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Biosynthesis of Silver nanoparticles using *Plumbago zeylanica* Leaves extracts and their bio-efficacy

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The present study was intended to optimize the protocol for the synthesis of silver nano particles of *Plumbago zeylanica* Linn. leaves extracts and evaluate their bioefficacy against six different human pathogens. The aqueous silver ions exposed to the leaves extracts and the silver nanoparticle synthesis was established by the change of colour of leaves extracts from greenish to reddish brown. The *in vitro* synthesised silver nanopeptides were further confirmed by using UV-Vis spectroscopy and examined their antibacterial activity against *S. aureus*, *B. subtilis*, *S. pyogenes* (Gram positive), *K. pneumoniae*, *M. morganii*, *P. aeruginosa* (Gram negative). To find the inhibition concentrations, the antibacterial activity was performed by agar well diffusion method with five different concentrations (10 – 50 µg/ml) against six different pathogens viz., *S. aureus*, *B. subtilis*, *S. pyogenes* (Gram positive), *K. pneumoniae*, *M. morganii*, *P. aeruginosa* (Gram negative). The zone of inhibition was directly consonance with the concentrations of *P. zeylanica* SNPS. 50 µl of *P. zeylanica* SNPs showed maximum zone of inhibition (25±0.5 mm) against *K. pneumoniae*, next to that, 23±0.5 mm zone of inhibition was observed against *M. morganii* and minimum zone of inhibition (9 ± 0.3 mm) was obtained aginst *S. pyogenes*. The results revealed that the AgNP's extract of *P. zeylanica* showed higher zone of inhibition against Gram negative bacteria than the Gram positive bacteria.

Keywords: Anti-bacterial; Silver nanoparticles; *Plumbago*.

Introduction

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment (Armendariz *et al.* 2004). Bio-nanotechnology has emerged as integration among biotechnology and nanotechnology for developing biological synthesis [illegible] hnology can

A number of nanoparticles based therapeutic have been accepted clinically for infections, vaccines and renal diseases (Malhotra, 2010). Silver nanoparticles have a great potential for use in biological including antimicrobial activity (Sap-Iam *et al.* 2010). Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices (Jones *et al.*, 2010). Silver nanoparticles are used as antimicrobial agents in most of the public places such as elevators and railway stations. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess anti-fungal, anti-inflammatory, anti-angiogenic and anti-

permeability activities (Kalishwaralal *et al.* 2009; Gurunathan *et al.* 2009; Sheikpranbabu *et al.* 2009). Primarily, silver nanoparticles are considered as an alternative to silver ions (obtained from silver nitrate), which were used as antimicrobial agents. Colloidal metal particles can be obtained by chemical synthesis but this method use toxic chemicals in the synthesis protocol, which raises great concern for environmental reasons (Lee and Jeon, 2004). Hence the present study is aimed to synthesis silver nanoparticles from leaves extract of *P. zeylanica* using silver nitrate. Further the synthesized silver nanoparticles were applied to act against common pathogens.

Materials and methods

Synthesis and characterization of silver nanoparticles

The aqueous leaves extract of *P. zeylanica* was used for the synthesis of AgNP's. Exactly 17 mg of $AgNO_3$ was dissolved in 100 ml distilled water (10^{-3}M) *P. zeylanica* leaves extract was added to $AgNO_3$ solution in 1:10 ratio for reduction of Ag^+ ions. After reduction incubated solution was centrifuged at 10,000 rpm for 15 min.

UV-Visible Spectral Analysis

The supernatant containing AgNP's was analyzed spectroscopically for further confirmation. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectra of the solution at 400- 450 nm using Shimadzu spectrophotometer and the characteristic peaks were detected.

