Green synthesis, Characterization, Antibacterial and Antifungal activity of Cassia Anceolata leaves extract mediated Silver Nanoparticles

K. Sowmiya, J. Thomas Joseph Prakash*

PG and Research Department of Physics, Government Arts College
(Affiliated to Bharathidasan University)
Tiruchirappalli-620 022, Tamil Nadu, India.

***Abstract*: Green synthesis of silver nanoparticles (CA-AgNPs) was achieved by reducing the bio availability of silver nitrate using Cassia anceolata leaf aqueous extract. The formation of CA-AgNPs was confirmed by UV-Vis spectroscopy. The synthesized silver nanoparticles were monitored by recording the surface plasmon resonance peak observed at 425nm. Functional groups of biomarkers in the extracts and their interaction with AgNPs were identified by FTIR analysis. FESEM and DLS analysis were used to confirm the mean particle size of CA-AgNPs at 31 nm, 35 nm and 155.3 nm, respectively. XRD and EDX analysis confirmed the nature and presence of silver. Combined CA-AgNPs showed significant antimicrobial (Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus) and mildew (Candida albicans and Candida vulgaris) activity. The method developed for the synthesis of silver nanoparticles using Cassia anceolata leaf extract is an eco-friendly and convenient method. In the future, integrated CA-AgNPs may be used in the biomedicine, biosensor and nanotechnology fields.**

***Keywords*: Antibacterial Activity; Antifungal Activity; Cassia anceolata; Silver nanoparticles.**

I. Introduction

In recent years, numerous approaches using chemicals or electrochemistry have been investigated for the preparation of silver nanoparticles (AgNPs). However, most of the techniques have difficulties during the refining phase as the used chemicals or the byproducts created are hazardous and require more energy to manufacture [1, 2]. Numerous materials are used in the preparation of nanoparticles such as metal oxide ceramics, metals, and silicate and non-oxide ceramics. Currently, the most widely used methods for the production of nanoparticles are reduction of chemical or light chemistry and electrochemistry [3]. Controlling the size / shape of nanoparticles and achieving monodispersity are common challenges faced by researchers [4]. To solve problems, eco-friendly approaches have been developed by applying biological principles such as microorganisms or plant extracts in the synthesis process [4]. Other methods used for the assembly of nanomaterials, such as Au, Ag, Pt and Pd, use hardness template [5], bacteria [4, 6], fungi [7, 8] and plants [9–13]. The main advantages of using plant materials for the synthesis of nanoparticles are that they do not require a complex process. Joint purification steps and maintenance of microbial cell cultures [14]. Most of the reported environmental harmless methods have various issues such as stability, growth of crystals and aggregation of particles. Silver products have been studied for centuries for the prevention and treatment of many diseases, especially infections caused by the strong inhibitory and bactericidal effects of silver [15]. Biodegradable silver nanoparticles have been identified to exhibit antimicrobial [16, 9], anti-inflammatory effect [17], antiviral activities [18], anti-angiogenesis [19] and antioxidant and platelet resistance activities[20]. Researchers used Cassia anziolata leaf extract at room temperature overnight to synthesize the AgNPs and reported their characterization. Two studies demonstrated the efficacy of AGNPs in the reduction of Cassia anceolataand capping agents. Cassia anceolatais a plant of the genus Cassia of the Fabaceae family. Cassia anceolatais used for irritable bowel syndrome, hemorrhoids and weight loss. The current system is eco-friendly, single-step, cost-effective and nontoxic to human health. The characterization of synthesized silver nanoparticles was performed using SEM, TEM, FTIR, DLS and XRD imaging.

II. MATERIAL and METHODS

Plant Material Source

Plant material leaves of Cassia anceolata (figure 1) were collected from Trichy, Tamilnadu.

Figure.1 Cassia anceolata Leaf

Preparation of plant extract

Fresh leaves of Cassia anceolata were gathered from Trichy, India. Silver nitrate was obtained from Sigma-Aldrich. Cassia anceolata leaves were washed with tap water to remove dirt, then washed with double distilled water and dried in the dark to remove moisture completely. 10 g of dried leaves grained in Mortar is added to 100 ml of double distilled water and boiled for 5 minutes at 60 ° C. After cooling, the plant extract was filtered done Whatman's Number 1 filter paper and the liquid was stored at 4 ° C for the synthesis of silver nanoparticles (AgNPs) (Figure. 2).

Biosynthesis of AgNPs

Bioenergetics of AgNPs, 10 mL of liquid extract was added to 100 mL of 5 mL of fluid$AgNO_3$ solution. The mixture was boiled for 10 min at 95 ° C. The reaction of silver nanoparticles was observed by a change in the color of the mixture during the temperature treatment. A control group was also developed without leaf extract. The solution of AgNPs was purified by repeated centrifugation at 10000 rpm for 20 min 4 °c. The resulting hole was suspended in distilled double distilled water and dried using Archive. The reduction of silver nanoparticles was observed by the change in color Reaction compound during temperature treatment [15].

