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Synthesis of Silver Nanoparticles from *Bauhinia acuminata* Aqueous Leaf Extract and Molecular Docking Analysis of Various Cancer Receptors

Divya Sebastian [1], Albin T. Fleming

P.G and Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034

Abstract: ***To synthesize silver nanoparticle from Bauhinia acuminate aqueous leaf extract under various concentrations. The characterization Bauhinia acuminate silver nanoparticle using PSA shows the nanoparticle size of 78, 89 and 110 nm. The BaAgNPs were effective against E.coli and S.aureus was found to be 900 and 750 µg using MIC assay. The molecular docking affinity of Caryophyllene oxide ligand binding to cellular receptors like 5DZL with -5.88 kcal/mol shows the effective nature of the aqueous leaf extract components. The BaAgNPs shows the effective antimicrobial inhibition against E.coli and S.aureus.***

Keywords: BaAgNPs, PSA, MIC, Molecular Docking

1. Introduction

The field of nanotechnology is currently observing a significant progress in ecofriendly method of nanosynthesis (Kumar et al., 2011). Plant crude extracts which contain novel secondary metabolites are an exciting possibility for nanosynthesis that is yet to be explored. The biomolecules present in the plant compounds are responsible for the formation and stabilisation of silver nanoparticles. The growing concern is especially devoted to new and emerging nanoparticles based on plant compounds in the field of drug synthesis. Bionanosynthesis of plant materials which contain phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds complementary to antibiotics are highly promising and reassuring the results. It shows a significant tool for antimicrobial agents in present and in a near future (Aromal and Philip, 2012). According to World Health organisation (WHO), the search for potent antibacterial agents has been shifted to plants.

Silver nanoparticles have been given prominence among metal nanoparticles as they are non-toxic to humans and have a great role of becoming next generation antimicrobials. Nowadays bacterial infections have a pivotal role in morbidity and transience. This environmentally friendly method of biological silver nanoparticles production provides rates of synthesis faster or comparable to those of chemical methods and can potentially be used as antimicrobial agents. Phytochemicals present in the plants can improve the quality of SNP against bacterial pathogens. According to Rai, et al., 2009 green synthesis of nanoparticles uses water commonly as an environmentally benign solvent, replacing toxic organic solvents. Many reports are available on the biogenesis of silver nanoparticles using plant extracts such as *Azadirachta indica* (Neem) (Tripathi, 2009). *Curcuma longa* (Sathish Kumar, 2010) *Lantana camara* (Siva kumar et al., 2011) *Azadirachta indica* (Lalitha et al, 2013), *Costeus igneus* (Vasantharaj et al., 2013), pineapple leaf (Elemike et al., 2014), Datura metel flower (Chandran et al., 2012), *Securinega leucopyrus* (Donda et al., 2013), *Rhinacanthus nasutus* (Pasupuleti et al., 2013), *Bauhinia purpurea* (Radha et al., 2016), *Ocimum sanctum* (Chung et al ., 2016). In the present study, using the leaves of *Bauhinia acuminata* an attempt was made to find out the bioprospect of plant-mediated synthesised BaAgNPs as a possible antimicrobial agent. *Bauhinia acuminata* otherwise known as dwarf white *Bauhinia* is a genus with more than 200 species of flowering plants in the subfamily *Caesalpinioideae* of the large flowering plant family Fabaceae, with a pantropical distribution. The bark, flower, and root of the *Bauhinia acuminata* are used for various skin diseases, skin diseases and ulcer, throat infection worms, tumours, and diabetes. Moreover, the leaf of *Bauhinia acuminata* is used to treat bladder stone venereal diseases, diabetes, leprosy, asthma and digestive diseases, decoction of the leaves have beneficial aspects on gonorrhoea. The bark and leaves of *Bauhinia acuminata* are used to treat biliousness a remedy recommended by the Indian vaidyas. The present study aims at biosynthesis and characterization with the help of *Bauhinia acuminata* leaf extract by reduction of Ag+ to Ag0 from silver nitrate solution and estimating the presence of nanoparticle by particle size analyser and also investigated the antibacterial activity of nanoparticles against gram negative pathogenic bacteria.

