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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING EPIPHYTES: *DENDROBIUM APHYLLUM* LEAF EXTRACTS AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT
Nanotechnology deals with the nanoparticles having a size of 1-100nm in one dimension used significantly concerning medical field, cosmetics, atomic physics and all other known fields. The silver nanoparticles have been prepared easily by different physical, chemical and biological approaches. The biological approach is most emerging because this method is easier than other methods, it is eco friendly, cost effective and less time consuming. The present work was aimed to the synthesis and characterization of silver nanoparticles from epiphytic plants *Dendrobium aphyllum* leaf extracts. A fixed amount of plant extract and metal ions are treated and the colour change was observed, which proved the formation of nanoparticles. The characteristics of silver nanoparticles were studied using UV-Vis absorption spectroscopy, FTIR, SEM analysis. The rapid reduction of silver ions was monitored using UV-Vis absorption spectroscopy and showed the formation of silver nanoparticles. FTIR analysis confirmed that the bio reduction of silver ions to silver nanoparticles is due to the reduction by capping material of plant extracts. SEM provided further insight in to the morphology and size details of the silver nanoparticles. The antibacterial activity of synthesized silver nanoparticles by plant is examined by well diffusion method.

KEYWORDS: Silver nanoparticles, *Dendrobium aphyllum* leaf extracts, UV-Vis absorption spectroscopy, FTIR, SEM, Antibacterial activity

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Biosynthesis of nanoparticles is an emerging highlight of the intersection of nanotechnology and biotechnology .The main studies discussed and focused here is aqueous extraction and biosynthesis of silver nanoparticles. Biological methods of nanoparticles synthesis using microorganisms (Mazzola, 2003), enzymes (Willner *et al*., 2006) fungus (Vigneshwaran *et al*., 2007), plants or plant extracts (Shankar *et al*., 2004) have been suggested as possible eco friendly alternatives to chemical and physical methods. The chemically synthesized metal nanoparticles are expensive, hazardous to environment and require high energy consumption. Sometimes syntheses of nanoparticles using plants parts are advantageous over other biological process by eliminating the process of maintaining the microbial culture. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternative tool towards chemical methods. The use of plants for synthesis of nanoparticles is rapid, low cost, eco friendly and a single-step method for biosynthesis process (Kumar V. and Yadav S K., 2009). Plant mediated nanoparticles synthesis is preferred as it is safe for human therapeutic use (Huang *et al*., 2008). Silver nanoparticles are nontoxic to human and most effective against bacteria, virus and other eukaryotic microorganisms at low concentration without any side effect (Jeong *et al*., 2005). Remarkable advances are made in the field of Biotechnology and Nanotechnology to harness the benefit of life science (Huangy *et al*., 2008) health care (Ahmad *et al.,* 2010) and industrial biotechnology (Elechiguerra *et al*., 2005). Nanomaterials may provide solutions to technological and environmental challenges in the area of solar energy conversion (Arango *et al*., 2000) catalysis (Tsujino K and Mastumura M. 2007) medicine (Jun *et al.,* 2005) and water treatment (Bao *et al*., 2007). This increasing demand must be accompanied by "green" synthesis procedures. New applications of nanoparticles and nonmaterials are emerging rapidly (C. J Murphy *et al*., 2008). Silver has inhibitory effects on microbes present in medicine and industrial process. The most important application of silver and silver nanoparticles in medicinal industry such as tropical ointments to prevent infection against burn and open wound (C. Lok; *et al*., 2007). This study deals with an eco friendly and biological synthesis of Silver nanoparticles by using leaf extracts of epiphytic plants and the characterization of these silver nanoparticles by using UV-Vis absorption spectroscopy, FTIR and SEM analysis methods and to analyze antibacterial properties against *E.coli* bacteria.

MATERIALS & METHODS

Plants used for extract preparation

FIGURE 1: *Dendrobium aphyllum*

Preparation of aqueous plant extracts.

Fresh plant materials are used for extract preparation. About 10 gram of fresh leaf weigh out and were thoroughly washed in distilled water, whipped slightly with tissue paper cut into small pieces and crushed in 100ml distilled water with the help of mortar and pestle. Then the mixture filtered through Whatman No: 1 filter paper.

Synthesis of Silver Nanoparticles

To synthesis silver nanoparticles, 1mM $AgNO_3$ solution was taken in a sterile conical flask and plant extract was added to it (1:9) for bio reduction into silver ions and kept at room temperature for overnight.

Characterization of silver Nanoparticles:

1. U.V- VISIBLE Spectrometry

Preliminary characterization of the silver nanoparticles was carried out using UV-Vis spectroscopy. The bio reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of reaction mixture (Mulvancy P, 1996).

2. FTIR – Analysis

FTIR was used to identify the possible functional groups responsible for the reduction of the Ag+ ions. In order to determine the functional groups and their possible involvement in the synthesis of silver nanoparticles, FTIR Analysis was carried out (Bankar AV, et al; 2009) by using FTIR Spectrometer [Perkin- Elmer L5 55- Luminescence spectrometer]. The samples were scanned using infrared in range of 4000-1000 $^{cm-1}$ using FTIR.

