International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-2539, Volume 3, Issue 4; October-December, 2016, pp. 242-246

http://www.krishisanskriti.org/Publication.html

Computational Study and Characterization of Green Synthesis of Silver Nanoparticles

Shreya Anand[1], Priyam Raut[2], Rashmi Gupta[3], Raghu Raja Pandiyan K[4] and Padmini Padmanabhan[5]

[1,2,3,5]*Department of Bio- Engineering BIT Mesra, Ranchi, INDIA*
[4]*Department of Chemical Engineering BIT Mesra, Ranchi, INDIA*
E-mail: [1]shreyaanand.30@gmail.com, [2]priyam.raut@gmail.com, [3]rashmi171990@gmail.com, [4]raghuchemraj@gmail.com, [5]padmini@bitmesra.ac.in

Abstract—*Nanotechnology has been heralded as a revolutionary field of research which leads to an alternative pathway for exploration and evolution of biological entities. Plants are the natural chemical factories which are economical and require minimal maintenance. They are non-toxic, environment friendly and compatible for pharmaceutical as well as biomedical application. The major mechanism of synthesis is plant-assisted reduction due to the presence of phytochemicals (phenols, alkaloids, flavonoids, steroids) which are directly involved in the reduction of the silver ions and the formation of silver nanoparticles. The objective of this research is to biosynthesize silver nanoparticles using leaf extract of Alternanthera sp. and the synthesized nanoparticles were characterized by UV-VIS spectroscopy, DLS, SEM. Using a host of computational biology tools such as ScanProsite, ASA view, CONSURF etc. we study the interaction of 4,5-dioxygenase like protein (betacyanin) with silver nitrate, analyze the structure, exposed amino acid residue and their solvent accessibility.*

1. INTRODUCTION

The biological approach of metal nanoparticles synthesis is a focus of interest because of its enormous potential in the field of Nanotechnology. Biosynthetic process involves the use of plant extract for the synthesis of silver nanoparticles [1]. The advancement of Green synthesis over other method provides easy scale up for large scale production of nanoparticles. The plant *Alternanthera ficoidea* is a species of Amaranthaceae family and is commonly used as ornamental edging plant. It is considered as environmental weed and shows anti-microbial activity. It has been suggested that the biomolecules present in the plants play important role in catalysing the synthesis [2]. The combination of phytochemicals – phenols, alkaloids, steroids in this plant helps in reduction of Ag (I) to Ag (0) ions.

Proteins contain various amino acids that act as a reducing agent. The protein-nanoparticle interaction either occurs through free amine group or cysteine residues in proteins and via the electrostatic attraction of negatively charged carboxylate groups [3]. The present study promoted us to investigate enzymes present in the *Alternanthera sp.* for the synthesis of metal-biomolecules hybrid. The protein, 4,5-dioxygenase like protein (betacyanin) is the protein which includes betalain pigment, a class of red and yellow indole-derived pigments found in plants of the Caryophyllales order [4].

Computational studies are used to understand the possible amino acid involved in the biosynthetic process [5]. This Green synthetic approach to silver nanoparticles will give insight to understand the chemistry of amino acids of the enzyme and its interaction with the metal ion.

2. EXPERIMENTAL

2.1 Materials and Methods

Alternanthera ficoidea leaves were collected from BIT, Mesra campus. Silver nitrate was procured from Merck, India. Phosphate buffer was prepared in laboratory at pH 8.5. All other chemicals and solvents used were of analytical grade.

2.2 Preparation of Extract

Extract was prepared by weighing 10gm of leaves and were thoroughly washed thrice in distilled water, surface sterilized using methanol, cut into fine pieces. Crushed using mortar pestle in phosphate buffer, centrifuged thrice and were properly filtered.

2.3 Synthesis of Silver nanoparticles

The biosynthesis of silver nanoparticles were carried out by incubating 50ml of leaf extract in 500µl (1M) silver nitrate. Solution was kept at 37°C and was monitored using UV-visible spectroscopy as well as Dynamic light scattering.

2.3 Characterization of silver nanoparticles

Dynamic light scattering (DLS) measurement was carried out by using Malvern instruments, UK, Nano ZS at 25°C for 12 cycles. Sample for Scanning electron microscopy were prepared by drop coating purified silver nanoparticle on carbon coated SEM grids. SEM measurement is performed on JEOL, Japan, JSM-6390LV instrument operated at an accelerating voltage of 20kV.

