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P. ZEYLANICA MEDIATED GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLES AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT
The synthesis, characterization and application of biologically synthesized nanomaterials have become an important branch of nanotechnology. *Plumbago zeylanica* is known as white chitrak, grows all districts of plains in Andhra Pradesh, Karnataka, Maharashtra. The purpose of this study was investigating experimentally about the synthesis of silver nanoparticles were carried out using the leaf of plant chitrak as a reducing agent. *P. zeylanica* was exposed in the synthesis of silver nanoparticles was investigated employing UV/Visible spectrophotometer, TEM (Transmission Electron Microscopy) & FTIR. After exposing the silver ions to leaf extract, rapid reduction of silver ions is observed leading to the formation of silver nanoparticles in solution. UV–VIS spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 420 nm. From Transmission electron microscopy (TEM) analysis, the size of the silver nanoparticles was measured 20-40 nm. Fourier Transmission Infra-Red Spectroscopy (FTIR) and energy dispersive spectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles. The above silver nanoparticles were effective against *S. aureus* and *P. aeroginosa*.

KEYWORDS: Nanoparticles, *Plumbago zeylanica*, UV–VIS spectrum, FTIR.

INTRODUCTION
Nano biotechnology is a burgeoning interdisciplinary field of research interlacing material science, bionanoscience, chemistry, physics, electrical engineering, material science and molecular biology.[1] The term Nano is adapted from the Greek word meaning "dwarf." When used as a prefix, it implies 10^{-9}. A nanometer (nm) is one billionth of a meter, or roughly the length of three atoms side by side. A DNA molecule is 2.5 nm wide, a protein approximately 50 nm, and a flu virus about 100 nm.[2]

Nanoparticles can be broadly grouped into two: namely organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) while some of the inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles.[3] Silver was known only as a metal until the recent advent of the nanotechnology era, when it became recognized that silver could be produced at the Nano scale. Silver has been 'oligodynamic', that is, its ions can cause a bacteriostatic or even a bactericidal impact.[4]

Silver has been exploited as antimicrobials from ancient period[4]. With the evolution of nanomedicine as a study for treating infections, metallic silver in the form of NPs has regained its significance.[5]

Several approaches have been employed to obtain a better synthesis of silver nanoparticles such as chemical and biological methods. Microbes and plants are currently used for nanoparticle synthesis. Recently, the synthesis of silver nanoparticles using plant extracts getting more popular. The use of plants for the fabrication of nanoparticles is a rapid, low cost, eco-friendly and a single step method for biosynthesis process.[6]

The bioreduction performance of different plants parts such as *Helianthus annus*, *Sorghum bicolour*, *Basella alba*, *Oryza sativa*, *Saccharum officinarium* and *zea mays* in the synthesis of Ag nanoparticles.[7] Based on this approach, the present study reviewed the green-chemistry type AgNPs synthesis processes using *Plumbaco zeylanica* a medicinally important herb belongs to family *Plumbaginaceae*.

Plumbaco zeylanica Linn. (Plumbaginaceae) is one of the well-known Ayurvedic drug. It is commonly known

as chitramula and chitrack. This is perennial herb grow in shady places in the garden and found in Sri Lanka (Ceylon) and parts India, which include Bengal, Uttar Pradesh and Southern India.[8]

Plumbago zeylanica root inhibits cell growth and potentiates in human gastric cancer cell[9], inhibits ultraviolet radiation induced development of squamous cell carcinomas[10] and genotoxic damage induced by potassium canrenoate in culture human peripheral blood lymphocytes.[11] Root also shows anti-fertility activity[12], Neo and 1epineo-isoshinanolones isolated from *Plumbago zeylanica* roots show the antimicrobial activity.[13]

In view of this, the present study was aimed at evaluating the synthesis of novel metal NPs of the leaves of *P. zeylanica* may synthesize exhibiting antibacterial activity.

MATERIALS AND METHODS

Synthesis of AgNPs

Plumbago zeylanica leafs were collected from TNAU, Periyakulam, Tamilnadu, India. The leaves were air dried for 10 days then leaves were kept in the hot air oven at 100°c for 24-48 hrs. The leaves were ground to a fine powder. 1 mM silver nitrate was added to plant extract to make up a final solution 200 ml and centrifuged at 18.000 rpm for 25 min. The collected pellet stored at -4°c.The supernatant was heated at 500°c to 950°c.A change in the color of solution was observed during the heating process.

