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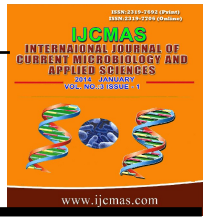

Original Research Article

Biological Synthesis of Silver Nanoparticles from *Nerium oleander* and its Antibacterial and Antioxidant Property

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A B S T R A C T

Keywords

Silver Nanoparticles; Nerium oleander; Antibacterial Activity; Antioxidant test.

Nanotechnology has now started to develop a new route for changing of our day to day life. Nanomaterials are mostly used for the production of efficient alternative energy sources. Silver nanoparticles (AgNPs) due to biocompatibility, antibacterial action and its various application in the field of electronics, optical, magnetic has attracted considerable attention. In this study crystalline silver nanoparticles were successfully produced from silver nitrate using *Nerium oleander* extract. This extract serves as a reducing agent. The AgNPs were characterized using UV-visible spectroscopy, scanning electron microscopy. Nanoparticles were determined at the range of 380 to420 nm. The antibacterial activity against different pathogen (*E.coli, B.subtilis*) and control were reported .The zone of inhibition is observed both in gram positive and gram negative bacterial strains. Using DPPH reagent & Hydrogen peroxide antioxidant property was also checked

Introduction

Nanotechnology is foreseen to significantly influence science, economy and everyday life in the 21stcentury and also to become one of the driving forces of the next industrial revolution (Klabunde *et al*., 2001). Nanoparticle synthesis has received considerable attention in recent years as a result of its optical, electronic, magnetic and chemical properties and their potential applications in subsequent technology development (Compagnini *et al.*, 1997).Recently, much effort has been devoted to the synthesis of silver nanorods (AgNR) and nanowires (Barun, *et al*., 1998). The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Nanomaterials such as Ag, Au, Ptand Pd have been synthesized by different methods, including hard (Zhou*et al*., 1999), using fungi (Sastry *et al*., 2003), plants (Justin Packia Jacob *et al*., 2012) and bacteria (Husseiny *et al*.,2007). Among these, silver nanoparticles play a significant role in the field of biology and

medicine due to their attractive physiochemical properties .The highly reactive metal oxide nanoparticles exhibit excellent bactericidal action against Gram-positive and Gram-negative bacteria (Stoimenov *et al*., 2002) .The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material. Sondi and Sondi (Sondi *et al*., 2004) studied antimicrobial activity of silver nanoparticles against *Escherichia coli* as a model of Gram-negative bacteria.This is particularly important for noble metals such as Au and Ag, which have strong surface Plasmon resonance oscillations. Tremendous applications are in the field of high sensitivity biomolecular detection and diagnosis, antimicrobials and therapeutics, catalysis, sensors, micro-electronics and filters. Chemical methods of nanoparticles synthesis are the most widely and traditionally used (Kasthuri *et al*., 2009).

Generally, the physical methods have low yields and the chemical methods cause contamination due to precursor chemicals, use of toxic solvents and the generation of hazardous by-products. Hence, there is a great need to develop high yield, safe, reliable, clean and eco-friendly methods for the preparation of nanoparticles. *Nerium oleander* is an evergreen shrub or small tree in the dogbane family Apocynaceae, toxic in all its parts. It is the only species currently classified in the genus *Nerium*. It is most commonly known as *Oleander,* from its superficial resemblance to the unrelated olive *Olea*. It is so widely cultivated that no precise region of origin has been identified, though southwest Asia has been suggested. The ancient city of Volubilis in Morocco took its name from the old Latin name for the flower. *Oleander* is one of the most poisonous of commonly grown garden plants.

Materials and Methods

Collection of Plant Materials

The fully matured leaves of *Nerium oleander* were collected from Okkilipatti village in Namakkal district of Tamilnadu, India. During the month of December, 2012.The leaves were thoroughly washed and shade dried for 5 days.

Preparation of Plant Extract

The *Nerium oleander* leaves after shade dried for a period of 5days were blended and made into fine coarse powder. 10gram of powder is mixed with 100ml of distilled water and boiled for 15 minutes. Then the extract is filtered using 25 µm and 0.6µm size of watt manfilter paper. Residue is removed and pure plant extract is obtained.

Synthesis of Silver Nanoparticles

1mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles.10ml of *Oleander* extract was mixed with 90ml of aqueous solution of 1mM silver nitrate for the reduction into Ag+ ions and incubated overnight at room temperature in dark.

