RESEARCH PAPER

# Facile Approach to Synthesize and Characterization of Silver Nanoparticles by Using Mulberry Leaves Extract in Aqueous Medium and its Application in Antimicrobial Activity

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## ABSTRACT

There is a huge demand of silver nanoparticles in the global market due to their special properties and applications in different fields such as nanomedicine , dentists , nanocatalysis, nanoelectronics, textile field, waste water treatment.The major cons of top down and Bottom up methods are the synthesis processes are highly costly, time consuming and many harmful chemicals are used. To reduce these problems Green chemistry comes to play a very important role for making of silver nanoparticles. Use of various plant extracts like leaves, fruits for synthesis of biogenic silver nanoparticles referred as Green Nanotechnology. In the present work we reported the green synthesis of silver nanoparticles using by Mulberry leaves extract without using any toxic chemicals. The Mulberry leaves extract act as reducing agent as well as a stabilizing agent in green nanotechnology process. The making of silver nanoparticles was determined by the change of shade from white to brownish by the addition of Mulberry leaves extract . UV-Vis absorption spectroscopy was used to monitor the measurable formation of silver nanoparticles showed a maximum peak at 440 nm. High resolution Transmission electron microscope confirmed the spherical nature and the highly crystallinity of silver nanoparticles on an average size 15 -25 nm .Antimicrobial activity of the biogenic Ag nanoparticles was performed by a well diffusion method. The nanoparticles exhibited enhanced anti-bacterial activity when incubated in Escherichia Coli and Bacillus Subtilis cultured plates at varied volumes. Current study thus presents a facile and innovative strategy for synthesis of silver nanoparticles.

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## INTRODUCTION

Research in the area of Metal nanoparticles has shown enormous growth due to their distinctive chemical and physical properties. Metal nanoparticles have fascinated researchers due to their impending applications in various fields like biomedical biomedical applications and sensitive diagnostic assays [1], radiotherapy enhancement, and thermal ablation [2, 3], gene delivery [4, 5]. As non toxic carriers for drug and gene-delivery applications [6], optical imaging and labeling of biological systems [7].

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Nano silver has been acknowledged for over 100 years as colloidal silver and used as an antimicrobial agent [8,9,10]. Synthesis of Ag NPs is generally carried out by various physical and chemical methods counting reduction in solutions, thermal decomposition of silver compounds, microwave assisted chemical and photochemical reactions in the reverse micelles, etc. The most communal methodology for synthesis of metal nanoparticles is chemical reduction by organic and inorganic reducing agents such as Sodium Borohydride[11] Hydrazine[12], Ascorbic acid[13] ,N-dimethylformaide, and Poly(ethylene glycol) [14] etc., which control the shape and size of the metal nanoparticles. For Ag NPs these reducing agents reduces the $Ag^+$ ions into Ag nanoparticles in aqueous or non- aqueous solution. These methods are quite simple and successful with the major disadvantage that they are expensive and hazardous to the environment. To lessen these detriments, Green nanotechnology comes into play where metal nanoparticles are synthesized without using any poisonous chemical[15]. Green nanotechnology or green synthesis is nothing but an organic synthesis of nanoparticles using plant extracts and the synthesized nanoparticles are then known as biogenic nanoparticles[16,17]. Also green synthesis offers numerous benefits over chemical and physical methods majorly because it is environmentally friendly. Apart from this, this method is cost effective and particles synthesized are more stable. The phytochemicals present in plant are terpenoids, aldehydes, flavones, ketones, carboxylic acids and amides. Eugenol, Flavones, organic acids, quinones are water-soluble phytochemicals that are responsible for immediate reduction of the ions[18] . In various studies huge number of medicinal plants such as Acorus calamus[19] Alternanthera dentate [20] , ,Ocimum Sanctum [21], Azadirachta indica [22] ,Brassica rapa [23], Coccinia indica [24] , Vitex negundo [25] , Melia dubia [38] are used have already been used to synthesize and stabilize metallic nanoparticles, very particularly biogenic silver (Ag) nanoparticles .

Mulberry is a perennial and woody plant, belongs to the family Moraceae and genus Morus native of China. Mulberry plant is one of the conventional herbs used in medicine Since time immemorial due to its chemical composition and pharmacological function. Most parts of mulberry plants are used as medicine in Chinese and Indian medicine[27]Mulberry leaves and fruits contained many bioactive components such as alkaloids,anthocyanins and flavonoids.[28,29]

Herein we report for synthesis silver nanoparticles, where silver ions are reduced in the presence of silver nitrate solution, using an aqueous leaf extract of Mulberry leaf extract which facilitates their antibacterial activity. Here the plant extract as reducing agent as well as capping agent.

