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Green Synthesis of Silver Nanoparticles Using *Passiflora foetida* Extract and Screening for Their Catalytic Activity

Vundela Reddy Swetha[1], Bonigala Bodaiah [1], Mangamuri Kiranmayi Usha [2], Poda Sudhakar [1], Kondapalli Kasturi [1*]

[1]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India - 522510

[2]Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India - 522510

ABSTRACT

Green synthesis is a zone of biosynthesis in which metallic nanoparticles are formulated using the extracts of plants as reducing and stabilizing agents. In the present study silver nanoparticles (AgNPs) were green synthesized using the aqueous extract of *Passiflora foetida*. When the plant extract was added to $AgNO_3$ solution, the amalgamated solution turned deep reddish brown in colour after 48 hours indicating the formation of AgNPs. Later in UV-Visible analysis the amalgamated solution has shown absorption maximum at 435.13nm which confirmed the formation of AgNPs. The green synthesized AgNPs were further characterized using dynamic light scattering, zeta potential analysis, X-ray diffractometer (XRD) and Transmission electron microscopy (TEM) to know their size, stability and crystalline nature. The plant mediated AgNPs were studied in flourescence transmission infrared spectroscopy (FTIR) to know the phytochemicals present in the plant extract which acted as reducing agents in the formation of AgNPs. Imperatively in the present study the plant mediated AgNPs were screened for catalytic activity in the presence of $NaBH_4$ in the degradation and removal of 4-Nitrophenol, methylene blue, methyl orange and methyl red. The obtained results proclaimed that the AgNPs of *P. foetida* acted as potential catalyzing agents in the degradation of 4-Nitrophenol, methylene blue, methyl orange and methyl red.

KEYWORDS: Green synthesis, *Passiflora foetida*, AgNPs, XRD, FTIR, Catalytic activity

[*]Corresponding author

Dr. Kondapalli Kasturi

Assistant Professor

Department of Biotechnology, Acharya Nagarjuna University

Nagarjunanagar, Guntur, Andhra Pradesh, India-522510

Email: kasturi.is.kondapalli21@gmail.com

Land line: 0866 -2494277, Mobile no: 9848546664

INTRODUCTION

Water pollution is a major issue in the present century because huge amounts of industrial effluents with remnant toxic chemicals are transported or thrown in to water bodies. The toxic pollutants have shown negative impact on human health and other living organisms[1]. Waste water treatment by physical and chemical methods requires high cost equipment which consume huge amount of energy. There is an urgent need to develop a new technology which can replace the physical and chemical methods for the removal of water pollution. According to research records metallic nanoparticles (MNPs) especially biosynthesized MNPs in addition to their potent biological activities have shown remarkable catalytic activity in the area of degradation and removal of toxic chemicals (for example 4-Nitrophenol) and synthetic dyes[2, 3]. Green synthesis is a sector of biosynthesis in which MNPs are synthesized in large scale using the extracts of plants in cost effective and ecofriendly manner. Among MNPs, silver nanoparticles (AgNPs) received high acknowledgements from the scientific world because of their remarkable antimicrobial, anticancer, insecticidal and catalytic activities etc[4].

4-Nitrophenol is a toxic synthetic chemical and polymorphic compound in crystalline state. It is colorless below pH 5.4 and yellow above pH 7.5 in solution form. 4-Nitrophenol is used to darken the leather and in the manufacture of drugs, fungicides, insecticides and dyes. Ingestion and inhalation of 4-Nitrophenol leads to nausea, drowsiness, headache and cyanosis[5]. Dyes are the major synthetic organic compounds used in textile industries for coloration of the fabrics. After completing the process i.e coloration of fabrics 15% of dye is wasted and released in to water bodies which ultimately results in to serious water pollution. Methylene blue or methylthioninium chloride is a synthetic heterocyclic aromatic dye and dark green powder when dissolved in water turns in to blue colour. It is a safe drug for therapeutic use in lower doses. Methylene blue has shown antimalarial activity and increases arterial pressure in septic shock. When exposed to higher doses it causes head ache, fever, diziness and mental confusion[6]. Methyl orange is a synthetic dye, pH indicator in acid-base titrations and causes irritation incase of skin contact and eye contact. Another one methyl red is an azodye and used in acid base titrations as pH indicator. It is prepared by diazotization of anthranilic acid and followed by dimethyl aniline[7].

