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GLIB GREEN ROUTE SYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACTS OF *CRESCENTIA CUJETE* L. AND ITS BACTERICIDAL ACTIVITY

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ABSTRACT

An easy, economical and eco-friendly approach of silver nanoparticles (SNPs) synthesis using aqueous dry leaf extract of *Crescentia cujete* L. has been reported in this paper. The formation and characterization of silver nanoparticles were carried out using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy, Dynamic light scattering, Scanning Electron Microscopy and Energy Dispersive X-ray analysis. The silver nanoparticles prepared by this tactic showed better antimicrobial activity against gram positive and gram negative bacteria than plant extract alone. On the basis of result obtained it can be said that the easy production of silver nanoparticle using green chemistry can be effectively utilized in pharmaceuticals and biomedical nanotechnology as well as combating harmful microbes.

KEYWORDS: Silver Nanoparticles, *Crescentia cujete*, FTIR, DLS, SEM, EDX, Antimicrobial Activity.

INTRODUCTION

Now a days nanoparticles of metals like silver, gold and copper are formed in a very simple and easy way using a green route i.e. reduction of metal salts to form nano particles of metals using aqueous plant extracts.[1,9] The aqueous extracts may be of the fresh plant parts or their dried powders. Generally dried leaf extracts are easy to be used. Silver nano particles (SNPs)

are put to various uses in therapeutics since long and these days in biomedical research.[10,15] Production of nano particles using a green course involves utilizing characteristic plant materials which is natural and hence expands incorporation of conditions that are neither too harsh nor leading to loss of vitality, moreover the whole process does not have to utilize high molecular weight and harmful chemicals thus rendering it eco-friendly and biocompatible.[16,22] Bio-enthused formation of nanoparticles gives headway over other harsh and physical approaches. Also it is quick and particular in objective towards some applications where particles can be used for their antimicrobial action.[23] These days, medicinal plants have been largely exploited as a part of extensive extents in view of the long term change against disorders after routine treatment.[24] In this research silver nano particles have been formed utilizing the green course *i.e*. from the aqueous leaf extracts of *Crescentia cujete* commonly known as Calabash tree. This tree is of rare distribution in India, native to central and South America and national tree of St. Lucia. The fruits of this tree are generally not edible. Juice of fruit is generally put to various medicinal uses as treatment of intestinal vagaries as well as cure for pneumonia. Crushed Leaves are used to clean wounds and to stop bleeding as well as to promote healing.[25,27]

MATERIALS AND METHOD

Fresh and healthy leaves of *Crescentia cujete* were hand plucked from the tree. Silver nitrate was purchased from Hi-Media and all chemicals were of analytical grade with 98-100% immaculateness measure as analytical reagents and chemical grade. Deionized and double distilled glass water was utilized throughout the experiment.

Preparation of Leaf Extracts

Fresh leaves of *Crescentia cujete* were washed with deionized water and after that with distilled water to ward off any grime material. These were then subjected to dry in shaded condition for around 7 days and then pulverised to fine powder utilizing a clean mechanized kitchen grinder with stainless steel cutting edges. This powder was stored in airtight bottles and was utilized for preparing the extracts. The extract was made by adding 1 g of leaf powder to 50 ml of double distilled water and left overnight in refrigerator. The crude extract was filtered and pure extract was utilized for formation of nanoparticles.

Synthesis of Ag nanoparticles

To 30 ml of 1mM $AgNO_3$, 10 ml of leaf extract was added and at interval of every 20 minutes colorimetric readings were taken at 410nm to find the presence of nano particles.

The colour change was an indication of formation of nano particles. These started forming within 20 minutes and continued up to 4-6 hours after that these particles were stable for about one month.

Characterization techniques

The biosynthesized silver nanoparticles were characterized by the following methods:

Visual Observation

A change of colour from light brown to dark reddish brown was observed in the solution after visible irradiation. "Fig.1".

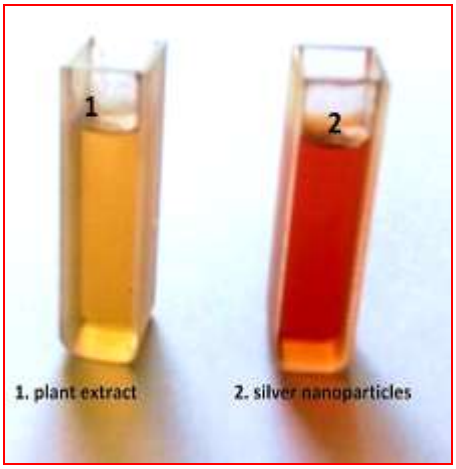

Fig. 1 Plant extract and Silver nanoparticles.

