Asian Journal of Innovative Research 2(1) 38-48 (2017)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH

Available online at http://www.asianjir.com

Research Article Chemistry

Received 10th Janu.2017;
Accepted 27 Feb. 2017
Online March 2017

SYNTHESIS OF NANOPARTICLES FROM *Ficus benghalensis* BARK AND EVALUATION OF ITS ANTIMICROBAL AND ANTIOXIDANTACTIVITY

M. Kavitha and V. Thirumurugan*

ABSTRACT

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of *Ficus benghalensis* it as reducing as well as capping agent. Nanoparticles are characterized using UV–Vis absorption spectroscopy and SEM analysis showed the average particle size of 20nm as well as revealed their cubic structure. Further these biologically synthesized nanoparticles are found to be highly toxic against different multi drug resistant human bacterial pathogens. Antioxidant activity is also confirmed by DPPH radical scavenging activity.

Keywords: Silver nanoparticles, Ficus benghalensis, Biosynthesis, SEM, Antimicrobial activity.

Citation: M. Kavitha and V. Thirumurugan (2017). Synthesis of nanoparticles from *Ficus benghalensis* bark and evaluation of its antimicrobal and antioxidant activity. Asian Journal of Innovative Research 2(1) 38-48 (2017).

M. Kavitha, Research scholar, PG &Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India.

***Corresponding author**
Dr. V. Thirumurugan, PG &Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur (Dt), Tamil Nadu, India.

1. INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology [1] and is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications [2].

Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution is reduced in conditions favouring the subsequent formation of small metal clusters or aggregates [3]. Recently, biosynthetic methods

employing naturally occurring reducing agents such as polysaccharides, biological microorganisms such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain AgNPs [4]. and 18% of women aged more than 60 years. Increases in life expectancy and aging populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020 (Woolf and Peger, 2003; Smith, 2005).

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity, they have various and severe adverse effects. Therefore, agents of natural origin with very little side effects are required as substitute of chemical therapeutics. Keeping this view the Phytochemicals and *in vitro* anti-inflammatory activity of the leaves of *Pergularia daemia*. was carried out The following aspects were analyzed to evaluate the anti-inflammatory activity.

In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. There are numerous work have been produced based on the plant and its extract mediated synthesis of nanoparticles. AgNP has been synthesized by using the plant broth from a wide variety of plants such as *Bacopa monnieri* [5] and *Catharanthus roseus* [6]. The medicinal value of study plant is *Ficus benghalensis*, belonging to the family of Moraceae and is commonly known as Banyan tree , Aalamaram in Tamil, Vata or Vada tree in Ayurveda. The main constituents in the *Ficus benghalensis* bark are carbohydrates, glycosides, tannins, steroids, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, leuco anthocyanidin, and leucoanthocyanin and gums & mucilage [7,8]. The present study to explore the novel approaches for the biosynthesis of silver nanoparticles using *Ficus benghalensis* bark.

2. MATERIAL AND METHODS

Chemicals

Materials used for the synthesis of silver nanoparticles is silver nitrate ($AgNO_3$), AR grade purchased from Merck, India.

Collection of plant materials

The fully mature bark of *Ficus benghalensis* were collected in April 2015 from river side trees Lalgudi, Trichy District, Tamil Nadu, India. The leaves were identified and authenticated by Botanist, Dr. S John Britto,Director, Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil nadu, India.

Preparation of extract

The barks of *Ficus benghalensis* were rinsed with water thrice to remove the fine dust materials. The barks were air dried for 15 days and then they were kept in air hot oven at 60°C for 36 hrs. The barks were ground to a fine powder. 50g of powdered material of sample were packed in soxhlet thimble and they were extracted using water as a solvent. The solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. Then, extract was kept in refrigerator at 4 ºC for future experiments.

Preparation of AgNPs and Standard solutions for the experiment

The dried bark aqueous extract of *Ficus benghalensis* was weighed (10mg/ml) and dissolved in sterile distilled water. Extract was mixed with silver nitrate(AgNO3) to make final volume concentration of 1mM solution. The reaction mixture was kept in dark room condition until the onset of colour change is observed. The colour changes in the reaction solution is watched carefully for the characterization of silver nanoparticles. Standard solution as Chloromphenicol (25mg/ml distilled water). They were kept under refrigerated condition unless they were used for the experiment.