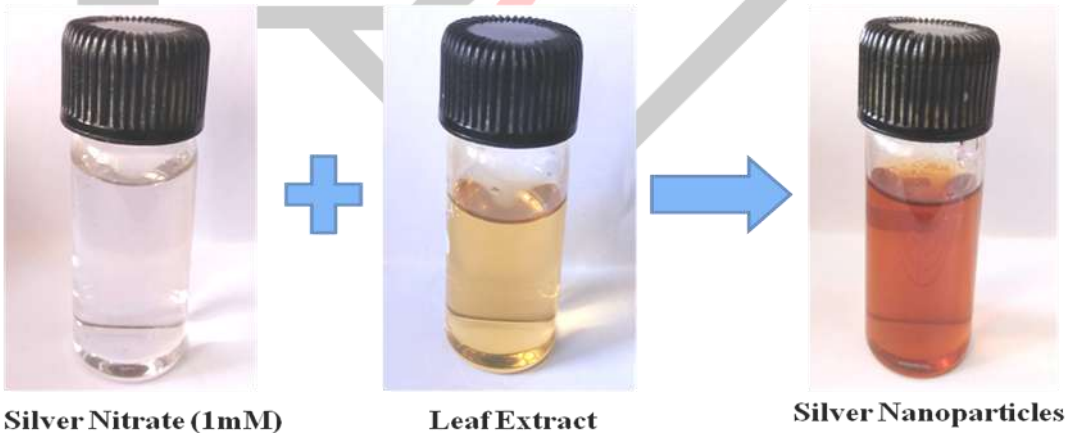


Figure.2 Color changed at brown to pale yellow

III Characterization

UV-Vis Spectra Analysis

Absorption spectrum of AgNPs and Plant Extract aqueous solutions were stored with UV-Vis Spectrophotometer (Perkin Elmer) in the range of 0–900nm at room temperature. Analysis Quartz was carried out with quartz. The reaction mixture was monitored spectroscopically from 0 to 150 min every 30 min. There was distilled water used as a blank solution.

FTIR Analysis

The presence of plant element on the surface of AgNPs was determined by Fourier transform infrared spectroscopy (FT-IR). Potassium bromide atoms were prepared for Plant Extract and AgNPs. This analysis helps determine which bonds are responsible for the synthesis and stability of silver nanoparticles. The biogeochemistry of NNPs was demonstrated by a comparative study of FTIR (Perkin Elmer 400 FTIR) spectroscopy in the range of 4000–500 cm^{-1}.

PXRD Analysis
X-ray diffraction (XRD) analysis was performed to study the crystal structure AgNPs. The XRD method was recorded using an embryon X-ray diffractometer.

DLS Analysis
Dynamic light scattering was used to determine the size distribution and average size of the synthesized Ag NPs

FESEM Analysis
The morphology of AgNPs was performed by scanning electron microscopy (FESEM) FEI QUANTA-250 FEG with a working voltage of 25 kV. For FESEM images, 2 drops of enrichment the aqueous solutions of AgNPs were placed on a carbon tape coated stub and allowed to dry overnight. The stubs were coated with gold using a sputter coater to create vivid images.

EDAX Analysis
Energy Dispersive X-Ray analysis (EDX) was used for the determination of the composition by BRUKER instrument.

Anti bacterial Activity

To examine the antibacterial activity of CA silver nanoparticles, one gram positive bacterial strains Staphylococcus aureus (MTCC 96). two gram negative bacterial strains Escherichia coli (MTCC 433) and Pseudomonas aeruginosa (MTCC 1688) were prepared as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

Antifungal Activity

Culture Media
The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.
Inoculums
The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^{5} CFU/ml.
Fungal strains used
The clinical fungal test organisms used for study are Candida albicans (*MTCC*-3498) and Candida vulgaris (MTCC 227), were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

IV RESULT AND DISCUSSION

UV-vis spectrum Analysis
The use of a UV-vis spectrophotometer induces localized surface plasmon vibrations in the metal, causing an electric field to resonate at a certain wavelength, causing strong beam dispersion at that wavelength. Assessment of spectrophotometric measurements at this level; Color or color calibration estimation is performed at different wave lengths. Surface plasmon resonance was induced by reducing the Ag + ion in the AgNPs complex by extracting the horse chestnut leaf and, consequently, quantifying the UV-Vis spectrophotometer. ccording to UV-Vis measurements, the formation of AgNPs in the 386 nm wavelength range is expected. Aggregation of acNPs in a liquid solution was observed by measuring the absorption spectrum in the 300–1100 nm (Fig. 3) wavelength range. The solution of silver nitrate became dark brown with the juice of the leaves. In the UV-vis spectrum; The single, strong and wide surface plasmon resonance peak was observed at 464 nm, which confirmed the synthesis of AgNPs.

