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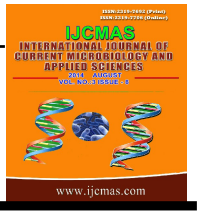

Original Research Article

Green synthesis of Silver Nanoparticles using *Vitex negundo* and its antimicrobial activity against human pathogens

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Keywords

Silver nanoparticles, Plant leaf extract, Nano-technology, Pathogens, Zone of inhibition

A B S T R A C T

The work is being carried out for the applications plant materials which would help in the prevention of human pathogens. We describe the synthesis of silver nanoparticles using plant *Vitex negundo* leaf extracts. A strong characteristic absorbance peak at around 420 nm was observed at different time intervals. This paper deals with a thorough investigation on the characterization of the silver nanoparticles by XRD, SEM and FTIR analysis and result reveals that, the average grain size of the silver nanoparticles formed in the bioreduction process is determined by XRD pattern of the silver nanoparticles formed in the experiment and is estimated to be 60 nm and spherical in shape. FTIR spectral analysis showed array of absorbance bands in 400 cm^{-1} -1500 cm^{-1} finally tested the biocompatibility by antimicrobial test against multi drug resistant human pathogens like *Staphylococcus aureus, Escherichia coli, Pseudomonous sp, Proteus vulgaris, and Salmonella typhi.* The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test sample. The result reveals that the maximum antimicrobial activity was observed against the test organisms when compared to the control

Introduction

The use of natural materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and cost effective for medical applications as they do not use toxic chemicals in the synthesis protocols. Chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have side effects in medical applications. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and eco-friendly and in this method there is no

need to use high pressure, energy, temperature and toxic chemicals. Silver nanoparticles are the metal of choice as they hold the promise to kill microbe's effectively (Sondi and Salopek-Sondi 2004). The silver nanoparticles act on a broad range of target sites both extra-cellularly as well intra-cellularly. In fact, microbes generally have a harder time developing resistance to silver than they do to antibiotics (Baker, et al. 2005 and Landsdown 2007) the oligo-dynamic effect that silver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation. Some well known examples are, synthesis of gold nanotriangles using Lemmon grass and tamarind leaf extract (Shankar et al 2005) synthesis of silver nanoparticles using geranium leaf (Shankar et al 2003), fungus (Ajaykumar et al 2001 and Ahmad et al 2003) soluble starch (Vigneshwaran 2006) silver tolerant yeast strain. (Kowshik 2003) synthesis of bimetallic Au core-Ag shell nanoparticles using Neem leaf broth (Shankar et al 2004) biosynthesis of silver based crystalline nanoparticles of well defined composition and shapes as equilateral triangles and hexagons within the periplasmic space of bacteria *Pseudomononas stutzery* isolated from silver mines.

The *Vitex* genus is members of Verbenaceae plant family growing upto 10 feet. *Vitex negundo* has lavender flowers is mainly native to europe and Asia and it is effectively used rhumatism and many ayurvedic formulations are available containing *Vitex negundo* for controlling diabetes. The purpose of the study is to obtain naturally synthesized nanoparticles which would be eco-friendly and cost effective. Herein, we report synthesis of silver nanoparticles, reducing silver ions present in the aqueous solution of silver nitrate complex by the plant extract of *Vitex negundo* This plant are traditional folk medicine and it can provide a new platform for value added nanotechnology product in future. The dried fruit, which has a pepper, like aroma flavor and it is used in herbal medicine preparations. Vitex is used to control acne in teenagers, young women and men. It also helps in regulating the ovulatory cycle in women.

Materials and Methods

Preparation of aqueous extract

Fresh plant materials of *Vitex negundo* was collected from Tiruchengode, Namakkal District of Tamil Nadu and the aqueous extract of sample was prepared using the freshly collected leaves (5g), by washing in running tap water and then in double distilled water, followed by boiling with 100 ml of distilled water, at 60˚C for 5 minutes. Then the extract is filtered through No1 what man filter paper and used for further experiments.

Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was purchased from Merck India Ltd, and used for the study. The 50ml of fresh leaf extract was added into the aqueous solution of 1mM Silver nitrate. Due to our interest to get much smaller particles, above solution was centrifuged at a rate of 12000 rpm for 15 minutes and investigated that particles present in the supernatant.

