



SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES USING SEED EXTRACT OF *TRICHOSANTHES CUCUMERINA* (SNAKE GOURD) AND ITS ANTIBACTERIAL AND PHOTOCATALYTIC ACTIVITY

Akshaya Kruthika J S¹, Shiny John Vairamon^{1*}

^{1,1*}Department of Chemistry, Stella Maris College (Autonomous), Chennai -600086, India

ABSTRACT

This study involves green synthesis of silver nanoparticles using 10mM AgNO₃ solution and the seed extract of *Trichosanthes cucumerina* (Snake gourd) as the reducing agent. The synthesis and characterisation were confirmed by UV-Vis spectroscopy, Fourier Transform Infra-Red spectroscopy (FTIR), Particle size analysis, SEM and X-ray Diffraction (XRD). This disc diffusion method was used to confirm the antibacterial activity. The aqueous medium containing silver nanoparticles synthesised using *Trichosanthes cucumerina* seed extract showed a peak at 450nm; FTIR analysis confirmed the role of reducing and capping agent. XRD analysis confirmed the crystallite size around 18nm for the silver nanoparticles using the seed extract of the snake gourd (SESG). Photocatalytic activity of the synthesised AgNP was studied using Congo red dye. It was found that the synthesised AgNP is a photocatalyst. The silver nanoparticles have shown antibacterial activity against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*.

Keywords: Antibacterial activity, Photocatalytic activity, Silver Nanoparticle, *Trichosanthes cucumerina*.

1. INTRODUCTION

The field of nanotechnology is one of the most active areas in modern materials science. Nanoparticles exhibit completely new or improved properties based on the specific characteristics such as size, distribution and morphology [1]. Silver nanoparticles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics. The silver metal has a great toxicity against wide range of microorganisms. Silver nanoparticles are found to be effective as anti-inflammatory, anti-angiogenesis, antiviral, anti-platelet activity and against cancer cells [2]. Green synthesis of AgNP (Silver Nanoparticle) using plant extracts containing phytochemical agents has attracted considerable interest [3]. The formation of silver nanoparticles via green synthesis is also studied by using *Phyllanthus amarus*,



Alternanthera sessilis, *Azardicainidca*, *Catharanthus roseus*[4].

The present study focuses on *Trichosanthes cucumerina* (Snake gourd) it belongs to the family *Cucurbitaceae*.

It is useful to maintain healthy heart and liver. It also counteracts respiratory problems, acidity, cancer and worms.

It provides relief from arthritis, promotes hair growth, boost immunity and promotes weight loss.

Trichosanthes cucumerina, the fruit of which is mainly consumed as vegetable as it has nutritional value. The dried seeds are used for its anthelmintic and antidiarrheal properties. Seeds have antibacterial, anti-spasmodic and insecticidal

properties[5]. Therefore a number of chemical, physical and biological approaches are available for the synthesis of silver nanoparticles. Biosynthesis is considered better than chemical and physical synthesis because the use of expensive and toxic chemicals is eliminated and it is a clean, eco-friendly method.

The present study focuses on the green synthesis of AgNPs using a cost effective, commonly and abundantly available vegetables *Trichosanthes cucumerina* (Snake gourd). The study aims to characterise the synthesised silver nanoparticles using UV-VIS, FT-IR, Particle Size Analysis, SEM and XRD. This study also focuses on the photocatalytic activity and antibacterial study of the synthesised silver nanoparticles against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*.

II. EXPERIMENTAL

Silver nitrate was obtained from ThermoFischer Scientific, India Pvt. Ltd, Mumbai. Seeds and vegetable of Snake gourd weighing 25g each were used to make aqueous extract. 10mM of 90mL silver nitrate solution was prepared. The silver nanoparticles (AgNP) was synthesized using 10 mL of Snake gourd vegetable extract and seed extract (AgNP- SESG) respectively for reduction of silver nitrate into elemental silver. The primary detection of synthesised silver nanoparticles was carried out in the reaction mixture by observing the colour change from colourless to dark brown[1].