Antibacterial assay

Preparation of the test organisms

Staphylococcus aureus (MTCC 737), *Streptococcus pyogenes* (MTCC 1928), *Bacillus aureus* (MTCC 6633), *Klebsiella pneumoniae* (MTCC 109), *Morganella morganii* (MTCC 662) and *Pseudomonas aeruginosa* (MTCC 1688) were commercially purchased from Institute of Microbial Technology, Chandigarh, India. Stock cultures of different bacteria were grown in nutrient broth at 30°C and were sub-cultured and maintained in nutrient broth at 4°C. Before swabbing, each culture was diluted (1:10) with fresh sterile nutrient broth.

The antibacterial activity was determined by the agar well diffusion method (Parekh and Chand, 2007). The Muller Hinton agar medium was prepared, sterilized and transferred to sterile petriplates and allowed for solidification. A suspension of the culture organism was swabbed above the solidified Muller Hinton agar medium at 45°C. Wells were made using sterile cork borer under aseptic condition. The silver nanoparticles *P. zeylanica* extracts were added to the wells in various concentrations viz., 10 µl, 20 µl, 30 µl, 40 µl and 50 µl. The fresh silver nitrate extract (25 µg /ml) was used as a standard to compare its effect on test organisms with the *P. zeylanica* nanoparticle extract. The plates were kept at room temperature for 2 h to allow diffusion of the test solution into the agar and were incubated for 24 h at 37°C. After the incubation period, the plates were observed and zone of inhibition was measured (mm) and the activities were recorded.

Result

UV Vis Analysis

UV Vis absorption spectra are known to be quite sensitive to the formation of AgNP's. Thus the presence of AgNP's in *P. zeylanica* leaves was characterized by using a UV-Vis spectrum. The AgNP's of *P. zeylanica* aqueous leaves extract showed a broad peak at 440 nm with the absorbance of 1.662 which corresponds to plasmon excitation of the AgNP's (Fig. 1). A control without the addition of the silver nitrate solution was also recorded and used as a blank.

