Orchid Plant Natural Source For The Synthesis Of Silver And Gold Nanoparticles With Antagonistic Analysis

Dr. Kalaiarasan. A*, Dr. Chinnappa. R[2]

[1.] Centre for Bioscience and Nanoscience Research, Coimbature, Tamil Nadu, India.
[2.] PG and Research Department of Botany, Arignar Anna Government Arts College, Musiri, Tiruchirappalli, Tamil Nadu, India.
*Corresponding Author: Email – myla_kalai@yahoo.com
Mobil no: +91 9943110536, 7092240630

Abstract: Nanoparticles are attractive in pharmacy and medicine simply because it is possible to control drug misdistribution and achieve therapeutic benefit with nanomedicines. Green synthesis of $AgNO_3$ and $HAuCl_4$ nanoparticles approach nanomedicines developing area investigation. The biomolecules found in orchid plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles and Au ions from chloroauric acid to gold nanoparticles. In this present investigation of silver and gold nanoparticles were biosynthesized from aqueous silver and gold nanoparticles through using *Bulbophyllum kaitense* pseudobulb-powder extracts. The chemical compound and morphology characterization of nanoparticles were done by using various instruments which included ultraviolet visible spectroscopy, Fourier transform infra red spectroscopy, scanning Electron microscopy, Transmission electron microscopy. The ultraviolet – visible spectrum of the plant aqueous extract containing silver nanoparticles showed on absorption peak at around 431nm and gold nanoparticles absorption peak at around 442nm. The silver nanoparticles synthesized were generally found to be slightly oval in shape with 70nm. Whereas the synthesized gold nanoparticles were found to be 60 nm. The energy Dispersive X-ray fluorescence spectra analysis confirmed the presence of elemental silver and gold signal. The results showed that the pseudobulb aqueous extract of *Bulbophyllum* is very excellent bioreductant for the synthesis of $AgNO_3$ and $HAuCl_4$ nanoparticles active against human pathogens Escherichia coli, Pseudomonas *aeruginosa*, *Salmonella typhi* and *Candida albicans*. In this plant world first report that orchidaceae family in nanoscience and Origin of in India

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Introduction

It has been noticed that the medicinally vehicle angiosperms have the greatest potential for the synthesis of metallic nanoparticles with respect to quality and quantity[1]. It has been carried out the synthesis of pure metallic nanoparticles of sivler and gold by the reduction of Ag^+ and $Au3^+$ ions using Neem (Azadirachtaindica) leaf broth. However, littlie has been carried out about engineering approaches such as rapid nanoparticles synthesis using plant extracts and size control of the synthesis for more than 90% reduction of Ag+ and Au3+ ions using Neem leaf broth were about 4 and 2h, respectively[2]. Nanoparticles usually referred as particles with size up to 100nm [3].

The several reports on synthesis of silver nanoparticles from plants and its antimicrobial activity[4-5].Have been suggested that the synthesis and characterization of Ag1 *Curcuma longa*. The Ag NPs were prepared using silver nitrated was a silver precursor and *C. longa* tuber- power water extract as reducing agent and stabilizer[6]. Earlier reported that they have demonstrated synthesis of gold nanotrianglesand silver NPs using *Aloe vera* plant extract[7]. Previoushy reported that the biosynthesis of Ag Nanopartricles using corundumsatiuvm extract as a reducing agent and demonstrate that this method yields faster and stable silver nanoparticles[8].Currently reported the phytochemical analysis of various solvent extracts of the *Bulbophyllm kaitense* psuedobulb. Different solvent extracts were tested to determine antibacterial activity[9]. Thirteen bioactive constituents in ethanolic extract were identified in *Bulbophyllum kaitense* root[10]. Preliminary Phytochemical analysis of various solvent extracts of *B.kaitense*leaf extract. The extracts alone contains phenolic compounds, coumarin, quinine, carbohydrate, plavonoids, Tannins, and antimicrobial activity[11].

Current investigation clearly indicated maximum activity was observed from the *B.kaitense* stem extract was potent against ten tested bacterial strains depended manner. There are effectiveness of traditional herbs against gram positive and gram negative microorganisms and as a result plants are still

recognized as the bedrock for modern medicine to treat infection diseases however, it was more active against fungal strains[12]. Gran negative bacterial organisms were highly sensitive the gram positive bacterial infested *B. kaitense* of ethanol extract showed highly sensitive activity (ranging from 12mm 23mm) respectively [13].

