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Green synthesis of silver nanoparticle using *Eucalyptus globulus* leaf extract and its antibacterial activity.

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ABSTRACT

Aqueous extract of dried leaves of *Eucalyptus globulus* is used as reducing agent for the environmentally friendly synthesis of silver nanoparticles at room temperature. The nanoparticles were characterized using UV-visible spectroscopy and Transmission electron microscopy (TEM). The silver nanoparticles synthesized are predominantly spherical in shape with diameter ranging from 9.18 to 32.39 nm. The antibacterial activity of the silver bio-nanoparticles was performed by a disc diffusion method. The synthesized silver nanoparticles exhibited antimicrobial activity against phyto-pathogenic bacteria.

Key words: Silver nanoparticles-*Eucalyptus globules*-Green synthesis-Antibacterial.

INTRODUCTION

Nanotechnology is considered to be the most exciting research field which deals with the materials having size varying from 1 to 100 nm. Research and development in this field is intensifying very rapidly. Due to quantum effect at nano level, the properties of materials are different from macro level. [7]. Metal nanoparticles have high specfic surface area and are intensely studied due to their unique optical, electronics, catalytic, anti-bacterial and magnetic properties [28,4,10,20].

A variety of method has been developed in order to prepare metal nanoparticles such as chemical reduction [16,25,23,27]. electrochemical reduction Liu and Lin, [12] photo-chemical reactions in reverse micelles [21] and via green chemistry route [2]. AgNPs are mainly synthesized by chemical methods involving the use of toxic chemicals such as thiophenol [17], marcaptoacetate [11] and thiourea [15], which create serious environmental problems. Biosynthesis of nanoparticles using plant extract is an advantageous method over physical and chemical as it is cost effective, environment friendly and there is no use of any toxic chemicals in the synthesis process. [19]. Other biological synthesis methods have also been used including bacteria and fungi [8,26,6]. But use of plant extract reduces the time and do not require any culture preparation [1,3].

In this study, the synthesis of silver nanoparticles carried out using the leaf extract of *Eucalyptus globulus* and characterized by using UV-visible spectra and Transmission electron microscopy. The antibacterial activity of the nanoparticles has been investigated against some phytopathogenic bacteria.

Materials and Methods

2.1. Materials:

Freshly collected leaves of *Eucalyptus globulus* were washed thoroughly with tap water, followed by distilled water and then cut into small pieces and shade dried. The fine powder was obtained from the dried leaves by using Mill (Glen Creston Stammore, England). Finally, the leaf powder was sterilized in an autoclave at a pressure of 15 Ib/sq inch and the temperature 121°C for 5 min. Silver nitrate analytical grade was purchased from Windsor Laboratories Limited, United Kingdom. All the aqueous solutions were prepared using distilled de-ionized water obtained from Water distiller LABCONCO water PROT.M PS LABCONCO Corporation, KANSAS City, Missouri 64132-USA.

2.2 Bacterial Strains:

Three phytopathogenic bacteria, *Agrobacterium tumefaciens*, *Erwinia amylovora*, and *Ralstonia solanacearum* were obtained from Bacterial Disease

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2.3. Synthesis of silver nanoparticles:

5.0 g of the leaf powder was boiled for 10 minutes in 100 ml deionized water and filtered through Whatman paper No.1. The filtered extract was stored in refrigerator at 4 ºC for further studies. For the biosynthesis of silver nanoparticles. 6.00 ml of plant extract broth was added to 44 ml of 2mM aqueous silver nitrate.

2.4. Characterization of silver nanoparticles:

2.4.1. UV-Visible Spectroscopy:

The reduction of pure Ag^+ ions was monitored by measuring a UV-Visible spectrophotometer (V-530, Japan Servoco., LTD. Indonesia). For the analysis, 0.1 ml of the sample in a cuvette and was diluted to 2 ml with denionized water.

