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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of size and photo variation on antioxidant and antibacterial activities of silver nanoparticles from *Dioscorea alata* L. aqueous Bulbil extract.

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ABSTRACT
Rapid and ecofriendly biosynthesis of stable silver nanoparticles was investigated by chemical route. The silver nanoparticles synthesized using bulbils extract shows tremendous antioxidant and antimicrobial activity and shows the significant source of therapeutic agents. The structural and optical synthesis material was characterized by XRD, UV-Visible spectrometer, HRTEM, HRSEM and bonding was analyzed by FTIR analysis. The enhanced band gap shows the particle size decreases with synthesizing parameter and shows that when particle size decrease it possess more antioxidant and antimicrobial activity. The PL spectra shows the defect states of the samples. The particle size decreases when more photon induced in the samples. The bulbil extract AgNPs possess antioxidant activity when compared with ascorbic acid. The optimizing AgNPs exhibits promising antibacterial effects against the selected human pathogens Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae and Escherichia coli.
Keywords: Antimicrobial; XRD; Band gap.

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INTRODUCTION

Nanotechnology is area that can manipulate the materials to the desired level. Nanotechnology in biological approach has great interest with its application in biomedical, pharmaceutical and optoelectronic field. Nanostructure has special attention in health care system as well as biomedical application due to their ultra-small particle. Semiconductor and metal material shows challenges properties when they come in nanometer level. The decrease in particle size from metal to semiconductor has exitonic effect and gains high chemical reactivity and physical activity. Among the material silver has particular interest because of distinguish properties like good conductivity, chemical stability, catalytic and antibacterial activities. Ag^+ ions and Ag^- based compounds are highly toxic to several microorganisms, which make them interesting candidates for multiple applications in the biomedical field [1, 2]. Due to the large surface to volume ratio the silver nanoparticles exabits efficient antimicrobial activity.

Prain and Burkill [3] reported that about 50 different *Dioscorea* in India, largely in the West, East and Northeast regions So far, 30 different *Dioscorea* species have been reported from North East India and identified *Dioscorea alata* is the most common and highly edible species [4]. Green synthesis is attractive and easy process synthesis of AgNPs by an environmentally friendly procedure involving the *in situ* reduction of Ag by *D. alata* L. bulbil extracts is the special attraction in our investigation. Antioxidant properties and antibacterial activity was measured with human pathogens Staphylococcus *aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* [5-10] .

MATERIAL AND METHODS

DPPH (2, 2-diphenyl-1-picryl-hydrazyl) and silver nitrate ($AgNO_3$) was purchased from Sigma–Aldrich (USA). Gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), ascorbic acid, Mueller Hinton agar andmethanol were used with high analytical grade.Three bacterial species *Staphylococcus aureus(MTCC 96), Bacillus subtilis (MTCC 441), Escherichia coli (MTCC 739) were tested. All bacterial culture were maintained at* -5^{o}C. Bulbils of *Dioscorea alata* were collected from Lowkhowa Reserve Forest of Nagaon

District and Kaziranga National Park of Golaghat District from India. The samples collected and dried in incubator at 40°C.

The reaction mixture was carried out at different conditions like sunlight irradiation, UV irradiation and room temperature. The colour change of the solution was checked periodically. After preparation of the sample, it was ready for UV-visible spectrophotometer, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The reaction mixture was then dried and stored at 4°C for antioxidant and antibacterial studies.

Determination of Antioxidant Activity and Phenolic Contents

The amounts of phenolic compounds in the extracts were determined according to the method of Waterman and Mole [11].Gallic acid was used as the standard phenolic compound with solution in appropriate solvent (0.1 mL) and 0.5 mL of Folin-Ciocalteu reagent was added with 2 mL of 20% sodium carbonate. The experiment was carried out in several times and the content of total phenolic compounds was calculated comparing with standard curve with gallic acid.