UV-Vis Spectroscopy

The bio reduction of Ag+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the sample with 2ml of DDW and subsequently measuring UV-Vis spectra, at the wave length of 190 to 800 nm in Beckman – Model No. Du - 50 spectrophotometer, at a resolution of 1nm.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device.

X – Ray Diffraction

The formation of single-phase compound was checked by X-ray diffraction (XRD) technique.

The XRD data were collected using Xray diffractometer (XPERT-PRO, PW3050/60) at room temperature, with CuKα radiation (λ = 1.5406 A°) between 90° and 300°.

EDX measurements

To carry out EDX analysis, the bark extracts reduced silver nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 N SEM instrument equipped with a Thermo EDX attachments.

Analysis of interaction of silver nanoparticles with Bacteria

Bacterial Growth Curve

The antibacterial activity of silver nanoparticles against *Pseudomonas aeroginosa, Klebsiella pneumonia, Proteus vulgaris, Bacillus subtilis, and Staphylococcus aureus* were analyzed. The MH media is supplemented with 10-50µg/ml silver nanoparticles and bacterial cultures were incubated at 37°C with continuous shaking at 150rpm.

RESULT

The formation of silver nanoparticles was confirmed by changes in the colour from green to dark brown after incubation with silver nitrate, while the controls retained the original colour of extract (Plate 1). UV-Vis spectra recorded from the aqueous silver nitrate - *P. Zeylanica* leaf broth. Strong characteristic absorbance peaks were observed at 454 nm (Fig. 1).

TEM analysis

TEM micrograph of silver nanoparticles obtained after 72 h of incubation. The micrograph showed nanoparticles with variable shape, most of the particles are monodisperse and mainly spherical in nature. The particles range from as low as 14 nm to as high as 39 nm (Plate 2). Majority of the silver nanoparticles were scattered with only a few of them showing aggregates of varying sizes as observed under TEM.

X-ray diffraction analysis

The XRD exhibited intense peaks in the whole spectrum of 2θ value ranging from 10 to 80 and this pattern was similar to the Braggs's reflection of silver nanocrystals. The Bragg reflections show samples of nanoparticles. The peaks were assigned to diffraction from the (111), (200), (220), (311) and (222) planes of face centered cubic (fcc) silver, which showed in good agreement with reference to the unit cell of the fcc structure with a lattice parameter of a = 4.077 A°. It was worth noting that the relative intensity of the 200 to 111 diffraction peaks of 0.50, 0.54 was higher than the conventional value (0.45). This result indicated that the synthesized silver NPs in our present study were enriched in {100} facets.

$L = k\lambda / \beta 1/2 \cos\Theta$ -------- (1)

k is the Scherrer constant, λ is the wavelength of the X-ray, β and Θ were the half width of the peak and half of the Bragg angle, respectively. Therefore, the size (L) of the particles could be easily estimated for about 15 nm.

FTIR analysis

The sample has shift of the bands from 3315.41 cm^{-1}, 3186.83 cm^{-1}, 2354.92 cm^{-1}, 1670.24 cm^{-1}, 1400.22cm^{-1}, 1332.72 cm^{-1}, 656.84 cm^{-1},752.19 cm^{-1}, 915.83 cm^{-}

[1],480.96 cm^{-1} is attributed to the binding of alkine strong and weak CH stretch, derivatives of C=C stretch, phenyl ring substitution overtones, chloroalkanes groups nanoparticles(Fig. 2).

EDX analysis

The energy dispersive X-ray analysis (EDX) (Fig 3) reveals strong signal in the silver region and confirms the formation of silver nanoparticles. Presence of oxygen indicates that the extracellular organic moieties were absorbed on the surface of metallic silver nanoparticles.

Antibacterial activity

The AgNPs exposure to six different test microorganisms resulted in formation of the zones of inhibition where as no zones were visible in the plant extract alone and DDW water with AgNO3. The efficient antibacterial property of AgNPs compared to the silver salts is due to their extremely large surface area, which provides better contact with the microorganisms.

Zone of incubation was measured in mm. *Pseudomonas aeroginosa* (25 mm), *klebsiella pneumonia* (14 mm), *proteus vulgaris* (17 mm), *Bacillus coagulans* (21 mm), *and Staphylococcus aureus*(27 mm).