2. Materials and Methods

2.1 Collection and Preparation of Plant Materials

Leaves of *Bauhinia acuminata Lin* were collected from Angamaly in the month of September and authenticated by Dr S.Jayaraman, Director of Plant and Anatomy Research Centre Chennai (Reg.No.PARC/2013/2189). Fresh leaves of *B.acuminata* were washed thoroughly in distilled water and, cut into fine pieces. Approximately 20 g of the leaves were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and filtered. The aqueous extract

was separated out using a Whatman No1 filter paper. The filtrate was collected and stored at 4°C for further use.

2.2 Synthesis and Lyophilisation of Nanoparticles:

Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich. An accurately weighed 0.017g of silver nitrate was dissolved with 100 ml of double-distilled water and stored in the amber colour bottle until further use. 1, 3 and 5 ml of plant extracts were added to the of 1 mM concentration of 10 ml of silver nitrate solution. Formations of sliver nanoparticles were monitored by using a UV-Visible spectrophotometer. UV- vis spectra were recorded as a function of reaction time on a UV1650CP Shimadzu spectrophotometer operated at a resolution of 1nm The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min followed by dispersion of the pellet in deionized water. The pellets obtained from the three concentrations of plant extracts were then lyophilized by using freeze dryer (Lyodel-Delvac Pumps Pvt. Ltd, USA) to enhance the stability of silver nanoparticles. After freeze drying of the purified silver particles, the structure and size were analysed by particle size analysers.

2.3 Physical Characterization

2.3.1 UV -Visible Spec Analysis
UV-Vis Spectroscopy plays an important role in the confirmation of formation of nanoparticles in an aqueous medium. The formation and completion of silver nanoparticles were characterised by UV-Visible spectroscopy by using Shimadzu UV-Visible spectrophotometer. After the addition of $AgNO_3$ to the plant extract, the spectra were recorded in the wave length, between 350 nm to 600 nm.

2.3.2 Particle Size Analyser:
Particle size analyzer has been used to detect the size of the synthesised nanoparticles. The Malvern Zeta sizer range provides both exceptionally high performance and entry level systems that incorporate combinations of a particle size analyser, zeta potential analyser, molecular weight analyser, protein mobility and micro-rheology measurements. Particles and molecules from less than a nanometer in size to several microns can be analysed by a range of variants to suit the applications and budget.

2.4 Antibacterial Assay

Mueller Hinton Broth E: 261415 and Nutrient Broth: 000239624 are collected from Micromaster, Sodium Chloride and Demineralized water are collected from Spectrum chemicals, Resazurin Dye and Ciprofloxacin were purchased from Himedia laboratories Pvt. Ltd. Mumbai, India. All other reagents used were of analytical grade.

2.4.1 Test Organisms
Escherichia coli (NCTC 12923) and *Staphylococcus aureus* (NCTC10788) National Collection of Type Cultures, United Kingdom The test sample was evaluated for antibacterial activity by MIC against *E. coli* and *S. aureus* at a different concentration ranging from 20000.976 µg. The MIC value of test substance was compared with the activity of standard antibiotic.

2.4.2 Preparation and Standardisation of Stock Cultures
A day prior to the experiment, a loopful culture of *E. coli* and *S. aureus*, were grown in NB at 37°C for 24 hours. The cultures were adjusted to 0.17 absorbance at 600 nm (corresponding 10^8 CFU/ml 0.5 McFarland standard) using a spectrophotometer. And further diluted to a concentration of approximately10^5 CFU/ml.

