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Production of nanodrug for *Bacillus cereus* isolated from HIV positive patient using *Mallotus philippensis*

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ABSTRACT

The present investigation was aimed to synthesis of silver nanoparticles (AgNPs) using *Mallotus philippensis* leaf extract and their antibacterial potential against *Bacillus cereus* isolated from HIV positive patient. In this, UV- Visible spectroscopy showed the high peak of absorption band at 450 nm. Based on XRD analysis, face centered cubic structure and average size of the AgNPs was around 16 nm. FTIR spectroscopy study revealed the seventeen functional groups of the AgNPs was observed. The morphology of AgNPs was spherical, oval shapes and diameter of the particle size ranges between 9 and 24 nm was measured using transmission electron microscopy (TEM). In addition to these green synthesized AgNPs were found to express the higher efficacy in inhibiting the growth of *Bacillus cereus (B. cereus)* isolated from the HIV-positive patient.

1. Introduction

Nanotechnology is an emerging field of design, fabrication, characterization and application of the particles at nanometer size (Krumov et al., 2009; Shah & Tokeer Ahmad, 2010; Yang, Lipowsky, & Dimova, 2009). It is an interdisciplinary area which, involves the physics, chemistry and biological fields of inventions at the nano level (Mohanpuria, Rana, & Yadav, 2008; Parashar, Saxena, & Srivastava, 2009). Silver ion has been used as a great potent antimicrobial agent against various human pathogenic organisms. For the last decades, the nanosilver particles are being used in the field of medicine. Synthesis of AgNPs using chemical approach is having some certain limitation which may overcome by biological methods of synthesis. Therefore, there is a need for developing various conventional methods includes biosynthesis (or) green synthesis of AgNPs (Krutayokav, Kudrinskiy, Olenin, & Lisichin, 2008) using plant diversity. Antibacterial activity of plant extract showed different inhibitory activity against the isolated opportunistic bacterial pathogen (Gudrun et al., 2013; Hanan, El-Kalek, & Mohamed, 2012; Muhammad, Samreen, Karam, & Muhammad, 2013; Sohail Zafar et al., 2014). *Mallotus philippensis* (Euphorbiaceae) is an ethanomedicinal plant and it

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is widely used for parasitic infections, aphrodisiac, skin infections and wound healing. Phytochemical of the plant is used to treat diarrhea, mouth inflammation, throat and injured skins (Idris, Ndukwe, & Gimba, 2009; Srivastava, Shankar, & Gupta, 2010). *Bacillus cereus* is a pathogenic bacterium, which produces hemolysin, enterotoxin and cytotoxin (Akinjogunla & Adegoke, 2009; Nataro et al., 1987; Smith et al., 2003). Among the toxins, one is diarrhea causing agent and another emetic toxin to vomiting. Human Immunodeficiency Virus (HIV) is the aetiological agent of the acquired immunodeficiency syndrome. Many authors have investigated the antibacterial activity of various medicinal plants extract against pathogenic bacteria, which (were) isolate from HIV positive parents (Georges & Georges-Courboj, 1990; Navaladian, Viswanathan, Varadarajan, & Viswanath, 2008; Prescott, Harley, & Klein, 1999; Talaro & Talaro, 1996). However, very few attempts might be done in antibacterial activity of biosynthesized silver nanoparticles against pathogenic bacteria isolated from HIV positive patients. Hence, the present study has aimed to biosynthesis, characterization and antibacterial potential of synthesized AgNPs using *M. philippensis* leaf extract. In addition, there is no report on synthesis, characterization and bioassay of AgNPs using *M. philippensis* leaf extract.

