

# Bioreduction of silver nanoparticles characterizations and their antimicrobial activity

Sarmad Moin<sup>1</sup>, A. Gowthami<sup>1</sup>, T. Uma Sankari<sup>2</sup> and A. Vinoth Kanna<sup>1\*</sup>

<sup>1</sup>Department of Biotechnology, Thanthai Hans Roever College (Autonomous), Perambalur – 621 212, Tamil Nadu, India.

<sup>2</sup>Department of Molecular Biology, School of Life Sciences, Madurai Kamaraj University, Palkalai Nagar, Madurai - 625 021, Tamil Nadu, India.

QR Code



## \*Correspondence Info:

A. Vinoth Kanna  
Department of Biotechnology,  
Thanthai Hans Roever College,  
Affiliated to Bharathidasan University,  
Perambalur – 621 212, Tamil Nadu, India

## \*Article History:

Received: 05/01/2017

Accepted: 21/01/2017

DOI: <https://dx.doi.org/10.7439/ijasr.v3i1.3889>

## Abstract

In this investigation we report the bioreduction of silver nitrate to silver nanoparticles using the leaves aqueous extract of *Alangium lamarckii*. The silver nanoparticles were characterized by UV–vis spectrophotometer, scanning electron microscope (SEM), DLS-Size and zeta potential analysis showed that the synthesized silver nanoparticles are varied from 60 - 70 nm and have the spherical shape. Further the prospect of protein as a stabilizing material in silver nanoparticles is shown by FTIR analysis and the XRD examination confirms monocrystalline phase of silver with FCC crystal structure. The antimicrobial activity of Ag nanoparticles was investigated against some pathogens. In these assessments, silver nanoparticles (AgNP) can stop microbial growth and even kill microbes, from now confirmed their antimicrobial importance.

**Keywords:** Bioreduction, *Alangium lamarckii*, silver nanoparticles, Antimicrobial activity.

## 1. Introduction

The use of silver is known since ancient times and already 3000 BC man were able to separate silver from lead. The electrical and thermal conductivity of silver is the highest of all metals, and it possesses the lowest contact resistance, which leads to its use as electrical contacts. Alloys of silver, like sterling silver, are widely used in jewellery and other silver ware [1]. Also silver salts are of high interest. Silver iodide for example is used in seeding clouds to produce rain [2]. Silver nitrate was extensively used in photography but its importance was reduced by upcoming of digital photography. In former times silver was widely used for coinage by many countries in the world. But this ended when the value of the coins gets greater than their exchange value. Bulk silver is not considered to be toxic but most silver salts show certain toxicity [3]. Already Hippocrates of Kos knew about the bioactivity of silver. Because of this biological activity silver was used for treatment of wounds as wound dressing until development of modern antibiotics in the 1940s.

The amount of nano- and microparticles can be so big, that it can be seen by satellites. The ashes produced in forest fire or an eruption of a volcano is also a source of nano- and microparticles [4]. These particles can reach the upper troposphere and can be found all over the world during years. Organisms like viruses and some bacteria also show sizes of a few nanometers up to a few hundred nanometers, which makes them part of the nanomaterial, which can be found in the air. But of course they are not nanoparticles. Not only outdoor nano- and microparticles can be found but especially indoor. Here the air can be ten times more polluted than outdoor, according to the US Environmental Protecting Agency [5]. In principal, nanoparticles can be found is a wide range of applications in biotechnology. In the field of diagnosis, they are used for labelling, sensing and imaging [6]. Therefore, antimicrobial silver is now used extensively to combat organisms in wounds and burns. It works because pathogens cannot mutate to avoid the antimicrobial effect of silver [3,7]. The importance of silver ions has been also found in the

treatment of burn wound by various researchers who studied the antimicrobial properties of silver nanoparticles against virulent pathogens [8]. The effect of the nanoparticles was found to be significantly more pronounced on MDR strains. For these reason, we investigated the *Alangium lamarckii* derived silver nanoparticles against for some human pathogens for their antimicrobial properties.

## 2. Materials and Methods

### 2.1 Collection of Plant Material

The plant materials, *Alangium lamarckii* have collected from Kolli kills, Namakal District of Tamil Nadu in India during the period of January to February 2016.

