www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.2, pp 725-733, 2014-2015

ICONN 2015 [4th -6th Feb 2015]
International Conference on Nanoscience and Nanotechnology-2015
SRM University, Chennai, India

Bioinspiredsynthesis of selenium nanoparticles using flowers of Catharanthus roseus(L.) G.Don.and Peltophorum pterocarpum(DC.)Backer ex Heyne – a comparison

B. Deepa[1] and V. Ganesan[2]*

[1]Dept of Botany, The Standard Fireworks Rajaratnam College for Women, Sivakasi, India.
[2]Centre for Research and Post Graduate studies in Botany, AyyaNadarJanakiAmmal College, Sivakasi, India.

Abstract : The present study is aimed to compare the bioreduction behaviour of flower broth of *Catharanthus roseus* (Family: Apocynaceae) and *Peltophorum pterocarpum* (Family: Caesalpiniaceae) in the synthesis of selenium nanoparticles. Stable selenium nanoparticles were formed by exposing the flower broths of aforesaid plants to the aqueous sodium selenate solution (reaction medium) within a week. The bio-synthesized selenium nanoparticles were characterized using a range of diverse techniques like UV-Visible (UV-Vis) Spectroscopy, Luminescence Spectroscopy (LS), Fourier Transform Infra-Red Spectroscopy (FT-IR), X-Ray Diffraction (XRD) analysis, Energy Dispersive X-ray Spectroscopy (EDAX), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).UV-Visible spectroscopic analysis showed the Surface Plasmon Resonance (SPR) vibrations with a λ_{max}at335nm and 325nm for the reaction medium prepared with the flower broth of *C.roseus*and *P.pterocarpum* respectively. Major peaks in the emission and excitation spectrum of both the reaction medium were very close to the peaks of SPR vibrations confirmed the synthesis of selenium nanoparticles.FT-IR analysis substantiated the role of esters, secondary and tertiary amides derived from the flower of *C.roseus*in the synthesis and stabilization of selenium nanoparticles whereas, *P.pterocarpum* flower reduced the sodium selenate with the help of ketones and primary amides. XRD studies confirmed the formation of face-centered cubic (fcc) phase of selenium nanoparticles with an average size of 32.02nm and 40.2nmwith the flower broth of *C.roseus*and *P.pterocarpum* respectively.The EDX and Scanning Electron Microscopic studies confirmed the formation of elemental spherical selenium nanoparticles. Further, TEM analysis reported the formation ofhollow spherical selenium nanoparticles with an average size of 23.2±6.06nm and 30.44±2.89nm using the flower broth of *C.roseus*and *P.pterocarpum* respectively. Availability of flowers throughout the year and synthesis of smaller sized nanoparticles by the *C.roseus* proved it as a better novel biomaterial for the synthesis of selenium nanopartilces than the *P.pterocarpum*. This eco-friendly approach employed for the synthesis of selenium nanoparticles is simple, amenable for large scale production and biomedical applications.

Key words: Bioinspired synthesis, *Catharanthus roseus,Peltophorum pterocarpum*, selenium nanoparticles.

Introduction

Recent years have witnessed the overwhelming growth in the field of Nanotechnology with a rapid stride synthesis and commercialization of nanomaterials[1]. Astounding efforts taken by the researchers to develop an efficient methodology for the large sale synthesis of nanoparticles includes physical, chemical and biological methods. The last is the latest and most preferred way for the synthesis of nanoparticles as it has many advantages such as process scaling up, economic viability and safe way to produce nanoparticles[2]. Biosynthesis of nanoparticles can be achieved with the help of various biomaterials like bacteria[3], fungi[4], algae[5], bryophytes[6], gymnosperms [7] and higher plants[8,9]. Among these biomaterials, plants are extensively employed for the reduction of metal ions into metal nanoparticles as it is rapid, easy, cost-effective and does not require special conditions[10].

Selenium (Se^0) is a chemical element that was discovered as a by-product of sulfuric acid[11]. Nano selenium possesses some unique mechanical, optical, electrical, biological and chemical properties as compared with bulk materials[12].It has an excellent antioxidant[13,14],and antifungal[15]properties.Various strategies so far employed for the synthesis of nanoselenium includes pulsed laser ablation[16], solution phase approach[17], electro-kinetic techniques[18], vapour deposition route [19], wet chemical method[12]*etc.*,These methods need elevated temperature and high pressure that are hazardous to the environment [20].Toxic effects produced by the use of reducing and stabilizing agent in the synthesis protocol also affect the technological applications of selenium nanomaterials[12].To overcome these hurdles, microorganisms and plant extracts were exploited as a possible green alternative to physical and chemical methods[21].

Microorganisms such as *Pseudomonas alcaliphila*[20]; *P.aeruginosa* strain JS-11[21]; *Lactobacillus casei*, *L.acidophilus, Bifidobacterium, Klebsiella pneumonia*[22]; *Bacillus cereus*[23]; *Pantoeaagglomerans*[14]; *Aspergillusterreus*[24]; *Saccharomyces cerevisiae*[21] *etc*., were already employed in the synthesis of selenium nanoparticles. Very few studies have been reported the synthesis of selenium nanoparticles using higher plants. The biomaterials of higher plants used for the synthesis of selenium nanoparticles were *Capsicum annum*[25], dried raisin extract[10], leaves of lemon[26], *Terminaliaarjuna*[27] and seed extract of fenugreek[28].In that line, the present investigation is aimed to compare the ability of bioreduction behavior of flower broth of *Catharanthu sroseus*(Family: Apocynaceae) and *Peltophorum pterocarpum*(Family: Caesalpiniaceae) in the synthesis of selenium nanoparticles.

