



## Facile synthesis of silver nanoparticles (AgNPs) using the leaf extracts of *Aristolochia tagala* L. for its photocatalytic and antimicrobial activity

M Thilagam<sup>1</sup>, B Esakkiammal<sup>1\*</sup>, P Mehalingam<sup>2</sup>

<sup>1</sup> PG and Research, Department of Zoology, Sri Parasakthi College for Women Courtallam (An Autonomous Institution Affiliated to Manonmaniam Sundaranar University), Tirunelveli, Tamil Nadu, India

<sup>2</sup> Research department of Botany, VHN Senthikumara Nadar College (Autonomous), Virudhunagar, Tamil Nadu, India

### Abstract

In the present study, a direct approach to fabricating silver nanoparticles (AgNPs) via the leaf extract of *Aristolochia tagala* has been demonstrated. The synthesized Ag-NPs were characterized by UV-visible, X-ray diffraction (XRD), Fourier-transform infrared (FT-IR), Scanning electron microscopy (SEM), Energy dispersive x-ray (EDX), DLS and ZETA Potential. Formation of silver nanoparticles was authenticated by the disappearance of colour from pale yellow to dark brown in colour after incubation. These results confirmed that the appearance of AgNPs was analyzed by UV- visible spectrum around the peak 420 nm. XRD (X-ray diffractometer) demonstrated the formation of crystalline AgNPs with FCC structure having an average crystalline size of 23 nm from XRD profile. The average grain size of the synthesized silver nanoparticles was about 8nm. FT-IR analysis revealed the presence of different functional groups in the synthesized silver nanoparticles. Antimicrobial activity of the synthesized silver nanoparticles was evaluated against Gram positive and Gram negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungus *Candida albicans*. Both the leaf extract of *Aristolochia tagala* and synthesized silver nanoparticles from the leaves of *Aristolochia tagala* showed moderate antimicrobial activity. In addition to antimicrobial activity, these synthesized silver nanoparticles were studied for the potential role in photocatalytic performance of effective degradation of MO dye under visible light irradiation conditions.

**Keywords:** facile synthesis, *Aristolochia tagala*, AgNPs, antimicrobial activity, photocatalyst

### Introduction

Nanobiotechnology is simultaneously emerging as an interdisciplinary and eco-friendly technology deals with various fields such as biology, chemistry, physics, biomedicine and material engineering [1, 2]. Over the past decade to the humans have discovered in many chemicals possess the antibiotic for treated to the threatened disease which including diarrhoea also [3]. Natural product from the plants has a major role in considered antibiotics among entered into the pathogenic bacteria to treating the diarrhoea [4]. The field of nanotechnology research is captured in great attention of the science. It is evident from the previous studies that metal nanoparticles were predominantly used in various research areas such as medicine, science, engineering, technology, biosensing and forensic science [5, 14]. The phytomediated synthesis of metal nanoparticles was performed as silver, gold, palladium, platinum [15, 16]. Silver nanoparticles are fabrication in reduction of Ag<sup>+</sup> ions to silver atoms and reducing agent as silver ions [17].

Few works were carried out for the green synthesis of silver nanoparticles and characterization techniques in various taxa such as *Euphorbia antiquorum* L. [18], *Melia azedarach* L. [19], *Averrhoa bilimbi* [20], *Silybum marianum* [21], *Calendula officinalis* [22, 23], *Eucalyptus camaldulensis* [24], *Ulva flexuosa* [25], and *Withania somnifera* [26]. A perusal of literature revealed that synthesis and characterization of silver nanoparticles were not executed in *Aristolochia tagala* L. (Family: Aristolochiaceae). It is commonly known as ishwari, nakuli and gandhanakuli in the Asian countries. The physical characteristics of the synthesized silver

nanoparticles were examined by various analytical tools such as XRD, EDX, TEM, FTIR, DLS and ZETA Potential, UV-visible spectroscopy and SEM. The Ag nanocomposites were observed on the interfaces by TEM, which could play a vital role in enhancing photocatalytic performance. The as prepared Ag nanocomposites were used as a highly visible light driven photocatalyst towards the degradation of MO dye. The synthesized AgNPs showed an excellent antimicrobial activity against human pathogenic bacteria and a fungus.

