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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM *HAMELIA PATENS* AQUEOUS LEAF EXTRACT

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ABSTRACT

The current study reports a safe method of synthesis of silver nanoparticles (AgNPs) from aqueous leaf extract of *Hamelia patens*.a medicinally important plant. The initial confirmation of biosynthesis of silver nanoparticles were characterized by UV–Vis spectrophotometer and the characteristic surface plasmon resonance peak was identified to be 435 nm. The AgNPs were observed to be roughly spherical in shape and size ranged 10–100 nm as seen in TEM analysis. X-ray diffraction (XRD) was employed to ascertain the crystalline nature and purity of the silver nanoparticles which implied the presence of (111) and (220) lattice planes of the face centered cubic (fcc) structure of metallic silver. Fourier transform infrared spectroscopy (FTIR) was used to key out the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the leaf extract. The synthesized AgNPs showed strong inhibitory activity against microorganisms like *Salmonella ebony* (MTCC 3384), *Bacillus subtilis*(MTCC 10619), *Klebsella pneumonia*(MTCC 432), *Pseudomonas aeruginosa*(MTCC 1688). These findings would find applications in the development of new antibacterial drugs.

INTRODUCTION:

Hamelia patens is a large perennial shrub in the coffee family, Rubiaceae, that is native to the American subtropics and tropics.The plants are used in folk medicine against a range of ailments. A number of active compounds have been found in *Hamelia patens*, but no scientific study of its medical usefulness has been conducted yet. People in Tropical America use extracts of the leaves and stems to treat various types of skin diseases such as rashes, skin fungus, sores and insect stings. Other ethno botanical uses include treatment for menstrual cramps, headache, rheumatism, fever, and dysentery. Effective immuno-stimulants have been identified in *Hamelia patens* extracts and studies with rats have shown that firebush has analgesic, diuretic and hypothermic activities. Nanoparticles have found lot of application in biology area. Silver nanoparticles (AgNPs) have been studied extensively as they exhibit different properties of importance such as biological physical, optical absorbance and chemical. These properties have created an opportunity for application in different industries and medical fields for drug delivery, preparation of medicines etc. The ratio of surface area to volume is more in silver Nanoparticles hence it has increased the interest of scientists. Silver Nanoparticles can be synthesized by chemical and physical methods.The chemical approaches involves the use of

reductants which are toxic in nature such as sodium borohydride, trisodium citrate and dimethyl formamide. There is an increasing need to develop an eco-friendly and nontoxic AgNPs in which the process is cost-effective and requires no high end equipments. Biosynthesis of nanoparticles using various plant part extracts gives approximately uniform size of the nanoparticles and they donot contain any contaminants and it can be scaled up (Mittal et al., 2014). Plant extracts have various primary and secondary metabolites which act as both reducing and capping agents (Tavakoli et al., 2015). The synthesized AgNPs of *Hamelia patens* leaf extracts were characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM) and X-ray diffraction method (XRD). The antimicrobial activity of the AgNPs was evaluated against human pathogens such as,*Salmonella ebony*(MTCC 3384), *Bacillus subtilis*(MTCC 10619), *Klebsella pneumonia*(MTCC 532), *Pseudomonas aeruginosa*(MTCC 1688) to check for their biomedical importance. Survey of Literature showed no reports on synthesis of silver nanoparticles using the aqueous leaf extract of *Hamelia patens*. Therefore, the objective of the present study was to synthesize and characterize the silver nanoparticles using aqueous leaf extract of *Hamelia paten* and screen for antibacterial activity using crude aqueous leaf extract and synthesized silver nanoparticles.

MATERIALS AND METHODS

Collection of plant sample: Fresh leaves of *Hamelia patens* were collected from Idupulayapaya region of Y.S.R.Kadapa district,Andhra Pradesh. Leaves were washed thoroughly with tap water and then followed with Distilled water and shade dried.

Preparation of the plant sample and its leaf aqueous extract: The dried leaves of *Hamelia patens* were ground finely. 20gms of the leaf powder was taken in to thimble made of Watman filter paper No.1 and taken into Soxhlet(Techinico) for extraction with MilliQ water used as solvent. The collected extract was further concentrated using Rotavapour (Heidolph) and this concentrated filtrate was used for the present study.