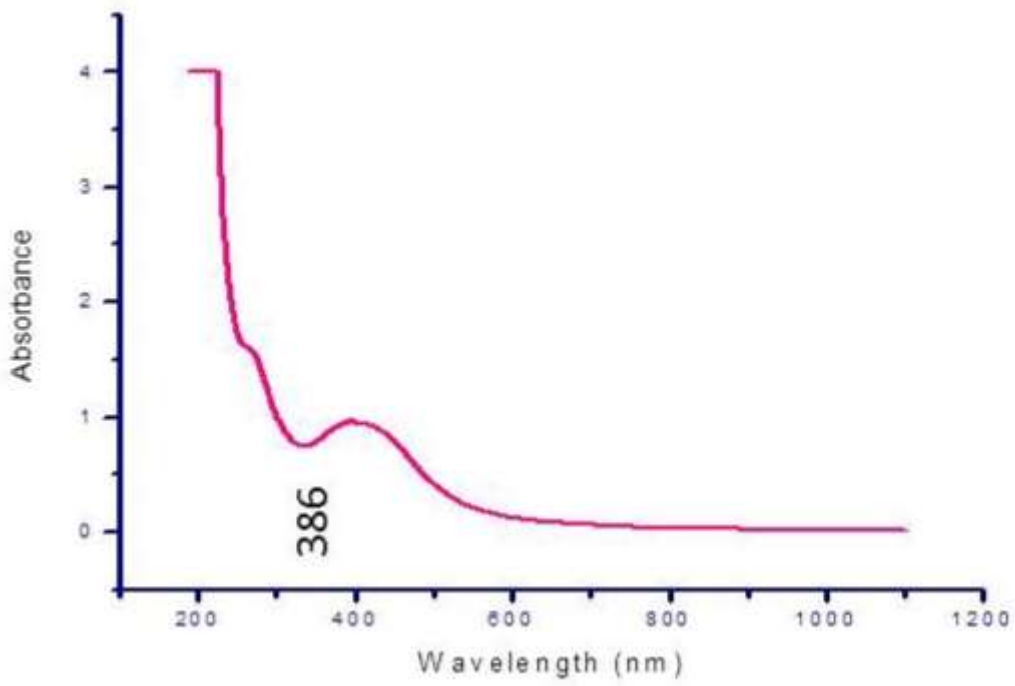


Figure.3 UV-Vis spectrum of AgNPs Using Cassiaanceolata Leaf extract

FTIR Analysis

The FTIR spectrum confirmed the interaction between silver nanoparticles and capping agents. FTIR spectra of 3381, 2925, 2865 cm^{-1} (phenolic OH) and 1598 cm^{-1} (aromatic N-H stretches) in speciosa leaf extracts (Figure S3 (a)). , 1384 cm^{-1} (–OH Stretch), 1072 cm^{-1} (Aromatic C-H Stretch), 914 cm^{-1} (Protein Binding due to Carbonyl Stretching in Proteins), 830 cm^{-1} (Aromatic ring Stretch), 667 cm^{-1} (C-C bond or set group II of the aromatic ring), 607 cm^{-1} (aromatic secondary amine CN stretching. which indicates the reduction of the corresponding functional groups (figure. 4). The results obtained show that the polyphenols in Cassia anceolata leaf extracts are extended in the C-C and CN-resonance.

