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Ecofriendly synthesis of silver nanoparticles using the bark of *Putranjiva roxburghii* and screening for their catalytic activity

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Abstract

Green synthesis is a bottom-up approach in which metallic nanoparticles are synthesized using the extracts of plants as reducing and capping agents in cost effective and ecofriendly manner. In the present study silver nanoparticles (AgNPs) were synthesized using the bark extract of *Putranjiva roxburghii*. The amalgamated mixture of plant extract and silver salt solution turned deep reddish brown in colour after 48hrs incubation. Later in UV-Visible analysis surface plasmon resonance band for amalgamated solution was observed at 421nm confirming the formation of AgNPs. For further characterization the plant mediated AgNPs were purified in centrifugation and studied in X-ray diffractometer (XRD), Flourescence transform infra red spectroscopy (FTIR) and Transmission electron microscope (TEM). Crucially in the present study the biosynthesized AgNPs were screened for their catalytic activity in the degradation and removal of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red. The results obtained in reduction reactions revealed the potential catalytic activity of *P. roxburghii* AgNPs in the degradation and removal of respective toxic and synthetic chemicals.

Keywords: *Green synthesis, Putranjiva roxburghii, AgNPs, $NaBH_4$, Catalytic activity*

1. Introduction

Nanobiotechnology is an interdisciplinary and applied science involves the synthesis, characterization and application of materials and devices on the nanoscale. In this field of science new and unique materials with dimension less than 100nm are created and used in different applications. The emergence of nanobiotechnology has exhibited promising results in recent years with various other branches of science and developed impact on all forms of life (Agarwal *et al*., 2017). In general nanomaterials are synthesized by two basic approaches i.e. Top-down and Bottom-up methods. In top-down method bulk materials are broken down in to fine particles through size reduction in to various lithographic techniques i.e. grinding, milling, and sputtering and thermal/laser ablation. But in bottom-up approach nanomaterials are formulated from smaller entities for example by joining atoms or molecules (Vijayaraghavan and Ashokkumar, 2017).

Biosynthesis is a bottom-up approach in which metallic nanoparticles are synthesized using the extracts of bacteria, fungi, algae and plants etc. In this process the secondary metabolites in the extracts of biological entities acts as reducing and stabilizing agents in the formulation of nanoparticles. Biosynthesis is a cost effective and ecofriendly process when compared to physical and chemical methods that are used for the synthesis of nanoparticles. More over in biosynthesis nanoparticles of homogenous chemical composition are synthesized (Sathishkumar *et al*., 2016). Because of their small size and high surface to volume ratio as well as the presence of bioactive secondary metabolites biosynthesized nanoparticles were shown to exhibit potential antimicrobial, anticancer and antiplasmodial activities (Kuppusamy *et al*., 2016). In addition biosynthesized nanomaterials have displayed significant results in electronics, drug delivery, catalysis, biological labeling, chemical sensing and imaging, cosmetics and environmental remediation (Vijayaraghavan and Ashokkumar, 2017).

Green synthesis is one of the research areas of biosynthesis in which plant and their extracts are used

in the formulation of metallic nanoparticles. According to the recent literature nanoparticles of different metals i.e silver, gold, platinum, palladium, zinc, copper and iron were synthesized by green route method (Khan *et al*., 2017). Among them green synthesized silver nanoparticles attracted the attention of researchers and ignited the minds of scientists because of their long term stability and remarkable biomedical activities and catalytic activity in the degradation and removal of synthetic and toxic chemicals from polluted water (Santhoshkumar *et al*., 2017; Khan *et al*., 2017). In the present study AgNPs were synthesized using *Putranjiva roxburghii* aqueous bark extract. It is an evergreen tree, grows up to 12 meters height and belongs to the family Puthranjivaceae. The leaves of the plant are simple, dark green, elliptic-oblong, distantly serrated and alternately arranged. Fruits are ellipsoid or rounded drupes with only one seed, stone pointed and very hard. In pharmacognostical analysis the leaves, fruits, stem and roots of the plant have shown the presence of many therapeutic active compounds includes saponins, glycosides, triterpenes, ellagic acid, gallic acid and flavanoids (Gupta, 2016). The synthesized AgNPs were characterized using different advanced techniques and further screened for their catalytic activity in the degradation and removal of 4-Nitrophenol, methylene blue, methyl orange and methyl red from water.