The results obtained indicate the *B.kaitense* psuedobulb has anti-inflammatory activities that supports the folk medicinal use of the plant. The world first report in the plant[14].*B.kaitense* root property is curing different diseases to determine the possible thirteen bioactive constituents in ethanol extract were identified[15]. GC/MS determination of Bioacccctive components of *B. Kaitense* leaves extract[16]. In this present investigation details with *Bulbophyllum kaitense* plant extract mediated synthesis of chemical reaction compound and morphology with characterization of silver and gold nanoparticles. The synthesized nanoparticles activity of nanomedical application.

Plant Material And Methods

The *Bulbophyllam kaitense* (Tamil vernacular name: Oruethalelai) belongs to the family orchidaceae was first identified at Sethurpattinadu 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 (Figure1).

***Bulbophyllumkaitense*Reichib**

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

Green Bio-Synthesized Silver And Gold Nanoparticles

Chemical

Silver nitrate ($AgNO_3$), Chloroauric acid ($HAuCL_4$) and other components were purchased from Himedia, Mumbai, India.

Preparation of plant extract

The pseudobulb 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.

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 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 10 minutes and the pellet was responded in small amount of sterilized double distilled 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 Bio reduction of Silver and Gold

Synthesis of 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.

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 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 calledmulti channelanalyze 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 a 25 KV accelerating voltage, objective aperture # 3 and 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.

Results:

Biosynthesis Of Silver And Gold Nanoparticles

Find out the extract was subjected to $AgNO_3$ and $HAuCl_4$, the biosynthesis reaction started with in few minutes and the color reaction was carried out the in which clear plant extract yellowish color added $AgNO_3$ solution changed into red color whereas yellowish plant extract added $HAuCl_4$ solution color changed turned dark block colored solution which indicates that the formation of silver and gold corresponding nanoparticles (Figure 2).

UV-visible spectroscopy analysis

The formation of silver and gold nanoparticles by reduction of the silver and gold ions during exposure of Blbophyllum kaitense pseudobulb aqueous extract microwave irradiation may be easily followed b VU-Vis spectroscopy. The VU-Vis spectra of silver and gold nanoparticles synthesized of *B. kaitense* are shown in fig 2a, The Plasmon band distinct peak observed at 431nm, That is surface Plasmon resonance of the silver nanoparticle whereas, after the addition of *B. kaitense* plant synthesis of gold nanoparticles is the aqueous extract. A broad band peak was observed at 442 nm in gold nanoparticles contrary, silver and gold nanoparticles showed the maximum band beak value 431-442nm (figure 3 and Table 1).

Characterization of silver and gold nanoparticles

Spectroscopy Analysis Fourier Transform carried out to the FT-IR spectroscopic investigation to find possible bio reducing component present in the plant extract. The spectra of extracts were recorded before and after added silver nitrate, (b) chloro auric acid and (c) plant extract (Figure 4 and Table 2). The information regarding the chemical change in bioreduction can be assessed. The infrared spectra usually have sharp features that are characteristic of specific groups of molecular vibrations, making the spectra useful for sample identification FT-IR analysis were recorded of the silver nanoparticles prominent peaks observed at 621, 717, 1639, 2075, 3418 cm^{-1} whereas FT-IR analysis were regarding of the gold nanoparticles broad strong bands peaks observed at 667, 1385, 1639, 2113, 3445, 3925cm^{-1}*B.kaitense* extract showed strong bands comparing the FT-IR spectra. The spectrum of partially *B.kaitense* pseudo bulb aqueous silver and gold nanoparticles showed broad intense absorbtion band at 621 -699cm^{-1} indicating the presence of chloalkanes and sharp intense band at 700-717cm^{-1} strongly suggesting methlene (CH2) vibrations.

