IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

Ecofriendly synthesis, physicochemical characterization and catalytic activity of gold nanoparticles using plants

Bodaiah Bonigala[1], Anupama Ammulu Manne[1], Yamini Saraswathi Yeragorla[1], Usha Kiranmayi Mangamuri[2], Pavani Bai Bondili[1], Sudhakar Poda[1*]

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

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

Abstract

Green synthesis is an ecofriendly, cost effective and time saving process for the synthesis of metallic nanoparticles. In the present study gold nanoparticles (AuNPs) were synthesized using the aqueous extracts of *Cascabela thevetia* (leaves), *Wrightia tomentosa* (leaves), *Rauvolfia serpentina* (Roots) and *Stemona tuberosa* (whole plant) in separate reactions. The green synthesized AuNPs of *C. thevetia* and *R. serpentina* have exhibited ruby red colour where as the AuNPs of *W. tomentosa* and *S. tuberosa* appeared dark purple in colour after 48hrs of incubation. The formation of AuNPs were studied and confirmed in UV-Visible spectroscopy and the absorption maximum was recorded at 543.14nm, 540.32nm, 546.80nm and 538.14nm respectively. The AuNPs of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* were analyzed in Dynamic light scattering and the mean size of the particles was recorded as 2.9nm, 2.5nm, 152.5nm and 16.2nm. Energy dispersive X-ray spectroscopy (EDX) results revealed the presence of pure gold in the green synthesized AuNPs. Scanning electron microscopic (SEM) images indicated that the green synthesized AuNPs were irregular in shape. Further the biosynthesized AuNPs were screened for their catalytic activity in the degradation and removal methylene blue. The AuNPs of all four plants showed catalytic activity but AuNPs of *S. tuberosa* Lour have exhibited greater activity than the AuNPs of other plants.

Key words: *Green synthesis, AuNPs, EDX, SEM, Methylene blue, Catalytic activity*

1. Introduction

Biosynthesis of metallic NPs has attracted attention of scientific world due to their remarkable physiochemical and biological properties. Moreover it is ecofriendly and cost effective approach for the synthesis of metallic NPs when compared to physical and chemical methods. In biosynthesis the extracts of bacteria, fungi, algae and plants etc are used as reducing agent to convert metal ions in to metal nanoparticles (Vijayaraghavan *et al*., 2017). The plant extract mediated synthesis (Green synthesis) of metallic NPs when compared to other biological extracts involves very safe and easy handling procedure because preparation of bacterial and fungal extracts requires complicated procedures for culturing and maintenance of cells (Bar *et al*., 2009). Among various metallic nanoparticles, gold nanoparticles were shown to exhibit unique physical, chemical and biological properties. Gold nanoparticles were used in catalysis, biochemical sensors, photo thermal therapy, drug delivery and tissue or tumor imaging (Rajan *et al*., 2017).

Synthetic dyes are used extensively in textile, cosmetics, food and paint industries as well as research laboratories (Khan *et al*., 2016). For example Methylene blue, a cationic and thiazine dye are used in the textile industries as colouring agent of fabrics (Small and Hintelmann, 2007; Xu *et al*., 2009). The remnants of the dyes that are used in these industries are not suitable for further use and are released in to water bodies which lead to water pollution (Rita, 2012). The physical and chemical methods for of removal of synthetic dyes from water bodies require high energy and are not cost effective (Kharub, 2012). Hence new treatment methods are required to eradicate this environmental issue or pollution.

Cascabela thevetia is an evergreen tropical shrub, cultivated throughout the world as ornamental plant and commonly called as Yellow oleander in English. The

leaves of the plant contains secondary metabolites like alkaloids, phenols, flavanoids, reducing sugars and steroids which have important role as antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities (Shannon and Paul, 1996). *Wrightia tomentosa* is an endangered medicinal tree and grows in India and the plant parts are used in the treatment of stomach ache, toothache, fever, haemorrhage and snake bite. The leaf extract has shown the presence of biologically active constituents like alkaloids, ellagic acid, iridioids, lignans, methylene dioxy compounds, steroids, tannins and triterpenoids (Srinivas *et al*., 2013). The plant *Rauvolfia serpentina* is a native plant of Indian subcontinent and East Asia, commonly called Indian snake root or Sarpagandhi. The extracts of Sarpagandhi are used in the treatment of various disorders like high blood pressure, traumas and epilepsy. The roots of the plants have shown the presence of high level of indole alkaloids and are generally used as antidote to snake bite (Deshmukh *et al*., 2012). All three plant discussed above (*Cascabela thevetia, Wrightia tomentosa and Rauvolfia serpentina*) belongs to the family Apocyanaceae. *Stemona tuberosa* Lour is an herbaceous plant and belong to the family Stemonaceae. The plant is seen in Central China, Indochina, Taiwan and India. The tuberous roots of the plant contain stemonine alkaloids and used to treat bacterial and helminthic diseases (Bharali *et al*., 2014). In the present study AuNPs were synthesized using the aqueous extract of *Cascabela thevetia* leaves, *Wrightia tomentosa* leaves, *Rauvolfia serpentina* roots and *Stemona tuberosa* Lour whole plant extracts in separate reactions. The synthesized AuNPs of the selected plants were characterised using UV-Visible spectrophotometer, Energy dispersive X-ray spectroscope and Scanning electron microscope. Further the AuNPs of all the plants synthesized were evaluated for the catalytic activity in the degradation and removal of methylene blue.