2. Materials and Methods

2.1 Collection of the plant material

The stem bark of *Putranjiva roxburghii* was collected from the premises of Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. The plant was taxonomically identified and authenticated by Prof. M. Vijayalakshmi, Dean and Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

2.2 Preparation of *P. roxburghii* extract

The collected material of *P. roxburghii* 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 100ml of molecular grade (Milli Q) water, boiled at 100^0C for 10 minutes and the extract was filtered with Whatman No 1 filter paper to remove impurities.

2.3 Green synthesis of *P. roxburghii* AgNPs

To study the effect of plant extract concentration on AgNPs formation 2ml, 5ml, 10ml, 15ml and 20ml of filtered plant extract was added to 198ml, 195ml, 190ml, 185ml and 180ml of 1mM Silver nitrate ($AgNO_3$) solution and kept for incubation. The effect of $AgNO_3$ concentration on AgNPs formation was analyzed by adding 15ml of plant extract to 185ml 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.

2.4 Characterization of AgNPs

The formation and stability of *P. roxburghii* AgNPs was confirmed by UV-Visible spectroscopic studies after 48hrs using $AgNO_3$ 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 (15ml of plant extract + 185ml of $AgNO_3$) was analyzed using UV-Visible spectrometer for every 1hr time intervals.

Later the AgNPs were purified from their solution by repeated centrifugation at 10,000 rpm for 15 min. The pellet of AgNPs was transferred into a 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 of *P. roxburghii* 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 4 cm^{-1} in order to find out the secondary metabolites in *P. roxburghii* extract which are responsible for reduction process in the AgNPs synthesis. Transmission electron microscope inspection was executed to know the morphology and particle size distribution of silver nanoparticles. The grid for TEM analysis was formulated by placing a drop of nanoparticle suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was then examined in a Hitachi Japan Model 7500 TEM machine.

2.5 Evaluation of catalytic activity AgNPs

In the present study AgNPs of *P. roxburghii* were utilised as catalyst 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

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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 *P. roxburghii* AgNPs to the second reaction and analyzed in UV-Visible spectroscopy after 1 minute (Bodaiah *et al*., 2017).

3. Results and Discussion

Surface plasmon resonance (SPR) is an elegant optical aspect displayed by nano silver particles due to vibration of the conducting metal surface electrons in resonance with the non-particulate radiation. Addition of *P. roxburghii* plant extract with aqueous solution of silver nitrate led to the observable colour change from yellowish to dark reddish brown solution (Ravichandran *et al*., 2016; Swarnavalli *et al*., 2017) after 48 hrs incubation (Fig.1d) due to Surface Plasmon Resonance indicating the formation of AgNPs.

3.1 Characterization of AgNPs

UV-Visible analysis

The amalgamated solution of 15ml plant extract + 185ml $AgNO_3$ have shown absorption maximum at 421nm (Fig. 2a) in UV-Visible spectroscopic analysis after 48 hrs incubation which confirmed the formation of AgNPs (Jasim *et al*., 2017). The amalgamated reactions that kept for incubation to know the effect of plant extract concentration were studied in UV-Visible spectroscopy. AgNPs formation was confirmed in the reactions with 5ml, 10ml, 15ml and 20ml of plant extract only as shown in the Fig. 2a. Further 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 0.1mM, 0.5mM and 1mM concentrations of $AgNO_3$ (Fig. 2b). When the amalgamated solution of 185ml $AgNO_3$ + 15ml plant extract was examined in UV-Visible spectrophotometer at every 1hour time intervals AgNPs formation was observed exactly after 11 hours incubation and the results were depicted in the figure 2c.

