IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
October 2016 Vol.:7, Issue:3


# Fruit Mediated Synthesis of Silver Nanoparticles, Characterization and their Antimicrobial Activity using *Thunbergia alata* Bojer ex sims

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ISSN 2349-7203
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**Basavarajeshwari Hedaginal[1] and T. C. Taranath*[1]**

[1]*Department of Botany, Karnatak University, Dharwad – 580 003, Karnataka, India.*

**Submission:** 30 September 2016
**Accepted:** 5 October 2016
**Published:** 25 October 2016


**www.ijppr.humanjournals.com**

**Keywords:** Biogenic**,** *Thunbergia alata* Bojer ex sims , Silver nanoparticle, Antibacterial, antifungal, HR-TEM.

**ABSTRACT**

The multidrug-resistant strains are a major problem in the control of infections in hospitals. The smaller size of nanoparticles is gaining importance in research for the treatment of various diseases therefore, we tried to establish eco-friendly, cost efficient small sized silver nanoparticles (AgNPs) by using fruit extract of *Thunbergia alata* Bojer ex sims (*T. alata*). These AgNPs are powerful tool against multidrug-resistant bacteria. The synthesized silver nanoparticles were characterized by UV-visible spectroscopy, atomic force microscopy, energy dispersive spectroscopy and fourier transform infrared spectroscopy, high resolution transmission electron microscopy and X-Ray diffraction. The nanoparticles appeared to be spherical in shape and the size of the particles varied from 10 to 20 nm. Further antibacterial and antifungal activity of silver nanoparticles synthesized from *T. alata* against multidrug resistant strains was determined by agar well diffusion assay and minimum inhibitory concentration (MIC) was estimated.. In summary the *T. alata* Fruit mediated AgNPs showed acceptable size and possess very good antimicrobial activity which makes them a potent source of antimicrobial agent.

## 1. INTRODUCTION

Nanotechnology is a fast growing area in the field of science which is an interdisciplinary field of both science and technology that increase the scope of investing and regulating at cell level between synthetic material and biological system [1, 2]. A rapid step in synthesis and applications of nanomaterial's, in recent years has been invented in almost every domain of life including health care, cosmetics, biomedical, food and feed, drug - gene delivery, environment, electronics, mechanics, catalysis, energy science, optics, chemical and space industries [3]. Nanoparticles (NPs) of noble metals, such as gold, silver, platinum, and zinc oxide are widely used in medical and pharmaceutical applications, and in an array of consumer products [4]. Synthesis of NPs has been reported using various chemical and physical methods, such as sol-gel process, chemical precipitation, chemical vapor deposition, hydrothermal and microwave methods [5]. Although chemical & physical methods are very successful in producing well- defined nanoparticles, they have certain limitations such as increase cost of production, release of hazardous by-products, long time for synthesis and difficulty in purification. Global warming & climate change has induced a worldwide awareness to reduce the toxic & hazardous waste materials, thus, the green synthesis route has raised actively the progress in the fields of science & industry [6]. Biosynthesis of nanoparticles as the name indicates help in the synthesis of very complex reaction within a fraction of minutes has now taken up the attention towards synthesis protest the need of environmentally benign technologies in material science. Use of biological organisms such as microorganism, plant extracts and biomass could be a best alternative method of physical and chemical method for synthesis of nanoparticles because the biological or green synthesis route is very spontaneous, economic, environmental friendly and non-toxic. The major biological systems involved in this are bacteria; fungi [7] and plant extract [8]. In recent years, the biosynthesis of nanoparticles using plant extracts has gained more significance. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, practical, scalable, nontoxic and avoidance of maintaining the microbial culture [9]. In most cases, they provide broad variety of metabolites which can aid in the reduction of silver ions and are quicker than microbes in the synthesis method. Different plants have been successfully used for the synthesis of biogenic metal nanoparticles [10]. Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer diagnosis and treatment [11, 12]. Biomedical applications; being added to wound

dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities. They are even being projected as future generation antimicrobial agents.

In the present investigation, we report the easy synthesis of silver nanoparticles by an environmental friendly method by using *T. alata* fruit extract and the evaluation of their antimicrobial activity against various human pathogenic microorganisms.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Microorganism

Analytical grade chemicals were used - Silver nitrate and sodium hydroxide. All glass wares were washed with sterile water and dried in an oven before use. Experimental plant *Thunbergia alata* Bojer ex Sims (Black-eye Susan vine) fruits were collected from the Karnatak University campus Dharwad, Karnataka, India. It is a flowering evergreen vine of the Acanthaceae family Native to tropical and southern Africa. (Fig.1.). Saponins, steroids, tannins and phenolic compounds present in *T. alata*

**Fig. 1. Experimental plant *Thunbergia alata* Bojer ex Sims and its fruits**

### 2.2. Preparation of fruit extract

Fruits of *Thunbergia alata* Bojer ex Sims were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities. Fruits were shade dried to remove the residual moisture and about 25gm were cut into small pieces and boiled in glass beaker

containing 250ml of sterile distilled water for 20 minutes. The aqueous extract was separated by filtration with whatman no. 1 filter paper and stored in refrigerator at $4^0$C for further use.

### 2.3. Phytosynthesis of Silver nanoparticles

For reduction of silver ions, 10 ml of fruit extract was added to 90 ml of 1mM aqueous $AgNO_3$ solution taken in erlenmeyer flask (250ml). Simultaneously, the reaction mixture was adjusted to pH 8 by using 1 N. NaOH. Then the flask containing reaction mixture was incubated at 40-$60^0$C, resulting in the formation of pale yellow to dark brown solution indicating the synthesis of silver nanoparticles.

