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Green Synthesis of Silver Nanoparticles Using Aqueous Extract of *Vanda Tessellate* Leaves and its Anti-oxidant and Antibacterial Activity.

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ABSTRACT

Vanda tessellata is an orchid that has been used in the traditional medicine for various ailments since ages. The aqueous extract of *V. tessellata* leaves was used as a reducing agent for the synthesis of nanostructure silver particles (AgNPs). Nanoparticles were characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and UV–Vis spectroscopy. The antibacterial activity of the synthesized silver nanoparticles was tested against pathogenic bacteria such as *Salmonella sps*, *Staphylococcus aureus* and *Escherichia coli*.The AgNPs synthesized were 15nm with size range 10 to 50nm with cubic and hexagonal shape. Antioxidant activity of AgNPs was studied by DPPH method. AgNPs showed enhanced antioxidant and antibacterial activity. Green synthesis of AgNPs with effective antioxidant and antibacterial activity may help in the development of cost effective therapeutic agent/s.

Keywords: *Vanda tessellata*, Eco- friendly, Antimicrobial, Antioxidant activity, Nanoparticles.

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INTRODUCTION

Silver nanoparticles (AgNPs) are gaining the attention of researchers in recent years due to their applications in drug delivery, diagnostics, imaging, sensing, catalysis, biomaterial production, artificial implants and tissue engineering. The size, shape and morphology of AgNPs influence their properties. AgNPs can be synthesized by chemical physical or biological methods. Green synthesis of AgNPs is an ecofriendly process and has many advantages over physical chemical methods and it is devoid of hazardous chemicals, high energy, high temperature and pressure [1-4]. Green synthesis of AgNPs is achieved using microorganisms [5-6] and plant extracts [7-11]. Synthesis of AgNPs from plant extract has additional advantages over microbial synthesis as culturing and maintaining the cells are not required [4].

Vanda tessellata Roxb. (Family: Orchidaceae) is a medicinal epiphytic perennial orchid found in the Indian subcontinent and Indochina. Indigenous medical systems such as *Ayurveda* and local traditional medical practices have used this plant for treatment of various ailments [12]. The leaves are used for the treatment of certain inflammatory conditions, bring down the fever and also as a remedy for Otitis [13]. The roots of *V. tessellata* were used to treat rheumatism, nervous problems, bronchitis and dyspepsia [14]. Unani practitioners use this plant as a laxative and tonic to the liver. The different parts of this plant are also used in hiccough, piles, and boils on the scalp, secondary syphilis and scorpion- sting. *V. tessellata* has been reported to contain various secondary metabolites such as alkaloids, flavonoids glycoside, tannins, β-sitosterol, γ-sitosterol and a long chain aliphatic compound, fatty oils, resins and colouring matters. Tetracosyl ferrulate and β-sitosterol-D-glucoside are found in roots [15]. *V. tessellata* roots were reported to possess antibacterial, antitubercular properties, anti-inflammatory activity and aphrodisiac activity [16-19].

Even though *V. tessellata* has been subjected for many medicinal properties, there is no report on the synthesis of nanoparticles from this plant. In the present work, we have synthesized AgNPs from *V. tessellata* leaves extracts. These particles were subjected to antibacterial and antioxidant activities.

MATERIALS AND METHODS

Healthy leaves of *V. tessellata* were collected from the Shimoga district, Karnataka, India and was identified and authenticated by Botany Department Kuvempu University, Shimoga. Silver nitrate used for the synthesis of silver nanoparticles was procured from E. Merck, Germany. Dehydrated Luria broth and Nutrient agar media used for bacterial growth study were the products of Hi Media, India. Cultures of *Escherichia coli*, *Salmonella* and *Staphylococcus* species were collected from the Department of Microbiology, Mangalore University.

Preparation of the Extract

Fresh leaves of *V. tessellata* were thoroughly washed with running tap water followed by distilled water. The leaf sample (25g) was grounded with 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, *V. tessallata* leaf extract and20 mM stock solution of silver nitrate ($AgNO_3$) were taken. For the reduction of Ag^+ ions, 10 ml of leaf extract was added drop wise to 10 ml of 1mM aqueous $AgNO_3$ solution. A distinct color change was observed after 24 hrs as the solution turned into dark yellow from normal colorless solution at room temperature suggesting formation of silver nanoparticles. The color became darker and turned to dark brown after 48 hrs. The reduction of Ag^+ was confirmed by UV–Vis spectrum of the solution. The nanoparticles were separated out from the mixture by centrifugation (10,000 rpm for 15min).The nanoparticles pelleted at the bottom of the centrifuge tube were dispersed in a 10mL of deionized water after carefully removing the liquid from the centrifuge tube.

