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Ecofriendly Synthesis of Silver Nanoparticles Using *Shorea tumbergaia* bark Extract and Screening for their Catalytic Activity

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ABSTRACT

Metallic nanoparticles synthesized by green route method have exhibited potential catalytic activity in the removal of toxic chemicals from polluted water. In the present study silver nanoparticles (AgNPs) were green synthesized using the aqueous bark extract of *Shoreatumbergaia*. When the bark extract was added to AgNO₃ solution, the amalgamated solution turned deep reddish brown in colour after 48 hours indicating the formation of AgNPs. Later the amalgamated solution was examined in UV-Visible spectrophotometer to confirm the formation of AgNPs. Surface plasmon resonance band for amalgamated solution was observed at 441.98nm after 48hrs which confirmed the formation of *S.tumbergaia* AgNPs. The green synthesized AgNPs were further characterized using X-ray diffractometer (XRD), Fluorescence transmission infrared spectroscopy (FTIR) and Transmission electron microscopy (TEM) to know their size, stability and crystalline nature. In further studies the plant mediated AgNPs have exhibited remarkable catalytic activity in the presence of NaBH₄ in the degradation and removal of 4-Nitrophenol, methylene blue, methyl orange and methyl red.

KEY WORDS: Green synthesis, *Shoreatumbergaia*, AgNPs, NaBH₄, Catalytic activity

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INTRODUCTION

In nanotechnology a wide variety of physical and chemical methods have been developed for the formulation of metallic nanoparticles. However these methods are expensive and involve toxic chemicals which are potentially harmful to the environment. Biosynthesis of metallic nanoparticles (MNPs) is a novel ecofriendly research approach of nanotechnology, gained prominence as an alternative to physical and chemical methods and attracted the attention of young researchers and scientists working in the field of life sciences especially biotechnology¹. In this cost effective process the extracts of bacteria, fungi, algae, actinomycetes, plants and viruses were used as reducing and stabilizing agents for the formulation of metallic nanoparticles. The special interest of researchers towards biosynthesis of MNPs was because of their potential antimicrobial, cytotoxic and insecticidal activities². In addition the biosynthesized MNPs were shown to exhibit significant catalytic activity in the degradation and removal of toxic chemicals from polluted water³. Green synthesis is a sector biosynthesis and a major research area of green chemistry in which plants and their extracts are used for the formulation of metallic nanoparticles. The biomolecules or secondary metabolites present in plant extract acts as reducing and stabilizing agents in the synthesis of MNPs. It is very cost effective and time saving process for large scale production of highly stable MNPs when compared to other biological methods. Currently the green synthesized MNPs were using in different biomedical applications because of their unique physical and chemical properties which are responsible for their biological properties⁴.

Till date different varieties of plants and their extracts were used for the formulation of MNPs. For example gold nanoparticles synthesized using the extracts of *Euphorbia hirta* L. leaves⁵; *Artocarpusheterophyllus* Lam. Fruits⁶ have exhibited potential antimicrobial activity. Later silver nanoparticles (AgNPs) synthesized from *CoffeaArabica* seed⁷; *Convolvulus arvensis* leaf extracts⁸ have shown significant antimicrobial activity. MNPs synthesized by green route method also shown cytotoxicity against cancer cell lines. For example *Momordicacymbalaria* fruit⁹; *Bauhinia Tomentosa* Linn leaf extract¹⁰ mediated silver nanoparticles were exhibited potential cytotoxic activity against selected cancer cell lines. However in addition to biological activities the green synthesized silver nanoparticles as well as gold nanoparticles have displayed catalytic activity in the degradation and removal of toxic chemicals. For example *Carica papaya* Peel extract mediated silver nanoparticles¹¹ have exhibited catalytic activity in the degradation of 4-Nitrophenol. In an interesting study silver nanoparticles were synthesized using the aqueous extract of *Stemonatuberosa* Lour, which have shown potential catalytic activity in the removal of methylene blue³. In another study gold nanoparticles were synthesized using the aqueous extract of same plant i.e. *Stemonatuberosa* Lour and screened for their

catalytic activity. The green synthesized gold nanoparticles were shown to exhibit remarkable catalytic activity in the degradation and removal of methylene blue¹².