2. Materials and Methods

Chemicals, reagents and plant source

The leaves of *Cascabela thevetia* were collected from Acharya Nagarjuna University campus, Guntur, India where as the leaves of *Wrightia tomentosa*, the roots of *R. serpentina* and whole plant of *Stemona tuberosa* Lour were collected from Tirumala hills, Tirupathi, India. The plants were taxonomically identified and authenticated by Prof. M. Vijayalakshmi, Dean and Professor, Dept of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. Gold (III) chloride trihydrate ($HAuCl_4$) solution of 99.5% purity and Methylene blue was purchased from Merck. Molecular grade water (Millipore, Milli Q) was used throughout the experimental studies. All the glassware used in the present study was carefully acid washed and rinsed with Milli Q water.

Preparation of aqueous plant extracts

The collected parts of selected plants were washed thrice with Milli Q water, shade dried, later chopped into small pieces and coarsely powdered with suitable pulveriser. 3gms of plant powder of all selected plants was mixed with 100ml of Milli Q water in separate reactions and boiled at 100^0C for 10 minutes and the extracts were filtered with Whatman NO 1 filter paper.

Green synthesis of AuNPs from the extracts of selected plants

2ml of aqueous plant extract was added to 48ml of 0.5mM $HAuCl_4$ solution and the suspension was stirred using a magnetic stirrer for 20 min. Later the amalgamated solution was kept for incubation for 48h at room temperature and change in the colour of the solution was observed.

Characterization of green synthesized AuNPs

The formation of gold nanoparticles was studied in UV-Visible spectroscopic analysis for 1hr time intervals using $HAuCl_4$ solution as blank to know the exact time for the formation of AuNPs from the respective plant extract and the final reading was taken after 48hrs incubation. Spectral analyses of biosynthesized AuNPs were studied using UV-VIS Double beam spectrophotometer (Thermo Fischer) and the values were recorded in the range of 400 to 700 nm. To know the size, distribution and particle's motion in the hydrodynamic medium the biosynthesized AuNPs were analyzed in *Dynamic Light Scattering* HORIBA Z100 Nanopartica at a scattering angle of 173°. The analysis was executed at 25°C in a standard mono dispersed medium maintained at a viscosity of 0.892m Pa.s.

Later Green synthesized AuNPs were centrifuged at 10000 rpm for 10 minutes and the pellet was shade dried in a china dish. The dried particles were washed with distilled water, dried again and the process repeated thrice. EDX analysis was performed to detect the presence of gold and other elemental composition as well as their concentration in the synthesized AuNPs. EDX analysis was completed using microprobe mounted scanning electron microscope. 10µl of the 100 fold diluted purified AuNPs were placed on the carbon stub and air dried. The spectrum was obtained at an operation of 20kV. Completion of the mapping represents the two-dimensional spatial distribution of energy emissions of the chemical elements present in the sample. Scanning electron microscopic (SEM) analysis reveals the surface morphology of nanoparticles. For Scanning Electron Microscopy analysis, thin films of purified AuNPs suspension were prepared and a small amount was dropped on the carbon coated copper grid. Excess sample was wiped out with blotting paper and the film is dried for 5 min using a mercury lamp and the images were taken in Zeiss Scanning electron microscope.

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

Catalytic Activity of biosynthesized AuNPs

The catalytic activity of the synthesized AuNPs of all plants was studied in individual experiments using milli Q water as blank and the absorbance values were recorded using UV-Visible spectrophotometer. 1ml of 0.1mM methylene blue was mixed with 2ml of milli Q water and the absorbance maximum was recorded. In the first reaction 1ml of 0.1mM methylene blue was mixed with 0.2ml of plant extract and 1.8ml of milli Q water and kept for incubation. In the second reaction 1ml of methylene blue of same concentration was mixed with 0.2ml of plant extract and 1.8ml of synthesized AuNPs and kept for incubation. In all the reactions the total volume was made up to 3ml. The absorbance maxima of incubated first and second reactions were recorded after 30, 40 and 50 minute intervals. The values obtained were compared with the absorption maximum of pure methylene blue (Ashokkumar *et al*., 2014).