The C Signal and H vibrations were observed at 1385 cm-1 for all exo polysaccharides. The peaks found at 1638 and 1639cm^{-1} can be attributed to the C-C in alkenes rings and C=C stretch of aromatic rings respectively. It frequency is found in the range between 2075 and 2113cm^{-1} it can be seen that spectra for in the –COOH group for –OH, i.e., the hydroxyl group, the band beak appeared at 3418cm-1 in the raw material and encapsulation of nanoparticles. The peak intensity was distinct at 3445 to 3922cm^{-1} the intermolecular OH sharp band appearance also for the –C- of the carboxylic group after encapsulation of nanoparticles. H bands can be formed between the amide groups. The spectrum has shown presence of carbohydrates, protains, DNA and quantity as strong evidence by the appearance of difference in shape and absorbance intensity. As the plant *B.kaitense* molecules became absorbed onto the surface of the green biosynthesized silver and gold nanoparticles.

Morphology Analysis Of Silver And Gold Nanoparticles

Energy Dispersive X-ray spectra analysis

Basis on the analysis through energy dispersive x-ray (EDAX) spectrometers confirmed the presence of for silver and gold SEM, TEM nanoparticles.

The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in K ev. 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 has been correctly identified as show in (Figure 5a,b,c,d).

Scanning electron microscopy silver nanoparticles

Analysis of the bio reduced green – synthesized silver and gold nanoparticles by SEM confirmed that they were in the plant powder particles are spherical shaped nanoparticles in size range of 70nm are shown in gold nanoparticles. (Figure 5a).

The morphology and size of the bio green synthesized gold nanoparticles find out the TEM observe formed were small grinds shape but it's the range of 90nm as shown in (Figure 5b). According to investigation results suggested that the silverand nanoparticles are synthesized due to the action of plant extract *B.kaitense* which act as well performance bioreductant for bio green synthesis

Transmission electron microscopy silver nanoparticles

Transmission electron microscopy (TEM) was find out to visualize the morphology and shape with size of the silver nanoparticles represent the silver nanoparticles, which were synthesized by using *B. kaitense* the histogram obtained from the enlarged TEM image close visualize showed the round shape and particle size range in 60nm as shown in (Figure 5c).

Gold nanoparticles. The morphology and size of the biogreen – synthesized gold nanoparticles determined by TEM visualize are Cleary shown in (Figure 6 d). The particles found in spherical shape but its size range of 90nm.

Antagonisticactivity

The antagonistic activity of the bioreduced aqueous *B.kaitense* extracts was investigated. The *E. coli* is highest zone of inhibition that that against *S. typhi*, *Pseudomonas aeruginosa* in silver extract The moderate activity of *P. aeruginosa* and S.typhi maximum activity in both samples whereas gold nanoparticles determined antagonistic activity against *P. aeruginosa* is higher against *E.coli, S.typhi* in synthesized pseudobulb gold extract. The maximum activity of *E.coli, S.typhi* in both samples silver nanoparticles as high zone of antifungal activity against *Candida albicanse* that synthesized gold nanoparticles as shown in Table 3.

Discussion

Green Biosynthesis of silver and gold nanoparticles observe the studies formation of $AgNO_3$ by reduction of silver nitrate during exposure to tulsi leaf extract can be easily monitored from the change in colour of the reaction mixture silver nanoparticles bear a characteristic yellow brown colour due to the excitation of surface Plasmon vibrations[17]. It has been reported A change in color of the synthesis of silver and gold nanoparticles. The irradiation the colour of the solution was changed from watery to yellowhish brown color due to reduction of ion. Which indicates the formation of silver nanoparticles[18]. On another reported compared to the extract was subjected to $AgNO_3$ and $HAuCl_4$. The biosynthesis reaction started within few minutes and the color reaction was observed in which clear $AgNO_3$ solution changed into brown color whereas pale yellowish $HAuCl_4$ solution turned ruby red colored solution which indicates the formation of corresponding nanoparticles[19].

Previouly reported it is well known that nanoparticles exhibit yellowish brown in aqueous solution to excitation of surface Plasmon vibrations in silver nanoparticles[2,20,7]. It has been reported the band observed at UV spectrum corresponding to surface Plasmon resonance occurs at 422nm and clearly indicates the formation of nanoparticles[21]. Similar spectra it is observed that the silver surface Plasmon resonance (SPR) occurs at 450nmnoticed that the evolution of the absorbance spectra emanating from the silver nanoparticles over time showed increasing sharp absorbance at around 440nm with increasing reaction time[17,18].