Passiflora foetida is a native plant of Southwestern United states and Mexico, belongs to the family Passifloraceae and has been introduced to the tropical regions of the world. The plant stem is thin and wiry with minute yellow hairs in younger stage and becomes woody later. The leaves are three to five lobed and the flowers are white to pale cream colored. The fruits of the plant are globose, 2-3cm in diameter and when riped appear yellow-orange to red in colour. The aqueous and

ethanolic extracts of plant has shown the presence of various secondary metabolites like terpenoids, phenols, saponins, flavanoids, steroids, alkaloids, oils and cardioglycocides[8, 9]. In the present study AgNPs were synthesized using the aqueous plant extract of *P. foetida* and characterized using different advanced techniques. Further the green synthesized AgNPs were screened for their catalytic activity in the degradation and removal of 4-Nitrophenol, Methylene blue, Methyl orange and methyl red.

MATERIALS AND METHODS

Collection of the plant material

The aerial parts of *Passiflora foetida* were collected from Tirumala hills, Tirupathi, Andhra Pradesh, India. The plants were taxonomically identified and authenticated by Prof. M. Vijayalakshmi, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Preparation of the aqueous extract of P. foetida

The plant material collected was washed thrice with distilled water to remove the dust and dried under the shade to remove the moisture. The dried plant material was then cut in to pieces and crushed in to fine powder with a suitable pulveriser. 3 grams of finely crushed dried powder was mixed to 100 ml of molecular grade water, boiled at 100^0C for 10 minutes and the extract was filtered with Whatman No 1 filter paper to remove impurities.

Green synthesis of AgNPs from the extract of P. foetida

2ml, 10ml, 20ml, 30ml and 40ml of filtered plant extract was added to 198ml, 190ml, 180ml, 170ml and 160ml of 1 mM Silver nitrate ($AgNO_3$) solution respectively and kept for incubation. The effect of $AgNO_3$ concentration on AgNPs formation was analyzed by adding 10ml of plant extract to 190ml of 0.1mM, 0.5mM, 1mM, 1.5mM and 2mM concentrations of $AgNO_3$ in separate reactions. Later the suspension was kept for incubation at room temperature.

Characterization of plant mediated AgNPs

The formation of AgNPs using *P. foetida* extract was confirmed by UV-Visible spectroscopic studies after 48hrs using $AgNO_3$ solution as blank and the values were recorded within the range of 200 to 800 nm. To know the effect of time on AgNPs formation, the amalgamated solution containing 10ml of plant extract + 190ml of AgNO3 of $AgNO_3$ was analyzed using UV-

Visible spectrometer for every 1hr time intervals. Later the plant mediated AgNPs were examined in HORIBA particle size analyzer and zeta potential analyzer to know the particle size and stability of AgNPs respectively.

The AgNPs were purified by repeated centrifugation at 10,000 rpm for 15 min. The pellet of AgNPs were transferred into china dish and kept for shade evaporation. The dried nanoparticles were washed with distilled water, allowed for shade drying and the process was repeated thrice. The purified and dried nanoparticle samples were collected and used for further characterization. The purified AgNPs were studied using Philips X'pert pro XRD with an operation voltage of 40KV and current of 30mA with CuKα radiation (1.540 °A) between 2θ° angles (30°-80°) for analysing peak data and crystal structure. Flourescence transmission infrared (FTIR) analysis of the AgNPs was carried out through potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/ IR- 6300 FTIR equipped with JASCO IRT-7000 Intron Infrared microscope (JASCO, Tokyo, Japan) using transmittance mode operating at a resolution of $4cm^{-1}$ in order to find out the phytochemicals in *P. foetida* extract which are responsible for reduction process in the AgNPs synthesis. Transmission electron microscope study was carried out to know the morphology and particle size distribution of AgNPs. The grid for TEM analysis was prepared by placing a drop of *P. foetida* mediated AgNPs suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing AgNPs was scanned by a Hitachi Japan Model 7500 TEM machine.