3. Results and Discussion

When 2ml of plant extract is added to 48ml of 0.5mM $HAuCl_4$ solution and incubated for 48hrs the amalgamated solutions of *C. thevetia* and *R. serpentina* showed ruby red in colour after 48hrs incubation. But the amalgamated solutions of *W. tomentosa* and *S. tuberosa* exhibited dark purple colour after 48hrs incubation. In general AuNPs exhibit ruby red to dark purple in colour in aqueous solutions due to excitation of surface plasmon vibrations (Singh *et al*., 2016; Umamaheswari *et al*., 2018). Thus the formation of ruby red or deep purple colour in the reaction mixtures indicates the formation of AuNPs (Fig. 1).

Fig. 1: Green synthesis of AuNPs (a) *C. thevetia* extract (b) *W. tomentosa* extract (c) *R. serpentina* extract (d) *S. tuberosa* extract (e) $HAuCl_4$ solution (f) *C. thevetia* AuNPs (g) *W. tomentosa* AuNPs (h) *R. serpentina* AuNPs (i) *S. tuberosa* AuNPs

Characterization of green synthesized AuNPs

UV-Visible analysis

When the amalgamated solutions of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* were analyzed in UV-Visible spectroscopy after 48hrs incubation time. The AuNPs of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* have shown absorption maximum at 543.14nm, 540.32nm, 546.80nm and 538.14nm respectively due to surface plasmon resonance. The obtained results were in agreement with the UV-Visible results of the green synthesized AuNPs of different plants (Lee *et al*., 2015; Philip *et al*., 2011) and confirmed the formation of AuNPs from selected plants (Fig. 2). UV-Visible analysis also revealed that the aqueous extracts of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* acted as reducing and stabilizing agents in the synthesis of respective AuNPs.

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

Dynamic light scattering (DLS) analysis

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 (Barbara, 2001). The graphs obtained in DLS analysis have shown almost equal size distribution of AuNPs and the mean size of 2.9nm, 2.5nm, 152.5nm, 16.2nm to *C. thevetia*, *W. tomentosa*, *R. serpentina*, *S. tuberosa* AuNPs respectively (Fig. 3). 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. The synthesized AuNPs of selected plants have shown a PDI value less than 0.5 which clearly indicates that the particles are in mono dispersed phase with very low chances of aggregation (Bodaiah *et al*., 2016).

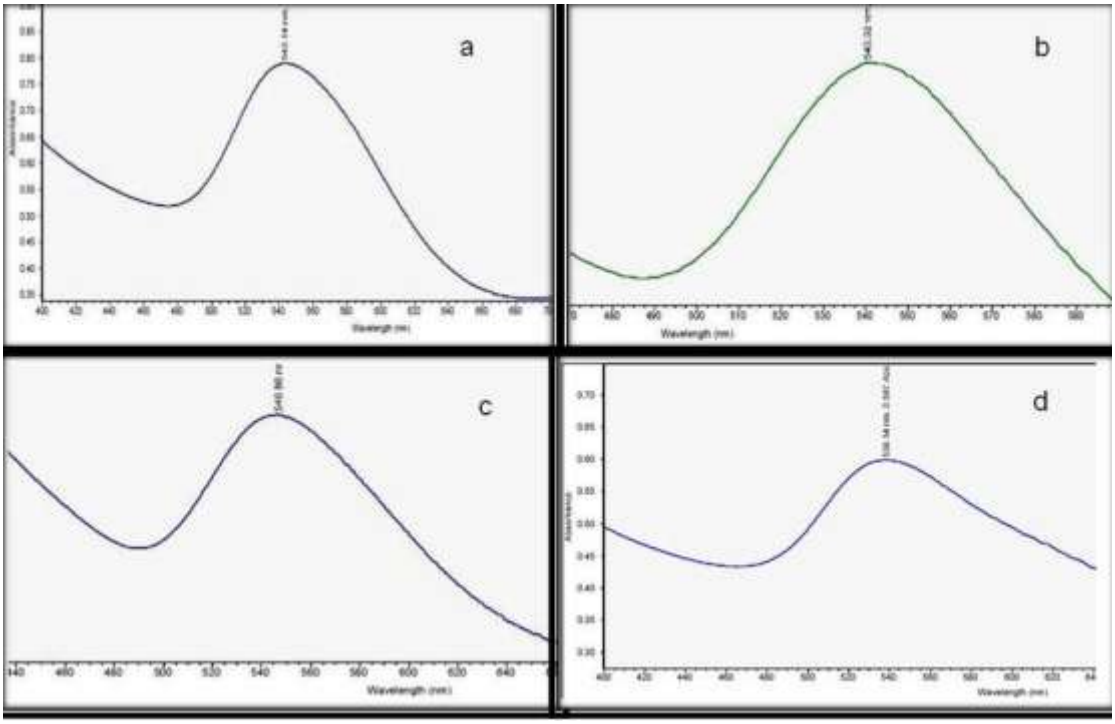

Fig. 2: UV-Visible analysis of (a) *C. thevetia* AuNPs (b) *W. tomentosa* AuNPs (c) *R. serpentina* AuNPs (d) *S. tuberosa* AuNPs