3. COMPUTATIONAL

Since crystal structure of any of the enzymes of the plant (*Alternanthera ficoidea*) do not exist, the sequence of **4-5,di-oxygenase like protein** (a putative betacyanin protein) was retrieved from NCBI database (ncbi.nlm.nih,gov) with **GENBANK ID AKI33720.1**. The sequence was submitted to **I-TASSER** [6] server for protein structure prediction. The PDB was modeled then **Ramachandran plot** was plotted to check the stability of the conformation using **Discovery Studio 2016** [7]. The PDB structure was later submitted to **IonCom**, **ASA view** [8] and **CONSURF** [9] for predicted binding residues, Absolute surface area and conserved amino acid sequences respectively.

4. RESULTS AND DISCUSSION

The *A.ficoidea* is a herbaceous plant which are considered to be a weed. It has antimicrobial activity and the species of *Alternanthera* contains active compounds including Betacyanin, flavonoids, saponins, phenols, alkaloids, steroids. Betacyanin is a type of hydrophilic pigment responsible for coloration of leaves and other plant parts. In this study, *A.ficoidea* leaf extract was used for *in vitro* synthesis of silver nanoparticles. When the leaf extract was mixed with freshly prepared aqueous solution of silver nitrate, the color of mixture turned to yellowish brown. **Fig. 2** shows the UV-Vis spectra of silver nanoparticles with the respect to time. The absorption centered between 400-450 nm Surface Plasmon Resonance (SPR) of silver nanoparticles increased in intensity up to 12 hrs.

The 280 nm region peak is also observed during the synthesis of silver nanoparticles (**Fig. 3**).The shape of the silver nanoparticles was observed by SEM. **SEM** images **Fig. 4** showed that the silver nanoparticles are spherical in shape. **EDX** confirmed that these nanoparticles are composed of silver (**Fig. 5**). Particles size distribution and estimation of silver nanoparticles were determined by **DLS** (**Fig. 6**) and the sizde of nanoparticle synthesized was 158nm. The observed **Zeta potential** for the synthesized nanoparticle was found to be **-25.6mV.** It indicates that the surface of the synthesized silver nanoparticle has negative charge (**Fig. 7**).

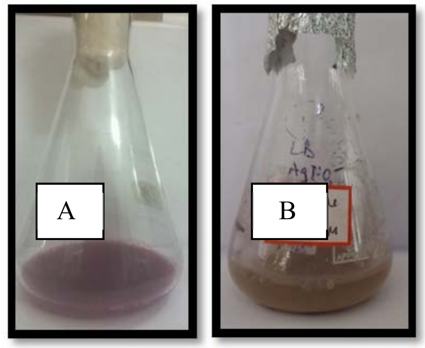


Fig. 1: Visual observation of silver nanoparticles formation (A) beginning of the reaction (B) after 12 hr.

