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Research Article

Biosynthesis of gold nanoparticles (AuNPs) from *Curculigo orchioides* and study their antimicrobial efficacy

S. Saranya*

Department of Microbiology, Thanthai Hans Roever College, affiliated to Bharathidasan University, Perambalur, Tamil Nadu – 621 212, India.

***Correspondence Info:**
S. Saranya
Department of Microbiology,
Thanthai Hans Roever College,
Affiliated to Bharathidasan University,
Perambalur, Tamil Nadu – 621 212, India
E-mail: saranyasuriya1415@gmail.com

Abstract

In this study, silver nanoparticles were synthesized using the *Curculigo orchioides* plant extract as a reducing agent. The bioactive phytochemicals/ secondary metabolites present in the plant and were responsible for the quick reduction of silver ion (Au^{+}) to metallic silver nanoparticles (Au^{0}). The reduced silver nanoparticles were characterized by UV–vis spectroscopy, Scanning electron microscopy (SEM), Fourier Transform-Infra Red Spectroscopy (FT-IR), Dynamic light scattering (DLS) and Zeta potential analysis. The UV-VIS spectroscopic studies revealed the presence of beard peaks at around 540 nm. SEM studies showed spherical-shaped gold nanoparticles at 90 nm in higher densities. The microbial derived gold nanoparticles were also showing more inhibition activity in both bacterial and fungus strains. In bacteria, gram negative strains are highly affected by the test samples than gram positive. In fungal strains, the highest effect was noticed in *Aspergillus niger* while less effect was observed in *Candida albicans*. The plant materials mediated synthesis of silver nanoparticles have comparatively rapid and less expensive and wide application to antibacterial therapy in modern medicine.
Keywords: *Curculigo orchioides*, Gold nanoparticles, SEM, Bioactive compounds.

1.Introduction

Nano refers to a nanometer (nm), i.e. one nanometer is a millionth of a millimeter or about one eighty thousandth the width of a human hair. At this dimension, materials exhibit novel properties that differ from both the isolated atom and bulk material, and largely depend on size of the particles [1]. It is interdisciplinary involving chemistry, physics, biology, engineering, toxicology, etc. Common nanoparticles that are distributed in nature are such as proteins, enzymes and nano-sized particles present in the atmosphere that are useful for the survival of organisms [2]. Nanobiotechnology is the multidisciplinary integrating biotechnology, nanotechnology, physical methodology and system engineering that is leading into production of biochips, molecular motors, nano crystals, and nanobiomaterials [3].

Synthesis of nanoparticles can be carried out using various deals of efforts that have been put up for the search of methods utilizing the biological systems such as microorganisms and plants for the synthesis of metal nanoparticles [4]. Various microorganisms such as bacteria, fungi, and yeasts have come-up as nano factories that synthesize metal nanoparticles which have attracted interest because of the unique optical, thermal, electrical, chemical, and physical properties [5]. The use of environmentally benign materials like plant leaf extract, bacteria

and fungi for the synthesis of gold nanoparticles offers numerous benefits of eco-friendliness and bio-medical application as they do not use toxic chemicals in the synthesis. Chemical synthetic methods lead to the presence of some toxic chemical species adsorbed on the surface that may have effects in medical applications [6].

Tanaka [7] in 1999 defined as gold nanoparticles have been used for more than 400 years for the treatment of certain illness, and the staining of glass enamels. But nowadays, the preparation of nano-scaled gold materials has become very important due to their unique properties of medicinal usages. Gold (Au) is known as a powerful disinfecting agent for killing unicellular microorganisms and has the strongest antimicrobial effects. Also, it is known to exhibit superb inhibitory effects of algal growth [8]. Presently available gold-based inorganic antimicrobial agents are produced in the forms of gold-supported inorganic powders, gold colloids, metal gold powders [9]. The gold-supported inorganic powders are the most used and thus are representative of a typical inorganic antimicrobial agent.

In the present study, single step synthesis of the gold nanoparticles (AuNPs) is presented by reduction of chloroauric acid ($HAuCl_4$) at room temperature with *C. orchioides* leaf extract. The biological method is not causing any toxic effect and preparation cost is very low. The second important objective is to find the antimicrobial efficacy of *C. orchioides* mediated AuNPs.

2. Materials and methods

2.1 Collection of plant material

The plant *C. orchioides* was collected from Tiruchirappalli District of Tamil Nadu in India during the period of March to May 2015.