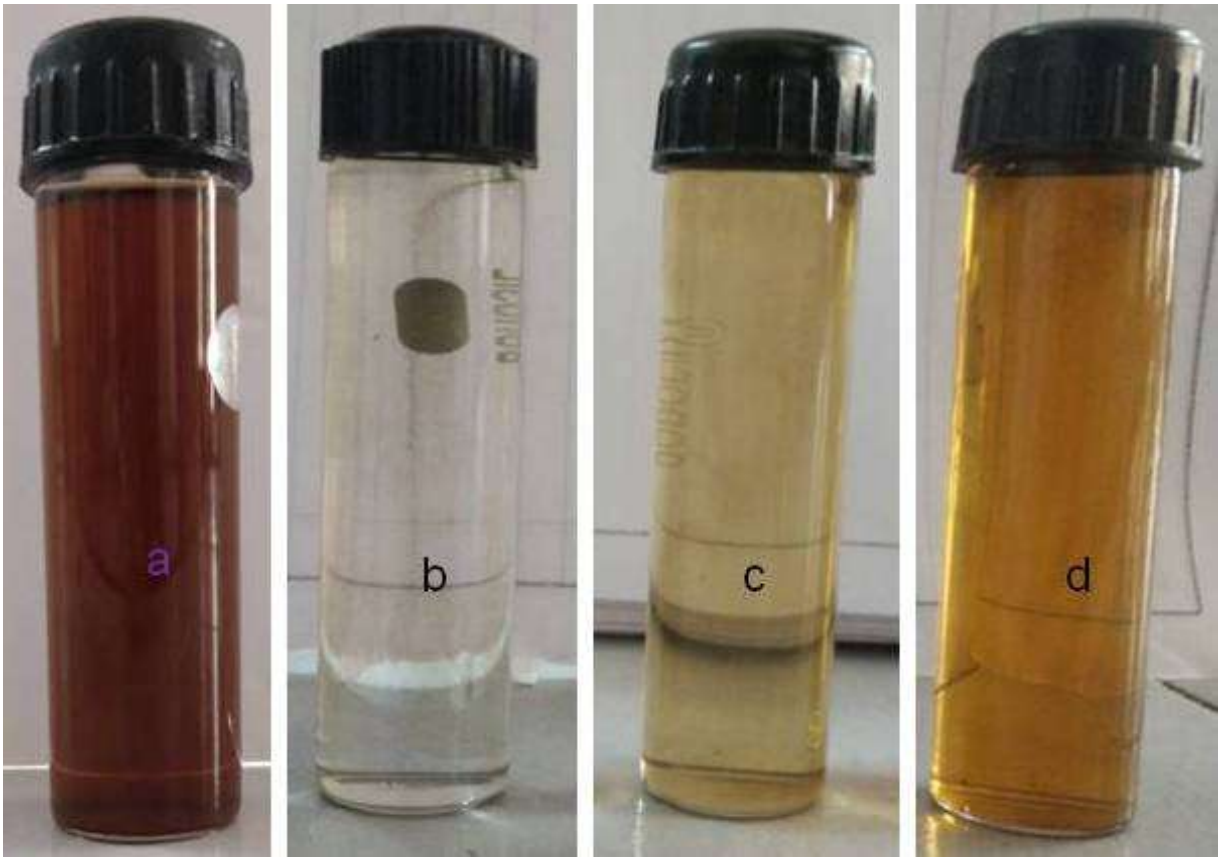

Fig.1: Green synthesis of AgNPs (a) Plant extract (b) 1mM $AgNO_3$ solution (c) $AgNO_3$ solution + Plant extract at the start of incubation (d) *P. roxburghii* AgNPs

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XRD Analysis

X-ray powder diffraction spectrum of green synthesized AgNPs have shown Bragg peaks (angle 2θ) at 27.54^0, 31.98^0, 37.89^0, 45.97^0, 54.64^0, 57.29^0, 64.30^0 and 77.25^0 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 and the XRD pattern (Fig. 3a) was in agreement with earlier XRD reports of green synthesized AgNPs (Suman *et al*., 2013; Ravichandran *et al*., 2016). 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 and the average particle size of *P. roxburghii* AgNPs was obtained as 16.50nm.