EDX analysis

The EDX study results of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* AuNPs were depicted in the fig. 4. The respective EDX spectrum of all plants have shown highest peak at 2.15 keV and confirmed the presence of pure gold in the biosynthesized AuNPs. The other peaks present in the figures are to the protein capping over AuNPs or may be the element composition of glass that holds the samples or the other elements in the respective salts. The above results were quite similar with the previous reports of biosynthesized gold nanoparticles (Tahir *et al*., 2015).

SEM studies

The SEM images of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* AuNPs were shown in the fig. 5. From the SEM images it can be known that the AuNPs of all four selected plants were in different shapes i.e some are spherical, some are rectangular, some are square and some are irregular in shapes. The SEM images also reveal the monodispersive nature of biosynthesized AuNPs (Isaac *et al*., 2013). However the mean diameter of biosynthesized AuNPs varied from plant to plant. The obtained results clearly indicate that *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* AuNPs were in the range of 20-50nm, 30-70nm, 20-70nm and 30-50nm respectively.

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

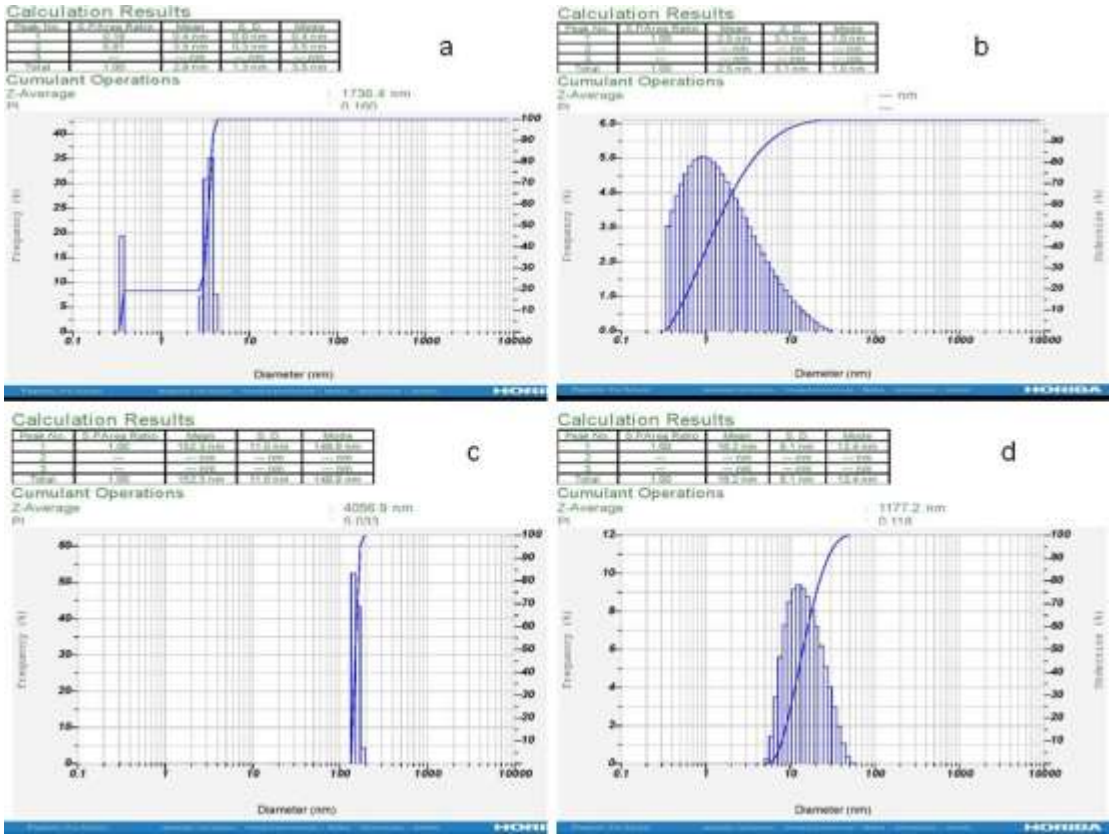

Fig. 3: DLS analysis of (a) *C. thevetia* AuNPs (b) *W. tomentosa* AuNPs (c) *R. serpentina* AuNPs (d) *S. tuberosa* AuNPs