### Materials and methods

#### Preparation of *Aristolochia tagala* L. leaves extract

Fresh leaves of *Aristolochia tagala* were collected from Pothigai hills, Tenkasi District, Tamil Nadu, India. 25g of the fresh leaf powder was mixed with 100 ml of double distilled water and transferred into the 500 ml beaker and boiled at 100°C for 40 minutes and then brought down to room temperature. Subsequently, the extract was subjected to filtration through Whatman No.1 filter paper and stored at 4°C in air tight containers.

#### Microorganisms

The microbial strains such as bacteria *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Streptococcus faecalis* (MTCC 5383), *Klebsiella pneumoniae* (MTCC 4030), *Pseudomonas aeruginosa* (MTCC 741), *E.coli* (MTCC 443) and fungal *Candida albicans* (GRIBI 03) were collected from Department of

Botany, Gandhigram Rural Institute - Deemed University, Gandhigram, Tamil Nadu. Microbial strains were incubated at 37°C in Mueller- Hinton Broth.

### Phytomediated synthesis of Silver nanoparticles using leaf extracts of *Aristolochia tagala*

10 ml of leaf extract of *Aristolochia tagala* was added to 1mM AgNO<sub>3</sub> (90 ml) aqueous solution. The reduction process of Ag<sup>+</sup> to Ag<sup>0</sup> was followed by the change of colour pale yellow to dark brown in colour which indicates the formation of AgNPs.

### Characterization of AgNPs

The absorption spectrum of the synthesized silver nanoparticles from the leaves of *Aristolochia tagala* was analysed by UV Visible spectroscopy-1800v (Shimadzu, Japan) at the wavelength ranging from 200-700 nm. The external morphology of the synthesized silver nanoparticles was observed on scanning electron microscope (SEM) at GRI and elemental composition of synthesized AgNPs was confirmed by EDAX analysis. Transmission electron microscopy (TEM) studies were performed by morphological investigations by JEOL-JEM 1200. Electron microscope was operating at an accelerating voltage of 90 kV. For the TEM measurements, a drop of solution containing the silver nanoparticles was deposited on a copper grid covered with amorphous carbon. FT-IR spectra revealed that perkin Elmer spectrum 400 FT-IR transmission mode 4000-400 cm<sup>-1</sup>. DLS and Zeta potential measurements were carried out on same instrument HORIBA SZ-100 which was used to evaluate particle size and surface charge of the synthesized AgNPs. The average grain size was calculated by image Joint Committee on Powder Diffraction Standards (JCPDS) software. The crystalline structure of the synthesized AgNPs was illustrated that XPERT-PRO through powder X-ray diffraction using Cu K $\alpha$  radiation with and theta angles at 10° C to 80° C.

### Antimicrobial activity

Antimicrobial activity of the synthesized silver nanoparticles was carried out by using *Bacillus subtilis* (GRIBI 01), *Staphylococcus aureus* (GRIBI 05), *Streptococcus faecalis* (GRIBI 08), *Klebsiella pneumoniae* (GRIBI 02), *Pseudomonas aeruginosa* (GRIBI04), *E.coli* (GRIBI 06) and *Candida albicans* (GRIBI 03) agar well diffusion method. Mueller hinton agar medium 30.4g MH medium was mixed with 800 ml of distilled water and sterilized in autoclave at 20 minutes. The sterilized medium was allowed to pour into the Petri dishes. The solidified plates were poured to cork borer with the help of 6 mm diameter. The plates were plant extract, silver nitrate and different concentrations of synthesized AgNPs 100µg/ml, 150µg/ml, and 200µg/ml for antimicrobial studies.