DPPH- Radical Activity

The stable free radical scavenging activity was determined by the 1, 1-diphenyl-2-picryl-hydracyl (DPPH) method of Shyur *et al*., [12]. 3mL reaction mixture containing 2mL of 0.1mM DPPH methanol solution, 0.9 mL of 50 mM tris-HCl buffer (pH 7.4) and 0.1mL of test extract at different concentrations. The mixture was performed with absorption at 527 nm. Radical activity is estimated by-

$$\% \text{ inhibition} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}}] \times 100 \quad (1)$$

Antibacterial activity

100 µL of each bacterial suspension was spread on a Mueller Hinton agar plate along with 100 µl of AgNPs solution. Aqueous Bulbil extract and 1mM $AgNO_3$ were used as negative control and antibiotic (amoxicillin 1mg/mL) were used as positive controls. The wells were maintained at 37°C for 24 hrs for microbial growth. After incubation time zone of inhibition was measured with high resolution travelling microscope.

RESULTS AND DISCUSSION

UV-Vis Spectra analysis

The UV-vis spectra peaks were recorded at 430-480nm at different time intervals: 5min at sunlight (A), 10min at UV (B) and 30min at room temperature (C) are shown in Fig. 1. UV-Vis spectra for the bulbil extract alone showed no absorption in the spectral window between 400-700nmThe reduction of pure Ag^+ ions to Ag^0 were monitored from spectrum.

The colour of the aqueous silver nitrate solution changed within 5 mins of mixing *D. alata* Bulbil extract and gradually to reddish brown, finally giving colloidal brown, indicating the formation of AgNPs.

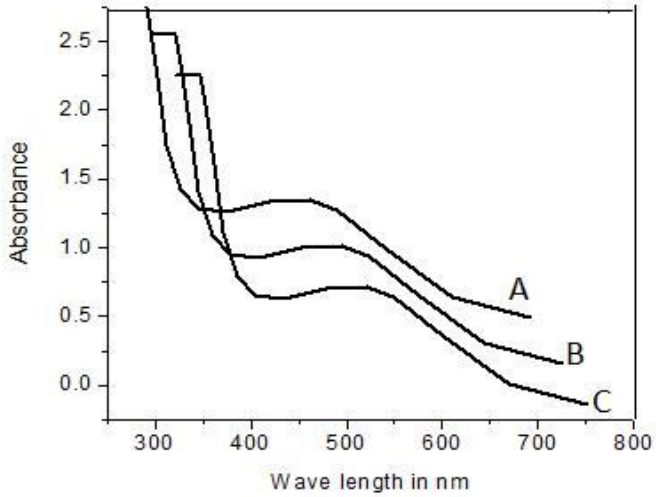


Fig. 1. UV-vis spectra showing absorption of AgNPs synthesized by *D. alata* aqueous Bulbil extract. A: 5 mins 5min at Sunlight B: 10mins at UV C: 30 mins at room temperature

It was observed that in presence of sun light the peaks are blue shifted indicating the band gap of the material increases (shown in Fig2). The band gap of the samples was calculated using a graph $(\alpha h\upsilon)^2$ versus $h\upsilon$. From the graph corresponding band gaps were obtained by extrapolating the straight-line to $(\alpha h\upsilon)^2 = 0$. From the shifting of band gap, the crystal radius (r) is calculated using the (EMA) effective mass approximation method

$$E_{gn} = [E^2_{gb} + 2\hbar^2 E_{gb}(\pi/R)^2 / m^*]^{1/2} \quad (2)$$

Where, m* represents the effective mass of the specimen, E_{gb} is bulk material band gap and E_{gn} is the sample band gap energy. It is observed that when the sample is expose with sun light the band gap increases comparative with UV and room temperature. The particle size was found in the range 5-9 nm which is below the de Broglie wave length and shows exitonic effect [13-15]. Further it was observed when size of the particles decreases zone inhibition of microbial effect increases. Table.1. represent the blue shift energy of the sample in different conditions and found that optimum value of pH was 1.6.