### 2.2 Preparation of Plant Aqueous Extraction

The plant materials were collected individually, washed thoroughly thrice with distilled water, shade-dried up to 5 days and prepared fine powder by grinding. The fine powder of the plant material was sterilized at 121°C 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°C for further studies to avoid microbial contamination.

### 2.3 Biosynthesis of nanoparticles

For the synthesis of silver nanoparticles, silver nitrate prepared at the concentration of  $10^{-3}$  M with pre-sterilized Milli Q water was used respectively. A quantity of 1.5 ml of each extract was mixed with 30 ml of  $10^{-3}$  M of silver nitrate for the synthesis of silver nano particles. Silver nitrate has taken in similar quantities of 1.5 ml each without adding plant extracts to main respective controls. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of silver ions, incubated at room temperature under dark condition and observations were recorded at 15, 30, 60, 120 mins.

### 2.4 Characterization of Nanoparticles

#### 2.4.1 UV-VIS Spectroscopy

After the prescribed incubation, the solutions were characterized on the basis of spectroscopic and microscopic analyses. The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behavior of Ag nanoparticles. The scanning range for the samples was 200-800 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "Origin 6.5".

#### 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  $\text{cm}^{-1}$ . The spectra of the extracts taken after the biosynthesis of nanoparticles were analyzed.

#### 2.4.3 Dynamic Light Scattering Particle size analyser

A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie-scattering theory. In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor [Vibronics, model: VPLP1]. Then experiment was carried out in computer controlled particle size analyzer [ZETA Sizers Nanoseries (Malvern Instruments Nano ZS)] to find out the particles size distribution.

#### 2.4.4 Dynamic Light Scattering Zeta Potential Measurement

Zeta potential describes the electrical potential in the double layer of ions surrounding a particle at the boundary of the particle surface and the adsorbed ions in the diffuse layer (Ives, 1956; Henderson, 2008). Zeta potentials were determined with a Zetaphoremeter IV (CAD, France), which subjects a sample to an applied electric field and uses digital image analyzing software to measure the resulting particle travel distances as electrophoretic mobility (EM). The software uses EM to calculate the zeta potential according to the Smoluchowski equation, which is valid when  $\kappa a \gg 1$ , as is the case with most microalgae (Henderson, 2008). If necessary, samples were diluted with filtered media to obtain an analyzed particle number of 30-200. A minimum of three replicate measurements were performed for each sample.  $EM = \frac{\epsilon \zeta}{\mu}$ ;

$\epsilon$  = permittivity;  $\mu$  = viscosity;  $\zeta$  = zeta potential

#### 2.4.5 Scanning electron microscope (SEM)

In this research work, Jeol JSM-6480 LV SEM machine were used to characterize mean particle size, morphology of nanoparticles. The freeze dried sample of Ag NPs solution was sonicated with distilled water, small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDX normally reveals the presence of phases.

## 2.5 Antimicrobial activity of *Alangium lamarckii* derived silver nanoparticles

### 2.5.1 Testing of antimicrobial activity

Microbial strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus sp.* MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). tested for antimicrobial sensitivity using the disc diffusion method [9]. The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. This method was used to evaluate in vitro antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the standardized bacterial suspension (test culture suspensions prepared in sterile 0.85% saline matching an optical density of 0.5 McFarland standards corresponding to 108 CFU/ml) on surface of agar Figure rotating the Figure every 60° to ensure homogeneous growth. The 15 and 30 µL of test solutions were poured in each disc, separately. One separate disc was used for control study by taking sterile triple distilled water (without test sample). The Figures were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler/HiAntibiotic ZoneScale-C. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

## 3. Results and Discussion

### 3.1 Biosynthesized nanoparticles

The present study was carried to analyze the *Alangium lamarckii* mediated Silver (Ag) nanoparticles was synthesized, characterized and their application also studied Biological systems have a unique ability to be self-organized and synthesize molecules that have highly selective properties. These properties make them a prospective tool that can be used to synthesize nanoscale sensors and Nano devices [10]. Many biological systems are able to create an interface with these materials to use them.

### 3.2 Metal-phyto Interaction

During silver nanoparticles synthesis, the change of colour from pale green to brownish colour suggested the formation of silver nanoparticles [11]. The tubes were observed periodically for change in colour from green to different shades of brown by silver nanoparticles, it was found that aqueous silver ions when exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver nanoparticles [12].

