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A NOVEL APPROACH TOWARDS GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *BIGNONIA VENUSTA* (L.) FOR THEIR BIOCOMPATIBILITY STUDIES.

MANOJ SHARMA*, SEEMA INDORA[1] AND R.D. AGRAWAL[1]

Department of Botany, University of Rajasthan, Jaipur -302004, India.

ABSTRACT

In recent years, the green synthesis of nanoparticles using various plant extracts has attracted great attention as these methods are simple, inexpensive and, eco-friendly. In the present investigation an attempt has been made for green synthesis of silver nanoparticles using plant extracts of *Bignonia venusta* (L.). In this study, it was observed that the plant extracts lead to the formation of crystalline silver nanoparticles. *Bignonia venusta* (L.) is known as a rich source of flavonoids, steroids, tannins and other phenolic compounds. The AgNPs prepared by green synthesis methods were monitored by measurements of FT-IR. Further morphology and size were determined by SEM and TEM. The size of AgNPs varied between 50 nm and 100 nm. XRD was done to study diffraction which denotes crystal structure of AgNPS. The AgNPs were tested for antimicrobial, antioxidants activity. Maximum antimicrobial activity was observed against *Streptomyces grisveus* at 40 µg/ml (20 mm) while in fungus no activity was observed.

KEYWORDS: ***Bignonia venusta (L.), silver nanoparticles, SEM , TEM, XRD, antimicrobial, antioxidants***

MANOJ SHARMA*

Department of Botany, University of Rajasthan, Jaipur -302004, India.

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INTRODUCTION

Nanobiotechnology is the integration of biotechnology, nanotechnology, chemical processing, physical methodology and systems engineering into biochips, nanocrystals and nanobiomaterials. In the last decade, biosynthesis of nanoparticles has introduced as a new emerging concept of the nano-biotechnology and is receiving great interest due to a growing need to develop environmentally benign technologies in material synthesis[1]. Chemical & physical methods are very successful to produce well defined nanoparticles, but they have certain limitations such as, increase cost of production, release of hazardous byproducts, long time for synthesis and difficulty in purification[2]. These disadvantages paved way for the development of nanoparticles using novel and well refined methods. This led to the exploration for new benign green routes for synthesizing high-yield, low cost, non-toxic and environment friendly nanoparticles. Due to the presence of various phyto constituents in various medicinal plants, they are used for green synthesis of eco-friendly nanoparticles. The Green (using extracts of *Bignonia venusta* leaves) synthesis of nanoparticles is a challenging concept which is very well known as green synthesis, has gained growing attention of researchers because of its rapid, economical, eco-friendly, nontoxic, large scale production, act as reducing and capping agent and it provides a single step process for the nanoparticles synthesis[1]. From the various studies it can be concluded that efficient nanoparticle synthesis depends on the reducing and capping capacity of the presence of various biomolecules such as polyphenols and other heterocyclic compounds[3]. *Bignonia venusta* (L) commonly called as 'Flame vine' belongs to family Bignoniaceae, *B. venusta* (L.) is most commonly found among *Bignonia binata* ,*Bignonia bracteomana* etc. The plant is native to northern India. *B. venusta* (L.) and has been commonly used in the traditional Brazilian medicine as a general tonic , treating skin infections (leukoderma, vitiligo), as well as a treatment for diarrhea, cough and common respiratory diseases related to infections, such as bronchitis, flu and cold. *B. venusta* (L.) leaves and stems are used in folk medicine as a tonic or antidiarrheal agent, while its flowers are used in treatment of patches on body, such as leucoderma and itiligo. Besides it is also used in cough and common diseases of respiratory system related to infections, such as bronchitis, flu, and cold[4, 5, 6].

MATERIALS AND METHODS

Synthesis of Nanoparticles

Silver nitrate ($AgNO_3$) was obtained from Merck Company for this study. All glassware were washed in dilute HNO3 acid and rinsed with distilled water and dried in a hot air oven before use. Fresh leaves of *Bignonia venusta* (L.) (˜10 g) were collected & washed several times with distilled water and subsequently macerated in 100 ml of distill water. 10 ml of supernatant was added in $AgNO_3$solution prepared by adding 0.01gm salt of $AgNO_3$ and kept on magnetic stirrer for 24 hrs to observe the change in colour which confirms the formation of nanoparticles. The change in colour from light to dark colour indicates reduction of Ag+ to Ag .