UV-Spectra Analysis

Silver nanoparticles (AgNPs) are soluble in distilled water and the colour changes were observed visually. Adark brownish colouration indicates the formation of silver nanoparticles. The reduction of pure Ag+ions was monitored by measuring the UV-spectrum of the reaction medium after

overnight incubation, after diluting a small aliquot of the sample into distilled water. UV-spectral analysis was done by using Systronics UV double-beam spectrophotometer, at a resolution of 1nm, between 200 and 600nm using 10nm quartz cuvettes.

FT-IR

FT-IR is a technique which is used to obtain an infrared spectrum of absorption emission photoconductivity of a solid gas or liquid.One of the most important tasks is to characterize the spectrum of a light source.

Antibacterial Activity

The antibacterial activity of *Nerium oleander* plant extract was evaluated by disc diffusion method. Muller Hinton agar medium was prepared and poured into the petriplates and allowed to solidify. Then it was inoculated with a swab of culture and spread throughout the medium uniformly with a sterile cotton swab. Two wells are bored in a plate and in which one wellare induced with100µl of plant extract (sample) and in another well 100µl of distilled water (control) were loaded and antibiotic (gentamicin) was also placed. The test bacterial *E.coli* and *Bacillus subtilis* were included in this study to assess the susceptibility pattern of the compounds. The plate was incubated 37ºC for 24hrs for observing inhibition rate.

Antioxidant Property

Antioxidant analysis was checked using two assays, namely DPPH and Hydrogen peroxide assay.

DPPH Assay

The Scavenging activity for DPPH free radicals was measured according to the procedure described by *Bliosetal.* Methanol solution of the sample extract at various concentration (0.6g, 1.2g, 1.8g, 2.4g & 3.0g) was added separately to each 5mL of 0.1mM methanolic solution of DPPH and allowed to stand for 20min at 27□C . After incubation, the absorbance of each solution was determined at 517nm using spectrophotometer. Ascorbic acid was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

Percentage of inhibition= [(control OD-sample OD) / (control OD)]*100

Hydrogen Peroxide Assay

Phosphate buffer was prepared in 0.01 ml of H_2O_2. This prepared solution was taken and it is added with leaf extract. 0.6g, 1.2g, 1.8g, 2.4g & 3.0g of powdered leaf was taken. It is then mixed with 6ml of ethanol. Then it is incubated in water bath for 10mins at 30□c. Then added with 0.6ml of H_2O_2 and it is incubated for 30mins in Room Temperature. After 30mins of incubation Optical Density was taken at 510nm. Phosphate buffer served as control.

Percentage of inhibition= [(control OD - sample OD) / (control OD)]*100

Results and Discussion

The chemical reduction of aqueous solution of silver nitrate is the most widely used method for the synthesis of silver nanoparticles. In the present study the formation of silver nanoparticles by using *Nerium oleander* was determined. The appearance of the dark brownish colour suggested the formation of silver nanoparticles.

