

Green Synthesis of Silver Nanoparticles Using Bauhinia Variegata Aqueous Leaf Extract and their Enhanced Antimicrobial Activities

S. Divya Barathi¹, K.R. Padmapriya², M. Muthumari³, M. Pandeewaran⁴

^{1,2}Research Scholar, Chemistry, GTN Arts College (Autonomous), Dindigul

^{3,4}Associate Professor, Botany, GTN Arts College (Autonomous), Dindigul

Abstract

Silver nanoparticles (AgNPs) were synthesized by the reduction of aqueous solution of silver nitrate using an aqueous leaf extract of Bauhinia variegata (Mantharai) with different extract concentrations and interaction time at room temperature. The green synthesized Bauhinia variegata silver nanoparticles (BV-AgNPs) were analyzed and characterized by spectroscopic techniques viz. SEM, FT-IR and UV/Visible Spectrometry. The UV-visible spectra showed the maximum absorbance at 426 nm, which confirmed the formation of AgNPs. The BV-AgNPs showed the enhanced antimicrobial activities against gram positive bacteria (streptococcus aureus and staphylococcus) and a gram negative bacterium (Escherichia coli) isolated from the garden soil. These results demonstrated that Bauhinia variegata is a potential plant that can be used to produce ecofriendly, low cost AgNPs and used as an efficient antimicrobial agent for various biological applications.



Keywords: Silver nanoparticles (AgNPs), Mantharai leaf extract, Bauhinia variegata, Antibacterial activity; Green synthesis.

1. Introduction

Chemistry celebrated the end of the 20th century with a remarkable result of strategic importance it has approached the threshold of being able to detect a single molecule and identify its physical and chemical behavior. As a result a new field of chemistry has appeared, viz., the chemistry of single molecule. This new field defines precisely the upper boundary of yet another field of chemistry is called nano chemistry

. Which studies small ensembles of molecules (or atoms) comprising more than one species.

The AgNPs which are less likely to cause ecological damage have been identified as potential replacement of synthetic chemical insecticides, hence the need to use green synthesized AgNPs for the control of disease vectors. The AgNPs may be released into the environment from discharges at the point of production, from erosion of engineered materials in household products (antibacterial coatings and silver-impregnated water filters) and from washing or disposal of silver containing products [1]. Silver has been known to exhibit strong toxicity to a wide range of microorganisms and has been used extensively in many antibacterial applications [2]. The green synthesis of AgNPs by various plants has been reported, the potential of plants as biological materials for the synthesis of nanoparticles are yet to be fully explored [3].

Recently, AgNPs have emerged as promising antimicrobial agents and have been widely applied in cosmetic, textile, medical and food industries [4]. In particular, it has received much attention to develop the biosynthesis of AgNPs via different biological systems, such as bacteria, fungi, yeasts, algae and plants. Compared with the conventional chemical and physical methods, the green biosynthesis has many more advantages. In particular, during the biosynthesis of AgNPs, the components in the extracts from living organisms can act as reducing and stabilizing agents. Thus the use of toxic chemicals as the reducing, stabilizing and capping agent could be avoided. Moreover the bioactive compounds in the extracts are supposed to make nano particles. The AgNPs have unique properties and improve their characteristics [5-6].

In recent years, increasing antibiotic resistance by microbes is imposing serious threat in the health sector. Nanoparticles have proved to be a likely candidate as antimicrobial agent since their large surface to volume ratio ensures a broad range of attack on bacterial surface [7]. One of the most promising nanoparticles which acts as a highly effective antimicrobial agent is silver. Various investigations on silver nanoparticles have been done to study its antimicrobial activity. AgNPs exhibited significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and antifungal activity against *Candida* [8]. Considering the advantages of green synthesis over other methods, this study aims at the synthesis of AgNPs using aqueous *Bauhinia variegata* (BV) leaf extract (Mantharai or Segappu Mantharai in Tamil). It focuses on the study of the effects of various physico-chemical parameters on AgNPs. We also attempt to investigate the antimicrobial effect of the synthesized nanoparticles. *Bauhinia variegata*, which is a common plant known as Mantharai or Segappu Mantharai is found abundantly in India and in nearby Indian subcontinents. It belongs to Fabaceae family and is known for its various applications especially its medicinal property.