In the present study taking the above findings in to consideration silver nanoparticles were synthesized using *Shoreatumbuggaia* stem bark extract and characterized using different advanced techniques. *S.tumbuggaia* is a native plant of Andhra Pradesh and Tamilnadu and belongs to the family Dipterocarpaceae. The plant is mainly grown for its timber and therapeutic purposes. The bark of the plant is used in the treatment of stomach ulcers where the leaf juice is used as ear drops¹³. 4-Nitrophenol is a toxic and hazardous chemical which is often used in pharmaceutical industries and agricultural industries in the manufacturing of drugs and pesticides or fertilizers respectively. It is found in agriculture and industrial waste waters as a pollutant¹⁴. But methylene blue, methyl orange and methyl red are synthetic organic dyes often used in textile industries for the coloration of the fabric^{15, 16}. The remnant of these dyes is left in to waste water cause severe health problems to humans and other living organisms if ingested¹⁷. Further in the present study the plant mediated 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 stem bark of *S. tumbuggaia* was collected from Tirumala hills, Tirupathi, Andhra Pradesh, India. The plant was taxonomically identified and authenticated by Prof.M.Vijayalakshmi, Department of Botany and Microbiology, AcharyaNagarjuna University, Guntur, Andhra Pradesh, India. All the materials used in the experiment were analytical grade. Silver nitrate (AgNO₃) was procured from Merck. Molecular grade Milli Q water was used throughout the experimental studies. All the glassware used in the present study was carefully acid washed and rinsed with Milli Q water.

Green synthesis of AgNPs using S. tumbuggaia extract

The collected material of *S.tumbuggaia* 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. To 100 ml of molecular grade (Milli Q) water 3 grams of finely crushed dried powder was mixed, boiled at 100⁰C for 10 minutes and the extract was filtered with Whatman No 1 filter paper to remove impurities. 2ml, 5ml, 10ml, 15ml and 20ml of filtered plant extract was added to 198ml, 195ml, 190ml, 185ml and 180ml of 1mM Silver nitrate (AgNO₃) solution and kept for incubation. The effect of AgNO₃ 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 AgNO₃ in separate reactions. Later the suspension was kept for incubation at room temperature.

Characterization of plant mediated AgNPs

The formation and stability of AgNP using *S.tubuggaia* extract was confirmed by UV-Visible spectroscopic studies after 48hrs using AgNO₃ as blank and the values were recorded within the range of 200 to 800 nm. In another study to know the effect of time on the formation of AgNPs the mixed solution of 10ml of plant extract +190ml of AgNO₃ was analyzed in UV-Visible spectroscopy for 1hr time intervals. Later the AgNPs were purified by repeated centrifugation from their solution 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 characterization. The purified biosynthesized AgNPs were studied using Philips X'pert pro XRD with an operation voltage of 40KV and current of 30mA with CuK α radiation (1.540 \AA) between 20 $^\circ$ angles (30 $^\circ$ -80 $^\circ$) for analysing peak data and crystal structure. Fluorescence 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 phytochemicals in *S.tubuggaia* 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.

Evaluation of catalytic activity of plant mediated AgNPs

In the present study AgNPs of *S.tubuggaia* were utilised as catalyst in the degradation and removal of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red by NaBH₄. The procedure of degradation of a respective synthetic chemical or dye (4-Nitrophenol or Methylene blue or Methyl orange or Methyl red) by NaBH₄ in presence of AgNPs or AuNPs 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 NaBH₄ was added to first reaction to prepare second reaction and analyzed in UV-Visible spectroscopy. The third

reaction is prepared by adding 20 μ L of green synthesized *S.tumbuggaia* AgNPs to the second reaction and analyzed in UV-Visible spectroscopy after 1 minute¹⁸.

RESULTS AND DISCUSSION

Addition of *S.tumbuggaia* plant extract with aqueous solution of silver nitrate led to the observable colour change from yellowish to dark reddish brown solution after 48hrs incubation (Fig.1d) due to Surface Plasmon Resonance indicating AgNPs formation¹⁹.



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

Characterization of plant mediated AgNPs

UV-Visible analysis

The amalgamated solution of 190ml AgNO₃ + 10ml of *S. tumbuggaia* have shown absorption maximum at 441.90nm (Fig. 2c) in UV-Visible spectroscopic analysis after 48 hrs incubation and confirmed the formation of AgNPs²⁰. 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 10ml, 15ml and 20ml of plant extract only (Fig. 2a). In the reactions of effect of molar concentrations AgNPs formation was observed in 0.5mM, 1mM, 1.5mM and 2mM concentrations as shown in the figure 2b. When the amalgamated solution (10ml of plant extract +190ml of AgNO₃) studied in UV-Visible spectroscopy AgNPs formation was observed after 14hrs and the result was depicted in the figure 2c.