2.4.3 Preparation of Resazurin and Standard Antibiotic Solution
The stock Resazurin solution was prepared by dissolving 2.7 mg in 4 ml of sterile saline. The further working solution was prepared by dissolving 1 ml of stock solution in 5ml of sterile saline. The standard antibiotic i.e., ciprofloxacin solution at 1% concentration was prepared in sterile.

2.4.4 Preparation of Test Sample
The test sample was prepared at a 30 mg/1.5 ml concentration by dissolving 30mg of the test sample in 1.5 ml of MHB. The sample was mixed using cyclomixer for 5min and sonicated for 5min. Sample was mixed thoroughly before using for experiment.

2.4.5 Determination of MIC
Experiments were performed in triplicate under aseptic condition. A volume of 50 µl respective sterile MHB was added to all 96 wells except first three wells of the microtitre plate to which only 100 µl test product was added. From first three wells of the plate, 50 µl of the test product was double diluted to the wells containing test material 10 µl bacterial suspension of approximately 10^5 CFU/ml was added. A growth control (bacterial cell suspension + 50 µl broth medium) and broth control (only broth medium 50 µl) was kept in the 96 wells plate. A positive control that consists of the ciprofloxacin was also placed on the plate. The plates were incubated at 37° C for 24 hours. After incubation, 10 µl of working solution of resazurin dye was added to all wells. The plates were wrapped with aluminium film and incubated at 37°C for 1hr. The colour change was then assessed visually. Any colour change from purple to pink or colourless was recorded as positive growth. The lowest concentration at which there is no colour change occurred was taken as the MIC value.

2.5 Molecular Docking

According to Vipinlal Vasudevan et al., 2013 GC-MS analysis of leaf distillation extract showed the presence of β-caroyphyllene (13.87 %) and caryophyllene oxide (3.15 %), were the other major constituents taken for molecular docking analysis using cancer receptors. The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The 3D structure of cancer Biomarkers from *Homo sapiens* was

retrieved and the cancer receptors are 3OQ9, 4OFB, 4WP7 and 5DZL. The molecular docking methodology was followed by Arumugam Madeswaran et al., 2012 and Gowri Shankar Krishnan et al., 2013.

3. Results and Discussion

The bioreduction of the pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours (complete colour change) following the dilution of a small aliquot of the sample in distilled water. The reduction of silver ions in the aqueous solution of nanoparticles could be correlated with the respective UV-Vis spectra of the colloidal solution which exhibited a strong absorption at 1:10, 3:10, 5:10, corresponding the value 395,410,420 shown in Figure 1.

The particles are analysed based on the mass median diameter which indicates the 50% diameter of the particle comprising of sub nano particles. The result obtained as reported by the size of the silver nanoparticles represented by Xiangqian Li et al., 2011. On analysing the results the particle synthesised range starts for 1:10, 3:10 and 5:10 mM concentration has been started with 78, 89 and 110 nm respectively are shown in Figure 2.

Formation of multidrug-resistant strains facade the risk, which necessitated the production of novel and potential antimicrobial agents in a persistent way. Pure natural constituents which are present in the plants could be used to bioreduce and stabilise silver nanoparticles. The positive charge on the Ag ions is suggested vital for antimicrobial activities. According to Cao et al., 2001 the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells. Nanoparticles have been shown to pile up inside the bacterial membrane and consequently penetrate into the cells causing lysis of bacteria. In analogy with other fellow research workers AgNPs Sathish kumar et al., 2010 *Curcuma longa* showed minimum bactericidal concentration (MBC) for *Escherichia coli* BL-21 strain was found to be 50 mg.L Wady et al., 2014 showed a prominent zone of inhibition when the synthesised nanoparticles were tested against *Staphylococcus aureus*. Number of theories for antimicrobial actions of colloidal silver solution have been proposed. There are a number of researches Negi et al., 2012 and Rashid, et al., 2014 reported that green particles from other *Bauhinia* species *B.variegata*, *B.purpurea* is well known for its antimicrobial activity and *B. tomentosa* have shown potential activity against cancer (Mukundan et al., 2015). In the current study, AgNPs synthesised with *B.acuminata* were effective against *E.coli* and *S.aureus* was found to be 900 and 750 µg respectively are shown in Table 1. A green method using naturally occurring precursors such as vitamins, sugars, plant extracts, biodegradable polymers and microorganisms as reductants and capping agents are aenvironmental friendly and it has good antimicrobial efficacy against most pathogenic bacteria. The antimicrobial activity of the biosynthesis of leaf extract of *Bauhinia acuminata* in the present study exhibited satisfactory activity towards *Staphylococcus aureus* and *Escherichia coli*.