Found that the band at 1,021cm^{-1} corresponded to C-N stretching vibrating of amine. 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 amide (11) band. The weaker band at 1,634cm^{-1} corresponded to amide I, arisen due to carbomy stretch in protein[22].

Obtained similar reported that the scanning micrograph the morphology of the silver and gold nanoparticles was observed and approximately spherical, in which the silver nanoparticles is in aggregated form[19]. Priviously observed that the maximum absorbances of pure gold and silver occur at 544 and 420nm, respectively[23]. However, a high concentration of phyllanthin leads to strong interaction between biomolecules and surfaces of the shaped NPs, Preventing nascent gold nanocrystals from rapid sintering[24]. It was suggested that the silver nanoparticles has shown antibacterial activity against all tested microorganism, *E.coli, Enterococcus faccalis, Bacillus subtitles, Klebsiella pneumonia, staphylococcusaurens, salmonella typhi, Vibrio cholera* and maximum zone of inhibition was found against vibrio cholere, reported [21].

Similar reported that the antibacterial activity of silver nanoparticles against of silver nanoparticles against *E.coli*is higher than that against S.aureous is due to the variation in the cell wall composition

between gram positive and negative bacteria whereas in gold nanoparticles showed antibacterial activity against E.coli not in S.aureous[19]. Reported that the silver and gold nanoparticles have an antimicrobial effect on *P.aeruginosa, S.pyogene, Enterococcus facials, Proteusvultgaris, P.mirabilis, litrobacterfrenudi, E.coli*[18]. Noticed that the growing microbial resistance against antibiotics and the development of resistant strains[25].

To summarize, we succeeded in the green bioreduction of silver and gold nanoparticles using *B.kaitens*pseudobulb extracts. Silver and gold nanoparticles were synthesized in component conditions and characterization of synthesized nanoparticles was carried out the UV-vis spectroscopy, FTIR and nanoparticles size, morphology analysis of SEM, STEM equipped with EDAX. It is excellent stability photochemical present in the extract of *B.kaitense* has reduction the silver and gold ions into crystal nanoparticles present investigation the synthesized silver and gold ions exhibited a excellent antagonistic activity against both *E.coli, P.aeruginosa, S.typhi* and *Candida albicans*. In the future using such plant extract to develop bio nanomedicine against various human pathogens and as well as food, cosmetic with drinking water purified industries.