2. MATERIALS AND METHODS

A. Chemicals

Silver nitrate (AgNO_3) was purchased from Sigma-Aldrich, India. All solutions were prepared in Millipore water and all apparatus were rinsed with aqua regia (3:1 solution of HCL, HNO_3) and then washed with Millipore water before use.

B. Preparation of BV aqueous extract by Microwave Method

Bauhinia variegata leaves were thoroughly washed and sliced into small pieces. About 10 gm of the leaves were soaked in 100 mL of Millipore water and irradiated with Microwave for 10 minutes. The extract was then filtered and stored at 4°C which is used for further experiments.

C. Phytosynthesis of BV –AgNPs

For the phytosynthesis of AgNPs, about 5 mL of BV aqueous extract was added to 5 mL of 0.1 M AgNO_3 aqueous solution and kept in microwave oven at Micro level of 60°C with continuous microwave irradiation for 90 seconds. Rapid reduction of Ag^+ ions to Ag^0 was observed by the change in the color of the solution from yellowish brown to dark brown and BV-AgNPs synthesized were taken up for further study.

D. Characterization of BV Extract-AgNPs

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 **JASCO V730 UV–Visible spectrophotometer**. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 3000 rpm for 10 minutes and the resulting suspension was redispersed in 10 ml distilled water. The centrifuging and redispersing processes were repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by **JASCO FT-IR 400**. Scanning Electron Microscopic (SEM) analysis was done using **VEGA 3 TESCAN** machine. Thin film of the sample were prepared on a glass plate 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 minutes

E. Antibacterial Activity Study

The antibacterial activity of AgNPs synthesized from *Bauhinia variegata* leaf extract was investigated against pathogenic bacteria viz., the gram positive bacteria *Staphylococcus aureus*, *Streptococcus* and the gram negative bacterium *Escherichia coli* by using agar well diffusion assay method (9). Agar well diffusion method was used to determine the antibacterial efficacy of the synthesized compounds. The synthesized silver nano particles dissolved in DMSO were added to the wells at a concentration of 10mg/ml. the petriplates were incubated at 37°C for 24 hours. The zone of inhibition formed around the wells was measured with the help of antibiotic measurement scale and expressed in millimeters. The negative growth zones were measured only after 24 hours to avoid misleading results (10).

3. RESULT AND DISCUSSION

In order to determine completion of the reaction, AgNO_3 was reacted with BV aqueous extract and spectra were recorded. Fig.2 shows the UV- Vis spectra of the synthesized silver nanoparticles. The colour reduction of AgNO_3 into nanoparticles was visibly evident from the colour change. leaf extract was added into a silver nitrate solution. Within few minutes the appearance of brown colour was observed which indicates the formation of AgNPs (Fig 1). The color was changed from dark brown to light brown.

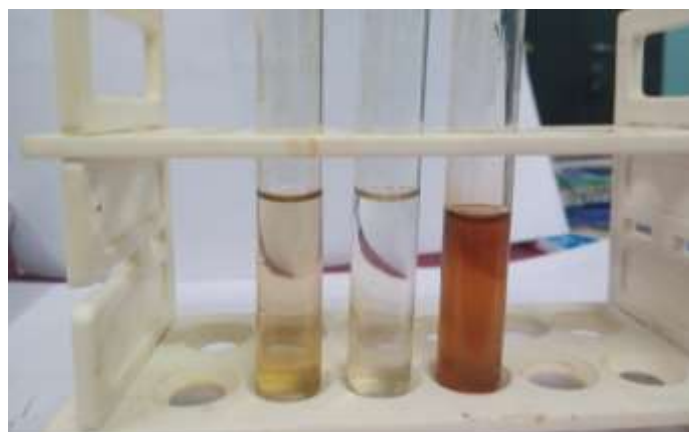


Fig.1 Colour change in reaction mixture of BV leaf extract + silver nitrate

This colour change indicates the formation of AgNPs. The dark brown colour of AgNPs is attributed to Surface Plasma Resonances (SPR) appearing at 426 nm.

