

## Investigation of Biological Activities Catalytic Activity Antioxidant Activity of Silver Nanoparticles Synthesized By Using Mulberry (Morus) Leaf Extract



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### Abstract

Few decades ago, almost no one understood the importance of remarks "There is plenty of room at the bottom" expressed by Robert Feynman until its reality appeared in the form of technology called nanotechnology, which has left its footprints in almost every field of science and technology. Nanotechnology is based on two approaches i.e. Top-Down Approach and Bottom-up Approach. These two approaches include different physical, chemical and biological methods for the production of materials at Nano scale. Biological method especially use of plant parts (green method) is strongly recommended nowadays due its unique features i.e. simplicity, biocompatibility, cost-effectiveness and eco-friendliness. In the present study, we have synthesized silver nanoparticles through green method by using Mulberry leaves. The green synthesized silver nanoparticles were characterized by techniques such as Uv-visible spectrophotometry, Scanning Electron Microscopy, Dynamic light scattering, Fourier transformer-infrared, X-ray Diffraction. The nanoparticles were studied for their different activities i.e. antimicrobial activity, antidiabetic activity, antioxidant activity, catalytic activity and cytotoxic activity.

**Key Words:** - Silver nanoparticles, antidiabetic activity, antioxidant activity, catalytic activity and cytotoxic activity.

### 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.Singh, J. et al 2018, physical 2. Yilmaz, A. & Yilmaz et al 2020 and optical properties 3.Scholes, G. D and 4.Susarrey-Arce, A. et al 2011. Intensive research is being done on silver nanoparticles (AgNPs) owing to their wide range of applications in medical devices 5.He Y, et al 2013, pharmaceuticals 6.Kumar V.G, et al 2011, clothing 7.Vigneshwaran N, et al 2007, water purification 8. Lin S, et al 2013 and also in adsorption of metals

and pesticides 9.Asthana A, and 10.Das SK, et al 2012 etc. NPs can be synthesized with physical, chemical and biological methods 11. Chen, H et al 2008. These methods have unique advantages and disadvantages depending on their application 12.Smetana, A and 16. Kholoud, M et al 2010. These 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.Toisawa, K and 18.Iravani, S et al 2010. The critical environmental issues had led the scientific community towards green produc-

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tion of nanoparticles using living systems such as plants, fungi, and bacteria 19. Krishnaraj C et al 2010. These green synthesized nanoparticles are much better than nanoparticles synthesized from chemical procedure 20. Baker. S et al 2013. The green synthesized nanoparticles have fast, environmental-friendly, and 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 reaction 21. Makarov VV et al 2014. Silver nanoparticles have been synthesized using parts of plants i.e. leaf 22. Prakash P, et al 2013, bark 23. Sathishkumar M, et al 2009, seeds 24. Bar H, et al 2009, roots 25. Suman T, et al 2013, fruit extract of *Embolica officinalis* 26. Ankamwar, Be et al 2005, leaves extract of *Citrus limon* 27. Vankar, P. S., and Shukla, D et al 2012, green tea (*Camellia sinensis*) 28. Nakhjavani, M et al 2017, *Coffea Arabica* 29. Dhand, V et al 2015, neem (*Azadirachta indica*) 30. Ahmed, S et al 2016, *Acalypha indica* 31. Krishnaraj, C et al 2012, Aloe vera plant extract 32. Tippayawat, P et al 2016, latex of *Jatropha gossypifolia* 33. Borase, H et al 2014, *Phoenix dactylifera* 34. Oves, M et al 2018, inflorescence extract of *Mangifera indica* 35. Qayyum, S et al 2017 etc. In the present work, we synthesised the silver nanoparticles using Mulberry leaf extract through green method and also studied some of their applications.

## Materials and Methods

### Materials required

Leaves of Mulberry plant, glass beaker, filter paper, test tubes, magnetic stirrer, funnel, burette, burette stand, syringe

### Chemicals required

Silver nitrate, 1,1-Diphenyl-2-picryl hydroxyl (Dpph),  $\alpha$ -amylase, sodium borohydride, ortho-nitrophenol, para-nitrophenol, congo red, methylene blue.

## Methodology

### Preparation of leaf extract

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. 10gm

of leaf powder was taken in a glass beaker and 100ml 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.