2.2 Preparation of leaf extracts

The leaves were collected individually from the plant, washed thoroughly thrice with distilled water; shade dried up to 5 days and ground into fine powder. The fine powder of the plant material was sterilized at 121 ℃ for 15 min and weighed. Sterilized fine powder, 20 g each was taken, mixed with 200 ml of Milli-Q water and kept in boiling water bath at 60 °C for 10 min. The extracts were filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4℃ for further studies to avoid microbial contamination.

2.3 Biosynthesis of nanoparticles

The aqueous solution of gold chloride (1 mM) was mixed with above said filtrate and the flasks were agitated at 37^0C. The saline bottles were tightly covered with aluminum foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations were recorded. Periodically, Aliquots of only those isolates which showed color change from white to purple were subjected to UV–visible absorption spectrophotometry, Fourier Transform Infra-Red (FT-IR) Spectroscopy and SEM studies. Control without gold chloride was also run along with the experimental flasks.

2.4 Characterization of silver nanoparticles

2.4.1 UV-VIS Spectroscopy

A small quantity of biosynthesized nanoparticles was characterized by UV-VIS spectroscopy. After color development, a small aliquot of the solution was absorbed between 200 and 900 nm under UV-VIS spectroscopy.

2.4.2 Fourier Transform-Infra Red (FT-IR) Spectroscopy

The analysis of bio-reducing agent present in each of the extracts was measured by FT-IR. After the reaction, a small aliquot of the concentrated reaction mixture was measured in the transmittance mode at 400 to 4000 cm^{-1}. The spectra of the extracts taken before and after the biosynthesis of nanoparticles were analyzed.

2.4.3 Scanning Electron Microscopy (SEM)

The aqueous solution containing gold nanoparticles was subjected to cooling centrifugation at 6000 rpm for 10 min. Supernatant solution was decanted and the remains present as thin-layer solid material was collected, dried in hot air oven at 60℃ until complete drying and examined under scanning electron microscopy (MODEL JEOL, JSM-5610) at different magnifications (10,000 X and 40,000 X).

2.4.4 Dynamic light scattering (DLS) and Zeta potential analysis

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of gold nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 25 ^{0}C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the

particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs).

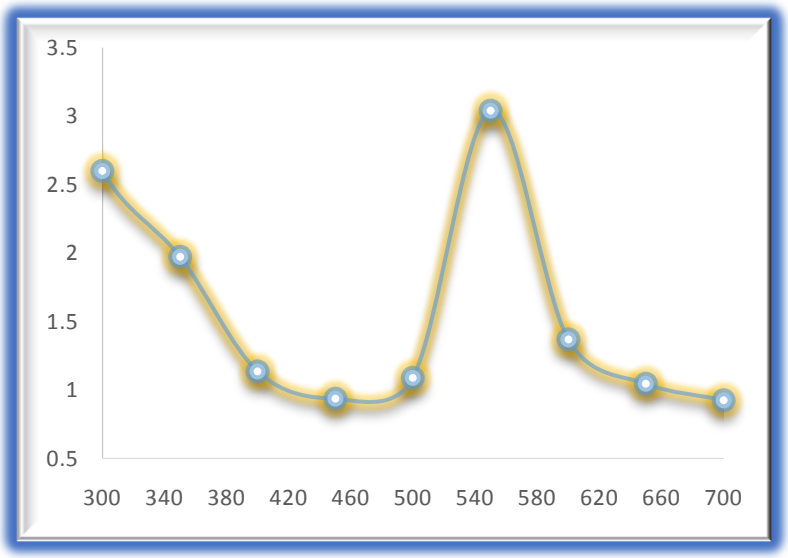
2.5 Assay of antimicrobial activity

The antimicrobials activity of synthesized gold and silver nanoparticles was analyzed against the human pathogens such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 96) (gram positive bacteria), *Klebsiella pneumoniae* (MTCC 432), *Salmonella paratyphi* (MTCC 735) (gram negative bacteria), fungal strains of *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 227), *Microsporum gypseum* (MTCC 2819) and *Trichophyton rubrum* (MTCC 296). These microbes were procured from Microbial Type Culture Collection (MTCC), Chandigarh, and tested against biosynthesized gold nanoparticles following agar well-diffusion method [10-12]. The plant *C. orchioides* derived gold nanoparticles and *Bacillus cereus* derived gold nanoparticles solution was taken in this experiment. The four well-formed to the region of Muller Hinton agar (MHA) plates for bacteria and potato dextrose agar (PDA) plates for fungi [13,14]. Biosynthesized gold nanoparticles were inoculated into the well. Then, the culture plates were incubated at 37 °C for 24 h for bacterial strains and 28^0C for 48-72 h for fungal strain and then observations were recorded after incubation period [15].