Experimental

Reaction medium

All the reagents used in the present study were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Fresh and healthy flowers of two higher plants like *Catharanthus roseus* (L.) G.Don.(**Fig.1a**) and *Peltophorum pterocarpum*(DC.)Backers ex Heyne (**Fig.1b**) were selected for the present study. The flowers were air dried for three days under shade.

Figure 1a: Flower of *Catharanthus roseus* **Figure 1b: Flower of *Peltophorum pterocarpum***

After three days the flowers were cut into small pieces. 10 gm of the flowers were taken in a separate conical flask containing 100 ml of sterile distilled water. They were kept in water bath at 90^0Cfor five min.After five min, the solutions were filtered through the cheese cloth. 10ml of the freshly prepared flower broth was

added to 90ml of 10mM aqueous solution of sodium selenate to prepare the reaction medium. This reaction medium was kept in an incubator cum shaker (Orbitek) with 250rpm at 36^{o}C for a week.

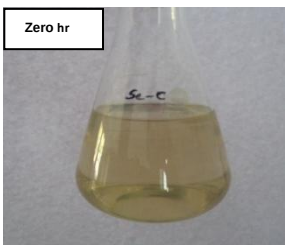
Characterization techniques

Formation of selenium nanoparticles in the reaction media were analysed by diluting small aliquots of sample with sterile distilled water using UV-visible spectrophotometer (Labomed Model UV- D3200) and itsoptical property was evaluated by recording emission and excitation spectrum with an aid of Luminescence Spectrophotometer (Perkin Elmer – Model LS45).After the complete reduction, the reaction media were centrifuged at 15000 rpm for 10 min and the aliquots were undergone repeated centrifugations using sterile water. Thereafter the collected aliquots were completely dried and analyzed by FT-IR spectrophotometer (Shimadzu) using KBr pellet method with a spectral range of 4000- 400 cm^{-1}.The XRD analysis was performed using a X'Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in θ- 2 θ configurations and its crystalline domain size was calculated from the width of the XRD peaks using the Scherrer's formula[29]. Scanning Electron Microscope (Quanta FE G250)coupled with Energy Dispersive X-ray Spectroscopy (Ametek) was used to assess the shape and percentage of the synthesized selenium nanoparticles respectively. Transmission Electron Microscopic (TEM) measurements were performed on a Philips-Techno 10 TEM operated at an acceleration voltage of 200 kV with a resolution of 0.3nm to find out the exact size and nature of the synthesized selenium nanoparticles.

Results and Discussion

Visual observation

The colour of the reaction medium prepared with the flower broth of *C.roseus* was initially pale green and turns to pinkish red (**Fig.2a**) due to the reduction whereas, the*P.pterocarpum* reaction medium shows a visible colour change from transparent orange to light brown (**Fig.2b**). This variation in the final colour of the reaction medium may be due to the variation in the size of the nanoparticles. Like selenium nanoparticles, the final colour of the reaction medium of gold nanoparticles also varied from deep red to purple based on its size [30]. Interestingly both the flowers took a week for the completion of reduction. While the reduction of selenious acid with the dried raisins produces selenium nanoparticles of brick red colour within 6-12 min. [10]. Similarly the bacteria *Pantoeaagglomerans*[14] and *Pseudomonas acaliphila*[20] also produced red colour selenium nanoparticles with an incubation period of 24 hr and 48 hr respectively.



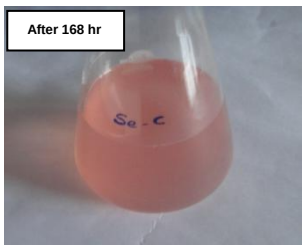


Figure 2a: Colour change of *C.roseus* reaction medium

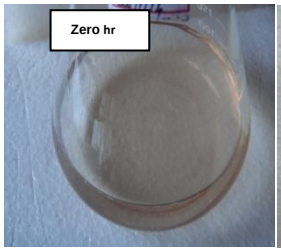
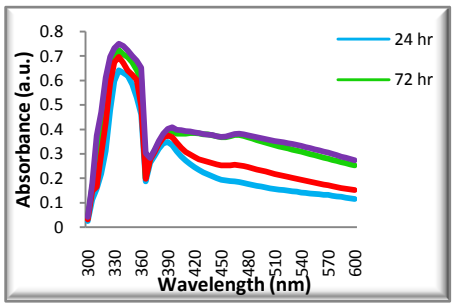


Figure 2b: Colour change of *P. pterocarpum* reaction medium

UV-Visible spectroscopic analysis

The UV-visible spectra of the Surface Plasmon Resonance (SPR) of selenium nanoparticles in the reaction medium were recorded periodically at24, 72, 120 and 168 hr in the wavelength range of 300 to 600nm. Selenium nanoparticles prepared with the flower broth of *C.roseus* showed SPR vibrations with a λ_{max}at 335nm(**Fig.3a**) and for the reaction medium prepared with the flower broth of *P.pterocarpum*s howed λ_{max}at 325nm (**Fig.3b**).