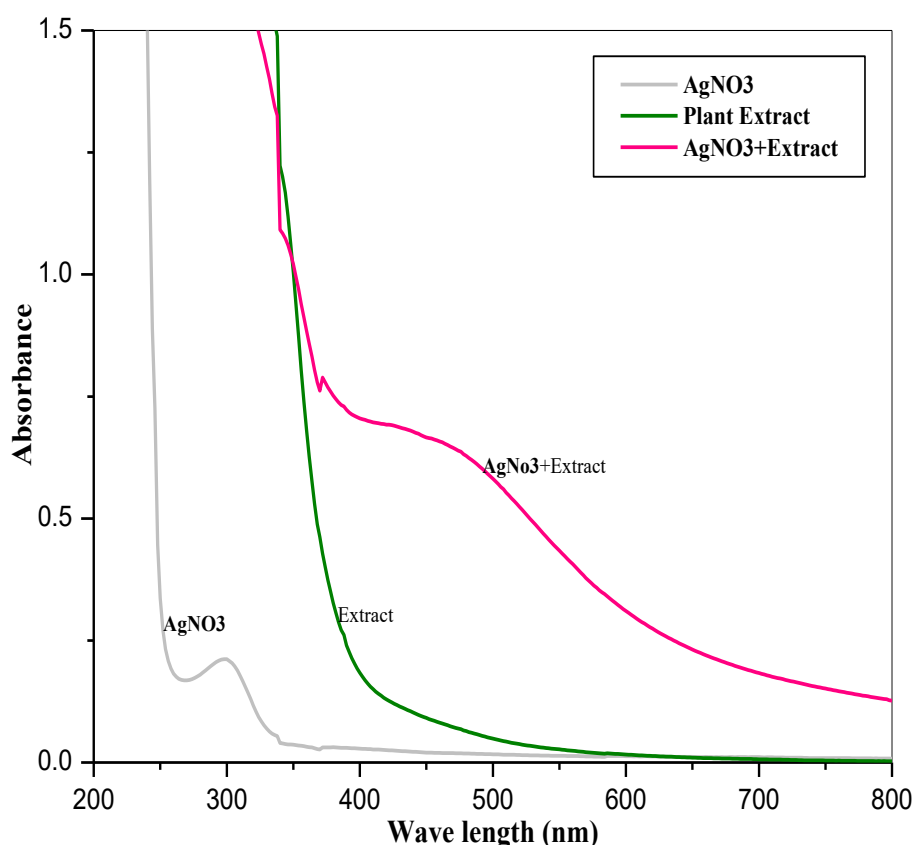


Fig.2. UV-Visible absorption spectra of AgNPs Synthesized by BV leaf extract

Fig. 3 and Fig. 3.1. As the concentration of leaf extract is increased, more number of bio molecules are available in the leaf extract which will be helpful for the metal reductive process.