2. Materials and methods

The plant leaves of *M. philippensis* were collected from Alagar hills, Madurai and identified by the Rabinet Herbarium, St. Joseph's College, Trichy. The herbarium specimen is maintaining at Department of Botany, J. J. College of Arts and Science, Pudukkottai. The leaves were washed in running water and rinsed with double distilled water to remove the exogenous materials and shade-dried at room temperature for 10 days. The fully dried leaves were powdered with a sterile electric blender. The powdered samples were preserved in an airtight container and away from the sunlight for further use. Qualitative phytochemical screening of the plant aqueous leaf extracts was performed (Abou El-Nour, Eftaiha, Al-Wart, & Ammar, 2010). Two grams of leaf powdered was mixed with 100 ml of de-ionized sterile water. The mixture was heated up to 100 °C for 30 min and the mixture was filtered by Whatman No.1 filter paper, finally the residue was re-extracted using vacuum pumps. Silver Nitrate ($AgNO_3$) stock solution was prepared by dissolving 6.299 g of $AgNO_3$ in 100 ml of de-ionized water. 2 ml of leaf extract was mixed with 20 ml of $AgNO_3$ solution and kept at room temperature (Song & Kim, 2009). Nanoparticles synthesis was read by the absorption spectrum of the reaction mixture at room temperature with different time intervals using UV–Vis spectrophotometer (Hitachi-U-2001) from 300 to 800 nm at 1 nm resolution. The residue containing silver nanoparticles solution was dispersed in sterile de-ionized water to remove the biological impurities. The pure residue was dried in oven at 70 °C overnight. For study of functional groups of synthesized nanoparticles was performed using Fourier Transform Infrared (FTIR) spectroscopy measurement. The study sample was prepared by freeze-dried and diluted with potassium bromide in the ratio of 1:100 and recorded in Perkin–Elmer instrument, resolution of 4 cm^{-1} in the transmission mode of 4000–400 cm^{-1}. For the XRD pattern, the silver nanoparticles solution was dropped on glass on XPERT-PRO, D-8, with 30kv, 40 mA with Cu kα radians at 2θ angle and used for phase identification of a crystalline material and can provide information on unit-cell dimensions. The crystallite size was calculated from the width of the XRD peaks and using the Scherrer's formula, $D = K\lambda/\beta\cos\theta$. The size distribution and shape of NPs was estimated based on TEM micrographs. For the TEM study, the sample was placed over a carbon tape and dried. A pinch of dried sample was coated with a thin layer of copper coated with copper grid and the microscopy was taken by using JEM 1011, JEOL, Japan. The antibacterial activity of green synthesized AgNPs and the *M. philippensis* leaf extract was tested against *B. cereus* isolated from HIV-positive patient by Kirby Bauer disk diffusion method (Bauer, Kirby, Sherris, & Turck, 1966). The bacterial strain was swabbed uniformly on Muller Hinton Agar (MHA) plates using sterile cotton swabs. Sterile filter paper disks (8 mm diameter) were separately impregnated with 5, 10, 20, 40 and 80 mg/ml of biosynthesized AgNPs and *M. philippensis* leaf extracts separately. The Chlorophenicol was used as positive control against the pathogen. The inoculated plates were incubated at 37 °C for 24 h and after the incubation time, the diameters of the inhibitory zones were measured in millimeter.

3. Results and discussion

The phytochemical investigation of the *E. agallocha* was performed. The aqueous extract which contained alkaloids, saponin, phenol, tannin and flavanoid (Deepa & Padmaja, 2014; Jayanta Kumar, Tapan, Sakti, & Nabin., 2009). The mixture of silver nitrate ($AgNO_3$) and *M. philippensis* leaf extract color was changed from colorless solution to dark brown in color which indicates the formation of AgNPs. There was a color change from colorless to yellow when the plant crude extract mixed with AgNO3, after the incubation period the yellow color was changed in dark brown in color, which designates the formation of AgNPs. The color change was a result of a formation of AgNPs and the maximum absorption peak observed at 450 nm without any shifting, which designates the Surface Plasmon Resonance (SPR). The SPR band increased intensity when the reaction time increases with time intervals without peak wavelength shifting (Fig. 1) (Mohd & Suhail, 2014). The XRD pattern clearly indicated the crystalline nature of synthesized AgNPs. The spectrum peak 2θ values (35.89°, 40.30°, 64.52° and 76.09°) were corresponding to the plane values (100), (102), (220) and (114) respectively. So, based on the pattern of the particles was face centered cubic crystal structure. The highest degree of crystallinity was observed at 64.52° (Fig. 2) and the results also supported by Shameli et al. and Sanjenbam et al. The average particle size of AgNPs can be calculated using Debye-Scherrer's formula,

$$D = \frac{K\lambda}{\beta\cos\theta}$$

Where D is the particle diameter size, K is a constant equal to 1, λ is X-ray wavelength (0.1541 nm), β is full width