3. SEM – Analysis.

The morphological features of synthesized silver nanoparticles from plant extract were studied by scanning electron microscope (JEOL JSM – 6390). The diameter of synthesized nanoparticles was identified using SEM analysis. SEM analysis done after drying the extract and dried powder was used for SEM images.

4. Antibacterial Assay

Agar-Well Diffusion Method

Principle

Antimicrobial activity was measured using well diffusion method.

Reagents

Nutrient Agar Medium (1L)

The medium was prepared by dissolving 28g of the commercially available Nutrient Agar Medium [Hi media] in 100ml of distilled water.

Nutrient Broth (1L)

One litre of nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (Hi media) in 100ml distilled water.

Gentamycin (Standard antibacterial agent, concentration 20mg/ml)

PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with 24 hr culture of bacterial strains such as *E. coli*. Wells of approximately 10mm was bored using a well cutter and bored for bacterial strain, control, 50 µl and 100µl respectively. Sample concentration made 100 mg in 1 ml sample. The plates were then incubated at 37^0C for 24 hr. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Wells with Gentamycin alone were served as positive control.

RESULTS & DISCUSSION

Dendrobium aphyllum are the epiphytic plants used for the extract preparation. *Dendrobium* is a huge genus of orchids. The synthesis of silver nanoparticles occurred during the exposure of epiphytic leaf extract to aqueous silver nitrate solution. The colour change from greenish yellow to brown is a visual indication of the formation of silver nanoparticles through the reduction process, which is confirmed by UV-Vis spectroscopy, FTIR analysis and SEM analysis. Antibacterial studies were also carried out with *E.coli* bacteria. Silver nanoparticles exhibit yellowish brown colour in aqueous solution. As the *Dendrobium aphyllum.* leaf extracts was mixed individually in the aqueous solution of silver ions, it start to change the colour from greenish yellow to yellowish brown due to the reduction of silver ions, which indicate the formation of silver nanoparticles. Further the colour changes to dark brown due to the increased concentration and growth of silver nanoparticles. After sometimes there was no significant colour change occurred which indicate the completion of reduction reaction. In *Dendrobium aphyllum* the colour change observed from greenish yellow to dark brown within 10 minutes (figure 2).

FIGURE 2: Aqueous *Dendrobium aphyllum* leaf extract as control and bio reduction of silver nanoparticles as yellowish brown colour.

UV-Visible spectroscopy is used to examine the size and shape of nanoparticles in aqueous suspension. It is a widely used technique for the structural characterization of silver nanoparticles. The synthesized aqueous solution of samples where measured through UV-Vis spectrometer. UV-Vis spectrometer at a range of 300-700nm were performed to observe the reduction rate of silver ions in Dendrobium *aphyllum.* In *Dendrobium aphyllum* absorption peak of UV-Vis spectra of silver nanoparticles observed at 472nm (figure 3)

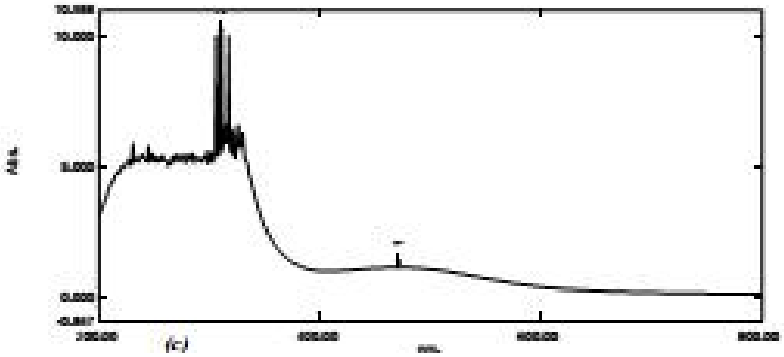

FIGURE 3:- UV-Vis absorption spectra of silver nanoparticles synthesized by *Dendrobium aphyllum*

FTIR analysis confirmed that the bio reductions of silver ions to silver nanoparticles are due to the reduction by capping materials of leaf extracts. For the characterization of the leaf extracts of *Dendrobium aphyllum* reaction with silver nitrate resulted as silver nanoparticles and characterization and observation were possible through FTIR analysis. In *Dendrobium aphyllum* the absorption bands showing the bio reduced silver ions observed by the absorption bands at the region 4000-1000cm^{-1} are 2372.44, 1238.3, 1219.01, 1145.72, 1047.35 and 1001.06cm^{-1} (figure 4).