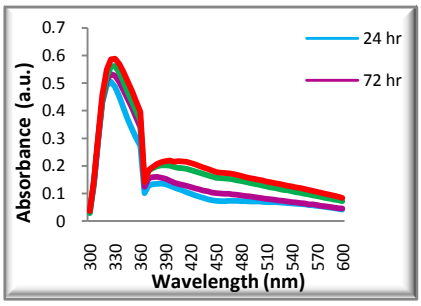


Figure 3a: UV-Vis spetrum of *C.roseus* reaction medium

Figure 3b: UV-Vis spetrum of *P. pterocarpum* reaction medium

The peak in spectrum is developed due to the excitation of the localized surface plasmons which causes strong light scattering by an electric field at a wavelength where resonance occurs[31]. The absorbance of *C.roseu* smediated reaction medium (0.751) was found to be higher than the reaction medium (0.588) prepared with the flower of *P.pterocarpum*. This variation in the position, shape and intensity of the SPR peaks was due to variation in the size, composition of the surrounding media and interaction between stabilizing ligands and the nanoparticles [32,33]. Further the single peak in the SPR band indicates that the synthesized nanoparticles were spherical [34].

Luminescence spectroscopic analysis

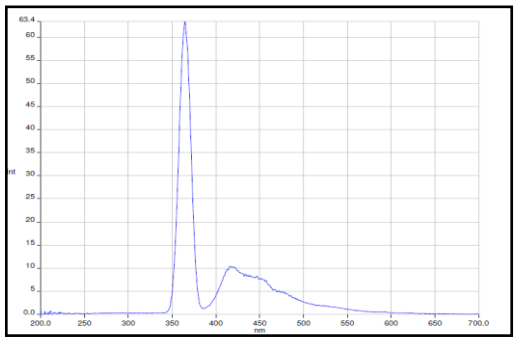


Figure 4a1: Excitationspetrum of *C.roseus* reaction medium

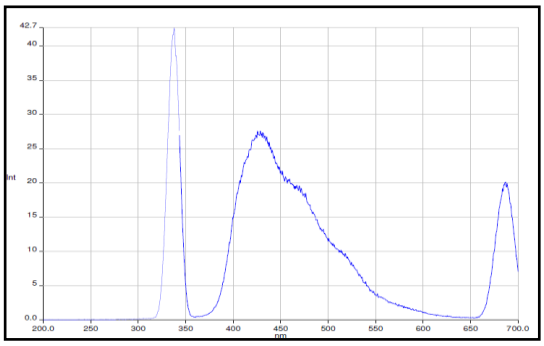


Figure 4a2: Emissionspetrum of *C.roseus* reaction medium

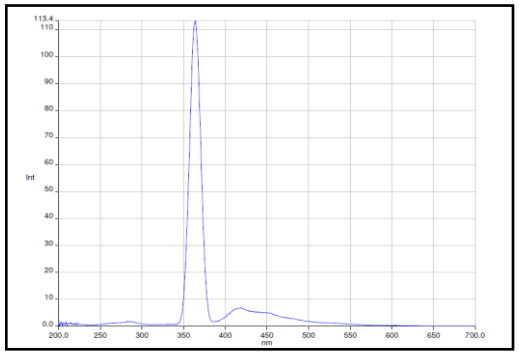


Figure 5a1: Excitation spetrum of *P. pterocarpum*reaction medium

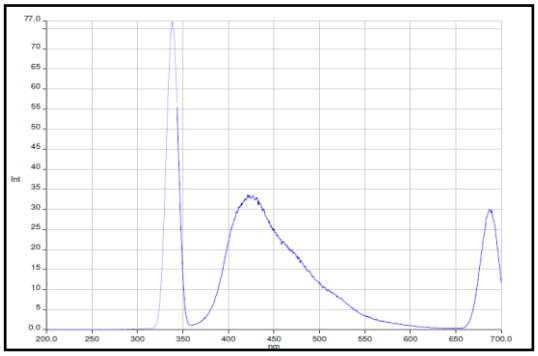


Figure 5a2: Emission spetrum of *P. pterocarpum* reaction medium

Luminescence spectroscopic analysis was also conducted to confirm the formation of Se nanoparticles. In the excitation spectrum (**Fig.4a1**) recorded for *C.roseus* flower mediated reaction medium, the $\lambda_{max}$was obtained at 364.5nm. The emission spectrum (**Fig.4a2**) shows a major peak at 338nm and minor peaks at 435.5 and 687 nm. The reaction medium prepared with the flower of *P.pterocarpum* showed excitation peak at364nm (**Fig.5a1**)and major emission peaks at 338.5nm and minor peaks at 427 and 689nm (**Fig.5a2**).However, selenium nanoparticles coated with GA nanofibres developed excitation and emission peak at 597and 682 nm

respectively [13].Selenium colloidal solution synthesized by the aqueous leaf extract of lemon plant exhibited an absorption maximum at 395nm and produced an emission maximum at 525nm[26].The nature of emission band indicates that the proteins bound to the nanoparticle surface and those present in the solution exist in the native form [35,36].