### 3.3 UV- Vis spectrum analysis

Biosynthesized AgNPs particles were confirmed by analyzing the excitation due to the applied electromagnetic field of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 420 nm and the peak was observed between 380-480 nm. Figure 1 show the UV absorption peaks of *Alangium lamarckii*. It clearly indicating the formation of spherical AgNPs through the plant extract. The change in colour is due to the excitation of surface plasmon vibration, which is indicated by the formation of silver nanoparticles at different time intervals. During each time interval, the peak became distinct and rising. This peak rising clearly denoted the increasing nanoparticles synthesis as the time increases. Similarly, the colour also became intensified as the time increases. The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions [12]. Optical response was recorded under UV-Vis spectroscopy in relation to increase in time duration [14]. The observation of brown and red colours is a characteristic feature for the surface plasmon resonance (SPR) band due to the formation of different sizes of silver nanoparticles in the respective solutions [15]. 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 [16]. This observation could be attributed to the excitation of surface plasmon vibrations and it has resulted in the formation of silver nanoparticles. Koperuncholan [17] reported formation of silver nanoparticles when constant aqueous AgNO<sub>3</sub> at 50 ml, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Cinnamomum camphora*.

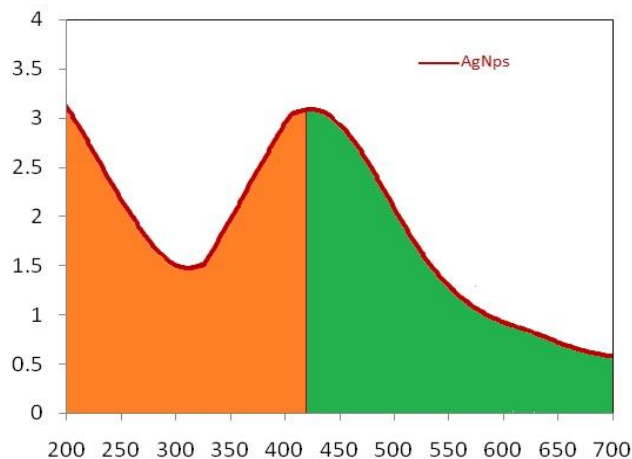


Figure 1: UV characterization of AgNPs

### 3.4 Scanning Electron Microscopy (SEM)

SEM absorption of the products was recorded as synthesis of nanoparticles spherical in structure of about 70 nm in diameter in the case of silver nanoparticles (Figure 5). The energy dispersive spectroscopy is an analysis or chemical characterization of a sample. The *Alangium lamarckii* is a promising one for the development of silver nanoparticles. SEM studies showed spherical-shaped silver nanoparticles at 70 nm (Figure 2) in higher densities. The SEM image showing silver nanoparticles synthesized using *Alangium lamarckii* extract confirmed the development of silver nanostructures.

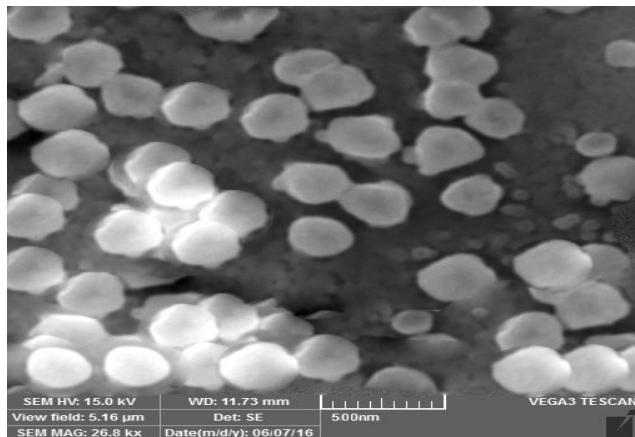


Figure 2: SEM characterization of AgNPs

### 3.5 Energy Dispersive Spectroscopy (EDS)

EDS revealed the presence of pure silver (Figure. 3) nanoparticles in higher percentages. Silver peak is higher than other peak. The EDX reading proved that the required phase of silver (Ag) is present in the sample. This is probably due to the presence of substrate over which the NP sample was held during SEM microscopy. Alfalfa biomass using SEM micrographs and a corresponding elemental composition of Na, Mg, K, S, Ca, P, Fe, Co, Mn, Cu, Mo, B, Cl as well as traces of Zn, Si, Ni and Pb analysis by EDS. They reported the highest being Ca, Mg and S with 63.5%, 11.9% and 10.72% respectively and the absence of Fe although it is found in very low proportions. As EDS equipment works at low 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 [18, 19].