1.1 CHARACTERISATION TECHNIQUES 2.1.1 UV-Visible spectroscopic analysis

The bioreduction of 10mM silver nitrate to silver nanoparticle using SESG was analysed after a time interval of 5 hours for the absorbance by the UV-Vis against double distilled water as blank. UV-Visible spectral analysis was done by using JASCO V-750 UV-VIS Spectrometer[4].

1.1.2 FTIR analysis

To remove any free biomass residue or capping ligand of the nanoparticles, the residual solution of 100mL after the reaction was centrifuged at 10,000 rpm for 10 minutes. The resulting suspension was dispersed in 10 mL sterile distilled water. The centrifuging and dispersion was repeated three times. The purified suspensions was dried in a vacuum desiccator. The FTIR Analyser (BRUKER ALPHA-T) was used to analyse the dried silver nanoparticles. [4]

1.1.3 Particle size analysis

This technique was used to study the size distribution of nanoparticles. The particle size of the residual solutions were studied using 2 mL of each of the solution fed into the Particle size analyser (MALVERN MODEL).

1.1.4 SEManalysis

The morphological characterisation of the samples were done using SEM-JOEL MODEL (JSM-6390 LV).The extract of reduced silver nanoparticle was dried and drop coated onto carbon tape and performed on SEM(JOEL MODEL JSM 6390LV). In this analysis an electron beam is focused into affine probe and subsequently scanned Over a small rectangular area. As the beam interacted with the sample it created various sign also that were detected [4].After the reduction process, the silverna no particles were separated by centrifuging the solution at 10,000 rpm for 15mins. Silver nanoparticles were purified by repeated centrifuging for three times. It was dried in a vacuum desiccator. The dried powder was used to identify the shape and morphology of thenanoparticles.

1.1.5 XRDanalysis

Thesilvernanoparticleswerepurifiedbyrepeatedcentrifugationofabovesynthesisedbrownsuspensionat10,000 rpm for 10 minutes followed by drying in a vacuum desiccator. The dried nanoparticles were analysed by using BRUKER D8 advanced powder X-ray diffractometer operation at a voltage of 40Kv and the intensity of the diffractedx-raysismeasuredasafunctionofthediffractionangle 2θ .Thecrystallinedomainsizewascalculated from the width of the XRD peaks, using Schererformula:

$$D = 0.9\lambda/\beta\cos\theta(1)$$

Where D is the average crystalline domain size perpendicular to the reflecting plane, λ is the X-ray wavelength, β is the full width half maximum (FWHM) and θ is the diffraction angle [6].

2.2 APPLICATION 2.2.1Antibacterialstudy

Antibacterial activity of the sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton agar (MHA) medium was poured in to the petriplate. After the medium was solidified, theinoculumswerespreadonthesolidplates withsterileswabmoistenedwiththebacterialsuspension.The disc were placed in MHA plates and added 20 μ L of the sample (Concentration: 1000 μ g, 750 μ g, 500 μ g) and 20 μ l of the standard (S) ampicillin (1mg/mL) onto the disc. The plates were incubated at 37 $^{\circ}$ C for 24 hrs. The antimicrobial activity was determined by measuring the diameter of zone of inhibition[7].

2.2.2 Preliminary study on photocatalytic activity insunlight

The photocatalytic activities of the materials were studied by examining the decolourisation reaction of Congo red (CR) in sunlight. ThesynthesisedAgNP from SESG was dispersed in 10mL of 10ppm CR dye. The photo decolourisation studies of these solutions were analysed by placing it in sunlight for one hour, samples were withdrawn at various time intervals, centrifuged and quantitative determination of CR was performed by measuring its absorption using UV-Visible spectrophotometer (MODEL 2373).



2.2.2.1 Study on photocatalytic activity using Photoreactor

The photolysis of CR was carried out in the UV photoreactor. 100 mL capacity Quartz tubes were used as reaction cell, the reaction solution was illuminated by a 8W mercury vapour lamp emitting 365 nm wavelength. Aerator along with the connecting tubes, were introduced into reaction system for effective mixing of the samples.