### 2.4. Detection of silver nanoparticles

A number of different measurement techniques were used for detection of Ag-NPS., including UV-Vis spectroscopy, Fourier Transform Infrared (FTIR), Atomic Force Microscopy (AFM), High-Resolution Transmission Electron Microscopy (HR-TEM), X–Ray Diffraction (XRD) and Energy dispersive spectroscopy (EDS).

### 2.5. Characterization of nanoparticles

The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution according to the method of Mie (1908), by the sampling of aliquots (3ml) of the aqueous component. The silver nanoparticles were measured in a wavelength ranging from 200-800nm. The UV-Vis spectroscopy measurement of silver nanoparticle was recorded on UV- Vis spectroscopy (Jasco V- 670 UV-Vis NIR spectrophotometer) operated at resolution of 1nm. The solution containing reduced silver ions was centrifuged at 3000 rpm for 40 min to remove the unwanted biomass residue; the resulting suspension was then dispersed in 10ml of double distilled water and centrifuged again at the same condition. Redispersion and centrifugation process was repeated for 2-3 times to obtain silver nanoparticles free from any biomass residue. A sample taken from pellet was dispersed on a slide and dried slide was observed on contact mode of AFM. The pellet thus obtained was redispersed in double distilled water and oven dried at $60^0$C to obtain the powder. The powder was used for FTIR and HRTEM (TECNAI 20 G2-electron microscope), X-ray diffraction (XRD) analysis and SEM with EDX analysis (Fei Quanta 200 SEM EDAX Genius Xm 4).

### 2.6. Antibacterial activities

The silver nanoparticles synthesized using *Thunbergia alata Bojer ex Sims* fruit extract were tested for antimicrobial activity by agar well diffusion method against human pathogenic *Staphylococcus aureus , Bacillus subtilis, Bacillus polymyxa, Escherichia coli, Salmolella typhi, , Vibrio cholera, Aspergillus niger and Candida albicans*.This method depends on the radial diffusion of an antibiotic from the well through semisolid agar layer in Petri plate, which prevents the growth of bacteria in a circular area or the zone around the well. The pure cultures of bacteria were sub-cultured on nutrient broth at 35 $^0$C. The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm were made on nutrient agar plates using gel puncture. Different concentrations of silver nanoparticles (25, 50, 100, 200, 400 µl) solution were poured on to four wells and in one well 400 µl of plant extract poured as control on all plates using micropipette. After incubation at 37 $^0$C for 24h for bacterial strains and 96h for fungal strains, the diameter of zone of inhibition was measured in millimeters and tabulated.

## 3. RESULTS

### 3.1. Characterization of silver nanoparticles

Addition of fruit extract to $AgNO_3$ the colour of the reaction mixture changes from pale yellow to dark brown (Fig. 2) within few seconds and after incubation time (24 hours) the walls of the Erlenmeyer flask (which contains reaction mixture) showed mirror like illumination, it clearly indicates the formation of silver nanoparticles in the reaction mixture. The UV-visible spectroscopic studies on the synthesis of silver nanoparticles (Fig. 3) have shown an absorbance at 420 nm due to surface plasmon resonance (SPR).

### 3.2 Study of effect of physicochemical parameters on the nanoparticles synthesis

Based on UV–Vis spectroscopy the effect and interaction of various physicochemical parameters were optimized which would increase the yield of nanoparticle synthesis. Various parameters such as concentration of the fruit extract and $AgNO_3$, pH, temperature and incubation time were optimized for the reduction of $Ag^+$ ions to AgNPs using *Thunbergia alata* Bojer ex sims extract. The maximum yield of AgNPs is 1 mM, this concentration was selected for further studies. Among the various parameters, pH is one of the fundamental

factors in nanoparticle synthesis. Among 8, 9, 10 pH, the reaction started rapidly at pH 8 of the reaction mixture (as observed by the change in color). The optimal pH for nanoparticle synthesis was preferred to be pH 8.