Characterisation of Silver Nanoparticles

Silver nanoparticles were characterized by UV-Vis schimadzu 1600 spectrophotometer. The bio reduction was monitored in the UV absorption spectrometer from 300 to 700 nm range. Then the solution was centrifuged at 18,000 rpm for 30 min at room temperature to precipitate the nanoparticles . The resulting pellet was dissolved in deionised water and filtered through whatman filter paper No: 42. An aliquot of this filtrate containing silver nanoparticles were used for Fourier transmission Infrared spectroscopy (FTIR).

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using VEGA3 LMU machine. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Microorganisms

Escherichia coli, *Staphylococcus aureus* and *Candida albicans* were the microorganisms used and they were obtained from the Rontgen Diagnostic Laboratory, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Rontgen Diagnostic Laboratory, and Thanjavur.

Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare four discs approximately 6 mm in diameter, which were placed in hot air for sterilization After sterilization, each discs were loaded with 30µl of plant extract, $AgNO_3$ solutions. AgNPs and Standard solution as Chloromphenicol respectively and again kept under refrigeration for 24 hrs.

Antimicrobial assay

Antibiogram was done by disc diffusion method [10, 11] using plant extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab was dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli, S. aureus* and *Candida albicans* on Nutrient agar plates for bacteria and potato agar for fungi. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, $AgNO_3$ solutions. AgNPs and Standard solution as Chloromphenicol were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for fungi strains. Each sample was tested in triplicate.

Measurement of zone of inhibition

The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale. The diameter sizes in mm of the zone of inhibition are shown in the table 1.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, [12]. 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

Radical scavenging activity (%)

$$= 100 - \left(\frac{A_C - A_S}{A_C} \right) X\ 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

3. RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the leaf extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles The appearances of yellowish-brown colour (Figure 4**)** in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [13].

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants [14, 15]. Among them the plant mediated nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, *Ficus benghalensis* extract was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned to brown colour in 30 min with the addition of leaf extract (Figure 1 shows - $AgNO_3$ and AgNPs), indicating the formation of AgNPs in the reaction solution probably as a result of the excitation of surface plasmon resonance (SPR) bands [16]. The control tubes ($AgNO_3$) showed no change in colour when incubated in a similar condition.

SEM Analysis

SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density poly dispersed spherical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the bark extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size around 20nm as well the cubic, face-centered cubic structure of the nanoparticles (Figure 2 and 3).

Energy dispersive X –Ray spectroscopy analysis

Elemental analyses of silver nanoparticles were performed using Energy Dispersive X-ray Spectroscopy (EDX) equipped on SEM. The EDX spectrum (Figure 4) shows the presence of pure silver with the peaks.

UV spectral analysis

It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions**.** Figure 5 shows the UV-Vis spectra recorded from the reaction medium after 5 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 436nm, broadening of peak indicated that the particles are polydispersed.

FTIR analysis

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. FTIR spectrum of *Ficus benghalensis* extract shows bands at 696, 1124, 1401, 1566, 1637, 2086 and 3410 cm^{-1}. The FTIR spectra of the *Ficus benghalensis* is given in the Fig. 6 which show the presence of silver nanoparticles, peak at 3410cm^{-1} which are assigned as –OH stretching in alcohols and phenolic compounds [17]. The band appeared at about 1637 cm^{-1} is assigned for aromatic rings. The strong broad band appearing at 3410 cm^{-1}can be associated to the stretching vibrations of alcoholic and phenolic O–H. At 1124cm-1a peak is observed that could be for plant ascribed to multiplet C=O group.

Antimicrobial activity

Silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [18]. In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses [19]. Reduction of the particle size of the materials is an efficient and reliable tool for improving their biocompatibility that can be achieved using nanotechnology.

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extract has a new awareness for the control of disease, besides being safe and no phytotoxic effects [20]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The SNPs of *Ficus benghalensis* shows highest antimicrobial activity was observed against *E. coli*, *S. aureus* and *C. albicans*. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 were comparable with standard antimicrobiotic viz. chloromphenicol.