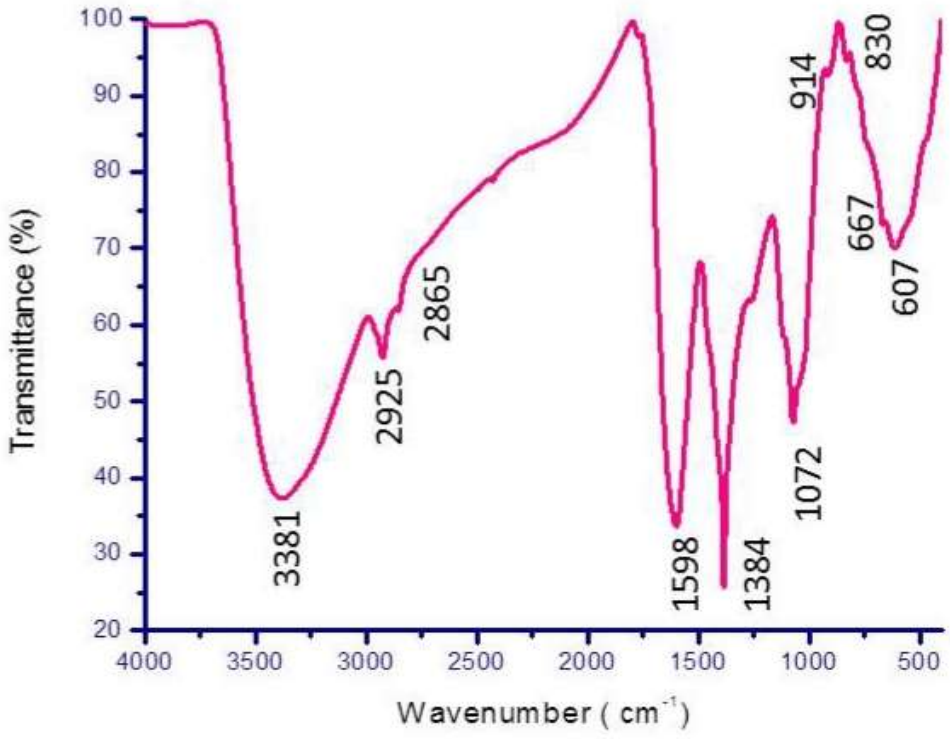


Figure.4 FT-IR spectrum of AgNPs Using Cassia anceolata leaf extract

PXRD Analysis

The crystalline of silver nanoparticles was confirmed by analysis of XRD shape as shown in Figure 3. Five different difference peaks in the values of 38.03 °, 44.29 °, 64.49, 77.49 ° and 81.51 °. which can be indexed to the (1 1 1), (2 0 0), (2 20), (3 1 1) and (2 2 2) can be coded with reflective planes of the face-centered cube structure (Figure 5). The intensity of 100% was found to be 20 with 38.03. These peaks are caused by existing organic compounds that cause the extraction and reduction of silver ions and the stabilization of the resulting nanoparticles.