The aqueous solution of 10 ppm CR over 10 mg AgNP obtained using SESG were analysed in the dark for 30 minutes to ensure the adsorption equilibrium. The concentration of the dye in the reaction system was monitored spectrophotometrically by measuring the adsorption intensity at the wavelength range of 400-800 nm with a calibration curve. Samples of about 3 mL were withdrawn at specific time intervals of dark reaction and of illumination and the absorbance of resulting solution were measured. The following parameters were analysed [8].

a) Percentage Adsorption

% Adsorption were calculated using the formula: $((C_{\text{blank}} - C_{\text{dark}}) / C_{\text{blank}}) \times 100$ (2)

C_{blank} = initial concentration

C_{dark} = concentration after adsorption equilibrium

b) Percentage Decolourization

% Decolourisation were calculated using the formula: $((C_0 - C_t) / C_0) \times 100$ (3)

Where, C_0 = initial concentration

C_t = final concentration at time 't'

III. RESULTS AND DISCUSSION

1.2 UV-Visible spectroscopic analysis

UV-Visible spectral analysis was done by using JASCO V-750 UV-VIS Spectrometer. The bio reduction of Ag^+ ions on the solution was monitored. The synthesised silver nanoparticle using the seed extract of snake gourd had an absorption band at λ_{max} 450 nm (Fig. 1), broadening of the peak indicated the particles were polydispersed [4].

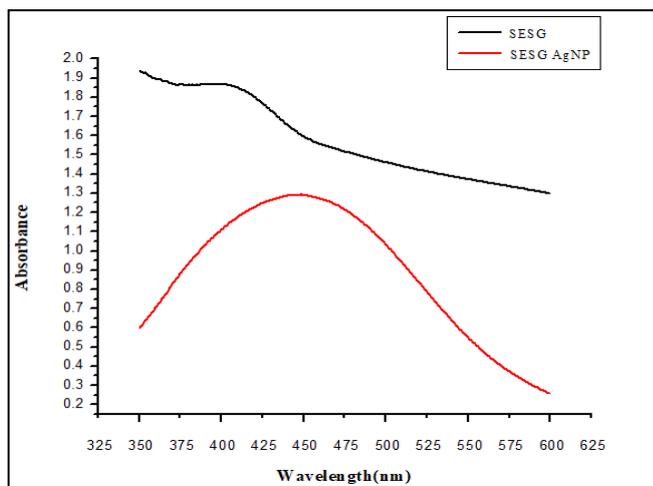


Fig 1: UV-Visible spectrum of SESG and AgNP- SESG

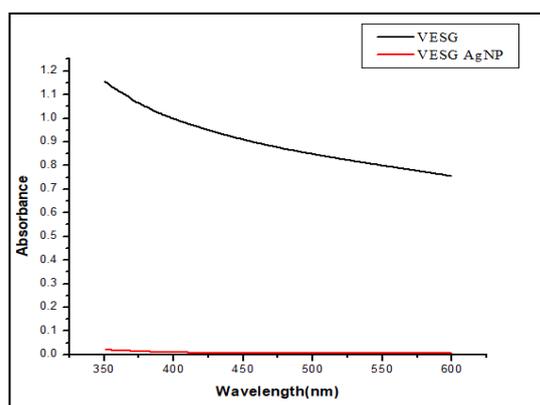


Fig 2: The synthesised silver nanoparticles using the snake gourd vegetable extract.

Hence standardisation has to be done for vegetable of Snake Gourd as it did not show evidence for formation of AgNP in this concentration. The present study thus focuses on green biosynthesis of AgNP using only the seed extract of snake gourd.

The band gap of SESG can be calculated using Plank's formula:

$$E = hc / \lambda_{\max} \quad (4)$$

Where, E is the energy band gap, 'h' is plank's constant ($6.626 \times 10^{-34} \text{Js}$), 'c' is the velocity of light ($3 \times 10^8 \text{ m/s}$) and $1\text{J} = 6.242 \times 10^{18} \text{ eV}$. The energy band gap for the SGS from the solid UV-Vis spectrum was found to be 2.75 eV.