Habitat of lithophytes *Bulbophyllum kaitence* orchid plant

Focusing of Bulbophyllum kaitence orchid plant

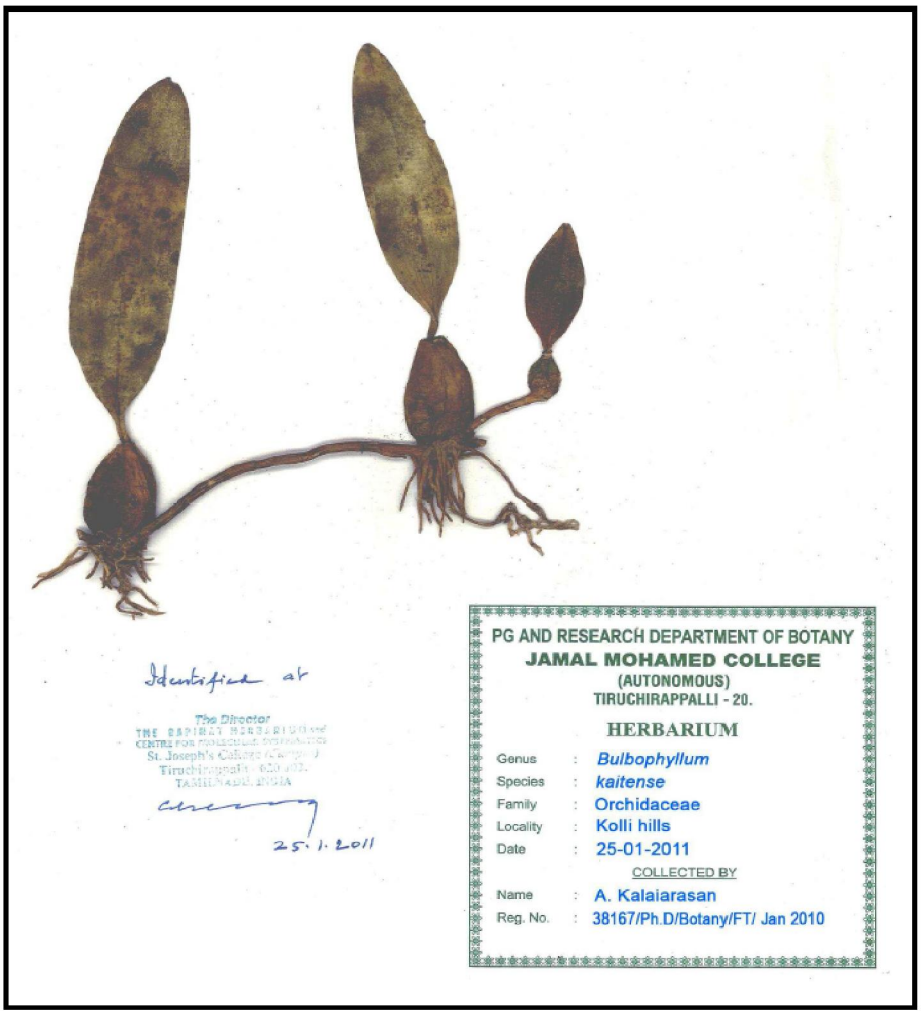

Figure 1: Herbarium of *Bullbophyllumkaitens*

