International Journal of Engineering, Science and Mathematics
Vol. 6 Issue 8, Decemer 2017,
ISSN: 2320-0294 Impact Factor: 6.238
Journal Homepage: **http://www.ijesm.co.in, Email: ijesmj@gmail.com**
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Antibacterial and Photocatalytic activities of silver nanoparticles synthesized by *Paeonia emodi* leaves extract

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Abstract

In the present study, an eco-friendly and facile method for biogenic synthesis of silver nanoparticles (AgNPs) has been developed using leaves extract of *Paeonia emodi.* The addition of plant leaves extract to silver nitrate solution results in the colour change of the solution, indicating the formation of silver nanoparticles. The synthesized nanoparticles were characterized by UV-Vis spectroscopy, X-ray diffraction(XRD),Dynamic light scattering (DLS),FTIR and Transmission electron microscopy(TEM).The XRD analysis shows that the AgNPs are of face centered cubic(FCC)structure.TEM analysis showed that the synthesized AgNPs have spherical shape with the particles size varying from5-40 nm. The synthesized AgNPs possess significant antimicrobial potential against gram's positive and gram's negative bacteria. The photocatalytic activity of the synthesized silver nanoparticles was examined by degradation of methylene blue under sunlight irradiation.

Key-words: *Paeonia emodi*, silver nanoparticles, XRD, TEM, Antimicrobial activity.

Introduction

Nanotechnology involves various areas of science which includes not only medicine but also, chemistry, physics, molecular biology, biochemistry etc. Now days, a large numbers of approaches are being used for the synthesis of nanoparticles (NPs).

Green synthesis of noble metal nanoparticles is evolving into an important branch of nanotechnology. Many metals have been used for the synthesis of nanoparticles such as gold, platinum, titanium, copper, zinc, cobalt, magnesium, nickel and silver [1-9].Among the various noble metals, silver is very important metal due to their diverse properties especially high antimicrobial and catalytic nature [10-11].Plant-mediated biological

synthesis of nanoparticles is gaining importance due to its simplicity, cost effectiveness and eco-friendliness [12].

Many researchers reported the plant mediated green synthesis of silver nanoparticles using extracts of different plant parts such as root, stem, bark, leaf, fruit, bud, and latex as natural resources [13-16]. The reduction and stabilization of metal ions is done by the various biomolecules such as tannins, terpenoids, polysaccharides, polyphenols, proteins and amino acids present in the plant extracts [17-18].

Paeonia emodi belong to the family Paeoniaceae (Peony family), commonly known as Himalayan Peony. The tubers of Himalayan Peony are a useful medicine for the treatment of hypertension, palpitations ,asthma hysteria, convulsions, colic ,uterine diseases, bilious obstructions and has also been used as an anti coagulant.

Different classes of natural products like triterpenoids, mono terpenoids, phenolics and tannins have been isolated from the species. These compounds possess wide therapeutic profile like cardiovascular and airway relaxant lipoxygenase and beta-Glucuronidase inbitory and free radical scavenging properties [19-20].

In the present research work, we discuss the green-synthesis of AgNPs using *Paeonia emodi* and its application for catalytic degradation of organic dyes. Finally, its antibacterial activity was investigated by the disk diffusion method.

MATERIALS AND METHODS

2.1Materials

The healthy leaves of plants were collected from Garhwal region of Uttarakhand and identified by Department of Botany, Srinagar, Uttarakhand, India. All the analytical reagents were purchased from Fisher chemicals. The deionized water is used throughout the experiment.

2.2. Preparation of Plant extract

The fresh plant material of *Peony emodi* was collected and shade dried at room temperature. The leaves extract was prepared by taking 10gm thoroughly washed with running water, followed by distilled water. Dried and powered plant material was boiled with 100ml of deionized water at 60^{o} for 30 min. This extract was cooled at room temperature and filtered by whatman filter paper No.1 and finally stored at 4^{o}C for further experiment.