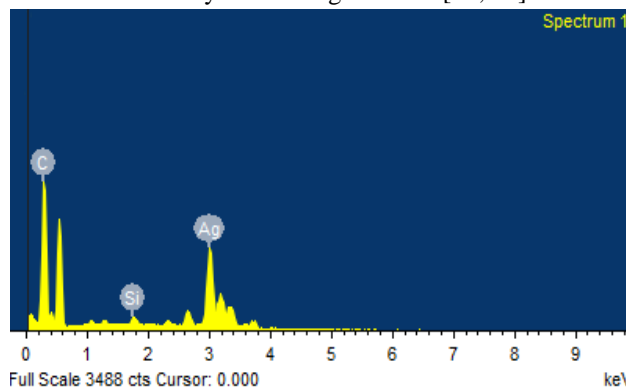


Figure 3: EDAX characterization of AgNPs.

### 3.6 Dynamic Light Scattering (DLS) analyzer

#### 3.6.1 Particle size study

Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly disparity index of particles in a colloidal suspension. Figure 4 show the particle size of the nanoparticles samples. After analyzing data, it was found that Ag nanoparticles size were in the range of 50-100nm. The highest fraction of AgNPs present in the solution was of 55 nm. From the plot it was evident that the solution was consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis [20].

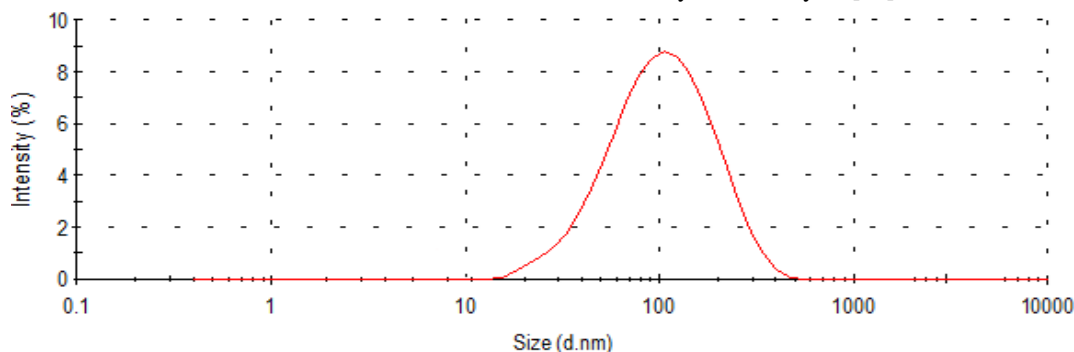


Figure 4: DLS Size distribution characterization of AgNPs.



### 3.6.2 Zeta potential study

Zeta potential measures the potential stability of the particles in the colloidal suspension. Silver nanoparticles generally carry a negative charge. The synthesized silver nanoparticles from the plant showed

negative charge and were stable at room temperature. DLS-zeta potential showed negative charge (-32.65) which indicated that the sample is moderately stable at room temperature. (Figure. 5)

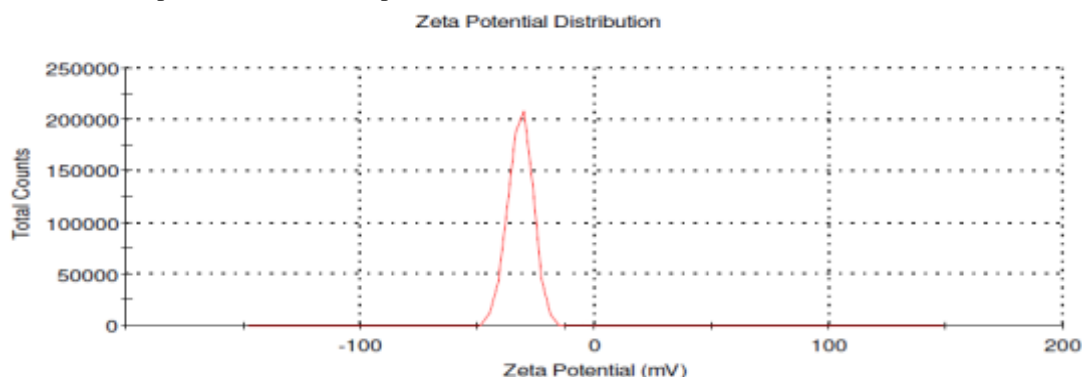


Figure 5: DLS Zeta potential characterization of AgNPs.

