BIOSYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *ACALYPHA FRUTICOSA* FORSSK.

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ABSTRACT*:* The nanoparticles are used in different fields of scientific research. In the present study, biosynthesis of silver nanoparticles and its antibacterial activity were investigated. Silver nanoparticles were synthesized using leaf extract of *Acalypha fruticosa* Forssk and the formation of nanoparticles was observed by colour change from light yellow to dark brown. The results obtained from UV–visible spectrum, Fourier transform-infra red spectroscopy (FT-IR*)* and X-ray diffraction (XRD) support the biosynthesis and characterization of silver nanoparticles. Further, Antimicrobial activity of *Acalypha fruticosa* was studied using human pathogenic bacterial strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella*sp. The antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against *Escherichia coli* and *Bacillus subtilis*.

K**ey words**: *Acalypha fruticosa*, AgNPs, FTIR, XRD, Anti bacterial activity

I. INTRODUCTION

Biosynthesis of nanoparticles as a developing highlight of the intersection of biotechnology and nanotechnology has received increased attention due to rising need to develop environmentally benign technologies in bio material synthesis (Bhattacharya et al., 2005). A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants (Mohanpuria et al. 2007, Farooqui et al., 2010).

Silver nanoparticles exhibit strong optical features making the nanoparticles suitable for biological sensing and imaging (Jain et al., 2008). Due to their high conductivity, silver nanoparticles are applied in conductive inks, adhesives and pastes for a range of electronic devices (Park et al., 2008); further used as catalysts in several chemical reactions such as the oxidation of styrene (Jiang et al., 2005; Xu et. al., 2006); it might exhibit additional antimicrobial capabilities not exerted by ionic silver, because its small size and large surface to volume ratios, which lead to both chemical and physical differences in their properties compared with their bulk counterparts.

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as 'Chinnichedi' and 'Birch-leaved Acalypha' is a strong smelling and bushy shrub and used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. In Yemen, leaf and stem have been used to treat malaria, wound and skin diseases. In the present work, we investigated the synthesis of silver nanoparticles with the bioreduction method using leaf extract of *Acalypha fruticosa* Forsskand further evaluated their antibacterial effect against four human pathogenic bacteria.

METHODOLOGY

COLLECTION OF PLANT MATERIALS

Acalypha fruticosa Forssk. belonging to the family Euphorbiaceae was collected from the campus of St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India during the month of December, 2018 and identified and confirmed by the flora of the presidency of Madras (Gamble, 1919). The plant leaf were placed on plastic sheet and spread out at room temperature under the shade for drying; grounded to fine powder using a tissue blender and then stored in the refrigerator for further use.

Figure.1

Silver Nanoparticles Synthesis

Two gram dried plant powder was taken in a 100 mL Erlenmeyer flask with 30 mL of sterile distilled water and then boiled for 2 minutes. After boiling, the compound was filtered with Whatman No.1 filter paper. 5 mL of plant extract was mixed with 25 mL of 3 mM silver nitrate. The formation of reddish brown colour was observed and λ max at different time intervals were taken for 8 hours using a UV–visible spectroscopy. Then the solution was maintained in room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours centrifugation of the reaction mixture, supernatant was discarded and 1 mL of distilled water added to the pellet and washed. Pellet was collected by using acetone/ethyl acetate/alcohol and dried in the watch glass and stored the nanoparticles.

UV–Visible Spectra Analysis The bio reduction of pure silver ions was observed by measuring the UV–visible spectrum of the reaction at different time intervals using 1 mL of the sample, compared with 1 mL of 3 mM silver nitrate used as blank. UV–visible spectral analysis has been one by using 'Elico spectrophotometer' at a resolution of 1 nm from 200 to 1100 nm.

FTIR Analysis

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} was used. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier transform infrared (FTIR) for the analysis of the nanoparticles.

XRD Analysis

X-ray diffraction (XRD) analysis of drop coated films of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticles by XPERT-PRO software and X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation.

Antimicrobial assay

Antimicrobial activity of *Acalypha fruticosa* was performed using a standard disc diffusion assay on nutrient agar against the selective Gram (+ and −) bacterial strains. Two Gram (+) (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram (−) bacteria (*Escherichia coli* and *Klebsiella*sp) were used for the determination of antimicrobial activity against different concentrations as described by Hossain et al. (2014). Four concentrations, 2, 1, 0.5 and 0.25 mg/ml of each fraction were prepared with dimethyl sulphoxide (DMSO) and incorporated onto 6 mm sterile disc in this experiment. Positive control discs with amoxicillin antibiotic (3 mg/ml) and negative control discs with DMSO solvent were also used. Different concentrations of fractions, positive and negative control were loaded on the inoculated agar plates seeded with test pathogen separately. Plates were incubated micro aerobically at 37 °C for 24 h until the culture developed. Antibacterial activity expressed in terms of the diameter of the zone of inhibition was measured in millimeter against the tested bacteria.