2.3 Preparation of silver nanoparticles

For the synthesis of silver nanoparticles, 10 ml of aqueous leaf extract was added to 90 ml of AgNO3 (2mM) solution and kept in dark for 48 hours for the formation of silver nanoparticles.

The colour changes of leaf extract indicating the formation of AgNPs. The reaction mixture was centrifuged at 10,000rpm for 15minutes.Pellets was collected followed by redispersion of pellets of AgNPs in deionzed water to get rid of any uncoordinated biological material.

2.4 UV-VIS spectra analysis

The UV-VIS spectrum of synthesized AgNPs was analyzed by spectrophotometer (UV-Visible Perkin Elmer Lambda 25).The stability and formation of AgNPs by bio reduction of Ag^{+} to Ag^{0} using *Peony emodi* leaves extract was easily monitored using UV-VIS spectroscopy. The AgNPs show the Plasmon resonance at 430nm. From the study it has been found that silver nanoparticles shows the characteristic SPR at a wavelength in the range of 400-450nm [21-22].

2.5. X-ray diffraction analysis of AgNPs

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of silver nanoparticles. The particles size of synthesized AgNPs was determined using Debye Sherrer's equation.

$$D= 0.94\lambda/\beta\cos\theta$$

Where D is the average crystalline domain size perpendicular to the reflecting planes, λ is the wavelength of X-rays, β is the full width at half maximum (FWHM), and θ is the diffraction angle [23-24].

2.6. TEM analysis of synthesized AgNPs

TEM are capable of imaging at a significantly highly resolution than the light microscope. Its give higher resolution images of inner structure of the sample compares to the SEM. Hence the size, shape and phase composition of particles were studied by TEM.

2.7. FTIR

The FTIR measurement were carried out to identify the possible biomolecules responsible for the reduction of Ag^+ ions and capping of the silver nanoparticles synthesized by leaf extract.

2.8. Study of Photocatalytic action of prepared AgNPs against Methylene Blue

1mg of methylene blue was added to100mL of deionized water used as stock solution. About 20mg of biosynthesized silver nanoparticles was added to 50mL of methylene blue solution [25]. The resultant solution was mixed in magnetic stirrer for 30 min. Finally the solution was kept in sunlight. At specific time intervals, aliquot (2 ml-3ml) was withdrawn. The concentration of MB during degradation was determined by UV-Vis spectrophotometer. Percentage of dye degradation was estimated by the following formula:

$$\%\text{Decolourization} = 100 \times (C_0 - C)/C_0$$

Where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

2.9. Antibacterial property of synthesized AgNPs

The antibacterial property of the silver nanoparticles was estimated against the pathogenic bacteria viz. gram's positive bacteria such as Staphylococcus aureus and bacillus subtilis and gram's negative bacteria such as Escherichia coli and Salmonella typhi. The antimicrobial activity of synthesized AgNPs and standard were tested by disk diffusion method. All the plates were incubated at 37°C for 24hours and the zone of inhibition of bacteria was measured.

1RESULTS AND DISCUSSION

3.1 UV-VIS spectroscopic analysis

The addition of plant leaves extract to silver nitrate solution result in colour change of the solution from greenish brown to reddish brown .This is due to the excitation of surface Plasmon vibration with silver nanoparticles.[1] after 48hours of incubation of the aliquot, the sample was analysed by UV-VIS spectroscopy which show that SPR occurred at 450nm. UV-Vis absorption spectrum of synthesized AgNPs using *Paeonia emodi* leaves extract is shown in fig.1. The maximum absorption peaks for synthesized AgNPs was observed at 450 nm which clearly indicating the formation of AgNPs [26].