### Photocatalytic activity

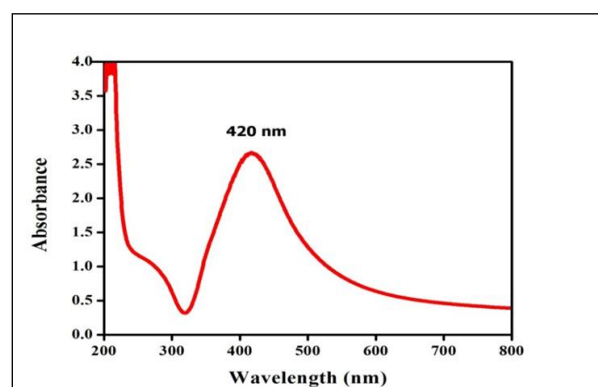
It is mandatory to carry out the photocatalytic degradation of Methyl Orange (MO) dye against synthesis of AgNPs. The photocatalytic performances of the synthesized silver nanoparticles from the source of UV-Visible light irradiation. The NPs synthesis via aqueous leaves extract of *Aristolochia tagala* was confirmed to MO dye using a photocatalytic performance. On the other hand, the synthesized silver nanoparticles suspension of 25 mg was added into 60 ml of methyl orange dye solution. Then, the mixture was allowed to constant stirring condition at 20 min after the reaction mixture was undertaken to visible light irradiation with temperature is going on 30°C from at certain time duration. The absorption spectrum of the suspension mixture was read accordingly to UV- Visible spectrophotometer absorption band maximum at 465 nm (Shimadzu UV-1800 Japan) after centrifugation at MO dye degradation.

## Results and discussion

### Characterization

#### UV-visible spectrum

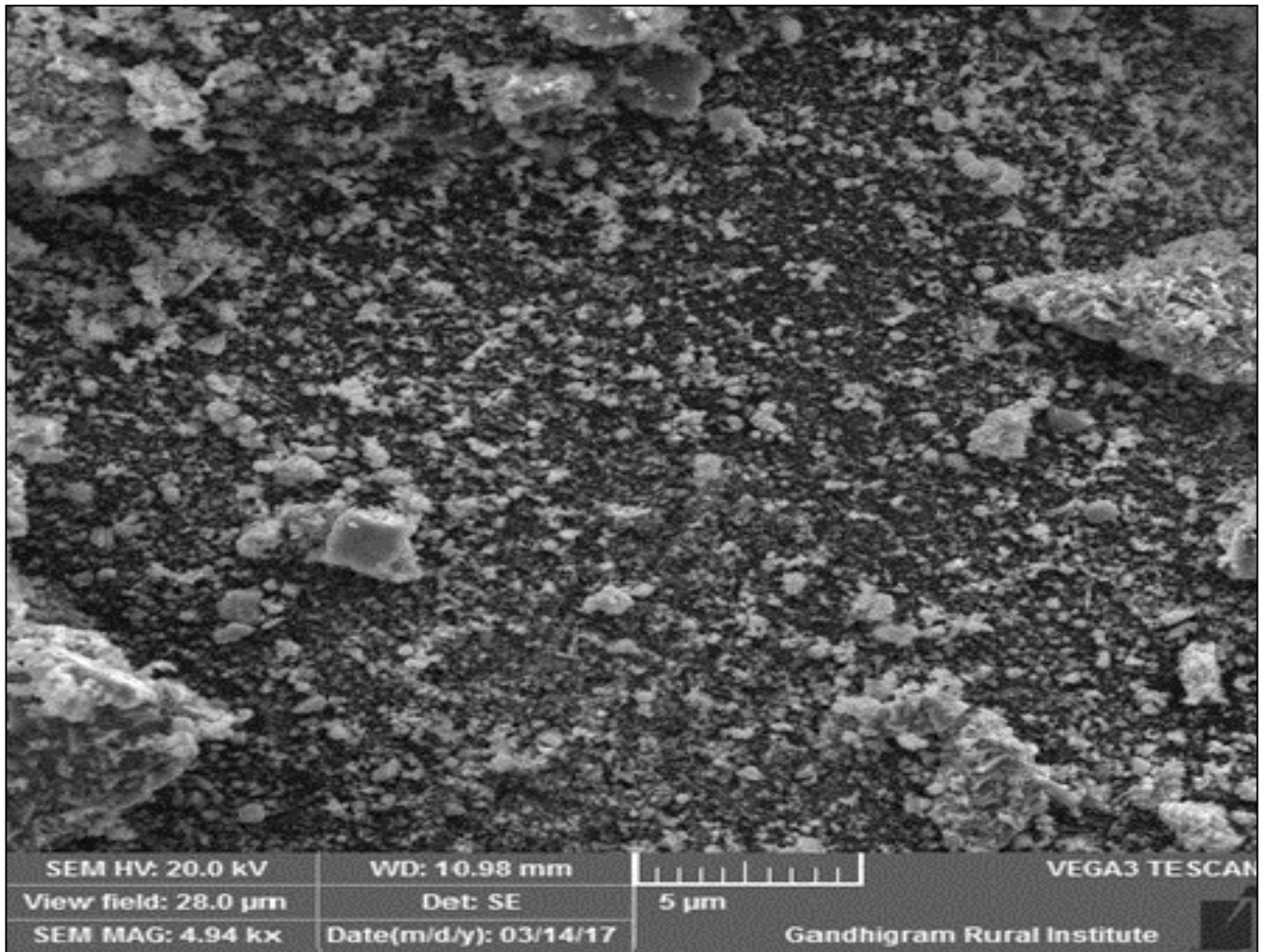
The UV-visible spectrum of the samples (Figure 1) showed the aqueous leaves extract and AgNPs was respectively. The synthesized AgNPs (420nm) was notified. The colour of the mixture of aqueous leaves extract of *Aristolochia tagala* was noted and AgNO<sub>3</sub> turned pale yellow to dark brown which indicates the formation of AgNPs. The formation of the synthesized AgNPs was allowed to determine the SPR (Surface Plasmon Resonance) interaction between the particles of the medium.