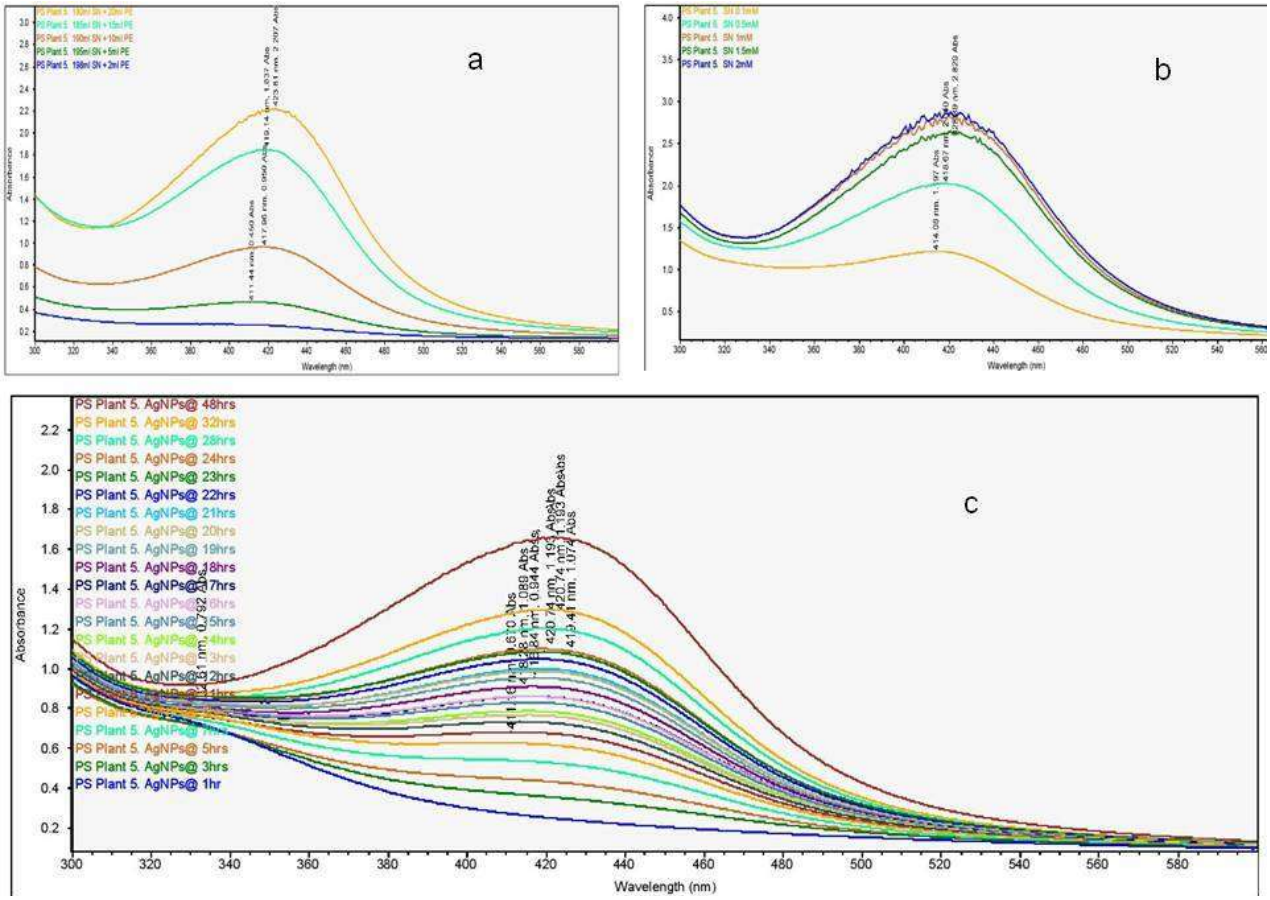

Fig. 2: UV-Visible analysis of *P. roxburghii* (a) Effect of plant extract concentration on AgNPs formation (b) Effect of $AgNO_3$ concentration on AgNPs formation (c) Influence of time on AgNPs formation (SN: $AgNO_3$ solution; PE: Plant extract; hr: Time in hours)

FTIR study

The AgNPs of *P. roxburghii* displayed a number of peaks in the FTIR spectrum (Fig. 3b) and portrayed their complex nature. The strong and broad peak at 3430.14cm^{-1} was formed because of O-H bond stretching of alcohols. The peak formed at 2930cm^{-1} and 2854cm^{-1} is due to the characteristic stretching vibrations of C-H stretch of alkanes. The C=O stretch of esters formed a medium peak at 1743cm^{-1}. The medium and strong peak formed at the 1632cm^{-1} denotes the C=O stretching vibrations of Amides (Ahmad *et al*., 2016). The peak formed at 1534cm^{-1} is because of N-H stretch of amides. The (CH_3) C-H bend of alkanes and alkyl groups formed a medium peak at 1368cm^{-1}. The peak at 1222cm−1 was formed due to C-F stretch of alkyl halides. The C-H bend of alkenes was formed a medium peak at 965cm^{-1} while the C-Cl stretch of alkyl halides formed weak peak at 743cm^{-1} but the weak peaks at 583cm^{-1} was formed because of C-Br stretch (Suman *et al*., 2013). These shifts in peak positions reveal that different phytochemicals were present in the plant extract of *P. roxburghii* and hence it can be concluded that bioorganic compounds present in the plant extract acted as reducing and stabilizing agents in the AgNPs formation.

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TEM studies

TEM analysis revealed the presence of spherical shaped AgNPs with size in the range of 10-20 nm (Fig. 3c). It was also found that the nanospheres of *P. roxburghii* are bounded with thin layer of biomolecules blanket on their surface which acts as stabilizing agent (Moldovan *et al.*, 2016). Therefore, the particles were polydispersed without direct contact and reliable for longer periods of time (Jang *et al.*, 2016).

3.2 Catalytic activity of 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. roxburghii* as catalysts was shown in the Fig. 4a. The reaction of 4-Nitrophenol when monitered in spectrophotometer the absorption maximum 1.066 was observed at 316 nm. On addition of $NaBH_4$ to first reaction the solution appeared bright yellow in colour because of the formation of sodium phenolate and the absorption maximum of 1.252 was recorded in UV-Visible analysis and shifted to 400 nm (Young *et al.*, 2018). Later 10µL plant mediated AgNPs were added to second reaction, the solution turned colourless suddenly and the absorption maxima decreased from 1.252 to 0.622 in UV-Visible analysis which confirmed the complete degrdation of 4-Nitrophenol (Shahriary *et al*., 2018).