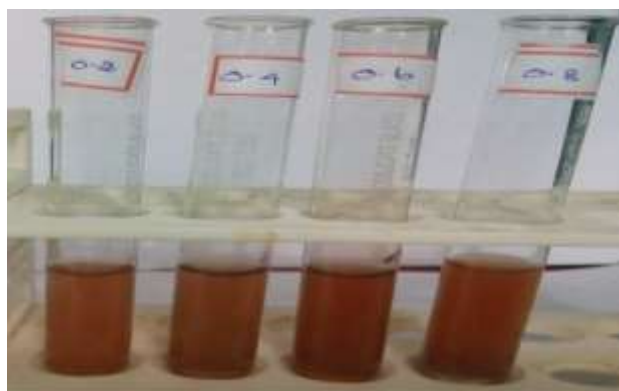


Fig.3 Concentration variation of plant extract

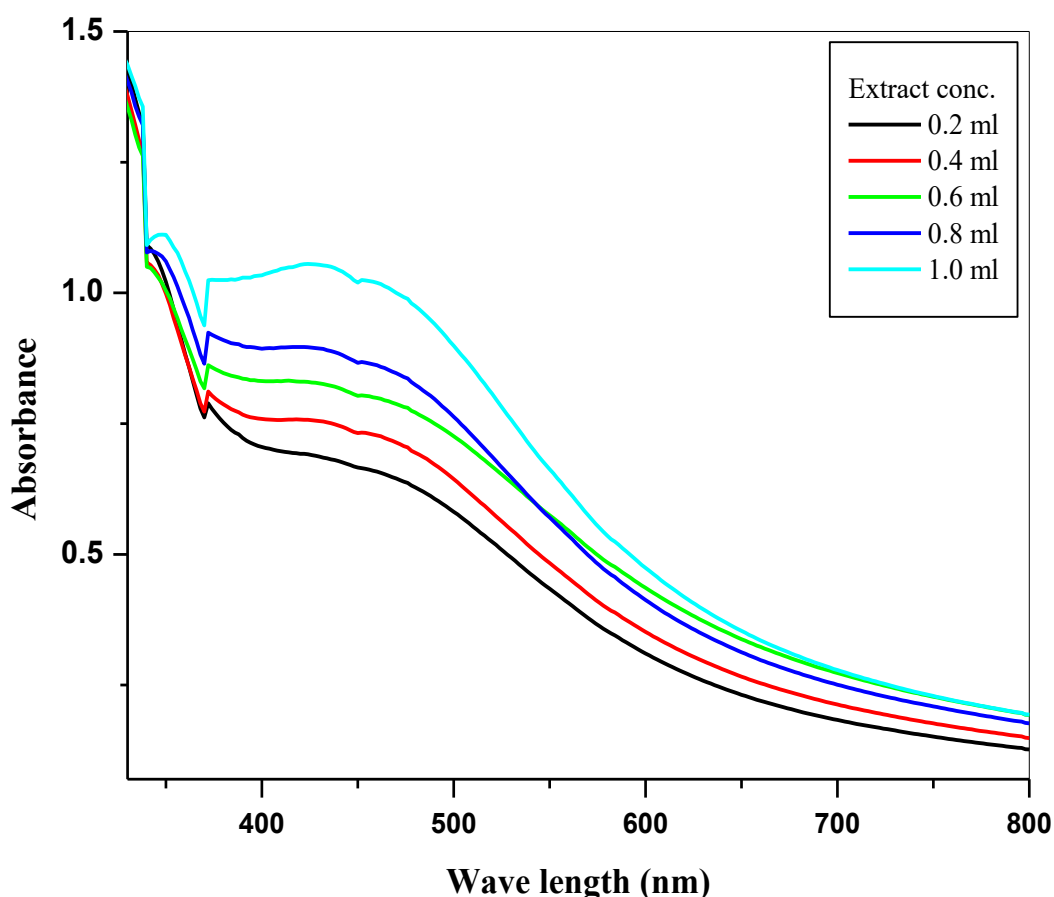


Fig.3.1 UV-Visible absorption spectra for concentration variation of plant extract of AgNPs Synthesized from BV leaf extract

Absorbance intensity increases with increasing extract quantity and increasing time (Fig. 4) and it is observed that the surface plasmon peak occurs at 426 nm with a slight shift in the peak towards shorter wavelength. Reduction of silver ions present in the aqueous solution of silver composite during the reaction with the ingredients present in the leaves of BV extract observed by the UV-vis spectroscopy revealed the presence of AgNPs which may be correlated with the UV-vis spectra.