In this study, to evaluate the antimicrobial effects Ag nanoparticles against various microorganisms, such as *S. aureus*, *E. coli* and *C. albicans*. There were distinct differences among them. When Ag nanoparticles were tested they effectively inhibited bacterial growth. In this results, Ag nanoparticles showed antimicrobial activity against *E. coli* (Figure 7) that was similar to that found by [21]. In contrast, the inhibitory effect of Ag nanoparticles was mild in *S. aureus* and *C. albicans* (Figures 8 and 9) as compared with other microorganisms; these results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms is inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria [22]. We think that the lower efficacy of the Ag nanoparticles against *S. aureus* may derive from the difference as a point of membrane structure. To confirm this hypothesis, further comparative study between various gram-negative and gram-positive bacterial species is needed. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent. The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications.

DPPH Radical scavenging activity

The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for in vitro antioxidant study to understand the pharmacological role of medicinal plant and it's isolate. *In vitro* techniques have been used for detection of antioxidants, which are based on the ability of compounds to scavenge peroxy radicals [23, 24].

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in

absorbance. The antioxidant activity of *Ficus benghalensis* and AgNPs are shown in Figure 10. The AgNPs exhibited a significant dose dependent inhibition of DPPH activity. AgNPs possess probable antioxidant activity as compared with plant extract.

DPPH free-radical scavenging activity Free radicals are harmful by-products generated during normal cellular metabolism, which could initiate oxidative damage to body[25]. Antioxidants are believed to play a significant role in the body's defence system against free radicals. Recently, numerous reports have described antioxidants and compounds with radical-scavenging activity present in fruits, vegetables, herbs and cereals extracts [26]. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants [27].

XRD

The X-ray diffraction measurement of the bioreduced silver nanoparticles drop coated onto glass substrates are done for the determination of the formation of silver by an X-ray diffractometer instrument operated at a voltage of 40 kV(Figure.11). XRD spectrum of synthesized silver nanoparticles showed five intense peaks at the 2θ values of 32.5º, 38.4º, 48.5º,64.8 and 77.7º these five peaks corresponded to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of crystalline silver, respectively. The crystallite domain size was calculated from the width of the XRD peaks, using the Scherrer formula. The average crystallite domain size of the particle is 13.2nm.

$$D = \frac{K\lambda}{B_{1/2}\cos\theta}$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, **λ**- is the X-ray wavelength, β - is the full width at half maximum (FWHM) and θ is the diffraction angle.

The present study concluded that the bio-reduction of silver ions through *Ficus benghalensis* extract and testing for their antimicrobial activity. The aqueous silver ions exposed to the extract, the synthesis of silver nanoparticles are confirmed by the change of colour of plant extract. These environmentally benign silver nanoparticles are further confirmed by using SEM. The SEM analysis showed the particle size around 20nm as well the cubic structure of the nanoparticles. The results indicated that silver nanoparticles have good antimicrobial and activity against different microorganisms such as *S. aureus, E. coli* and *C. albicans*. It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. The Antioxidant activity was also confirmed by DPPH radical scavenging activity. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

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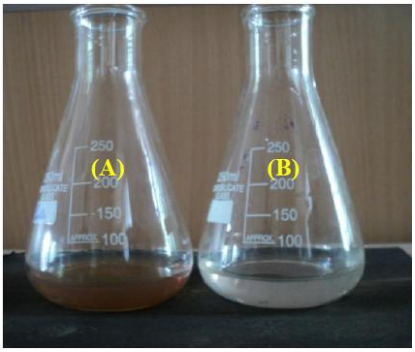
Source of support: Nil; Conflict of interest: None declared

Figure 1 Colour changes after (AgNPs) the process of reduction of Ag^+ to Ag nanoparticles and control ($AgNO_3$)



(A) = 1 mM $AgNO_3$ with *Ficus benghalensis* after 5 hrs of incubation (Brown colour)

(B) = 1 mM $AgNO_3$ without *Ficus benghalensis* extract.

Figure 2 High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs around 20nm.

