Orchid Leaf Mediated Biosynthesis of Silver And Gold Nanoparticles with Antagonistic Activity against Human Pathogens.

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Abstract: Most important application of silver and gold nanoparticles is in medical industry such as topical ointments to prevent infections green biologically synthesized silver and gold nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and inter collation material for electrical batteries, as optical receptors catalysts in chemical reactions, biolabelling and biosensors. Biosynthesis of nanoparticles is due to wide biomedical approach and research inspiration in nanotechnology. Bioreduction of silver nitrate (AgNO₃) and chloroauric acid (HauCl₄) for the synthesis of silver and gold nanoparticles respectively with plants extract. Bulbophyllum kaitense (Orchidaceae). The plant leaf extract is mixed with AgNO₃ and HauCL₄, incubated and carried out synthesis of nanoparticles using UV-Vis spectroscopy. The nanoparticles were characterized by FT-IR, scanning Electron Microscopy, Transmission Electron Microscopy equipped with Energy Dispersive x-ray fluorescence spectrometry. The scanning electron micrograph, the morphology of the silver and gold nanoparticles synthesize were generally found to be slightly oval shaped but its size range of 110nm, whereas the synthesized gold nanoparticles were found to be round spike shape nanoparticles electron to visualize size and shape. The transmission electron micrograph visualise and shape in silver and gold synthesized nanoparticles. The nanoparticles synthesized found to be spherical shaped but its size range-in 90nm, whereas the synthesized gold nanoparticles were found to be granules shaped but its size range is 140nm. The results showed that the leaf extract of Bulbophyllum kaitense is very excellent biosreductant for the synthesis of silver and gold nanoparticles and synthesized nanoparticles antagonistic active against human pathogens, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Candida albicans.

Keywords: Silver nitrate (AgNO₃), Chloroauric acid (HauCl₄), Energy Dispersive x-ray fluorescence spectrometry, Scanning electron micrograph, Transmission Electron Microscopy.

1. Introduction

Nanotechnology has a wide range of approaches in the fields of biology, Medicine, optical, electrical, mechanical, opted electronics, photochemical, son chemical, biolabelling, biosensors and the most widely used known approaches of nanoparticles are applicable in water purified industry currently there is growing need to develop environmentally benign silvers and gold nanoparticles synthesis processes that don’t use toxic chemical in the synthesis protocol. A large number of plants are being currently investigated for their role in the synthesis of silver and gold nanoparticles nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled disparity and their potential use for human benefits [1].

As a result observed that the concentration of Phyllanthis extract is the reaction medium. The change of hexagonal shaped gold NP to spherical particle was greater and the size of the NPs can be modulated accordingly infrared (NIR) region can be easily turned. The prominent disparities of shapes control between gold and silver NPs [2]. Biosynthesis of NPs found that the considerable attention due to the growing need to develop clean, nontoxic chemicals, environmentally benign sowings and renewable materials [3].

Another research noticed that the demonstrated synthesis of gold nanotriancles and silver nanoparticles using Aloe vera plant extract. [4] In addition, they had

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also synthesized gold nanoparticles using tamarind leaf extract and studied their potential application in vapor sensing [5] reported that the also explored the mechanism of formation of gold nanotriangles by lemongrass extract. They found that the nanotriangles seemed to grow by a process involving rapid reduction assembly and room temperature sintering of liquids like spherical gold nanoparticles [6]. Recently reported that the biosynthesis of silver nanoparticles using lemon leaves extract and its application for antimicrobial finish on fabric [7].

In the last decade, carryout the various biosynthetic approaches, the use of plant extracts has advantage such as easily available, safe to handle and possess a broad viability of metabolites it has been reported that medicinally valuable angiosperms have the greatest potential for the synthesis of metallic nanoparticles with respect to quality and quantity [8, 9] The synthesis of silver nanoparticles of varying size using aqueous leaf extract of cassia articulate different concentrations. The synthesized nanoparticles were evaluated for its antimicrobial activity against human pathogens bacteria as well as fungi. [10].Very recently Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder [11].


2. PLANT MATERIAL

The Bulbophyllum 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 Bulbophyllum 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 (Figure 1).

