEM equipped with EDX. The synthesized silver and gold inhibited a deep antimicrobial activity. The reaction for the synthesis of nanoparticles in magnification using these available plant extract material. The plant kolli hills people using various diseases but local people the cultivated forest export to medicinal practitioners and Ayurvedda medicine. So in the expensively produce for large amount of growing plant in Indian reserve forest. The as well as development of plant tissue culture and molecular genetically in the plant. In the endemic Indian flora and origin of India, only growing Kolli hills but not growth for other state and country.

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