The UV-Vis spectra of the AgNP of the vegetable extract of the Snake Gourd is shown in Fig.2. The synthesised silver nanoparticle using the vegetable extract of snake gourd showed no characteristic absorption band which might be due to the absence of reducing agent in the vegetable.

1.3 FTIR Spectroscopic analysis

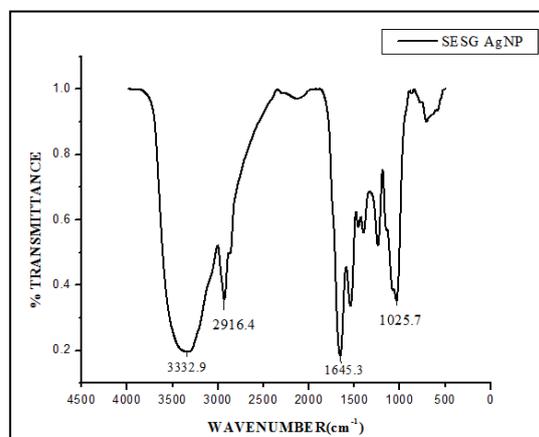


Fig 3: FTIR Spectrum of AgNP-SESG

Silver nanoparticles synthesised from the seeds of snake gourd shows strong absorption bands at 3332, 2916, 1645, 1025 cm^{-1} (Fig.3). The absorption peak at 3332 cm^{-1} is assigned to O-H stretch of alcohols and phenolic compounds. The absorption band at 1645 cm^{-1} may be due to amide bonds of proteins arising from carbonyl stretching in proteins and 1025 cm^{-1} can be assigned to the C-N stretching vibrations [4]. These FT-IR spectrum values showed the bonding of Silver nanoparticles with some groups in the compound from the vegetable extract.

These compounds may cap the silver nanoparticles and form a layer on the surface on them which could result in the stabilising of NPs in aqueous medium. The FTIR spectrum confirmed the presence of amine and proteins be strongly attached to the metal particles and the role of protein is to prevent the agglomeration and thereby stabilise the nanoparticles. These indicate the function of biological compound has performed a dual function of formation and stabilisation of metal nanoparticles in an aqueous medium [9][10].

1.4 Particle size analysis

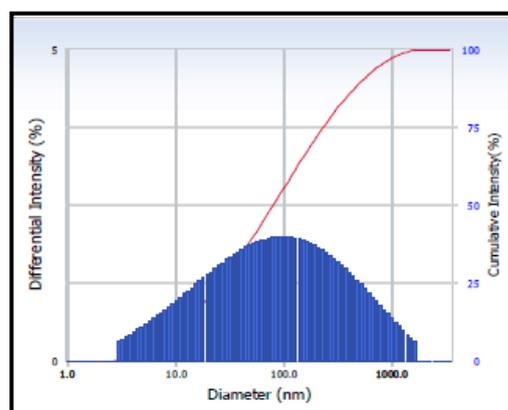


Fig 4: Particle size analyser spectrum of AgNP-SESG

The particle size of AgNP ranged between 5nm-1010nm with the mean size of 204.2nm by using seed extract of snake gourd (Fig.4). The average size is higher than XRD and this can be due to agglomeration on long standing in the liquid phase.