## MATERIALS AND METHODS

Mulberry leaves were collected from the nearby garden and washed methodically with distilled water to remove the dust particles and were then air dried for one hour to eliminate all the water content. The dried leaves were then cut into smaller pieces. For performing the experiment, cultures of test bacterial strains-Escherichia Coli (Gram negative bacterium) and Bacillus subtilis(Gram positive bacterium) , from the biotechnology department of Sri Guru Granth Sahib World University.

### *Preparation of Leaf Extract*

In a 250 mL beaker, 100 mL distilled water was poured and about 3g of the finely sized leaves were added into the beaker. It was then boiled for about one hour. The extract was then filtered thoroughly by using Whatman No: 1 filter paper to eradicate particulate matter and to get a clear solution which is further stored in the refrigerator to be used for various characterizations and future work.

### *Preparation of Silver Nitrate Solution*

In 100 mL of distilled water, $10^{-3}$ M silver nitrate was added to get a fresh solution of Silver Nitrate (Stock solution).

### *Synthesis of Silver nanoparticles*

For reduction of $Ag^+$ ions into $Ag^0$, Mulberry leave extract was added into $10^{-3}$M silver nitrate($AgNO_3$) solution with continuous stirring for one hour at room temperature. Due to the Surface Plasmon Resonance Phenomena, the color of the solution changed from colorless to pale yellow and finally reddish brown, approving the complete reduction of $AgNO_3$.

### *Characterization of Bioreduced Silver nanoparticles*

UV-vis spectra were measured in a quartz cuvette using Shimadzu-UV 2600 spectrophotometer by analyzing the sample in the range of 200-800nm. The peak of the Silver nanoparticles prepared by green synthesis lies between the range 420-480nm. The particle size profile and polydispersity index (PDI) was measured by Zetasizer Nano (Malvern-ZEN-1690). To examine the role of molecules and presence of capping agent in the synthesis of silver nanoparticles, the FTIR spectra were measured in the range of 4000-400cm$^{-1}$ in Bruker Alpha-T spectrophotometer. Morphology and size of synthesized biogenic silver nanoparticles were investigated by High-resolution transmission electron microscope (HR-TEM) at Jeol 2100 operated at an acclerating volatage of 300KV.

*Assessment of antimicrobial assay*

The conventional well plate diffusion technique was used to determine the antibacterial activity of biogenic silver NPs.. The strain was grown and preserved in the culture media. In this study, the Nutrient Agar medium was used which supports the growth of bacteria such as Escherichia coli and Bacillus subtilis. For 100ml Nutrient agar medium, 2.8 g nutrient powder was added in 100 ml distilled water. The medium kept in cotton-plugged glass container was sterilized in an autoclave at 121$^0$ C for 15 mins. After preparing a medium, the freshly grown liquid culture of Escherichia coli was poured in agar plates. Then synthesized Silver NPs were added were added in plates and kept in an incubator at 37$^0$ C for 24 hrs.

## RESULTS AND DISCUSSION

The reduction of silver ions to silver nanoparticles was confirmed by UV-visible spectroscopy analysis as shown in Fig. 2. When the plant extract was added into the $AgNO_3$ solution the pale yellow colour was obtained. After 10 min the colour changes from pale yellow to dark reddish brown.This colour occurs due to the observable effect of surface Plasmon excitations in metal nanoparticles[30]. The absorption spectra of bioreduced Ag NPs were observed the sharp band around 440 nm in UV- vis spectra. The development of biogenic silver nanoaprticles was determined at various time interval inset in Fig. 3.