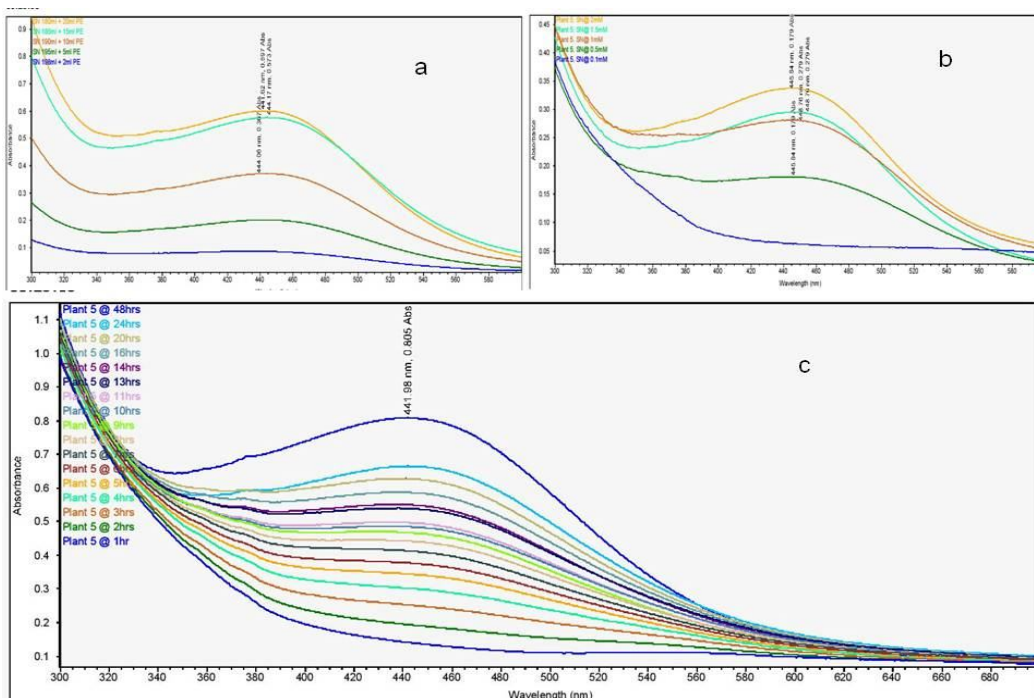


Fig. 2 UV-Visible analysis of(a)Effect of plant extract concentration on AgNPs formation (b) Effect of AgNO₃ concentration on AgNPs formation (c) Influence of time on AgNPs formation (SN: Silver nitrate solution; PE: Plant extract; hr: Time in hours)

XRD Analysis

X-ray powder diffraction spectrum of green synthesized AgNPs have shown Bragg peaks (angle 2θ) at 27.25° , 31.86° , 37.77° , 45.90° , 54.56° , 57.17° , 64.16° and 77.10° 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 crystal²¹. 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 $(\pi / 180) * \text{FWHM}$ and ' θ ' is the Bragg angle. The average particle size of *S.tumbuggaia* AgNPs was obtained as 16.75nm and the XRD pattern (Fig. 3a) was in agreement with earlier XRD reports of green synthesized AgNPs²².

FTIR study

The biosynthesized AgNPs displayed a number of peaks in the FTIR spectrum (Fig. 3b) and portrayed the complex nature of particles. The strong and broad peak at 3383.14 cm^{-1} was formed because of O-H bond stretching of alcohols²³. The peak formed at 2933.73 cm^{-1} is due to the characteristic stretching vibrations of C-H stretch of alkanes. The medium and strong peak formed at the 1639.49 cm^{-1} denotes the C=O stretching vibrations of Amides²⁴. The (CH₃) C-H bend of alkanes and alkyl groups formed a medium peak at 1370.50 cm^{-1} . The peaks at 1238.30 cm^{-1} , 1141.86 cm^{-1} and

1053cm⁻¹ were formed due to C-F stretch of alkyl halides. The C-H bend of alkenes was formed a medium peak at 991.41cm⁻¹ and the C-Cl stretch of alkyl halides formed weak peak at 833.25 cm⁻¹. The weak peaks at 599.86 cm⁻¹ and 526.57 cm⁻¹ were formed because of C-Br stretch where as the weak peak formed at 418 cm⁻¹ was due to C-I stretch of alkyl halides²⁵. These shifts in peak positions reveal that different phytochemicals were present in the plant extract of *S.tumbuggaia* and hence it can be concluded that bioorganic compounds present in the plantextract acted as reducing and stabilizing agents in the AgNPs formation.