Evaluation of catalytic activity of plant mediated AgNPs

In the present study AgNPs of *P. foetida* were utilised as catalysts seperately in the degradation and removal of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red by $NaBH_4$. The procedure of degradation of a respective synthetic chemical or dye (4-Nitrophenol or Methylene blue or Methyl orange or Methyl red) by $NaBH_4$ in presence of AgNPs as catalyst involves 3 reactions. All the reactions were studied in Thermoscientific UV-Visible spectrophotometer using milli Q water as blank. The first reaction is prepared by adding 1.5mL of 1mM of a synthetic chemical or dye to 1.5mL of milli Q water, mixed well and analyzed in UV-Visible spectroscopy. Later 1mg of solid $NaBH_4$ was added to first reaction to prepare second reaction and analyzed in UV-Visible spectroscopy. The third reaction is prepared by adding 10µL of green synthesized nanosolution to the second reaction and analyzed after 1 minute in UV-Visible spectroscopy[7].

RESULTS AND DISCUSSION

Addition of *P. foetida* plant extract with aqueous solution of silver nitrate led to observable Colour change from yellowish to dark reddish brown solution after 48 hrs incubation due to Surface Plasmon Resonance indicating AgNPs formation[10] and the result was depicted in the Fig.1d.

Characterization of *Passiflora foetida* AgNPs

UV-Visible analysis

The amalgamated solution of 190ml AgNO3 + 10ml plant extract have shown absorption maximum at 435.13 (Fig. 2c) in UV-Visible spectroscopic analysis after 48 hrs incubation[11, 12] which confirmed the formation of AgNPs. When the amalgamated solutions that kept for incubation to know the effect of plant extract concentration studied in UV-Visible spectroscopy AgNPs formation was confirmed in the reactions with 2ml, 10ml and 20ml of plant extract only (Fig. 2a). Further when the amalgamated solutions that kept for incubation to know the effect of $AgNO_3$ concentration studied in UV-Visible spectroscopy AgNPs formation was confirmed in the reactions with only to 1mM, 1.5mM and 2mM concentrations of $AgNO_3$ (Fig. 2b). In another study when the amalgamated solution of 190ml AgNO3 + 10ml plant extract was studied every 1hr time intervals in UV-Visible spectroscopy *P. foetida* AgNPs formation was observed after 27hrs (Fig. 2c).

Dynamic light scattering (DLS) study

In DLS study the particles exhibit Brownian motion when dispersed in the medium which is measured by the fluctuations in the intensity of scattered light in the system from which translational diffusion co-efficient is calculated by applying Stokes-Einstein equation which determines the hydrodynamic size[13]. The graph has shown almost equal size distribution of *P. foetida* AgNPs and their mean size was recorded as 99.3nm (Fig. 3a). Polydispersity index (PDI) represents the ratio between different sizes to total number of particles. A PDI value more than 0.5 refers to the aggregation of the particles[14]. The green synthesized AgNPs have shown zero PDI value which clearly indicates that the particles are in mono dispersed phase with no aggregation.

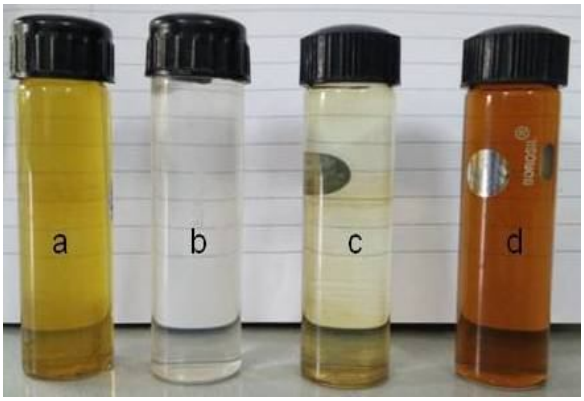

Fig. 1 Green synthesis of AgNPs (a) Aqueous plant extract (b) Silver nitrate solution (c) 1mM silver nitrate+plant extract at the start of incubation (d) *P. foetida* AgNPs

Zeta potential analysis

The Zeta potential of *P. foetida* AgNPs solution is measured by applying an electric field across the dispersion. Particles within the dispersion with a zeta potential will migrate towards the electrode of opposite charge with a velocity proportional to the magnitude of zeta potential. It should be noted that the particles with zeta potential values more positive than +30 mV or more negative than -30 mV are considered to be stable[15]. The zeta potential of *P. foetida* AgNPs was recorded as -20.8mV in the zeta potential analysis as shown in the Fig. 3b and from the obtained result it can be known that the synthesized nanoparticles are moderately stable[16].