### 3.6.3 Fourier Transform Infra-Red Spectroscopy

FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic AgNo<sub>3</sub> to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the Biofabrication of silver nanoparticles mediated by the plant extracts. (Figure 6) *Alangium lamarckii* petal extract, in AgNo<sub>3</sub> peaks were observed at recorded in the region between 4000 and 400 cm<sup>-1</sup>. They

include 3306 cm<sup>-1</sup>, 2124 cm<sup>-1</sup>, 1637 cm<sup>-1</sup> which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the Biofabrication of the nanoparticles by the action of the protein or phytochemicals. Figure 6 clearly illustrates the biofabrication of the AgNPs by the action of the phytochemicals such as phenols, flavonoids and alkaloids in *Alangium lamarckii*.

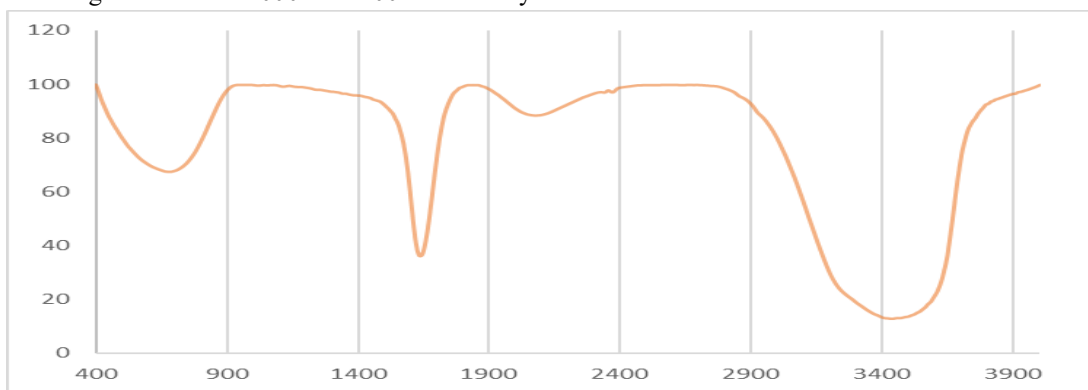


Figure 6. FTIR characterization of plant broth and AgNPs.

### 3.7 Antimicrobial screening

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities are summarized. The two tested concentrations such as 15 and 30 μL /disc produce zone of inhibition on MHA and PDA Figures for bacteria and fungi, respectively. In the present study, higher (30 μL/disc) concentration of sample got greater sensitivity than (15 μL/disc) lower concentration in all the tested microorganisms. Koperuncholan co-workers (2010) stated that the solvent extraction of plant was affected the

bacterial strains in the higher concentration such as 2.5 and 5.0 mg/well. But in this study, we conformed that the low concentrations (15 and 30 μL/disc) of the *A. lamarckii* derived AgNPs were highly affect the microbial growth. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus sp.* MTCC 7076 (F2) (Figure 7).

All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6 (Figure 7). There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. The spherical shaped silver nanoparticles having size in range of 16–28 nm were achieved using this extract with antibacterial property

observed by Kirby-Bauer method against multi-drug resistant bacteria such as *Streptococcus pyogens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [21]. A stable and spherical shaped silver nanoparticle was synthesized using extract of *Abutilon indicum*. These nanoparticles show high antimicrobial activities against *S. typhi*, *E. coli*, *S. aureus* and *B. subtilis* microorganisms [22].