The goal of ligand- protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures (Mittal et al., 2009). The binding affinity of the four cancer receptors with the ligands was measured by kcal/mol. The docking scores for Caryophyllene oxide ligand binding to cellular receptors like 5DZL with -5.88 kcal/mol as a target and interacted in the regions with two bonds (bond distance) of C:GLU37:OC (2.6) and C:THR101:OH (2.1) with 11.52 RMSD and 48.77 Estimated Inhibition Constant, Ki (µM) respectively are shown in Table 2 and Figure 3.

4. Conclusion

Biosynthesis of NPs using plants is very cost effective and thus can be used as an economic and valuable alternative for the extensive production of NPs. The current study revealed that silver nanoparticles can be synthesised by a simple method using *B.acuminata* leaf extract. The resourcefulness of this plant has dispelled the fear of therapeutic failure of antibiotic to life-threatening infectious diseases for the willing pharmaceutical industries to explore the use of the plant as drug against antibiotic-resistant pathogens.

References

[1] Kumar, V., Yadav, S. K., 2011. Synthesis of variable shaped gold nanoparticles in one solution using leaf extract of *Bauhinia variegata L.,* Digest Journal of Nanomaterials and Biostructures, 6(4), 1685-1693.
[2] Aromal, S. A., Philip, D., 2012. Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size dependent catalytic activity. Spectrochim Acta A, 97:1–5
[3] Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.
[4] Shankar, S.S., Rai, A., Ahmad, A., Sastry, M., 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloid Interface .
[5] Tripathi, A., Chandrasekaran, N., Raichur, A.M., Mukherjee, A., 2009. Antibacterial applications of silver nanoparticles synthesized by aqueous extract of *Azadirachta Indica* (Neem) leaves. J. Biomed. Nanotechnol. 5, 93.
[6] Sathish kumar, M., Sneha, K., Yun, Y.S., 2010. Immobilization of silver nanoparticles synthesized using *Curcuma longa* tuber powder and extract on cotton cloth for bactericidal activity. Bioresour. Technol. 101, 7958–7965.
[7] Sivakumar, P, Chandran Nethradevi, Sahadevan Renganathan., 2012. Synthesis of silver nanoparticles using Lantana camara fruit extract and its effect on pathogens. Asian Journal of Pharmaceutical and Clinical Research 5.
[8] Lalitha, A., Subbaiya, R., Ponmurugan, P., 2013. Green synthesis of silver nanoparticles from leaf extract

Azhadirachta indica and to study its anti-bacterial and antioxidant property. Int. J. Curr. Microbiol. Appl. Sci. 2, 228–235.

[9] Vasantharaj, S., Sathiyavimal, S., Hemashenpagam, N., n.d., Biological and Chemical Sciences Antimicrobial Activity of Silver Nanoparticle Synthesized by Using *Costeus igneus*. Research Journal of Pharmaceutical. 4, 733–738.

[10] Elemike, E. E., Oseghale, C. O., Chuku, A., Labulo, A. H., Owoseni, M. C., Mfon, R., Enock, O. D., Adesuji, E. T., 2014. Evaluation of antibacterial activities of silver nanoparticles green-synthesized using pineapple leaf (Ananas comosus). Micron 57:1–5 Manipulation of Plant Tissue Culture. Monograph 16, British Plant Growth Regulator Group, Bristol.