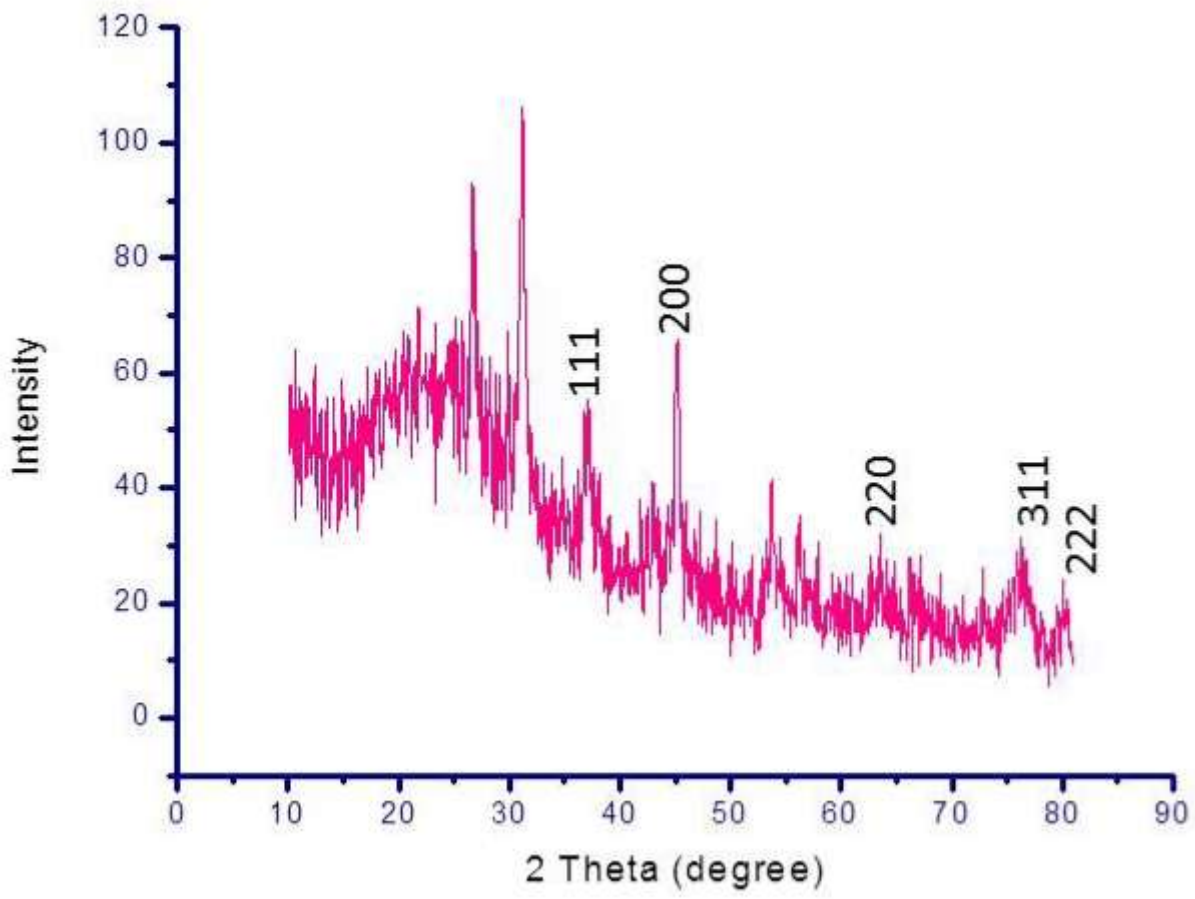


Figure.5 PXRD pattern of AgNPs synthesized using supernatant of Cassia anceolata leaf

Particle Size Analyzer (DLS) and Zeta potential

From DLS analysis the average hydrodynamic size and Zeta potential of CA -AgNPs were observed. The size distribution graph of CA -AgNPs show a single peak between 63.47nm and 159.2nm and its average size was found to be 136.9nm shown in Figure.6

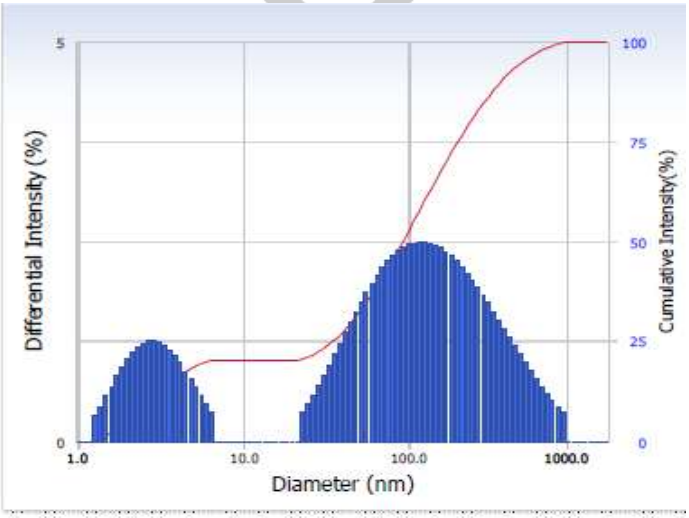


Figure.6 DLS spectra of silver -Nanoparticles

FE-SEM and EDAX Analysis

The FE-SEM and EDAX were used to study the morphology, size and elemental composition of biosynthesized silver nanoparticles. The synthesized nanoparticles at micro (10^{-6}) and nano (10^{-9}) can be identified by FE-SEM. The surface imaging technique, detection of particle shape, size, surface morphology and size distribution of nanoparticles [21-23]. The FE-SEM clearly shows that the particle is spherical and the size is found to be about 31 nm and 35 nm for CA-AgNPs as shown in Figure.7. The size of particles matches with Cassia anceolataleaves coat silver nanoparticles [24]. The elemental composition of silver nanoparticles is examined by Energy Dispersive X-ray spectroscopy and is a chemical analysis method combined with the FE-SEM [25]. In EDAX, the optical absorption peak at 3KeV is observed this shows that metallic silver nanocrystallitis which is due to surface Plasmon resonance [26]. The other element signals are due to phytochemicals or the protein present in stem extract and Si element is due to the glass wafer used for coating silver nanoparticles shown in Figure.8