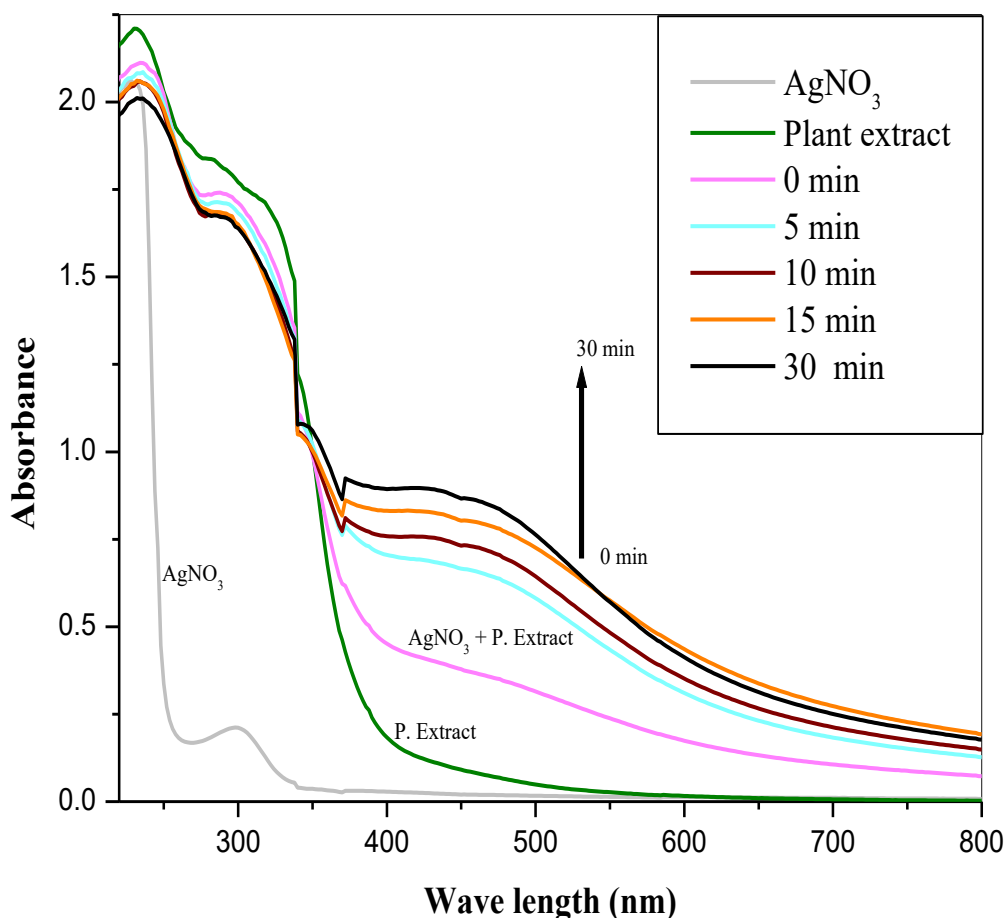


Fig.4 UV-visible spectra for time variation graph of BV-Ag nanoparticles, BV and AgNO_3 at room temperature.

FT-IR (Fig.5) spectrum of the extract, the absorbance bands at 650 , 1063 , 1408 , 1564 and 3424 cm^{-1} were associated with the stretch vibration of $-\text{C}-\text{O}$, $-\text{C}-\text{H}$, $-\text{C}=\text{C}$, CH_2 and $\text{O}-\text{H}$ respectively. These absorbance bands could be attributed to the reducing sugars, flavonoids, saccharides and proteins in the extract, while the absorbance band at 827 cm^{-1} could be regarded as a fingerprint of the biomolecules. In the present study, the band at 3383 cm^{-1} may be the result of the $-\text{C}-\text{O}$ groups of the reducing sugars, flavonoids, saccharides and proteins in the extract.