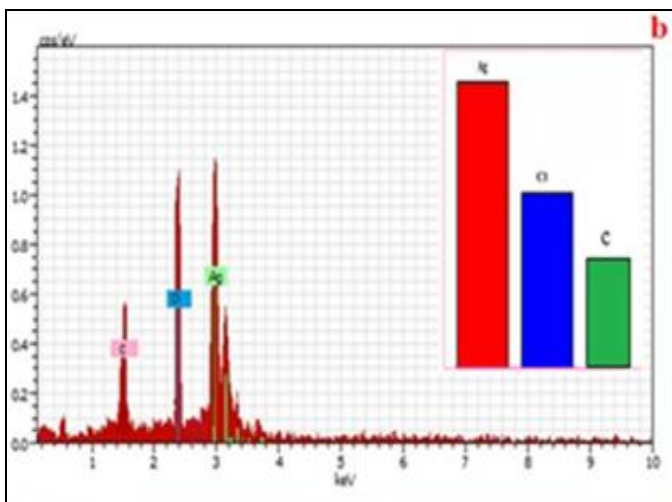
**Fig 1:** UV-visible spectrum of synthesized AgNPs using aqueous leaves extract of *Aristolochia tagala*

#### SEM with EDX analysis

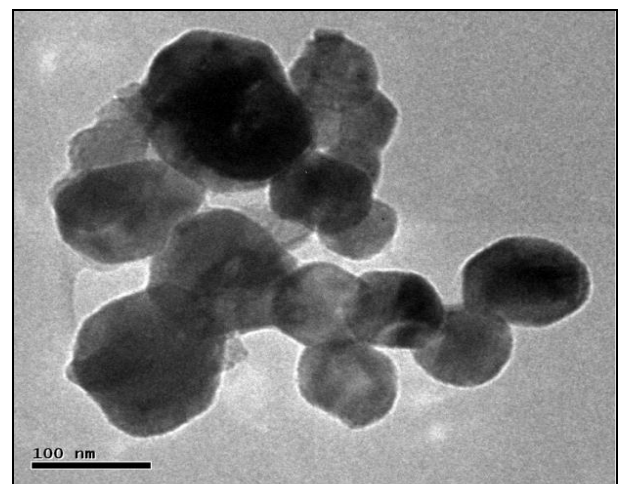
The size and morphology of the synthesized AgNPs was investigated by SEM. (Figure 2) shows that the synthesized nanoparticles consist of large number of sponge-like structures with the length of micrometer (20µm). The Figure 2 displayed the chemical composition of the synthesized silver nanoparticles by EDX. The result of the EDX analysis showed that the sample contain Ag, Cl and C without any impurities.