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. It was based on Mie-scattering theory. The prepared sample was dispersed in deionised water followed by ultra-sonication. Then solution was filtered and centrifuged for 20 minutes. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries,Malvern instrument Nano Zs). The study revealed that the Z- average size of Ag nanoparticles range 80.44 and PDI .0222 in the Fig. 4

The size and morphology of the as-prepared

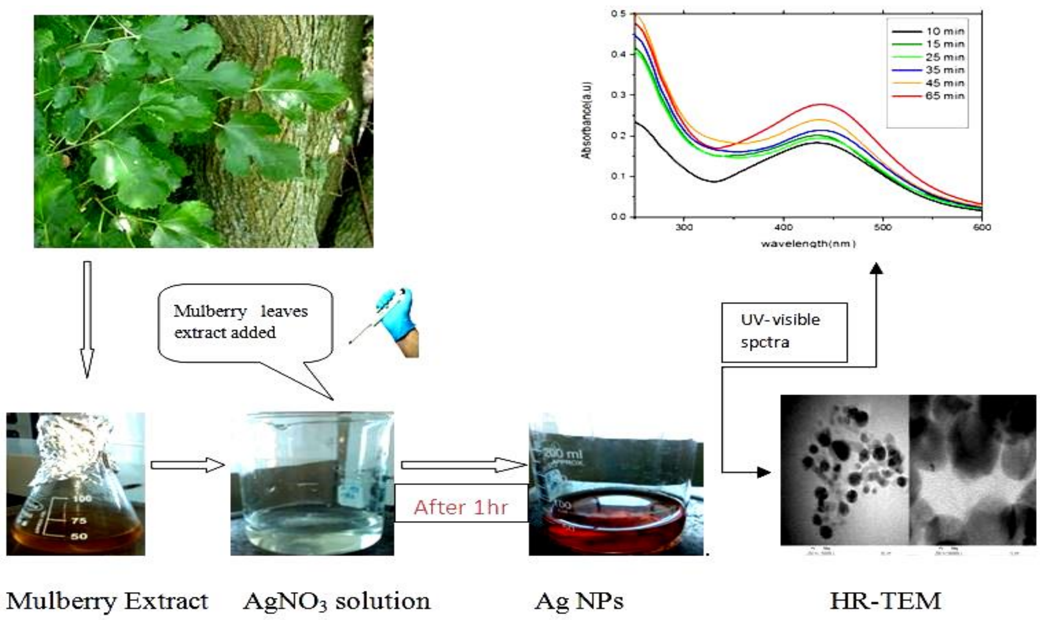


Fig. 1. Schematic representation of Ag NPs synthesis from leaf extract of mulberry in aqueous medium

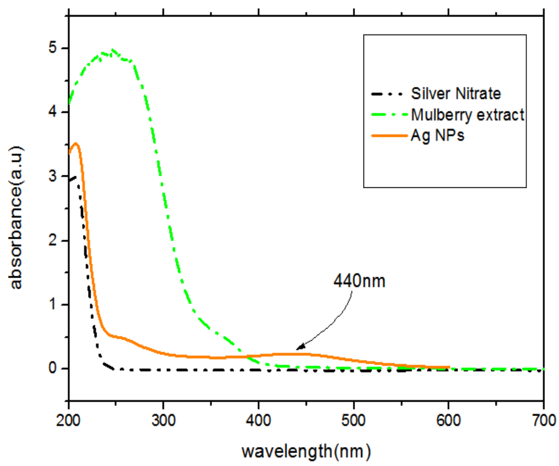


Fig. 2. UV-visible spectrum of a) $AgNO_3$ solution(Black dotted line), Mulberry extarct(green dotted line) and Ag NPs( orange line)

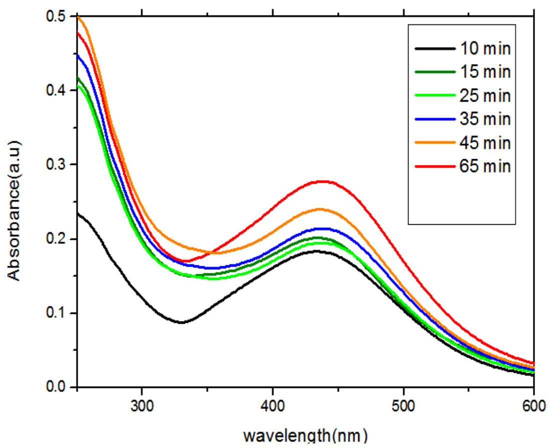


Fig. 3. UV-visible spectrum of bioreduced Ag NPs synthsized by 5ml mulberry extract at different time intervals

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 80.44 | Peak 1: | 95.62 | 98.3 | 39.82 |
| PdI: | 0.221 | Peak 2: | 4592 | 1.7 | 830.5 |
| Intercept: | 0.949 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality : | Good | | | | |