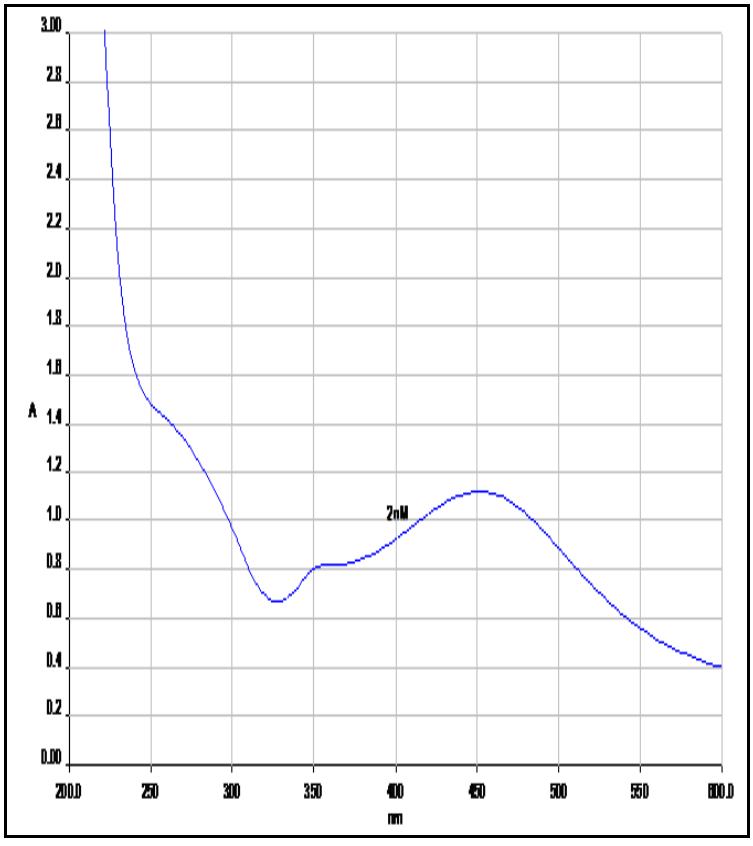

Figure 1: UV-Vis spectrum of AgNPs using leaves extract of *Paeonia emodi*

3.2 X-ray diffraction analysis of synthesized AgNPs

Intense peak occurring at a 2θ=38.45, 44.53 and 64.76 which indexed the lattice planes (111), (200) and (220) of the cubic face-centered silver. The lattice constant calculated from this pattern was a=4A^0. These Bragg's reflections are corresponding to the planes which are in good agreement with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file no.04-0783.The average grain size of the silver nanoparticles was determined using Debye-Scherrer formula ,d=(0.9λ×180^o/βcosθπ) and was estimated as 17.38nm.

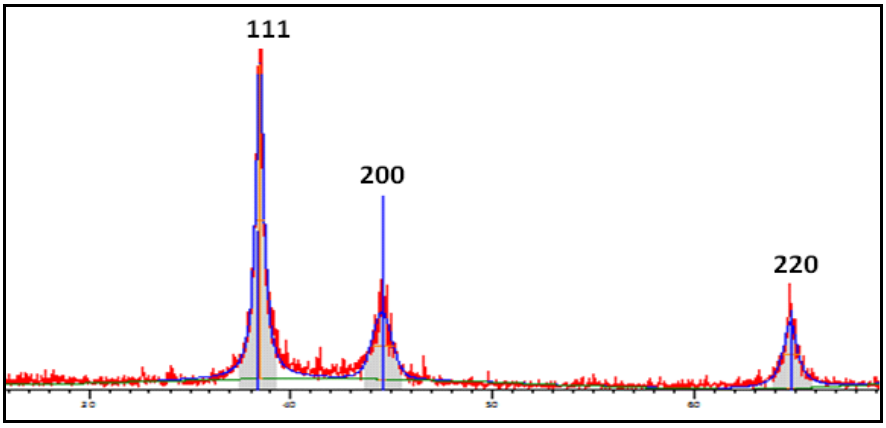

Figure 2: XRD spectrum of AgNPs using leaves extract *Paeonia emodi*

3.3. TEM analysis of synthesized AgNPs

TEM micrograph fig.3 revealed that the particles are spherical, oval and well dispersed without agglomeration. The particles size of synthesizes silver nano particles from *Peony emodi* leaves extract is in the range of 5-40 nm. Various reports have provided evidences of extracellular synthesis of silver nano particles by TEM images.