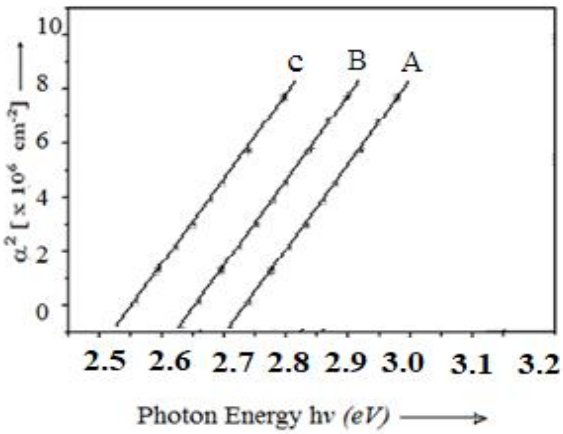


Fig. 2. Band gap of AgNPs synthesized by *D. Alata* aqueous bulbil extract. A: 5min at sunlight B: 10mins at UV C: 30 mins at room temperature

Table 1. Blue shift, band gap energy of the Synthesized particle at different condition

Sample at	pH of the sample	Band gap energy	Blue shift energy(eV)
5m at sunlight	1.6	2.71	0.23
10m at UV	1.8	2.68	0.17
30 m at room temperature	2	2.53	0.02

FTIR Analysis

In Fig. 3 the AgNPs shows five absorption peaks at 3443.95 cm^{-1}, 2074.68 cm^{-1}, 1638.18 cm^{-1}, 1018.22 cm^{-1} and 669.97 cm^{-1}. Peak at 3443cm^{-1} results due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The absorption peak at 2074 cm^{-1} is the functional and can be assigned to –CH stretching vibrations of $–CH_3$ and $–CH_2$. The shoulder peak at 1638 cm^{-1} assigned for C=O group of carboxylic acids. The weak band at 1018 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines. The main bands absorbed at 3443.95 cm^{-1}, 1638.18 cm^{-1} and 669.97 cm^{-1}indicates the presence of alcohols and phenols (O-H), carboxylic acids (C=O) and Chloro alkanes (CX) respectively [16].It was observed that in all cases the FTIR peaks remains same indicating same bond formation.

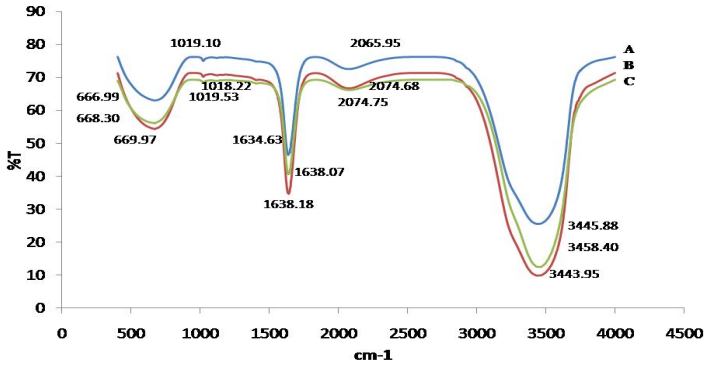


Fig. 3. FTIR spectrum of silver nanoparticles (AgNPs) synthesized by *D. alata* aqueous bulbil extract. A: Sunlight treatment B: UV treatment C: Room temperature

Structural Analysis by XRD:

X-ray diffraction (XRD) of silver quantum dot from Dioscorea *alata* L. aqueous bulbil extract is shown in Fig 4. The X-ray diffractometer was operated at a voltage of 40kV and a current of 30mA with Cu Kα

radiation.

XRD analysis shows distinct diffraction peaks of 27.5°, 38.1°, 44.3° and 64.4° at 2θvalues indexed to (110), (111), (200) and (220) the crystalline planes of the face centered cubic structure.

The intense peaks represent the good crystalline in structure. Again, it was observed that when the samples are exposed more time in sunlight the peaks are slightly shifted to higher diffraction angle indicating strain produced in the crystal. The size of the nanocrystal was determined using Scherer formula [17,18].

$$D = \frac{kl}{\mathrm{V}w_{2q}\cos q_B} \qquad (3)$$

Where, q_B is the Bragg angle in radian and K=0.9 for spherical shape. The average crystalline was calculated 27 nm which is larger than size obtained in TEM and optical model.

The sizes of the samples were calculated from different peak position. It is found that from three prominent peaks contains same grain size. It indicates that particles are symmetrical in shape. In the XRD pattern some extra peaks are also observed in lower diffraction angle. This may be arising due to bulbil extract as a capping agent. The Rietveld refinement analysis was done on the sample which is expose in sun light in 5 hours. It gives information about the lattice parameters-a and found value 4.11 Å in optimum condition. The lattice parameter was calculated from three peaks as shown in Fig.5 by applying Nelson and Riley plot. The increase of lattice parameters signifies that particle size decreases in presence of sunlight compare with other UV and room temperature.