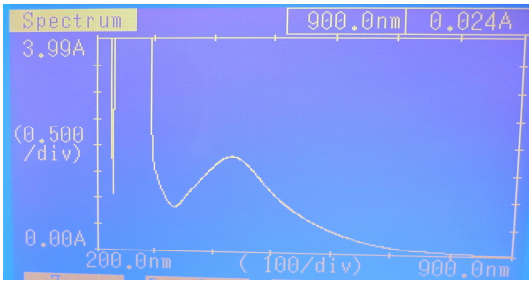

Fig. 1: UV-Vis spectrum of *P. zeylanica* AgNP's

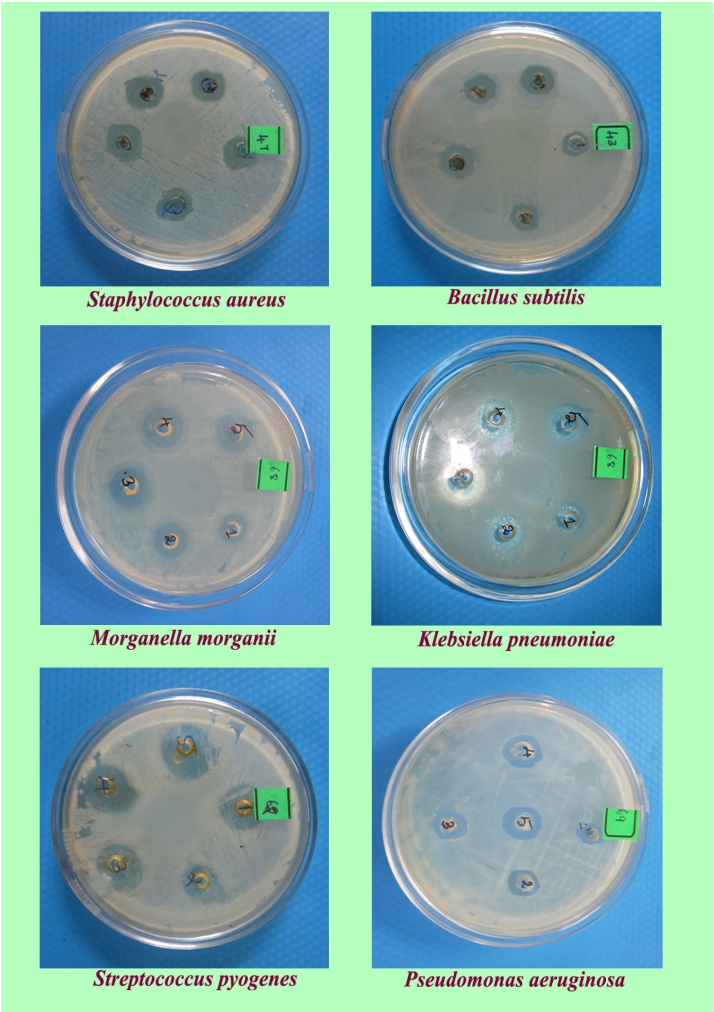

Fig. 2: Bioefficacy of *P. zeylanica AgNPs* against the bacterial pathogens

Antibacterial activity on AgNP's extract of *P. zeylanica*

Silver nanoparticles of *P. zeylanica* leaves extracts demonstrated broad spectrum of antibacterial activities (Fig. 2) that inhibited the growth of pathogens with the maximum zone of inhibition 25±0.5 mm for *K. pneumoniae* (50 µl), 21±0.5 mm for *P. aeruginosa* (50 µl) which was higher than that of the fresh silver nanoparticles. Following these, the AgNP's extract of *P. zeylanica* revealed zone of inhibition viz., 23±0.5 mm for *M. morganii* (50 µl),

19±0.5 mm for *B. subtilis* (50 µl), 15±0.3 mm for *S. aureus* (50 µl) and 9±0.3 mm for *S. pyogenes* (50 µl).

Table 1: Bioefficacy of *P. zeylanica AgNPs*

Pathogens	Concentration of silver nano particles of *P. zeylanica*					
	10 µl	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**Amikacin (30 µg)**
S. aureus	06±0.2	08±0.3	10±0.3	12±0.3	15±0.3	17
B. subtilis	10±0.3	13±0.3	15±0.3	17±0.3	19±0.5	19
S. pyogenes	03±0.3	05±0.5	07±0.3	09±0.3	09±0.3	11
K. pneumoniae	12±0.4	15±0.5	18±0.3	22±0.3	25±0.5	23
M. morganii	11±0.4	13±0.5	17±0.3	20±0.3	23±0.5	25
P. aeruginosa	09±0.4	11±0.5	15±0.3	19±0.3	21±0.5	20

Discussion

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phyto-toxic effects (Torresdey, 2003). The efficiency of various silver based antimicrobial fillers in polyamide toward their silver ion release characteristics in an aqueous medium was also investigated and discussed in number of plants including algae, yeast and fungi (Arya, 2010; Linga Rao and Savithramma, 2012). UV–visible spectroscopy is an important technique to determine the formation and stability of metal nanoparticle in aqueous solution. The reaction mixture changes the colour by adding various concentrations of metal ions. These colour changes arise because of the excitation of surface plasmon vibrations in the silver nanoparticle (Mulvaney, 1996). It shows yellowish to dark brown in colour. The dark brown colour of silver colloid is accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromagnetic field (Song and Kim, 2004). Salunke *et al.* (2014) studied the antibacterial property of AgNP's root extract of *P. zeylanica* against *A. baumannii, S. aureus* and *E. coli* which showed higher zone of inhibition against *S. aureus* (98%). AgNP's root extract of *P. zeylanica* also showed higher zone of inhibition with the highest of 88% in *A. baumannii*. In the present study, AgNP's leaves extract of *P. zeylanica* revealed moderate antibacterial activity against *S. aureus* 15±0.3 mm in 50 µl of AgNP's extract. The results of silver nano particles leaves extracts of *P. zeylanica* demonstrated broad spectrum of activities that inhibited the growth with the maximum zone of inhibition 25±0.5 mm for *K. pneumoniae* (50 µl), 23±0.5 mm for *M. morganii* (50 µl), 21±0.5 mm for *P. aeruginosa* (50 µl), 19±0.5 mm for *B. subtilis* (50 µl), 15±0.3 mm for *S. aureus* (50 µl) and 9±0.3 mm for *S. pyogenes* (50 µl).