[11] Chandran, N., Padmanaban, S., Sahadevan, R., 2012. Green synthesis of silver nanoparticles using datura metel flower extract and evaluation of their antimicrobial activity. Int J Nanomater Biostruct 2:16–21.

[12] Donda, M.R., Rao, K., Alwala, J., Miryala, A., Sreedhar, B., Pratap, M.P., 2013. Synthesis of silver nanoparticles using extracts of Securinega leucopyrus and evaluation of its antibacterial activity. Int J Curr Sci 7, 1–8.

[13] Pasupuleti, V.R., Prasad, T.N.V.K. V, Shiekh, R.A., Balam, S.K., Narasimhulu, G., Reddy, C.S., Rahman, I.A., Gan, S.H., 2013. Biogenic silver nanoparticles using Rhinacanthus nasutus leaf extract: Synthesis, spectral analysis, and antimicrobial studies. Int. J. Nanomedicine 8, 3355–3364

[14] Radha, R., Murugalakshmi,M., Kokila, S., 2016. Eco friendly synthesis and characterization of Gold Nanoparticles from Bauhinia purpurea flower extract.Imp.J. Interdiscip. Res. 2, 306–310.

[15] Chung, I.-M., Park, I., Seung-Hyun, K., Thiruvengadam, M., Rajakumar, G., 2016. Plant-Mediated Synthesis of Silver Nanoparticles: Their Characteristic Properties and Therapeutic Applications. Nanoscale Res. Lett. 11

[16] Li, X., Xu, H., Chen, Z., Chen, G., 2011. Biosynthesis of Nanoparticles by Microorganisms and Their Applications. J. nan 2011.

[17] Cao, Y. W., Jin, R., Mirkin, C. A., 2001. DNA-modified core–shell Ag/Au nanoparticles. J Am Chem Soc. 123:7961–2.

[18] Ayurvedic Medicinal Plants: Bauhinia acuminata (http://www.toxicologycentre.com/English/Plants/Botanical/vellamandram.html).

[19] Wady, A.F., Machado, A.L., Foggi, C.C., Zamperini, C.A., Zucolotto, V., Moffa, E.B., Vergani, C.E., 2014. Effect of a Silver Nanoparticles Solution on *Staphylococcus aureus* and *Candida spp.*

[20] Mukundan, D., Mohankumar, R., Vasanthakumari, R., 2015. Green Synthesis of Silver Nanoparticles using Leaves Extract of *Bauhinia tomentosa Linn* and its invitro Anticancer Potential. Mater. Today Proc.

[21] Sakthivel, M., Palani, P., 2016. Isolation, purification and characterization of antimicrobial protein from seedlings of *Bauhinia purpurea L*. Int. J. Biol. Macromol. 86, 390–401. doi:10.1

[22] B.S. Negi, B.P. Dave, Y.K. Agarwal, Indian J. Med. Microbiol. 52 (2012) 360–365.

[23] Arumugam Madeswaran, Muthuswamy Umamaheswari, Kuppusamy Asokkumar, Thirumalaisamy Sivashanmugam, Varadharajan Subhadradevi, Puliyath Jagannath, 2012. Computational drug discovery of potential phosphodiesterase inhibitors using in silico studies. Asian Pacific Journal of Tropical Disease, S822-S826.

[24] Gowri Shankar, K., Albin Fleming, T., Vidhya, R., Sophy Nirmal. 2013. In-Silico Molecular Docking Analysis of Cancer Biomarkers with Bioactive Compounds of *Tribulus terrestris*. International Journal of Novel Trends in Pharmaceutical Sciences. 3(4).

[25] Vipinlal Vasudevan, Joseph Mathew, Sabulal Baby. 2013. Chemical Composition of Essential Oil of *Bauhinia acuminata* Leaves. Asian Journal of Chemistry, 25(4), 2329-2330.