UV-Vis Spectra analysis

The reduction of pure Ag+ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

XRD measurement

The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag^+ nanoparticles by an x –ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu kα radiation in a θ- 2 θ configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula. D= 0.94 λ/ β Cos θ →(1) where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample. B corrected = (FWHM2sample- FWHM2si)1/2 → (2), This modified formula is valid only when the crystallite size is smaller than 100 nm [20].

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

FTIR analysis

To remove any free biomass residue that is not the capping ligand of the nanoparticles, the residual solution of 100ml centrifuged at 5000 rpm for 10 min and the resulting residue suspension was redispersed in 10ml sterile distilled water. The centrifuging process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally the dried nanoparticles were analyzed by FTIR.

Antioxidant activity

In vitro antioxidant activity of the silver nanoparticles was measured by DPPH radical scavenging method based on the procedure of Brand- Williams *et al* [21] with modifications. 1ml of the extract (in methanol) was added to 0.5ml of 0.15mM DPPH solution (in methanol). The contents were mixed vigorously and incubated at 20^0c for 30min. The absorbance was read at 517nm. Butylated hydroxytoluene (BHT) was used as standard antioxidant agent.

The percentage anti-oxidant activity (AA%) or scavenging activity was calculated as follows

AA% = (Absorbance of Control - Abasorbance of Sample) / Absorbance of Control x 100

Antibacterial activity

Antibacterial activity was assayed by using a standard well diffusion method against pathogenic bacteria like *Escherichia coli, salmonella and staphylococcus* species. Nutrient agar was used for cultivation of the bacteria. 100µl of cultures of the bacteria were spread on Petri plates containing nutrient agar. Wells were punched in the nutrient agar with the aid of sterile borer. 50µl of the plant extract, solution containing nanoparticles and standerd antibiotic drug (ampicillin) was inoculated in the wells and the plates were incubated at 37^0c overnight. The zone of inhibition was measured after 24hrs.

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RESULTS AND DISCUSSION

Visual inspection

The color of the reaction mixture was turned to yellowish brown and then to dark brown after mixing silver nitrate (1mM) to aqueous extract of *V. tessallata* leaves (Figure 1). The color change in the mixture confirmed the formation of AgNPs. Our observation is in agreement with the other studies reported earlier [10]. Surface plasmon vibrations in silver nanoparticles are believed to be responsible for the dark brown color.

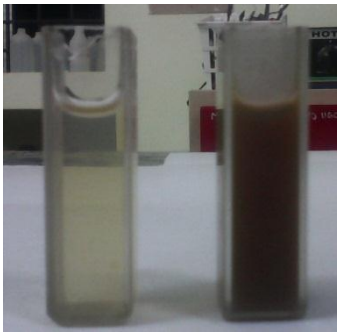

Figure 1 Color of reaction mixture before and after formation of AgNPs from aqueous extract of *V. tessallata* leaves.

UV-Vis Spectral analysis

It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions [22]. Figure 2 shows the UV-Vis Absorption spectra of silver nanoparticles formed in the reaction media. Absorbance peak at 420 nm confirmed the formation of silver nanoparticles form the plant extract. Broadening of peak indicated that the particles are polydispersed. The monodespersity of AgNPs in solution can be achieved by optimizing various factors such as substrate concentration, electron donor, reaction or incubation time, pH, temperature, buffer strength, mixing speed, and light [10].

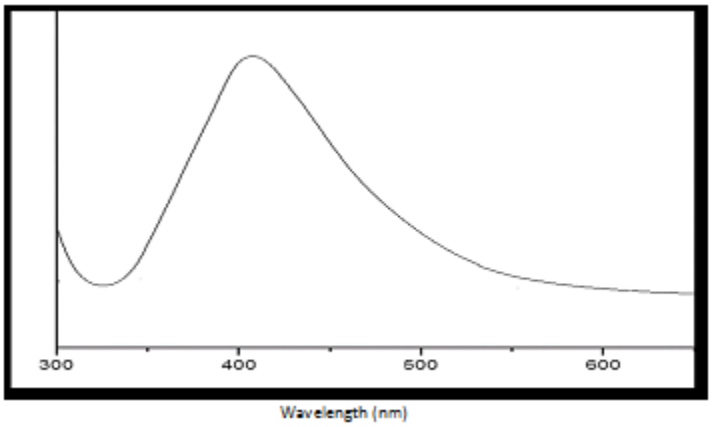

Figure 2 UV-Vis absorption spectrum of AgNPs synthesized by *V. tessellata* leaves extract.