Bulbophyllum kaitense Reichb (Figure 2)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unranked</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>Unranked</td>
<td>Monocots</td>
</tr>
<tr>
<td>Order</td>
<td>Asperagales</td>
</tr>
<tr>
<td>Family</td>
<td>Orchidaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Bulbophyllum</td>
</tr>
<tr>
<td>Species</td>
<td>kaitense</td>
</tr>
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</table>

Figure 1: Herbarium of Bulbophyllum kaitense

Figure 2: Habitat of Bulbophyllum kaitense

Habitat of Lithophyte (top)
Habitat of epiphyte (down)
3. Green Bio-Synthesized Silver And Gold Nanoparticles

**Chemical**

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

**Preparation of plant extract**

The leaves, pseudabulb, stem and root 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 powerd was taken and mixed with 200 mL of Milli Q water and kept in boilingwater 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.

**Biosynthesis of silver and gold nanoparticles**

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 FTIRs 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 10 minutes and the pellet was resuspended in small amount of sterilized doubledistilled water and then small amount of suspension was sprayed on glass slide to 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.

**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 aliququots (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.

**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 biosynthesis plant extract with 200 mg of dry potassium bromide, ground well in mortar under an IR lamp for 30 minutes and then pressing in a mold. The IR spectrum of Silver and Gold nanoparticles plant extract from 400 to 4000 cm-1 was obtained using a Perkin-Elmer spectrum GX.

**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 digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the X-rays together with processing electronics to analyses the spectrum.

**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.

**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. Results

1. Biosynthesis of Silver and Gold nanoparticles

Bring out the extract was subjected to AgNO$_3$ and HauCl$_4$, biosynthesis reaction started within few minutes and the color reaction was observed in which clear AgNO$_3$ solution changed into brown color. It is well known that silver nanoparticles exhibit yellowish to brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. Whereas yellowish plant extract solution turned to dark brown colored solution due to reduction of gold ion which indicates that formation of gold nanoparticles (Figure :4)

![Figure 4: Bio synthesis of silver and gold nanoparticles](image)

2. UV- Vis spectra Analysis

The formation of silver and gold nanoparticles by reduction of the aqueous silver ions and gold ions during exposure of Bulbophyllum kaitense leaves extract. The UV- Vis spectra of silver and gold nanoparticles synthesized in B.kaitense are shown in Figure 5a. The distinct peak observed at 467 nm, that is surface Plasmon resonance of the silver nanoparticles. Whereas, after the addition of B.Kaitense extract, the color of HAUCL$_4$ changed from yellowish to dark brown intimated the synthesis of gold nanoparticles in the aqueous solution. A broad peak was observe at 205 nm is gold nanoparticles Figure 5b.

![Figure 5: UV-Vis spectroscopy analysis of before and after B.kaitense Leaves, silver and god extract](image)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Leaves Plant extract (nm)</th>
<th>Leaves silver nanoparticle (nm)</th>
<th>Leaves gold nanoparticle (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>193</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>205</td>
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<td>3</td>
<td>-</td>
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<td>4</td>
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<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>467</td>
<td>-</td>
</tr>
</tbody>
</table>

Table :1 UV – spectrum functional group of B. Kaitense before and after silver and gold synthesized Leaves extract
Table 2. FTIR Functional group of B. kaitense before and after silver and gold synthesized leaves extract

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Leaves Plant extract (nm)</th>
<th>Leaves silver nanoparticle (nm)</th>
<th>Leaves gold nanoparticle (nm)</th>
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<tbody>
<tr>
<td>1</td>
<td>Chloroalkanes</td>
<td>694</td>
<td>-</td>
<td>658</td>
</tr>
<tr>
<td>2</td>
<td>CH2</td>
<td>709</td>
<td>704</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Aromatic Ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>C–O–C Arylalkyl asymmetrical</td>
<td>-</td>
<td>-</td>
<td>1385</td>
</tr>
<tr>
<td>5</td>
<td>CO</td>
<td>1638</td>
<td>1639</td>
<td>1638</td>
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<tr>
<td>6</td>
<td>Methylene</td>
<td>-</td>
<td>1411</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>C-H</td>
<td>2075</td>
<td>2074</td>
<td>2078</td>
</tr>
<tr>
<td>8</td>
<td>Alkynes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Phosphines</td>
<td>-</td>
<td>2385</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>OH or N-H group</td>
<td>3398</td>
<td>3438</td>
<td>-</td>
</tr>
</tbody>
</table>