**Fig 2:** SEM image of phytomediated synthesis of Silver nanoparticles from the leaf extract of *Aristolochia tagala*



**Fig 3:** EDX phytomediated synthesis of AgNPs from the leaf extracts of *Aristolochia tagala*



**Fig 4:** TEM image of AgNPs using the leaf extract of *Aristolochia tagala*

**TEM**

The TEM analysis revealed that the synthesized silver nanoparticles were spherical in shape (Figure 3). The overall morphology of the synthesized silver nanoparticles produced by reduction of Ag<sup>+</sup> ions with 1mM AgNO<sub>3</sub> nanoparticles.

Furthermore, the capping ability of *Aristolochia tagala* leaves extract was also observed.

**FT-IR**

FT-IR spectroscopy of synthesized AgNPs were shown in Figure 4 and was mainly used to identify the functional groups that were bound to be specifically to silver surface. Further, it revealed that possible functional groups which were responsible for the reduction of nanoparticles. The IR spectrum of the *Aristolochia tagala* leaves extract and synthesized AgNPs were investigated ranging from 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup>. FTIR spectrum of the *A.tagala* leaves extract showed beak at 1107.06 cm<sup>-1</sup>, 1383.83 cm<sup>-1</sup>, 1585.38 cm<sup>-1</sup>

and 2923.92  $\text{cm}^{-1}$ . The strong peak at 2360.71  $\text{cm}^{-1}$  of the synthesized AgNPs was ascribed to the strong C=N stretching vibrations of functional groups at nitriles. The corresponding peaks at 1585.38  $\text{cm}^{-1}$  and 2923.92  $\text{cm}^{-1}$  were mainly due to the N-H bend amides and Hydrogen bonded O-H stretching vibrations at carboxylic acids. The band at

1383.83  $\text{cm}^{-1}$  N=O bend was noticed due to the existence of nitro groups. The weak band peak at 1107.06  $\text{cm}^{-1}$  were due to the presence of both C-O stretching mode and the functional group at ethers. The functional components in *Aristolochia tagala* leaves extract were reduced to the  $\text{Ag}^+$  and stabilize the AgNPs.

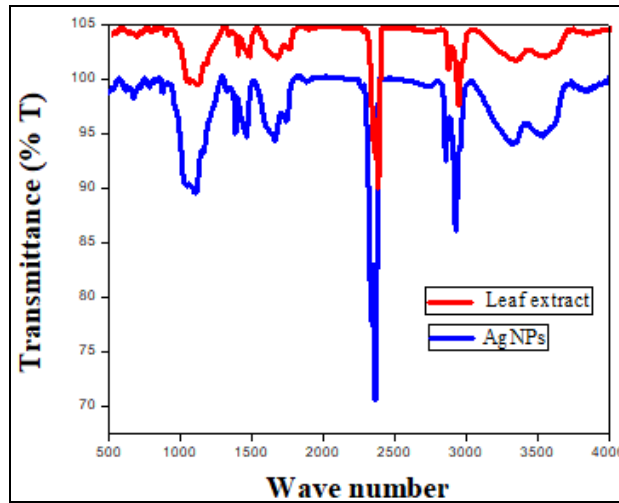


Fig 5: FT-IR spectrum of leaf extracts of *Aristolochia tagala* and synthesized AgNPs

**Particle size distribution and Zeta potential stability**

The size of the particles and distribution of the synthesized AgNPs was determined by using DLS (Dynamic Light Scattering) as given in Figure 5a and calculated the particle

size from 48.9 nm with polydispersity index (PDI) as 0.680 that corroborates with SEM result. The average Zeta potential value was -54.8 mV (Figure 5b) and showed in higher stability of synthesized AgNPs.

