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Antimicrobial efficiency of biologically synthesized nanoparticles using root extract of *Plumbago zeylanica* as bio-fertilizer application

H. Patel*and R. Krishnamurthy
C. G. Bhakta Institute of Biotechnology, Uka Tarsardia University, Bardoil- 394601, Surat, India

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Abstract: The synthesis of nanoparticles using biological system is in wide research due to the potential applications in Nano medicines. The biological synthesis of nano-particles is less expensive & eco-friendly. In the present study, the silver nanoparticles are synthesized rapidly due to the root extract of medicinal plant *Plumbago zeylanica*. After assessing the formation of silver nanoparticles, these phyto-synthesized silver nanoparticles were tested for their antibacterial activity against test cultures of *Bacillus cereus, Straptococcus aureus, Serratia marcescens* and *Escherchia coli*. The antibacterial property of silver nanoparticles was analyzed by measuring Zone of Inhibition. The silver nanoparticles synthesized from root extract of *Plumbago zeylanica* showed toxic effect on *Bacillus cereus, Straptococcus aureus, Serratia marcescens* and *Escherchia coli*. The outcome of this study will help in future to develop value added drug from medicinal plant for biomedical and nanotechnology based industries.

Key words: Medicinal Plant-*Plumbago Zeylanica*; Silver Nanoparticles; ZOI; Antimicrobial Activity

INTRODUCTION

Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines and renal diseases (Malhotra, 2010). One of the fields in which nanotechnology finds extensive applications is Nano medicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job; the materials and devices designed at the level of Nano scale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health (Naik and Selukar, 2009). The antibacterial activity of silver species has been well known since ancient times (Shrivastava *et al.,* 2007). Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronics and pharmaceuticals (Sanskar *et al.,* 2010). And in biomedicine especially for antibacterial agent (Rai *et al.,* 2009) and antiviral agent (Elechiguerra *et al.,* 2005). Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it have lethal effects spanned across all microbial domains (Prabhu *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 (Marambio-Jones and Hoek, 2010). The application of silver of silver nanoparticles in drug delivery, drug discovery and new drug therapies have declare war on many dead full diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells. (Balaji, 2010). The most important application of silver and silver nanoparticles is in medical in medical industry such as tropical ointments to prevent infection against burn and open wounds (IP *et al.,* 2006. Biologic synthesis of nanoparticles by plant extract is at present under exploitation as some researchers worked on it (Calvo *et al.,* 2006; bhyan *et al.,* 2007) and testing for antimicrobial activities (Saxena *et al.,* 2010., Khandelwal *et al.,* 2010., Savithramma N. *et al.,* 2011).

One of the highly useful plants in the indigenous systems of medicine is *Plumbago zeylanica* system of medicine. A native of South Asia, the species is distributed throughout most commonly known as Ceylon, Leadwort, Chitra, Chitrak and Chitramoolan. *P. zeylanic* is multipurpose medicinal herb of family Plumbaginacease. One of the common plants used in Indian traditional of the tropics and subtropics; growing in deciduous woodland, savannas and scrub lands from sea level up to 2000m altitude (Paras *et al*, 2014). It is a tropical shrub, it grow wild as a garden plant in India. The root is used as laxative, expectorant, astringent, abortifacient and in dysentery. Tinchure of root bark is used as antiperiodic. The leaves are caustic and used in treatment of scabies. Plumbagos are chemically characterized by the presence of naphthoquinones, flavonoids, terpenoids and steroids, many of them being responsible for several biodynamic activities. (Paras *et al*, 2014), *P. zeylanica* root is powerfully poisonous and its internal use is attended with great danger, it causes abortion. The root is sometimes given internally but more commonly employed as local irritant to the uteri (Sweta *et al*, 2015).