RESULTS

UV-visible Spectrum Analysis

The addition of aqueous leaf extract of *Acalypha fruticosa* Forssk into 3mM silver nitrate solution led to the appearance of a brown color solution indicating the formation of Ag nanoparticles. The color changes due to the excitation of surface plasmon vibrations with the silver nanoparticles. The SPR of silver nanoparticles produced a peak centered near 420 nm. After few minutes there was no significant color change, which indicated the completion of reduction reaction.

FT-IR Spectrum

The FT-IR spectrum obtained for carob leaf extract (Figure 2) displayed a number of absorption peaks, reflecting its complex nature. This spectrum showed the presence of bands at 1088.74, 1118.64, 1197.71, 1400.22, 1461.94, 1715.56, 2850.59, 2920.03 and 3161.11cm^{1}. The bands at 1715.56cm^{1}corresponds to tertiary amides C=O band, the band at 1118.64 cm^{1} was assigned to methylene scissoring vibration from the protein in the solution and the band at 1400.22 cm^{1} were assigned to C–O stretching vibration of the proteins. The positions of these bands were close to that reported for native proteins. The FT-IR spectroscopic study also confirmed that the protein present in carob leaf extract acts as a reducing agent and stabilizer for the silver nanoparticles and prevents agglomeration.

X-Ray Diffraction Studies

XRD pattern taken using powder X-ray diffractometer instrument (XRDML) in the angle range of 10—80 °C of the AgNPs at 2θ, scan axis: Gonio. A number of Bragg reflections corresponding to 18.01, 19.17, 27.84, 32.31, 46.27, 57.43 and 76.96sets of lattice planes were observed which can be indexed to face-centred cubic silver (Figure 5). The peaks were identified as AgNPs

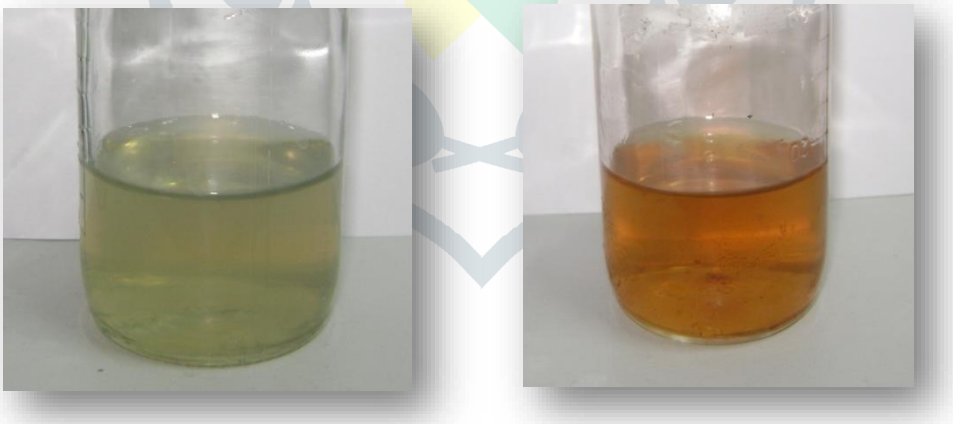
according to XPERT-PRO software (PDF#030921). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

Antimicrobial activity

The activity of aqueous leaf extract of *Acalypha fruticosa* was observed to have a range of zone inhibition patterns against different selected Gram (+ and −) microbes. Invariably it has shown significant antibacterial activity against all employed cultured bacterial strains within the range of 7–12mm. Different zone of inhibition was exhibited at different concentration of 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml; activity increased with the increasing concentration. The highest zone of inhibition was found to be 12mm against *Staphylococcus aureus* at 2mg/ml and least against *Bacillus subtilis* (7mm) at 0.25 mg/ml. The maximum zone of inhibition was observed in 2mg/ml.

Table.1: antibacterial activity of extracts

Pathogens	*Acalypha fruticosa*			
	Zone of Inhibition (mm)			
	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2mg/ml
Staphylococcus aureus	9	11	11	12
Bacillus subtilis	7	8	8	9
Escherichia coli	8	8	10	11
Klebsiella sp	9	10	10	11



A ***B***

Fig. 2.Plant Extract With Silver Nitrate Solution Before (A) and After the Formation of Silver Nanoparticles (B).