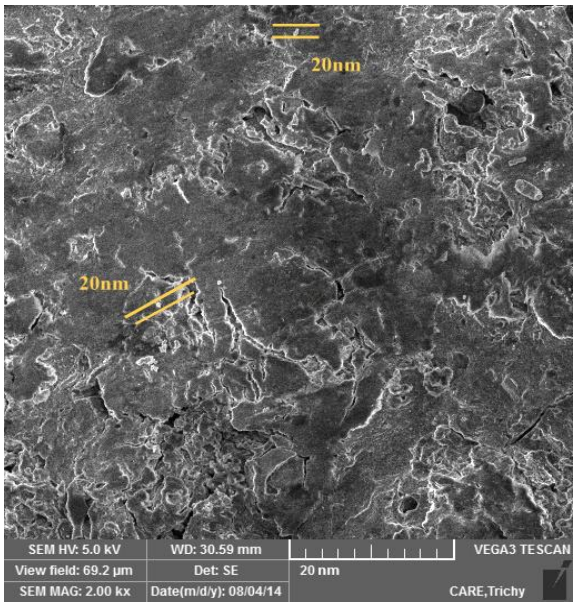


Figure 3 Capturing a high resolution scanning electron microscopic image of Ag nanoparticles

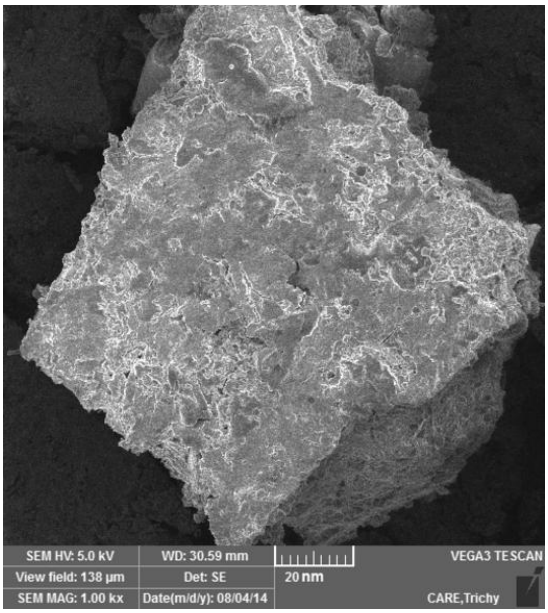


Figure 4 EDAX pattern of Ag nanoparticles with *Ficus benghalensis* extract.

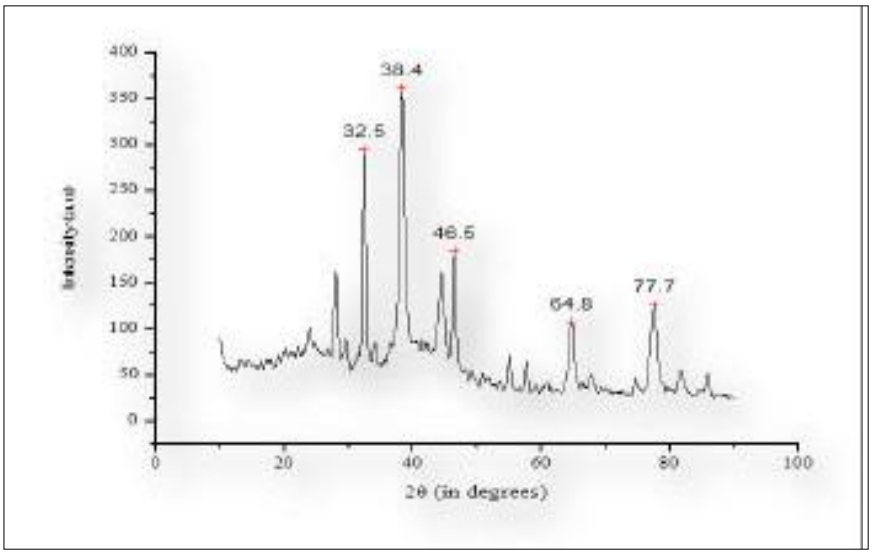


Figure 5 UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Ficus benghalensis* extract.