### Synthesis of silver nanoparticles

Mulberry leaves extract (5ml) was taken in 250ml beaker and kept on magnetic stirrer which was adjusted at 700rpm and 70°C. 50ml of 0.001M AgNO<sub>3</sub> were added drop-wise by burette to the beaker containing leaf extract. The mixture was kept at same rpm value and temperature until the colour change was observed i.e. light yellow to greyish brown which is first indication of formation of silver nanoparticles.

### Characterisation

The spectrophotometric techniques used for characterisation are: (i) Uv-visible spectrophotometer (nano spectrostar (BMG LAB TECH) (ii) scanning electron microscope (SEM) equipped with an energy-dispersive X-ray (EDX) (JFC1600 instrument (JEOL, Ltd., Tokyo, Japan) (iii) Dynamic Light Scattering (Malvern Zeta sizer) and (iv) Fourier transform infrared spectroscopy (FTIR) analysis using a Spectrum One FTIR spectrophotometer (PerkinElmer, Inc.) and X-ray diffractometry. The instruments were used to determine various parameters such as formation of nanoparticles, shape, size, zeta potential, stability, elemental composition, functional group and crystalline nature of synthesized AgNPs.

### Encapsulation Efficiency

#### Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Amalgamation of materials of same or different structure to assist the fabrication of requisite characteristics is known as Encapsulation. Metal load efficiency in the mother solution of nanoparticle was determined by Inductive coupling plasma with optic emission spectrometry technic (ICP-OES). It was achieved with atomic emission spectrophotometer ICP-OES Varian 725-ES using argon as carrier gas. Sample ashing was carried out at 550°C in furnace. Collected ash of sample was then dissolved in 0.5M nitric acid. The solution was then filtered by Ashless filter paper. Finally, filtered solution (sample) was

made upto the volume of 50ml. Sample solution was then put into ICP-OES [36]. The results obtained are expressed as mg of copper per gram of leaf extract. The encapsulation efficiency percentage is calculated by following formula

$$\% EE = \frac{C_i}{C_0}$$

Where,  $C_i$  is the real concentration calculated by ICP. and  $C_0$  is theoretical concentration of product involved into the process.

#### **Anti-diabetic ( $\alpha$ -amylase assay)**

The inhibition of  $\alpha$ -amylase was carried out by the method [37] with slight modifications. 20  $\mu$ l of alpha amylase (.5mg/ml) were taken in test tubes. Different concentrations i.e. 20 $\mu$ l, 40 $\mu$ l, 60 $\mu$ l of test samples (plant extract, silver nanoparticles) were taken in test tubes and 10 $\mu$ l of 0.02m phosphate buffer (ph 6.9) were added to each test tubes and the incubation of mixture was done for 10minutes. 1ml of 1%starch solution was added to the mixture and again incubated for 20minutes. Finally 400 $\mu$ l of DNS reagent was added to stop the reaction and then the reaction mixture was boiled for 5minutes. Control was prepared wherein amylase was not added. Absorbance was measured at 540nm.

#### **Antimicrobial (Agar well diffusion)**

The antibacterial assay was performed by agar well diffusion method on Escherichia coli (E.Coli), Staphylococcus, Bacillus cereus which were obtained from Food Microbiology, Defence Food Laboratory Mysore. The bacterial culture medium used, was nutrient agar medium. The dissolved medium was autoclaved at 15 lbs pressure at 121 °C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten [38]. After 30 minutes, the cultured medium was inoculated with the test organisms Petriplates containing 20 ml Muller Hinton medium were seeded with 24hrs culture of bacterial strains. Wells were cut and 100  $\mu$ l of the silver nanoparticles were added along with the standard antibacterial agent were placed onto an agar plate. 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.