TEM study

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 AgNPs of *S.tumbuggaia* are bounded with thin layer of biomolecules blanket on their surface which acts as stabilizing agent. Therefore, the particles were polydispersed without direct contact and reliable for longer periods of time²⁶.

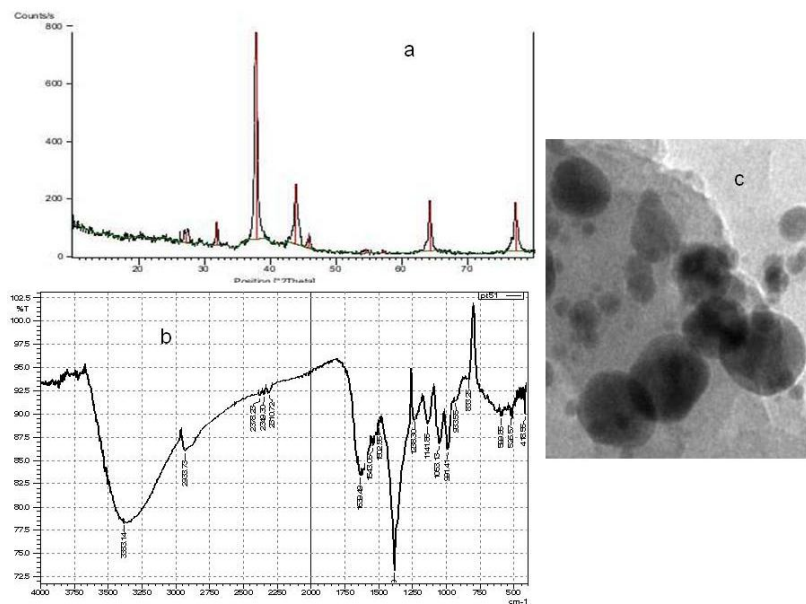


Fig.3(a) XRD spectrum(b) FTIR spectrum(c) TEM image of *S. tumbuggaia* AgNPs

Catalytic activity of *S. tumbuggaia* LourAgNPs

The synthetic chemical or dye degradation reactions were monitored 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 NaBH₄ with *S. tumbuggaia* AgNPs as catalysts was shown in the Fig. 4a. The reaction of 4-Nitrophenol when monitored in spectrophotometer

the absorption maximum 1.369 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. Later $20\mu\text{L}$ plant mediated AgNPs were added to second reaction, the solution turned colourless suddenly and the absorption maximum decreased from 1.252 to Nil in UV-Visible analysis which confirmed the complete degradation of 4-Nitrophenol^{18, 27}.