Synthesis of silver nanoparticles: Biological synthesis of AgNPs was carried out by preparing 1 mM aqueous solution of silver nitrate ($AgNO_3$)(purchased from Molychem). 20 ml of aqueous *Hamelia patens*.L leaf extract was added to 180 ml of aqueous 1 mM silver nitrate solution for reduction of Ag+ ions .This was left overnight at room temperature (Vivek R *et al*. 2012).

Bio-reduction mechanism: The biochemical reaction of AgNO3 reacts with plant broth leads to the formation of AgNPs by following reaction (Tripathy et al., 2010). Fig. 2 explains the proposed mechanism of biological synthesis of nanoparticles

$Ag^{+}NO3^{-}$ + plant extract ⟶ AgNPs + byproducts

Characterization of synthesized silver nanoparticles: The synthesis of silver nanoparticles by reduction of silver was monitored using UV-visible spectrometer, at the wavelength of 200–700 nm. The nano particle formed in plant extract solution was centrifuged at 8000 rpm for 10 min. The supernatant was again centrifuged at 10,000 rpm for 20 min, and the pellet of silver nanoparticles was obtained. The pellet were resuspended into 1 mL of deionized water . Characterization of nanoparticles was carried out in the mid IR region of 400–4000 cm−1 by Fourier transform infrared spectroscopy (FTIR) analysis. X-ray diffraction (XRD) analysis was carried out to study the nature of AgNPs. X-ray diffractometer (Bruker, D8 advance, Germany) was operated at a voltage of 40 kV and a current of 30 mA at a wave length of 1.5406Å. Elemental Detection Analysis(EDA) was used to detect the presence of elemental silver. Transmission Electron Microscope was used to observe image and size of Silver Nanoparticles (Santhosh S. B. *et al.* 2015).

ANTI BACTERIAL ACTIVITY ASSAY

The pure cultures of *Salmonella ebony* (MTCC 3384), *Klebsiella pneumonia* (MTCC 432), *Bacillus subtilis* (MTCC 10619) and *Escherichia coli* (MTCC 443) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. All the cultures were revived aseptically in freshly prepared Nutrient broth medium and incubated at 37°C for 18-24 hrs for bacterial growth as per instructions. The antimicrobial activity was carried out with 24 h active cultures by employing disc diffusion method (Ghassan *et al*. 2013). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hours old broth culture of human pathogenic bacterial strains. Sterile discs impregnated with 20 µl of AgNPs solution at a concentration of 100µg/ml were then placed on the surface of the inoculated medium. Standard antibiotic ampicillin was used as a positive control. The agar plates were incubated at 37°C for 24 h. The diameter of the inhibition zone (mm) was measured after 24hrs of incubation. Triplicate plates for each condition and the experiment was repeated thrice.

RESULTS AND DISCUSSION

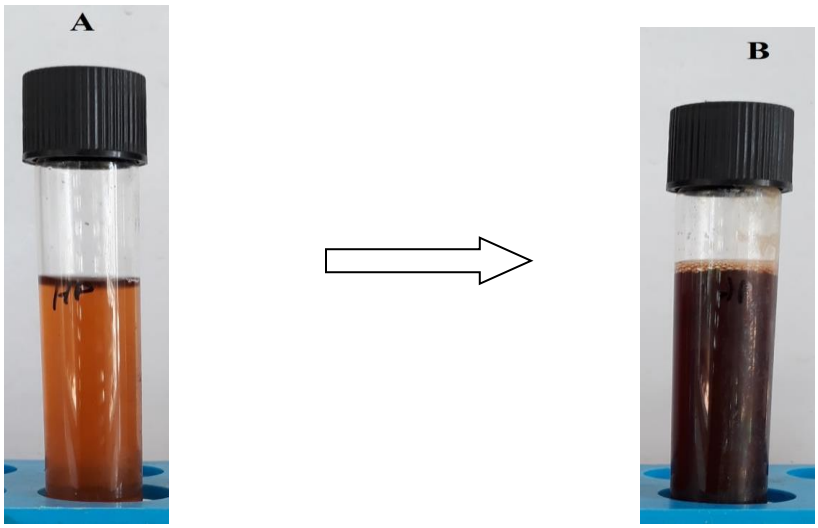
synthesis of silver nanoparticles: Light brown aqueous leaf extracts of *Haemelia patens* on addition to aqueous silver nitrate solution changed colour from light brown to dark brown with overnight incubation period(Fig.1).The change in color is because of excitation of surface plasmon resonance in silver nanoparticles (Veerasamy *et al*., 2011). The change in coloration of plant extract is due to formation of silver nanoparticles. Spectra of Plasmon resonance band observed at 435 nm similar to those reported in literature (Obaid *et .al*.2015).