#### **In vitro cytotoxicity by MTT assay**

##### **Cell lines and culture medium**

MCF-7 cancer cell line was purchased from the national centre for cell sciences (NCCS), Pune, India. Dulbecco's modified eagle's growth medium (DMEM)-high glucose (#AL111, himedia) with 10% fetal bovine serum (#RM10432, hi media) was used in order to culture the cancer cells and incubation was done at a temperature of 37 $\pm$ 0.2 °C. MTT reagent (5mg/ml) #4060) was purchased from hi media. Cytotoxic activity of silver nanoparticles against MCF-7 was evaluated by MTT(3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazoliumbromide) colorimetric assay. Key aim of the assay is the reduction effect of the NAD (P)H dependent oxidoreductase enzymes of viable cells on yellow coloured water-soluble tetrazolium dye to give a formazan product with deep purple colour . The cytotoxicity assay was completed by seeding 100  $\mu$ l cell suspension in a 96-well plate at required cell density (10,000 cells per well) and incubated for 24 h at 37 °C [39]. Different concentrations (15, 30, 50, 90 and 120  $\mu$ g/ml) of test sample (silver nanoparticles) were taken and incubated for 24 h at 37 °C. Cancer cells without test compound were used as control. Exhausted media was taken out after the incubation period and MTT reagent was added to a final concentration of 0.25 mg/ml of total volume and incubated again. After incubation MTT reagent was removed and finally 50 $\mu$ l solubilized solution of dimethyl sulfoxide (DMSO) was added to get the purple formazan crystals for complete dissolution. The colorimetric assay is analysed and noted the absorbance at 570 nm using an spectrophotometer [40]. Cell viability and cytotoxicity percentage were calculated using the following equation [41, 42]:

$$\text{Cell Viability \%} = \frac{\text{Test OD}}{\text{Control OD}} \times 100 \dots\dots\dots (1)$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability \%} \dots\dots\dots (2)$$

##### **Catalytic Activity**

1ml of 0.2M freshly prepared sodium boro-hy-

dride was taken in a cuvette and 1.9ml of 0.2Mm of dye was added to the cuvette containing sodium boro-hydride. Cuvette was shaken and placed in the uv-visible spectrophotometer to record the absorbance[43].The cuvette was removed and 0.1ml of test sample was added and shaken vigorously and kept in Uv-visible spectrophotometer and absorbance was recorded.

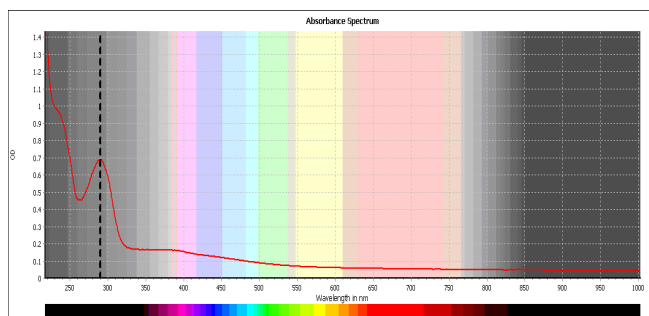
### Antioxidant ( Dpph assay)

DPPH free radical scavenging of plant extract and silver nanoparticles was examined by following procedure described by [44] 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, silver nanoparticles) were taken in test tubes. To each sample 800µl of methanol and

ples were incubated for half an hour in dark and then absorbance was measured at 517nm [45].

### Results and Discussions Ultraviolet-Visible Spectroscopy

The absorption spectra of the green synthesized silver nanoparticles was taken by Uv -visible spectrophotometer (NANO STAR SPECTRA BMG Lab tech). Colour change indicates the formation of silver nanoparticles [46]. Phytochemicals like Flavones, amides, carboxylic acid, aldehydes, ketones, terpenoids, quinines, and anthraquinones play prominent part in the preparation of nanoparticles [47]. The wavelength set between 200nm and 800nm produce absorption band of silver nanoparticles [48]. An absorption peak (fig.1) was observed at 295nm which confirms the formation of silver nanoparticles. The results obtained are in accordance with previous reports.

