International Journal of Applied Chemical and Biological Sciences (2021), 2(5), 59-69
ISSN 2582-788X

RESEARCH ARTICLE

Article Identifier: https://identifier.visnav.in/1.0001/ijacbs-21i-24009/

Characterization and investigation of biological catalytic antioxidant activities of Silver nanoparticles synthesized by using mulberry leaf extract

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ABSTRACT

Until few decades ago no one would had imagined that a technology is around the corner that would definitely show its influence in every sphere of life. That technology is called nanotechnology. Nanotechnology has adopted different methods for the production of materials at Nano-scale. Among the methods green synthetic approach is most preferred because of eco-friendliness, biocompatibility and cost-effectiveness. In the present work we have synthesized silver nanoparticles through green method by using Mulberry leaves. The synthesized silver nanoparticle were subjected to characterization by UV-visible spectrophotometry, Dynamic light scattering , Scanning Electron Microscopy, Fourier transformer-infrared, X-ray Diffraction. The nanoparticles were further studied for their antimicrobial, anti-diabetic, antioxidant, catalytic activities.

Keywords: Silver nanoparticles, anti-diabetic activity, antioxidant activity, catalytic activity

1. INTRODUCTION

Nanotechnology involves the generation of nanoparticles which are in size range 1 to 100 nm. Nanoparticles possess different shapes which give them unique chemical [1], physical [2] and optical properties [3-4]. Intensive research is being done on silver nanoparticles (AgNPs) owing to their wide range of applications in medical devices [5], pharmaceuticals [6], clothing [7], water purification [8] and also in adsorption of metals and pesticides [9-10]. NPs can be synthesized with physical, chemical and biological methods [11]. These methods have unique advantages and disadvantages depending on their application [12-16]. The methods can be time-consuming and are restricted to particular requirements like high temperature or pressure, which might result in wastage and damage to equipment and associated cost [17-18]. The critical environmental issues had led the scientific community towards green production of nanoparticles using living systems such as plants, fungi, and bacteria [19]. These green synthesized nanoparticles are much better than nanoparticles synthesized from chemical procedure [20]. The green synthesized nanoparticles have fast, environmental-friendly, low-cost production strategies, and are biocompatible. Phytochemicals of plants like carbohydrates, fats, enzymes, polyphenols, alkaloids, flavonoids and terpenoids act as stabilizing, reducing and capping agents in the metabolic reaction [21]. Silver nanoparticles have been synthesized using parts of plants like leaves [22], bark [23], seeds [24], roots [25], fruit extract of *Emblica officinalis* [26], leaves extract of *Citrus limon* [27], green tea (*Camellia sinensis*) [28], *Coffea Arabica* [29], neem (*Azadirachta indica*) [30], *Acalypha indica* [31], *Aloe vera* plant extract [32], latex of *Jatropha gossypifolia* [33], *Phoenix dactylifera* [34], inflorescence extract of *Mangifera indica* [35] etc.

The study aims to synthesised Silver nanoparticles and evaluate the biological, catalytic, antioxidant activities of synthesized by using mulberry leaf extract.

2. METHOD AND MATERIAL

2.1. Sample Collectionand preparation

Mulberry leaves were collected from department of sericulture University of Mysore (Mysuru) India. Leaves were oven dried and pounded to powder. The powder was collected and stored for further use.

10 gm of leaf powder was taken in a glass beaker and 100 ml of water was added. The mixture was boiled for 30 minutes at 70°C and then filtered. The filtrate was kept in a beaker with a label of Mulberry leaf extract and then kept at 4°C in refrigerator.

2.2. Synthesis of Silver nanoparticles

Mulberry leaves extract (5ml) was taken in 250ml beaker and kept on magnetic stirrer which was adjusted at 700rmp and 70°C. 50ml of 0.001M $AgNO_3$ were added dropwise in the beaker with leaf extract. The mixture was kept at same rmp value and temperature until the colour change was observed. The product so obtained was subjected to characterization.