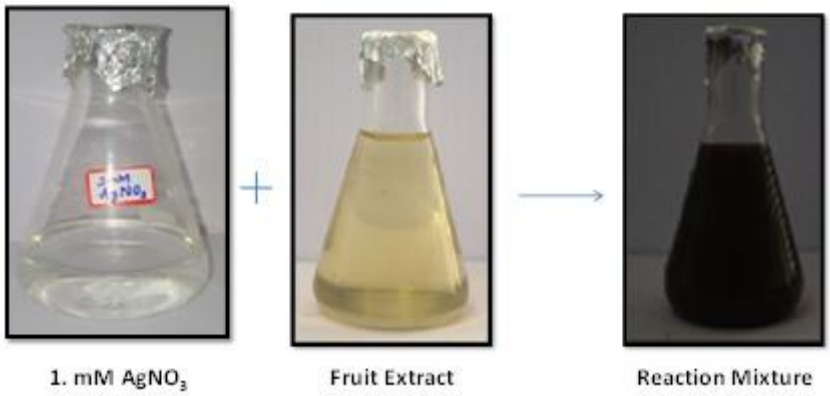


**Fig. 2. Visual observation of the formation of silver nanoparticle synthesis**

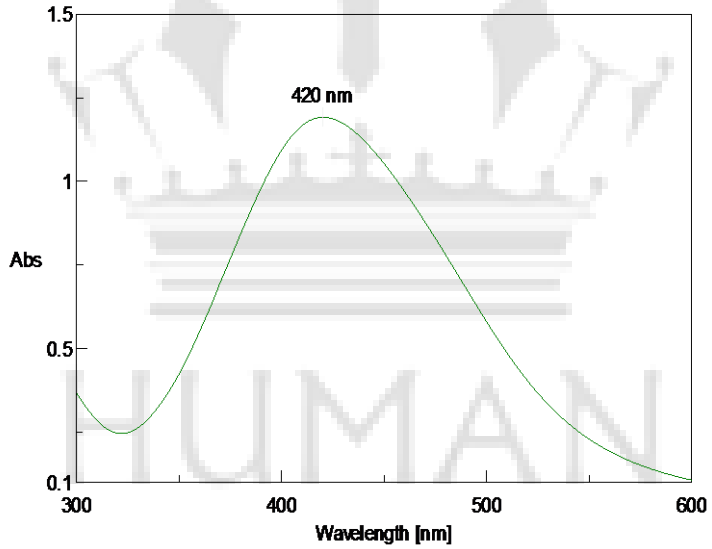


**Fig. 3. UV-Vis spectrum of AgNPs in an aqueous solution.**

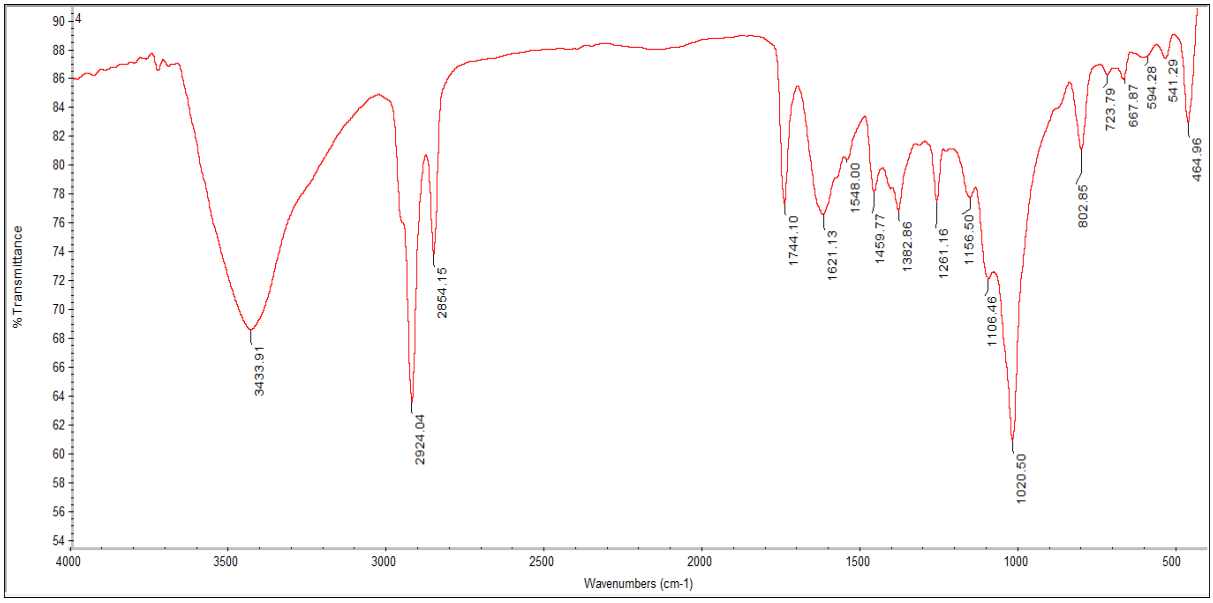


**Fig.4. FTIR spectrum of Ag-NPs synthesized from fruits of *Thunbergia alata* Bojer ex sims**

**Table 1. FTIR absorption peaks and their functional groups of Silver nanoparticles synthesized from fruits of *Thunbergia alata* Bojer ex sims**

| Sl. No. | Absorption peaks ($cm^{-1}$) | Functional groups. |
|---|---|---|
| 1 | 3433 | Indicative of OH stretching H-bonded alcohols and phenols (O-H Amide N-H Stretch) |
| 2 | 2924 | O-H stretch Carboxylic acid, Alkyl C-H Stretch |
| 3 | 2854 | C-H stretch region for the aldehyde, aldehyde hydrogen bond(-CHO). |
| 4 | 1744 | C=O aldehyde saturated aliphatic |
| 5 | 1621 | N-H, C=C |
| 6 | 1459 | Nitro group show strong bands & overlaps the aromatic ring region. Stretching & bending of $1^0$ & $2^0$ amines & amides takes place. |
| 7 | 1382 | C-H, C-N alkyl halide and aliphatic amine |
| 8 | 1261 | C-Cl strongly stretches in aliphatic chlorides, S-O |

| | | |
|---|---|---|
| | | stretch strongly |
| **9** | **1156** | Aromatic amines |
| **10** | **1020** | C-N stretching vibrations of aliphatic amines |
| **11** | **602** | Chloride, Alcohols, Ethers, Esters, Carboxylic acids, Anhydrides present. |