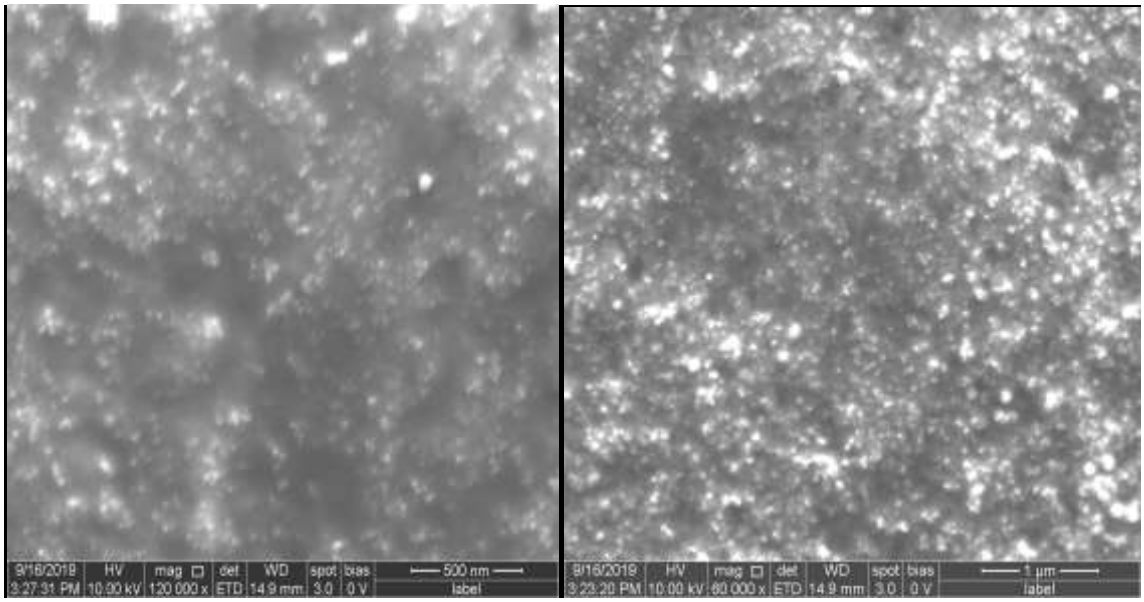


Figure.7 The typical FESEM image of the silver nanoparticles of Cassia anceolata leaf

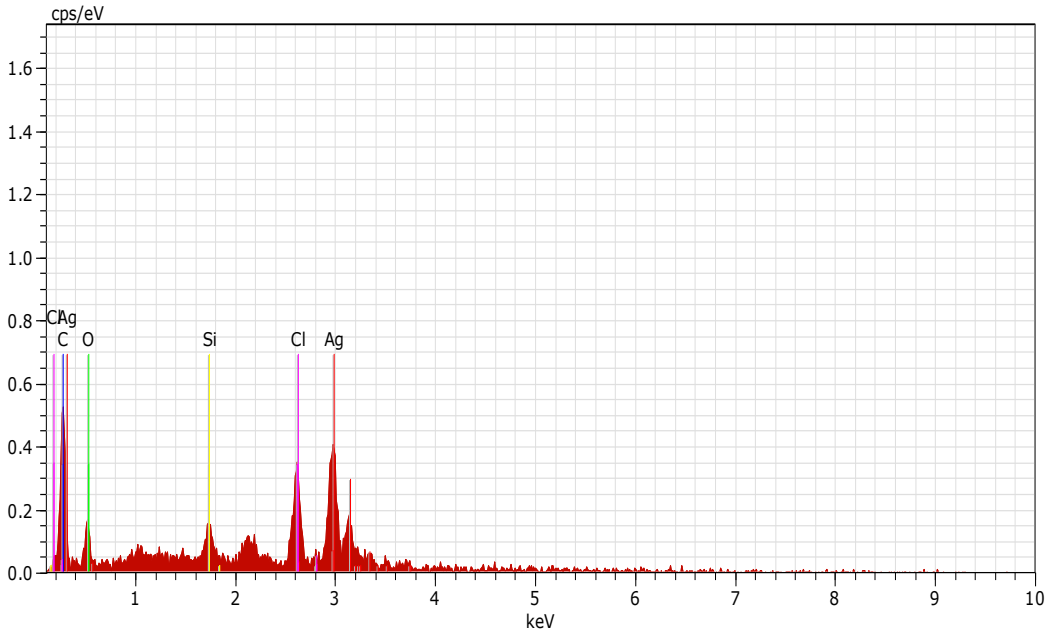


Figure.8 The EDX of silver nanoparticles of Cassia anceolata leaf

Antibacterial activity of silver nanoparticles **(disc diffusion method)**

Antibacterial activity of CA-silver nanoparticles was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Amoxicillin as standard antibiotic

disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

The results of anti-bacterial activity of various samples by disk diffusion method were tested against pathogens (Table 1). The model showed growth inhibitory activity against D. Escherichia coli (7 mm). All four bacteria in the C sample exhibited antibacterial activity, but it was more susceptible against Pseudomonas aeruginosa (4 mm) and Staphylococcus aureus (5 mm). However, crude extract and synthesized nanoparticles showed excellent preventive action against pathogens (Figure 9).