S. aureus (Plate 3) shows better result when compared with other bacteria's. After that *P. Aeroginosa* was highly resistance to silver nanoparticles.

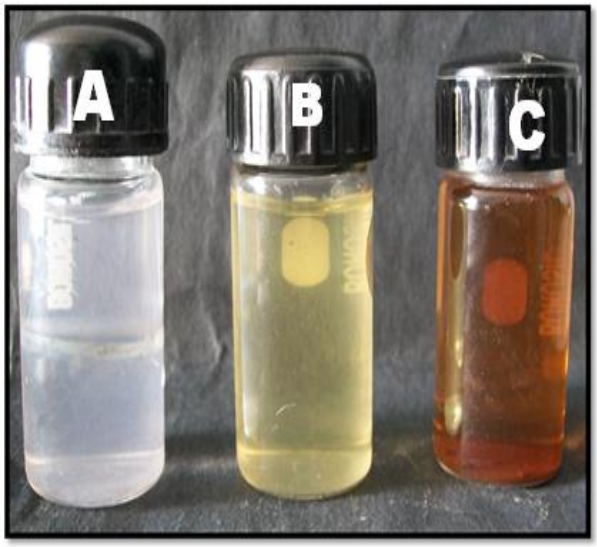

Plate 1: Phytoreduction of Silver Nanoparticles.

A - Aqueous Silver Nitrate solution
B - Plant leaf sample
C - Bio reduced Ag Nano ($AgNo_3$ + Leaf extract)

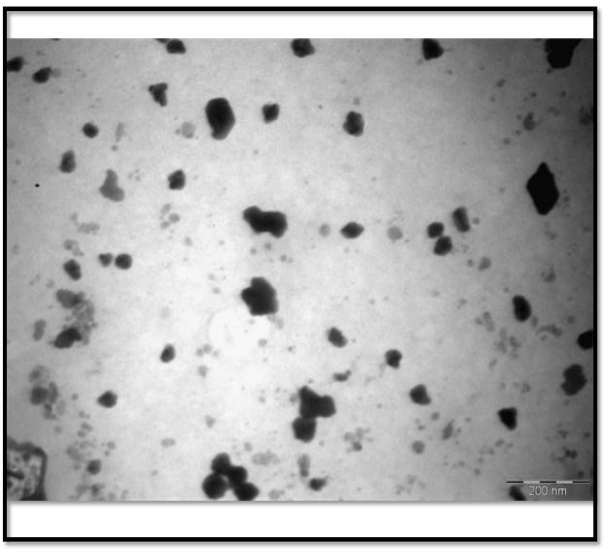

Plate 2: Image of Silver Nanoparticles synthesized using Leaf extract in TEM.

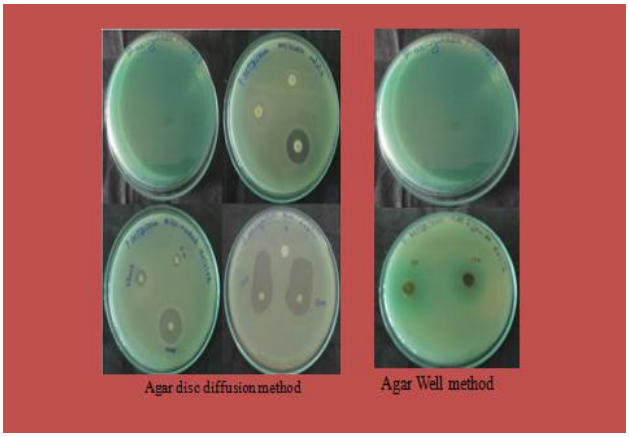

Plate 3: Antibacterial activity of Silver nanoparticles synthesized using leaf extract.