UV Analysis: It is generally recognize that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions.There is increase in intensity of absorption peaks and the colour intensity increased with the duration of incubation. The UV–Vis absorption spectra of the AgNPs showed the absorbance Surface plasmon resonance(SPR) peak at 435 nm, which confirms the synthesis of AgNPs(Fig.2a,2b). Similar results have been reported in literature showing absorbance at 445 nm of silver nanoparticles synthesized by *Cochlospermum religiosum* extract (Sasikala *et.al* 2014) and by *Pithophoraoe dogonia* extract (Sinha *et.al*, 2014).

The different molecules present in the aqueous plant extracts help in capping and stabilization of AgNPs formed. This characteristic SPR peak also confirms the spherical shape of AgNPs (Kotakadi *et al*. 2014)

FTIR Analysis: The bands at wave number of 3272.32 cm-1, 1639.9cm-1, 1327.31cm-1 and 1095.4cm-1 correspond to N–H stretching of the secondary amide, carboxyl –C=O stretching, C–N stretching and C–O stretching respectively respectively (Fig.3). FTIR results show the presence of different functional groups on the nanoparticles surface which indicates that stability of indicating the potential of *Hamelia patens.*L.,leaf aqueous extract in synthesizing AgNPs. The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticle. From FTIR results, it can be concluded that some of the natural compounds from *Haemelia paten* extract formed a strong coating/capping on the nanoparticles.

XRD Analysis: The XRD analysis of the synthesized AgNPs showed four distinct diffraction peaks at 38.26, 44.33,64.53and 77.62 (Fig.4) corresponding to (111), (2 2 0) and (3 1 1) lattice planes of the face centered cubic (fcc) lattice of silver. This also revealed the crystalline nature of AgNPs. The mean average size of the particle was calculated as 22nm using the f Deseherrer formula (Avg D = $0.9\lambda/\beta\cos\theta$).



A. *Hamelia patens* aqueous leaf extract (Light Brown colour)

B.*Hamelia patens* Synthesized AgNps (Dark Browncolour)

Fig.1 Synthesis of Nanoparticle synthesis from aqueous *Hamelia patens* leaf extract in presence of Silver nitrate solution. A. Initial Light Brown colour of plant extract B. Dark Brown colour formed after synthesis of silver nano particles.

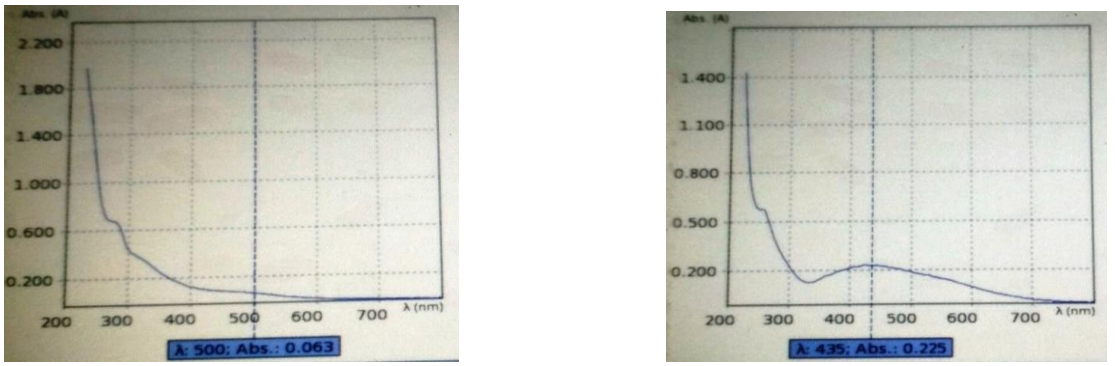


Fig.2 (a)*Hamelia patens* Aqueous Leaf extract (b) Synthesized Silver nanoparticles