Size Distribution by Intensity

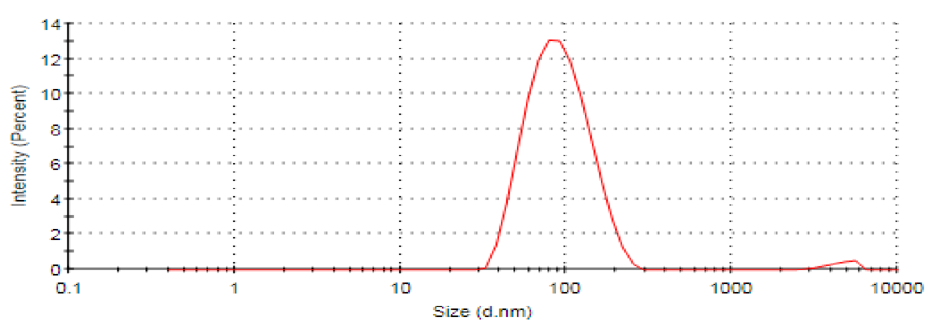


Fig. 4. Zetasizer of Biogenic silver NPs

biogenic silver nanoparticles was determined by HR-TEM. Fig. 5. showed HRTEM images of Bioreduced silver NPs prepared by 5ml Mulberry extract .The HR-TEM studies depicts that the prepared Ag NPs have spherical in nature and average size is about10-15 nm.

The compositional study was done using a technique called Energy-dispersive X-ray spectroscopy (EDS).The above data was collected using EDX700. Fig. 6 (a) and Table 1. shows noticable peaks.The highest peak is of silver and another peak shows minor traces of oxygen element also present in the final sample. The Spectra and the presence of Ag peak indicates the formation of silver nanomaterials.The oxygen peak is also present which maybe due to the fact that during the synthesis process a trace amount of oxygen from the environment would have combined with the sample which is the environmental oxygen present in the environment because the experiment of making silver nanoparticles from Mulberry leaves was done in open environment.Here Mulberry leaves was used as the main material,thus the above above spectroscopy peak confirms the formation of silver nanomaterial.

Table1. Show of weight% and atomic % for EDX of Biogenic Silver Nanoparticles .

| Element | Weight % | Atomic % |
|---|---|---|
| O K | 24.16 | 68.23 |
| Ag L | 75.84 | 31.77 |

The disc diffusion method was used for the determination of the Biogenic silver Nanopartilces antimicrobial activity against one Gram (+) ve (B. subtilis) bacteria and one Gram (-) ve (Escherichia coli (E. coli)) bacteria . The wells were poured with different concentration of Biogenic Silver nanoparticles 10, 20, 40, and 80µl. Distinct zone of inhibition were observed in all the wells filled with Biogenic NPs suspension there is a linear increment in the diameter of zone of clearance with increasing volume of the Silver NPs suspension added to the culture plates (Fig. 7). Hence the anti-microbial screening test confirmed that the biogenic Ag NPs possess enhanced the antimicrobial property against E. coli.bacteria and B.Subtilis.