XRD Analysis

X-ray powder diffraction spectrum of green synthesized AgNPs (Fig. 4a) have shown Bragg peaks (angle 2θ) at 27.48^0, 31.90^0, 37.78^0, 43.80^0, 45.91^0, 57.15^0, 64.25^0 and 76.56^0 which corresponds to the indexed planes of 210, 122, 111, 200, 142, 241, 220 and 311 miller indices of face centered cubic (FCC) structure of a regular silver crystal[17]. Using Debye -Scherrer equation the average particle size of biosynthesized was determined [$d = K\lambda / \beta \cos \theta$] where'd' is the mean diameter of the particle; 'K' is the shape factor (0.9); 'λ' is the X-ray radiation source (0.154 nm); 'β' is ($\pi / 180$) ∗ FWHM and 'θ' is the Bragg angle. The average particle size of *P. foetida* AgNPs was obtained as 14.30nm and the XRD pattern was in agreement with earlier XRD reports of green synthesized AgNPs[18].

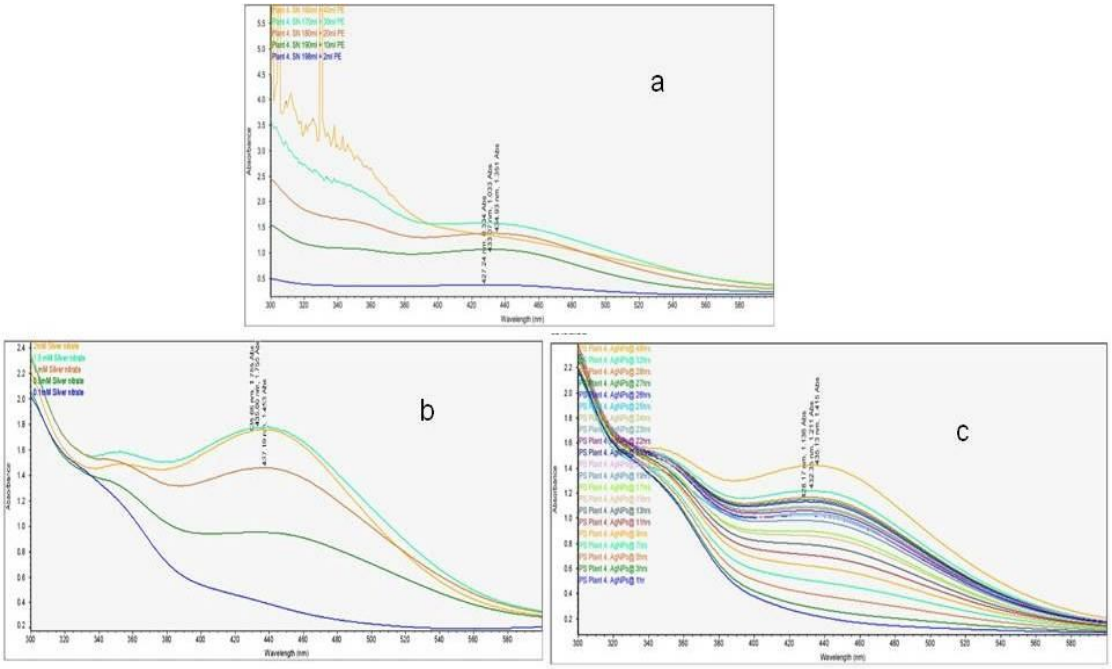

Fig. 2 UV-Visible analyses of (a) Effect of plant extract concentration (b) Effect of $AgNO_3$ concentration (c) Influence of time on *P. foetida* AgNPs formation