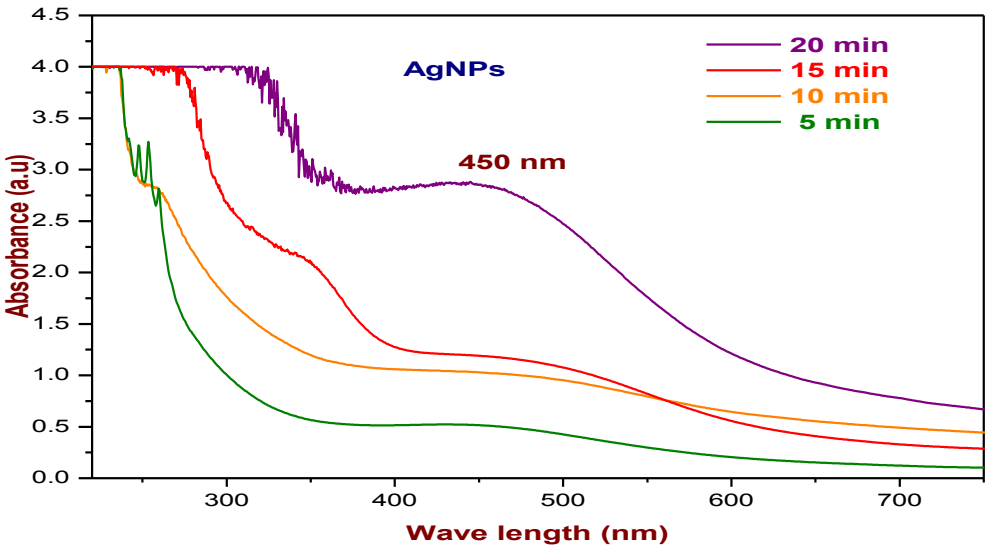

Fig. 1 – UV–Vis spectrum of biosynthesized AgNPs and its plasmon excitation upon the interaction by *M. philippensis* leaf extract with different time intervals.

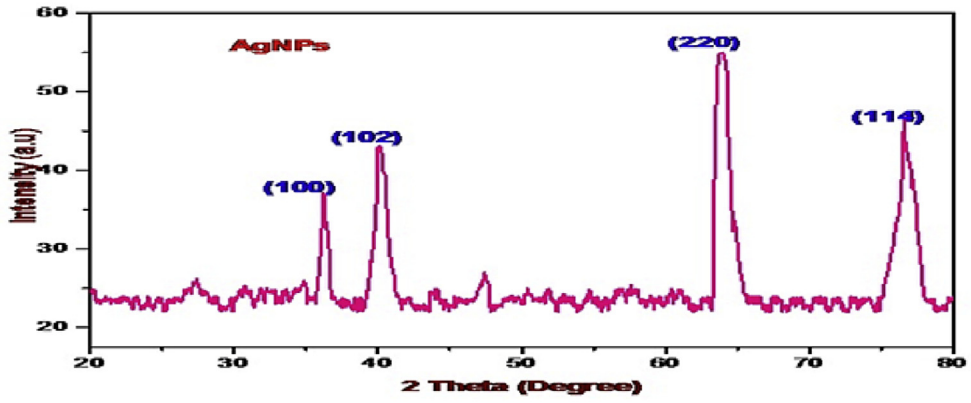

Fig. 2 – XRD pattern of synthesized AgNPs using *M. philippensis* leaf extract.