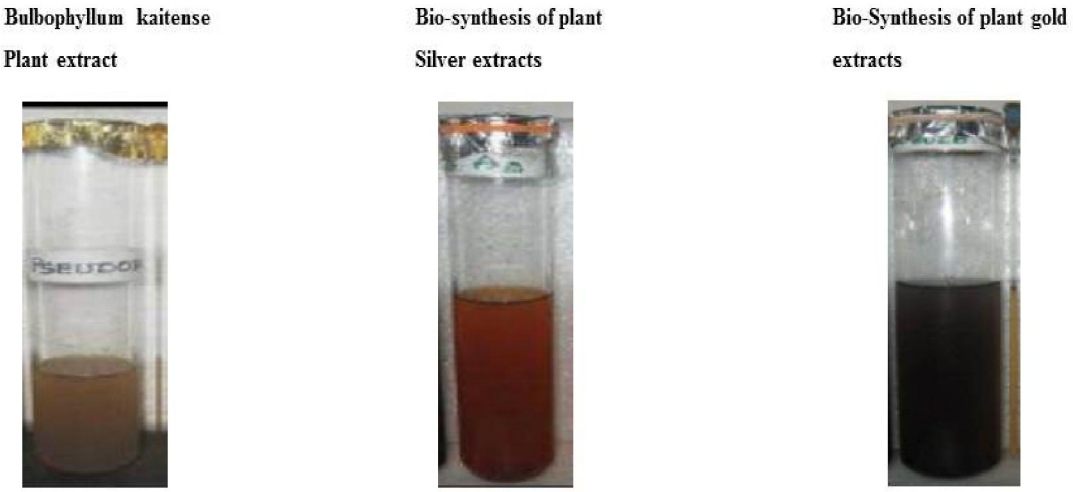

Figure 2: Bio synthesis silver and gold nanoparticles

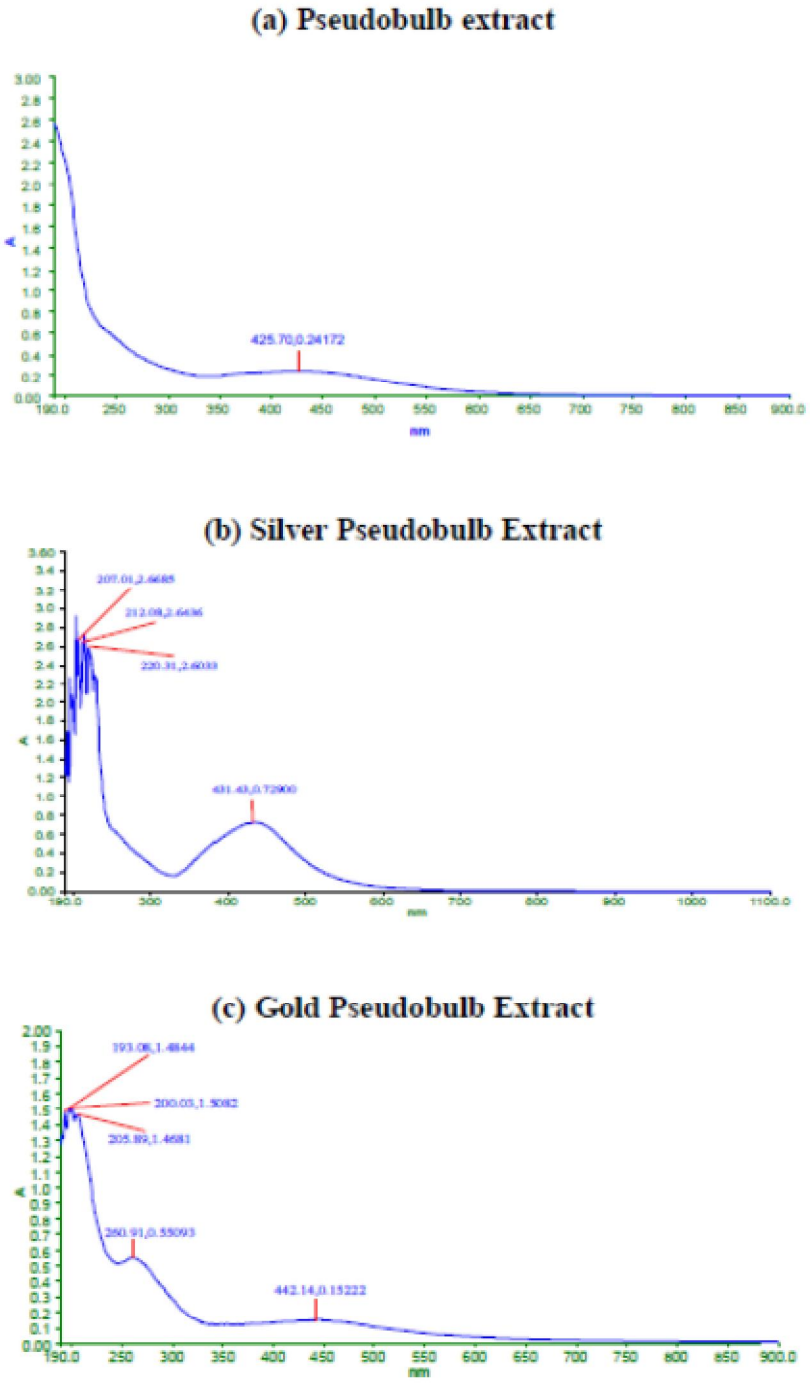

Figure 3: UV-Vis Spectroscopy analysis of *B. Kaitense* Pseudobulb, silver and gold extract