UV-Vis spectra analysis

The bioreduction of Ag^+ in aqueous solution was monitored by measuring the UV-Visible spectrum of the reaction medium at different time interval and different nanometer (280-580). UV-Vis spectra were

recorded at 2min, 30min, 1hr and 24hrs. This UV-Vis spectral analysis has been done by using a Perkin-Elmer Lamda-25 spectrophotometer.

Characterization of silver nanoparticles
SEM Analysis

This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized using aqueous extract of *Vitex negundo*, and recovered the silver nanoparticles by ultra-centrifugation for characterization. The 100ml of fresh leaf extract was added into the aqueous solution of 1mM Silver nitrate. Due to our interest to get much smaller particles, above solution was centrifuged at a rate of 25000 rpm for 15 minutes and air dried under hot air oven (Shanker *et al* 2008). The dried silver nanoparticles were subjected to SEM analysis.

XRD Measurements

The air dried nanoparticles were coated on XRD grid and analyzed for the formation of Ag nanoparticles by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray generator operated at a voltage of 40kV and a current of 30mA with Cu Kα1 radiation. The diffracted intensities were recorded from 10˚ to 80˚ of 2θ angles.

FTIR Analysis

The dried silver nanoparticles were subjected to FTIR analysis. The chemical groups present in the nanoparticles have been studied using FTIR (Perkin Elmer, USA).

Antibacterial activity

Silver nanoparticles synthesized using aqueous leaf extract of *Vitex negundo*, was tested for its potential antimicrobial activity against few human pathogens. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion Techniques as described by (Agarry *et al*., 2005). Wells of 6 mm diameter were cut on sterile Muller Hinton agar plates and swabbed with an overnight broth culture of the organism. Each well was loaded with 40µl the solutions in the following order: water, solution of silver nanoparticles, silver nitrate solution and aqueous plant leaves extract of *Vitex negundo* containing nanoparticles and incubated at 37°C ± 0.2 C. Antimicrobial activity in terms of zones of inhibition (mm) was recorded after 24 h of incubation. The antagonistic action of plant leaf extracts of *Vitex negundo*, was tested against test organisms in triplicates.

Test organisms

Pure cultures of bacteria namely *Staphylococcus aureus, Escherichia coli, Pseudomonous sp, Proteus vulgaris,* and *Salmonell typhi* and they cause most of the hospital infections. The evaluation of agar diffusion test was made on the basis of zone of inhibition of bacteria around the test sample.

Result and Discussion

The formation of silver nanoparticles in the solution of 1mM silver nitrate and aqueous extract of the *Vitex negundo* plant sample was confirmed by change in the color to yellowish after incubation with silver nitrate, while the controls retained the original color. (Fig.1).

UV-Visible Spectral Analysis

The bioreduction of Ag^+ in the aqueous extract was monitored by periodic sampling

of the reaction mixture at regular intervals by using UV- Visible spectroscopy. The silver nanoparticles exhibits yellowish color in water and this arises due to excitation of surface plasmon vibrations in the metal nanoparticles (Mulvaney et al., 1996). The UV-Visible spectra recorded from the aqueous silver nitrate - *Vitex negundo* leaf broth. A strong characteristic absorbance peak at around 420 nm was observed at different time intervals (Fig.2). Scanning electron microscopic analyses of the silver nano particles reduced form of silver nitrate solution through bioreduction are clearly distinguishable owing to their size difference. It is clear from the SEM pictures that silver particles in the bio-reduced colloidal suspensions measured 40- 70 nm in size. The particles are spherical in shape, well defined, separated as much as possible (Fig. 3a and 3b). This may be due to the reduction in liquid solution and some chelating action also available in the solution. Due to this silver particle nucleation is higher than the particle agglomeration.

XRD Analysis

X-Ray Diffraction studies of the two samples are shown (Fig. 4). XRD analysis shows three distinct diffraction peaks at 28.3˚, 32.4˚, 38.7˚, 46.0˚ and can be indexed 2θ values of (220), (311), (111), (420) crystalline planes of cubic Ag. Sample have peak at 38.6^{o} (2θ) and the h k l value for this is Ag particle is (111). Corresponding JCPDS value are presented in Fig The average grain size of the silver nanoparticles formed in the bioreduction process is determined using Scherr's formula, d=(0.9λ*180˚)/β cosθπ and is estimated to be 60 nm shows the XRD pattern of the silver nanoparticles formed in our experiment (Fig.5).