Fourier Transform Infra-Red Spectroscopic (FT-IR) analysis

FT-IR analysis of synthesized selenium nanoparticles were carried out in order to explore the biological compounds responsible for the synthesis and stability of the particles. The results ofFT-IR spetrum of Se nanoparticles synthesized by *C.roseus* (**Fig.6a**) shows significant absorbance bands at 1736cm^{-1}corresponding to -CO-O- stretching vibrationof esters, 1659cm^{-1}corresponding to -CONH- stretching vibration of tertiary amide, 1566cm^{-1}corresponding to -N-H- plane bending of secondary amide and 1165 cm^{-1}corresponding to higher esters were the compounds involved in the synthesis. Whereas, FT-IR spetrum of Se nanoparticles synthesized by *P. pterocarpum*(**Fig.6b**) shows vital absorbance bands at 1775cm^{-1} corresponding toprimary amide and 1620cm^{-1} corresponding to –C=O- stretching vibration of ketones were involved in the reduction of sodium selenate. In contrast to this, the raisin extract used lignin (a phenolic compound) for the reduction and stabilization of the selenium nanoballs[10]. The role of other functional groups such as carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) derived from the lemon plant extract in the synthesis of selenium naoparticles was also reported[26].

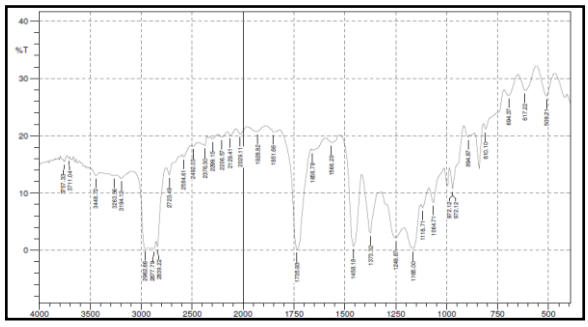


Figure 6a: FT-IRspetrum of Se nanoparticles synthesized by *C.roseus*

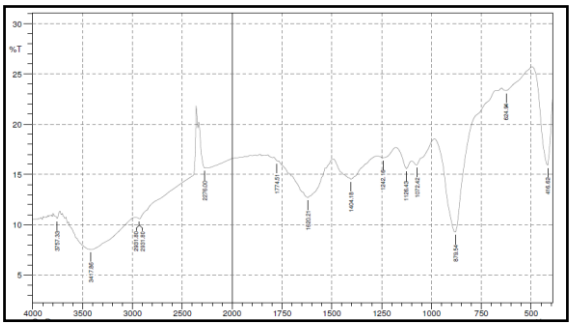


Figure 6b: FT-IR spetrum of Se nanoparticles synthesized by *P. pterocarpum*

X-Ray Diffraction (XRD) analysis

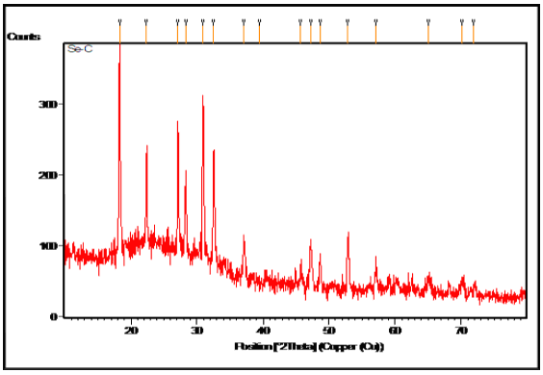


Figure 7a: XRD pattern of Se nanoparticles by *C.roseus*synthesized

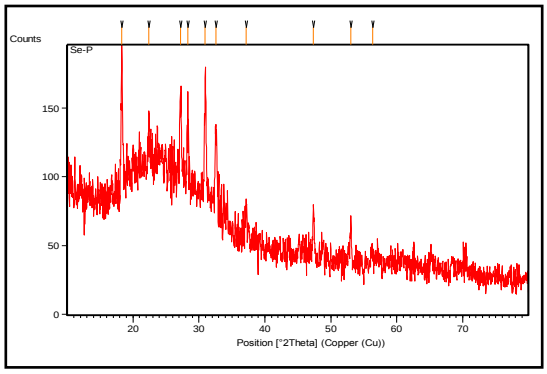


Figure 7b: XRD pattern of Se nanoparticles synthesized by *P. pterocarpum*

The crystal structure, phase composition and the particle size of selenium nanoparticles were determined by XRD analysis. The XRD pattern of se nanoparticles synthesized using the flower broth of *C.roseus*(**Fig.7a**)shows clear peaks corresponding to selenium nanoparticles at 30.89^0, 32.5^0, 45.79^0, 47.28^0, 52.86^0, 57.08^0 and 65.08^0 with an average particle size of 32.02nm. While the XRD pattern of se nanoparticles synthesized using the flower broth of *P. pterocarpum*(**Fig.7b**)shows peaks at 30.9^0, 32.5^0, 47.32^0, 53^0 and 56^{0}with an average particle size of 40.2nm. The sharpening of peaks in both the pattern clearly indicates that the particles are in the nanoregime[37] corresponding to 101, 111, 201, 112 and 210 planes of se nanoparticles. These

sets of lattice planes had been indexed on the basis of the face centered cubic structures (fcc) of standard selenium PDF card -00-001-0848.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) spectroscopic analyses

The SEM images ofselenium nanoparticles synthesized by *C.roseus* and *P. pterocarpum*showed the formation of spherical selenium nanoparticles (**Fig.8a and 8b**).