2.3. Characterization of synthesied Silver nanoparticle

The synthesised AgNPs were first was subjected to various parameters for the confirmation of size, shape and structure. The UV-vis absorption spectra of the green synthesized AgNPs was taken by UV–visible spectrophotometer (NANO STAR SPECTRA BMG Lab tech). Dynamic light scattering (DLS) was used to determine the particle size distribution and average particle size of synthesied AgNPs. Morphology i.e. shape and size of synthesized AgNPs was studied by Scanning Electron microscopy (SEM). FTIR spectroscopic analysis was used to determine the functional groups present in the synthesized AgNPs. The crystallinity, phase structure and purity of the silver nanoparticles nanoparticles were determined by its typical powder XRD diffraction patterns.

2.4. Anti-diabetic (α-amylase assay)

The inhibition of α-amylase was carried out by the method by Malik CP and Singh MB (1980) [36] with slight modifications. 20 µl of alpha amylase (0.5mg/ml) were taken in test tubes. Different concentrations of 20µl, 40µl, 60µl of test samples (plant extract, AgNPs) were taken in test tubes and 10µl of 0.02m phosphate buffer (pH 6.9) were added to each test tubes. The tubes were incubated for 10 minutes. 1ml of 1% starch solution was added to the mixture and again incubated for 20 minutes. Finally 400µl of DNS reagent was added to stop the reaction and boiled for 5 minutes. Control was prepared wherein amylase was not added. Absorbance was measured at 540 nm.

2.5. Antioxidant (DPPH assay)

DPPH free radical scavenging of plant extract and silver nanoparticles was examined by following procedure described by Basavegowda N *et al.,* and Patra JK & Baek K-H (2017) [37-38] was followed with a little modification. A stock solution of DPPH (7mg in 10ml of methanol) was prepared. To 1ml of stock solution 10ml of methanol was added and was labelled as working solution. Different concentrations of test samples (plant extract, AgNPs) were taken in test tubes. To each sample 800µl of methanol and 400µl of DPPH was added. DPPH solution without test sample was used as control. All the samples were incubated for half an hour in dark and then absorbance was measured at 517 nm.

2.6. Antimicrobial (Agar well diffusion)

The antibacterial assay was performed by agar well diffusion method on *Escherichia coli* (ATCC 10536), *Staphylococcus* (ATCC 11632), *Bacillus cereus* (ATCC 14579) which were obtained from Food Microbiology, Defence Food Laboratory Mysore. The sterile nutrient medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate). Wells were cut and 100 µl of the silver nanoparticles were added along with the standard antibacterial agent containing disc were placed onto an agar plate. After 30 minutes, the cultured medium was inoculated with the test organisms Petriplates containing 20 ml Muller Hinton medium were seeded with 24 hrs culture of bacterial strains. Silver salt solution was used as a standard antibacterial agent. The plates were then incubated at 37°C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well. The inhibition cleared zone around the sample decides the efficiency of the antibacterial agent to inhibit the growth of bacteria.

2.7. Catalytic Activity

1 ml of 0.2M freshly prepared sodium boro-hydride was taken in a cuvette and 1.9ml of 0.2Mm of dye was added to the cuvette containing sodium boro-hydride. Cuvette was shaked and placed in the UV-visible spectrophotometer to record the absorbance. The cuvette was removed and 0.1ml of test sample was added, shaken vigorously. The observation was made on the UV-visible spectrophotometer and recorded.