FTIR

FTIR spectral analysis showed array of absorbance bands in 400 cm^{-1} -1500 cm^{-1}. Organic functional groups are available in the air dried silver nanoparticles. The spectral bands were prominent for *Vitex negundo* at 2358 cm^{-1} (Si-H silane), 1683 cm^{-1} (C=O), 1510 cm^{-1} (Nitro Compounds), 1458 (C=C, Aromatic Ring), 1238 cm^{-1} (C-O), 1066 cm^{-1} (Alcohols, ether, carboxylic acid, esters), 887 cm^{-1} (Alkenes). This organic group presence is due to Silver Particles reduction through biological sources. Fig. 5 shows the FTIR spectrum of the silver nanoparticles. Ultra pure Ag Particle can be prepared by removing the functional groups through chemical modification in future studies. (Fig 5).

Fig.1. Nanoparticles synthesized by *Vitex negundo* leaf extract.

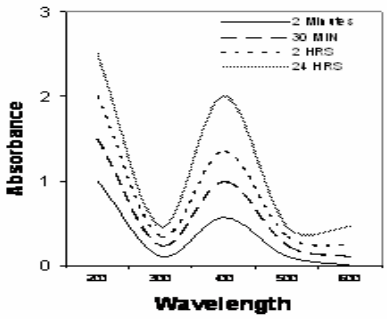


Fig.2. Absorbance of silver nanoparticles at different time interval and wavelength (280- 580 nm).

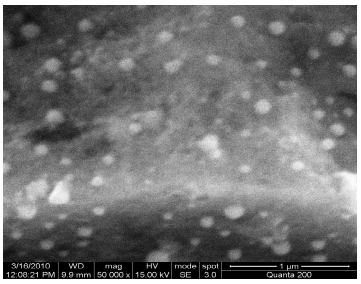


Fig.3a. SEM analysis shows that size and shape of the silver nanoparticles synthesized by *Vitex negundo* at 1µm.

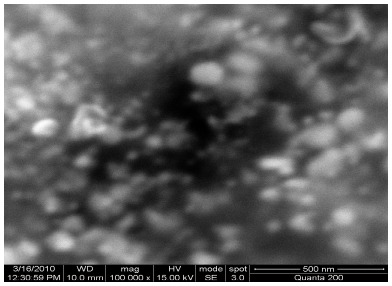


Fig. 3b. SEM analysis shows that size and shape of the silver nanoparticles synthesized by *Vitex negundo* at 500nm.

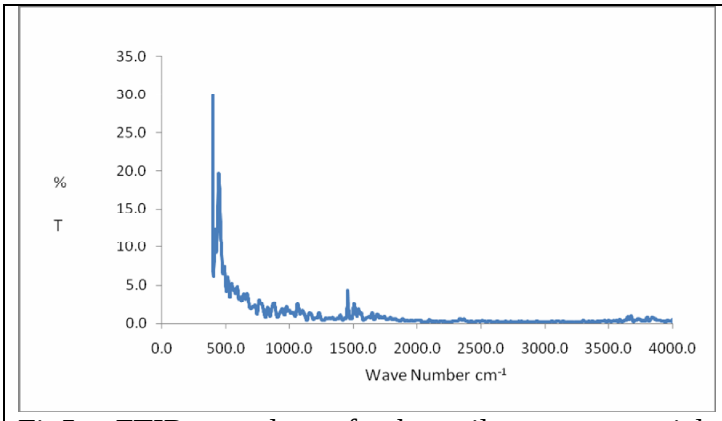


Fig5. FTIR results of the silver nanoparticles synthesized by *Vitex negundo* leaf extract

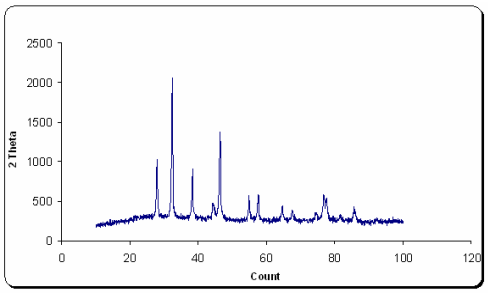
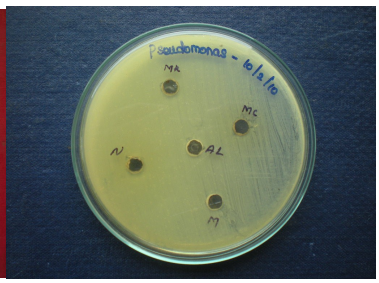