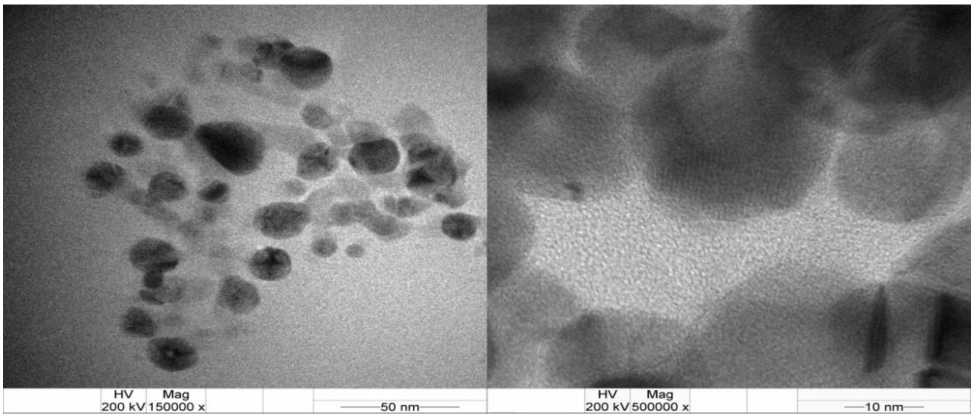


Fig. 5. HR-TEM images of Ag NPs

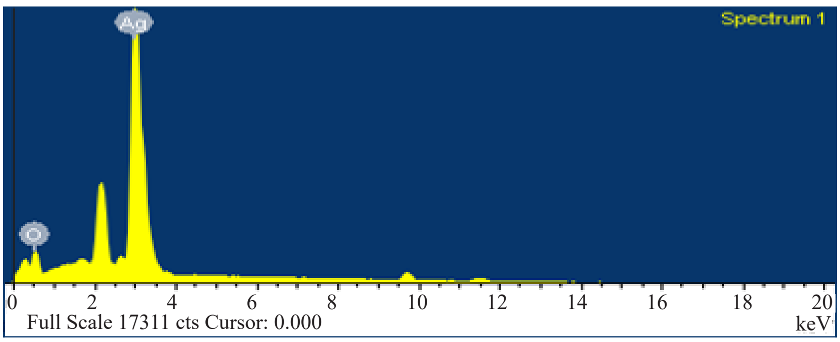


Fig. 6. Showing EDX of biogenic silver nanoparticles

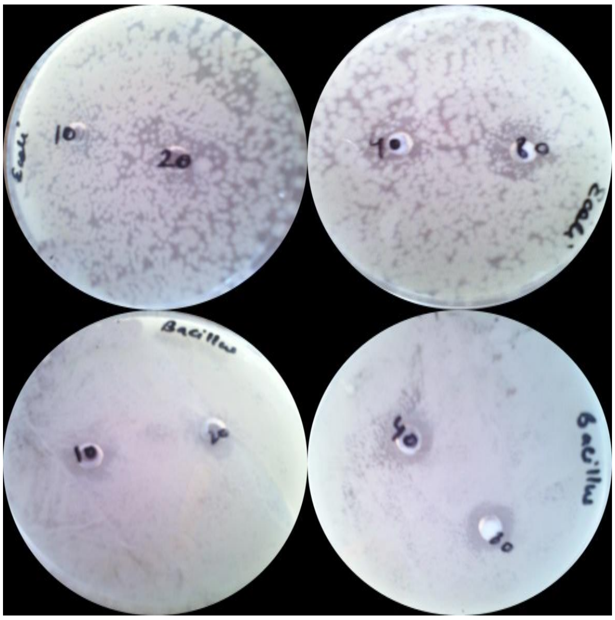


Fig. 7. Plates showing diameters of zones of inhibition of E. coli. (gram -ve)(at top) and B. subtilis (gram –ve)bacteria(at bottom)

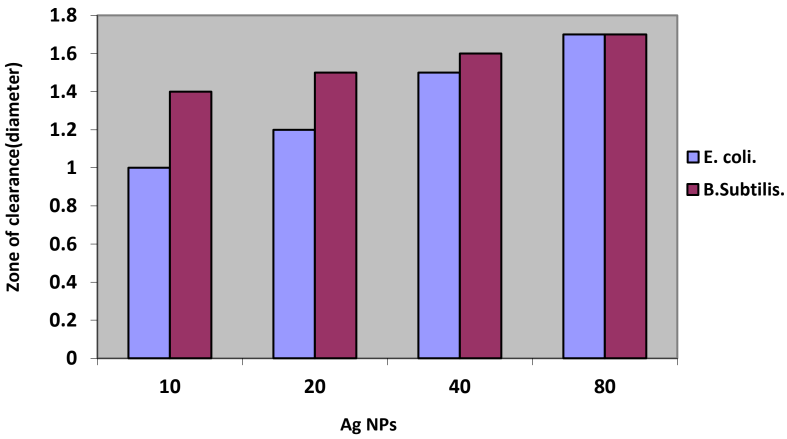


Fig. 8. Histogram of zone of clearance vs concentration of Ag NPs

## CONCLUSION

In this work, stable silver nanoparticles with 10-15 nm were produced using a simple one-pot synthesis method. The synthesized silver nanoparticles were found to be efficient in terms of their stability using Mulberry as the reducing agent which is eco-friendly and compatible with the environment rather than other chemicals used which are even toxic in nature The synthesised silver nanoparticles showed enhanced antimicrobial activities against both E. coli and B. subtilis method proved to be an efficient method for the preparation of Silver nanoparticles in terms of cost, environment affable and stability. Also, no external chemical for reduction is essential for the production of the same. Therefore, this synthesis can be a tough competent for various other ongoing chemical methods and have a potential application in biomedical, opto-electronics, medical devices and wastewater treatment.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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