1.5 SEManalysis

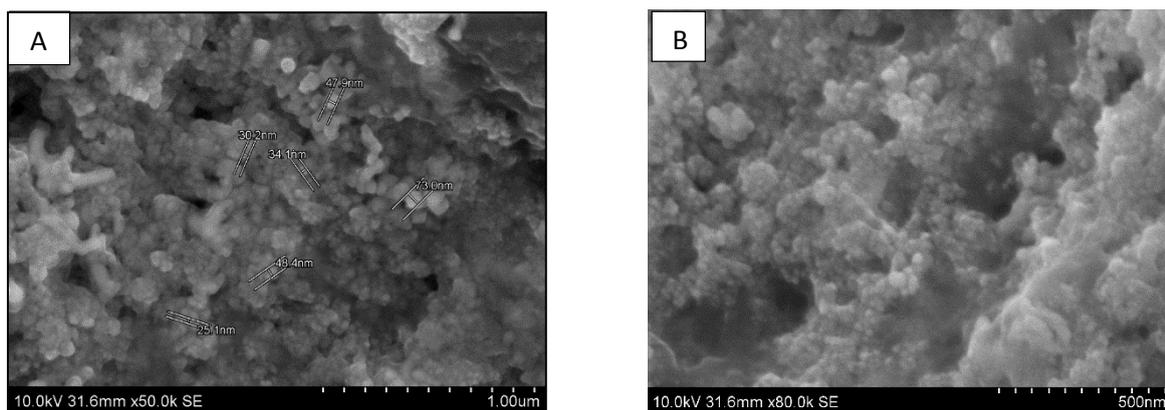


Fig 5: SEM morphology of AgNP-SESG

The SEM pictures shows relatively spherical shaped nanoparticles formed with the diameters that ranged between

25.1 nm to 73.0 nm for AgNPsynthesised using seed extract of snake gourd (Fig.5).

3.5 XRDAnalysis

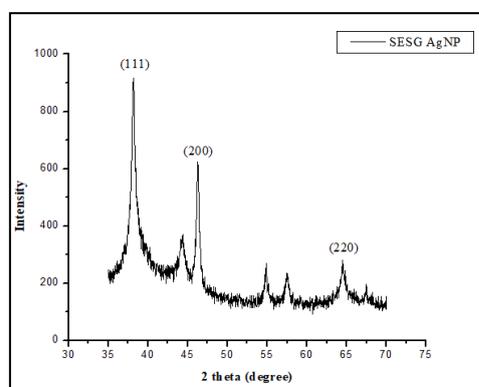


Fig 6: XRD spectra of AgNP-SESG

Three distinct diffraction peaks at 38.16°, 44.29° and 64.51° were obtained for the AgNPsynthesised from seed

extract of snake gourd (Fig.6). These were indexed with the planes (111), (200) and (220) for the face centered cubic silver as per the JCPDS card No:40-0783. The crystallite size calculated from XRD analysis were found to be 16.98 nm, 15.77 nm and 19.94 nm for the synthesised AgNP using seed extract of snake gourd.

3.6 ANTIBACTERIAL STUDIES

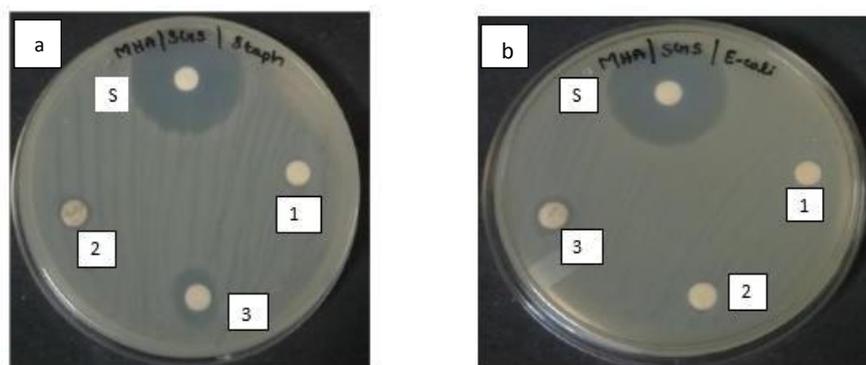


Fig 7: Antibacterial activity of the synthesised SESG silver nanoparticles at three different concentration (500µg/ml, 750µg/ml, 1000 µg/ml) against a) *S aureus* b) *E coli*

[S- Ampicillin (20µl/disc); 1) 500 µg/ml of SGS; 2) 750 µg/ml of SGS; 3) 1000µg/ml of SGS]

The maximum zone of inhibition was formed against gram-positive bacteria *Staphylococcus aureus* when loaded with 1000µg of silver nanoparticles synthesised using seed extract of snake gourd (Fig.7). The small size of AgNP provides better contact and interaction with bacterial cell. The action of AgNP on the bacteria may be due to its attachment on the surface of the bacterial cell membrane by interacting with thiol group found in the respiratory enzymes of bacterial cells. This may inhibit the respiratory process in bacteria resulting in the bacterial cell death.

