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Bio-Fabrication, Characterization of Silver Nanoparticles and their Evaluation of Catalytic, Antioxidant and Antimicrobial Efficacy

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Abstract: *The present investigation was aimed to synthesis of silver nanoparticles (AgNPs) using Dodonaea viscosa (D.viscosa) leaf extract by an eco-friendly method. The leaf extract worked as bio- reductant and stabilizer. The biosynthesized AgNPs was characterized by Ultraviolet visible (UV-vis) spectrophotometer, the leaf extract worked as reductant and stabilizer. The surface plasmon resonance (SPR) peak was observed at 440nm. Fourier Transmission Infrared Spectroscopy (FTIR) exhibited the various functional groups which, were used for capping of nanoparticles (NPs). Field Emission Scanning Electron Microscope (FESEM) and Transmission electron microscopy (TEM) analysis measured the size of the AgNPs ranged from 22.9 to 41.5nm. The X-ray Diffraction (XRD) pattern conformed the face centered cubic (fcc) crystalline structure of AgNPs. The bio-reduction of the silver ions in solution was monitored by Energy dispersive X-ray spectroscopy (EDX). The synthesized AgNPs showed good catalytic activity by reducing the methlylene blue (MB) also expressed antioxidant and antimicrobial activity than plant leaf extract. So, the present study was assessed the usage of the plant derived AgNPs in the field of drug design.*

Keywords: *Antimicrobial activity, Catalysis, Green synthesis, Silver Nanoparticles, Surface Plasmon Resonance.*

I. Introduction

Nanotechnology is an emerging field to discover, describe and manipulate the unique properties of matter, especially metals at the nano-scale in order to develop new capabilities with applications across all fields of science, engineering and medicine [1]; [2]. AgNPs synthesized by chemical method is having some limitations like chemical precursors and generating toxic by-products [1]; [2]; [3]. So, there is need of AgNPs by eco-friendly cost-effect and less harmful to the society. Since, the method of green synthesis provides less expensive, non-toxic and eco-friendly [4]. *Dodonaea viscosa* Jacq. (Sapindaceae) is weed plant and also used to treat various disorders like fever, sore throat, cold, dressing for skin diseases, hemorrhoids [5]; [6]; [7]; [8]. Recent times, multi drug resistant pathogens are very big challenge to the field of medicine. Thus, the NPs may be employed due to their large surface area and high possibility of host interaction. Antioxidants are chemically unstable atoms or molecules that can cause extensive damage to cells as a result of imbalance between the generations of Reactive Oxygen Species (ROS). 2, 2-diphenyl-2- picrylhydrazyl (DPPH) shows their antioxidant activity is mainly due to their redox potential, which allow them to act as reducing agents and hydrogen donors [9]; [10]. The synthesized AgNPs induces the generation of free oxygen radicals. Catalytic activity was demonstrated by MB reduction assay. The present investigation was focused on synthesis, physical, chemical and biological characterization of synthesized AgNPs using *D.viscosa* leaf extract.

II. Materials And Methods

The fresh leaves of *Dodonaea viscosa* was collected from Sivapuram, Pudukkottai District, Tamil Nadu, India and confirmed with the Department of Botany, J.J. College of Arts and Science, Pudukkottai. The collected leaves were washed in running water and and shade-dried at room temperature for 10 days. The fully dried leaves were powdered with a sterile electric blender. The powdered samples were preserved in an airtight container and away from the sunlight for further use. Two grams of powdered leaf was mixed with 100ml of de-ionized water, heated to 100°C for 30min and filtered by Whatman No.1 filter paper followed by the residue was re-extracted using vacuum pumps. Qualitative phytochemical screening of *D. viscosa* leaf extract was performed using standard procedure [11]. Silver Nitrate ($AgNO_3$) stock solution was prepared by dissolving 6.299gm of $AgNO_3$ in 100ml of de-ionized water. *D.viscosa* leaf extract (2ml) was mixed with 20ml of $AgNO_3$ solution [12]. The absorption spectrum of the reaction mixture was recorded at room temperature using UV-vis spectrophotometer (Hitachi-U-2001) from 300-800nm at a resolution of 1nm for detection of AgNPs formation. FTIR studies, the dry powder of the NPs was by centrifuged at 10,000rpm for 15min. The residue containing AgNPs solution was dispersed in sterile de-ionized water twice to remove the biological impurities. The pure