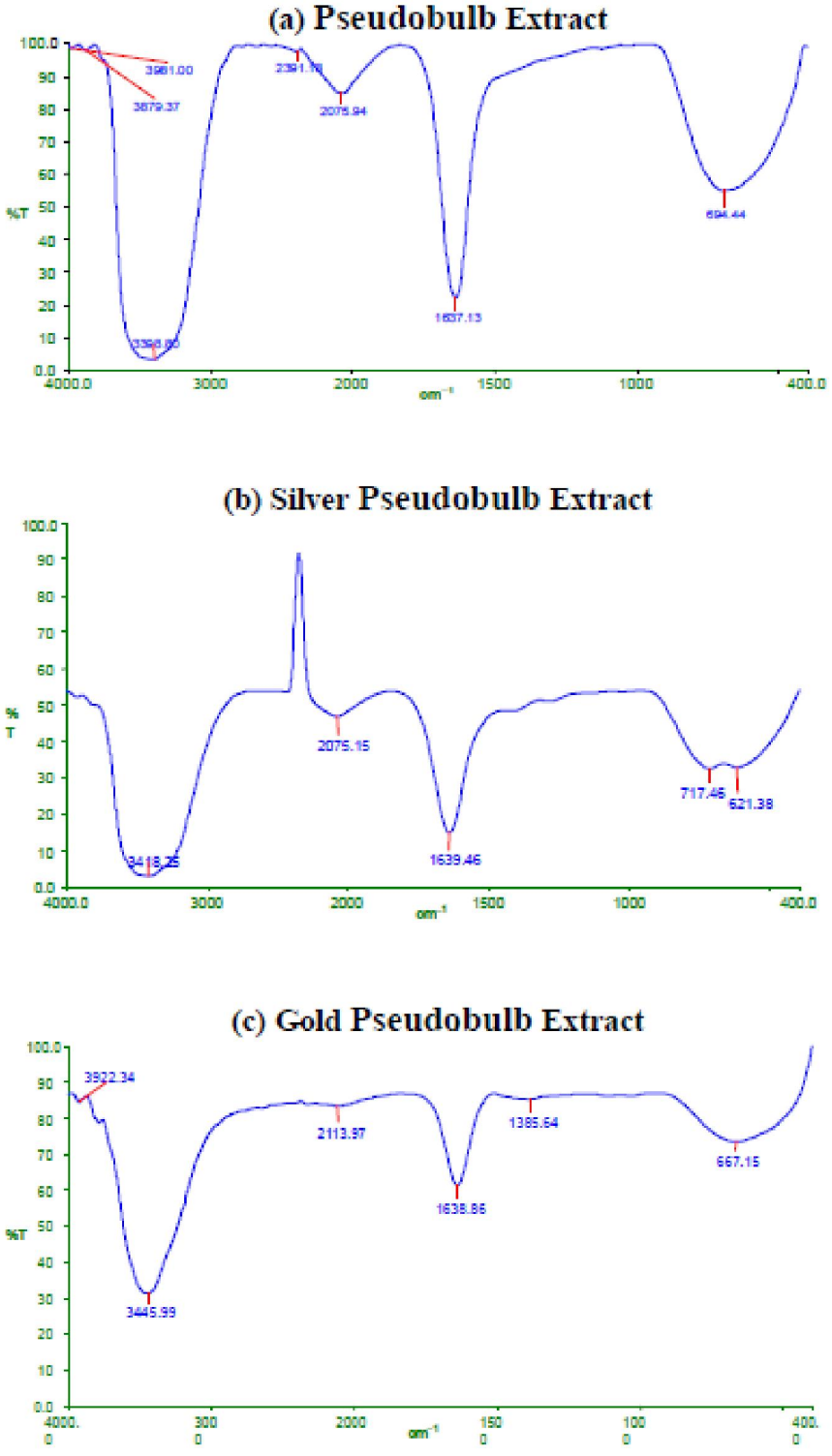

Figure 4: FT-IR Spectroscopy analysis of *B. kaitense* Pseudobulb, silver and gold extract