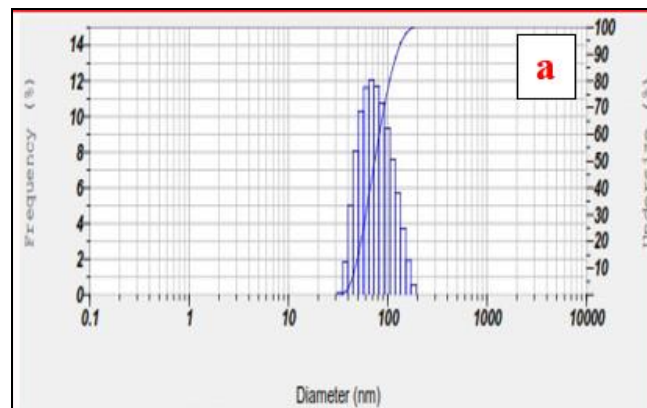


Fig 6: DLS and Zeta potential results for synthesized AgNPs showing Z -Average at 48.9 nm

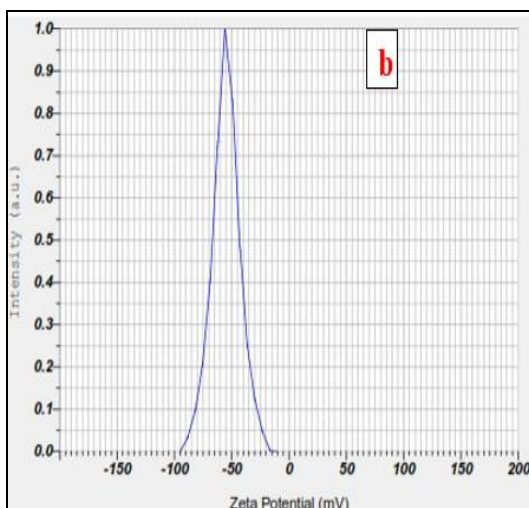


Fig 7: Zeta potential value of synthesized AgNPs showing -54.8 mV.

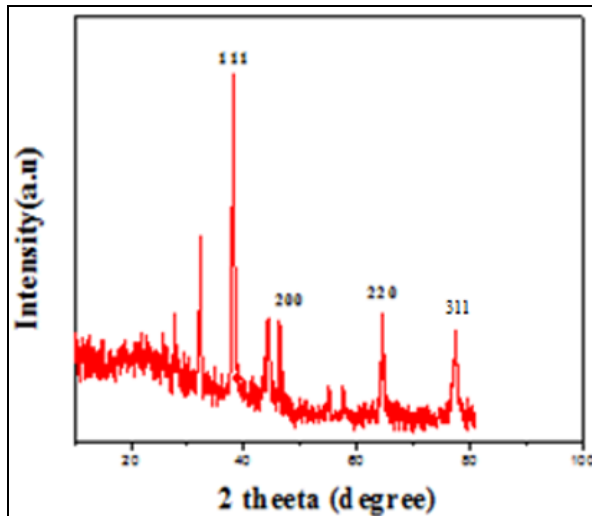
**XRD**

The XRD pattern of the synthesized silver nanoparticles from the leaf extract of *Aristolochia tagala* exhibited the distinct peaks indexed to the crystalline nature of the face centered cubic structure (Figure 6). The diffraction peak angle noted at  $2\theta$  degree was 38.14, 44.11, 64.51 and 77.45 corresponding to (111), (200), (220) and (311) reflection planes respectively. These results confirmed by (JCPDS card no 87-0718). The peak was clearly indicated to the synthesized silver nanoparticles. Similar observations were reported at earlier researchers. The average particles size of the synthesized AgNPs was illustrated by using Scherer's equation.

$$D = K\lambda/\beta\text{Cos}\theta.$$

Where, D is the average nanocrystalline size (nm), k- is the constant (0.94), d - is the mean diameter of the

nanoparticles,  $\lambda$ - is the wavelength of X- ray (1.542 Å),  $\beta$ - is the width of FWHM of the X-ray diffraction peak at Bragg's diffraction angle  $\theta$ . The average size of the synthesized AgNPs is estimated to be around 29 nm using Scherer's equation.