Identification of the biomolecules involved in the formation of silver nanoparticles was done using FTIR. The FTIR spectrum of silver nanoparticles synthesized from fruits of *Thunbergia alata* Bojer ex sims (Fig. 4) Showed absorption peaks located at 3433 $cm^{-1}$, 2924 $cm^{-1}$, 2854 $cm^{-1}$, 1744 $cm^{-1}$, 1621 $cm^{-1}$, 1459 $cm^{-1}$, 1382 $cm^{-1}$, 1261 $cm^{-1}$, 1156 $cm^{-1}$, 1020 $cm^{-1}$ and 602 $cm^{-1}$. The peak at 3433 $cm^{-1}$ is indicative of OH stretching H-bonded alcohols and phenols (O-H Amide N-H Stretch), the peak at 2924 $cm^{-1}$ is due to the O-H stretch carboxylic acid, Alkyl C-H Stretch, the band at 2854 $cm^{-1}$ is responsible for the C-H stretch region for the aldehyde, 1744 $cm^{-1}$ is responsible for the C=O aldehyde saturated aliphatic, 1621 $cm^{-1}$ is due to N-H and C=C bonds, 1459 $cm^{-1}$ is formed by nitro group and stretching of $1^0$ & $2^0$ amines takes place, 1382 $cm^{-1}$ is responsible for the C-H, C-N alkyl halide & aliphatic amines and 602 $cm^{-1}$ is due to chloride, alcohols, ethers, esters and carboxylic acids. Absorption peaks value and their functional groups are represented in table no. 1

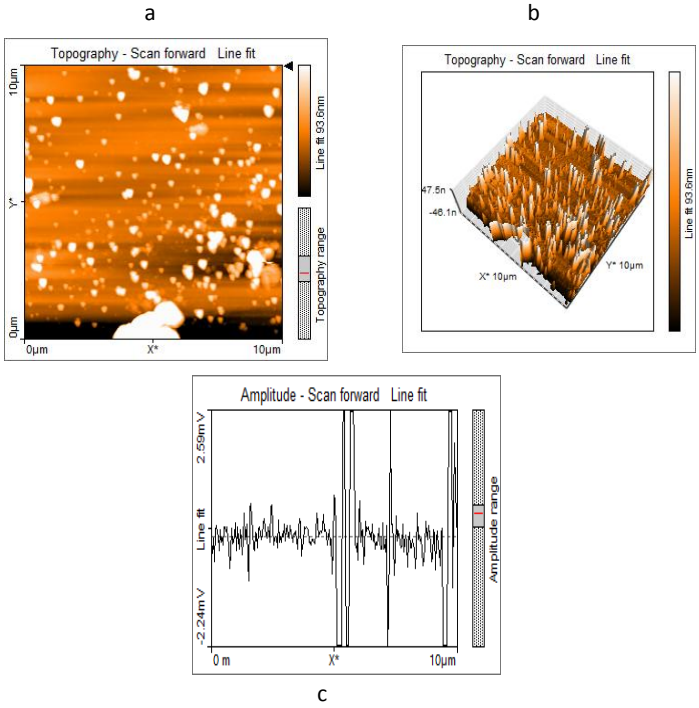


**Fig.5. AFM images of silver nanoparticles synthesized from *Thunbergia alata* Bojer ex sims**

AFM data reveals that the particles are monodispersed and spherical in shape and that the size ranges from 10 nm 60nm (Fig. 5a, b) in 2D and 3D structures of the nanoparticles with a distance of 25 to 30 nm from each other (Fig.5 c).

The silver nanoparticles were further characterized by HR-TEM micrograph, these Silver nanoparticles showed spherical shape with the majority size range from 10 to 20 nm (Fig. 6). Further, it also shows that the biomolecules of fruit extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles.

The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000). The XRD pattern of the biosynthesized silver nanostructure produced by the fruit extract of *T.alata* represented in figure 7 and was further confirmed by the characteristic peaks observed in the XRD image. The XRD data showed intensive diffraction peaks at a 2θ value of 37.88º from the (111) lattice plane of face centered cubic (fcc) silver unequivocally indicates that the particles are made of pure silver. The additional broad bands are observed at 44.05º (2θ), 64.12º (2θ), 77.06º (2θ) and 81.21º (2θ) they Correspond to the (200), (220), (311) and (222) planes of silver respectively (Fig. 22). Other spurious diffractions are due to crystallographic impurities. In the spectrum obtained the Bragg peak position and their intensities were compared with the standard JCPDS files 89-3722. The software gave the information about the face centered cubic (fcc) structure of silver nanoparticles. The average size of the nanoparticles is 15 nm. It can be estimated using the Debye–Scherrer equation.

EDX analysis was conducted to confirm the elemental composition of the sample. The EDS images (Fig.8.) confirmed the presence of significant amounts of elemental silver along with other elements, which may be originate from the biomolecules that are bound to the surface of nanosilver.