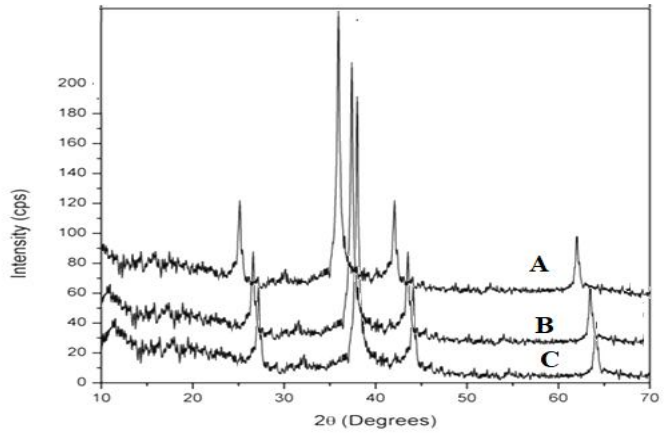


Fig. 4. XRD pattern of silver nanoparticles (AgNPs) synthesized using *D. Alata* aqueous bulbil extract. A: Sunlight treatment B: UV treatment C: Room temperature

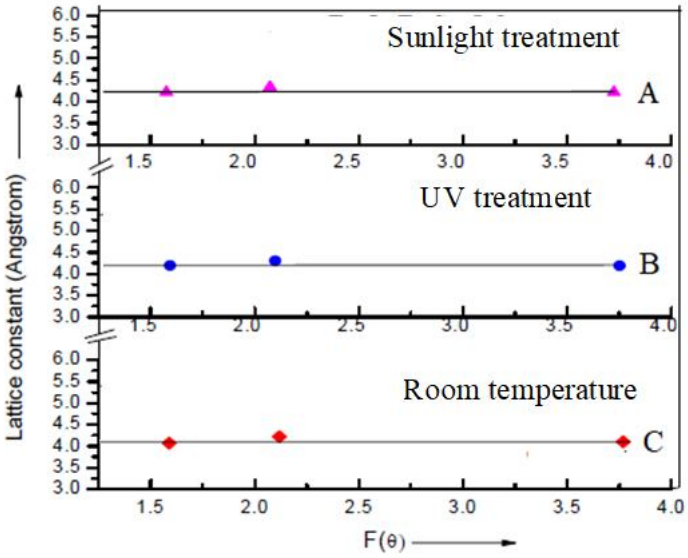


Fig.5.Nelson Relay plot of three samples A: Sunlight treatment B: UV treatment C: Room temperature

DPPH radical scavenging activity

Fig. 6 shows the dose-response curve of DPPH activities of the extracts when it was expose 5 hours in sunlight. It was found that that both the AgNPs and aqueous bulbil extract exhibited a potential free radical

activity. The half inhibition concentration (IC_{50}) of the radical scavenging activity of the AgNPs was 200µg/ml and the bulbil extract was found to be 300µg/ml. The DPPH activity of the AgNPs extract was found to increase in increasing concentration. The results shows that highest effective radical scavenging activity was the AgNPs, followed by the aqueous bulbil extract.

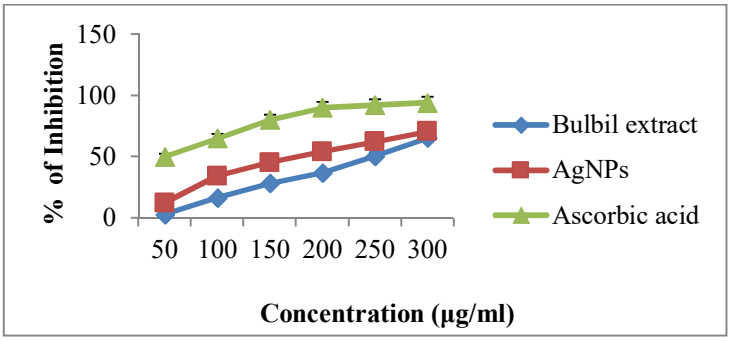


Fig. 6. DPPH Radical Scavenging assay of *D. alata* aqueous bulbil extract, AgNPs and Ascorbic acid (standard)

Antibacterial activity

The antibacterial activity was performed with pathogenic bacteria *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli.* Fig. 7 represents the zone of inhibition (diameter) 17mm, 15mm, 11mm and 13mm in *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli* bacterial suspension respectively. When the positive control, amoxicillin (1mg/ml) was tested for the antimicrobial activity against *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli,* the zone of inhibition was observed 32mm, 34mm, 32mm and 37mm respectively [19-25].