residue was dried in oven at 70°C overnight. The dried, powdered NPs were subjected to FTIR analysis on a Perkin-Elmer spectrum on the instrumental resolution of $4cm^{-1}$ in the transmission mode of $4000\text{-}400cm^{-1}$ in KBR pellets. XRD measurement of AgNPs solution, a drop-coated on glass slide was done on XPERT-PRO, D-8, with 30kv, 40mA with Cu kα radians at 2θ angle. The particles size was calculated using the Debye Scherrer's formula,

$$D = K\lambda/\beta\cos\theta \quad \text{———} \quad (1)$$

Where, D is the particle size, K is constant (shape factor), λ is the wavelength of X-ray (0.1541nm), β is the Full Width Half Maximum (FWHM) and θ is the diffraction angle corresponding to the lattice plane. The morphology of AgNPs was analysed using Field Emission Scanning Electron Microscopy (Joel JSM-6701 FESEM). Thin films of the samples were prepared with aluminium foil by dropping a small amount of the sample onto the copper grid and the EDX analysis also done with same sample by FESEM (BRUKER-INDIA, FESEM) equipped with an EDAX attachment. The size distribution and the average size of the AgNPs was estimated on the basis of TEM micrographs. The samples were placed over a carbon tape and dried and pinch of dried sample was coated with a thin layer of platinum in an auto fine coater. TEM was performed using JEM 1011, JEOL, Japan. The antimicrobial activity was investigated against human pathogenic organisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, E.coli, Klebsiella pneumonia* and *Candida albicans* using the disk-diffusion method. The pure cultures were collected from the Raja Muthiah Medical College, Annamalai Nagar, Tamil Nadu and subcultures in Muller-Hinton Agar (MHA). The pathogens were swabbed uniformly onto culture medium using sterile cotton swabs. To evaluate the antibacterial activity of the AgNPs, 25, 50 and 100µl of AgNPs solutions were dropped over the disk. All the petri plates containing organism and AgNPs solutions were incubated at 37°C for 24hrs, the diameter of the zone of inhibition was measured in millimeter. If the NPs has any effect against pathogenic organisms in certain concentrations, then no organism will grow in around the area of disk. The diameter of the zone of inhibition indicates the efficacy of the AgNPs. Different concentrations of AgNPs (10, 20, 30, 40 and 50µl/ml) was prepared, from the each concentrations 3ml of AgNPs solution was mixed with 1ml of DPPH solution (prepared using methanol) and for the control ascorbic acid was used as standard antioxidant. The tubes were incubated in dark place for 30 min and absorbance was measured at 517nm using UV–visible spectrophotometer [9].

$$\% \text{ of inhibition} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}})}{\text{Absorbance}_{\text{control}}} \times 100 \quad \text{———} \quad (2)$$

The catalytic activity of AgNPs was performed by two reactions were evaluated in standard quartz cell using the MB reduction assay. In the first reaction, 1ml of MB (1×10^{-4}M) was mixed with 0.4 ml of leaf extract and 1.6 ml of distilled water. In the second reaction, 1ml of MB (1×10^{-4} M) was added to 0.4 ml leaf extract with 1.6 ml of synthesized AgNPs and the absorption spectra were recorded at three different intervals (30, 45 and 60min) by UV-vis spectrophotometer.