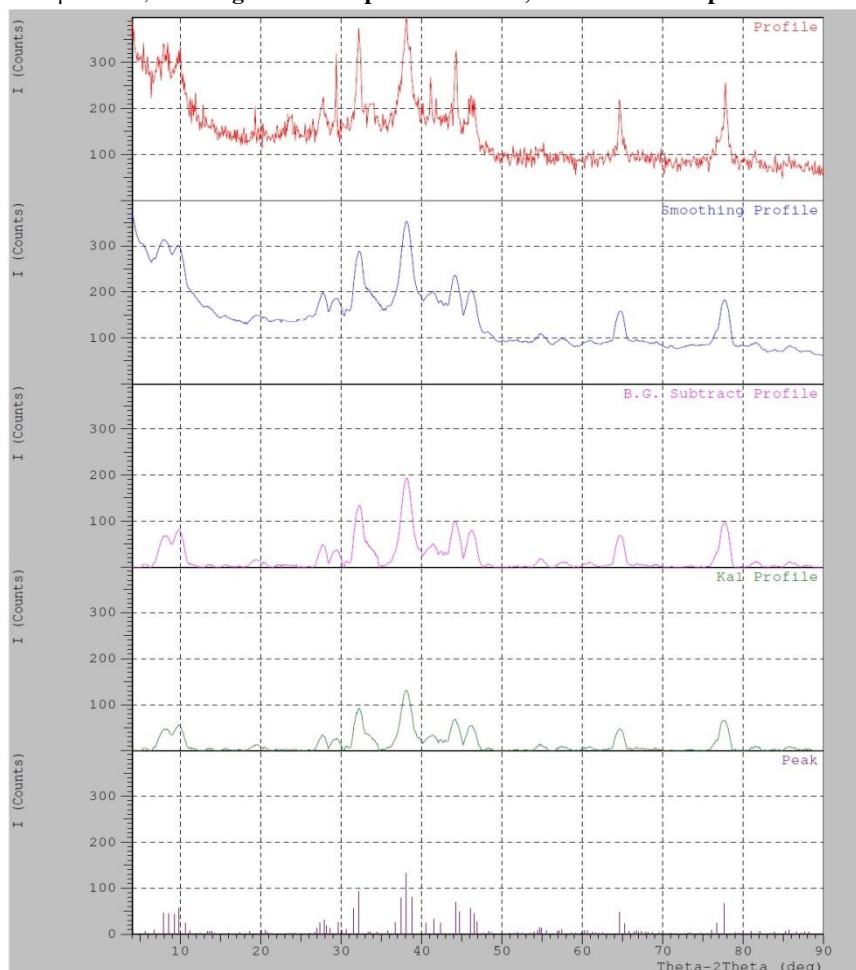
**Table 1: Antimicrobial screening**

S. No	Test Microorganisms		Zone of inhibition (mm)				Diseases	Route of Transmission
			Sample (15 & 30) µL / disc	15µL	30µL	PC		
<b>Bacteria</b>								
1.	<i>Aeromonas liquefaciens</i>	B1	7	9	14	< PC	Wound Infections / Gastroenteritis	Water / Food
2.	<i>Enterococcus fecalis</i>	B2	10	13	8	> PC	Endocarditis / Bladder, Prostate	Water / Food
3.	<i>Klebsiella pneumonia</i>	B3	9	14	28	< PC	Acute diarrhea / Dysentery	Water / Food
4.	<i>Micrococcus luteus</i>	B4	7	10	38	< PC	Skin & Pulmonary infections	Soil / Dust / Water
5.	<i>Salmonella typhimurium</i>	B5	13	14	0	> PC	Typhoid	Water / Food
6.	<i>Vibrio cholerae</i>	B6	8	11	16	< PC	Cholera	Water / Food
<b>Fungi</b>								
7.	<i>Candida albicans</i>	F1	10	11	10	> PC	Skin (Integument) Infections /	Airways / Wound /
8.	<i>Cryptococcus sp.</i>	F2	9	13	9	> PC	Cryptococcal disease /	Airways / Wound /
9.	<i>Microsporium canis</i>	F3	8	12	9	> PC	Tinea capitis / Ringworm	Airways / Wound /
10.	<i>Trichophyton rubrum</i>	F4	8	14	7	> PC	Tinea corporis / Tinea cruris / Tinea	Airways / Wound /

The result indicate mean value of the result (3 replicates)

PC -Positive Control (Using antibiotic disc; **Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)**)

Samples - 15 µL / disc & 30 µL / disc; > PC – greater than positive control; < PC – less than positive control



**Figure 7: XRD characterization of AgNPs**

## 4. Conclusion

The prospective benefits of nanotechnology in biomedical applications have become widely accepted and are the most promising sector for the generation of new applications in medicine. From the present study even at very small concentration (in µg/ml) *Alangium lamarckii* derived AgNPs possess very good antimicrobial activity which makes them a potent source of antimicrobial agent against some human pathogens. Due to the structural difference in the composition of the cell walls of Gram-positive and Gram-negative AgNPs have significantly less effect on the growth of Gram-positive bacteria. Also, green synthesis of AgNPs can potentially eliminate the problem of chemical agents that may have adverse effects, thus making nanoparticles more compatible with the eco-friendly approach. Moreover, the synthesized AgNPs enhance the therapeutic efficacy and strengthen the medicinal values of *A. lamarckii*.