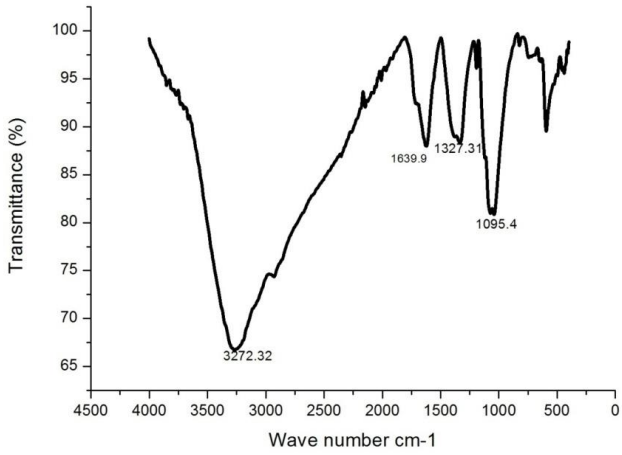


Fig.3 FTIR spectra of Silver nanoparticles produced by Leaf extract of *Hamelia patens*

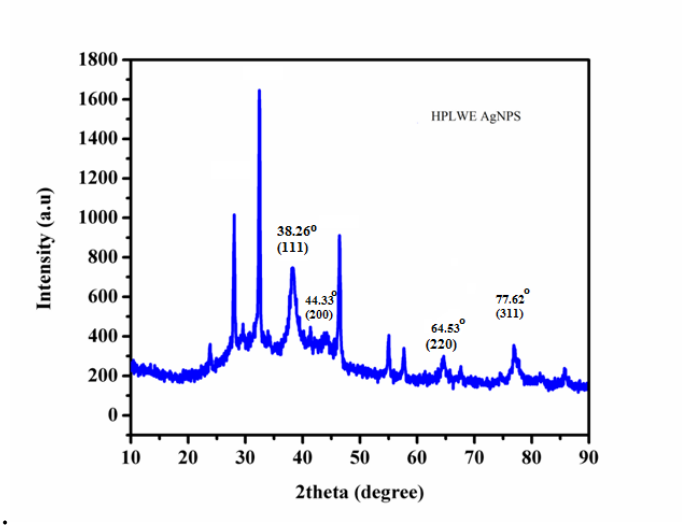


Fig.4. The XRD pattern of Silver nano particles synthesized using aqoeous leaf extract of *Hamelia patens*

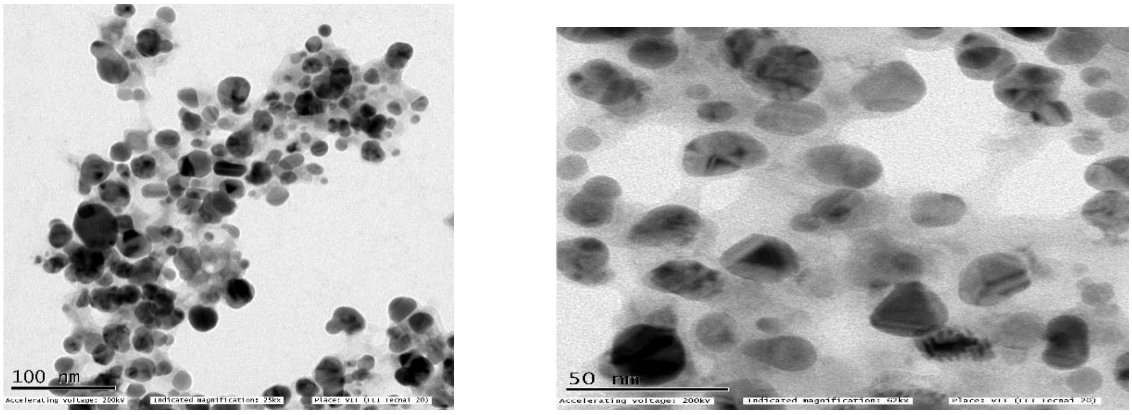


Fig.5. TEM micrograph of *Hamelia patens* Silver nanoparticles size ranging 100nm and 50nm

ELEMENTAL DETECTION ANALYSIS

Elemental Detection Analysis (Fig.6.) shows the constituents of the synthesized nanoparticles, containing Ag,C and Cu in the sample(Table1.)