FTIR study

The biosynthesized AgNPs displayed a number of peaks in the FTIR spectrum (Fig. 4b) and portrayed the complex nature of particles. The strong and broad peak at 3371 cm^{-1} was formed because of O-H bond stretching of alcohols and phenols[19]. The peaks formed at 2923 cm^{-1} and 2854 cm^{-1} due to the characteristic stretching vibrations of C-H stretch of alkanes. The medium and strong peak at the 1611 cm^{-1} is the N-H stretching vibrations of Amides[18]. The medium peak at 1382 cm^{-1} was formed because of $-(CH_3)_3$ bend of alkanes and alkyl groups. The strong peak formed at 1222 cm^{-1} due the stretching vibrations of alkyl groups. The peak at 1055 cm^{-1} was formed due to the C-O stretch of alcohols. A strong peak was formed at 986 cm^{-1} because of the =C-H bend of alkenes. The C-H bend of aromatic compounds formed a weak peak at 708 cm^{-1}. These shifts in peak positions revealed the presence of different secondary metabolites in the plant extract of *P. foetida*. Hence it can be concluded that the phytochemicals or secondary metabolites of plant extract acted as reducing and stabilizing agents in the AgNPs formation[5].

TEM analysis

The TEM image revealed that most of the AgNPs were spherical in shape with an average size of 20nm (Fig. 4c) and is in agreement with results produced in XRD analysis[20]. The TEM examination also confirmed the presence of biomolecule blanket on the surface of AgNPs which acts

as capping agents. Therefore the AgNPs of *P. foetida* were polydispersed without direct contact with the other and stable for longer periods of time.

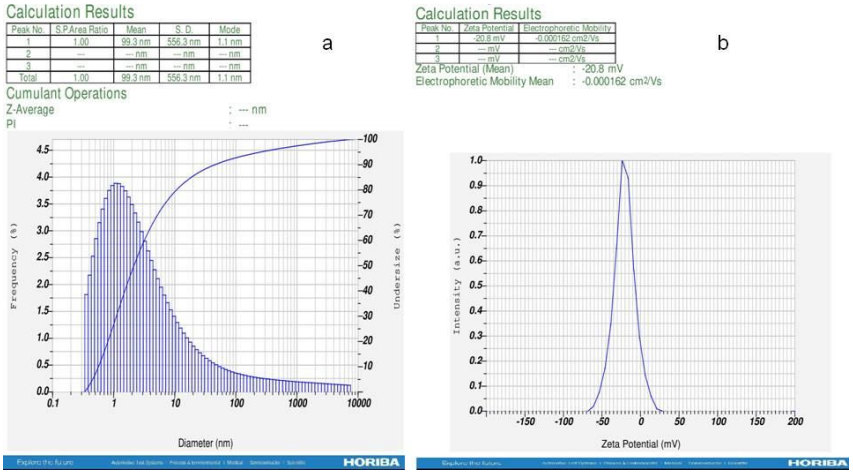

Fig. 3 (a) Dynamic light scattering (b) Zeta potential analysis of *P. foetida* AgNPs