UV Spectrophotometric analysis

The characterization technique involved ultra-violet and visible spectroscopy. UV-Vis absorption spectra were measured using Systronic UV-117 spectrometer from 300nm to 700nm continuously and the leaf powder extract was used as the reference for the baseline correction.

Fourier Transform Infrared Spectroscopy Analysis

FTIR analysis was carried out to determine the functional groups present in leaf extract and their possible involvement in the formation of silver nanoparticles. FTIR analysis were carried using a FTIR SHIMADZU 8300 instrument. The results were compared for shift in functional peaks. A FTIR graph can be useful for preliminary investigation of surface chemistry of biogenic nanoparticles (i.e. those chemicals that contain carbon). This technique is widely used for identification of chemical residues such as amine, carbonyl and hydroxyl functional groups in a molecule, also FTIR is an effective tool in detecting the shape of

nanometer sized materials, and it can effectively be used to measure the particle formation. It is found that the width and intensity of peaks in an IR spectrum have explicit dependence on the particle size. As particle size increases, the width of the peak decreases and intensity increases.[28] The FTIR analysis was performed with reduced silver nanoparticles. All measurements were carried out in the range of 400-4,000 cm^{-1} at a resolution of 4 cm^{-1}. For this fresh sample were sent for FTIR Analysis at Gujarat Laboratory, Ahmedabad. Samples with total of volume 1-2 ml were given in aqueous form formed by producing SNPs using the reduction reaction of 9 ml of 1mM Silver Nitrate solution through 3 ml of plant extract.

Dynamic Light Scattering

These studies were carried out to know the particle size distribution in the solution. The particle size comes out to be 67nm and hence this can be further verified from EM analysis.

SEM & EDX Analysis

The surface morphology of silver nanoparticles was examined using a scanning electron microscopy (6010 LA, JEOL). The elemental composition of the synthesized silver nanoparticles was analysed using Energy Dispersive X-Ray Spectrometer attached with Scanning electron microscope.

Determination of antimicrobial activity

A. Microorganisms

The bacterial pathogens namely *Staphylococcus aureus, Streptococcus pneumonia, Escherchia coli* and *Salmonella typhi* were obtained from the Department of Microbiology, Gujarat Arts and Science College, Ahmedabad. These human pathogens were used to study the antibacterial activity. The nutrient broth, nutrient agar were used growing the test bacterial strains and were maintained on corresponding agar slants at 4°C.

B. Preparation of inoculums

The bacterial pathogens were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours until the culture attained a turbidity of 0.5 McFarland units. The final inoculum was standardized to 105 CFU/ml by diluting fresh cultures with sterile distilled water.

Colonies were suspended in 5 ml of sterile 0.85% saline. The resulting suspension was vortex and the turbidity was adjusted to yield 2×10^6 cells/ml (≅0.5 McFarland standards).

C. Antibacterial activity

Antibacterial activity of SNPs was determined by the agar disc diffusion method. Plates of Nutrient agar were evenly streaked across the complete surface throughout the petri plate so as to get a loan growth of the inoculums with the help of spread plate technique with a known volume of 0.01 ml of active young culture with approximate microbial count as 105 CFU/ml. Sterile filter paper discs (5 mm diameter) were immersed in the 50 µl of synthesized SNPs (10, 20, 30, 40, 50 µg/ml) and allowed to dry at room temperature and were placed over the Nutrient agar plates. Streptomycin 10 mcg/disc was used as positive control and the disc immersed in distilled water was used as negative control. The plates were incubated overnight at 37 ºC and the zone of inhibition around each disc was measured. Experiments were done in triplicate and mean values of zone diameter were taken.