III. Results And Discussion

Phytochemical investigation revealed the alkaloids, saponins, phenols and flavonoids are present, while the glycosides, tannins and cardiac glycosides are absent. The reaction-mixture composed of $AgNO_3$ and *D. viscosa* leaf extract showed a color change from colorless to dark brown in colour which might be is due to excitation of the Surface Plasmon Resonance (SPR). The high intense peak was observed at 440nm (Fig.1) by UV-vis spectrophotometer, where the NPs formation was authenticated and similar result was obtained by Njagi et al. [13]; Basavegowda and Lee [14]. FTIR analysis was performed to identify the available bio-molecules responsible for capping and stabilizing the synthesized AgNPs. The synthesized AgNPs revealed strong bands (fig.2) at $3374cm^{-1}$, $2920cm^{-1}$, $2352cm^{-1}$ $1641cm^{-1}$, $1391cm^{-1}$ and $1036cm^{-1}$. The band at $3374cm^{-1}$ was corresponding with O-H band stretching. The peak at $2920cm^{-1}$ was corresponding with the vibration of C-H band of aliphatic groups, the results coincidence with Ashokkumar et al., [15] on *Gloriosa superba* leaf extract. The other bands 2352, 1641, 1391 and $1036cm^{-1}$ were corresponding with N-H stretching vibration of amines groups, C=C stretching of amide groups, –C-N- stretching vibration and C-O stretch vibration of alcohols, carboxylic acid, protein respectively. XRD pattern was indicated the particle structure and calculated the size of AgNPs. i.e peaks of AgNPs at 38.03º, 44.01º, 46.11º and 65.02º were corresponding to (111), (200), (100) and (220) plane values (Fig.3). The intense peak at 38.03° indicates a high degree of crystallinity. In overall XRD indicates the face centered cubic (fcc) crystalline structure and similar result has been reported by Basavegowda and Lee [14]; Shenny et al. [16]. The size of the AgNPs was calculated, it found to be ranged between 8.95 to 46.71nm and the average size was around 22.18nm. TEM image was declared that the particles are mostly spherical in shape and size was found varying from 22.9 to 41.5nm (fig.4). FESEM (Fig. 5.a) clearly exhibit the particles were spherical in shape, some were irregular and mostly aggregated, few particles were present

individually. This result of FESEM coincides with Njagi et al. [17]; Jeeva et al. [18]. The major element compositions of the AgNPs were studied using EDX, the sharp peaks indicate the reduction of $AgNO_3$ to AgNPs (Fig. 5.b). The antibacterial activity of biosynthesized AgNPs against human pathogens were measured by a zone of inhibition and compared with controls viz., $AgNO_3$ and commercial antibiotic disk (Fig.7.a-f) (Table.1). The biosynthesized AgNPs were found to have the highest antibacterial activity against *S.aureus* (15mm, Fig.7.a) and *B. Subtilis* (13mm, Fig. 7.b) in 100µl/ml while, the minimum antibacterial activity Fig.7 was recorded against *K. pneumonia* (12.6mm, Fig.7.d), *E.coli* (11.2mm, Fig.(7.e), *C. albicans* (10.10mm, Fig. 7(f)) and *P. aeruginosa* (10mm, Fig.7.c). Logeswari et al., [19] stated the NPs affect the respiratory chain, cell division of the organisms and finally lead to cell death. While, release silver ions into the microbial cells enhance the bio-cidal activity [20]; [21]. The antioxidant potential of biosynthesized AgNPs was measured by DPPH assay. Biosynthesized AgNPs shows stronger DPPH activity than the plant extract [22] (Fig.8). As shown in fig. 6, MB exhibits a main absorption peak (λmax) at 750nm. After 30 min, MB absorbance was gradually decreased and was shifted to higher wavelength. The decrease of absorbance is indication of the ability of Phytoextract to degrade the MB. The reduction of MB in the presence of AgNPs showed greater catalytic activity and their composites without any reduction agent for MB dye reduction. The reaction mixture containing plant extract, synthesized AgNPs and MB at the end of 30 min time interval showed a decrease in the absorbance of MB and increase of SPR peak of AgNPs, similar result agrees with Pal et al. [23].