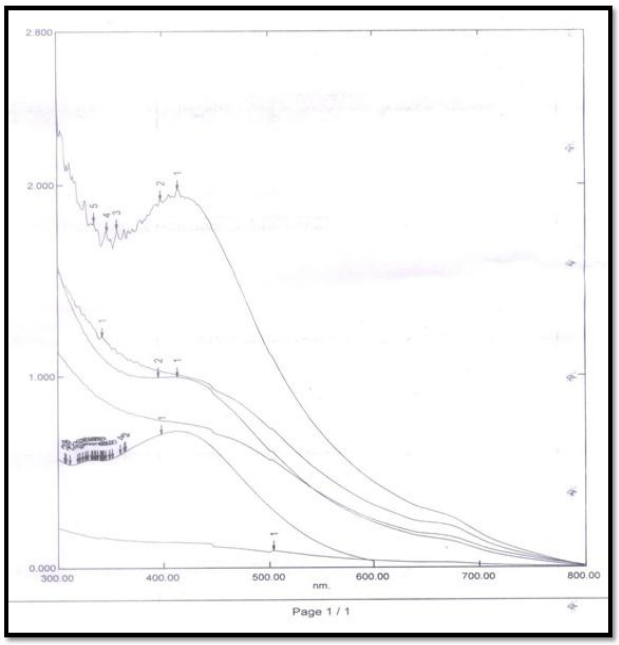

Fig. 1: UV-Visible analysis of extract of *P. zeylenica.*

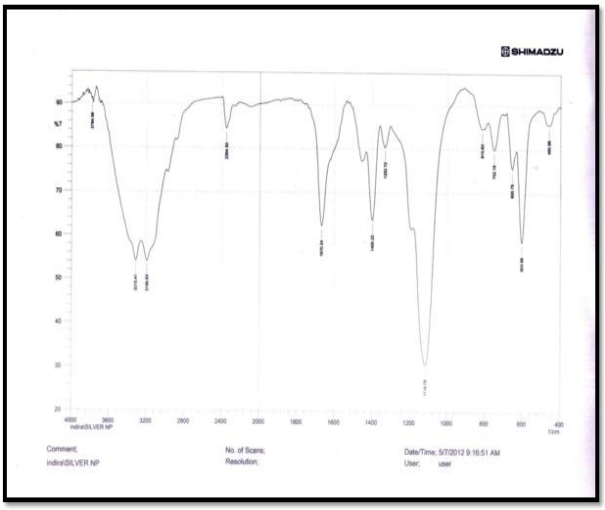

Fig. 2: FTIR analyses of Leaf extract synthesised Silver nanoparticles.

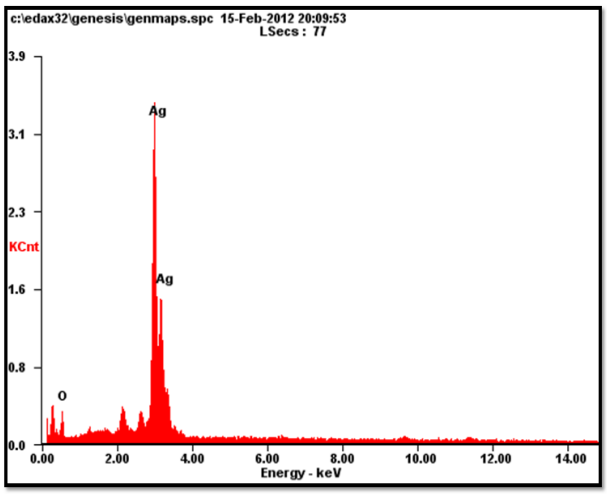

Fig. 3: EDX analysis of Leaf extract of *P. zeylenica* synthesised Silver Nanoparticles.

DISCUSSION

Silver nanoparticles by using the extract of *P. Zeylanica* at 24 h of incubation. Similarly, silver nanoparticles were synthesized using leaves extract of *A. indica*. Interestingly, silver nanoparticles were synthesized rapidly within 30 min of incubation period.[14] Cycas leaf mediated synthesis shows that the silver surface Plasmon resonance was observed at 449 nm, which steadily increased in intensity as a function of time of reaction.[15]

In present work, the particles range from as low as 14 nm to as high as 39 nm. Majority of the silver nanoparticles were scattered with only a few of them showing aggregates of varying sizes as observed under TEM. Silver nanoparticles formed were polydisperse, predominantly spherical with some nanotriangles in the range of 20-80 nm[16], some small particles in the regime of 10-20 nm are also present.[17]

FT-IR spectra of apiin extract shows bands at 1618 and 1718cm−1 along with an intense broad band at 3080cm−1. The two bands observed at 1618 and 1718cm−1were assigned to the stretching vibrations of the C-C bond and C-O group, respectively, while the peak observed at 3080cm−1 corresponds to stretching vibration of –OH bond.[18]

EDS spectrum showed high for silver signals. The vertical axis shows the counts of the X- ray and the horizontal axis shows energy in keV. The strong signals of silver correspond to the peaks in the graph confirming presence of silver nanoparticles.[19]

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