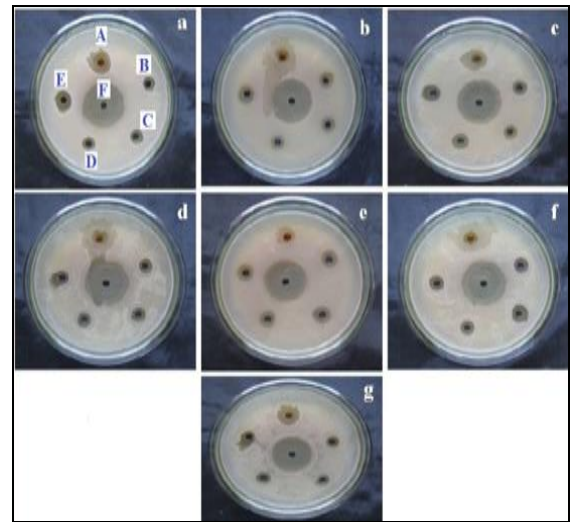


**Fig 8:** X-ray diffraction profile of phytomediated synthesized AgNPs from leaf extracts of *Aristolochia tagala*.

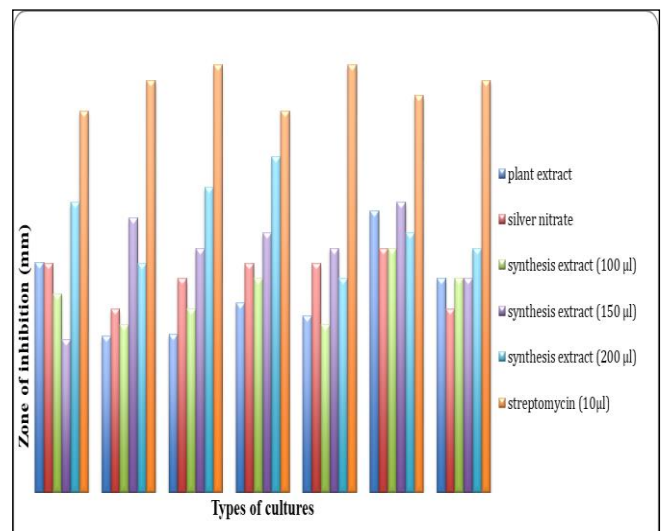
#### Antimicrobial activity

Antimicrobial properties of synthesized Ag nanoparticles were evaluated by well diffusion method: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli* and *Candida albicans*. About 20 ml of Mueller Hinton Agar media was poured into sterilized Petri dishes. The sterilized Petri dishes were kept over night at room temperature to check out sterility. The agar wells (6mm) diameter with the help of Cork borer were used to load the samples. As the preliminary, 6 wells were bored, first well is loaded with aqueous leaves extract of *Aristolochia tagala* (10 $\mu$ l). Second well is loaded with AgNO<sub>3</sub> (10 $\mu$ l), third well is three different concentrations (100 $\mu$ g/ml, 150 $\mu$ g/ml, 200 $\mu$ g/ml) of synthesized Ag nanoparticles. (Figure 7a). Finally, sixth well is loaded with reference sample at streptomycin (10 $\mu$ g). Then, the plates were subjected to incubation at 37° C for 24 hrs. Then, the zone of inhibition around the wells were measured.

The present study clearly indicated that the maximum zone of inhibition was reported against *Streptococcus aureus* (22mm), *Streptococcus faecalis* (20mm) and *Bacillus subtilis* (19mm) respectively. However, the least effective zone of inhibition was observed *Klebsiella pneumoniae* (11mm) and *E.coli* (11mm). From this study, the synthesized Ag nanoparticles (Figure 7b) were showing potential antimicrobial activity against various microbial strains.



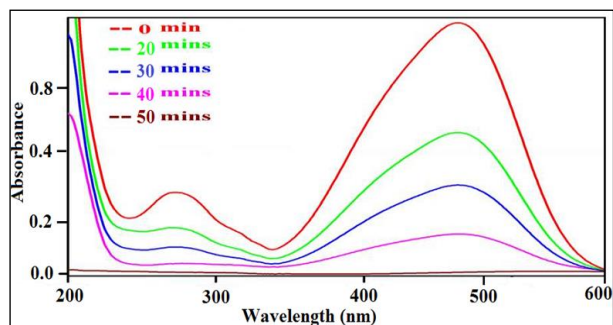
**Fig 9:** Zones of inhibition of different microbial strains