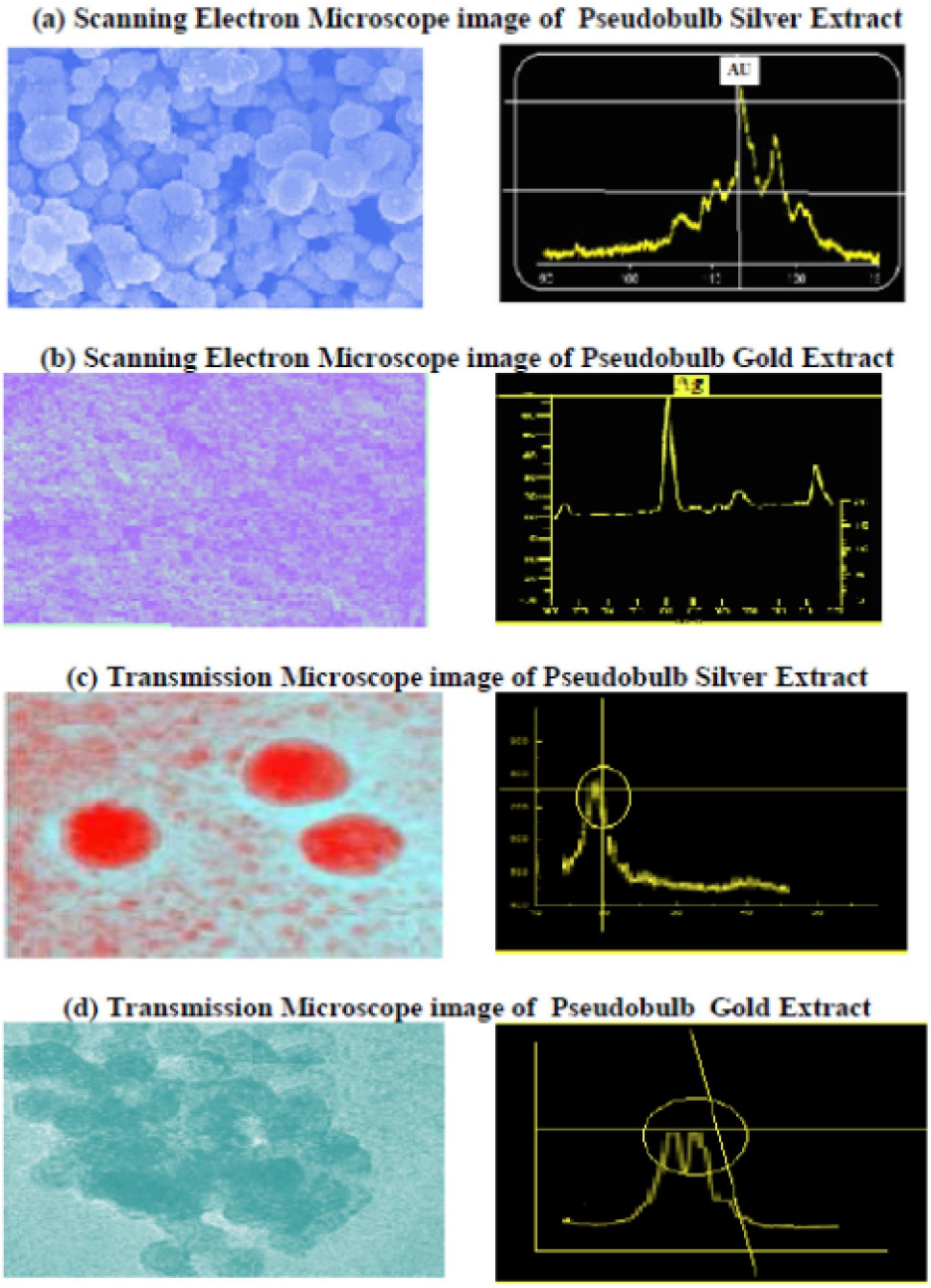

Figure 5: Scanning electron microscope, Transmission microscope and Energy dispersive X-ray fluorescence spectrometry spectra of *B. kaitense* Pseudobulb silver and gold extract

Table 1. UV – spectrum functional group of *B. Kaitense*before and after silver and gold synthesized extract

S. No	Pseudobulb Plant extract (nm)	Pseudobulb silver nanoparticle (nm)	Pseudobulb gold nanoparticle (nm)
1	-	-	193
2	-	207	-
3	-	212	-
4	-	220	-
5	-	-	-
6	425	431	442

Table 2. FTIR Functional group of *B. kaitense*before and after silver and gold synthesized extract

S. No	Group	Pseudobulb Plant extract (nm)	Pseudobulb silver nanoparticle (nm)	Pseudobulb Gold nanoparticle(nm)
1	Chloroalkanes	694	621	667
2	CH2	-	717	-
3	Aromatic Ether	-	-	-
4	C–O–C Arylalkyl asymmetrical	-	-	1385
5	CO	1637	1639	1638
6	Methylene	-	-	-
7	C-H	2075	2075	-
8	Alkynes	-	-	2113
9	Phosphines	2391	-	-
10	OH or N-H group	3398	3429	-

Table 3. Antagonistic activity of silver and gold nanopalticles against human bacterial pathogens

S. No.	Microbes	Pseudobulb		
		Synthesis silver nanoparticle extract	Synthesis gold nanoparticle extract	Plant pseudobulb extract
1	*Escherichia Coli*	11	16	10
2	*Pseudomonas aeruginosa*	12	10	9
3	*Salmonella Typhi*	9	9	11
4	*Candida albicans*	Nil	Nil	Nil

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Corresponding Author:

Dr. A. Kalaiarasan M.Sc., M.Ed., M. Phil., Ph.D.
Assistant Professor
Centre for Bioscience And Nanoscience Research
Coimbatore- 641 021
Tamilnadu, India.
E-mail: myla_kalai@yahoo.com

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