[26] Mittal, R. R., McKinnon, R. A., Sorich, M. J., 2009. Comparison data sets for benchmarking QSAR methodologies in lead optimization. J Chem Inf Model. 49:1810-20.

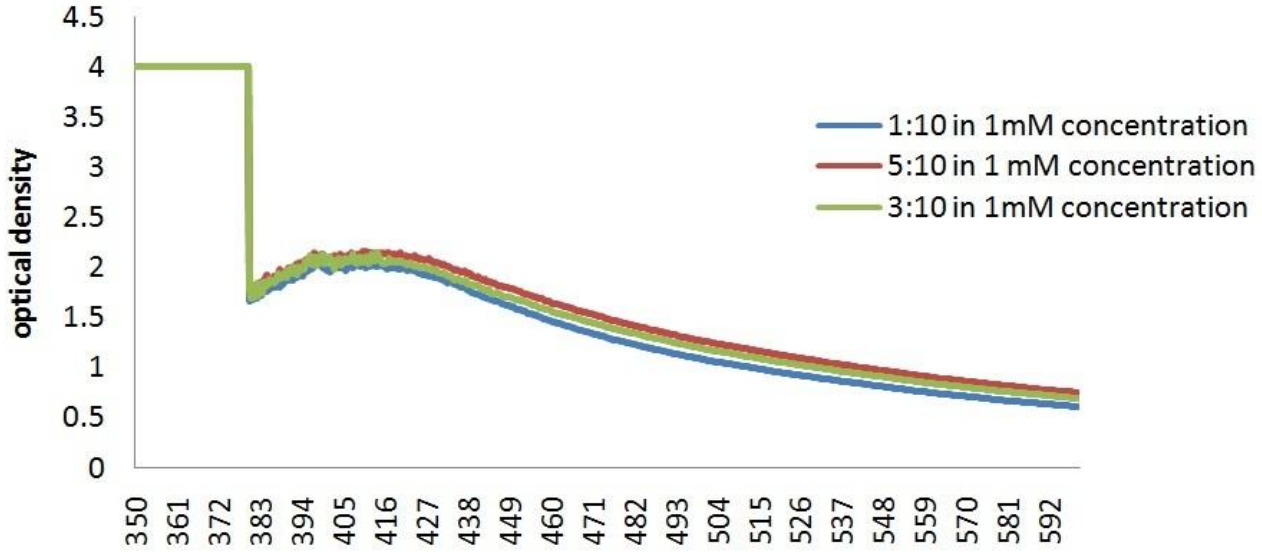

Figure 1: Visual observations and UV–vis absorption spectra of *B.acuminta* (a) at different with varying concentrations of sliver nitrate(mM) [(A) 1.10, (B) 5.10, (C) 3.10