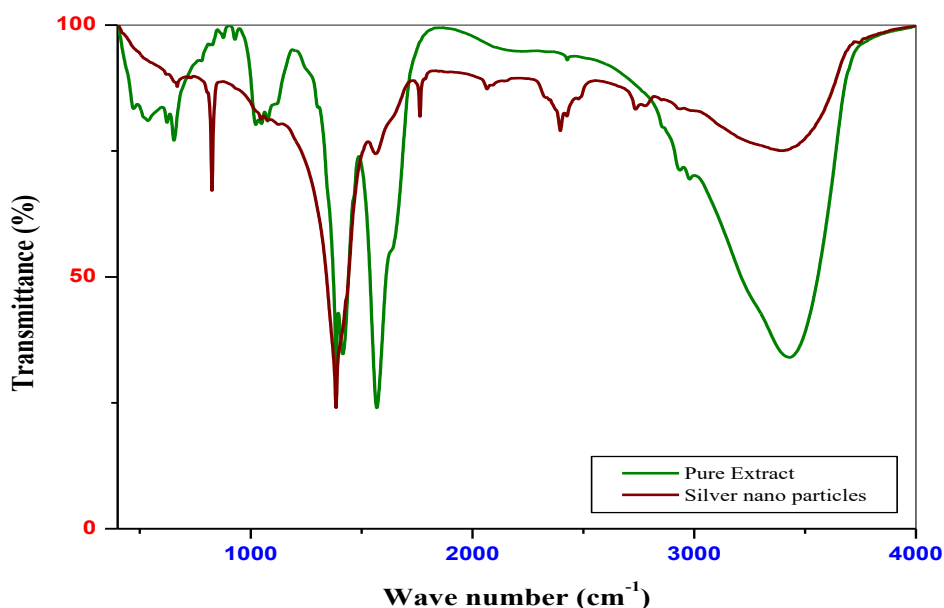


Fig.5 FT-IR spectra for the BV extract and BV-Ag nanoparticles

SEM image showing the high density silver nanoparticles synthesized by BV aqueous extract further confirmed the development of silver nanostructures Fig.6a, 6b, 6c and 6d.