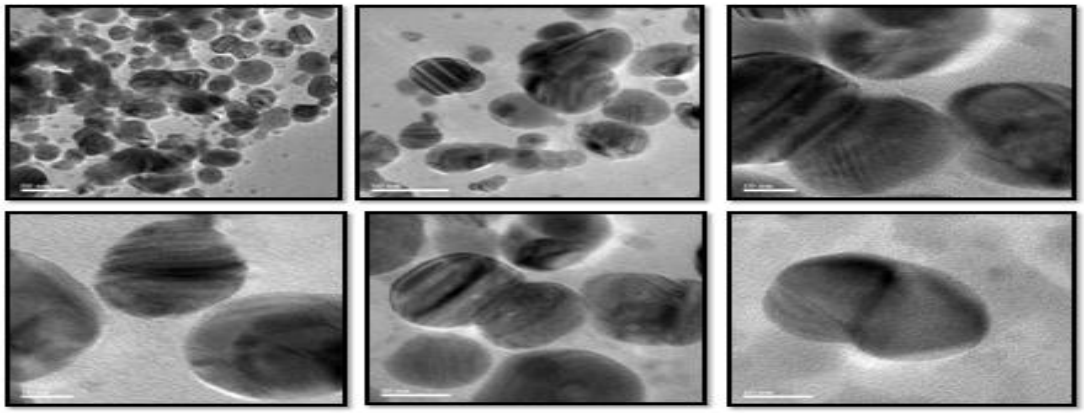

**Fig. 6. HR-TEM image of silver nanoparticle synthesized from *Thunbergia alata* Bojer ex sims**

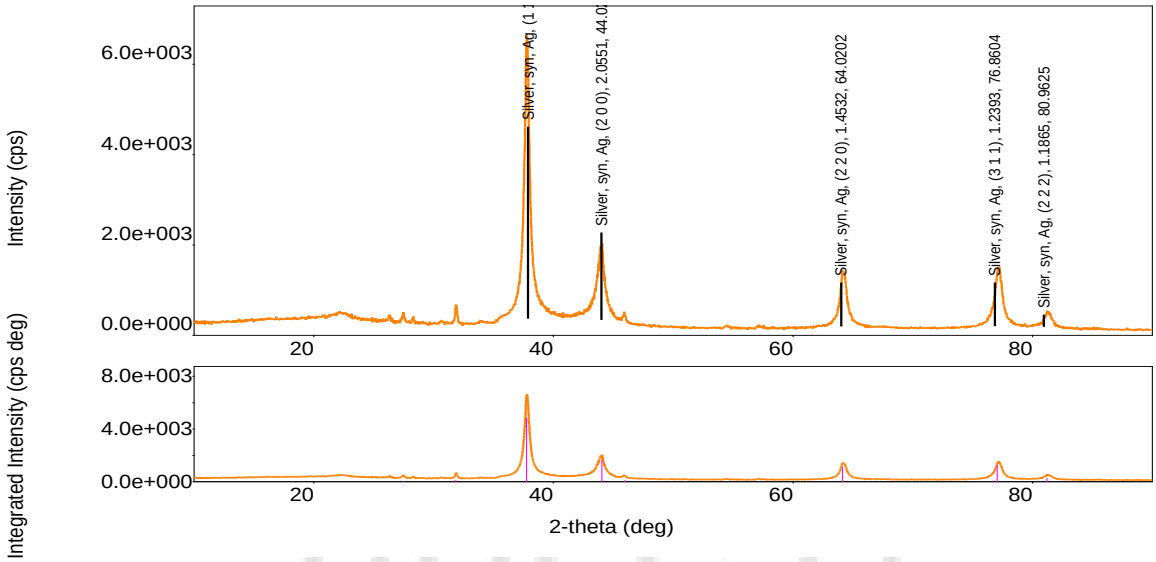


**Fig. 7. X-ray Diffraction Spectrum of Silver Nanoparticles from fruits *Thunbergia alata* Bojer ex sims**

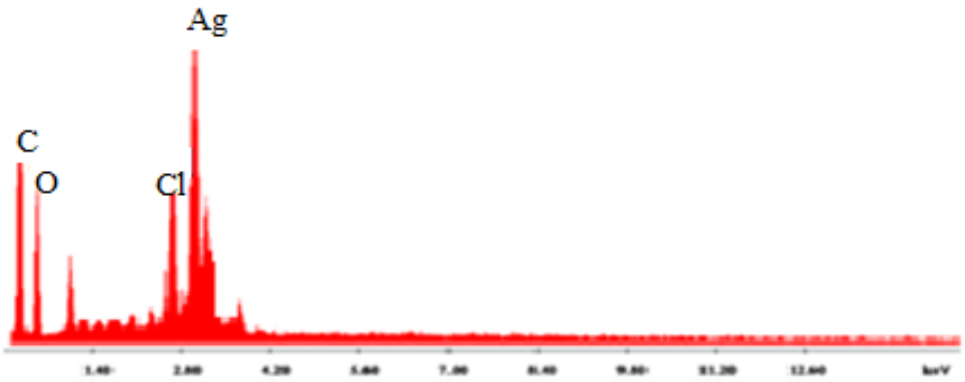


**Fig. 8. EDS spectra of silver nanoparticles synthesized by fruit extract of *Thunbergia alata* Bojer ex sims**

### 3.3 Antimicrobial activity of silver nanoparticles

Synthesized silver nanoparticles exhibited antibacterial activity against gram-positive *Staphylococcus aureus* , *Bacillus subtilis, Bacillus polymyxa* and *gram*-negative *Escherichia coli, Salmonella typhi, , Vibrio cholera* and antifungal activity against *Aspergillus niger and Candida albicans* (Fig. 9 & 10), while control ( fruit extract) didn't show any antimicrobial activity. The antibacterial and antifungal effect of silver nanoparticles at different concentrations (25-400 µl) were quantitatively assessed on the basis of the zone of inhibition (Table 2 & 3). The antibacterial activity of silver nanoparticles against *S. aureus* showed an inhibition zone of 3, 7,9,12 and 16 mm for concentration of 25, 50, 100, 200 and 400 µl respectively. 2, 6, 10 and 12 mm for concentration of 50, 100, 200 and 400 µl respectively against *B. Subtilis.* 5, 9 and 12mm for concentration of 100, 200 and 400 µl respectively against *B. Polymyxa.*9, 10, 13 and 15mm for concentration of 50, 100, 200 and 400 µl respectively against *E. Coli.* 6, 8 and 12mm for concentration of 100, 200 and 400 µl respectively against *S. typhi.