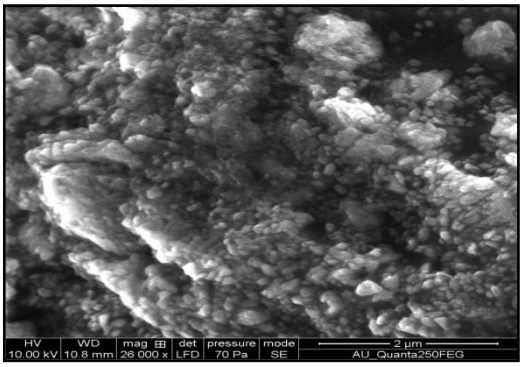


Figure 8a: SEM image of Se nanoparticles synthesized by *C.roseus*

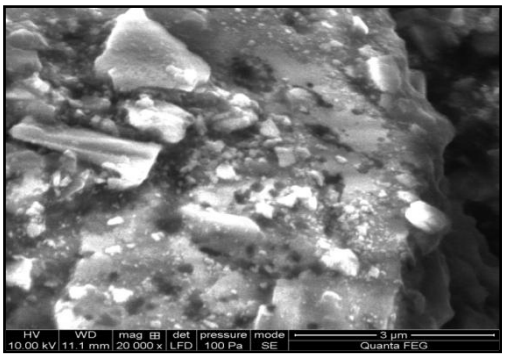


Figure 8b: SEM image of Se nanoparticles synthesized by *P. pterocarpum*

When compared to *P. pterocarpum*, the flower broth of *C.roseus* produced more amounts of selenium nanoparticles of smaller size.Most of the microbial mediated synthesis of selenium nanoparticles reported so far produces particles of larger size when compared to the present study. *Pseudomonas alcaliphila* produces spherical Se nanoparticles with diameters ranging from 50-500 nm [20]. The probiotic yogurt bacteria produces elemental nano-size selenium of 100-500nm through a fermentation procedure [38]. Elemental selenium nanoparticles synthesis by *Klebsiella pneumonia* (39) and *Bacillus cereus* strain CM100B (23) were also produces particles of 245nm and 150-200nm respectively.

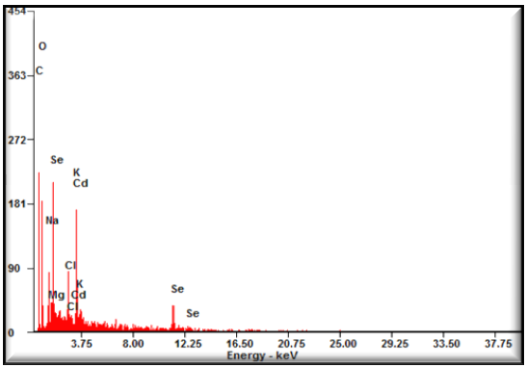


Figure 9a: EDX spectrum of Se nanoparticles synthesized by *C.roseus*

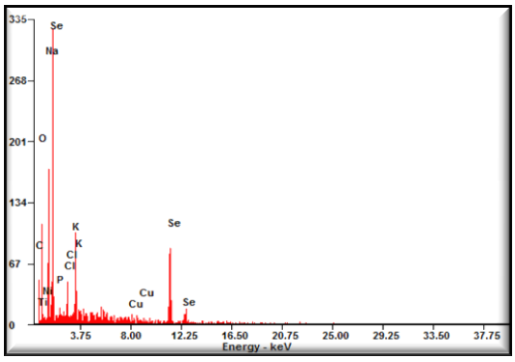


Figure 9b: EDX spectrum of Se nanoparticles synthesized by *P. pterocarpum*

Analysis through EDX spectrometer confirmed the synthesis of elemental selenium nanoparticles by the flower broth of *C.roseus* (**Fig.9a**)and*P. pterocarpum*(**Fig.9b**). The reported identification lines for the major emission energies for SeLα, SeKα and SeKβare 1.37, 11.22 and 12.49 keV respectively [23]and these correspond with peaks in the spectrum of our study, thus giving confidence that selenium has been correctly identified.Our report is also in consistent with the reports of earlier studies which indicated the peaks for SeKα at 11.22 keV[40,41]

The EDX spectrum of selenium nanoparticles synthesized with *C.roseus* shows 32.57 percent of selenium with signals of C, O, K, Na, Cd, Mg and Cl while *P. pterocarpum* flower mediated synthesis shows the presence ofonly 26.14 percent of selenium with signals of C,O,K,P,Na, Ti, Cu, Ni and Cl.The signals for other elements may be from the carbohydrate / protein / enzymes present in the mixed precipitates of plant extract[42].