AgNP may also prevent DNA from replication and the cells from reproduction [7]. Thus the synthesised AgNP using SESG shows antibacterial activity.

3.7 PHOTOCATALYTIC ACTIVITY

Photodecolourisation of Congo red over 10 mg of AgNP-SESG was studied with 10 ppm concentration of the dye. The preliminary study of the photodecolourisation of the dye by AgNP-SESG in presence of sunlight showed a decrease in the intensity of absorption at λ_{max} 497 nm with time. Hence further preliminary study on the photocatalytic activity of AgNP-SESG was carried out using UV photoreactor.

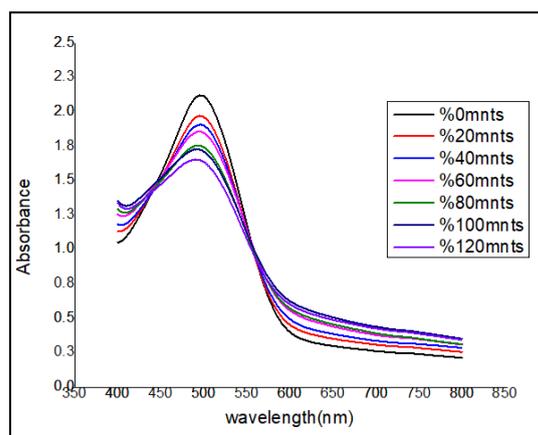


Fig 8: Photocatalytic degradation of Congo red using AgNP-SESG

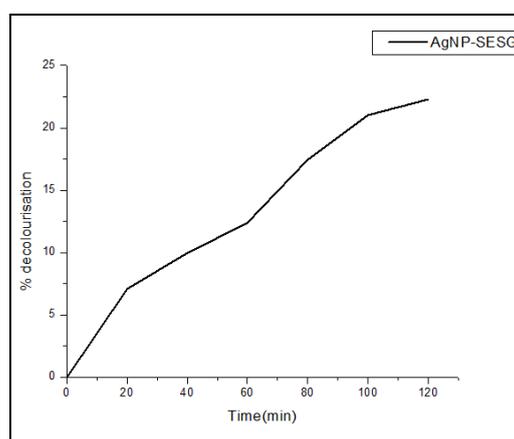


Fig 9: % Decolourisation of Congo red over AgNP-SESG

The aqueous solution of 10ppm CR over 10mg AgNP-SESG also showed a decrease in the absorption intensity from 2.11 to 1.64 at λ_{max} 497 nm using a UV photoreactor when monitored over the wavelength range of 400-800 nm and time period from 0 min to 120 mins (Fig.8). The % decolourisation of CR at 120 mins was found to 22.3 % (Fig.9). Thus, the present study shows new finding that the synthesised silver nanoparticle using the seed extract of snake gourd exhibits photocatalytic activity as it decolourised the Congo red dye.

IV.CONCLUSION

The present study helped to develop a fast, eco-friendly, and convenient green method for the synthesis of silver nanoparticles from silver nitrate using seed extract of snake gourd (AgNP-SESG) at ambient temperature. Colour changes occurred due to the Surface Plasmon Resonance because of the reduction of Ag^+ ions to Ag^0 by the biomolecules present in the extract resulting in the formation of silver nanoparticles. The green synthesis of the silver nanoparticles was confirmed by UV-Vis, FT-IR, Particle size analysis, XRD analysis and SEM. UV-Vis