Catalytic Activity of green synthesized AuNPs

The plant mediated AuNPs of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* were studied for catalytic activity in the reduction of methylene blue in separate reactions. Pure methylene blue of 0.1mM concentration showed a lambda max value at 664nm (Suvith and Philip, 2014). When first reaction of *C. thevetia*, *W. tomentosa* and *S. tuberosa* Lour were analyzed in UV-Visible spectrophotometer the absorbance gradually decreased and shifted to higher wavelengths compared to pure methylene blue. But the first reaction of *R. serpentina* when studied in UV-Visible spectrophotometer have shown neither decrease in absorption and nor shifted to higher wavelength. It indicates that the extracts of all three plants except *R. serpentina* have ability to degrade methylene blue. The second reaction of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* Lour was also analyzed for lambda max value after 30 minutes. The absorption further decreased and shifted to higher wavelengths when compared to first reaction except the second reaction of *R. serpentina*. The second reaction of all plants again screened for absorption maxima after 40minutes as well as 50 minutes incubation. The absorption is gradually decreased and shifted to higher wavelength after 40 and 50 minutes incubation. The results were depicted in the fig. 6 and the graphs obtained clearly indicate that AuNPs of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* Lour have shown catalytic activity in the degradation and removal of methylene blue (Das and Velusamy, 2014; Khan *et al*., 2017; Khan *et al*., 2016). From the obtained results it can be known that AuNPs of *S. tuberosa* Lour exhibited greater catalytic activity where as the AuNPs of *W. tomentosa* and *C. thevetia* exhibited moderate catalytic activity. But the AuNPs of *R. serpentina* have shown poor catalytic activity in degradation and removal of methylene blue because of their larger size.

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

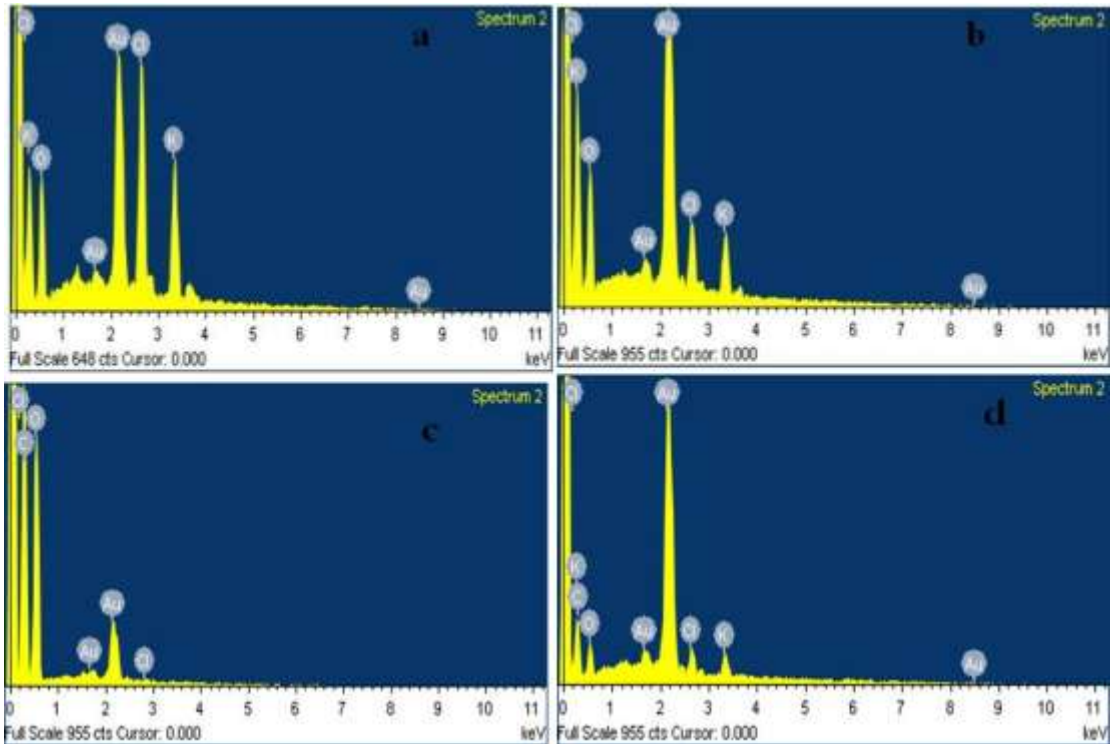

Fig. 4: EDX spectrum of (a) *C. thevetia* AuNPs (b) *W. tomentosa* AuNPs (c) *R. serpentina* AuNPs (d) *S. tuberosa* AuNPs