IV. FIGURES AND TABLES

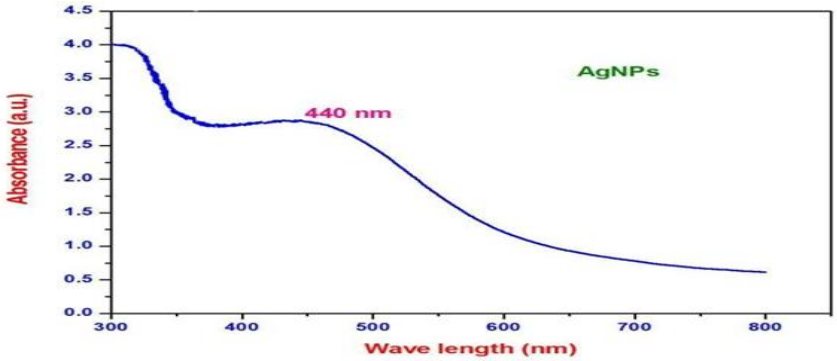

Figure.1 UV-Visible spectrum of biosynthesized AgNPs using *Dodonaea viscosa* leaf extract.

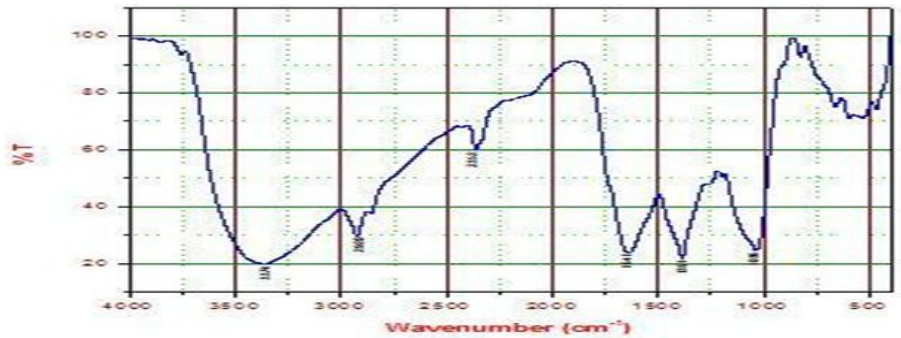

Figure.2 FTIR spectrum of biosynthesized AgNPs using *Dodonaea viscosa* leaf extract.

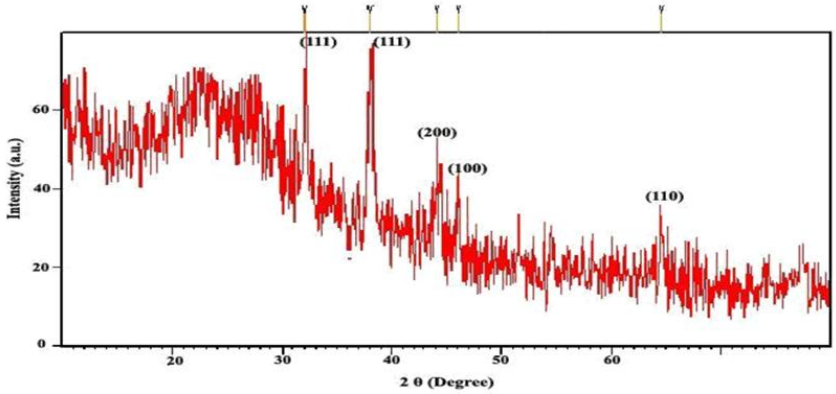

Figure.3 XRD pattern of biosynthesized AgNPs using *Dodonaea viscosa* leaf extract.