Characterization of Silver nanoparticles

The crystalline nature of Silver nanoparticles was investigated by XRD analysis. X–ray diffraction data of AgNPs were obtained using a Philips– X'Pert Pro MPD with Agkλ radiation (k = 1.54 A°) in the 2Θ range of 20^0 to 80^0 and with a steps size of 0.02^0 at 40 kV and 30 mA. The size of the Silver nanoparticles was examined by TEM. Furthermore, SEM study was carried out to investigate the shape, size and the surface area of the AgNPs.

Antimicrobial Activity

Antimicrobial activity of silver nanoparticles was studied by well diffusion with four bacterial and fungal strains.

Microorganisms Used

Clinical laboratory bacterial isolates of *Staphylococcus aureus* (MTCC-3381), *Escherichia coli* (MTCC-443), *Bacillus subtilis* (MTCC-10619), and *Streptomyces grisveus* (MTCC-4734) and fungal isolates viz. *Aspergillus niger* (ATCC-9029) *Fusarium oxysporium* (MTCC-62506), *Penicillium funiculosum* (ATCC-11797) and *Trichoderma ressei* (ATCC-13631) were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India.

Determination of Antibacterial Assay

In vitro antibacterial activity of synthesized of the samples were studied against gram positive and gram negative bacterial strains by the agar well diffusion method[7]. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The nanoparticles were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 1 mg/mL. The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the ciprofloxacin and ketokenazole commercial control antibiotics and streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the synthesized nanoparticles was investigated by agar well diffusion method[8]. The fungal strains poured onto Potato dextrose agar, PDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile Phosphate buffer saline (PBS) and adjusted to a concentration of 106 cells/ml. Dipping

a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C for 48 h and the bio activity as determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

Antioxidant activity

FRAP Assay (Ferric reducing ability of Plasma)

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. The method was performed using established protocol[9].

Reagents

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x H_2O and 16 mL conc. Acetic acid per 1 mL of buffer solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$x 6 H_2O in distilled water

FRAP working solution: 25 mL acetate buffer (1), 2.5mL TPTZ solution and 2.5mL $FeCl_3$ x 6 H_2O solutions. The working solution must be always freshly prepared. Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100-1000µ mol/L).

Assay : Blank: FRAP reagent

Sample : FRAP reagent -1.5mL, plant extract- 50mL

Procedure

Nanoparticles at 1mg/ml was prepared and 9 mL cool 0.1M phosphate buffer was added. (pH 7.6, containing 0.1mM EDTA). This mixture was filtered through a filter paper and centrifuged at 15,000 rpm for 10 min. The supernatant was used for the measurements. The volume was make up to 5mL and O.D. was taken at 593nm.

Calculation

The relative activities of samples were assessed by comparing their activities standard curve of ferrous sulphate.

Peroxidase Assay (POXA)

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20°C.

Pyrogallol+ H_2O_2 $\xrightarrow{\text{Peroxidase}}$ Purpurogallin

Reagents and Test sample solutions

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (125µmol, pH- 6.8)
- Pyrogallol (50µmol)
- H_2O_2 (30%)

Procedure

Plant samples (200mg) was homogenized with 10mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 minutes. The clear supernatant was taken as the enzyme extract. The activity was assayed after the established method as described[10] with the following modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H_2O_2 was added. The amount of purpurogallin formed was determined by taking the absorbency at 420nm immediately after adding 0.1mL enzyme extract.

Catalase (CAT) assay

2H2O2 $\xrightarrow{\text{CAT}}$ 2H2O + O2

Reagents and Test sample solutions

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (50M, pH- 7)
- H2O2 (24 mM)
- Na2EDTA (0.1mM)
- PVP (1%)

Procedure

The clear supernatant solution of nanoparticles was taken as the enzyme extract. The activity was assayed using established protocoal[11] with the following modifications. 2.0mL of phosphate buffer, 0.8mL of H2O2 was added and finally 0.2 mL enzyme extracts then immediately absorbance taken at 240nm.