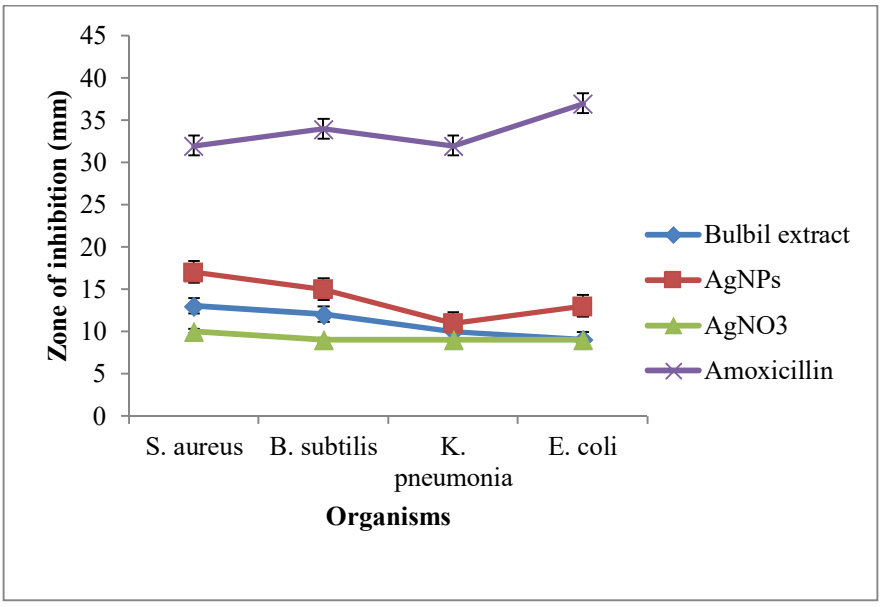


Fig. 7. Antibacterial activity of *D. Alata* aqueous bulbil extract, AgNPs, $AgNO_3$ and amoxicillin (standard) showing zone inhibitions in four bacterial cultures

PL Studies

For PL analysis samples were excited by wavelengths between 250 nm to 400 nm. Figs 8. show the luminescence spectra of silver nanoparticles (AgNPs) synthesized using *D. Alata* aqueous bulbil extract under optimum growth condition at A: Sunlight treatment B: UV treatment C: Room temperature. All luminescence spectra clearly show that prepared samples have emission peak with high intensity at longer wave length. Another peak of smaller intensity is also observed at higher wave length. The small emission peak of low intensity is observed almost all the samples near 560 nm, which is expected due to the plant extract.