## Acknowledgement

The authors thank the Biospark Biotechnological Research Center (BBRC), Tiruchirapalli, Tamil Nadu, India for characterizations of nanoparticles.

## Reference

- [1] Ahmed John S and Koperuncholan M. Antibacterial Activities of various solvent extracts from *Impatiens balsamina*. *International Journal of Pharma and Bio Sciences*, 2012; 3:401-406.
- [2] Beevi, M.H., Vignesh, S, Pandiyarajan, T, Jegatheesan, P, Arthur James, R, Giridharan, N.V., Karthikeyan, B. Synthesis and antifungal studies on CuO nanostructures. *Advanced Materials Research*. 2012; 488-489, 666 - 670.
- [3] Fazal Mohamed MI, Arunadevi S, Koperuncholan M and Seeni Mubarak M. Synthesis and antimicrobial activity of some naphthyl ether derivatives, *Der Chemica Sinica*, 2011; 2 (2):52-57.
- [4] Sinthiya A and Koperuncholan M. *In-silico* characterization for Multiple sclerosis: A special emphasis on Tetrakis (4-aminopyridine-kN1) dichloridocopper (II) monohydrate with sphingosine 1-phosphate lyase, *Crystal Research*, 2015; 89: 36824-36826.
- [5] Ramesh V, Ahmed John S and Koperuncholan M. Impact of cement industries dust on selective green plants: A case study in Ariyalur industrial zone, *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 2014; 4:152-158.
- [6] Koperuncholan M, Sathish Kumar P, Sathiyarayanan G, Vivek G. Phytochemical Screening and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. *International Journal of Medicobiological Research*, 2010; 1: 48-59.
- [7] 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.
- [8] Ahmed John S and Koperuncholan M. Direct Root Regeneration and Indirect Organogenesis in *Silybum marianum* and Preliminary Phytochemical, Antibacterial Studies of Its Callus. *The International Journal of Pharmaceutics*; 2012; 2: 52-57.
- [9] Koperuncholan M and Ahmed John S. Antimicrobial and Phytochemical Screening in *Myristica dactyloides* Gaertn. *Journal of Pharmacy Research*; 2011; 4: 398-400.
- [10] Koperuncholan M and Ahmed John S. Biosynthesis of Silver and Gold Nanoparticles and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. *IJPI'S Journal of Pharmacognosy and Herbal Formulations*, 2011; 1(5):10-15.
- [11] Pandiyarajan T, Udaybaskar 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.
- [12] Muthukumar K, Vignesh S, Dahms HU, Gokul MS, Palanichamy S, Subramanian G, Arthur James R. Antifouling assesments on biogenic nanoparticles: A filed study from polluted offshore platform. *Marine Pollution Bulletin* 2015. <http://dx.doi.org/10.1016/j.mar.bul.2015.08.033>.
- [13] Koperuncholan M and Manogaran M.. Edible plant mediated biosynthesis of silver and gold nanoflakes against human pathogens, *World Journal of Pharmaceutical Research*, 2015; 4(1):1757-1775.
- [14] 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.
- [15] 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.
- [16] 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.
- [17] Koperuncholan M. Bioreduction of chloroauric acid (HAuCl<sub>4</sub>) for the synthesis of gold nanoparticles (GNPs): A special empathies of pharmacological activity, *International Journal of Phytopharmacy*, 2015; 5 (4):72-80.
- [18] 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.
- [19] 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.
- [20] Vignesh S, Karthikeyan B, Udayabhaskar R, Arjunan V, Muthukumar K, Ashok M, Narayana Kalkura S, Arthur James R. 2014. Antimicrobial activity of biological green synthesized silver nanoparticles. *Asian Journal of Physics* 2014; 23(6): 1025-1030.
- [21] Vignesh S, Muthukumar K, James RA. 2012b. Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. *Marine Pollution Bulletin* 2012; 64:790–800.
- [22] Vignesh S, Raja A, Arthur James R. Marine Drugs: Implication and Future Studies. *International Journal of Pharmacology* 2011; 7: 22 – 30.