half maximum (FWHM) and θ is the diffraction angle. The size of the particles ranged between 9.35 and 23.57 nm and average size of the particle is found to be around 16 nm. FTIR spectrum was done to identify the possible biomolecules responsible for reducing and capping of bioreduced AgNPs. The major peaks in the FTIR spectrum of AgNPs (Fig. 3) were observed at 3777.15 cm^{-1} (medium N–H Stretching), 3170.26 cm^{-1} (medium bond, O–H stretching, carboxylic acids group), 2829.15 cm^{-1} (medium bond H–C=O; C–H stretching alkanes group), 2616.71 cm^{-1} (very strong bond, C≡N stretching, nitriles group), 2123.19 cm^{-1} (weak bond, –C≡C– stretching, alkanes group), 1608.13 cm^{-1} (medium bond, N–H bending, amines group), 1517.95 cm^{-1} (strong bond, N–O assymmtric stretching, nitrocompounds group), 1408.86 cm^{-1} (medium bond, C–C stretching, aromatics group), 1331.24 cm^{-1} (strong bond, C–N stretching, aromatic amines group), 1112.59 cm^{-1} and 1031.25 cm^{-1} (medium bond, C–N stretching, aliphatic amines group), 895.86 cm^{-1} (strong N–H bending, amines group), 775.21 cm^{-1} (medium bond, C–Cl stretching, alkyl halides group), 695.76 cm^{-1} (strong C–H bending, alkynes group), 606.77 cm^{-1} (medium bond, C–Br stretching, alkyl halides group) and 503.86 cm^{-1} (strong COO- stretching) respectively (Bhumi, Linga Rao, & Savithramma, 2015; Marcato et al., 2015; Metuku et al., 2014). The morphology and size of the synthesized AgNPs were determined by transmission electron microscopy (TEM) study. The TEM image (Fig. 4) of AgNPs was spherical and oval in shape with ranges between 9 and 24 nm. *In vitro* antibacterial activity of aqueous leaf extract of *M. philippensis* and synthesized AgNPs was done using Kirby Bauer agar diffusion method. Antibacterial activity of AgNPs was measured by the diameter of the zone of inhibition (mm) against *B. cereus* in various concentrations (5, 10, 20, 40 and 80 mg/ml) of AgNPs solution and *M. philippensis* leaf extract. The Table 1 shows the zone of inhibition of synthesized AgNPs ranged from 11.0 ± 0.36 to 27.0 ± 0.45 while, the mean zone of inhibition of the *M. philippensis* leaf extracts ranged from 4.0 ± 0.35 to 12.0 ± 0.40 against *B. cereus*. The maximum zone of inhibition (27 mm) expressed at 80 mg/ml of AgNPs where as the leaf extracts showed 12 mm in 80 mg/ml. The positive control also indicated 23 mm at 80 mg/ml. Overall, AgNPs exhibits