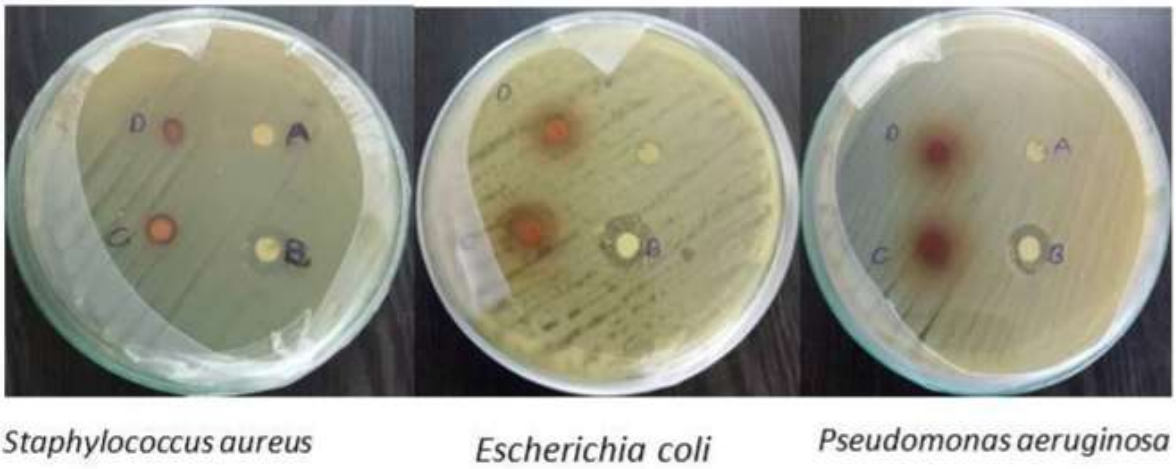


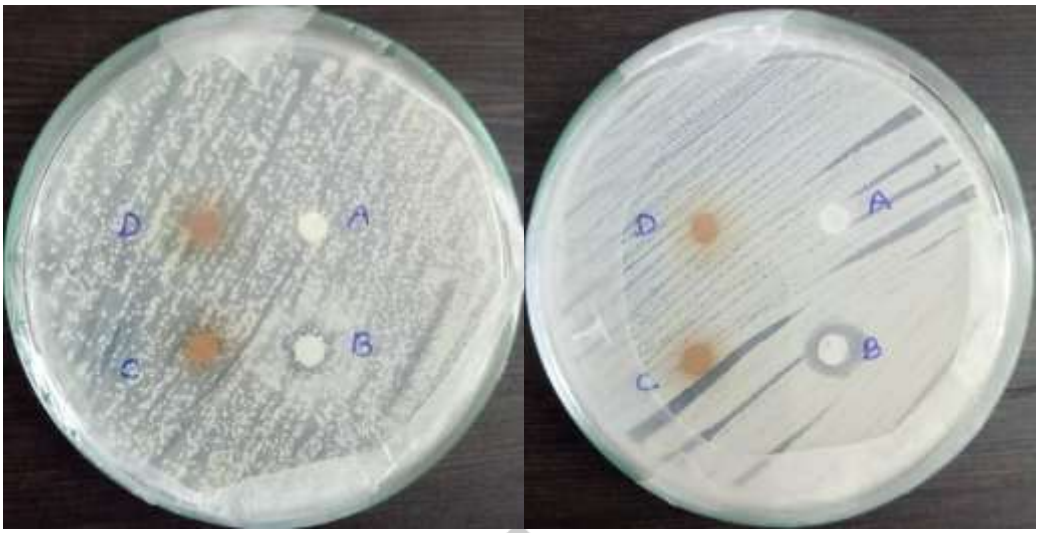
Figure.9 Antibacterial image of $AgNO_3$ nanoparticles

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)		
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
A (silver nitrate)	10	0	0	0
B (Amoxicillin)	10	9	9	9
C (Plant extract)	10	7	5	2
D (Nanoparticles)	10	6	4	0

Table 1: Antibacterial activity of silver nanoparticles

Antifungal Activity

Antifungal activity of sample was determined using the disc diffusion method Thepetridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Fluconazole as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.
Table 2 shows the results of the fungicide susceptibility test against different samples and test organisms. As a result, the sample D was most effective and demonstrated the highest activity against Candida albicans (2 mm inhibition zone).



Candida albicans Candida vulgaris

Fig.10 Antifungal image of $AgNO_3$ nanoparticles

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)	
		Candida albicans	*Candida vulgaris*
A (silver nitrate)	10 µl	0	0
B (Fluconazole)	10 µl	8	8
C (Plant extract)	10 µl	3	1
D (Nanoparticles)	10 µl	4	0

Table 2: Antifungal activity of silver nanoparticles

V Conclusions

The biocompatible synthesis of silver nanoparticles ($AgNO_3$) was achieved by applying a simple and novel green chemistry procedure involving the use of Cassia anceolata leaf extract as a reducing and capping agent. The biology of silver nanoparticles was carried out at room temperature via hydroxide precipitation, followed by calculations at 450 C. Successful formation of silver nanoparticles was confirmed by UV, FTIR, XRD, DLS, FESEM and EDX analyzes. XRD results confirm the average crystallite size observed from the intense plane of (111) was measured as 38.03 nm. The biosynthesized silver nanoparticles exhibited strong levels of antibacterial activity against Escherichia coli, Pseudomonas aeruginosa (gram positive bacteria) and Staphylococcus aureus(gram negative bacteria). *Candida vulgaris*exhibited stronger antifungal behavior than the *Candida albicans*strain. Biosynthesized silver nanoparticles were found to protect against bacterial and fungal phyto pathogens, suggesting that they may be used as effective antimicrobial and anticancer agents for commercial biomedical applications.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

References

[1] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silvernanoparticles. Nanomedicine 2007;3:95–101.