5. Characterization of Silver and Gold nanoparticles.

3.1. FT-IR analysis

For the FTIR absorption spectra studies were briefly investigated to find out possible bio reduction agent present in the extract. The spectra of extracts were recorded before and after adding silver nitrate and chloroauric acid, (c) plant extract (Fig3) The suggest information regarding the chemical change in bio reduction can be assessed. The infrared spectra usually have sharp features that are characteristic of specific groups of molecular vibration, making the spectra useful for sample identification. The IR spectrum of partially silver and gold nanoparticle showed broad intense absorption band at 658 -704 cm\(^{-1}\) indicating the presence of chloroalkanes vibrations secondary alcohol, phenols and aromatic ethers characteristic bands 1283 cm\(^{-1}\) were found in B. kaitense silver and gold synthesized nanoparticles respectively. The band between 1400 cm\(^{-1}\) to 1750 cm\(^{-1}\) related variation of carbohydrates, lipids and proteins. Proteins are the largest group and the peptide group which is the structural repeat unit in proteins, gives up to 9 characteristic band Amide I and amide II bands are two major bands of the protein infrared spectrum. Amide I is the most intense absorption band in proteins. It is primarily governed by the stretching vibrations of the C = O (70-85%) and C-N groups (10-20%) It frequency is found in the range between 1411 cm\(^{-1}\) and 1638 cm\(^{-1}\) the exact band position is determined by the backbone conformation and the hydrogen bonding pattern. Amide II is found in the 1510 and 1580 cm\(^{-1}\) region and it is more complex than amide. The banding of OH is primary alcohol was observed 1411 cm\(^{-1}\) the presence of alkynes at a 2074 cm\(^{-1}\) to intermolecular OH sharp bands were noted at 3438 cm\(^{-1}\) and 3778 cm\(^{-1}\) is all the synthesized nanoparticles.

All the spectra has shown the presence of carbohydrates, proteins, DNA and lipids in varying composition and quantity as evidenced by the appearance of difference in both shape and absorbance intensity of B. kaitense synthesized silver and gold nanoparticles.

Figure 6: FT-IR Spectroscopy analysis of before and after B. kaitense Leaves, silver and gold extract

3.2. Morphology analysis of silver and gold nanoparticles energy dispersive x-ray spectrometers analysis.

3.2.1. Energy Dispersive X-ray (EDX) Spectrometry Analysis

According to the analysis through energy dispersive x-ray (EDX) spectrometry confirmed the presence of elemental signal of silver and gold nanoparticles summarized in Fig 4a,b) The vertical axis displays the number of x-ray counts whilst the K.e.v. 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 silver and gold nanoparticles has been correctly identified.

3.3. Scanning Electron micrography analysis

Scanning Electron micrography analysis of silver and gold nanoparticles Bring out scanning electron micrograph. The morphology of the silver and gold nanoparticles was observed and reveals that the extract powder particles are oval shaped, in which the silver nanoparticles is suggested from (Fig5a) The powder particles are its size range of 110nm and the closed view of oval shaped nanoparticles had showed. The is a micrograph of gold nanoparticles indicating that they are also powder particles are round spike shaped nanoparticles in size range of 110nm and the closed view of round spike nanoparticles has showed (Fig 5b) Above investigation results suggested that the silver and gold nanoparticles are synthesized due to the action of plant extract.

3.4 Transmission electron micrography analysis

Transmission electron micrography analysis of silver and gold nanoparticles overall, observed to the Transmission electron microscope (TEM) was find out to visualize the morphology of the silver and gold nanoparticles Represented the silver nanaparticles, which were synthesized by using extract powder. The histogram obtained from the enlarged transmission electron micrograph visualize showed the extract powder nanoparticle spherical shape but its size range in 90nm (Fig 6a) whereas gold extract powder particles visualised showed the nanoparticles granules shaped buy its size ranga in 140nm (Figs 6b) which act as ell excellent performance bioreductant for biosynthesis of silver and gold nanoparticles.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Microbes</th>
<th>Pseudobulb</th>
<th>Silver nanoparticle extract</th>
<th>Gold nanoparticle extract</th>
<th>Plant leaves extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas aeruginosa</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Salmonella typhi</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Candida albicans</td>
<td>Nil</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

6. Discussion

Suggested that the extract was subjected to AgNO₃ and HAuCl₄. The biosynthesis reaction started within few minutes and the color reaction was observed in which clear AgNO₃ solution changed into brown color whereas pale yellowish HAuCl₄ solution turned to ruby red colored solution which indicates that formation of corresponding nanoparticles (12). It is well known that silver nanoparticles exhibit yellowish brown color in
aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (21). Researchers reported the persimmon leaf extracts it is known that gold and silver nanoparticles exhibit ruby-red and yellowish color in aqueous solution due to excitation of surface Plasmon vibrations in the metal nanoparticles (14) noticed that similar report UV –Vis spectra of silver and gold nanoparticles synthesized by M.piperita the distinct peak observed at 4.50nm, that is surface Plasmon resonance of the silver nanoparticles (12). It has been reported that the UV-Visible absorption spectra *Curcuma longa* was reduced silver ions and it was revealed that the 415 nm range of peak was observed (11).