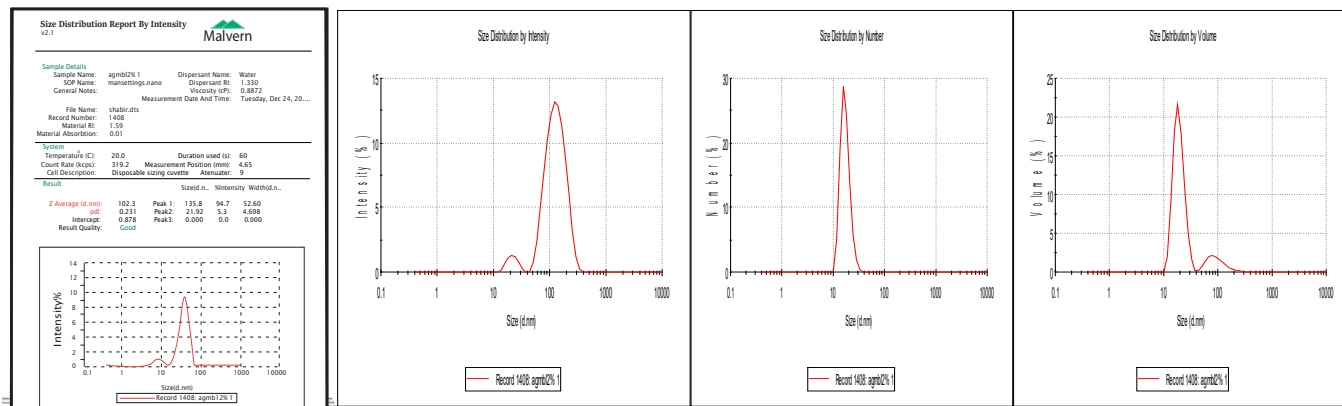


**Figure. 1**  
**Uv spectrum of silver nanoparticles**

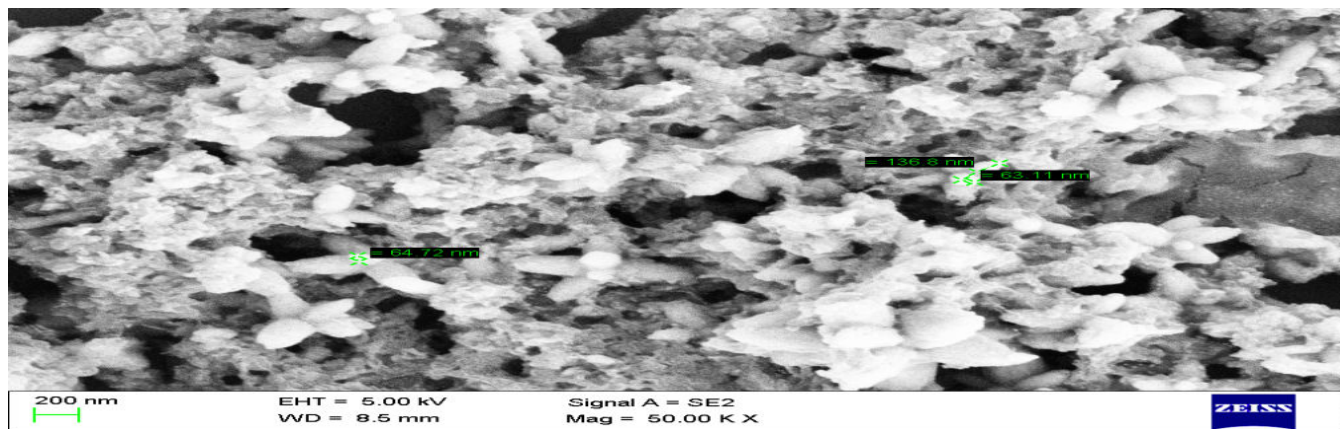
400µl of dpph was added. Dpph solution without test sample was used as control. All the sam-

### 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 102nm. Poly-distribution index was found 0.231. Morphology i.e. shape and size of green synthesized silver nanoparticles was studied by Scanning Electron microscopy. The SEM image (fig.3) shows green synthesized AgNPs with different size i.e. 63nm, 64, 135nm, etc. The morphology of the silver nanoparticles made using Mulberry leaves were spherical in shape. Aggregations or impurities were also



**Figure.2**  
**Scanning Electron Microscopy**

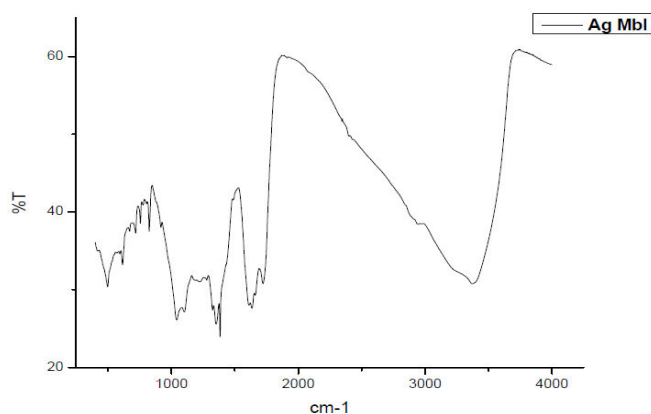


**Figure. 3**  
**SEM image of silver nanoparticles**

observed. Particles could be seen well dispersed. These bigger particles are either waste plant material, or bigger particles formed due to agglomeration.