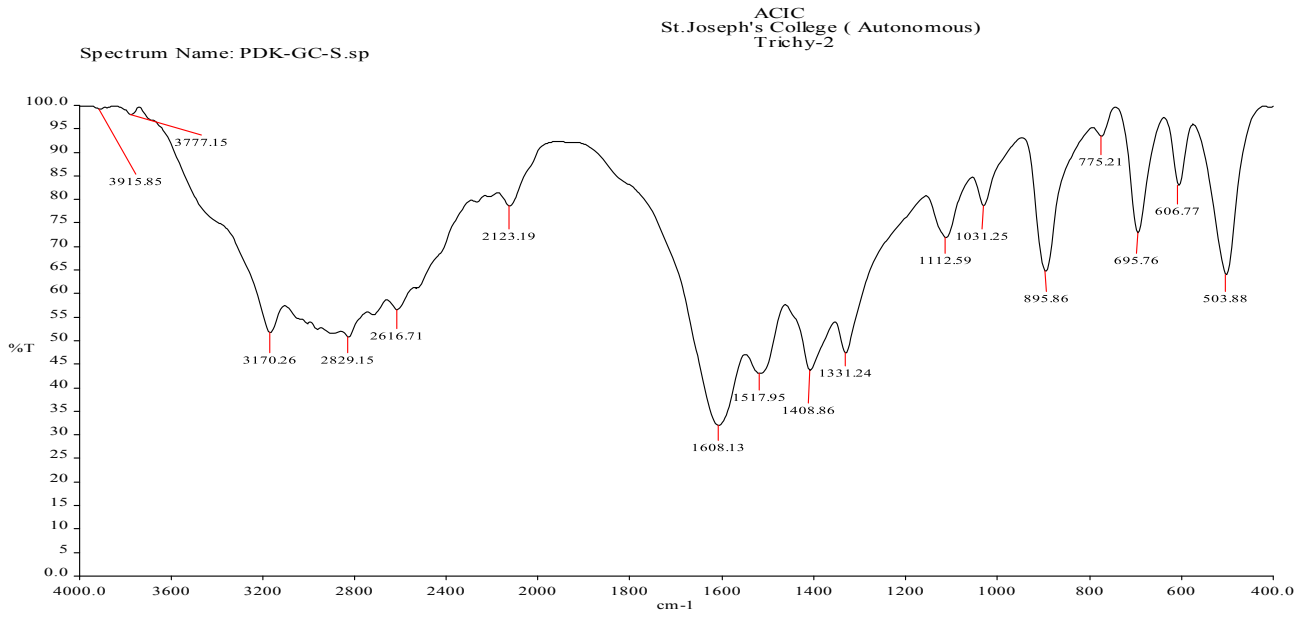

Fig. 3 – FTIR spectrum of green synthesized AgNPs using *M. philippensis* leaf extract.

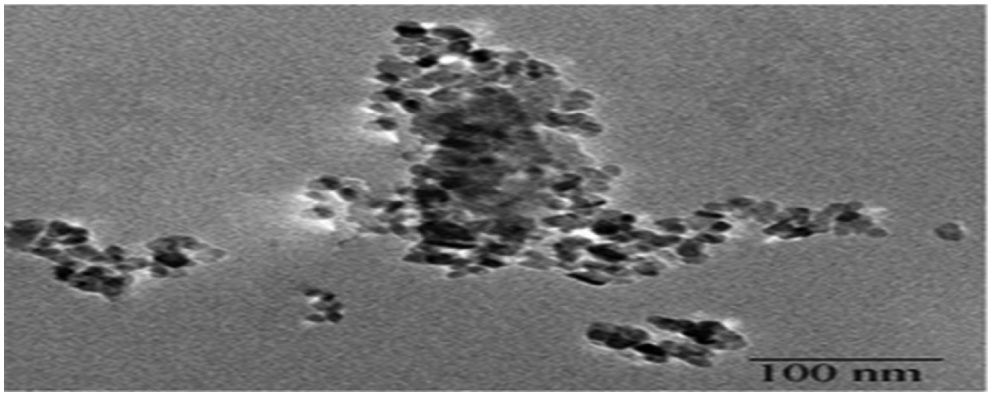

Fig. 4 – TEM image analysis of biosynthesized AgNPs using *M. philippensis* leaf extract.

Table 1 – Antibacterial activity of AgNPs and leaf extract against *B.cereus* isolated from HIV-positive patient.

Sl.No.	Different concentrations	Zone of inhibition in plant extract	Zone of inhibition in AgNPs	Positive control chloramphenicol
		Mean ± Standard deviation		
1.	5 mg/ml	4.0 ± 0.35	11.0 ± 0.36	10.21 ± 0.42
2.	10 mg/ml	6.0 ± 0.25	12.0 ± 0.15	12.5 ± 0.24
3.	20 mg/ml	8.0 ± 0.43	14.0 ± 0.31	14.5 ± 0.35
4.	40 mg/ml	10.0 ± 0.43	19.0 ± 0.29	21.01 ± 0.24
5.	80 mg/ml	12.0 ± 0.40	27.0 ± 0.45	23.0 ± 0.36

higher antibacterial activity by attaching the bacterial wall. Since, the bacterial plasma membrane is the site of respiratory chain components and transport of molecules and ions. Here, the changes in the bacterial membrane system by contacting the AgNPs would ultimately result in inhibition of pathogen growth (Gopinath et al., 2012; Jayachandra, Rani, Arvind Kumar, & Sudha, 2014). Hence, the synthesized nanoparticles expressed a higher zone of inhibition. Here, the phenomenon is well known that attributed to the large surface area and size of synthesized AgNPs plays a vital role in their interaction with bacterial cells.

4. Conclusion

We conclude that the bio-synthesis, characterization and antibacterial activity of AgNPs against *B. cereus* isolated from HIV-positive patients using *M. philippensis* leaf extract.

Phytochemical screening of aqueous leaf extract of *M. philippensis* showed many phytochemical constituents availability. The biosynthesized AgNPs exhibited excellent antibacterial activity against the isolated opportunistic bacterial pathogens from HIV patient. Hence, this study report that biosynthesized AgNPs will create social awareness in the field of drug design. Further studies are required to fully characterize this organism in a zone of inhibition from biosynthesized AgNPs.

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