XRD Measurement and SEM analysis of AgNPs

The green synthesis of silver nanostructure by employing *V.tessellata* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Figure 3) and the structural view under the scanning electron microscope. The XRD pattern showed three intense peaks in the whole spectrum of 2θvalue ranging from 10 to 80. Average size of the particles synthesized was 15nm with size range 10 to 50nm with cubic and hexagonal shape (Figure 4). The typical XRD pattern (Figure 3) shows that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 15 nm derived from the FWHM of peak corresponding to 111 plane.

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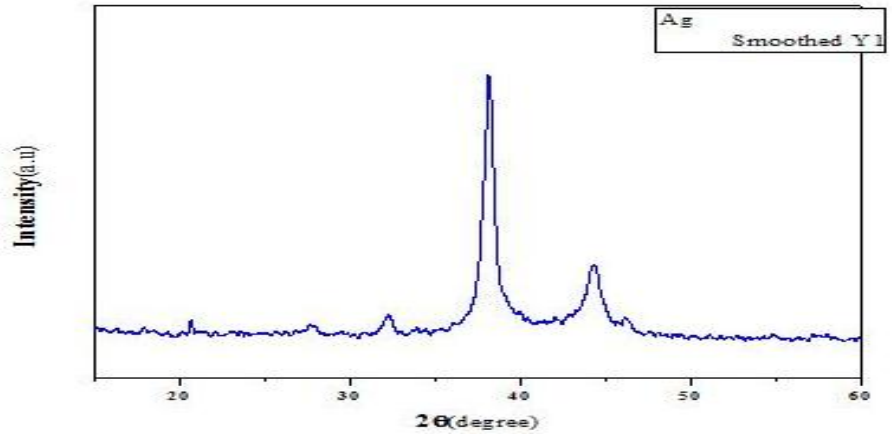

Figure 3: XRD pattern of AgNPs synthesized from *V.tessellata* leaf extract.

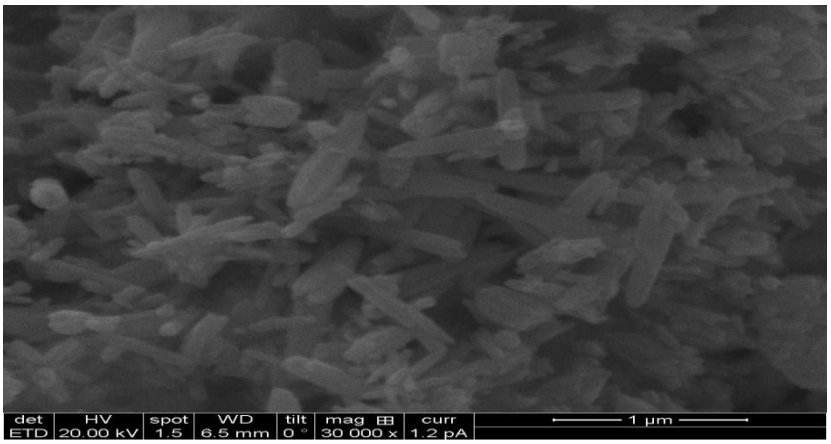

Figure 4: SEM image of AgNPs synthesized from *V.tessellata* leaf extract.

FTIR analysis of AgNPs

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles FTIR absorption spectra of water soluble extract before and after reduction of Ag ions are shown in Figure 5. Absorbance bands are observed in the region of 3500–500 cm-1 are 3018, 1750, 1514, 1355, 1226,1023 cm-1. These absorbance bands are known to be associated with the stretching vibrations for –C-H (alkane H), C=O stretch in esters, –C C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic],and C–O (polyols), respectively [4]. In particular, the 1226 cm-1band arises most probably from the C–O group of polyols such as hydroxyflavones and catechins.

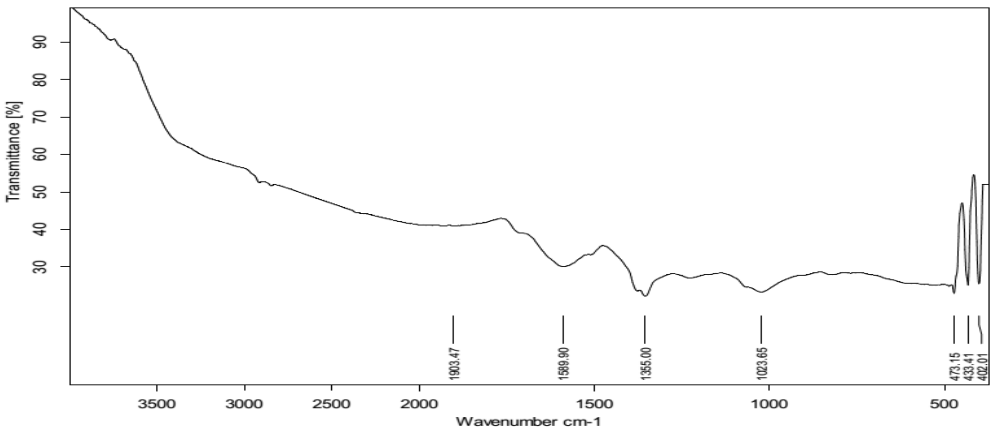

Figure 5: FT-IR spectrum of AgNPs synthesized from *V.tessellata* leaf extract.