RESULTS AND DISCUSSION

The *Bignonia venusta* (L.) plant extract was employed for the green synthesis of AgNPs. After the addition of the plant leaf extract to the silver solution, it was observed that the color of the reaction mixture was gradually changed from light yellow to dark brown, indicating the formation of silver nanoparticles.

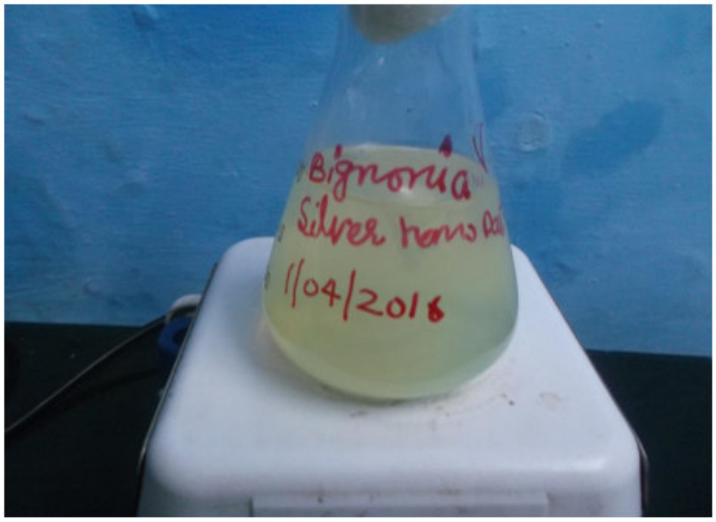

Figure 1
Bignonia venusta (L.) $AgNO_3$Day 1st

Figure 2
Bignoniavenusta (L.) $AgNO_3$Day 2nd

Characterization of synthesized nanoparticles
XRD analysis

The X-ray diffraction analyses were done to determine the phase pattern of the silver nanoparticles. XRD pattern of the dried synthesized AgNPs by *Bignonia venusta* (L.) extract has been reported (Fig. 3). The diffraction peaks data obtained were in accordance with the reports of FCC structure from Joint Committee Powder Diffraction Standards (JCPDS) file No. 04–0783. The mean grain crystalline size of green synthesized AgNPs was calculated by employing Debye–Scherrer formula. It was observed that 2Θ were in range of 28.4 to 66.5 ^{0}C.

$$D = K\lambda / \beta \cos \Theta$$

where D is the average crystalline diameter size (A°), K is a constant (0.9), 'λ' is the X-ray wavelength used (k = 1.54 A °), 'β' is the angular line width at the half maximum of diffraction (radians) and 'Θ' is the braggs angle (degrees)[12]. A small number of unassigned peaks (marked with stars) were also recorded that might be due to the crystallization of bioorganic phases present in *Bignonia* extract on the surface of the silver nanoparticles. Similar results were also obtained for other nanoparticles synthesized by the beetroot extract and *Ixora coccinea* leaves extract[13].

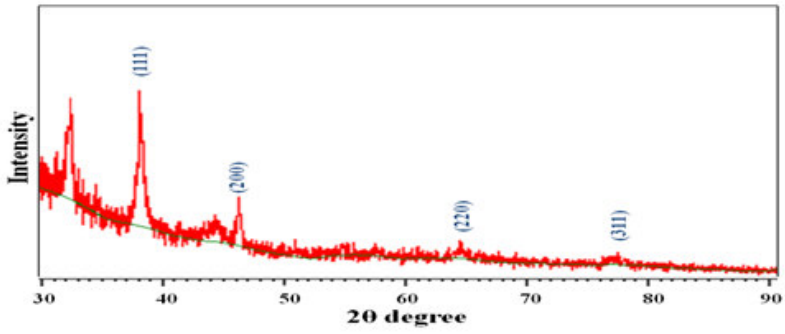

Figure 3
Bignonia venusta (L.) X-ray diffraction analyses.