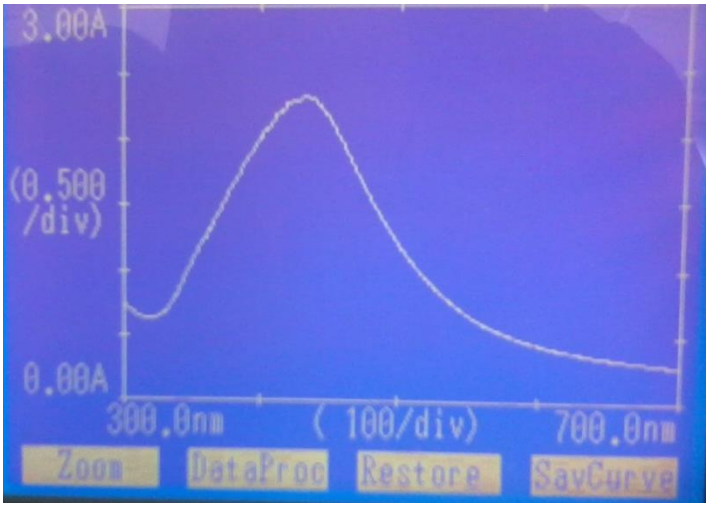


Fig. 3. UV-visible Spectra Analysis of Silver Nanoparticles

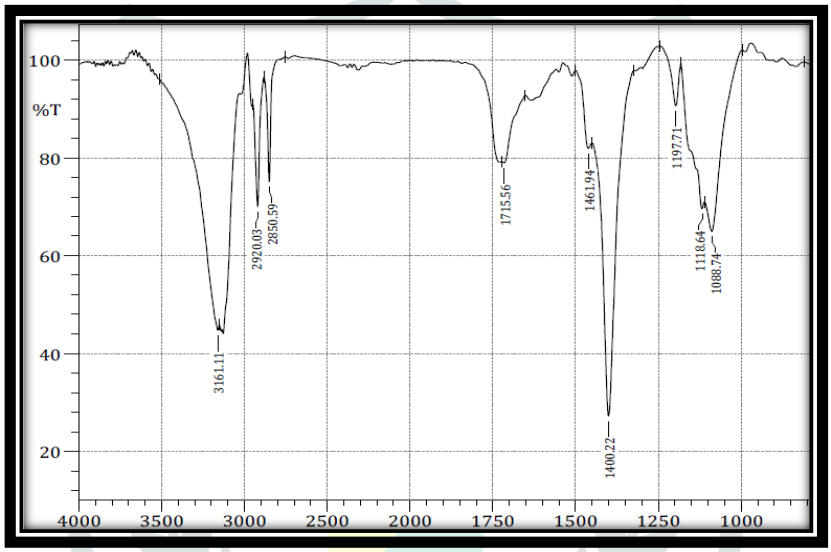


Fig. 4.FTIR Spectrum of Silver Nanoparticles Using Acalypha fruticosa.

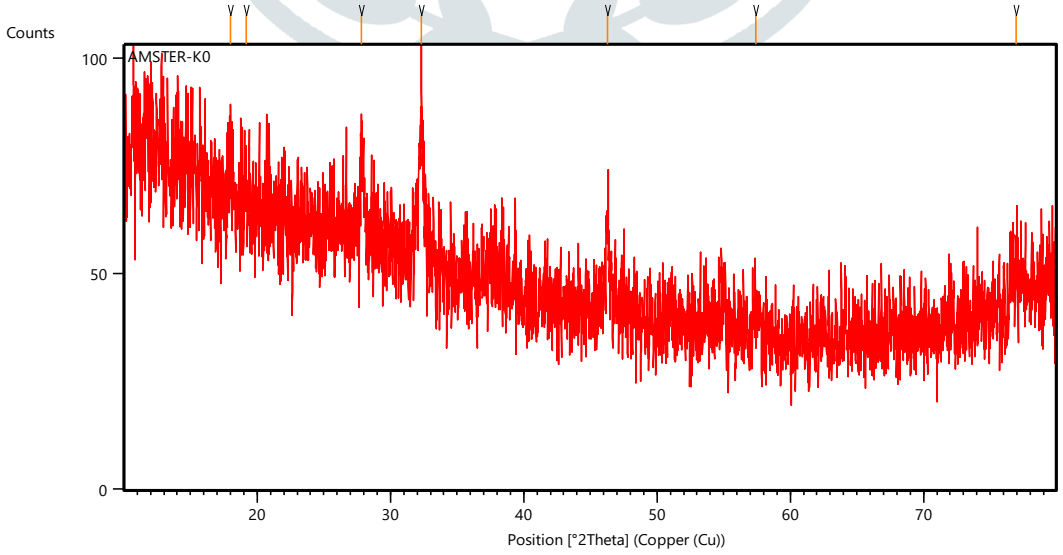


Fig. 5.*XRD Analysis of Silver Nanoparticles Using Acalypha fruticosa.*

CONCLUSION

Our results suggest that the presence of active metabolites in the leaf of *Acalypha fruticosa* with appreciable antimicrobial activity from the disc diffusion assay.The results of this study clearly demonstrated that the colloidal Ag NPs inhibited the growth and multiplication of the tested bacteria, including highly multidrug-resistant bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella* sp.

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