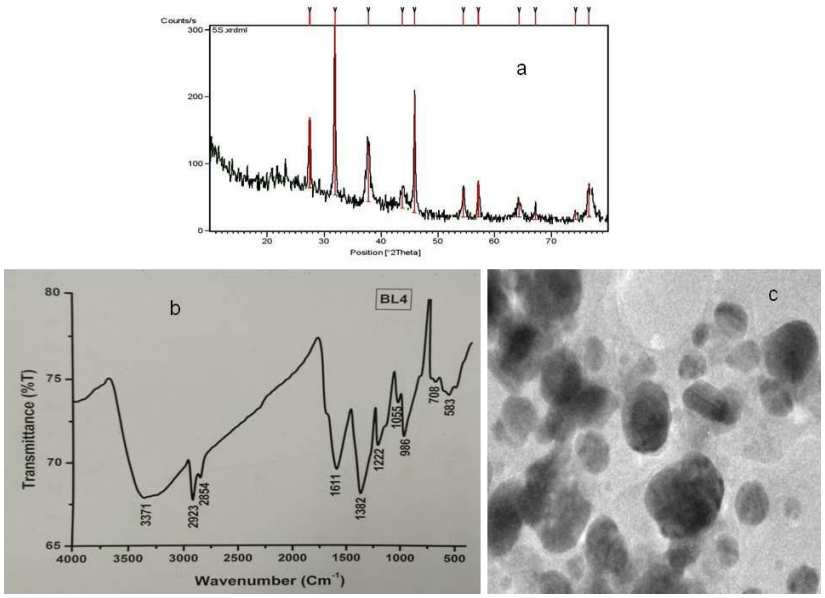

Fig. 4 (a) XRD sppectrum (b) FTIR spectrum (c) TEM image of *P. foetida* AgNPs

Catalytic activity of *P. foetida* AgNPs

The synthetic chemical or dye degradation reactions were monitered and depicted in the following order 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red. All reactions were analysed by UV-Visible spectrophotometer.

Reduction reactions of 4-Nitrophenol

UV-Visible analysis of 4-Nitrophenol degradation using $NaBH_4$ with *P. foetida* AgNPs as catalysts were shown in the Fig. 5a. The reaction of 4-Nitrophenol when monitered in

spectrophotometer the absorption maximum (1.252) was observed at 318 nm. On addition of $NaBH_4$ to first reaction the solution appeared bright yellow in colour because of the formation of sodium phenolate, no change in the absorption maximum with respect to time was observed and shifted to 400 nm[21]. With the addition of plant mediated AgNPs the solution turned colourless and the absorption maximum was suddenly decreased from 1.252 to 0.823 indicating the complete degradation of 4-Nitrophenol[22].

Reduction reactions of Methylene blue

The degradation and removal of methylene blue by $NaBH_4$ in the presence of biosynthesized AgNPs as catalyst were analyzed and the UV-Visible results were illustrated in Fig. 5b. For pure 1mM methylene blue absorption maximum of 2.009 was initially observed at 664 nm[23]. When 1mg of $NaBH_4$ added the absorption maximum was recorded at same wavelength with a slight change. With the addition of green synthesized AgNPs the solution turned colourless and the absorption maximum was completely decreased from 2.009 to Nil indicating that methylene blue was completely degraded[24].

Reduction reactions of Methyl orange

Fig. 5c illustrates the results related to the degradation and removal reactions of methyl orange by $NaBH_4$ in the presence of *P. foetida* AgNPs as catalysts. In UV-Visible analysis for pure methyl orange the absorption maximum was found to be 0.752 at 462.22nm and when 1mg of $NaBH_4$ added the absorption maximum was found to be 1.673 at 461.11nm. After adding AgNPs, the solution turned colourless with decrease in absorption maximum from 1.673 to 0.647[25]. From the above result it is clearly known that the AgNPs of *P. foetida* completely degraded methyl orange in presence of $NaBH_4$.