[2] Mukunthan KS, Elumalai EK, Patel TN, RamachandraMurty FV. Catharanthusroseus: a natural source for the synthesis of silver nanoparticles, Asian Pacific. J Trop Biomed
2011;1:270–4.

[3] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 2009;145:83–96.

[4] Saifuddin N, Wong CW, NurYasumira AA. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E-J Chem2009;6:61–70.

[5] Satishkumar G, Titelman L, Landau MV. Mechanism for the formation of tin oxide nanoparticles and nanowires inside the mesopores of SBA-15. J Solid State Chem2009;182:2822–8.

[6] Kalishwaralal K, Deepak V, Pandian SRK, Kottaisamy M, Kartikeyan B, Gurunathan S. Biosynthesis of silver and gold nanoparticles using Brevibacteriumcasei. Colloids Surf, B 2010;77:257–62.

[7] Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanyan RH. Biological synthesis of silver nanoparticles using the fungus Aspergillusflavus. Mater Lett 2007;61:1413–8.

[8] Gajbhiye M, Kesharwani J, Ingle A, Gade A, RaiM. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotech Biol Med 2009;5:382–6.
[9] Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N.Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf, B 2010;76:50–6.
[10] Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS. Cinnamon zeylanicumbark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids Surf, B 2009;73:332–8.
[11] Parashar V, Parashar R, Sharma B, Pandey AC. Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Digest J NanomatBiostruct2009;4:45–50.
[12] Jain D, Kumar Daima H, Kachhwaha S, Kothari SL. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Digest J NanomatBiostruct 2009;4:557–63.
[13] Song JY, Jang HK, Kim BS. Biological synthesis of gold nanoparticles using Magnolia kobusand Diopyros kaki leaf extracts. Process Biochem 2009;44:1133–8.
[14] Narayanan KB, Sakthivel N. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci 2011;169:59–79.
[15] Ramirez IM, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. Colloids Surf, B 2009;73:185–91.
[16] Panacek A, Kolar M, Vecerova R, Prucek R, SoukupovaJ, Krystof V, et al. Antifungal activity of silver nanoparticles against Candida. Biomat 2009;30:6333–40.
[17] Nadworny PL, Wang J, Tredget EE, Burrell RE. Anti-inflammatory activity of nanocrystallinesilver in a porcine contact dermatitis model.Nanomed Nanotech BiolMed 2008;4:241–51.
[18] Rogers JV, Parkinson YW, Choi JL, Speshock SM, Hussain M. A Preliminary assessment of silver nanoparticles inhibition of monkeypox virus plaque formation. Nanoscale Res Lett 2008;3:129–33.
[19] Gurunathan S, Lee K, Kalishwaralal K, Sheikpranbabu S, Vaidyanathan R, Eom S. Antiangiogenic properties of silver nanoparticles. Biomat 2009;30:6341–50.
[20] Shrivastava S, Dash D. Agrifood nanotechnology: a living revolution in food and agriculture. J Nanotech 2009;12:240–3.
[21] Ajitha B, Ashok Kumar Reddy Y, Reddy PS. Biogenic nano-scale silver particles by *Tephrosiapurpurea* leaf extract and their inborn antimicrobial activity. SpectrochimicaActa, Part A: Molecular and BiomolecularSpectroscopy 2014; 121:164-72.
[22] Christy J, Dharaneya D, Vinmathi V, Justin Packia Jacob S. A Green Nano-Biotechnological Approach For The Synthesis of Silver Nanoparticles Using The Seed Coat of *TamarindusIndica*. Study of Its AntibaCterial and Anticancer Activity.International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7:192-4.
[23] Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. Applied Nanoscience 2016; 6: 399-408.
[24] Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids and Surfaces B: Biointerfaces 2008; 65:150-3.
[25] Joshi SC, UtkarshKaushik, AproovaUpadhyaya, Priyanka Sharma. Green Technology Mediated Synthesis of Silver Nanoparticles from *MomordicaCharantia* Fruit Extract and Its Bactericidal Activity. Asian Journal of Pharmaceutical and clinical research 2017; 10:196-200.
[26] Lin L, Qiu P, Cao X, Jin L.Colloidal silver nanoparticles modified electrode and its application to the electroanalysis of Cytochrome c. ElectrochimicaActa 2008; 53:5368-72.