#### Fourier Transformer-Infrared

FTIR spectroscopic analysis was used to determine the functional groups present in the synthesized silver nanoparticles from Mulberry leaf extract. FT-IR spectrum of dry powder of synthesized AgNPs is shown in (Fig.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  $\text{cm}^{-1}$  which are due to presence of functional groups such as alkyl halides  $\text{N}-\text{C}=\text{O}$  amide,  $\text{C}-\text{H}$  stretch of aldehydes,  $\text{C}-\text{H}$  of alkanes,  $\text{O}-\text{H}$  of alcohol,  $\text{N}-\text{H}$  of amines,  $\text{C}=\text{O}$  of carboxylic acid/ aldehydes or ester,  $\text{H}-\text{C}=\text{O}:\text{C}\equiv\text{N}$  stretch of nitriles [49-50].

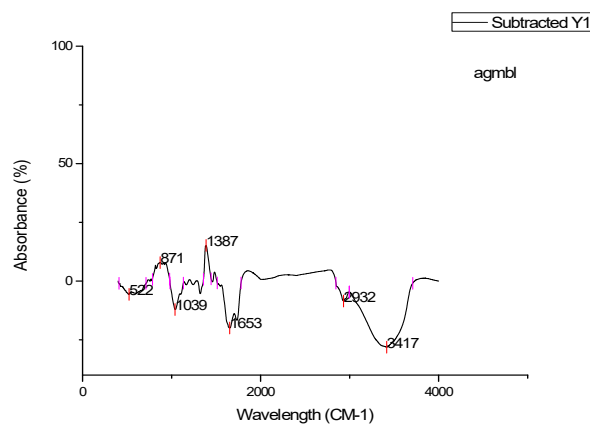


#### X-Ray Diffraction

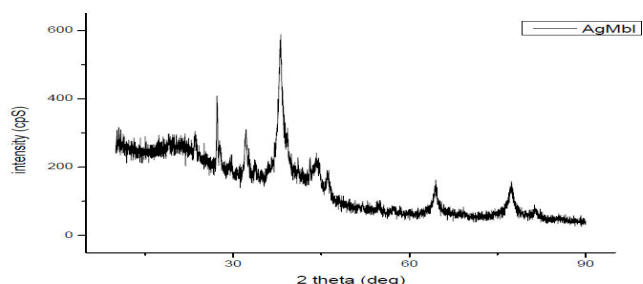
The formation of AgNPs nanoparticles was detected by X-ray diffraction (XRD). The crystallinity, phase structure and purity of the silver nanoparticles nanoparticles were determined by its typical powder XRD diffraction patterns. All the diffraction peaks corresponds to the lattice planes of (110), (111), (200) and (211) in between  $2\theta$  values:  $38.17^\circ$ ,  $47.29^\circ$ ,  $66.42^\circ$  and  $78.71^\circ$  (Fig.4) 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.

#### Encapsulation Efficiency

The ICP-OES spectroscopy was used to determine the Ag ion concentration encapsulated by phytochemicals of plants ex



**Figure. 4**  
**FT-IR spectrum of silver nanoparticles**



**Figure. 5**  
**XRD spectrum of Silver nanoparticles**

xtract in the solution of AgNPs. Ag content in the solution was determined by ICP-OES equivalent to 1.45g which accounts for 72.5% of total amount of nanoparticles. Hence, it confirms the formation of high yield of silver nanoparticles.