SEM and TEM analysis

SEM technique was employed to determine the surface morphology and the topography of synthesized silver nanoparticles by both the methods. The size of silver nanoparticles from 50 to 100 nm, with an average size 85 nm. SEM image exhibited that the biosynthesized silver nanoparticles were mostly spherical in shape(Fig. 4). It was observed that when nanoparticles were prepared by heating method aggregation was higher as compared to particles which were prepared without heating. The morphology of reduced AgNPs were confirmed by TEM. The data revealed that (Fig.5) that the most AgNPs were found to be spherical and well dispersed, bearing size of 100 nm. The data was confirmed proper reduction and synthesis of nanoparticles

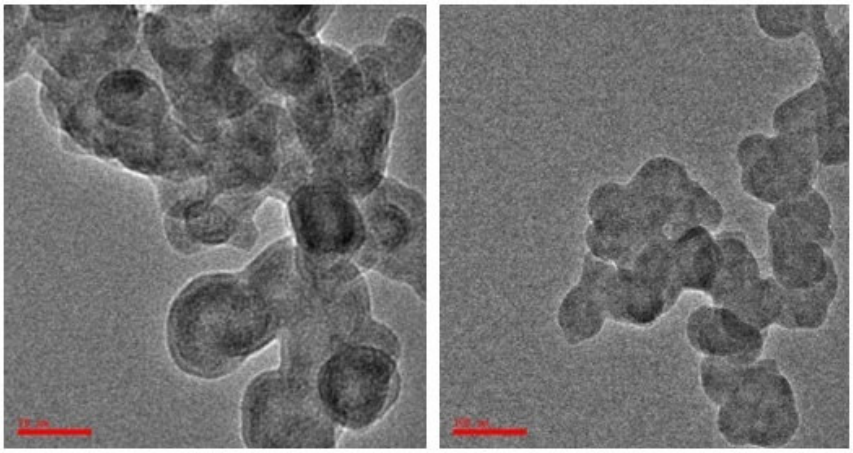

Figure 4

TEM images of Silver nano particles of Bignonia venusta (L.)

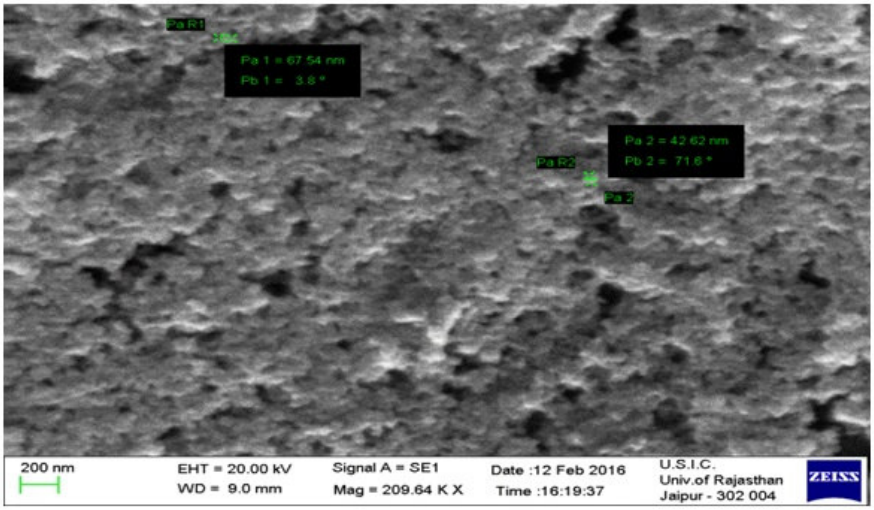

Figure 5

SEM images of Silver nano particles of Bignonia venusta (L.)

Antimicrobial activity

The antimicrobial activity was done using agar well diffusion method against clinically important microbes. It was observed that when nanoparticles were synthesized. Maximum activity was observed in *Staphylococcus aureus* and *Streptomyces grisveus* at concentration of 60 µg/ml (having inhibition zone of 10mm each) while minimum activity was in *Staphylococcus aureus* at 80 µg/ml. Both *E. coli* and *B. subtilis* were found to be resistant at all concentrations. When nanoparticles prepared by green synthesis method were applied, it was observed that maximum activity was observed in *Streptomyces grisveus* at 40 µg/ml (inhibition zone of 20 mm) which was at par with 80 µg/ml while minimum was in *Streptomyces grisveus* at 60 µg/ml (7mm). Against *E. coli* it was found to be 20 mm (at 20 µg/ml) and *B. subtilis* was found to resistant along with *S. aureus*. Activity was observed at 8 µg/ml (inhibition zone of 8 mm). In case of fungal strains it was observed that fungal strains were resistant (data not represented) AgNPs might have been attached to the surface of the cell membrane of microorganisms, leading to the disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. In general, small nanoparticles have a larger surface area for interaction with bacteria, as compared to that of bigger particles, due to greater antibacterial activity [14].(Table 1)

Table 1
Antibacterial activity of green synthesized Silver nanoparticles.