3. RESULTS AND DISCUSSION

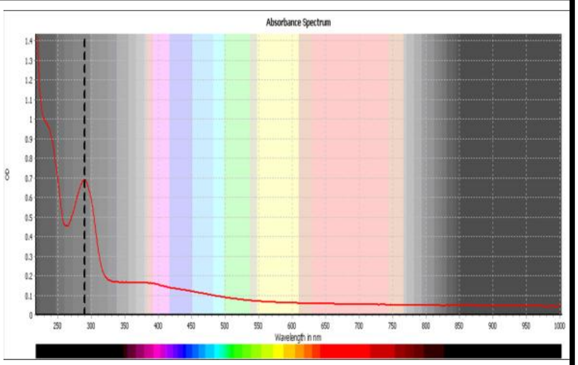

Figure 1. The UV vis characterization for the confirmation of synthesis of AgNPs

3.1. Characterization

3.1.1. Ultraviolet-Visible Spectroscopy

The colour change was observed i.e. light yellow to greyish brown, which is first indication of formation of silver nanoparticles [39-40]. Phytochemicals like Flavones, amides, carboxylic acid, aldehydes, ketones, terpenoids, quinines, and anthraquinones play prominent part in the preparation of nanoparticles [41-42]. The wavelength set between 200 nm and 800nm produces absorption band of silver nanoparticles [43]. An absorption peak was observed at 295 nm which confirms the formation of silver nanoparticles (figure 1). The results obtained are in accordance with previous reports [40].

3.1.2. Dynamic Light Scattering

Dynamic light scattering (DLS) was used to determine the particle size distribution and average particle size of all metal NPs at a scattering angle of 90°. The average particle size of silver nanoparticles was found 102 nm. Poly-distribution index was found 0.231 (figure 2).