Transmission Electron Microscopic (TEM)analysis

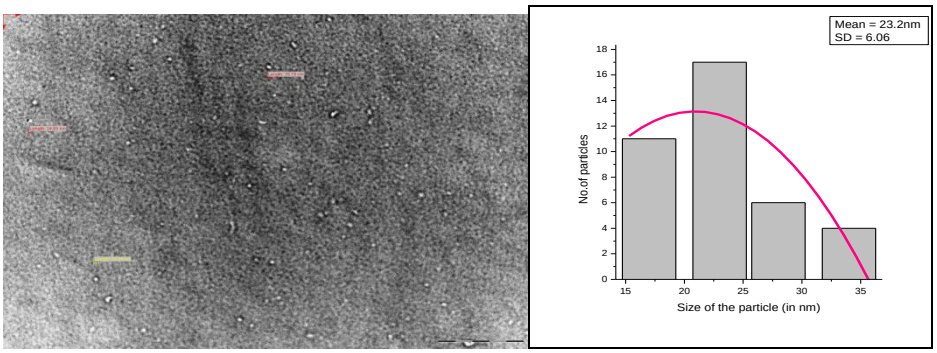


Figure 10a: TEM image and histogram of Se nanoparticles synthesized by *C.roseus*

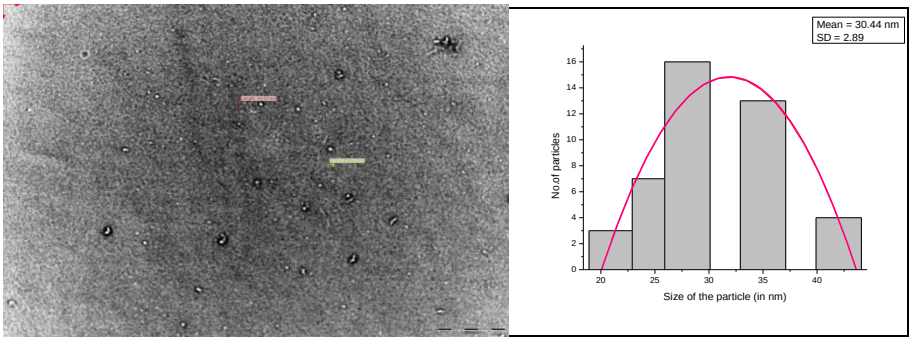


Figure 10b: TEM image and histogram of Se nanoparticlessynthesized by *P. pterocarpum*

TEM images were used to predict the morphology and size of the nanoparticles synthesized by *C.roseus*(**Fig.10a**)and*P. pterocarpum*(**Fig.10b**).Both the flowers had the ability to synthesize hollow spherical selenium nanoparticles. The particles synthesized by *C.roseus* were in the range of 17-34nm with an average size of 23.2±6.06nm, where as the particles synthesized by *P. pterocarpum* were in the range of 21-42nm with an average size of 30.44±2.89nm.There is a direct relationship between incubation time and size of the nanoparticle. Intracellular reduction of selenium IV by *Pantoea agglomerans* culture incubated for 15hr produces nanoparticles of varied size with an average between 90 and 110nm [14]. Similar results were obtained in the generation of selenium nanoparticles by *Shewanella* sp HN-41showing an increase of size and amount of nanoparticles in time [43]. The smaller sized hollow selenium nanoparticles synthesized by the present study will be highly beneficial for the drug loading and thereby it will do wonders in the field of biomedical applications.

Conclusion

In conclusion, the present study reported a novel bioinspired technology for the synthesis of selenium nanoparticles using the flower of *C.roseus*and*P. pterocarpum***.** The bioreduction of sodium selenate into selenium nanoparticles through the flower broths took a week and the position of peaks in UV – visible, excitation and emission spectrum confirmed its formation in the reaction medium. The nature and role of the various functional groups in the reduction and stabilization was elucidated with the FT-IRspectrum. Analyses through XRD, SEM and EDX proved the synthesis of more amounts of nano spherical selenium nanoparticles by *C.roseus*than *P. pterocarpum.* Further,TEM studies authenticated the formation of smaller sized hollow selenium nanoparticles of23.2±6.06nm by the flower of *C.roseus* than by the flower of *P. pterocarpum* which produces particles of 30.44±2.89nm.Availability of exceptionally good raw material and synthesis of smaller sized biocompatible hollow selenium nanoparticles by the flower of *C.roseus* proved this as a better source of biomaterial than the flower of*P. pterocarpum*. Selenium nanoparicles produced by this bioinspired way is a facile, environmentally benevolent and green alternate route to physical and chemical methods.

Acknowledgement

The authors acknowledge the financial support from Science and Engineering Research Board, Department of Science and Technology, Government of India, New Delhi. Authors also thank the Management and Principal of AyyaNadarJanakiAmmal College, Sivakasi for providing the facilities.