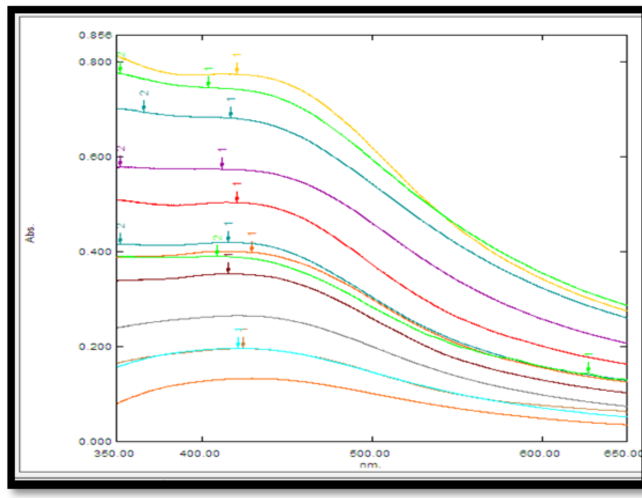


Fig. 2: UV-VIS spectra at 400-450 nm for silver nanoparticles

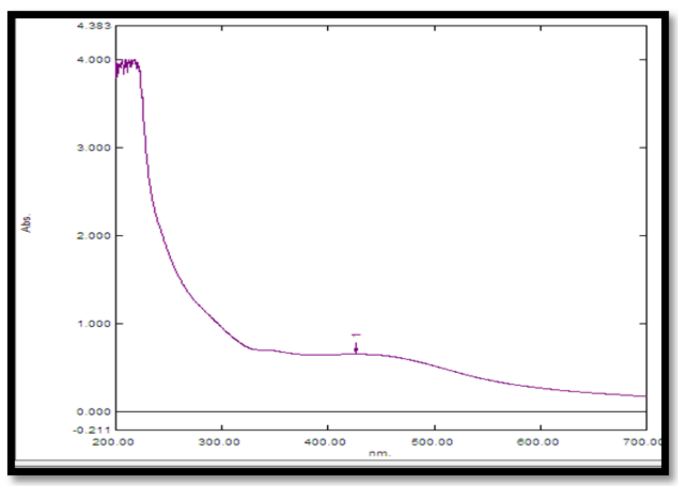


Fig. 3: UV-VIS spectra at 280 nm for silver nanoparticles

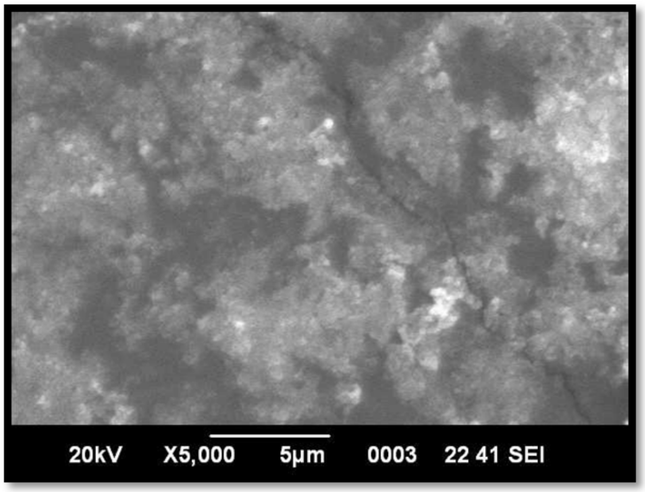


Fig. 4: Scanning Electron Microscopy (SEM) images of purified silver nanoparticles.