### Anti-diabetic Activity

Alpha-amylase is the main enzyme responsible for breakdown of starch and carbohydrates into sugars. 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, synthe

| Concentration (µl) | Control % | Lagerstroemia leaf % | Silver nanoparticles % |
|--------------------|-----------|----------------------|------------------------|
| 20                 | 0         | 56.18                | 77                     |
| 40                 | 0         | 63.14                | 81                     |
| 60                 | 0         | 68.01                | 87                     |

**Table. 1**  
**Inhibition percentage (%) versus different concentration of test samples**

sized silver nanoparticles was studied on enzyme. It was observed that silver nanoparticles showed strong inhibition i.e. the levels of enzymes are decreased, which are accountable for catalyzing the hydrolysis of complex carbohydrates and increased the utilisation rate of glucose than leaf extract. It was also observed that inhibition increased with increase in concentration until it reaches maximum. The percentage of inhibition of Mulberry leaf extract and silver nanoparticles is given (table. 1). The results obtained are

in agreement with previous studies [51-53].

### Antimicrobial Activity

Antimicrobial activity of Mulberry leaf extract, silver nanoparticles and silver nitrate (standard) was carried out against the pathogens such as Escherichia coli, Staphylococcus and Bacillus cereus. It was found that Silver nanoparticles show strong zone of inhibition against all pathogens

| Samples               | Zone of inhibition by well diffusion assay |                           |                            |
|-----------------------|--|---------------------------|----------------------------|
|                       | E.coli ATCC 10536                          | Staphylococcus ATCC 11632 | Bacillus cereus ATCC 14579 |
| Silver standard       | 16mm                                       | 18mm                      | 15mm                       |
| Mulberry Leaf extract | 14mm                                       | 16mm                      | No inhibition              |
| Silver nanoparticles  | 19mm                                       | 27mm                      | 23mm                       |

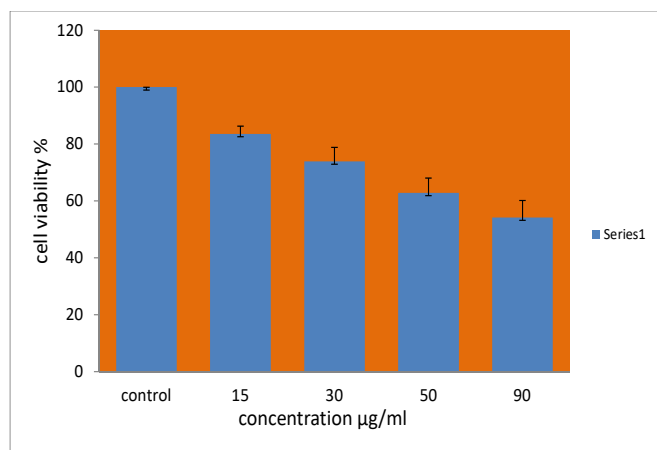
**Table. 2**  
**Zone of Inhibition versus different concentrations of test samples against pathogens**

used during study than plant extract, silver nitrate standard. The zone of inhibition of all test samples is given in the (table. 2). AgNPs show high antibacterial activity against bacteria in previous studies [54-55]. The phytochemicals which act as capping agents and stabilizing agent for synthesis of AgNPs are selective for bacterial strains changes. Conformational changes in the membrane structure of bacterial cell wall are thought to be generated by the action of AgNPs which increases the chances of permeability of membrane, and hence lead to bacterial cell death [56].

### In vitro anticancer activity

#### MTT Assay and Cell Morphology

In this experiment, the MTT assay was used to compute the anticancer capability of AgNPs on breast cancer cell line (MCF-7). From the study it was witnessed that the cytotoxicity against breast cancer cell line increases with increasing concentration of AgNPs. The IC50 of AgNPs was found at 92µg/ml against the MCF-7 cancer cell lines. It was revealed that 50% inhibition of cells was observed compared to untreated control. The proliferation of MCF-7 cancer cell line subjected to AgNPs was considerably inhibited in a dose-dependent manner as shown in (fig.5). The inspection of cytotoxic effect of synthesized AgNPs on cancer cells was done by checking