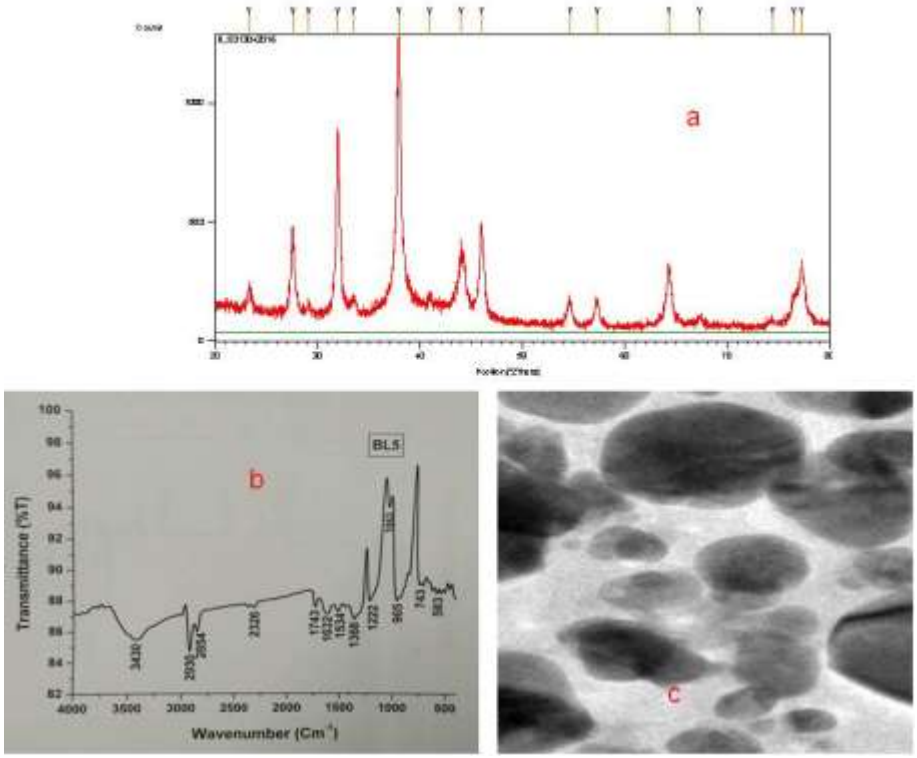

Fig. 3: (a) XRD spectrum (b) FTIR spectrum(c) TEM image of *P. roxburghii* AgNPs

Reduction reactions of Methylene blue

The degradation and removal of methylene blue by $NaBH_4$ in the presence of plant mediated AgNPs as catalyst was analyzed and the UV-Visible results were illustrated in Fig. 4b. For pure 1mM methylene blue absorption maximum of 2.009 was initially observed at 664 nm. When 1mg of $NaBH_4$ added the absorption maximum was recorded at 661nm with a slight change. With the addition of *P. roxburghii* AgNPs the solution turned colourless and the absorption maximum was decreased from 2.480 to 0.210 indicating that methylene blue was completely degraded (Rajan *et al*., 2015; Saha *et al*., 2017).

Reduction reactions of Methyl orange

In UV-Visible analysis for pure methyl orange the absorption maximum was found to be 0.752 at 462.22nm. When 1mg of $NaBH_4$ added the absorption maximum was found to be 1.673 at 464.11nm. After adding AgNPs of *P. roxburghii*, the solution turned colourless with decrease in absorption maxima from 1.673 to 0.511 as shown in the figure 5c. From the above results it can be known that AgNPs of *P.*

roxburghii completely degraded methyl orange (Varadavenkatesan *et al*., 2016; Saha *et al*., 2017).