RESULTS AND DISCUSSION

A comfortable, cheap and an applied tactic for eco-friendly synthesis of silver nanoparticles using aqueous leaf extract of *Crescentia cujete*, as both reducing and stabilising agent, under the prescribed condition of room temperature has been used without the use of harsh inducers or hazardous chemical additives and/or severe chemical reactions. The formation of SNP was predicted visually from a color change and confirmed using UV-visible spectroscopy (peak-458 nm) "***Fig.2"*** further validated by SEM "***Fig.3"*** and also EDX "***Fig.4"*** of SNP. Also using FT-IR Spectroscopy the characteristics of SNP was confirmed "***Fig.5".*** FTIR studies show peaks at c.a. 1600, 2200 and 3300 wave numbers confirming the formation of nano particles of silver. Size controlled was c.a. 67 nm revealed using DLS measurements "***Fig.6".***

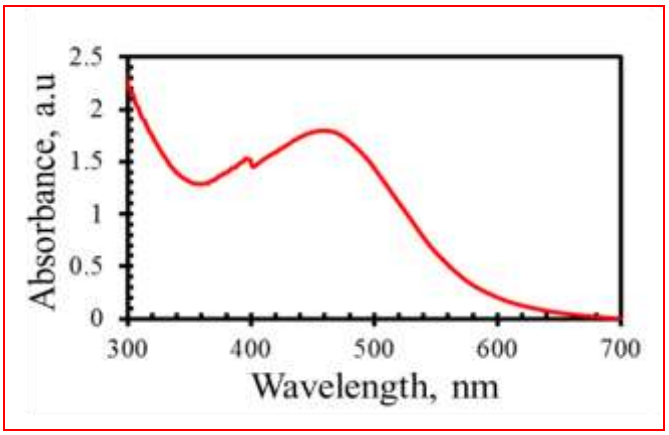

Fig. 2 UV-VIS scan of SNP.

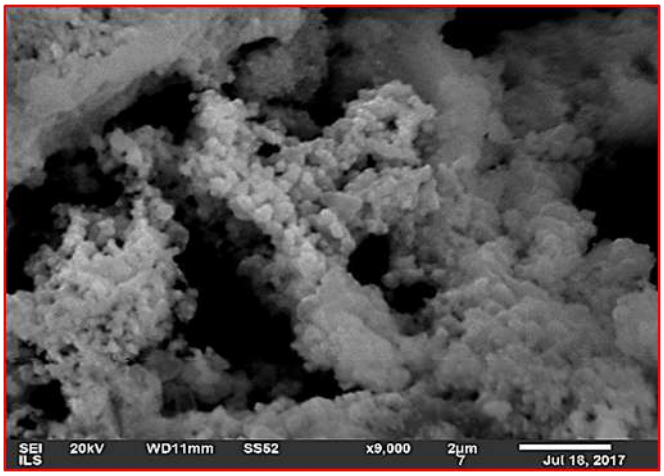

Fig. 3 SEM of SNP.

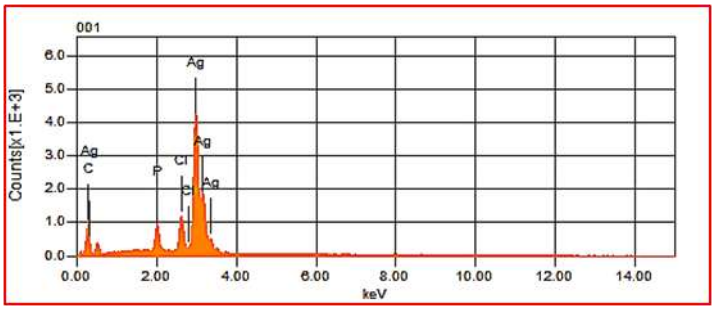

Fig. 4 EDX of SNP.

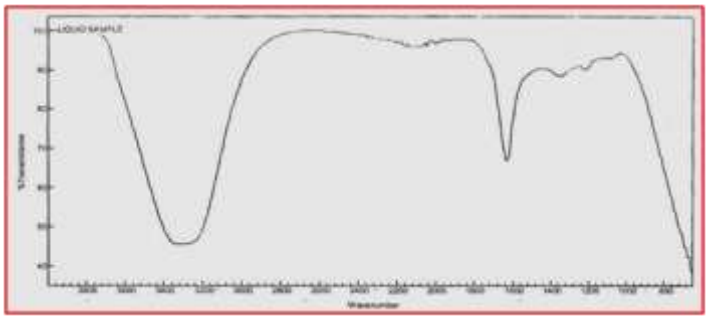

Fig. 5 FTIR of SNP.