* . It was noticed that the zone of inhibition increased with increased concentration of Ag NPs, while *V. Cholera* didn't show any antimicrobial activity (Fig. 9). The antifungal activity of silver nanoparticles also tested against *A. Niger* and *C. albicans. A. Niger* showed 3mm zone of inhibition only for 400 µl concentrations of silver nanoparticles while C. *albicans* showed 4, 9 mm for concentration of 200 and 400 µl. The antifungal activity of Fluconazole antibiotic (positive control) also tested against *A. Niger* and *C. albicans*, but comparatively, no zone of inhibition was noticed.

The minimum inhibitory concentration (MIC) studies showed varying concentrations of AgNPs against selected microbes. The gram-positive *S. aureus* showed a MIC of 25 µl, *E. Coli* and *B.subtilis* showed a MIC of 50 µl, *S. typhi* and *B. Polymyxa* showed a MIC of 100 µl. The fungal strains like *C. albicans* and *A. niger* showed a MIC of 200 and 400 µl respectively.

**Table 2. Antibacterial and antifungal activity of silver nanoparticles synthesized from fruits of *Thunbergia alatab* Bojer ex Sims**

| Bacterial species | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control (fruits extract) | Silver nanoparticle solution | | | | | |
| | **400** µl | **25** µl | **50** µl | **100** µl | **200** µl | **400** µl | **MIC** µl |
| *S. typhi* | 0 | 0 | 4 | 7 | 10 | 14 | 50 |
| *B. polymyxa* | 0 | 0 | 0 | 4 | 7 | 10 | 100 |
| *E. coli* | 0 | 0 | 0 | 1 | 5 | 11 | 100 |
| *V. cholerae* | 0 | 0 | 0 | 0 | 0 | 0 | NF |
| *B. subtilis* | 0 | 0 | 3 | 9 | 11 | 13 | 50 |
| *S. aures* | 0 | 0 | 6 | 8 | 11 | 15 | 50 |

**Table 3. Antifungal activity of silver nanoparticles synthesized from fruits of *Thunbergia alatab* Bojer ex Sims**

| Fungal species | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control (plant extract) | Silver nanoparticle solution | | | | | |
| | **400** µl | **25** µl | **50** µl | **100** µl | **200** µl | **400** µl | **MIC** µl |
| *A. niger* | 0 | 0 | 0 | 0 | 0 | 3 | 400 |
| *C. albicans* | 0 | 0 | 4 | 6 | 8 | 12 | 50 |