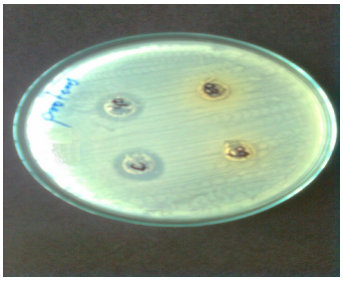
Fig4. XRD of the silver nanoparticles synthesized by *Vitex negundo* leaf extract

Escherichia coli

Staphylococcus aureus



Pseudomonas sp



Tabel.1 Antimicrobial activity by agar well diffusion method -Zone of inhibition range of silver nitrate and silver nanoparticles synthesized by *Vitex negundo* leaf extract

Compound	Silver nitrate (Control)	Silver nanoparticles
Staphylococcus aureus	5mm	8 mm
Escherichia coli	10 mm	17 mm
Proteus vulgaris	4 mm	6 mm
Pseudomonas sp	Nil	Nil
Salmonella typhi	3mm	9mm

Antimicrobial activity

The zone of inhibition in the well diffusion method showed that the silver nano-particles synthesized using aqueous leaf extract of *vitex negundo,* have the antibacterial activity against test pathogens namely *Escherichia coli, Staphylococcus aureus, Pseudomonous sp, Proteus vulgaris, and Salmonella typhi.* On comparison with the silver nitrate and plant extracts silver nanoparticles outperformed in the bactericidal effect (Fig 6).

Addition of the aqueous leaf extract of *Vitex negundo,* to 1Mm solution of silver nitrate led to the appearance of yellow color as resultant of formation of silver nanoparticles in the solution. Before reaction, the silver containing solution is colorless but changes to a yellowish brown color on completion of the reaction. The brown color of the medium could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Vigneswaran *et al.,* 2007). The UV-Vis absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 400-430nm. The rate formation is literally rapid, comparable to the chemical method of synthesis. The pale yellow color appears immediately after the addition of the aqueous plant extract, and the reaction is completed in about 2-24 hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles. The SEM studies confirmed the formation of silver particles in the size range of 40-100nm, a clear indication of the formation of silver nanoparticles. The characterization of silver nano particles by XRD studies revealed the three distinct diffraction peaks at 28.3˚, 32.4˚, 38.7˚, 46.0˚ and can be indexed 2θ values of (220), (311), (111), (420) crystalline planes of cubic Ag. Sample have peak at 38.6^{o} (2θ) and the h k l value for this is Ag particle is (111). The average grain size of the silver nanoparticles formed in the bioreduction process is determined using Scherr's formula, d=(0.9λ*180˚)/β cosθπ and is estimated to be 60 nm. 1238 cm^{-1} (C-O), 1066 cm^{-1} (Alcohols, ether, carboxylic acid, esters), 887 cm^{-1} (Alkenes). FTIR analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by bioreduction process. FTIR spectral analysis showed array of absorbance bands in 400 cm^{-1} -1500 cm^{-1}. Organic functional groups are available in the air dried silver nanoparticles. The spectral bands were prominent for *Vitex negundo* at 2358 cm^{-1} (Si-H silane), 1683 cm^{-1} (C=O), 1510 cm^{-1} (Nitro Compounds), 1458 (C=C, Aromatic Ring). This organic group presence is due to

Silver Particles reduction through biological sources. These data suggests that the stabilizing agents may be alkaloids, phenols, terpenes and polyols present in the aqueous leaf extract of *Vitex negundo.*

We have found that the silver nanoparticles synthesized in our study effectively inhibited the growth and multiplication of human pathogenic bacteria like *Staphylococcus aureus, Escherichia coli, , Proteus vulgaris, Serratia marscensus* and *Salmonella typhi* comparison with the silver nitrate and plant extracts silver nanoparticles out-performed in the bactericidal effect. The results of the investigation showed that silver nanoparticles synthesized from *Vitex negundo* leaf extract possess discrete antibacterial activity against clinically isolated pathogenic bacteria at a concentration of 20μg/ml. In this comparative study, *Vitex negundo* have higher antimicrobial activity.

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