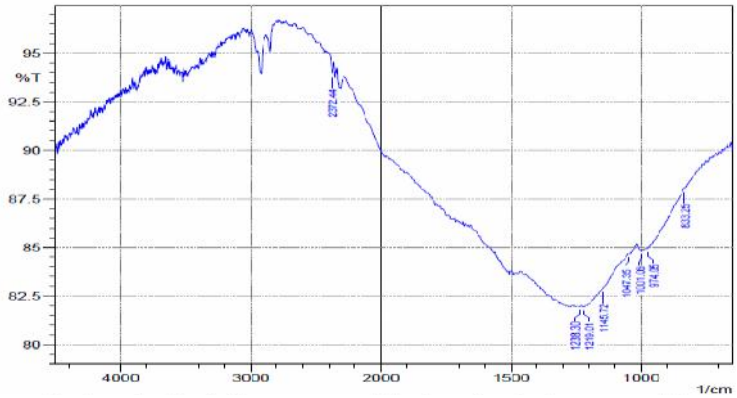

FIGURE 4:- FTIR result of synthesized silver nanoparticles by using leaf extracts of *Dendrobium aphyllum* .

The SEM images showing the morphological characters and the size of bio synthesized silver nanoparticles. SEM images showing the high density silver nanoparticles synthesized by *Dendrobium aphyllum*. The size of silver nanoparticles between the ranges from 2µ-10µ under the magnification range of 6000x (figure5).

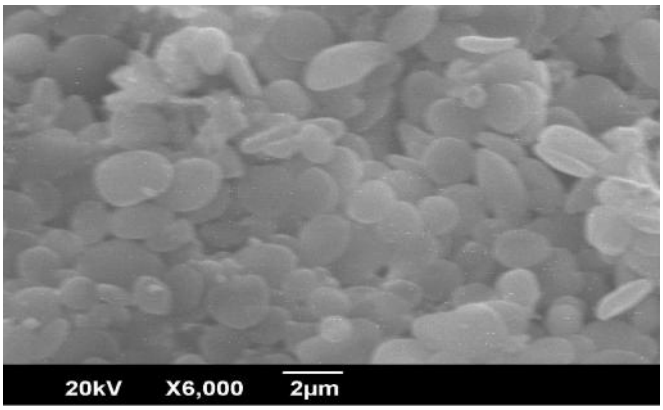

FIGURE 5:- SEM images of silver nanoparticles synthesized using *Dendrobium aphyllum*

Bactericidal effect on Hospital strain *E.coli* of silver nanoparticles synthesized by *Dendrobium aphyllum* leaf extracts shows synergetic effect with gentamycin. Synthesized silver nanoparticles shown zone of inhibition against the entire tasked microorganism. Zone of inhibition for *Dendrobium aphyllum* was found from the range of 4mm and 6mm with 50µl and 100µl concentrations respectively against *E. coli* (Fig-6) (Table-1).

TABLE 1-Effect of rang of synthesized silver nanoparticles on the inhibition growth of *E .coli* in mm

Sample	Concentration (µl)	Zone of inhibition
Dendrobium +Silver Nitrate	50	4mm
Dendrobium +Silver Nitrate	100	6mm

FIGURE 6: Antibacterial activity of *Dendrobium* silver nanoparticles against *E. coli*

CONCLUSION

The study shows that the leaf extracts of epiphytic plants such as Dendrobium *aphyllum* could be used as an effective reducing agent for the biosynthesis of silver nanoparticles. This study explined a easy, speedy and economical route to synthesize silver nanoparticles. In the present study we found that leaf extracts of *Dendrobium aphyllum* are also good source for the synthesis of silver nanoparticles. The colour change depicts the presence of silver nanoparticles in the reaction mixture. The appearance of dark brown colour is a clear indication of the formation of silver nanoparticles in the reaction mixture. The UV-Vis spectra analysis was carried out to examine the size and shape controlled nanoparticles in the reaction mixture. UV-Vis spectroscopy of *Dendrobium* the absorption peak observed at 472nm confirms that the synthesized particles are silver nanoparticles. FTIR analysis was carried out for the characterization of the reaction mixture. FTIR absorption spectra of bio reduced silver ions observed by the absorption bands at the regions of 4000-1000cm^{-1}. In *Dendrobium aphyllum* the absorption bands showing the bio reduced silver ions observed by the absorption bands at the region 4000-1000cm^{-1} are 2372.44, 1238.3, 1219.01, 1145.72, 1047.35 and 1001.06 cm^{-1}. SEM analysis was carried out to determine the morphological characters and size of the synthesized silver nanoparticles. SEM images showing the size of silver nanoparticles between the ranges from 1µ-10µ. In *Dendrobium aphyllum*, the size of silver nanoparticles between the ranges from 2µ-10µ under the magnification range of 6000. The silver nanoparticles synthesized using epiphytic leaf extract are pollutant free and eco friendly. So it can be used as a valuable alternative tool towards the chemical synthesis. The silver

nanoparticles also possess effective antibacterial properties against *E. coli* bacteria. Bactericidal effect on Hospital strain *E. coli* of silver nanoparticles synthesized by *Dendrobium aphyllum* found synergetic effect with gentamycin. Synthesized silver nanoparticles shown zone of inhibition against the entire tasked microorganism. Zone of inhibition for *Dendrobium aphyllum* was found from the range of 4mm and 6mm with 50µl and 100µl concentrations respectively against *E.coli* .

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