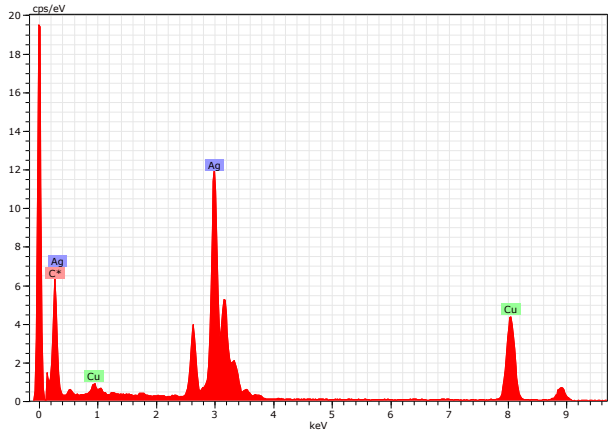


Fig.6. EDA showing the chemical constituents of the Synthesized Nanoparticles

Spectrum: Hamelia patens aqueous leaf extracts AgNPs

Element	Series	Net unn. [wt.%]	C norm. [wt.%]	C Atom. [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	16804	49.32	49.32	88.29	4.66
Copper	K-series	30997	11.59	11.59	3.92	1.14
Silver	K-series	8887	39.09	39.09	7.79	3.85
	Total:		100.00	100.00	100.00	

Table.1 Showing the % of elements found in the AgNPs

TEM Analysis: Transmission electron microscopy (TEM) has been used to identify the size, shape and morphology of nanoparticles. It reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, with size ranging from 10-100mm while some of the NPs were found to be having structures of irregular shape as shown in Fig. 5. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UV-visible spectrum. The TEM results are consistent with many earlier reports (Tran et al. 2013; Salprima 2013).

ANTIMICROBIAL ASSAY WITH SILVER NANOPARTICLES

Antimicrobial activity:

The anti bacterial properties of Silver are well known since ancient times. Silver nanoparticles have found wide applications industries such as health, textile, food and also used in preparation of skin ointments (Gao *et al*., 2014). The synthesized silver nanoparticles obtained from *Haemelia patens* extract were screened for antibacterial activity against human pathogens (*Salmonella. ebony, Bacillus subtilis, klebsiella .pneumonia* and *Peudomonas. Aeruginosa).* The anti bacterial activity of crude plant extacts were found to be less compared to the silver nanoparticles as seen by zones of inhibition(Fig.7.) and in Table1. The zone of inhibition was maximum for silver nanoparticles against *Pseudomonas aeruginosa(*4.13±0.08mm), followed by *Salmonella ebony (*3.56±0.12mm) and *Klebsella pneumonia(*3.34±0.08mm) and least was for *Bacillus subtilis(*2.9±0.1mm) From the zone of inhibition produced, synthesized silver nanoparticles show good antibacterial activity against *Pseudomonas aeruginosa* and *Salmonella ebony*. Though *Haemelia patens* being a medicinally important plant the plant extract alone shows very low activity this could be due the solvent in which it is extracted as well as lower concentration used during experimentation. The silver nanoparticles showed better antimicrobial property compared to plant extract this is due to their extremely large surface area which helps in close contact with cell wall of microorganisms (Ibrahim, 2015).

CONCLUSION

Biosynthesis of Silver nanoparticles using *Hamelia patens* leaf extract is reported for the first time. The plant metabolites induce the production of metallic nanoparticles in ecofriendly manner.The plant mediated nanoparticles have the potential to be used in various fields such as pharmaceuticals, therapeutics, sustainable and renewable energy and other commercial products.

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