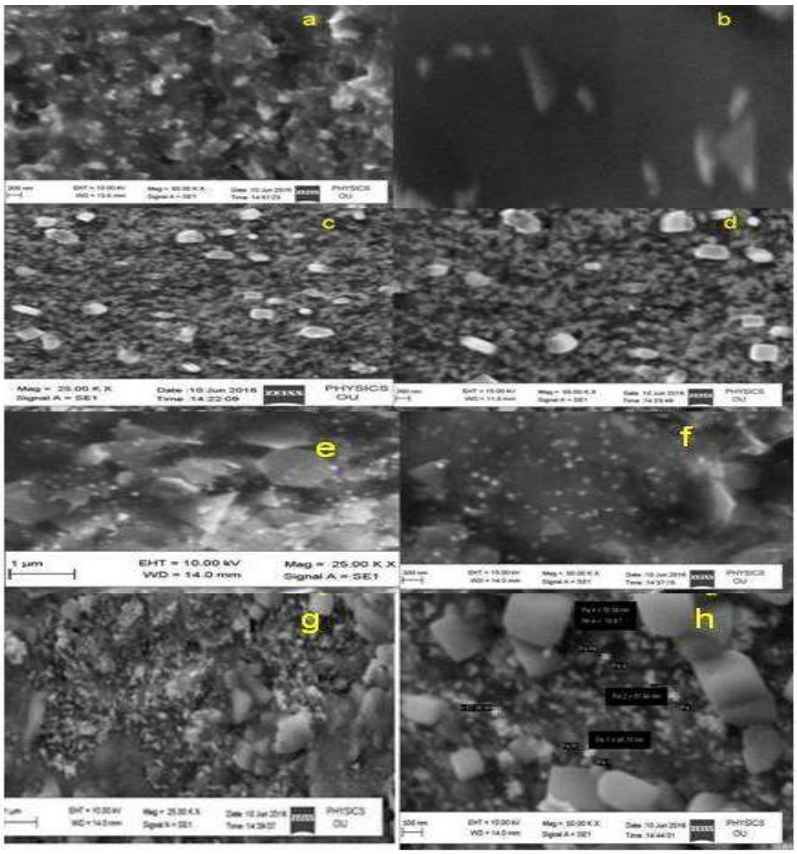

Fig. 5: SEM images of (a&b) *C. thevetia* AuNPs (c&d) *W. tomentosa* AuNPs (e&f) *R. serpentina* AuNPs (g&h) *S. tuberosa* AuNPs

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 3 Issue 8, Aug 2018

www.ijasrm.com

ISSN 2455-6378

4. Conclusion

In the present study gold nanoparticles were synthesized using the aqueous extracts of selected plants and evaluated for their catalytic activity in the degradation and removal of methylene blue from water. When the aqueous extracts of *Cascabela thevetia* leaves, *Wrightia tomentosa* leaves, *Rauvolfia serpentina* roots and *Stemona tuberosa* Lour whole plant were mixed with $HAuCl_4$ solution in separate reactions the amalgamated solutions of *C. thevetia* and *R. serpentina* were turned in to ruby red in colour where as the amalgamated solutions of *W. tomentosa* and *S. tuberosa* were turned in to dark purple in colour after 48hrs incubation indicating the formation of AuNPs. However the formation of AuNPs from the extracts of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* were confirmed in UV-Visible analysis with the absorption maximum recorded at 543.14nm, 540.32nm, 546.80nm and 538.14nm respectively. From the results obtained in dynamic light scattering analysis it can known that the mean size of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa* AuNPs was 2.9nm, 2.5nm, 152.5nm and 16.2nm respectively. The respective EDX spectrum of all plants have shown highest peak at 2.15 keV and confirmed the presence of pure gold in the biosynthesized AuNPs of *C. thevetia*, *W. tomentosa*, *R. serpentina* and *S. tuberosa*. The recorded images in Scanning electron microscopic (SEM) analysis revealed that the respective AuNPs of all plants are irregular in shape and their size varied 1-100nm. In further study the green synthesized AuNPs have exhibited potential catalytic activity in the degradation and removal methylene blue. In comparison the AuNPs of *S. tuberosa* Lour have exhibited greater activity but the AuNPs of *C. thevetia*, *W. tomentosa* have shown moderate activity. However the AuNPs of *R. serpentina* have shown to exhibit poor activity in the degradation and removal of methylene blue because of their larger size.