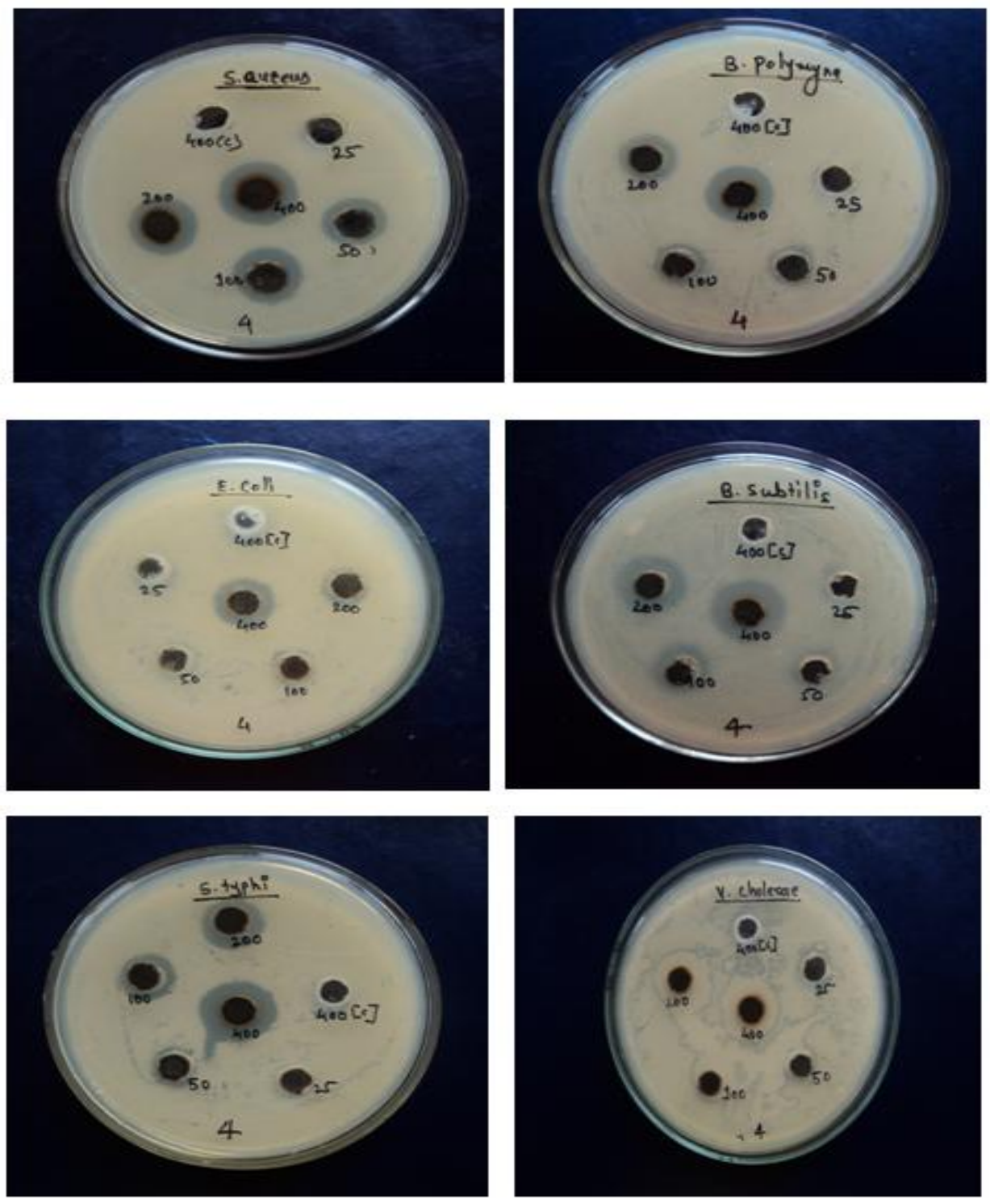


**Fig. 9 Antibacterial of silver nanoparticles synthesized from fruits of *T. alata* against gram positive *S. aureus , B. subtilis, B. polymyxa* and *gram*-negative *E. coli, S. typhi, V.cholera*.**

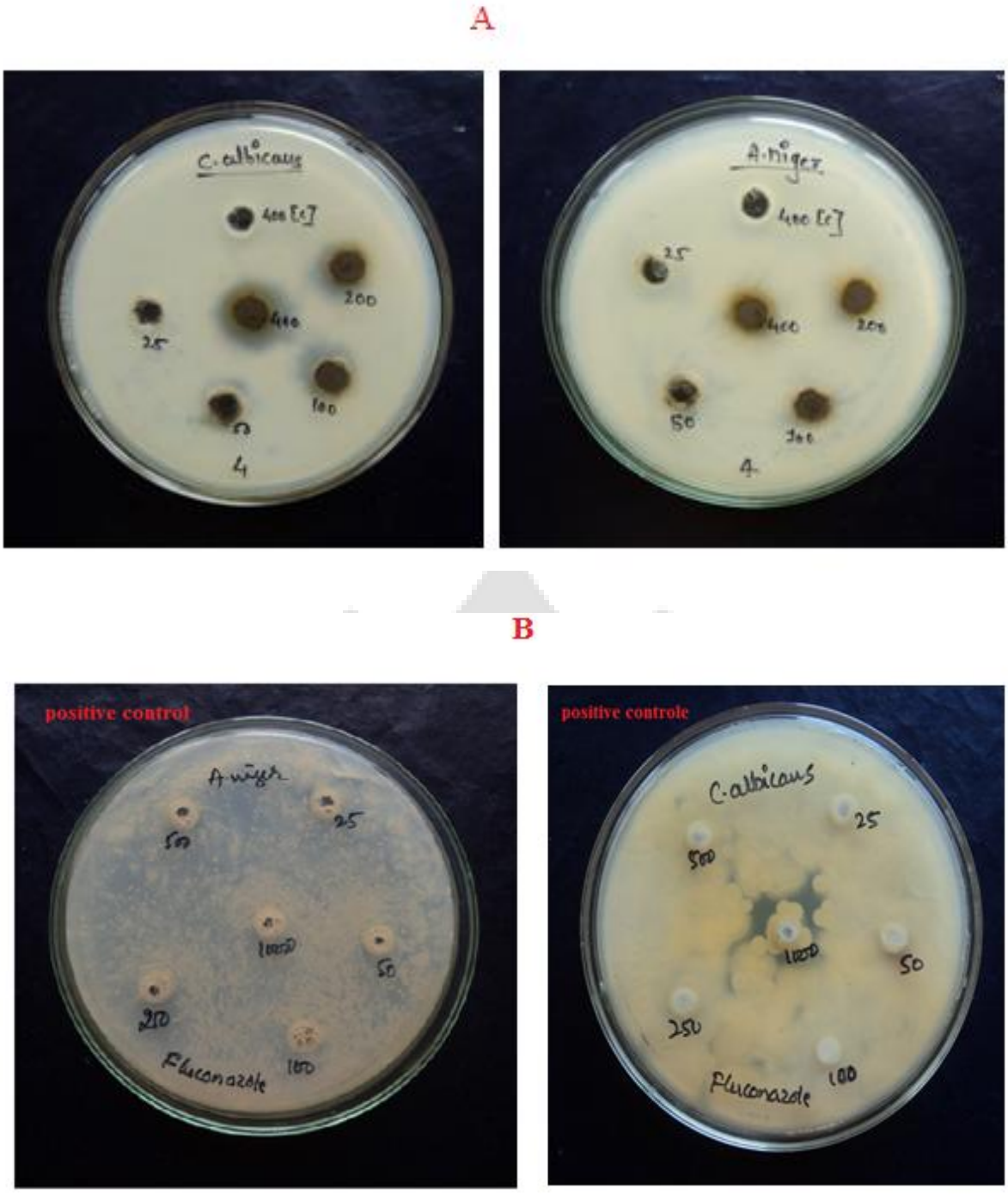


**Fig. 10 Comparison of Antifungal activity of [A] silver nanoparticles synthesized from fruits of *T. alata* and [B] Fluconazole antibiotic – a positive control against *A.niger* and *C. albicans***