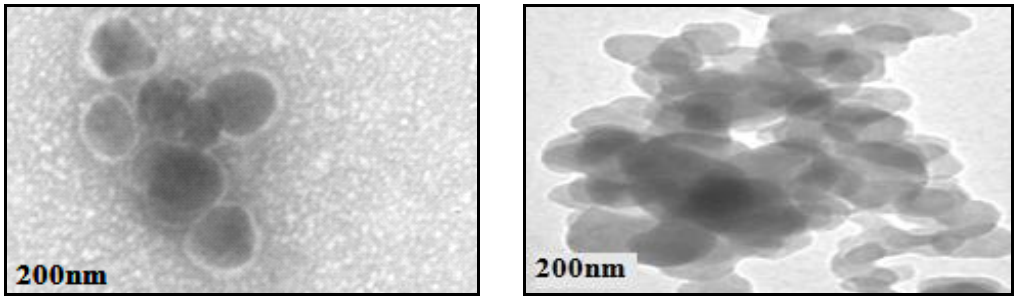

Figure 3: TEM micrograph of AgNPs using leaves of *Paeonia emodi*

3.4. Study of catalytic action of prepared AgNPs against Methylene Blue

Photocatalytic activity of silver nanoparticles was evaluated by the degradation of methylene blue, which was carried out in the presence of sunlight. The absorption spectrum peaks for methylene blue was decreased at different time intervals. The intensity of the absorption peaks at 660 nm for methylene blue was decreased gradually with the increase of the exposure time which shows the photocatalytic degradation reaction of methylene blue. The degradation percentage was increased as increasing the exposure time of dye silver nanoparticles solution in sunlight fig.4. Absorption peak for methylene blue was found at 660 nm in visible region which diminished and finally, it disappeared while increasing the reaction time. The percentage of degradation efficiency of silver nanoparticles was calculated as 87% at 48 h.

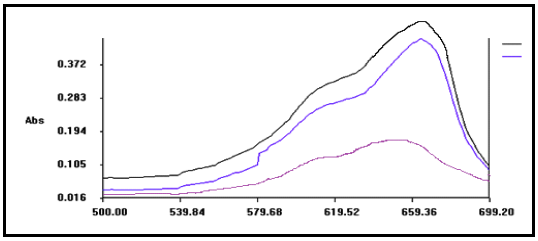

Figure 4: The absorption spectra of aqueous solution of methylene blue dye and AgNPs synthesized by using *Paeonia emodi* leaves extract at different time intervals

3.5. Antibacterial property of synthesized AgNPs

The synthesized AgNPs showed significant antimicrobial potential against gram's positive bacillus subtilis and Salmonella typhi and also found to be inactive against gram's negative Escherichia coli and gram's positive Staphylococcus aureus.

Bacteria	**Zone of inhibition (mm)**	**Positive Control (Streptomycin- 25 µg)**
Staphylococcus aureus	R	12mm
Bacillus subtilis	9.8	12mm
Escherichia coli	R	15mm
Salmonella typhi	10.5	15mm

Table.1Antimicrobial activity of Nanoparticles against gram's positive and gram's negative bacteria

Conclusion

Silver nanoparticles were successfully obtained by bioreduction of silver nitrate solution using *Paeonia emodi* leaves extract. The reduction of silver ions and stabilization of the AgNPs were thought to occur due to the surface Plasmon resonance with the ingredients present in the plants leaves extract which were characterized by UV-Vis, XRD, TEM and FTIR measurement. The biomolecules present in synthesized nanoparticles were acting as capping as well as reducing agents. The results showed that synthesized AgNPs presented significant antimicrobial potential against gram's positive bacillus subtilis and gram's negative Salmonella typhi.

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