3. Result and discussion

Electronic absorption or UV-visible spectroscopy is one of the simplest and yet most useful optical techniques for studying optical and electronic properties of nano materials. This technique is based on the measurement of light absorption by a sample, typically using commercially available spectrometers at reasonable cost. Most spectrometers cover the wavelength range from about 300 nm to 700 nm. The UV-VIS spectroscopic studies revealed the presence of beard peaks at around 540 nm. The plasmon resonance of the gold nanoparticles was recorded. When the precursor chloroauric acid solutions were mixed with the plant extracts/microbial broth they were reduced into gold (Au) nanoparticles (Figure 1). The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions [16].

Figure 1. UV-Vis spectrum of plasmon resonance of gold nanoparticles reduced by leaves in *C. orchioides*



Wavelength	AuNPs
200	3.987
250	3.922
300	2.599
350	1.972
400	1.135
450	0.936
500	1.088
550	3.041
600	1.369
650	1.046
700	0.924
750	0.769
800	0.657

Optical response was recorded under UV-Vis spectroscopy in relation to increase in time duration. The observation of brown and red colors is a characteristic feature for the surface plasmon resonance (SPR) band due to the formation of different sizes of gold nanoparticles in the respective solutions. The transverse plasmon resonance absorption peak appeared at 540 nm is slightly shifted to shorter wavelength along with increase in intensity. The observation of reduction of silver ions present in the aqueous solution of silver complex during reaction with the ingredients of the plant extract may be correlated by the formation of silver nanoparticles in the solution under UV-

Vis spectroscopy. This observation could be attributed to the excitation of surface plasmon vibrations and it has resulted in the formation of silver nanoparticles.

The FTIR result of the plant and plant mediated gold nanoparticle are presented in Figure 2a – 2b. The FTIR spectrum of the crude leaf extract wherein some pronounced absorbances were recorded in the region between 4000 and 400 cm^{-1}. They include 3432 (secondary amine, free, N-H asymmetric stretching), 2830 (alkyl ethers for C-H stretching), 2085 (isothiocyanates, aromatic N=C=S stretching), 1632 (β-dikeone, enolic form, C=O), 1381 (Alkanes, R-CH_3 symmetric bending), 1353 (Deformation bending for and 652 (C-S, R-C-CH_3 stretching for sulphur compounds) cm^{-1}. FTIR spectra of the plant extract with gold chloride solution after 5 hrs such as 3435 (Secondary amine (free) N-H asymmetric stretching), 2829 (Alkyl ethers, C-H stretching), 2728 (Aldehyde, C-H stretching), 2091 (Isothio-cyanates, Aromatic N=C=S stretching), 1631 (β-diketone - enolic form) C=O), 1353 (Deformation bending, R-C-CH_3) and 644 (Sulphur compounds, C-S stretching) cm^{-1}.

Figure 2a: FTIR analysis of vibration modes and function groups of *C. orchioides* plant extract with gold chloride solution