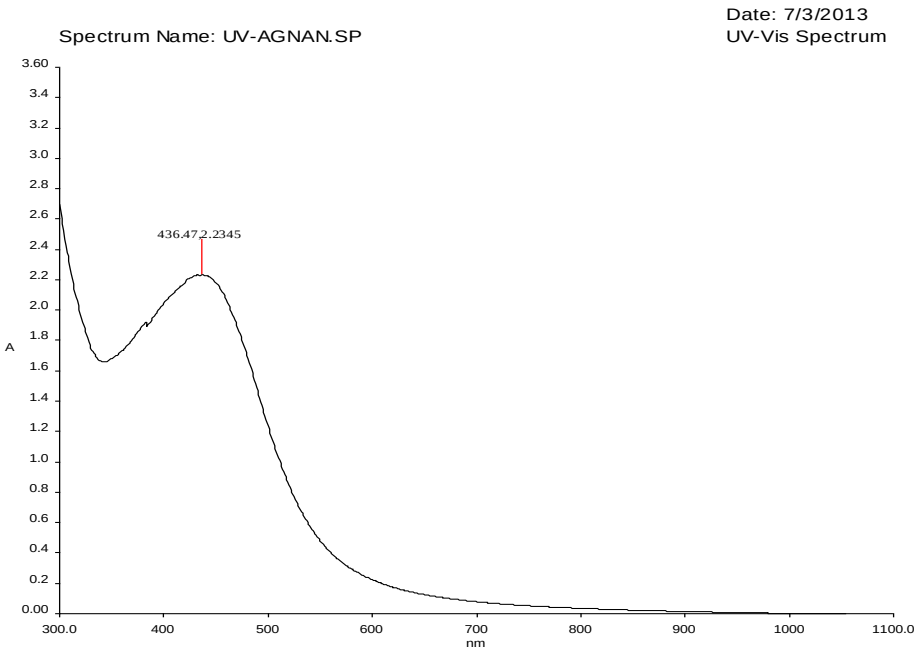


Figure 6 FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Ficus benghalensis* extract.

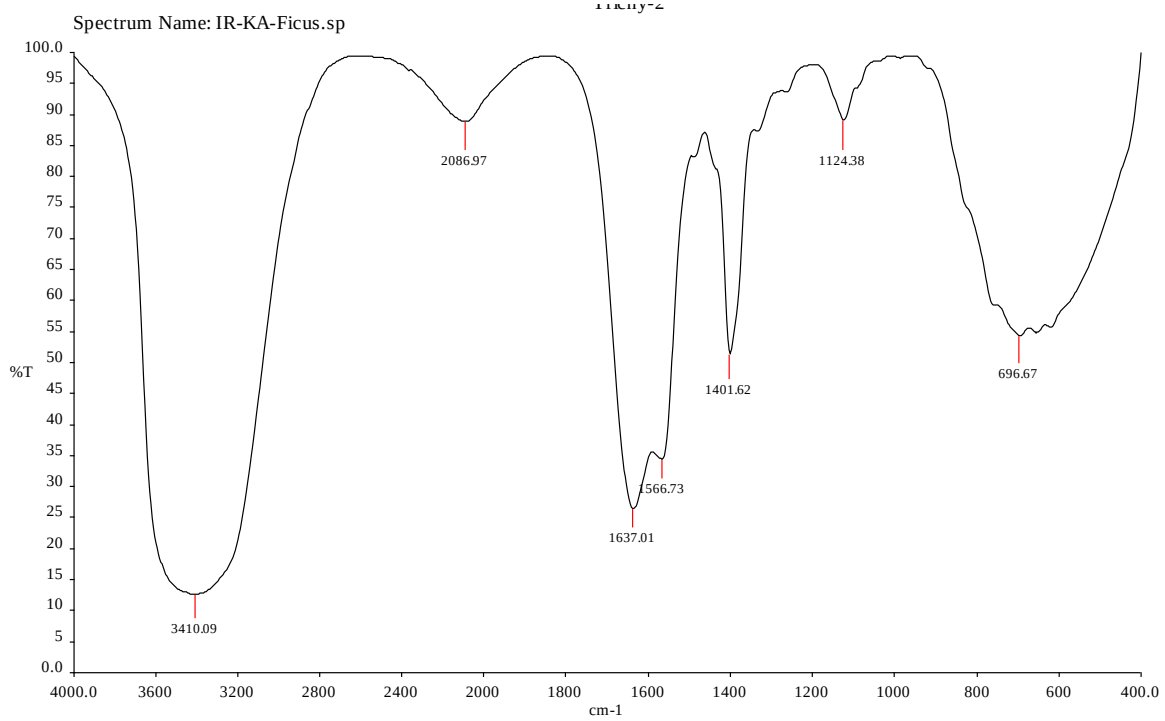


Table 1 Antimicrobial activity of AgNPs, $AgNO_3$ and *Ficus benghalensis* bark extract