2.4.2. Transmission Electron Microscopy:

The morphology and size of the biosynthesized silver nanoparticles were investigated by Transmission Electron Microscopy (TEM) using a JEM 2010 instrument at an accelerating voltage of 200 kV. The sample for TEM analysis was prepared by placing a drop of silver nanoparticles solution onto a carbon film supported on a copper grid, followed by water evaporation in air at room temperature.

2.5. Screening of antibacterial property in synthesized nanoparticles:

The silver nanoparticles synthesized using Eucalyptus *globulus* leaf extract was tested for antimicrobial activity against phytopathogenic bacteria *Agrobacterium tumefaciens, Erwinia amylovora and Ralstonia solanacearum* by the use of filter paper disc diffusion method. [24]. Sterilized filter paper discs (6 mm in diameter) were impregnated in silver nanoparticle solution. The discs were then placed on the surface of the plates containing nutrient glucose agar medium previously seeded with organisms (one flask for each isolate) using appropriate amounts (10ml for each flask containing 250 ml of sterilized NGA medium) of 24h old broth culture (10^8 CFU/ml) of microorganism as inoculums . Discs with sterilized water were used as a control. The plates were incubated at 28 ºC for 48 h, after incubation time, the diameter of inhibition zone (mm) was measured. The experiment was performed in three replicates. [14].

Results and Discussion

In the present study, formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV–vis spectral analysis. The tinge of yellowish brown colour was remarkable with the first appearance after minutes of addition of leave extract to aqueous silver nitrate solution due to the formation of silver nanoparticles. (Fig 1 and 2). Thereafter intensity increases with the reaction time thus confirming the formation of silver nanoparticles. After of 2h the intensity of brownish black colour did not increase confirming that the reaction has come to an end. The optical absorption spectra of the corresponding particles show a peak at around 412 nm. The peak at 412 nm indicating reduction of silver nitrate into silver nanoparticles.

With increase the time, the peak at 412 nm is shifted to higher wavelength, which further red shifted. In addition to the red shift, the width of the plasmon peak is also increased. The radiative damping that arises from spontaneous emission of radiation by induced dipole grows rapidly with particle size thereby increasing the plasmon linewidth [9].

The increase in bandwidth can also arise from the excitation of different multiple modes present in anisotropic particles that peak at different energies [13]. Thus, the observed line broadening and wavelength shift can be attributed to an increase in particle size.

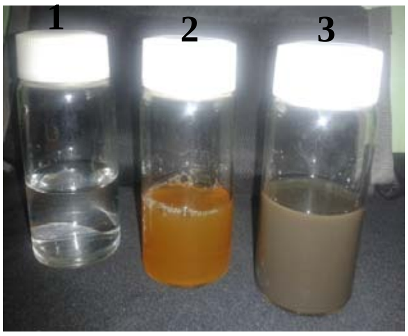

Fig. 1: Synthesis of AgNPs by *Eucalyptus globulus* leaf extract. The figure shows vials containing samples of the culture filtrate after exposure to Silver nitrate alone (1), *Eucalyptus globulus* leaf extract (2) and *Eucalyptus globulus* leaf extract with $AgNO_3$ (3). It is observed that the color of the solution turned from colorless to brown, indicating the formation of AgNPs.

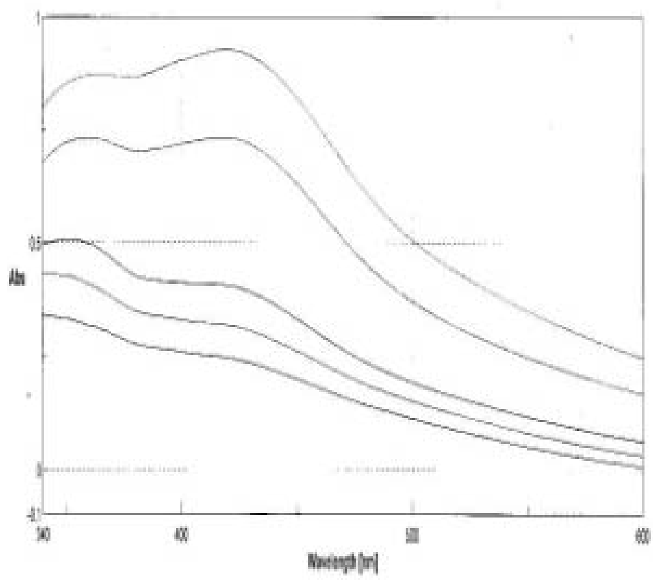

Fig. 2: UV–vis absorption spectra of AgNps.