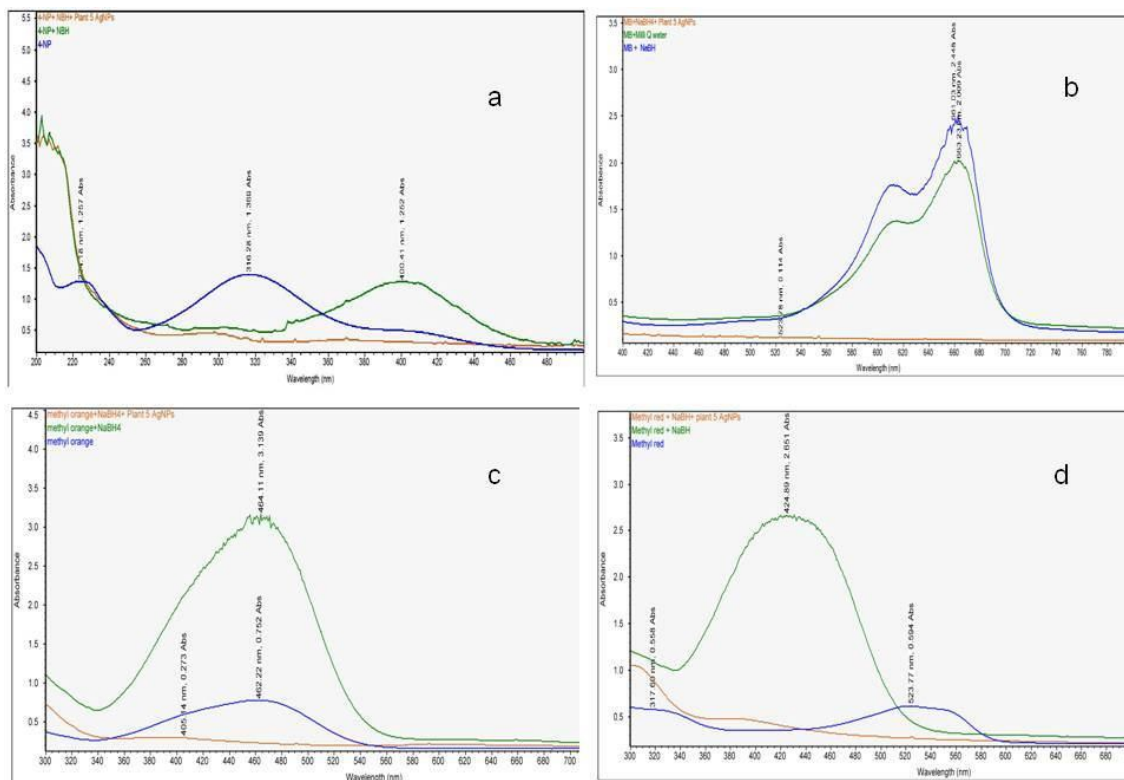


Fig. 4 Catalytic activity of *S. tumbuggaia* AgNPs on (a) 4-Nitrophenol (b) Methylene blue (c) Methyl orange (d) Methyl red (NaBH: Sodium tetra borate, AgNPs: Silver nanoparticles)

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 *S.tumbuggaia* AgNPs the solution turned colourless and the absorption maximum was completely decreased from 2.448 to 0.114 indicating that methylene blue was completely degraded²⁸.

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 NaBH₄ added the absorption maximum was found to be 3.139 at 464.11nm. After adding AgNPs of *S.tumbuggaia*, the solution turned colourless with decrease in absorption maximum from 3.139 to 0.273 as shown in the figure 4c. From the above results it can be known that AgNPs of *S.tumbuggaia* completely degraded methyl orange²⁹.

Reduction reactions of Methyl red

The UV-Visible analysis results related to the reduction reactions of methyl red by NaBH₄ in presence of AgNPs were illustrated in Fig. 4d. An absorption maximum of 0.594 was observed at 523.77nm to pure 1mM methyl red in UV-Visible analysis. After addition of 1mg NaBH₄ the absorption maximum of 2.651 was obtained at 424.89nm. With the addition of *S.tumbuggaia* AgNPs yellow colored solution turned colourless and zero absorption maximum was recorded in the third reaction indicating the complete reduction of methyl red^{18,30}.

Table: Absorption maxima of synthetic chemicals before and after addition of *S. tumbuggaia*AgNPs

Name of the synthetic chemical	Absorption Maximum before to the addition of AgNPs	Absorption Maximum after the addition of AgNPs
4-Nitrophenol	1.252	0
Methylene blue	2.448	0.114
Methyl orange	3.139	0.273
Methyl red	2.651	0

CONCLUSION

Green synthesis is a cost effective, ecofriendly and time saving process for the synthesis of metallic nanoparticles. In the present studyAgNPs were synthesized using the stem bark extract of *S.tumbuggaia*, a native plant of Andhra Pradesh and Tamilnadu. When 10ml of plant extract is added to 190ml of silver nitrate solution and incubated the amalgamated solution turned in to reddish brown in colour after 48hrs. Later in UV-Visible analysis the amalgamated solution has recorded absorption maximum at 441.98nm which confirmed the formation of AgNPs. The spectrum and data obtained in XRD analysis proclaimed that the synthesized AgNPswere crystalline and face centered cubic in structure. FTIR examination revealed the presence of various secondary metabolites in the bark extract of *S.tumbuggaia* and their role as reducing and capping agents in the formation of AgNPs. TEM analysis proclaimed the spherical structure of AgNPs and their average size. Most importantly in the

present study the catalytic activity of *S.tumbugaia* AgNPs in the degradation and removal of synthetic and toxic chemicals were analyzed and the results were discussed in the order 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red. After adding 20 μ L of AgNPs to the reductions of 4-Nitrophenol, Methylene blue, Methyl orange and Methyl red the absorption maximum decreased from 1.252 to Nil, 2.448 to 0.114, 3.139 to 0.273 and 2.651 to Nil respectively. From the obtained results it is known that plant mediated AgNPs have shown to exhibit remarkable catalytic activity in presence of NaBH₄ in the degradation and removal of respective synthetic chemicals.

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

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