Sample	Concentrations	*Escherchia Coli* (mm) (30µl)	*Staphylococcus aureus* (mm) (30µl)	*Candida albicans* (mm (30µl))
$AgNO_3$	30µl	8±0.56	4±0.28	3±0.21
Plant extract	30µl	5±0.35	6±0.42	1±.07
AgNPs	30µl	11±0.77	9±0.63	6±0.42
Standard (Chloromphenicol)	30µl	10±0.70	12±0.84	---
Standard (Flucanazole)	30µl	--	--	18±1.26

Values were expressed as Mean ± SD.

$AgNO_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

Figure 7 shows the antibacterial (*E. Coli*) activity of AgNPs and *Ficus benghalensis* aqueous extract $AgNO_3$ standard Chlorompenicol

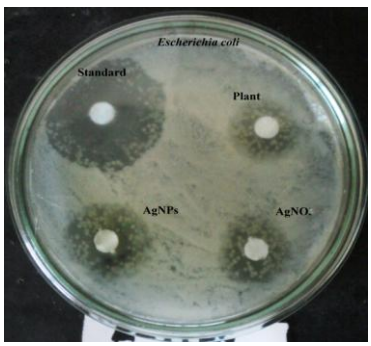


Figure 8 shows the antibacterial (*S. aureus*) activity of AgNPs and *Ficus benghalensis*

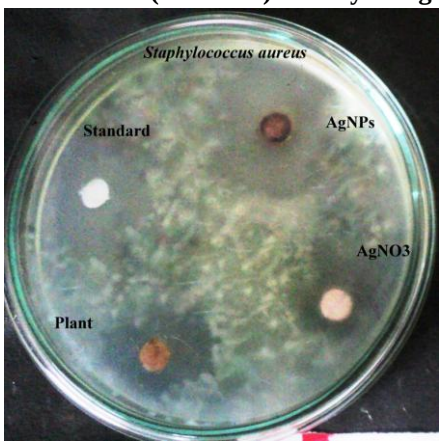


Figure 9 shows the antifungal (*Candida albicans*) activity of AgNPs and *Ficus benghalensis*

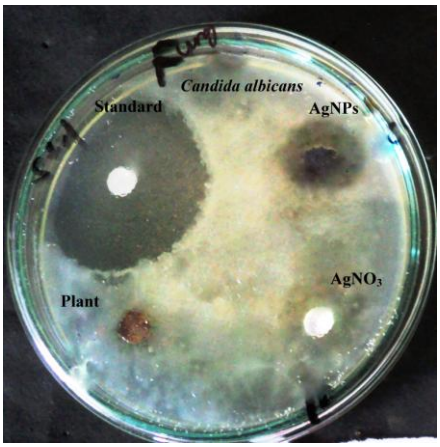


Figure 10 DPPH radical scavenging activity of *Ficus benghalensis* and standard (Ascorbic acid)

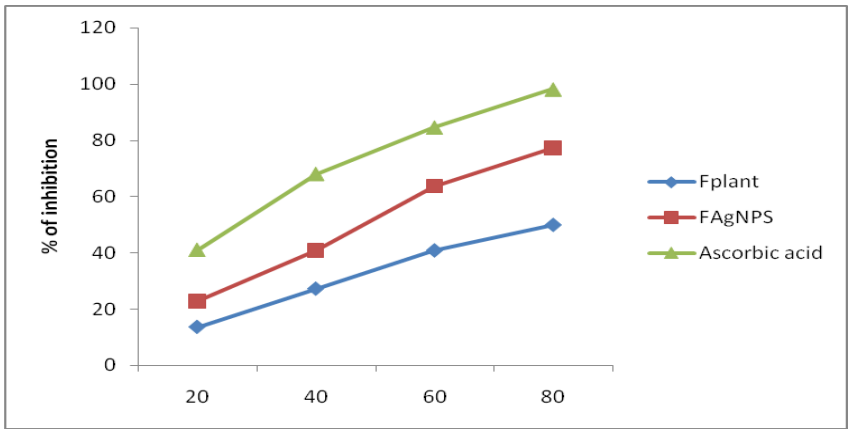


Figure 11 XRD analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Ficus benghalensis* extract.