**Fig 10:** Antimicrobial activities of leaf extract and synthesized AgNPs from leaf extracts of *Aristolochia tagala*

#### Photocatalytic activity

Photocatalytic performances of the synthesized silver nanoparticles were evaluated by using MO dye under visible light irradiation with *Aristolochia tagala* leaf extract. The UV-Visible spectrum of the aqueous solution of MO dye (initial concentration 20 ppm) with 25 mg of the synthesized AgNPs as a photocatalyst and UV-Visible light irradiation for different time durations were given in Figure 8. The results showed the absorption peak around the MO dye solution at 460 nm decreased completely at 50 minutes. Blank tests were carried out (MO without any catalyst) under visible light irradiation, a negligible amount of MO solution was degraded and which was indicated that the degradation of MO dye solution virtually does not occur without a photocatalyst. The synthesized AgNPs displaced the best photocatalytic activity.



**Fig 11:** Photodegradation of MO dye under UV-Visible light irradiation with 25 mg of the synthesized AgNPs.

### Conclusions

The present study illustrated a cost effective and convenient method for the phytomediated synthesis of AgNPs at room temperature using *Aristolochia tagala* leaves extract. The biogenic synthesis of AgNPs confirmed by UV-Visible spectroscopy and SEM. The crystalline nature and property of the synthesized silver nanoparticles was carried out by XRD and the multifunctional group was illustrated by *Aristolochia tagala* leaves extract and synthesized AgNPs confirmed by FTIR studies. The morphological investigations of the as synthesized silver nanoparticles were done by TEM. The antimicrobial activity of the synthesized AgNPs was evidenced by different microbial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli* and fungal *Candida albicans*. This is the first report on degradation of MO dye using AgNPs as a catalyst. Hence, this synthesis process may be green method which could be used in large scale production of nanoparticles.

### Acknowledgements

The authors are grateful to Madurai Kamaraj University, Madurai and Department of Biology, Gandhigram Rural Institute- Deemed University Gandhigram for Characterization studies and antimicrobial studies respectively.

### References

1. Arunachalam KD, Annamalai SK, Hari S. One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from *Memecylon umbellatum*. *International Journal of Nanomedicine*,2013;8:1307-1315.
2. World Health Organization: Antimicrobial Resistance Global Report on Surveillance, 2014, 257.
3. Oldfield EC, Wallace MR. The role of antibiotics in the treatment of infectious diarrhea. *Gastroenterol. Clin. North Am*,2001;30:(3):817-835.
4. Dar MA, Ingle A, Rai M. Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria* sp. evaluated singly and in combination with antibiotics. *Nanomed Nanotechnol Biol Med*,2013;9:105-110.
5. Godin B, Sakamoto JH, Serda RE, Grattoni A, Bouamrani A, Ferrari M. Emerging applications of nanomedicine for the diagnosis and treatment of cardiovascular diseases. *Trends Pharmacol Sci*,2010;31:199-205.

6. Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM. *et al.* Topical delivery of silver nanoparticles promotes wound healing. *Chem Med Chem*. 2007; 2:129-136.
7. Meng H, Liong M, Xia T, Li Z, Ji Z, Zink JI *et al.* Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. *ACS Nano*,2010;4:4539-4550.
8. Shipway N, Katz E, Willner I. Nanoparticle arrays on surfaces for electronic, optical and sensor applications. *Chem Phys Chem*,2000;1:18-52.
9. Yi KC, Hórvölgyi Z, Fendler JH. Chemical formation of silver particulates films under monolayers. *J Phys Chem*,1994;98:3872-3881.
10. Schmid G. Clusters and Colloids: From Theory to Applications. VCH, Weinheim, Germany. *Crystal Research and Technology*,1994;29(7):546.
11. Rao CNR, Kulkarni GU, Thomas PJ, Edwards PP. Metal nanoparticles and their assemblies. *Chem Soc Rev*,2000;29:27-35.
12. Tolaymat TM, Badawy AM El, Genaidy A, Scheckel KG, Luxton TP, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: a systematic review and critical appraisal of peer-reviewed scientific papers. *Sci Total Environ*,2010;408:999-1006.