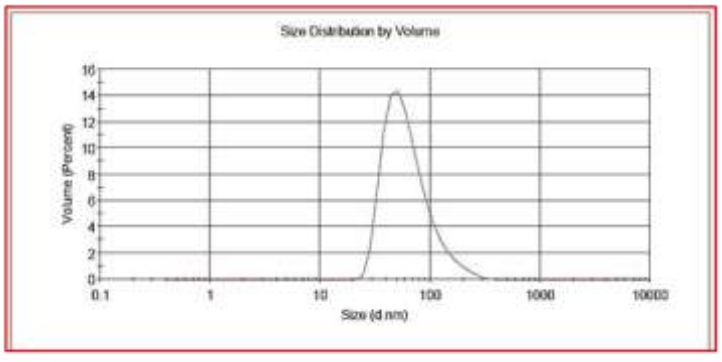

Fig. 6 DLS of SNP.

The prepared silver nanoparticles exhibited reasonable antibacterial activity "***Figs.7-8".*** The effects were more pronounced on Gram-negative bacteria *Salmonella typhii* (MTCC: 733) and *Escherichia coli* (MTCC: 425). The nanoparticles also showed prominent activity on Gram-positive bacteria S*taphylococcus aureus* (MTCC: 96) and *Bacillus cerus* (MTCC: 430). A bactericidal mode of action was observed more for both Gram-positive and Gram-negative bacteria by the nanoparticles as compared to plant extracts.

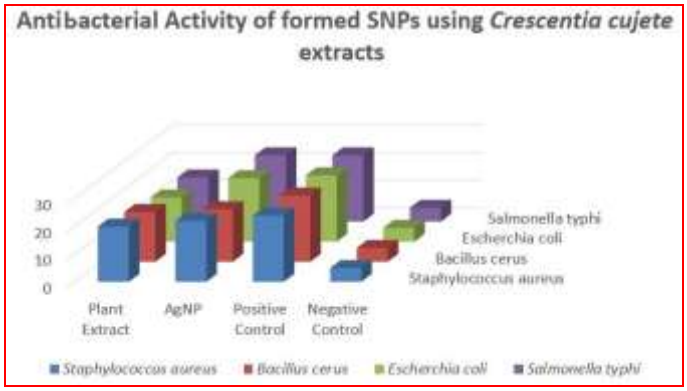

Fig. 7 Antibacterial Activity of formed SNPs using *Crescentia cujete* extracts.

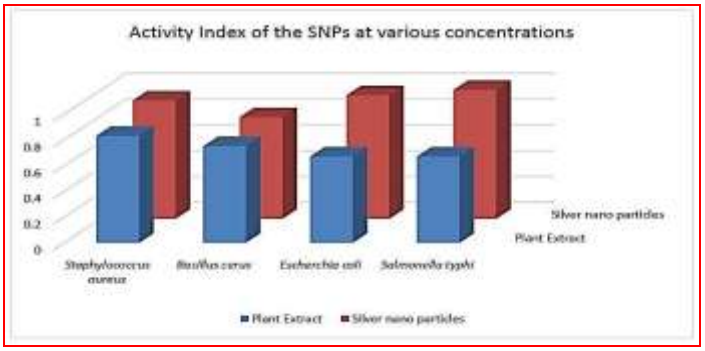

Fig. 8 Activity Index of the SNPs at various concentrations.

Note: Zone of inhibition by streptomycin as a standard drug = 24 mm (Mean Value).

$$Activity\ Index\ (A.I.) = \frac{\text{Mean of Zone of Inbition by SNPs}}{\text{Zone of Inhibition obtained for standard Antibiotic Drug}}$$

CONCLUSION

A distinctive glib, proficient and feasible method for the phytofabrication of silver nanoparticles using aqueous leaf extract of *Crescentia cujete*, under the normal influence of light and temperature can be used. The bio-fabrication of silver nanoparticles making use of such a traditionally important medicinal plant without applying any other chemical additives or harsh conditions of temperature etc., thus offers a cost-effective and environmentally

benign route for their large-scale commercial exploitation. The SNPs can be easily characterized by UV-visible, SEM-EDX and FT-IR spectrum. Synthesis of SNPs using green resources like *Crescentia cujete*, is a better alternative to chemical synthesis, since this green synthesis is pollutant free and eco-friendly. The biosynthesized SNPs have shown good antibacterial efficacy and hence has a potential to be used as antibacterial agent against Gram-negative bacteria *Salmonella typhii* (MTCC: 733) and *Escherichia coli* (MTCC: 425) and Gram positive bacteria S*taphylococcus aureus* (MTCC: 96) and *Bacillus cerus* (MTCC: 430) as well more effectively then the aqueous extracts of *Crescentia cujete*. These SNPs were proved to be powerful weapons as antibacterial.

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