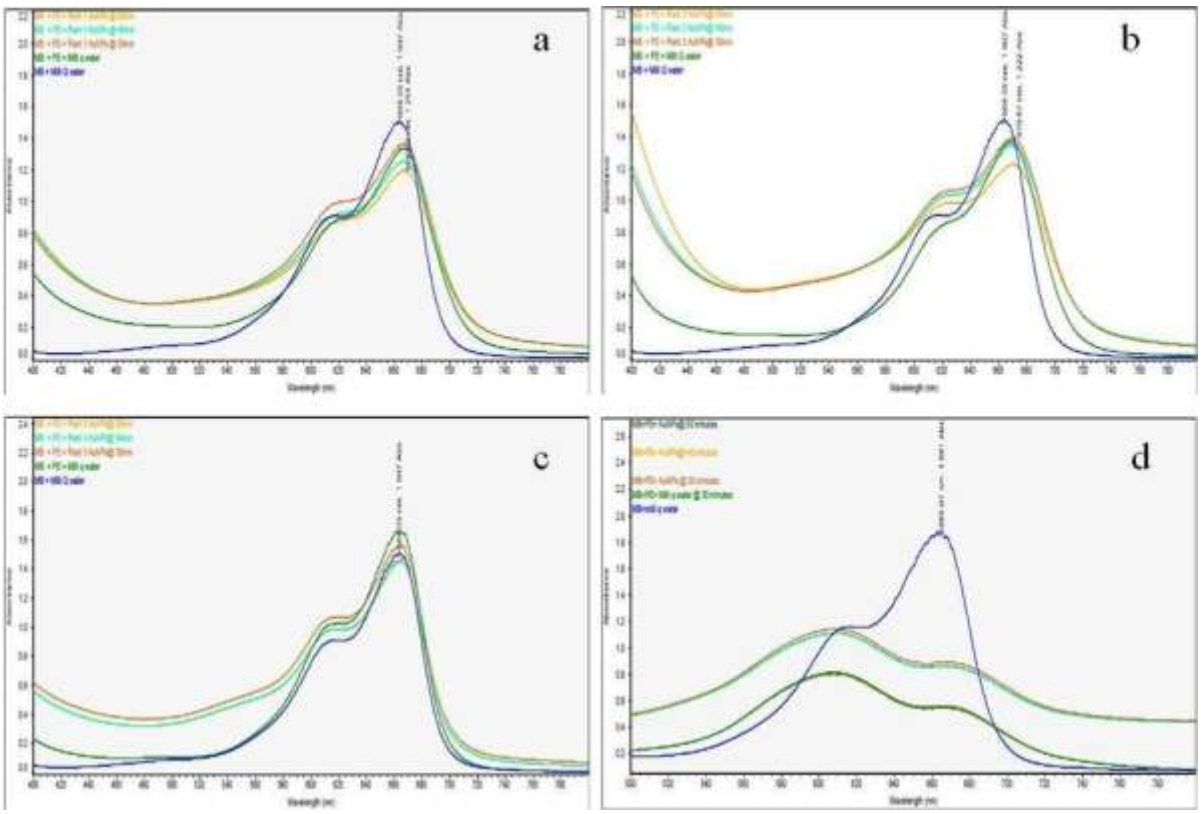

Fig. 6 Catalytic activity of (a) *C. thevetia* AuNPs (b) *W. tomentosa* AuNPs (c) *R. serpentina* AuNPs(d) *S. tuberosa* AuNPs on methylene blue

Acknowledgments

The authors are thankful to the University Grants Commission (UGC) RGNF's, Govt of India for providing financial support to the first author and Department of Biotechnology, Acharya Nagarjuna University, Guntur for providing research facilities.

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

5. References

1. Ashokkumar, S., Ravi, S., Kathiravan, V., Velmurugan S. Rapid biological synthesis of silver nanoparticles using *Leucas martinicensis* leaf extract for catalytic and antibacterial activity. Environ Sci and Pollu Resear 21: 11439–11446, (2014).

2. Bar, H., Bhui, DK., Sahoo, GP., Sarkar, P., De, SP., Misra, A. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. Colloids and Surfaces A: Physicochem. Eng Aspects 339: 134–139, (2009).

3. Barbara, JF. Revisiting the method of cumulants for the analysis of dynamic light-scattering data. Appl. Opt 40: 4087-4091, (2001).

4. Bharali, P., Paul, A., Dutta, P., Gogoi, G., Das, AK., Baruah, AM. Ethnopharmacognosy of *Stemona tuberosa* Lour., a potential medicinal plant species of Arunachal Pradesh, India. W J of Pharm and Pharm Scien 3(4): 1072-1081, (2014).