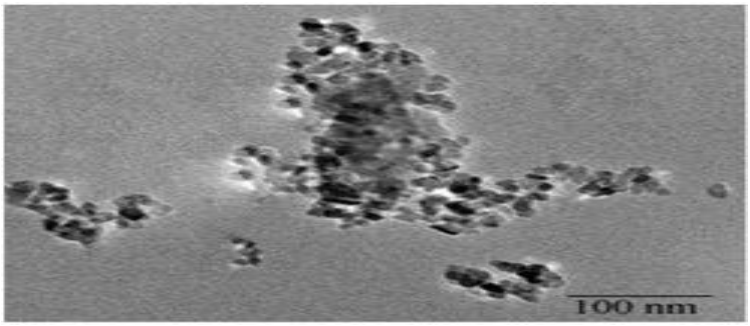

Figure.4 TEM image of biosynthesized AgNPs using *Dodonaea viscosa* leaf extract.

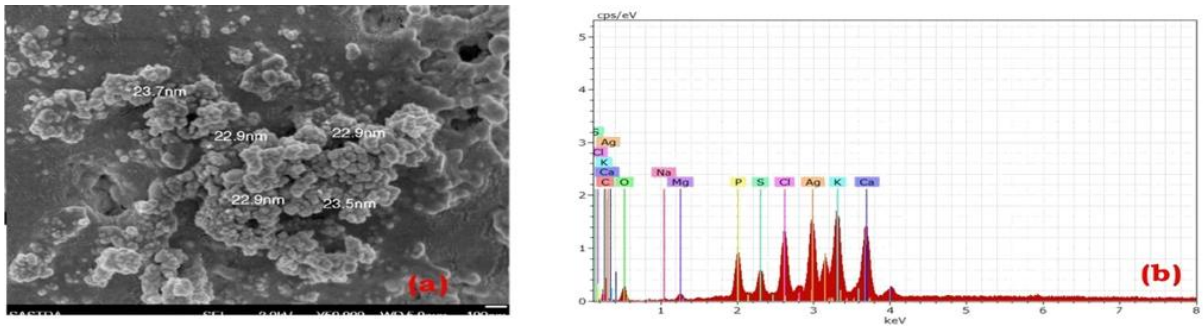

Figure.5. (a) Field emission SEM analysis and (b) EDX of silver nanoparticles synthesized using *Dodonaea viscosa* leaf extract