## 4. DISCUSSION

The inhibitory action of silver compounds and silver ions had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The inhibitory action of silver on bacterial cells is related to the strong interaction of silver with thiol groups present in key respiratory enzymes in bacteria [13] Whereas, Nanocrystalline silver shows the most effective inhibitory action with a rapid inhibition rate [14]. The aim of this study is based on exploring the potential of AgNPs from *T. alata* to provide the eco-friendly, cost-effective antimicrobial nanoparticles against multidrug-resistant human pathogenic microbes. The important findings of this study is that the biosynthesized silver nanoparticles from *T. alata* Ag NPs showed comparatively good antifungal activity than the Fluconazole antibiotic (positive control) against A.niger and *C. albicans* (fig.10) and the antibacterial effects of were also successfully investigated *against Staphylococcus aureus, Bacillus subtilis, Bacillus polymyxa, Escherichia coli, Salmonella typhi, Vibrio cholera, Aspergillus niger and Candida albicans*. Also, there are various reports which have been providing the evidence that silver nanoparticles were used as powerful tool against multidrug-resistant bacteria [15, 16]

The characterization of nanoparticles was done using various techniques. The results obtained from the techniques and antibacterial studies have coincided with the literature in the field of nanoparticle research [17, 18] and certainly gives proof about the nanoparticle synthesis and its efficiency as antibacterial agent [19]. The reduction of metal ions was primarily monitored by visual inspection of the reaction mixture [20]. The change in color has been attributed to excitation of surface Plasmon resonance of the metal nanoparticles [21]. UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanoparticles in aqueous solution [22]. The FTIR analysis was carried out to identify the possible interfacial groups between the capping agents and silver nanoparticles. Different functional groups indicate that the silver nanoparticles synthesized from the extract are surrounded by some proteins and metabolites such as terpenoids that have amine, alcohol, ketone, aldehyde and carboxylic acid functional groups [23].This result suggests that the biological molecules could probably perform a function involving the formation and stabilization of Ag NPs through free amine groups in the proteins [24]. The HR-TEM studies have given further inputs on the morphology and size of biosynthesized silver nanoparticles ranging between 10 to 50nm with a scale of 100 nm and histogram showing sizes of particles with spherical morphology (Fig. 6) [25, 26]. EDX analysis shows the presence of pure silver and other

elements confirming the biosynthesis of silver nanoparticles. EDX peak in the range of 3–4 keV is typical for the absorption of metallic silver nanoparticles [27].

The antimicrobial analysis of synthesized AgNPs showed profound antibacterial effect against both Gram positive and Gram negative strains. The sensitivity of microbial strains to AgNPs is increased by affecting the cell morphology and membrane permeability. It was noticed that the zone of inhibition increased with increased concentration of Ag NPs. Similarly, Dipankar and Murugan have reported dose-dependent inhibition by Ag NPs synthesized from *Iresine herbstii* fruit aqueous extract [28]. This might be due to the denaturation of bacterial cell wall, blocking bacterial respiration, destabilization of outer membrane, and depletion of intracellular ATP [29]. The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag+ bactericidal agent. Changes in the bacterial membrane structure bacteria as a result of the interaction with silver cations leads to the increased membrane permeability [30,31] Lin explained that in general, silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads into denaturation of protein and finally cell death [32]. Silver has a greater affinity to react with sulfur or phosphorus-containing biomolecules of the cell. Thus, sulphur-containing proteins the membrane or inside the cells and phosphorus-containing elements like DNA are likely to be the preferential sites for silver nanoparticle binding [33, 34]. The variation in MIC values of AgNPs could be due to the existence of different modes of action on individual microorganisms [35].

This Phytosynthesis approach appears to be a cost-effective, non-toxic, eco-friendly alternative to the conventional microbiological, physical and chemical methods, and would be suitable for developing a biological process for large-scale production. These silver nanoparticles are powerful tool against multidrug-resistant bacteria and also may be used in effluent treatment process for reducing the microbial load

## 5. CONCLUSIONS

The green synthesis and characterization of -AgNPs was done and confirmed by UV–visible spectrophotometer, FTIR, AFM, HR-TEM, XRD and EDX techniques. The nanoparticles appeared to be spherical in shape and the sizes of the particles varied from 10 to 60 nm, but amongst them, most of the particles obtained were sized in between 10 and 20 nm. Growth

studies of different microbial cultures were performed in the presence of nanoparticles to observe their effect on the growth profile. This study shows that *T.alata* fruit extract mediated silver nanoparticles have great promise as antimicrobial agent against human pathogenic multidrug-resistant microbes. Hence, our results are promising and prove to be an important step in this direction as it decreases the burden of multidrug resistance in patients and might act as long searched alternative and could be the answer to antibiotic resistance.

**Acknowledgements**

The authors are thankful to the Chairman P. G. Department of Botany, Karnatak University, Dharwad for the facilities. One of the author (B.R.H) thanks to the University for the Award of UGC – UPE Fellowship. Instrumentation facility at USIC (K.U. Dharwad) and DST Unit (HR-TEM) IIT Madras and MIT Manipal (XRD) is greatly acknowledged.

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