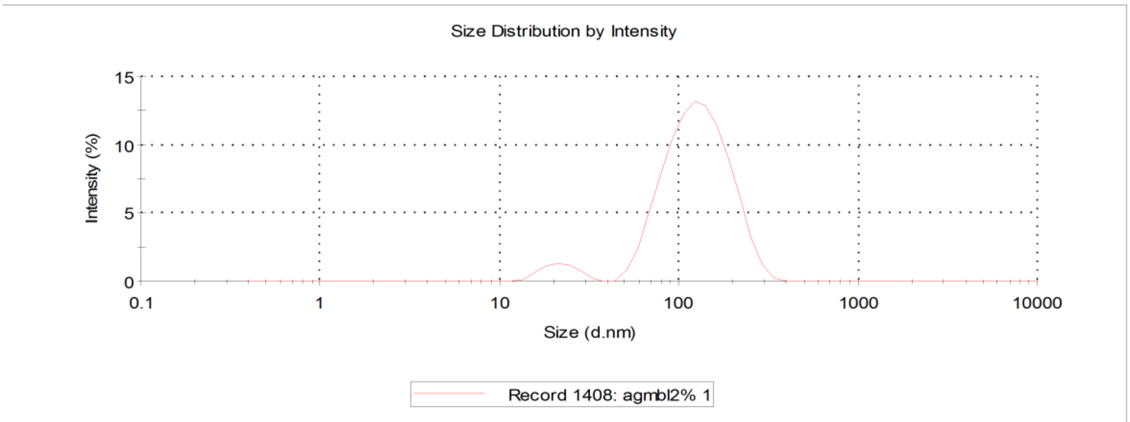

Figure 2. The Dynamic light scattering (DLS) of synthesised AgNPs

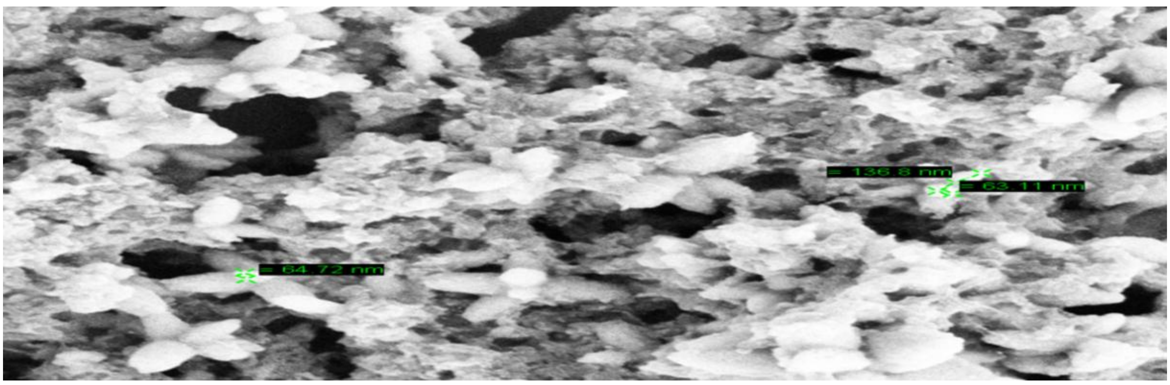

Figure 3. The Scanning Electron Microscopy (SEM) of synthesised AgNPs

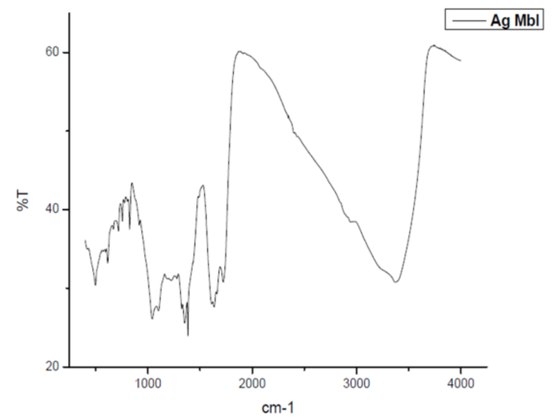

Figure 4. The FTIR of synthesised AgNPs

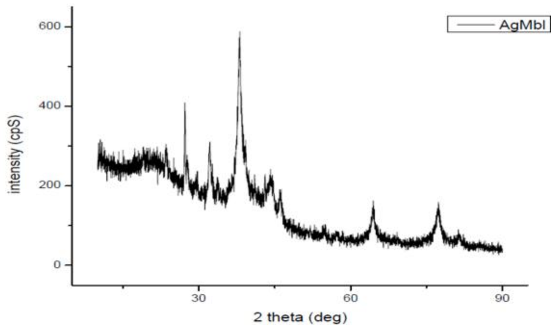

Figure 5. XRD of synthesised AgNPs

3.1.3. Scanning Electron Microscopy (SEM)

The SEM image (figure 3) shows green synthesized AgNPs with different size ranging from 63nm, 64, 135nm, etc. The morphology of the silver nanoparticles made using Mulberry leaves was spherical in shape. Aggregations or impurities were also observed. Particles could be seen well dispersed. These bigger particles are either waste plant material, or bigger particles formed due to agglomeration.

3.1.4. Fourier Transformer-Infrared (FTIR)

The FT-IR spectrum of dry powder of synthesized AgNPs is shown in figure 4. The IR-spectrum of the silver nanoparticles synthesized from Mulberry leaves showed absorption bands at 622, 871,1039,1387,1653, 2932, 3417 and 3700 cm^{-1} which are due to presence of functional groups such as alkyl halides N–C=O amide, C–H stretch of aldehydes, C–H of alkanes, O–H of alcohol, N–H of amines, C=O of carboxylic acid/ aldehydes or ester, H–C═O:C≡N stretch of nitriles [44-45].

3.1.5. X-Ray Diffraction (XRD)

The formation of AgNPs nanoparticles was detected by X-ray diffraction (XRD). All the diffraction peaks corresponds to the lattice planes of (110), (111), (200) and (211) in between 2θ values: 38.17º, 47.29º, 66.42º and 78.71º is in good agreement with the AgNPs which can be indexed on the basis of JCPDS card no. 65-2309. Sharp peaks in diffraction pattern show the crystalline nature of the particles (figure 5).

3.2. In-vitro Bioactivities

3.2.1. Anti-diabetic activity

Alpha-amylase is the main enzyme responsible for breakdown of starch and carbohydrates into sugars are Alpha-amylase [36]. The sugars enter into blood and increase the blood glucose level. This increase in blood glucose level is commonly known as diabetes. The inhibitory effect of this enzyme has strong effect on diabetes. The inhibitory effect on enzyme is produced by different agents. Here the inhibitory effect of Mulberry leaf extract, synthesized AgNPs was studied on enzyme. It was observed that AgNPs shows strong inhibition percentage than leaf extract. It was also observed that inhibition increased with increase in concentration until it reaches maximum. The percentage of inhibition