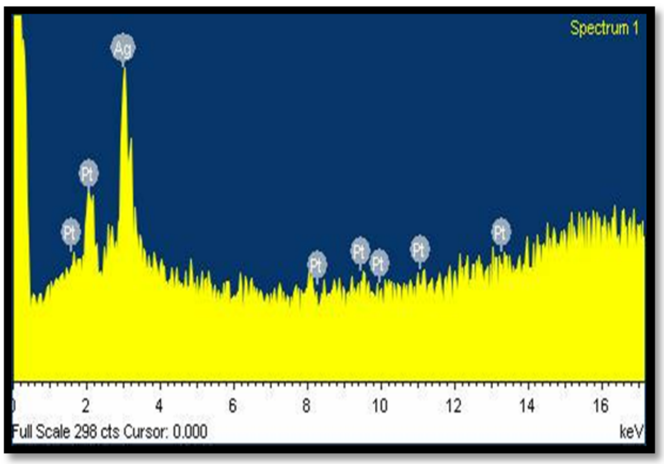


Fig. 5: EDX spectrum of silver nanoparticles

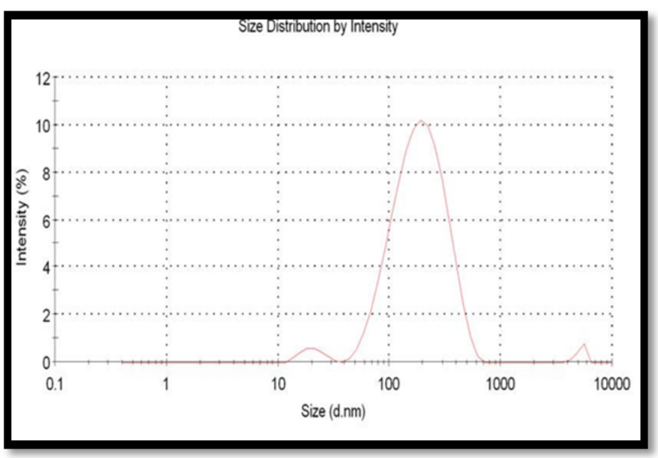


Fig. 6: DLS image of silver nanoparticles

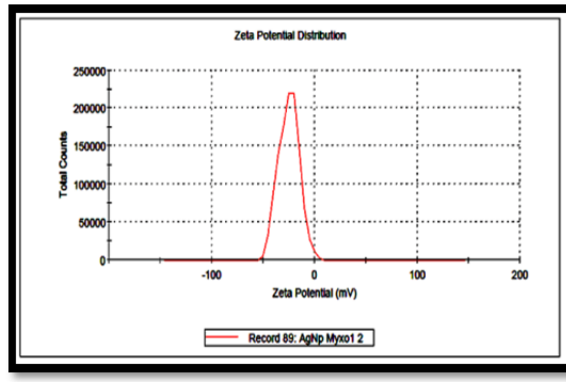


Fig. 7: Zeta potential image of silver nanoparticles

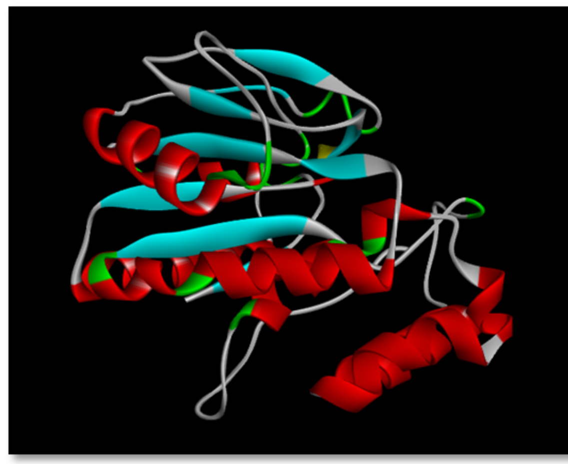


Fig. 8: PDB structure after modeling using I-TASSER server

The PDB structure with the best C score of 0.8 was selected using the **I-TASSER** server (**Fig. 8**). The Ramachandran plot and disulfide bridges between cysteine residues were obtained using **Discovery Studio 2016** to validate the protein structure of the modeled structure and check its stability (**Fig. 9**). The plot showed 92.1 % of the residues in favoured and allowed regions. The disulfide bonds are tabulated on the basis of their score (**Table 1**). The bond energy has to be less than 5 to be stable. The **ASAview** results showed the exposed residues present in the protein and the solvent accessibility of specific amino acids present (**Graph 1**). From it we can list the positively charged and polar uncharged amino acids which are more likely to interact with the silver cation.

When the protein sequence was uploaded to **ScanProsite** no hits were found but with the help of **IonCom** the binding residues of the sequences were derived. Using **CONSURF**, the conserved functional regions of protein were found out. In the amino acid sequence 81 out of 244 amino acids were fully conserved among homologues. By this we can infer that these 81 residues are functional. By using these various tools we can predict the binding site of the protein [10].

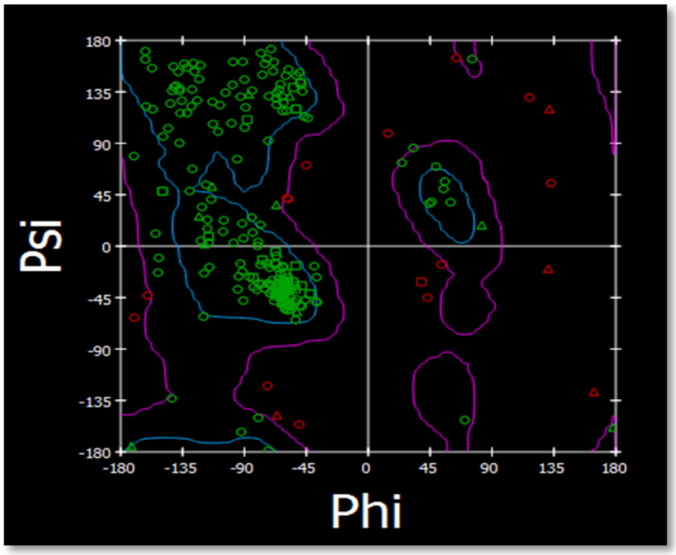
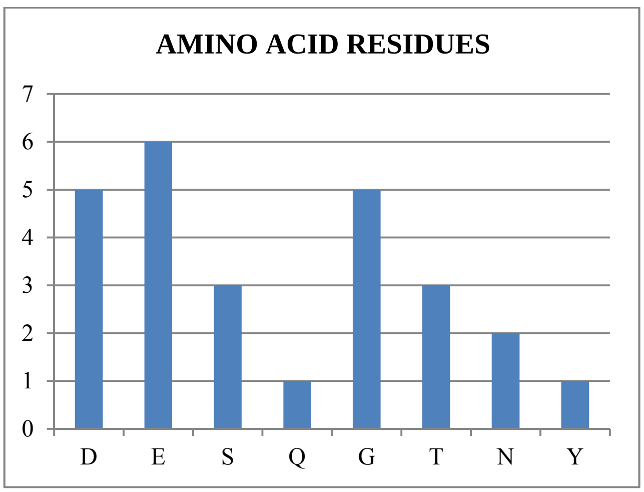


Fig. 9: Ramachandran plot using Discovery Studio 2016 of the structure of betacyanin after modeling.