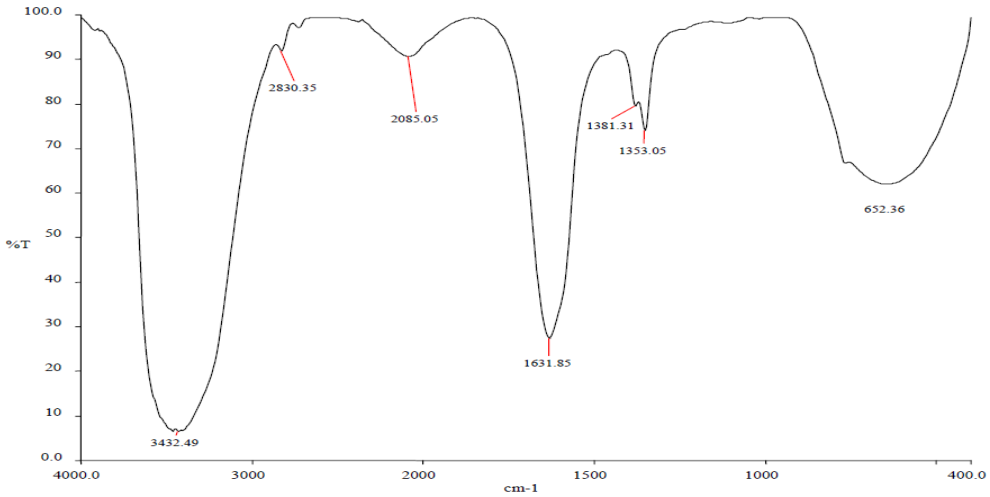
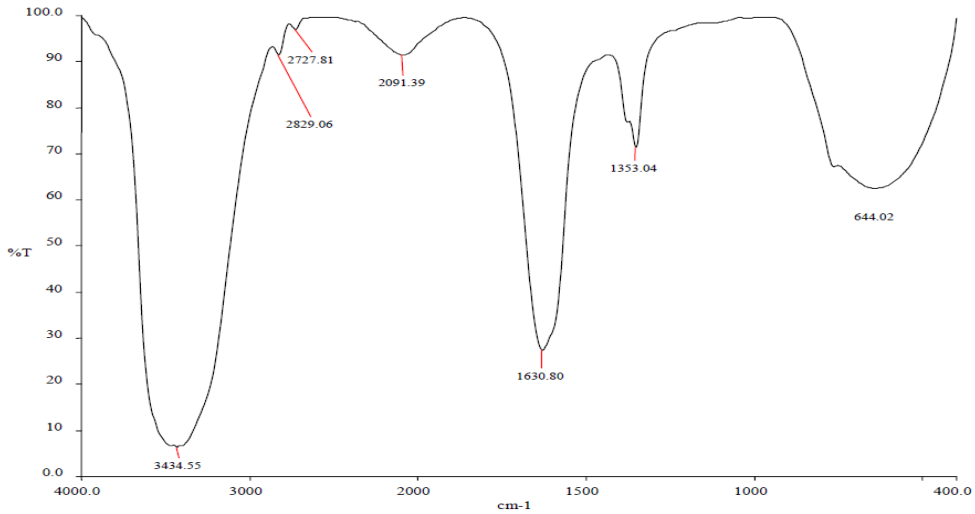


Figure 2b: FTIR analysis of vibration modes and function groups of *C. orchioides*

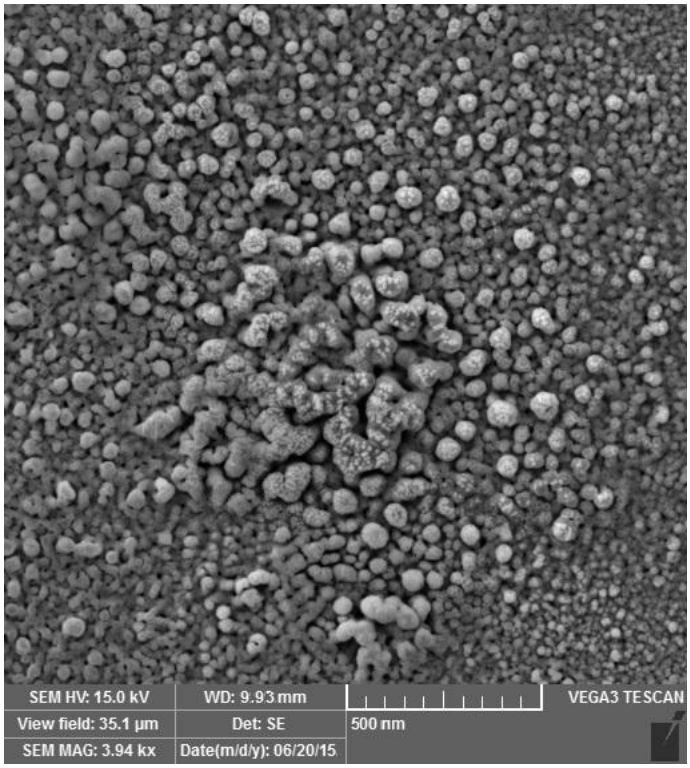


In this spectrum, it is found that disappearance of alkanes at 1381 (Alkanes, R-CH_3 symmetric bending) and appearance of C-H stretching of aldehyde at 2728 (Aldehyde, C-H stretching) and the functional groups were as that of the crude extract. The same solution has polymeric hydroxyl compounds showing O-H stretching at 3400. Aldehyde bond between 2728 and 2730 is present in that solution at 5 h. Only chloroauric acid ($HAuCl_4$) aqueous solution at 5 h has polychlorinated compounds showing C-Cl stretching. The mechanisms involved in the uptake of

metal ions may be intracellular accumulation and surface adsorption. The former one is an active process because the plant must be active to carry out. In the case of surface adsorption, it is a passive process because the chemical groups attached to the cell walls of the plant can bind with metal ions even though when the plant is dead.