References:

1. Dwivedi, S.,AlKhedhairy, A.A., Ahamed, M., Musarrat, J.,Biomimetic synthesis of selenium nanospheres by bacterial strain JS-11 and its role as a biosensor for nanotoxicity assessment: A novel Se-bioassay. PLoS ONE., 2013, 8:e57404.
2. Udayasoorian, C., Vinoth Kumar, K., Jayabalakrishnan, R.M.,Extracellular synthesis of silver nanoparticles using leaf extract of *Cassia auriculata.*Dig JNanomatBiostru.,2011, 6: 279-283.
3. Pugazhenthiran, N., Anandan, S., Kathiravan, G., UdayaPrakash, N.K., Crawford, S., Ashok Kumar,M., Microbial synthesis of silver nanoparticles by *Bacillus* sp. JNanopart Res., 2009, 1811-1815.
4. Sastry, M., Ahmad, A., Islam Khan, M., Rajiv, K.,Biosynthesis of metal nanoparticles using fungiand actinomycetes. Curr Sci., 2003, 85: 162-170.
5. Ganesan, V., Aruna Devi, J., Astalakshmi, A., Nima, P.,Thangaraja, V., Eco-friendly synthesis ofsilver nanoparticles using a seaweed,*Kappaphycusalvarezii*(Doty) Doty ex P.C.Silva. Int J EngAdvan Technol., 2013, 2: 559-563.
6. Srivastava, A.A., Kulkarni, A.P., Harpale, P.M., Zunjarrao, R.S.,Plant mediated synthesis of silver nanoparticles using a bryophyte: *Fissidensminutus*and its anti-microbial activity. Int J Eng Tech., 2011, 3: 8342-8347.
7. Anal,K., Jha, Prasad, K.,Green synthesis of silver nanoparticles using *Cycas*leaf. Int J Green NanotechPhy Chem.,2010,1: 110-117.
8. Ganesan, V., Deepa, B., Nima, P., Astalkshmi,A.,Bio-inspired synthesis of silver nanoparticles using leaves of *Millingtoniahortensis*L.f. Int J AdvanBiotechnolResear., 2014, 5: 93-100.
9. Nima, P., Astalkshmi, A., Ganesan, V., A comparative account on the green synthesis of silver nanoparticles using leaves and pods of *Peltophorumpterocarpum*(DC.) Backer ex Heyne. J Biopro Tech., 2013, 98: 308-317.
10. Sharma, G., Sharma, A.R., Bhavesh,R., Park, J., Ganbold,B., Nam, J., Lee,S., Biomolecule-mediated synthesis of selenium nanoparticles using dried *Vitisvinifera*(raisin) extract. Molecules.,2014, 19: 2761-2770.
11. Berzelius, J.J., Letter from Mr Berzelius to MrBerthollet on two new metals. Ann Phys (Paris).,1818, 7: 199-206.
12. Ingole, A.R., Thakareb, S.R., Khatic, N.T., Wankhaded, A.V., Burghateb, D.K.,Green synthesis of selenium nanoparticles under ambient condition. Chalcogenide Letters.,2010, 7: 485 – 489.
13. Barnaby, S., Sarker, N., Dowdell, A., Banerjee, I., The spontaneous formation of selenium nanoparticles on gallic acid assemblies and their antioxidant properties. The Fordham Undergraduate Research Journal., 2011, 1: 41-46.
14. Torres, S.K., Campos, V.S., Leo´n, C.G., Rodrı´guez-Llamazares, S.M., Rojas, S.M., Gonza´lez, M., Smith, C., Mondaca, M.A., Biosynthesis of selenium nanoparticles by *Pantoeaagglomerans* and their antioxidant activity. J Nanopart Res.,2012, 14:1236-1244.
15. Shahverdi, A.R., Fakhimi, A., Mosavat, G., Fesharaki, P.J., Rezaie, S.M., Rezayat, S.M.,Antifungal activity of biogenic selenium nanoparticles. World ApplSci J., 2010, 10: 918-922.
16. Quintana, M., Haro-Poniatowski, E., Morales, J., Batina, N., Synthesis of seleniumnanoparticles by pulsed laser ablation. Appl Sur Sci.,2002, 195:175–186.
17. Gates, B.,Mayers, B., Cattle, B., Xia, Y., Synthesis and characterization of uniformnanowires of trigonal selenium.AdvanFunct Mat., 2002, 12:219–227.
18. Wang, M.C.P., Zhang, X., Majidi, E., Nedelec, K., Gates, B.D.,Electrokinetic assemblyof selenium and silver nanowires into macroscopic fibers. ACS Nano.,2010, 4: 2607–2614.
19. Filippo, E.,Manno, D., Serra, A., Characterization and growth mechanism ofselenium microtubes synthesized by a vapor phase deposition route. CrystalGrowth and Design., 2010, 10: 4890–4897.
20. Zhang, W., Chena, Z., Liua, H., Zhang, L., Gaoa, P., Daping,L.,Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*. Colloids Surf B., 2011, 88:196–201.
21. Hariharan, H., Al-Dhabi, N.A., Karuppiah, P., Rajaram,S.K.,Microbial synthesis of selenium nanocomposite using *Saccharomyces cerevisiae*and its antimicrobial activity against pathogens causing nosocomial infection.Chalcogenideletters., 2012, 9: 509-515.
22. Sasidharan,S., Balakrishnaraja,R.,Comparison studies on the synthesis of selenium nanoparticles by various microorganisms. Int. J. Pure. Appl. Biosci., 2014, 2: 112-117.
23. Dhanjal, S., Cameotra, S.S.,Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil. Microbial Cell Factories., 2010, 9:52-61.