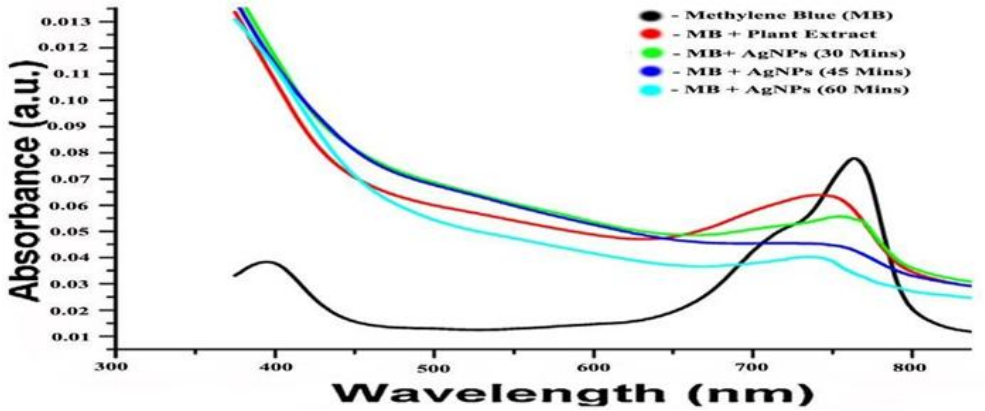

Fig. 6 UV–Vis spectra of methylene blue degradation test for catalytic assay

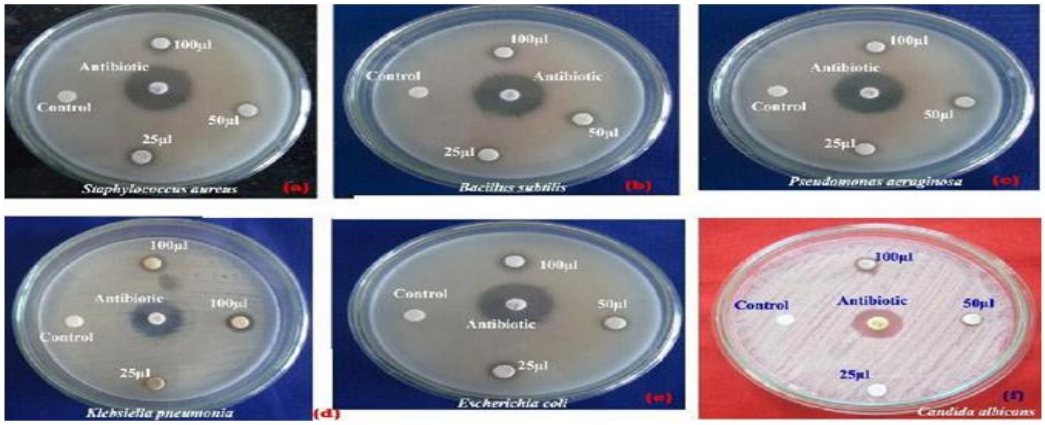

Figure.7 Antimicrobial activity of AgNPs using *Dodonaea viscosa* leaf extracts by disc diffusion method. (a) *Staphylococcus aureus* (b) *Bacillus subtilis* (c) *Pseudomonas aeruginosa* (d) *Klebsiella pneumonia* (e) : *Escherichia coli* (f) *Candida albicans*

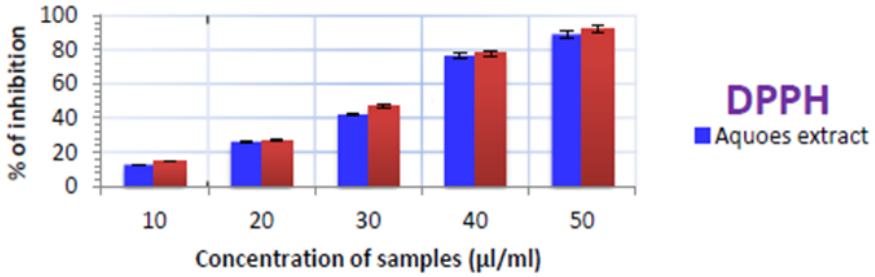

Figure.8 Estimation of DPPH in phytosynthesized AgNPs and plant leaf extract of *Dodonaea viscosa*.

Table.1. Zone of inhibition of synthesized silver nanoparticles using *Dodonaea viscosa* leaf extract against human pathogenic microorganisms

			Zone of inhibition (mm)		
Sl. No	**Organisms**	**Antibiotics**	**100µl**	**50µl**	**25µl**
1.	*Staphylococcus aureus*	24.2±1.01	**15.0±0.25**	**11.2±0.25**	**9.7±0.20**
2.	*Bacillus subtilis*	24.3±0.62	13.1±0.36	11.1±0.65	8.1±0.36
3.	*Escherichia coli*	**26.2±0.64**	11.2±0.52	10.1±0.36	8.9±0.64
4.	*Klebsiella pneumonia*	24.4±1.05	12.6±0.3	10.0±0.25	8.1±0.36
5.	*Pseudomonas aeruginosa*	27.2±1.05	10.01±0.30	9.7±0.25	7.2±0.25
6.	*Candida albicans*	14.4±0.56	10.10±0.40	8.2±0.32	7.3±0.40

V. Conclusion

Current global scenario on environmental protection from increasing pollutants and multidrug resistant pathogens is an immediate need for producing the viable phytoactive biosynthesized AgNPs. The present study indicates the purpose of using leaf extract of *D.viscosa* for the synthesis of 22.9-41.5nm sized AgNPs. Polyphenols, alkaloids, saponins and flavanoids in the leaf extract acted as reducing and capping agent, as stated by the FTIR analysis. AgNPs expressed a high purity and crystallinity by the XRD pattern. Biological activities like antibacterial, antioxidant and catalytic demonstrated strong response against their respective assay. By a well defined phyto-synthetic approach of this study may support a possible of biosynthesized AgNPs to be utilized in different disciplines.

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