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Antioxidant and antibacterial activity of AgNPs

The green synthesized nanoparticles from *V.tessellata* leaf extract was further subjected to antioxidant and antibacterial activity. The antioxidant activity was measured by DPPH scavenging method. The AA% in AgNPs (Figure 6) was higher than the plant extract alone and the activity was comparable to the standard antioxidant (BHT). AgNPs , BHT and plant extract showed 85.9, 94.3 and 73.8 AA% respectively at 100 µg. These results are in agreement with the antioxidant activity reported in various ornamental plants [23]. The antioxidant potential of AgNPs synthesized from *V.tessellata* leaf extract can be exploited in treating various disorders arising from oxidative stress.

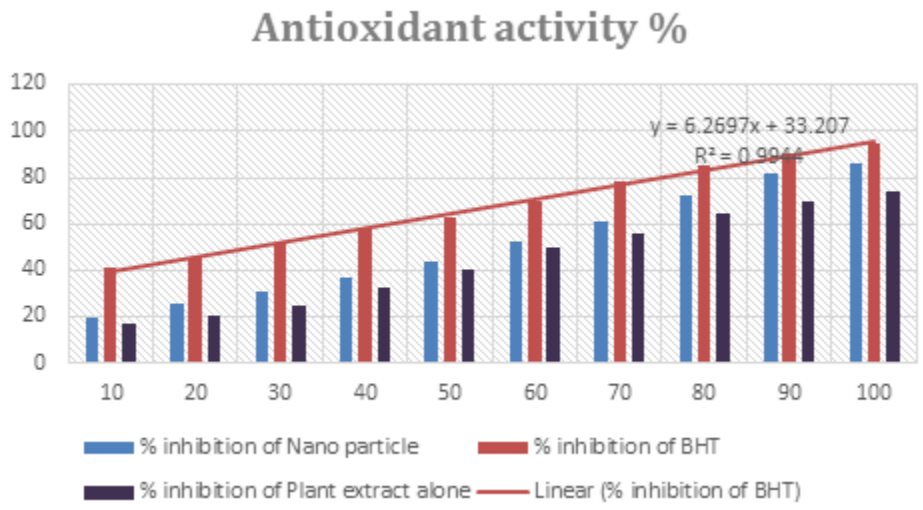

Figure 6: Antioxidant activities % (AA%) of AgNPs synthesized from *V.tessellata* leaf extract.

The AgNPs synthesized from *V.tessellata* leaf extract were also assessed for their bactericidal property. AgNPs were tested against *E.coli*, *salmonella sp* and *S. aureus* (Table 1) .The AgNPs synthesized using plant extract have been reported be highly toxic against gram positive as well as gram negative bacteria. Logaranjan et al [11] have shown an enhanced antibacterial activity in AgNPs synthesized using Ficus caricus fruit extract. Similar results obtained even our study. The antibacterial potential of AgNPs synthesized from *V.tessellata* leaf extract was comparable to the standard drug, ampicillin. Our study has clearly established the antibacterial activity of AgNPs synthesized from *V.tessellata* leaf extract.

Table 1 Antibacterial activity of AgNPs synthesized from *V.tessellata* leaf extract.

Test organism	Zone of inhibition in (mm)		
	Plant extract	**AgNPs**	**Ampicillin**
E.coli	5.0	9.0	14.0
Salmonella sp	6.0	10.0	13.5
S.aureus	5.8	11.2	15.0

CONCLUSIONS

We have developed a fast and convenient method for the synthesis of silver nanoparticles using *V. tessellatta* leaf extract which was confirmed by UV-Vis, XRD, SEM and FTIR spectroscopy. The AgNPs synthesized were 15nm with size range 10 to 50nm with cubic and hexagonal shape. The nanopartcles showed enhanced antioxidant activity. The antibacterial activity of biologically synthesized nanoparticles was evaluated against *E. coli*, *salmonella* species and *S. aureus* showing effective bactericidal activity. The antioxint and antibacterial activity of silver nanoparticles synthesized via green route provide a great potential in biomedical applications. The present study showed a simple, rapid and economical route to synthesize therapeutic silver nanoparticles.

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