MATERIALS AND METHODS

Biogenisis of silver nanopartilces

The root of *Plumbago zeylanica* were collected at time of harvest in the year 2013 from the Uka Tarsadia University experimental field plot after application of biofertilizer to the plant for its growth and development. The roots were air dried for 10 days and then were kept in the hot air oven at 55^{o}C for 24 hours. The dried roots were grounded to a fine powder. Using HPLC technique the concentration of Plumbagin was quantified. Azospirillum biofertilizer application showed highest concentration of Plumbagin content in the roots (0.026% w/w). This powder sample was carried further for preparation of silver nanoparticles. One mM silver nitrate was added to the extracts of to make up a final solution of 200ml. Centrifugation of the content at 18,000 rpm for 25 min was done and pellets were collected and stored at 4^{0}C. The supernatants were collected and heated at 50 to 95^{0}C.

***Corresponding Author:**
Hetal Patel,
C.G. Bhakta Institute of Biotechnology,
Uka Tarsardia University,
Bardoli, Surat, India.

Keep observing the change in the color of the solution to brown. The reduction of pure Ag^{+2} ions using UV-visible spectrophotometer was measured at 5 hours after diluting a small aliquot of the sample into distilled water. UV-VIS spectrophotometer UV-VIS (Shimadzu) was used.

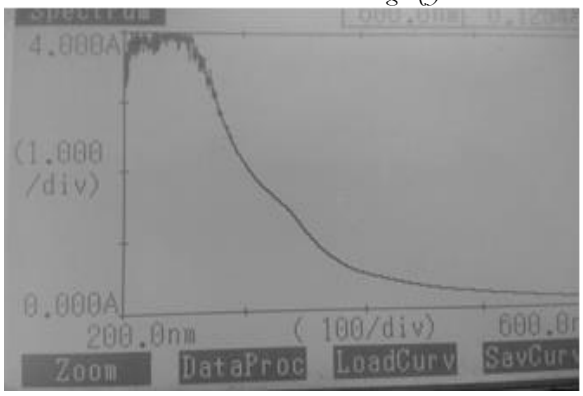
Antibacterial efficacy analysis

Pure cultures of *Bacillus cereus, Streptococcus aureus, Serratia marcescens* and *E. coli* were procured from the Department of Microbiology of Uka Tarsadia University, Bardoli, Surat. The experiments of antibacterial analysis was carried out in the C. G. Bhakta institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat. The sensitivity testing of the plant extracts were determined by using disc diffusion method. 17hr culture of *Escherichia coli, Streptococcus, Bacillus cereus, Serratia marcescens* was spread plate onto Muller Hiton agar media. 5 wells were bore on an agar media. 100µl of the samples without silver nanopartilces were loaded in 4 wells and one with DMSO as control loaded with human pathogenic bacteria. While 100µl of samples were loaded in 3 wells. Similar procedure was repeated with samples with silver nanoparticles. The plates were incubated for 24 hrs. The zone of inhibition was measured for each sample. The experiments were repeated thrice and mean values of zone diameter were presented.

RESULT AND DISCUSSION

Confirmation of metal-plant interaction: The aqueous silver ions when exposed to *Plumbago zeyalnica* root extract were reduced in solution there by leading to the formation of silver hydrosol. The root extracts were pale yellow in colour before addition of Ag (NO_3) and these were changed to brownish colour suggested the rapid formation of silver nanoparticles. Time duration of change in colour was 30mins. The time duration of change in colour varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanoparticles within 10 min whereas *Shorea tumbuggaia* took 15 min to synthesize silver nanoparticles (N. Savithramma *et al.,* 2011). The change of colour indicates the biosynthesis of silver nanoparticles exhibit brown colour in aqueous solution due to the surface plasmon resonance phenomenon. In the study the results obtained is clear in terms of identification of potential medicinal plants for synthesizing the silver nanoparticles. Though the synthesis of silver nanoparticles had been confirmed by measuring the UV-VIS spectrum of colloidal solutions of silver nanoparticles synthesized from *Plumbago zeyalnica* have absorbance peaks at about 250 nm (Figure-1) and the broadening of peak indicated that the particles are polydispersed. Silver nanoparticle synthesis using medicinal plant is reported by many researchers (Ek. Elumalai *et al.,* M. Linga *al*., 2010Roa *et al.,* 2011).