Graph 1. Relative solvent accessibility of Amino acid residues exceeding 50%.



D: Aspartic acid, **E**: Glutamic acid, **G**: Glycine,

N: Asparagine, **Y**: Tyrosine, **Q**: Glutamine,

S: Serine, **T**: Threonine

Table 1: Predicted disulfide bridges of predicted protein model using Discovery studio

S. No.	Disulphide bond link	Score	Energy
1	38-126	95.03	2.48
2	23-238	94.28	2.86
3	79-127	93.15	3.42
4	55-86	91.61	4.20
5	10-73	91.07	4.47
6	151-156	90.04	4.98

According to the **IonCom** Ligand Binding Site Prediction, the predicted binding residues are Glu-1, His-7, Gly-8, Lys-37, Ser-43, His-45, Trp-46, Glu-47, Asp-60, Asp-64, P78, Asp-103, Asp-109, His-110, Glu-122, Asp-124, Cys-128, His-141, G162, His-166, C174, Glu-187, His-212, Glu-216, His-217, His-238, Asn-239 AND Asp-242.

5. CONCLUSION

Silver nanoparticles were synthesized using leaves extract of *A.ficoidea.* It was studied that the plant has 4,5-dioxygenase like protein (betacyanin) and the structure of the protein was predicted. The amino acid involved in the synthesis as well as the relative solvent accessibility of those amino acid residues were studied by ASA view. Consurf was used to estimate the amino acids which are conserved, based on the phylogenetic relationship between the homologous sequences. IonCom ligand binding site predicted the binding residues of betacyanin with metal ligands. The computational study of the protein enables us to understand the interaction of the silver particle with the protein present in our plant sample.

6. ACKNOWLEDGEMENTS

The financial support was provided by the departmental funding for final year master's project. The authors are thankful for the Central Instrumentation Facility, centre of excellence, TEQIP-II, BIT Mesra for the letting us use the instruments at their disposal.

REFERENCES

[1] Mohanpuria, P.; Rana, N.K.; Yadav S.K. "Influence of Physiochemical Factors on Size of Gold Nanoparticles Synthesised Using Leaf Extract of Syzygium Cumini", *J. Nanopart. Res.* 2008, pp.*10*, 507.

[2] Mishra, A.; Kumar, K.N.; Sardar, M.; Sahal, D. "Biosynthesis and antibacterial activity of silver nanoparticles". *Colloids Surf. B* 2013. pp. *111*, 713.

[3] Mukherjee, P.; Senapati, S.; Mandal, D.; Ahmad, A.; Khan, M.I.; Kumar, R.; Sastry, M. "Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*." *ChemBioChem*, 2002, pp.*3*, 461.

[4] Rodrigues-Brandão, I., Kleinowski, A.M., Einhardt, A.M., Lima, M.C., Amarante, L.D., Peters, J.A. and Braga, E.J.B., 2014. "Salicylic acid on antioxidant activity and betacyan in production from leaves of Alternanthera tenella". *Ciência Rural*, *44*(10), pp.1893-1898.

[5] Mishra, A.; Singh, P.; Sardar, M. " Peroxidase assisted biosynthesis of silver and gold nanoparticles: Characterization and computational study". *Adv. Mater. Lett*. 2015. pp. 194-200

[6] Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J. and Zhang, Y.,. The I-TASSER Suite: protein structure and function prediction. *Nature methods*, 2015 ,*12*(1), pp.7-8

[7] Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.

[8] Ahmad, S.; Gromiha, M.; Fawareh, H.; Sarai, A.; "ASAView: database and tool for solvent accessibility representation in proteins".*BMC Bioinform.* 2004, pp. *5*, 51.

[9] Celniker, G., Nimrod, G., Ashkenazy, H., Glaser, F., Martz, E., Mayrose, I., Pupko, T. and Ben-Tal, N. "ConSurf: using evolutionary data to raise testable hypotheses about protein function". *Israel Journal of Chemistry*, 2013, *53*(3-4), pp.199-206.

[10] Shahin, G.; Hamidi A.; Behrouz L.; Amin M.; "Bioinformatics Prediction of Interaction of Silver Nitrate and Nano Silver on Catalase and Nitrat Reductase". *International Journal of Scientific Research in Environmental Sciences*".2013. pp. 26-35.