Table 1. The Anti-diabetic activity of AgNPs

Concentration (µl)	Control %	Lagerstroemia leaf %	AgNPs %
20	0	56.18	77
40	0	63.14	81
60	0	68.01	87

Table 2. The Anti-oxidant activity of AgNPs

Concentration (µl)	Control %	Lagerstroemia leaf %	AgNPs %
20	0	53.80	75
40	0	61.95	80
60	0	70.10	85

Table 3. The Anti-microbial activity of AgNPs

Samples	Zone of inhibition by well diffusion assay		
	E.coli	Staphylococcus	B. cereus
Silver standard	16mm	18mm	15mm
Mulberry Leaf extract	14mm	16mm	No inhibition
Silver nanoparticles	19mm	27mm	23mm

of Mulberry leaf extract and AgNPs is given in the table 1.

3.2.2. Antioxidant activity

DPPH is a stable free radical scavenger and shows a characteristic absorption at 517 nm wavelength and after reduction colour changes from violet to yellow [46]. The antioxidants react with DPPH and convert it to 1, 1- diphenyl-2-picryl hydrazine with decolourisation. The silver nanoparticles showed higher free radical scavenging power than plant extract. The free radical scavenging activity of AgNPs at higher concentration (60µl) was found higher than plant extract (table 2). This is due to the efficient oxidation of AgNPs. The AgNPs quenched the activity of DPPH by donating silver's electrons.

3.2.3. Antimicrobial activity

Antimicrobial activity of Mulberry leaf extract, AgNPs and silver nitrate (standard) was carried out against the pathogens such as *E. coli, Staphylococcus and B. cereus*. It was found that AgNPs shows strong zone of inhibition against all pathogens used during study than plant extract and silver nitrate standard. The zone of inhibition of all test samples is given in the table 3. Similarly antibacterial assay was performed in a study using Papaya fruit extract mediated silver nanoparticles on human pathogen, showing high toxicity against multidrug resistance bacteria [47]

3.2.4. Catalytic activity

The reduction of 4-nitrophenol (4-NP), 2-nitrophenol (2-NP) and dyes such as congo red, methylene blue was studied using $NaBH_4$ in the presence of synthesized AgNPs using Mulberry (Morus) plant leaves at room temperature and monitored by UV-Visible spectroscopy [48-49]. The reduction of 4-NP, 2-nitrophenol, congo red, methylene blue using aqueous NaBH4 is thermodynamically favourable but due to large energy barrier feasibility of reaction decreases. To overcome the energy barrier metal nanoparticles were used which help fast transfer of electrons from the donor – to acceptor thus catalysing the reaction and hence act as catalysts. Absorption peaks have been observed for pure 4-NP, 2-nitrophenol, congo red, methylene blue at 317nm, 355nm, 500nm and 600nm. Upon addition of $NABH_4$ change in colour and shift in absorption peaks to 400nm, 420nm 550nm and 650nm appeared which indicated the intermediate formation. No further change was observed until the addition silver nanoparticles. The addition of silver nanoparticles completely changed the colour i.e. coloured to colourless and further shifted the absorption peaks to 292nm, 290nm, 250nm, 255nm respectively. Thus, it was concluded that silver nanoparticles act as catalysts (figure 6).

4. CONCLUSION

Metal nanoparticles are gaining importance in different fields of science and technology because of their unique properties. Different methods have been used to synthesize the metal nanoparticles but the most preferred method is green synthetic approach. We have synthesized the silver nanoparticles through green synthetic method using Mulberry plant leaves. The synthesized silver nanoparticles were characterized by UV-visible spectrophotometer, DLS, SEM, XRD etc. The silver nanoparticles were studied for various activities like antimicrobial activity, antioxidant activity, anti-diabetic activity, catalytic activity and the results obtained showed strong activity.

5. ACKNOWLEDGEMENT

I am thankful to the Director of Defence food research laboratory, Mysore, India for providing laboratory facilities to carry out the study. I also express my gratitude to Farhath Khanum (HOD) of Nutrition Biochemistry and Toxicology Department for her assistance to complete this study well in time.

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

No source of funding

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