Conclusion

In the present investigation, a facile, environmentally benevolent green synthetic route is used for synthesis of silver Nanoparticle. The Phytofabrication of silver nanoparticles by using leaves extract of *P. zeylanica* not including with any toxic chemicals. Assessment on the antibacterial effect of nanosized silver colloidal solution against various Gram positive and Gram negative bacteria was studied. Comparitively, In the present analysis, the AgNP's leaves extract of *P. zeylanica* showed higher zone of inhibition against Gram negative bacteria than Gram positive bacteria, which reveals high efficacy of silver nanoparticle as a strong antibacterial agent. The present AgNP's leaves extract of *P. zeylanica* used as a source which is easily obtainable and extensively useful in biomedical application.

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References

Armendariz V, Gardea- Torresdey JL, Herrera MI, Jose-Yaceman JR, Peralta-Videa, Santigo P. 2004. Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. J of Nanoparticles Res 6(4): 377 – 382.

Arya V. 2010. Living system: Eco-Friendly nano factories. Digest. J. Nanomater. Biostruct: 5, 9-21

Bhattacharya D, Gupta RK. 2005. Nanotechnology and potential of microorganisms. Crit Rev Biotec., 24(4): 199.

Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M. 2003. *Alfalfa* sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19, 1357– 1361

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SRK, Muniyandi J, Hariharan N, Eom SH. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf B 74(1): 328–335.

Kalishwaralal K, Banumathi E, Pandian SBRK, Deepak V, Muniyandi J, Eom SH. 2009. Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. Colloids Surf B 73: 51–7.

Lee HJ, Jeon SH. 2004. Bacteriostasis of nanosized colloidal silver on polyester non-wovens. Text. Res. J 74: 430.

Linga Rao M, Savithramma N. 2012. Antimicrobial activity of silver nanoparticles synthesized by using stem extract of *Svensonia hyderobadensis* (Walp.) Mold – A rare medicinal plant. Research in Biotechnology, 3(3): 41-47.

Malhotra R. 2010. Mass spectrometry in life sciences, Curr. Sci. 98: 140-141.

Marambio-Jones C, Hoek EMV. 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J. Nanopart Res. 12: 1531- 1551.

Mulvaney P. 1996. Surface plasmon spectroscopy of nanosized metal particles. Langmuir, 12: 788-800.

Salunke GR, Ghosh S, Santosh Kumar RJ, Khade S, Vashisth P, Kale T, Chopade S, Pruthi V, Kundu G, Bellare JR, Chopade BA. 2014. Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant *Plumbago zeylanica* and their application in biofilm control. Int J of Nano Medicine 9: 2635-2653.

Sap-Iam N, Homklinchan C, Larpudomlert R, Warisnoicharoen W, Sereemaspun A, Dubas ST. 2010. UV irradiation-induced silver nanoparticles as mosquito larvicides. J. Applied Sci. 10: 3132 – 3136.

Sheikpranbabu S, Kalishwaralal K, Venkataraman D, Eom SH, Park J, Gurunathan S. 2009. Silver nanoparticles inhibit VEGF-and IL-1b-induced vascular permeability via Src dependent pathway in porcine retinal endothelial cells. J. Nano biotechnol 7: 8.

Song JY, Kim BS. 2008. Biological synthesis of bimetallic Au/Ag Nanoparticle using persimmon (*Diopyros kaki*) leaf extract. Korean J of Chem. Eng. 25: 808 - 811.