S.No.	Bacterial strains			
	Bacillus subtilis	***E. Coli***	***Staphylococcus aureus***	***Streptomyces grisveus***
20µg/ml	NIL	16 AI-0.8	NIL	IZ-8 AI-0.4
40µg/ml	NIL	NIL	8 AI-0.4	IZ-20 AI-1.0
60µg/ml	NIL	NIL	IZ-8 AI-0.4	IZ-7 AI-0.35
80µg/ml	NIL	NIL	NIL	IZ-8 AI-0.4
40µg/ml	20	20	20	20

Zone size is measured in mm, IZ- Inhibition zone, AI = zone of sample / zone of standard
Standard solution-Ciprofloxacin

Nanoparticles synthesized from plants produce a diverse array of secondary metabolites; many of them have antimicrobial reactions. However, some of these compounds are constitutively present in healthy plants in their biologically active forms [15,16] while some other compounds such as cyanogenic glycoside and glucosinolates, occur as inactive precursor and are activated in response to tissue damage or pathogen attack. This activation often involves plant enzyme, which are released as a result of breakdown in the cell integrity[17,18.] The literature indicates that *B. venusta* extract shows presence of biologically active compounds *viz*. anthraquinones, sennosides flavonoids and other natural phenolic compounds that possess antimicrobial properties [19.]

Table 2
Antioxidant activity of Silver Nano particles prepared by Bignonia venusta(L.)

S.No.	Activity	O.D.
1.	Catalase (CAT) Assay	116.24mM/L/g
2.	FRAP	73.02mM/L/g
3.	Peroxidase activity	1.65mM/L/g

Free radicals are generated in our cells when exposed to a variety of substances such as radiation, chemicals, pollution, smoke, drugs, alcohol, pesticides, sun rays and through various metabolic processes. A poor diet also aids in the formation of free radicals. Free radicals such as highly Reactive Oxygen Species (ROS) include hydrogen peroxide (H_2O_2), super oxide anion radical (O_2^-), hydroxyl radical ($OH^\cdot$), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and peroxyl radicals ($ROO^\cdot$). It causes damage of cellular DNA, especially in susceptible organs such as the eyes, lungs and the central nervous system. These damages ultimately cascade into a variety of different health problems and diseases i.e. coronary heart disease (CHD), cardiovascular diseases and even some forms of cancer, while in some cases it also accelerates the ageing. In the present investigation nanoparticles possessed significant antioxidant potential (Table 2) These oxidative damages could be retarded by endogenous defense systems such as catalase, superoxide dismutase and the glutathione peroxidase system. Further, to enhance our body's antioxidant defense, it is necessary to eat food that are rich in antioxidants. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Moreover, the plant sourced antioxidants i.e. vitamin C (ascorbic acid), vitamin E (tocopherol), vitamine A (β-carotenes), phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk.

CONCLUSION

In the present study, we reported the green synthesis of stable and nearly spherical silver nanoparticles using the plant extract of *B.venusta* leaves as a strong reducing and capping agent. The synthesized nanoparticles were found to possess strong antimicrobial activity against Gram-positive (*Staphlycoccus aureus*) and Gram-negative (*Streptomyces grisveus*) bacteria. We confirmed the formation of silver nanoparticles from XRD, SEM, TEM and FTIR. The synthesized AgNPs were found to have a crystalline structure as investigated by XRD method. The particle size of the Silver nanoparticles ranged in size from 50 to 100 nm, with an average size of 85 nm. Further the synthesized nanoparticles also showed potent antioxidant and antimicrobial activitiy. Thus green synthesis of Silver nanoparticles using plant material was found to be the most eco– friendly and conventional method, in comparison to chemical and physical synthesis method.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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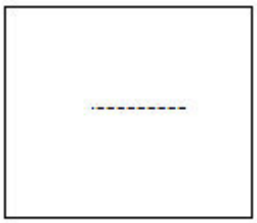

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Satya vihar, Jaipur, Rajesthan.

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