TEM image of silver colloidal solution synthesized by treating from $AgNO_3$ solution using of *Eucalyptus globulus* leaves extract is shown in Fig. (3). It illustrates that the Ag nano particles are predominantly spherical in shape with diameter ranging from 9.18 to 32.39 nm.This result also indicates that the Ag nanoparticles are monodispersed without aggregation.

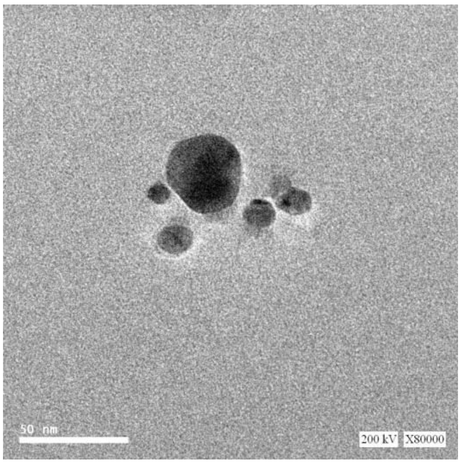

Fig. 3: TEM micrograph of silver nanoparticle.

Antibacterial Activity Study of Silver Nanoparticles (AgNPs):

We are interested to see the potential effect of the antibacterial activity of biologically synthesized AgNPs against *Agrobacterium tumefaciens, Erwinia amylovora* and *Ralstonia solanacearum*. The results of antibacterial activity inhibition zone are tabulated in Table (1). The maximum antibacterial activity was recorded for *Erwinia amylovora* (17 mm) followed by *Agrobacterium tumefaciens* and *Ralstonia solanacearum* (14 mm and 11 mm, respectively). The AgNp synthesized in this process was found to have antimicrobial activity against pathogenic bacteria. From the table, it is evident that the nanoparticles synthesized are good candidates their usage as antibacterial.

Table 1: inhibition zone (mm) of biologically synthesized silver nanoparticles against phytopathogenic bacteria.

S.No.	Test Organism	inhibition Zone of AgNps in mm
1	*Agrobacterium tumefaciens*	14
2	*Erwinia amylovora*	17
3	*Ralstonia solanacearum*	11

The mechanism of inhibitory action of silver nanoparticles on microorganisms is partially known. Silver nanoparticles are have positive charge, it will attach with the negative charged microorganisms by the electrostatic attraction in the cell wall membrane [5]. The silver nanoparticles closely associated with cell wall of bacteria by forming 'pits' finally it affects the permeability, and cause cell death [22]. The silver nanoparticles were small in size, so it easily enters into the bacterial cell and affect the intracellular processes such as DNA, RNA and protein synthesis. Silver nanoparticles were binding with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria thus it has more bactericidal activity than the larger sized nanoparticles [21].

Conclusion:

Green chemistry approach towards the synthesis of nanoparticles has many advantages such as, ease with which the process can be scaled up and economic viability. We have developed a fast, ecofriendly and convenient method for the synthesis of silver nanoparticles from *Eucalyptus globulus* leaf extract with a diameter ranging from 9.18 to 32.39nm. These particles are spherical. No chemical reagent or surfactant template was required in this study, which consequently enables the bioprocess with the advantage of being environmental friendly. Color change occurs due to surface plasmon resonance during the reaction with the ingredients present in the plant leaf extract results in the formation of silver nanoparticles which is confirmed by UV–vis and TEM. The silver nanoparicles synthesized in this process was found to have antimicrobial activity against pathogenic bacteria.

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