Figure.1 UV- VIS Spectroscopy

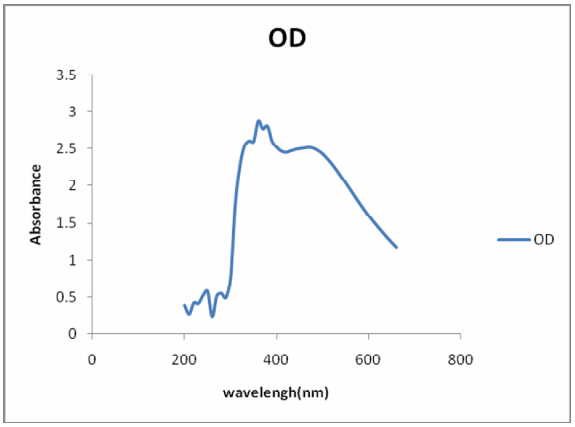


Figure.2 FTIR

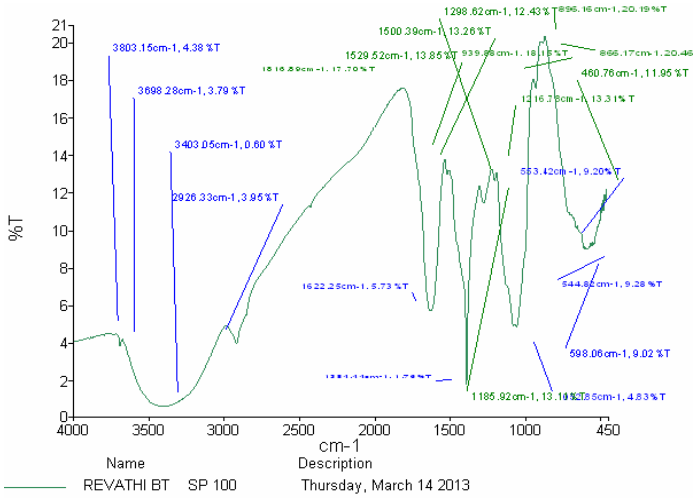


Figure.3 Antioxidant Property- DPPH Assay

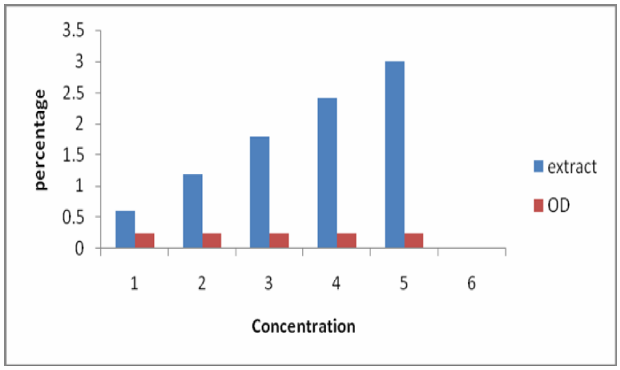
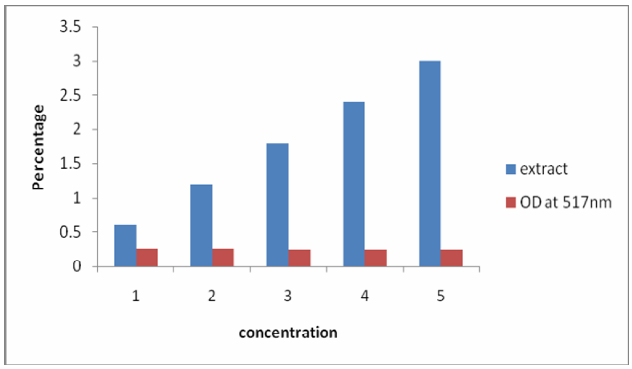


Figure.4 Antioxidant Property - Hydrogen Peroxide Assay



UV- VIS Spectroscopy

The plant extract after addition of aqueous 1mM silver nitrate was subjected to optical measurement using UV-visible spectrophotometer. From this analysis absorbance peak was found at 410nm which was specific for Ag nanoparticles.

FTIR

The silver nanoparticles synthesized using *Nerium oleander* showed strong bands at 3698.28cm-1 has a O-H functional group and the type of vibration is Stretch and free, 3403.05cm-1 has a O-H functional group and the type of vibration is Stretch and H-bonded, 2926.33 cm-1 has a C-H functional group and the type of vibration is Stretch, 1622.25cm-1 has a C=C functional group and the type of vibration is Stretch, 1500.39cm-1 has a C=C functional group and the type of vibration is Stretch, 1529.52cm-1 has a N-O functional group and type of vibration is Stretch and 1216.78cm-1 has a C-O functional group and type of vibration is Stretch.

Antibacterial Activity

Silver nanoparticles exhibit antibacterial properties against *E.coli* and *B.subtilis*. The antibacterial activity of AgNanoparticle produced *Nerium oleander* was studied against various

Gram positive and Gram negative strains. The zone of inhibition was found more at *B.subtilis* than at *E.coli*. The activity of silver nitrate, silver nanoparticles and culture were studied.

Antioxidant Property

DPPH Assay

The total antioxidant activity of DPPH was determined to be 38% equivalents for the ethanol leaf extracts of *Nerium oleander* which is comparable to that of the standard Ascorbic Acid (20%).

Hydrogen Peroxide Assay

The total antioxidant activity of Phosphate buffer was determined to be 32% equivalents for the ethanol leaf extracts of *Nerium oleander* which is comparable to that of the standard Phosphate buffer (22%).

From this study, it is suggested that the *Nerium oleander* extract is capable of producing nanoparticles in room temperature, without using additives, accelerants or any templates. TheHydroxyl ions present in *Nerium oleander* along with other alkaloids may be responsible for the synthesis of silver nanorods. It is concluded that the aqueous silver ions exposed to the plant extract were reduced in the structure level. Presence of nanoparticles were confirmed by colour change of media from pale yellow to dark brownish colour. The antimicrobial efficiency of *E.coli* was more than against *Nerium oleander*. The antioxidant activity of *Nerium oleander* was checked using DPPH and Hydrogen peroxide assay.Silver nanoparticles produced may be applied in display technologies, optoelectronics and flexible thin film computers.It is also applied in medical field.

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