5. Bodaiah, B., Usha Kiranmayi, M., Vijayalakshmi, M., Rama Krishna, GVS., Sambasiva Rao, KRS., Sudhakar, P. Green synthesis, characterisation and biological activities of silver nanoparticles by using *Wrightia tomentosa* leaves. J of Chem and Pharma Resear 8: 266-278, (2016).

6. Das, J., Velusamy, P. Catalytic reduction of methylene blue using biogenic gold nanoparticles from *Sesbania grandiflora* L. J Taiwan Inst Chem Eng 45: 2280-2285, (2014).

7. Deshmukh, SR., Ashrit, DS., Patil, BA. Extraction and Evaluation of Indole alkaloids from *Rauwolfia serpentina* for their antimicrobial and antiproliferative activities. Inter J of Pharm and Pharm Scien 4: 329- 334, (2012).

8. Isaac. RSR., Sakthivel, G., Murthy, Ch. Green Synthesis of Gold and Silver Nanoparticles Using *Averrhoa bilimbi* Fruit Extract. J of Nanotech Article ID: 906592, (2013).

9. Kharub, M. Use of various technologies, methods and adsorbents for the removal of dye. J of Environ Resear and Develop 6: 3A, (2012).

10. Khan, AU., Yuan, Q., Wei, Y., Khan, GM., Khan, ZUH., Khan, S., Ali, F., Tahir, K., Ahmad, A., Khan, FU. Photocatalytic and antibacterial response of biosynthesized gold nanoparticles. J of Photochem and Photobio, B: Biolo 162: 273–277, (2016).

11. Khan, ZUH., Khan, A., Chen, Y., Khan, AU., Shah, NS., Muhammad, N., Murtaza, B., Tahir, K., Khan, FU., Wan, P. Photo catalytic applications of gold nanoparticles synthesized by green route and electrochemical degradation of phenolic Azo dyes using AuNPs /GC as modified paste electrode. J of Alloys and Comp 725: 869-876, (2017).

12. Lee, KD., Nagajyothi, PC., Sreekanth, TVM., Park, S. Eco-friendly synthesis of gold nanoparticles (AuNPs) using *Inonotus obliquus* and their antibacterial, antioxidant and cytotoxic activities. J of Indust and Engin Chem 26: 67–72, (2015).

13. Philip, D., Unni, C., Aromal, SA., Vidhu, VK. *Murraya koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles. Spectrochimica Acta Part A 78: 899–904, (2011).

14. Rajan, A., Rajan, AR., Philip, D. *Elettaria cardamomum* seed mediated rapid synthesis of gold nanoparticles and its biological activities. Open Nano 2: 1–8, (2017).

15. Rita, K. Textile dyeing industry an environmental hazard. Natural science 4: 22-26, (2012).

16. Shannon, DL., Paul, JB. Oleander toxicity: an examination of human and animal toxic exposures. Toxicology 109: 1-13, (1996).

17. Singh, P., Singh, H., Ju Kim, Y., Mathiyalagan, R., Wang, C., Yang, DC. Extracellular synthesis of silver and gold nanoparticles by *Sporosarcina koreensis* DC4 and their biological applications. Enzyme and Microbial Tech 86: 75–83, (2016).

18. Small, JM., Hintelmann, H. Methylene blue derivatizations then LC–MS analysis for measurement of trace levels of sulfide in aquatic samples. Anal Bioanal Chem 387: 2881-2886, (2007).

19. Srinivas, P., Samatha, T., Valya, G., Ragan, A., Swamy, NR. Phytochemical Screening and Antimicrobial Activity of Leaf Extract of

Wrightia tomentosa. Int Research J of Biolo Scien 2: 23-27, (2013).

20. Suvith, VS., Philip, D. Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. Molecu and Biomolecu Spectro 118: 526-532, (2014).

21. Tahir, K., Nazir, S., Li, B., UllahKhan, A., UlHaqKhan, Z., Gong, PY., UllahKhan, S., Ahmad, A. *Nerium oleander* leaves extract mediated synthesis of gold nano-particles and its antioxidant activity. Materials Letters 156: 198–201, (2015).

22. Umamaheswari, C., Lakshmanan, A., Nagarajan, NS. Green synthesis, characterization and catalytic degradation studies of gold nanoparticles against Congo red and methyl orange. J of Photochem and Photobio, B: Bio 178: 33–39, (2018).

23. Vijayaraghavan, K., Ashokkumar, T. Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. J of Environ Chemical Engin 5: 4866-4883, (2017).

24. Xu, JZ., Dai, L., Wu, B., Ding, T., Zhu, JJ., Lin, H., Chen, HL., Shen, CY., Jiang, Y. Determination of methylene blue residues in aquatic products by liquid chromatography-tandem mass spectrometry. J Sep Sci 32: 4193-4199, (2009).