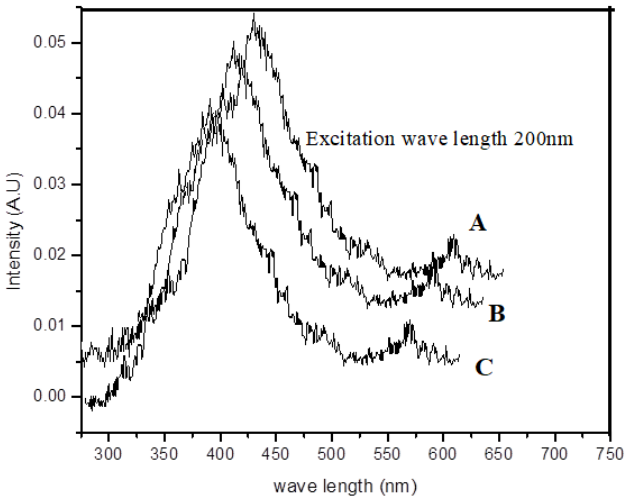
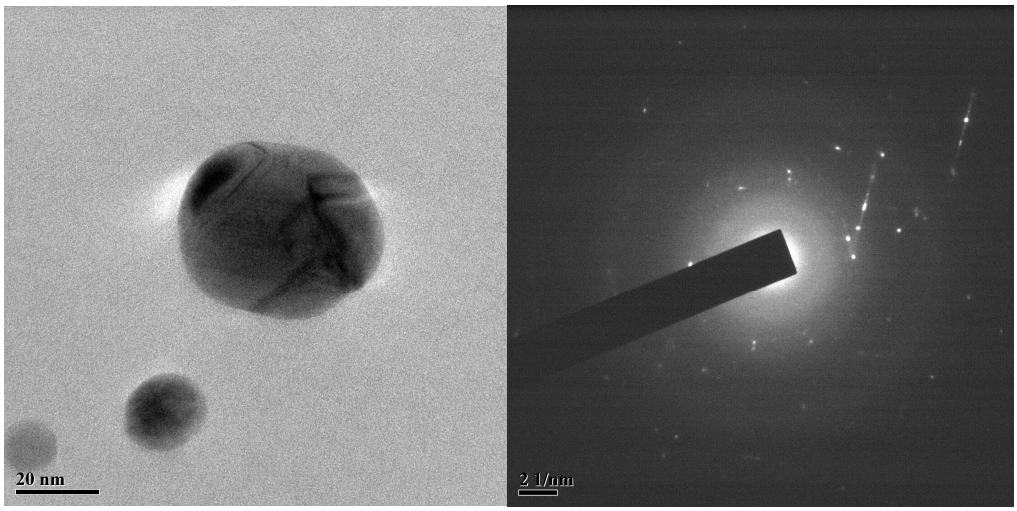


Fig8.PL spectra synthesized using *D. Alata* aqueous bulbil extract.
A: Sunlight treatment B: UV treatment C: Room temperature

These redshifted emissions are usually associated with trapped states such as vacancies, interstitials, impurities, and surface defects [2,30]. It is seen that when the size of the nanocrystallite decreases the luminance has been found to be dominated by the band impurity.

TEM and SEM studies



a **b**

Fig. 9. (a) HR TEM image of AgNPs, (b) SAED pattern

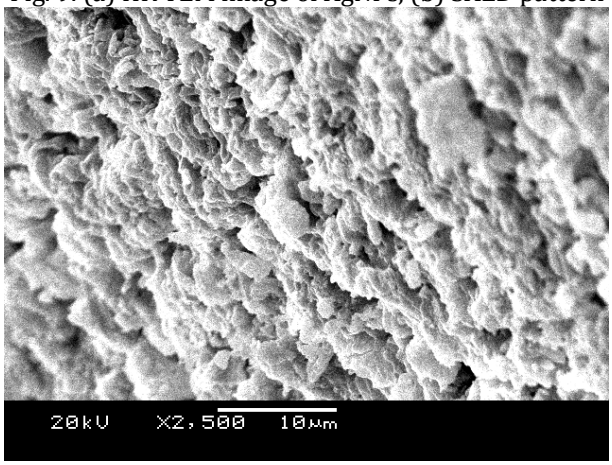


Fig. 10. HR-SEM Micrograph of AgNP

Fig. 9 a and b represent the HRTEM direct image of *D. alata* aqueous bulbil extract silver at three hours sunlight expose in our optimum antimicrobial effect. The size of the particle is uniformly distributed and spherical in shape. The minimum size found to be 12 nm which coincides the size obtained in optical model. Fig. 10 represent the SEM photograph synthesis bulbil extract silver nanoparticles at optimum condition. It is clear that grains are not overlap each other and uniformly distributed.

CONCLUSION

The silver nanoparticles synthesized using bulbils extract shows high and efficient antioxidant and antimicrobial activity in presence of sunlight. Rapid and ecofriendly biosynthesis of stable silver nanoparticles was observed in this study. Exposure of AgNPs to sunlight showed significant results, compared to UV and room temperature. Again, spherical and small particle exbibit better antimicrobial effect, which is confirm by XRD and TEM. Antimicrobial effect was observed against the important pathogens *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli.* Optical properties shows the increase of band gap 2.7 eV and particle size 12nm.PL studies shows the emission are red shifted.

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