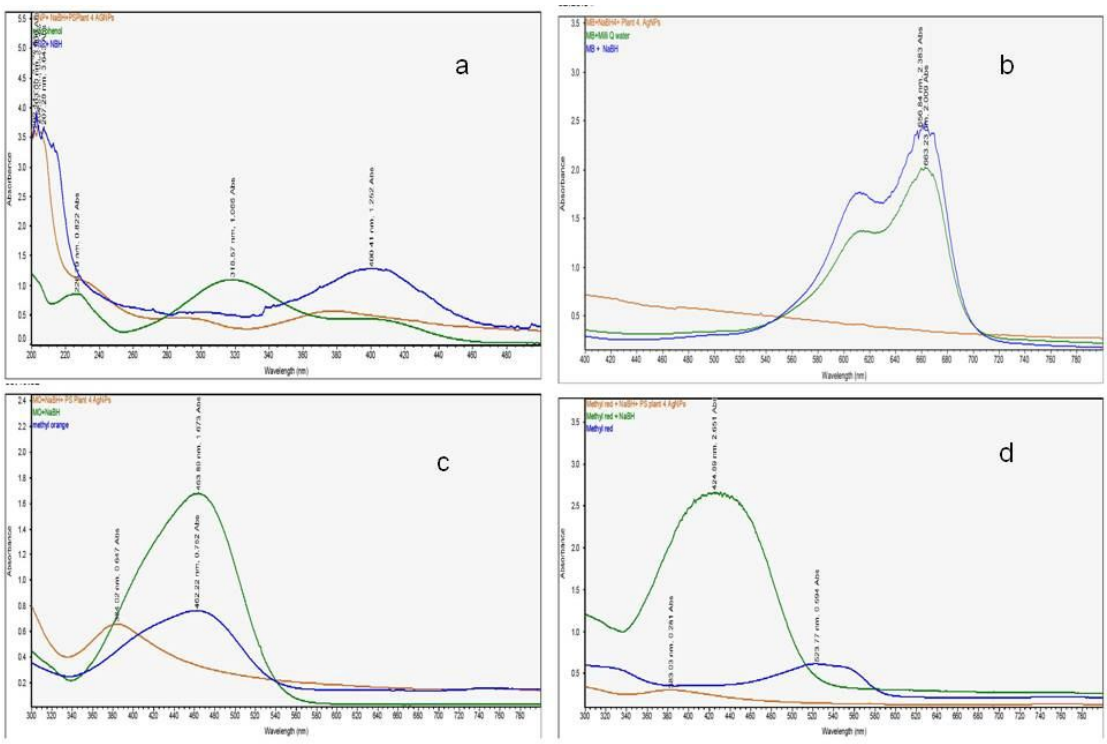

Fig. 5 Catalytic activity of *P. foetida* AgNPs on (a) 4-Nitrophenol (b) Methylene blue (c) Methyl orange (d) Methyl red (NaBH: Sodium tetra borate, AgNPs: Silver nanoparticles)

Table No. 1- Absorption maxima of synthetic chemicals before and after addition of AgNPs

Name of the synthetic chemical/ dye	Absorption maximum before the addition of AgNPs	Absorption maximum after the addition of AgNPs
4-Nitrophenol	1.252	0.822
Methylene blue	2.009	Nil
Methyl orange	1.673	0.647
Methyl red	2.651	0.281

Reduction reactions of Methyl red

UV-Visible analysis results related to the reduction reactions of methyl red by $NaBH_4$ in presence of AgNPs as catalysts were illustrated in Fig. 5d. An absorption maximum of 0.594 was observed at 523.77nm to pure 1mM methyl red. After addition of 1mg $NaBH_4$ the absorption maximum of 2.651was obtained at 424.89nm. With the addition of *P. foetida* AgNPs yellow colour

solution was turned colourless and absorption maximum of 0.281 was recorded for third reaction in the UV-Visible analysis indicating the complete reduction of methyl red[26, 27].

CONCLUSION

Biosynthesis gained huge fame in the scientific and research world for the synthesis of metallic nanoparticles because of its cost effective and ecofriendly nature. In the present study AgNPs were synthesized using the aqueous extract of *Passiflora foetida* aerial parts. The amalgamated solution of 10ml plant extract + 190ml AgNO3 solution turned deep reddish brown in colour and has shown surface plasmon resonance band at 435.13nm in UV-Visible analysis after 48hrs confirming the formation of AgNPs. In DLS study the AgNPs have shown equal size distribution and no aggregation with mean size of 99.3nm. The plant mediated AgNPs have exhibited the zeta potential value of -20.8mV which proclaimed their moderately stable nature. The spectrum obtained in the XRD analysis clearly indicates that the biosynthesized AgNPs of *P. foetida* were crystalline face centred cubic in structure. The peaks obtained in the FTIR spectrum of AgNPs acknowledged the presence different photochemical in the plant extract of *P. foetida* and their role as reducing and stabilizing agents in AgNPs formation. The TEM images revealed that most of the AgNPs were spherical in shape with an average size of 20nm produced in XRD analysis. Further crucially in the present study the plant mediated AgNPs were examined for their catalytic activity in presence of $NaBH_4$ in the degradation and removal of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red. After adding AgNPs to the redution reactions of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red the absorption maximum was decreased from 1.252 to 0.823, 2.009 to Nil, 1.673 to 0.647 and 2.651 to 0.281 respectively. The above results recorded in UV-Visible analysis confirmed that the AgNPs of *P. foetida* were shown to exhibit remarkable catalytic activity in the degradation and removal of respective synthetic and toxic chemicals.

CONFLICT OF INTEREST: All the authors declare that there is no conflict of interest

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