SEM absorption of the products was recorded as synthesis of nanoparticles spherical in structure of about 90 nm in diameter in the case of plant derived gold nanoparticles (Figure 3). The energy dispersive spectroscopy is an analysis or chemical characterization of a sample. Leaf extract of *C. orchioides* was promising one for the development of gold nanoparticles. SEM studies showed spherical-shaped gold nanoparticles at 90 nm in higher densities. Gardea-Torresdey and his co-workers [17] in 2008 evaluated *Alfalfa* biomass using SEM micrographs and a corresponding elemental composition of Na, Mg, K, S, Ca, P, Fecdx vacuum (1-270 pa) it allows to observe non-conducting samples without the need to cover them with a thin conductive film, and consequently no evidence of noise by the coating material.

Figure 3: SEM analysis of C. orchioides mediated AuNPs



The extracts were used a control and did not show any antibacterial and antifungal activity. The result reveal that the plant extracts may be used as therapeutically agents in biomedical application. The nanoparticles formed in the plant extracts have high toxicity to the pathogens of *S. epidermidis* (MTCC 435), *B. cereus* (MTCC 430), of gram positive bacteria *S. paratyphi* (MTCC 735) of gram negative bacteria and *Candida albicans* (MTCC 227), *Microsporum gypseum* (MTCC 2819) of fungus strains. The microbial derived gold nanoparticles were also showing more inhibition activity in both bacterial and fungus strains. In *Bacillus cereus, p*lant derived gold nanoparticles zone activity size between 12 – 20 mm. The plant derived gold nanoparticles have higher inhibitory activities than the previous report. In *Staphylococcus aureus, p*lant derived gold nanoparticles zone activity size between 8 – 21 mm.

The gram positive cocci is having less inhibitory effect than gram positive rods. The less concentration gave smaller effect against *S. aureus* while high concentration of the test sample gave good effect. So, concentration depend manner play an important role in the antibacterial activity studies. In *Klebsiella pneumoniae, p*lant derived gold nanoparticles zone activity size between 18 - 20 mm. The Plant derived gold nanoparticles also having more or less equal effect against *Bacillus cereus* effect. In *Salmonella paratyphi* (*A), p*lant derived gold nanoparticles zone activity size between 13 – 18 mm. This is also close similar to *Klebsiella pneumoniae* study. In bacteria, the highest effect was noticed in *Klebsiella pneumoniae* while less effect was observed in *Staphylococcus aureus*. In this study, gram negative strains are highly affected by the test samples than gram positive. It is explained that the gram

positive cell wall is strong than the gram negative and it also indicated that the test samples are not given the more or less equal result within the group. The variations of antimicrobial activity were observed in all the groups.

In *Aspergillus niger*, *p*lant derived gold nanoparticles zone activity size between 23 – 29 mm. Interestingly the fungal strains were highly susceptible to test samples than bacterial strains. *A. niger* is one the best witnessed strain for the above statement. *In Candida albicans*, *p*lant derived gold nanoparticles zone activity size between 12 – 20 mm. The veginal/ buckle cavity normal flora was not highly affected by the test samples. *In Microsporum gypseum*, *p*lant derived gold nanoparticles zone activity size between 17 - 22 mm. The Dermatophytic fungus was less sensitive against test samples than *Aspergillus niger*. *In Trichophyton rubrum*, *p*lant derived gold nanoparticles zone activity size between 20 – 21 mm. It is also one of the dermatophytic fungi and it gave the more or less similar effect against test samples like *M. gypseum*. In fungal strains, the highest effect was noticed in *Aspergillus niger* while less effect was observed in *Candida albicans*. It is indicated that the terrestrial strains were highly susceptible against test samples than the normal flora's and human body associated pathogenic microbes.