Carryout the strong evidence for the FT-IR absorption spectra of the dried biomass of lemon leaves before and after bio reduction. The chemical change of the functional groups involved in bio reduction can be assessed. The band at 1.101cm\(^{-1}\) which might be contributed by the C-C-O groups of the polyols such as flavones, terpenoids and polysaccharides is the biomass appeared as a signifycant peak. FT-IR analysis of the bio extract before and after the addition of silver solution revealed that strong bands at 1. 021, 1. 443, 1.634 and 3.428cm\(^{-1}\). The best at 1.021cm\(^{-1}\) corresponded to C-N stretching vibrations of anine. The band at 1.443cm\(^{-1}\) corresponded to C-H and OH bending and 3.428cm\(^{-1}\) was attributed to characteristic of -NH stretching of amid(II) band the weaker band at 1,364cm\(^{-1}\) corresponded to amid I, arisen due to carbony stretch in proteins (7) Both the interferrogram exhibit a broad from peptide linkage. Formation of C=C bonds is energetically favored over S=C bonds as the latter will impose severe geometrical constraints on the molecule more specific in thio-group and less in acidic as compared to alcohols and that makes elimination of hydrogen attached to sulfur group. There is a decrease in the concentration of the amide linkage is the aqueous solution after the formation of silver nanoparticles (12) It is all the spectra investigated that the *Bulbophyllum kaitense* leaves extract may reduce the silver and gold nanoparticles into metallic nanoparticles Researches previously reported the analysis energy dispersive spectroscopy of silver and gold nanoparticles in the presence of elemental metal signal was confirmed (12) on another reported compared the elemental analysis was done by EDAX form the EDAX spectrum a strong signals form silver atoms and weaker signals from atoms was observed. The weaker signals were preventions form protein / enzymes of the stem extract (34).

The distribution of the particle diameters showed as main peak located between 32 and 43nm and the sized ranging from 10 to 110nm with an average particles size of 37.5nm (2). Earlier reported that the sub-micro scale particles between 100 and 800nm were obtained with high concentrations of leaf broth more that 10%, suggesting that too many reducing agents cause aggregation of the silver particles synthesized possibly due to the interactions between capping molecules bound to the surface of particles and secondary reduction process on the surface of the performed nuclei. (14).

Very recently reported that the anbacterial activity of silver nanoparticles against *E.coli*, is higher than that against *S.aureus* is due to the variation in the cell wall composition between gram positive and negative bacteria whereas in gold nanoparticles antibacterial activity against *E.coli* not in *s.aureus*, in plant extract was not found any of antibacterial pattners. (12). It has been reported that the antibacterial activity of the silver nanoparticles was tested against the following bacterial culture *E.coli, Entrococcus faecalis, Balillus subtilis, klebsiella pneumonia, Staphtococcus aureus, Salmonella typhi, Vibrio cholera* and maximum activity of the silver nanoparticle was found to be against *Vibrio cholerae* (13)

Based on the silver synthesized nanoparticles has been used for its well known microbial properties since roman time however the advances in generating silver nanoparticles have made possible revival of the use of silver as a powerful antimicrobial efficacies.

7. Conclusion

To overcome the problem, we successful in the biological reduction of silver and gold nanoparticles aqueous leaves extracts of *Bulbophyllum kaitense* silver and gold nanoparticles were synthesized in component conditions and characterization of synthesized nanoparticles was bring out the UV-Vis spectroscopy, FT-IR scanning electron microscopy equippped with EDAX it is observed that secondary compounds present in the *B.kaitense* extract the present the silver and gold ions into metallic nanoparticles. The Synthesized silver and gold exhibited a excellent antagonistic activity. The assesses for the synthesis of nanoparticles in large scale using the plant extract may have commercial viability and to develop studies in the biology and nanoscience suggested that the needed outcome of this work will be the development of value added products from *B.kaitense* for nanoscience and nanomedical with cosmetic based industries. This is a first report that the orchidaceous plant extraction in nanoscience technology.

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Reference


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