24. Zare,B.,Babaie,S., Setayesh,N., Shahverdi, A.R.,Isolation and characterization of a fungus for extracellular synthesis of small selenium nanoparticles. Nanomed. J., 2013, 1: 13 -19.
25. Li, S.K.,Shen, Y.H.,Xie, A.J., Yu, X.R., Zhang, X.Z., Yang, L.B., Li, C.H.,Rapid, room-temperature synthesis of amorphous selenium/protein compositesusing *Capsicum annuum*L. extract.Nanotechnol., 2007, 18:405101–405109.
26. Prasad, K.S., Patel, H., Patel, T., Patel, K., Selvaraj, K., Biosynthesis of Se nanoparticles and its effect on UV-induced DNA damage.Colloids Surf B Biointerfaces.,2013, 103:261-266.
27. Prasad, K.S., Selvaraj, K., Biogenic synthesis of selenium nanoparticles and their effect on As(III)-induced toxicity on human lymphocytes.Biol Trace Elem Res., 2014, 157:275-283.
28. Ramamurthy,C.h., Sampath, K.S., Arunkumar, P., Kumar, M.S., Sujatha, V., Premkumar, K., Thirunavukkarasu, C., Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells.Bioprocess Biosyst Eng., 2013, 36:1131-1139.
29. Scherrer, P.,Bestimmung der Grösse und der innerenStruktur von KolloidteilchenmittelsRöntgensrahlen [Determination of the size and internal structure of colloidal particles using X-rays]. NachrGesWissGoettingen, Math-PhysKl., 1918, 98-100.
30. Klug,H.P., Alexander, L.E.,X-ray Diffraction Procedures for Polycrystalline and AmorphousMaterials. Wiley: New York, NY, USA, 1967.
31. Biradar, D., Lingappa, K.,Isolation and screening of gold nanoparticles by microbes. W J SciTechnol., 2012,2: 20-22.
32. Bharani, M., Karpagam,T., Varalakshmi, B., Gayathiri, G., Lakshmi Priya., K.,Synthesis and characterization of silver nanoparticles from *Wrightiatinctoria*.Int J ApplBiol Pharm Technol.**,** 2012,3: 58-63.
33. Lisiecki, Billoudet, F.,Pileni, P.,Control of the shape and the size of copper metallic particles. J PhysChem.,1996, 100: 4160-4166.
34. Kelly, L.K., Coronado, E., Zhao, L., Schatz, G.C.,The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape and Dielectric Environment.J Phys Chem., 2003, B 107:668-677.Nellorea,J., Cynthia Pauline, P., Kanchana, A.,Biogenic synthesis by *Sphearanthusamaranthoids*; towards the efficient production of the biocompatible gold nanoparticles. Dig J NanomateBiostru.,2012, 7: 123-133.
35. Durán,N.,Priscyla, D., Marcato**,** Oswaldo, L., Alves**,** De Souza GI., Elisa Esposito. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusariumoxysporum*strains. J Nanobiotech., 2005,3:3155-3-8.
36. Honarya, S., Barabadia, S., Gharaeifathabada, E.,Naghibib,F.,Green synthesis of copper oxide nanoparticles using *Penicilliumaurantiogriseum, Penicilliumcitrinum*and *Penicilliumwaksmanii*. Dig J NanomateBiostru., 2012,7: 999-1005.
37. Varshney, R., Mishra, A.N., Bhadauria, S., Gaur, M.S.,A novel microbial route to synthesize silver nanoparticles using fungus *Hormoconisresinae***.** Dig J NanomatBiostru., 2009, 4: 349-355.
38. Eszenyi, P., Sztrik,A., Babka,B., Prokisch,J.,Elemental., nano-sized (100-500 nm) selenium production by probiotic lactic acid bacteria. Int J BiosciBiochemBioinform., 2011, 1:148-152.
39. Fesharaki, P.J., Nazari,P., Shakibaie,M., Rezaie,S., Banoee, M., Abdollahi,M., Shahverdi,A.R., Biosynthesis of selenium nanoparticles using *Klebsiellapneumoniae*and their recovery by a simple sterilization process.Brazilian JMicrobiol., 2010, 41: 461-466.
40. Kessi, J., Ramuz, M., Wehrli, E., Spycher, M., Bachofen,R., Reduction of seleniteand detoxifixation of elemental selenium by the phototrophic bacterium*Rhodospirillumrubrum*. Appl Environ Microbiol 1999, 65:4734-4740.
41. Roux, M., Sarret, G., Paintrand, I.P., Fontecave, M., Coves, J., Mobilization ofselenite by *Ralstoniametallidurans* CH34. Appl Environ Microbiol., 2001,67:769-773.
42. Nagajyoti, P.C., Prasad,T.N.V.K.V., Sreekanth,T.V.M., Lee,K.D., Bio-fabrication of silver nanoparticles using leaf extract of *SaururusChinenis***.** Dig JNanomatBiostru., 2011,6: 121-133.
43. Tam, K., Ho, C., Lee, J., Min-Lai C., Rheem, Y., Chen, W., Myung, N.V.,Growth mechanism of amorphous selenium nanoparticles synthesized by *Shewanella* sp. HN-41. BiosciBiotechnolBiochem., 2010, 74: 696–700.

*****