4. Conclusion

In the present study reports the simple one step eco-friendly synthesis of AuNPs using *C. orchioides* plant extract. The extract acts as both reducing and stabilizing agent which was confirmed by FTIR studies. The reduction of Au+ ions to AuNPs was examined by certain techniques such as UV-vis spectroscopy, FTIR and SEM studies. The SEM reports revealed that synthesized AuNPs were crystalline in nature with an average particle size of 90 nm. This bioinspired AuNPs were found to be multifunctional with good antibacterial activities. The highlight of biosynthesized AuNPs is to stable for more than 5 months and is also best alternative for both chemical and other physical methods. Hence this method can be employed in large scale production and can be used in many medicinal and technological applications.

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References

[1] Vignesh S, Karthikeyan B, Udayabhaskar R, Arjunan V, Muthukumar K, Ashok M, Narayana Kalkura S, Arthur James R. Antimicrobial activity of biological green synthesized silver nanoparticles. *Asian journal of Physics*. 2014; 23(6): 1025-1030.

[2] Anitha R, Karthikeyan B, Pandiyarajan T, Vignesh S, Arthur James R, Vishwanathan K, Murari BM. Antifungal studies on bio-compatible polymer encapsulated silver nanoparticles. *Int J Nanosci*. 2011; 10:1-5.

[3] Storhoff JJ, Elghanian R, and Mucic RC. One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes. *J Am Chem Soc* 1998; 120:1959-64.

[4] Hussain Beevi M, Vignesh S, Pandiyarajan T, Jegatheesan P, Arthur James R, Giridharan NV and Karthikeyan B. Synthesis and antifungal studies on CuO nanostructures. *Advanced Materials Research*. 2012; 488-489: 666 - 670.

[5] Mocanu A, Cernica I and Tomoaia G. Self-assembly characteristics of gold nanoparticles in the presence of cysteine. *Colloids and Surfaces A: Physicochemical And Engineering Aspects*, 2009; 338:93-101.

[6] Pandiyarajan T, Udaybhaskar R, Vignesh S, Arthur James R, Karthikeyan B. Concentration dependent antimicrobial activities of CuO nanoflakes. *Material science and engineering C*. 2013; 33(4): 2020 – 2024.

[7] Tanaka, K. Nanotechnology towards the 21st century. *Thin solid films.* 1999; 341:120-125.

[8] Gericke, Mariekie, Pinches, Anthony. Microbial Production of Gold Nanoparticles. *Gold bulletin* 2006; 39:22-28.

[9] Sasha Md, Nasir and Hadi Nur. Gold Nanoparticles Embedded on the Surface of Poly Vinyl Alcohol Layer. *Journal of Fundamental Sciences* 2008; 34(4): 234-237.

[10] Vignesh G, Arunachalam S, Vignesh S, Arthur James R. BSA binding and antimicrobial studies of branched polyethyleneimine - copper (II) bipyridine / phenanthroline complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2012; 96: 108 - 116.

[11] Vignesh S, Muthukumar K, James RA. Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. *Marine Pollution Bulletin*. 2012b; 64:790–800.

[12] Lakshmi praba J, Arunachalam S, Riyazuddin R, Divya R, Vignesh S, Akbarsha A and Arthur James R. DNA/ RNA binding and anticancer/ antimicrobial activities of polymer-copper (II) complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013; 109:23 – 31.

[13] Vignesh G, Sugumar K, Arunachalam S, Vignesh S and Arthur James R. A comparative study on the binding of single and double chain surfactant–cobalt (III) complexes with bovine serum albumin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013; 113, 415 – 422.

[14] Vignesh G, Pradeep I, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Biological and protein-binding studies of newly synthesized polymer–cobalt (III) complexes. *Luminescence* 2015. DOI 10.1002/bio.2992.

[15] Vignesh G, Sugumar K, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Studies on the synthesis, characterization, human serum albumin binding and biological activity of single chain surfactant–cobalt(III) complexes. *Luminescence* 2015. DOI 10.1002/bio.2991.

[16] Wiley, B.J., Im, S.H., Mc Lellan, J., Seikkinin, A., Xia, Y. Maneuvering the surface Plasmon resonance of silver nanostructures through shape-controlled synthesis. *J of phys. chem*. B. 2006; 110:15666-15666.

[17] Gardea-Torresdey, J.L., Gomez, E., Peractavedea, J.R., Parsons, J.G., Troiani, H. and Jose-Yacaman, M. Alfalfa sprouts: A Natural source for the synthesis of silver Nanoparticles. *Langmuir*. 2003; 19:1357-1361.