spectra confirmed the presence of elemental silver nanoparticles shown by λ_{\max} at 450 nm. FTIR showed characteristic absorption peaks at 3332, 2916, 1645, 1025 cm^{-1} . FT-IR spectroscopic study indicated the carbonyl group of amino acid residues in seed extract had strong ability to bind with silver and serving as capping and stabilizing agent in the formation of silver nanoparticles. The particle size analysis, SEM and XRD confirmed the formation of silver nanoparticles. The particle size analysis showed the average mean size of 204.2 nm for silver nanoparticle using seed extract of snake gourd due to agglomeration. The crystallite size from XRD analysis were found to be 16.98 nm, 15.77 nm, 19.94 nm and the face centered cubic structure was confirmed. The SEM analysis showed the morphology of the silver nanoparticles to be spherical and the size ranging from 23.1 nm to 73.0 nm. The antibacterial activity of silver nanoparticles by green synthesis was evaluated against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*. It was found that AgNP exhibited greater antibacterial activity against gram positive *Staphylococcus aureus* at the concentration 1000 μg with 12 mm zone of inhibition. Photocatalytic activity of the as synthesised AgNP-SESG was studied using Congo red dye. It was found that AgNP-SESG acts as a photocatalyst and can be further used for treatment of waste water from industries. Thus, the silver nanoparticles obtained by the green synthesis from seed extract of snake gourd (SESG) had exhibited both bactericidal activity and photocatalytic activity which could be used for further research to study therapeutic and environmental applications.

V. ACKNOWLEDGEMENT

We thank Dr. Sr. Jasinthia Quadras, The Principal; The Department of Chemistry & CRIST, Stella Maris College (Autonomous), Chennai for all the support and facilities provided. We extend our thanks to Life Tech Research Centre, Chennai; NIT, Calicut; St. Joseph College, Trichy and SAIF, IIT Madras for the support in characterisation studies.

REFERENCES,

- [1] D. Jain, H.K. Daima, S. Kachhwaha, S.L. Kothari, Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities, *Digital Journal of Nanomaterial and Biostructures*, 4(3), 2009, 557-563.
- [2] S. Yasin, L. Liu, J. Yao, Biosynthesis of Silver Nanoparticles by Bamboo Leaves Extract and Their Antimicrobial Activity, *Journal of Fiber Bioengineering and Informatics*, 6(1), 2013, 77-84.
- [3] R.R. Bhosale, A.S. Kulkarni, Gilda, N.H. Aloorika, R.A. Osmani, B.R. Harkare, Innovative eco-friendly approaches for green synthesis of silver nanoparticles, *International Journal of Pharmaceutical Science and Technology*, 7(1), 2017, 2328-2335.
- [4] V. Aravindhan, C.S. Kumar, H.L.J. Kumari, K. Ruckmani, Characterisation studies of sunlight induced biosynthesis of silver nanoparticles using *Solanum melongena* (Eggplant), *Journal of Advanced Pharmaceutical Technology*, 2014, 50-57.
- [5] S. Sandhya, K.R. Vinodh, S.J. Chandrar, R. Aradhana, S.N. Vamshi, An updated review on *Trichosanthes cucumerina*, *International Journal of Pharmaceutical Science*, 1(2), 2010, 56-60.

- [6] B. K. Mehta, M. Chhajlani, B. D. Shrivastava, Green synthesis of silver nanoparticles and their characterisation by XRD, *Journal of Physics*, 836(1), 2017,012050-012054.
- [7] M. Smekalova, V. Aragon, A. Panacek, R. Pucek, R. Zboril, L. Kvitek, Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens, *The Veterinary Journal*, 209, 2016,174-179.
- [8] H.Jian,K.Yong,Wang,Rui,Xia,S.Shu,D.Ying,PhotodegradationofazodyeCongoredfromaqueous solution by the WO_3-TiO_2 / activated carbon (AC) photocatalyst under the UV irradiation, *Material Journal of Chemical Physics*, 115(1), 2009,303-308.
- [9] S. Li, Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, Q. Zhang, Green synthesis of silver nanoparticles from *Capsicum annum* leaf extract, *Journal of Green Chemistry*, 9, 2007,852-858.
- [10] S.S.Shankar,A.Ahmed,M.Sastry,GeraniumLeafAssistedBiosynthesisofsilvern nanoparticles, *Journal of Biotechnology*,19(6), 2003, 1629 -1631.