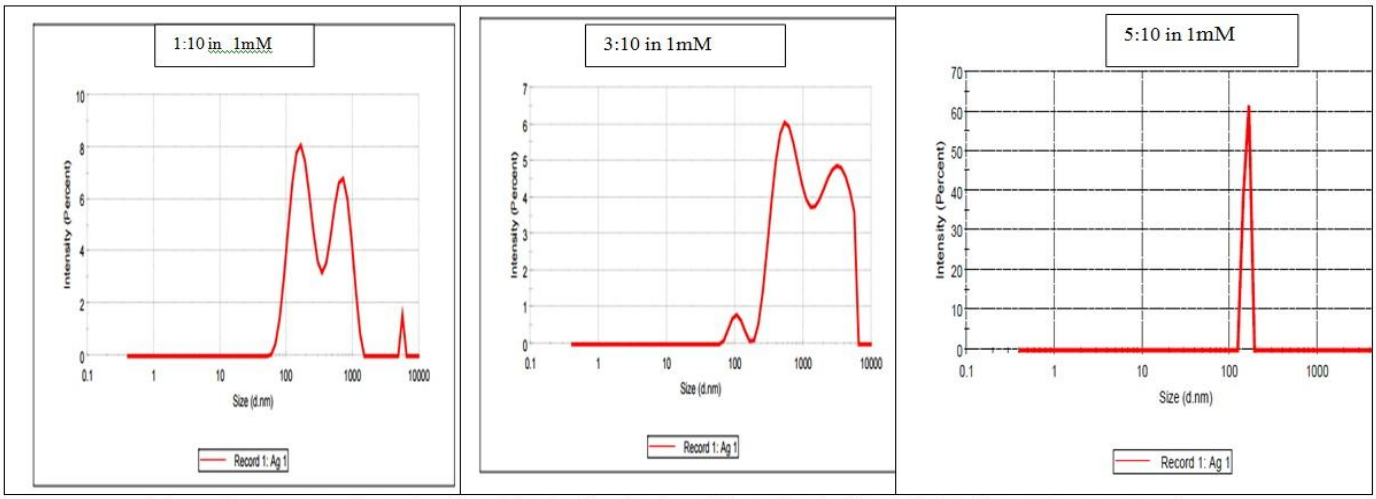

Figure 2: Percentage intensity of particle size distribution of biosynthesized BaAgNPs with varying concentration

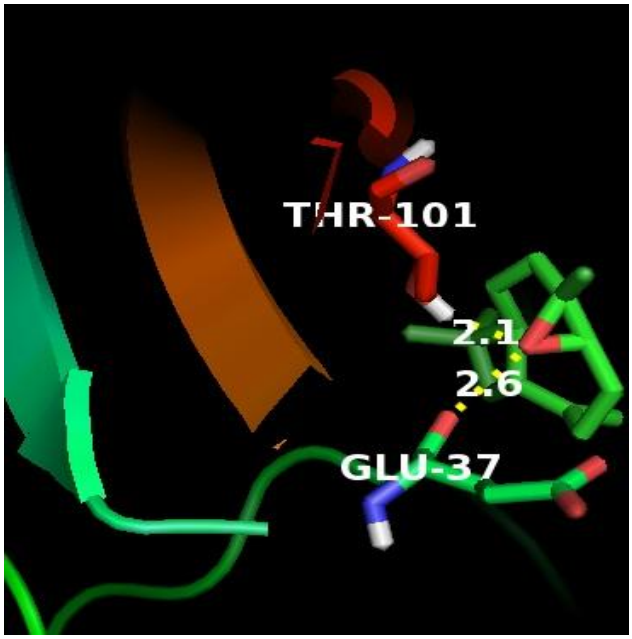

Figure 3: Binding orientations of 5DZL with the ligand Caryophyllene oxide.

Table 1: MIC of test sample against pathogenic bacteria

Test samples	Concentration Range (µg)	MIC (µg)	
		E.coli	*S.aureus*
BaAgNPs	1500-0.576	900	750
Ciproflaxin (std)	1000-0.488	<0.488	<0.488

Table 2: Molecular docking analysis of the ligands against various cancer receptors

Ligands	Parameters	Receptors			
		3OQ9	4OFB	4WP7	5DZL
Caryophyllene oxide	Binding energy kcal/mol	-	-	-	-5.88
	Number of bonds	-	-	-	2
	Interacted Amino acid residues	-	-	-	C:GLU37:OC, C:THR101:OH
	Bond distance Angstrom (Å)	-	-	-	2.6, 2.1
	RMSD (Å)	-	-	-	11.52
	Estimated Inhibition Constant, Ki (µM)	-	-	-	48.77
β- Caryophyllene	Binding energy kcal/mol	-	-	-	-
	Number of bonds	-	-	-	-
	Interacted Amino acid residues	-	-	-	-
	Bond distance Angstrom (Å)	-	-	-	-
	RMSD (Å)	-	-	-	-
	Estimated Inhibition Constant, Ki (µM)	-	-	-	-