Figure 1: UV-VIS absorption spectra of silver nanoparticle synthesized from root extract of *Plumbago zeylanica*



Antibacterial efficacy of SNPs

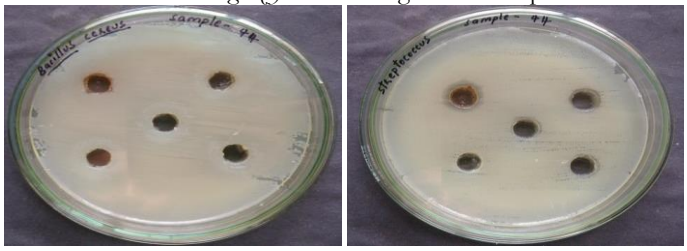
The ZOI of root extract with silver nanoparticles was observed in *Streptococcus, Escherichia coli* and *Serratia marcescens* grown plates. The zone of diameter was 15 mm, 16mm, and 14 mm for *Streptococcus, Escherichia coli* and *Serratia marcescens* respectively (Table 1). The maximum ZOI was observed for *Serratia marcescens* strain while minimum for *E. coli*. The silver nanoparticles synthesized from the extract of the roots are toxic to these strains. Similar results are observed in *Allium cepa* (Saxena *et al.,* 2010 and Savithramma N. *et al.,* 2011). There was no ZOI seen with *Bacillus cereus* shown in figure-2. The tested concentrations of root extract was 8.2 mg/100µ in *Plumbago zeylanica*.

Table 1: Zone of Inhibition in mm using sample with silver nanoparticles against test organisms

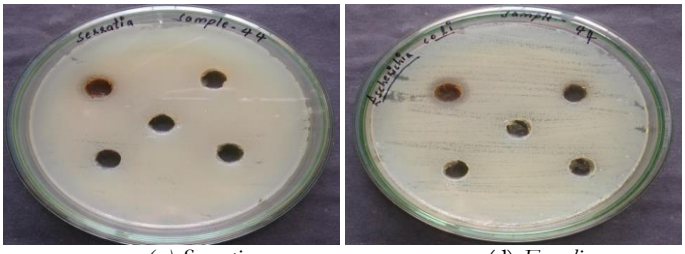
Sample	Concentration mg/100µl	Zone of Inhibition (mm)			
		Bacillus cereus	*Streptococcus*	*Serratia marcescens*	*E. coli*
Root extract with silver nanoparticles	8.2	No ZOI	15	16	14

The observation indicates that the root sample with silver nanoparticles is showing significant impact on the ZOI of microorganisms compare to root extract without silver nanoparticles. This may be due to the size variation. Martinez-castanon *et al.,* (2008) found that the antibacterial activity of the nanoparticles varies when their size diminishes. The use of plant extracts is effective against various microorganisms including pathogens (Mishra *et al.,* 2007). 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 photo-toxic effect (Gardea-Torresday *et al.,* 2003). The greatest surface area of silver Nano particles the greatest the antibacterial activity (Thiel *et al.,* 2007).

Figure 2: Zone of Inhibition of Test microorganisms with root extract of *Plumbago zeylanica* having silver nanoparticles



(a) *Bacillus cereus* (b) *Streptococcus*



(c) Serratia marcenscens (d) *E. coli*

CONCLUSION

The present study included the bio-reduction of silver ions by root extract of *Plumbago zeylanica* and their antibacterial activity. The study revealed that the plant is a good source for synthesis of silver nanoparticles at fast rate. The aqueous silver ions exposed to the extract, the synthesis of silver nanoparticles were confirmed by the brown colour formation within 30mins. The antibacterial efficacy against different species of bacteria confirmed that the silver nanoparticles are capable of rendering antibacterial efficacy and strengthening the medical value of the plant. The biosynthesis of silver nanoparticles is simple and convenient to handle and most advantage and eco-friendly.

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