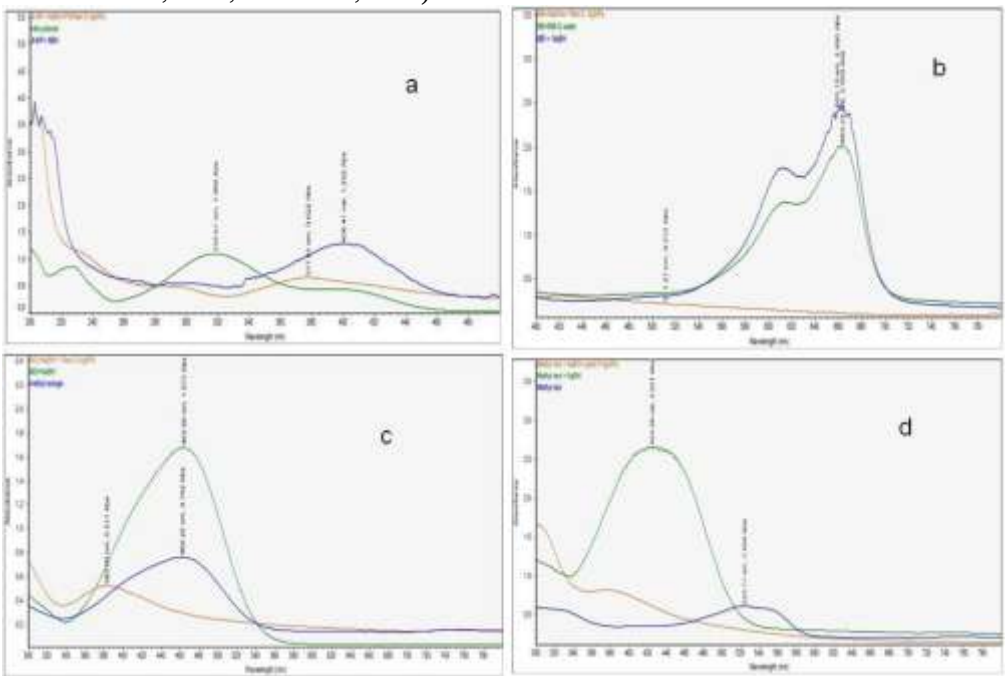

Fig. 4: Catalytic activity of *P. roxburghii* AgNPs on (a) 4-Nitrophenol (b) Methylene blue (c) Methyl orange (d) Methyl red (NaBH: Sodium tetra borate, AgNPs: Silver nanoparticles)

Table 1: Absorption maxima of synthetic chemicals before and after addition of *P. roxburghii* AgNPs

Name of the Synthetic chemical	Absorption Maximum before the addition of AgNPs	Absorption Maximum after the addition of AgNPs
4-Nitrophenol	1.066	0.622
Methylene blue	2.480	0.210
Methyl orange	1.673	0.511
Methyl red	2.651	Nil

Reduction reactions of Methyl red

The UV-Visible analysis results related to the reduction reactions of methyl red by $NaBH_4$ in presence of AgNPs were illustrated in Fig. 5d. An absorption maximum of 0.594 was observed at 523.77nm to pure 1mM methyl red in UV-Visible analysis. After addition of 1mg $NaBH_4$ the absorption maximum of 2.651was obtained at 424.89nm. With the addition of *P. roxburghii* AgNPs yellow colored solution turned colourless and zero absorption maximum was recorded in the third reaction indicating the complete reduction of methyl red (Bodaiah *et al*., 2017).

4. Conclusion

Biosynthesis of AgNPs using the extracts of plants attracted the attention of biotechnologists because of its cost effective, ecofriendly, time saving and easy handling nature. Moreover the green synthesized AgNPs were more stable and exhibited remarkable biological and catalytic activities. In the present study the mixture of *Putranjiva roxburghii* extract + silver salt solution changed in to reddish brown in colour after 48hrs incubation and in UV-Visible analysis have shown absorption maximum at 421nm confirming the formation of AgNPs. Further the peaks obtained in the XRD spectrum revealed the crystalline face centred cubic nature of biosynthesized nanoparticles and the average size of particles obtained as 16.50nm in calculation by using Debye - Scherrer equation. FTIR examination confirmed the presence of secondary metabolites in the plant extract and their role as reducing and capping agents in the AgNPs formation. From TEM analysis it is known that the plant mediated AgNPs were mostly spherical and in the range of 10-20nm size. In further studies i.e in catalytic activity evaluation after adding AgNPs the reduction reactions of 4-Nitrophenol, methylene blue, methyl orange and methyl red have recorded the absorption maximum reduction from 1.252 to 0.622, from 2.480 to 0.210, from 1.673 to 0.511 and from 2.651 to Nil respectively. The results obtained in the reduction reactions confirmed the significant catalytic

activity of AgNPs in the degradation and removal of 4-Nitrophenol, methylene blue, methyl orange and methyl red.

Conflict of Interest: All the authors declare that there is no conflict of interest.

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