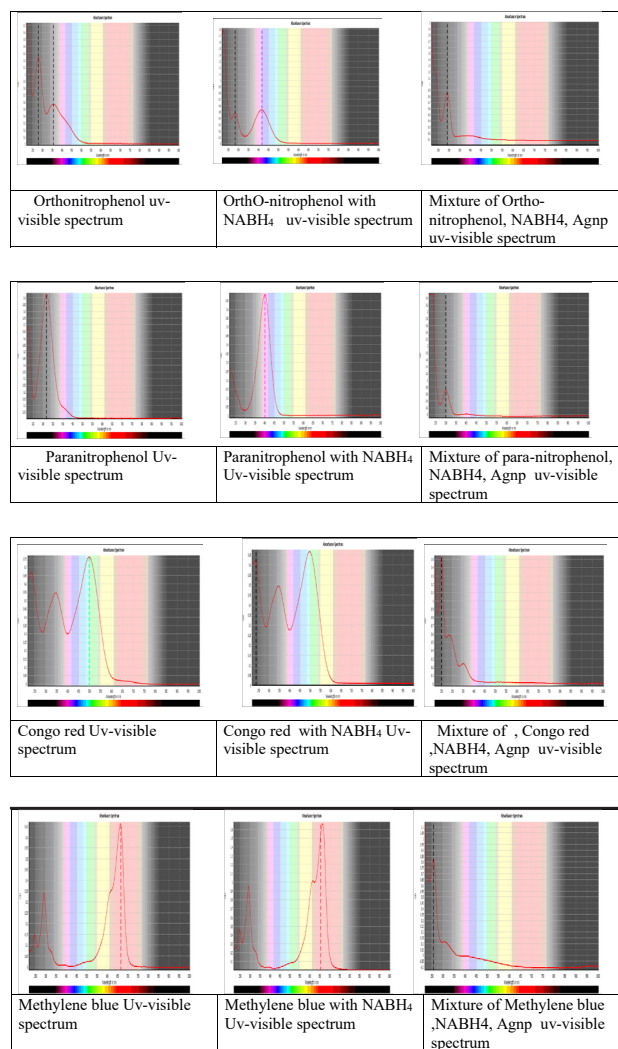
**Figure.6**

**Cell Viability % versus concentration µg/ml of test sample**

the morphology of all the cells under an optical microscope. It was established that the morphological evaluation of AgNPs treated cancer cells show apparent structural changes such as change in cells membrane surface, cell contraction and inhibition of cell growth. Therefore, validating that apoptosis has been induced in AgNPs treated MCF-7 cancer cells [57].

**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<sub>4</sub> in the presence of synthesized AgNPs using Mulberry (Morus) plant leaves at room temperature and monitored by UV-Visible spectroscopy [58-59]. The reduction of 4-NP, 2-nitrophenol, congo red, methylene blue using aqueous NaBH<sub>4</sub> 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 317, 355nm, 500nm, 600nm. Upon addition of NABH<sub>4</sub> change in colour and shift in absorption peaks to 400nm, 420nm 550nm, 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



**Figure .7**

further shifted the absorption peaks to 292nm, 290nm, 250nm, 255nm respectively. Thus, it was concluded that silver nanoparticles act as catalysts. The results obtained are given below.

**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 [60]. The antioxidants react with DPPH and convert it to 1,1- diphenyl-2-picryl hydrazine with decolourisation scavenging DPPH due to donation of hydrogen atom to stable the DPPH molecule [61-62]. 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.3). This is due to the efficient oxidation of AgNPs. The AgNPs quenched the activity of DPPH by donating silver's electrons.

| Concentration (µl) | Control % | Lagerstroemia leaf % | Silver nanoparticles % |
|--------------------|-----------|----------------------|------------------------|
| 20                 | 0         | 53.80                | 75                     |
| 40                 | 0         | 61.95                | 80                     |
| 60                 | 0         | 70.10                | 85                     |

**Table. 3**  
**Scavenging (%) versus different concentration of test samples**

### Conclusion

Metal nanoparticles are gaining importance in different fields of science and technology because of their peculiar properties. We have synthesized the silver nanoparticles through green synthetic method using Mulberry plant leaves because of its simplicity, cost-effectiveness and eco-friendliness. The synthesized silver nanoparticles were characterized by UV-VIS SPECTROPHOTOMETER, DLS, SEM, FT-IR, XRD etc. to determine the various parameters such as size, shape, stability, composition and nature of nanoparticles. The silver nanoparticles were studied for various activities like antimicrobial activity, antioxidant activity, antidiabetic activity, catalytic activity and cytotoxic activity by different assays and the results obtained showed strong activities.

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