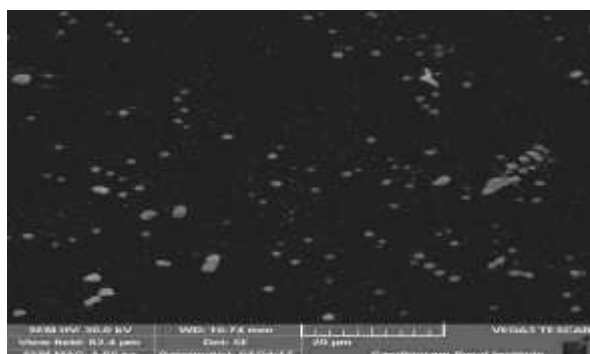


Fig.6a

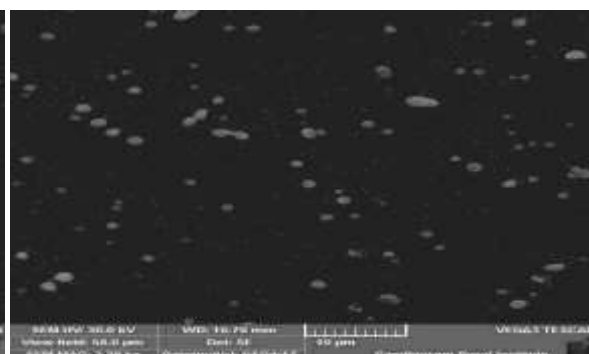


Fig.6b

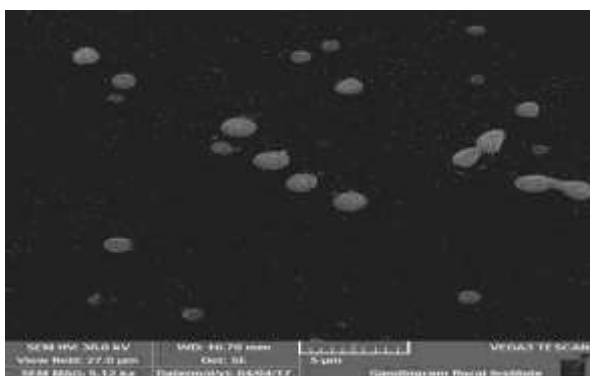


Fig.6c

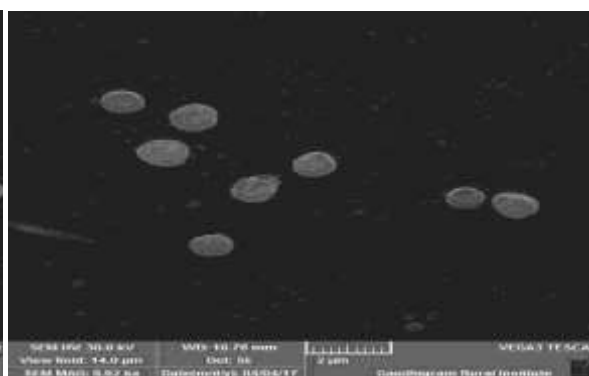


Fig.6d

TEM measurements and size distribution analyses of the AgNPs synthesized using BV leaf extract showed that the particles were spherical in shape with an average diameter of 25 nm and within 10 % of the size distribution, confirming that the particles were synthesized homogeneously in terms of their size and shape (Fig. 7 a-c). In a more detailed observation, it was seen that the BV-stabilized AgNPs. Furthermore, the synthesized NP solutions are well dispersed and stabilized over a long period of time. This is because of the high surface charges of the phyto-constituents hydroxyl groups as the coated stabilizer, their strong repulsive forces prevent the agglomeration of the AgNPs. The stability of the NP dispersion depends upon the balance of repulsive and attractive forces that exists between the NPs as they approach one another.

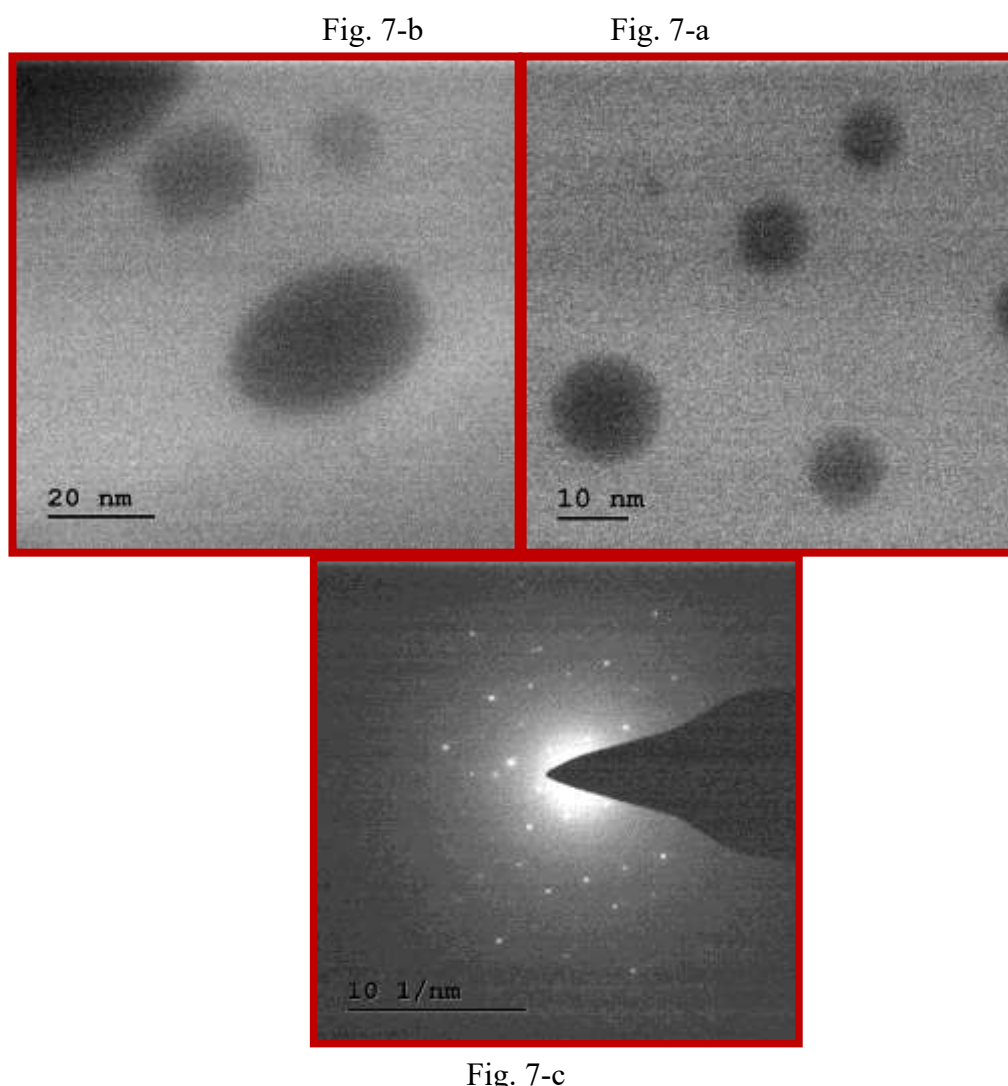


Fig. 7-c

Fig.7. TEM analysis of spherical silver nanoparticles fabricated by BV at magnification (a)100 nm, (b) 2 nm and (c) Selected area electron diffraction pattern of the AgNPs.

The results of antibacterial activity of the phytosynthesized silver nanoparticles with a larger surface area against both Gram-negative and Gram-positive microorganisms are presented in **Fig. 8a, 8b, 8c**. The details of inhibition zone around the disk is approximately 6 mm each with BV aqueous extract, BV-AgNPs and standard against the test strains at test concentration are shown in **Table 1**. Flavones,

polyphenols and isoflavones, were the major constituents of BV, and these compounds were most likely responsible for the antibacterial efficacy of the plant.

Table 1: Antimicrobial effect of synthesized silver nanoparticles from Bauhinia variegata leaf extract

S. No.	Name of the sample	Name of the organism and Zone of inhibition (mm)			
		Gram Positive		Gram Negative	
		Streptococcus	Staphylococcus aureus	E. coli	Candida (Fungi)
1.	Plant extract	4	3	4	4
2.	Plant extract + AgNPs	17	14	11	8

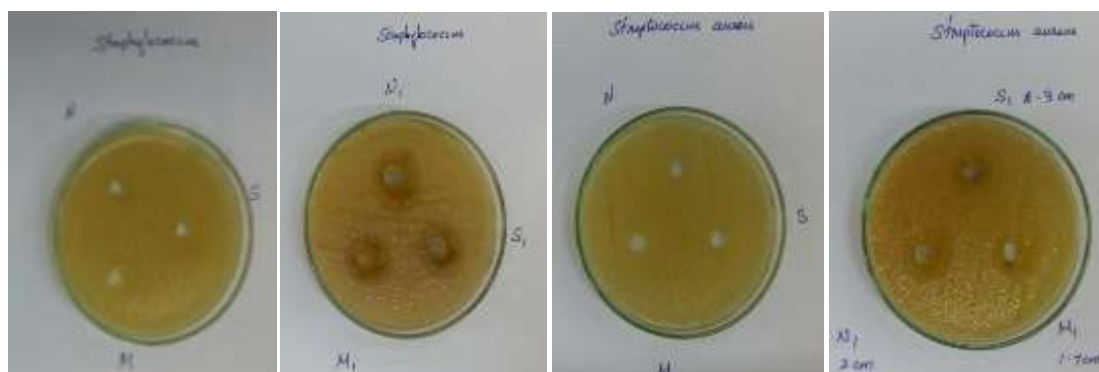


Fig.8a

Fig.8b

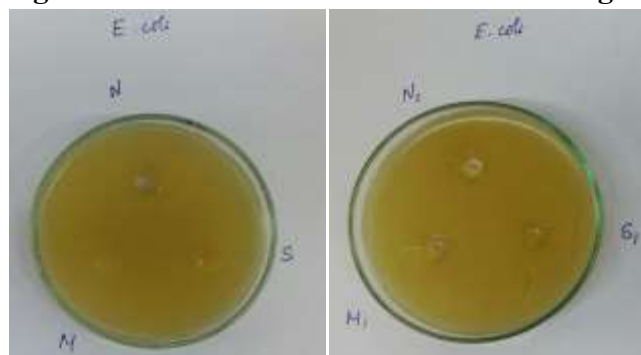


Fig.8c

CONCLUSION

In our study, it has been observed thus that BV – AgNPs synthesized under Microwave assisted condition using BV leaf extract were spherical in shape and they can be a good capping as well as a stabilizing agent for synthesis of BV – AgNPs. This green chemistry BV approach towards the synthesized of BV– AgNPs has many advantages such as, ease with which the process can be scaled up, high-yield, speed, and low cost etc.,. The evaluation of the antibacterial activities by agar well diffusion method, this investigation clearly shows microbial activity against gram positive bacteria staphylococcus aureus. In conclusion, a novel approach of synergistically enhancing the antimicrobial activity of antibiotics supplemented with AgNPs can be suggested. In future, this approach can also be used for suc

successful development of drug delivery.

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