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GREEN SYNTHESIS OF SILVER NANOPARTICLE WITH *PLUMBAGO CAPENSIS* L. AQUEOUS ROOT EXTRACT AND ITS ANTIFUNGAL ACTIVITY

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ABSTRACT

Plumbago capensis root extract can be effectively used in synthesis of silver nanoparticles. The minimum concentration of the extract required to synthesis was 100µl. The silver nanos so synthesized had a maximum absorption of 419nm at UV-VIS spectroscopy and FT-IR analysis indicates capping of the silver nanoparticles. The Scanning and Transmission electron microscopy revealed spherical to oblong shaped particles of 18.69 nm to 46.71nm in length. *Curvularia lunata* and *Aspergillus fumigatus* was inhibited by 100% at 1000 µg/ml silver nanoparticles. The MIC value for C. lunata was 300µg/ml and *A. fumigatus* was between 750-1000 µg/ml respectively.

KEYWORDS: Silver nanoparticles, *Plumbago capensis* root extract, Antifungal activity.

INTRODUCTION

Nanobiotechnology is a widely expanding branch of science which finds its applications in various fields. Nanoparticles are very characteristic with its size, morphology and distribution (khandelwal et al., 2010). A number of noble metallic nanoparticles have been synthesized. Among them synthesis of silver nanoparticle through green synthesis is cost effective, cheap and ecofriendly means of synthesis. Biosynthesis using plant extracts is the best method, since plants are widely distributed, easily available and most important feature being safe to handle .Earlier several plant extracts such as *Mollugo nudicaulis* (Anarkali et al.,2012), *Andrographis paniculata* (Panneerselvam et al., 2011), *Punica granatum* (Nehaad et al

2012), *Ocimum sactum* (Mallikarjuna,2011), *Aloe vera*, *Azadiracta indica* and *Datura metel* (Chandran et al.,2012), *Cassia auriculata* (Udayasoorian et al.,2011), *Tridax procumbens* (Dhanalakshmi, 2012),Orange peel (Manal et al.,2014) and several other Angiosperm and Gymnospermic plants have been reported in the biosynthesis of silver nanoparticle. Athithi et al.,2011 reported the synthesis of silver nanos using Anthoceros extracts. These silver nanoparticle find a lot of applications in the field of pharmaceutics and biological applications. Since these have potent antimicrobial activity they have been used in the preparation of ointments. Kalaiselvi et al., 2013 reported the antibacterial activity of silver nanoparticles. Panneerselvam et al., 2011 has reported its use as antiplasmodial agent.

Plumbago capensis (syn) *Plumbago auriculata* is commonly called blue plumbago an ornamental plant traditionally used in the treatment of wounds, warts and broken bones. Blackwater fever can be treated with a decoction of aerial parts or roots. The roots have emetic and syptic properties. All parts of *Plumbago capensis* contain the naphthoquinone plumbagin. Plumbagin possesses a variety of pharmacological activities such as anticancer, cardiotonic, antimicrobial, antiinsecticidal and antifertility. Hence, an attempt was made to synthesize silver nanoparticles in one step method with *Plumbago* extracts, characterize and evaluate the antifungal activity.

MATERIALS AND METHODS

Preparation of plant extract - Different parts of *Plumbago capensis* such as leaves, stem, root and flowers were collected , washed thoroughly with running tap water and blotted dried using filter paper. Each material (1gm) was weighed and extracted in mortor and pestle with 10ml sterile distilled water. The extracts were then filtered using whatmann No1 filter paper for further use.

Synthesis of Silver nano particles with different plant parts

The plant extract (leaves, stem, root and flower) was mixed with 1mM silver nitrate in the ratio 1:10 and boiled in a water bath for 5 minutes. Change in the colour of the reaction mixture was observed which indicated the completion of the synthesis.

Minimum concentration for the synthesis of silver noanoparticles

The root., stem and flower which were effective in the synthesis were selected for further use. The minimum concentration required to initiate the reduction of silver nitrate to metallic

silver was studied with 100, 200,300,400,500,600,700,800,900 µl of plant extract and a similar procedure was carried out for the sytnthesis.

UV-Vis Spectroscopy

The reaction mixture was analysed for its maximum absorption in UV-VIS Spectroscopy at wavelength range of 300-600nm and the corresponding peaks were recorded.

FT-IR Spectroscopy

In Fourier Transform Infrared(FTIR) spectroscopy measurements, the silver nanoparticles of *Plumbago capensis* root was mixed with potassium bromide in the ratio of 1:100.FTIR samples were recorded on Shimazdu IR Prestige-21 FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 650-4500cm-1 at a resolution of 4cm-1.This range was used to study the fundamental vibrations and associated rotational vibrational structure.

Scanning and Transmission Electron Microscopy

The reddish orange coloured complex of silver nanoparticles obtained from reaction of *Plumbago capensis* root extract was centrifuged at 10,000g for 30 min. The pellet thus obtained was washed thrice to remove any residual silver nitrate. Silver nanoparticles were dried under vaccum, loaded onto copper stub processed and observed under SEM and TEM .The shape and the size of the particle was observed and photographed.

Antifungal Activity of Silver Nanoparticles

The antifungal activity was tested by spore germination assay. Spores of the following fungi were selected *Curvularia lunata, Fusarium oxysporium, Aspergillus fumigatus,Aspergillus flavus* and *Trichoderma* sp. A spores suspension of $1x10^{-5}$ dilution of the test fungi was prepared and incubated along with 10µl of different concentration (1000 to 50 µg/ml) of silver nano particles in a moist chamber. After 24 hours of incubation the percentage of spore germination was determined. A corresponding control was maintained with 10 µl of sterile distilled water.

RESULTS

Synthesis of silve nanoparticles

On boiling the reaction mixture for 5 minutes a gradual change in colour was observed. The colour changes in the case of the whole plant extract, stem and root was reddish orange, while

it was chocolate brown with flower extract (Fig-1). The colour of the reaction mixture indicated the shape of the silver particles.

UV–VIS Spectroscopy

The formation of silver nanoparticle was monitored under UV-VIS spectroscopy. The maximum absorption was at 419nm. Each nanoparticle has a characteristic absorption maxima due to Plasmon resonance absorption band. Reduction of silver ions by the root extract was evident by the UV-Vis spectroscopy.

FT-IR Spectroscopy

The bands seen at 3435cm^{-1} and 2369 cm^{-1} were assigned to be stretching vibrations of primary and secondary amines respectively, while these corresponding bending vibrations were seen at 1641cm^{-1} and 1631cm^{-1} revealed the capping of silver nanoparticles (Fig-2).

Minimum concentration for the synthesis

All the extracts (root, stem, flower and leaves) when analysed for the synthesis of nano particles showed positive result (Table-1).Studies on the minimum concentration required for the synthesis revealed that root and flower extracts were efficient at 100 µl, leaves at700 µl and stem extract only at 800 µl .

Scanning and Transmission Electron Microscopy

The SEM images showed particles of varied shapes and sizes. The shape and size determine the efficacy of the particles. The smaller the particle size greater is their properties. The TEM studies revealed oblong to spherical silver nanoparticle of approximately 18.69 nm to 46.71nmin length. (Fig-3).

Antifungal activity of silver nanoparticles

Among the test fungi *Curvularia lunata and Aspergillus flavus* were inhibited 100%, while *Trichoderma* sp and *Aspergillus fumigatus* was inhibited the least at 1000µg/ml concentration(Fig-4). However at least concentration of 50 µg/ml concentration *A.fumigatus* alone exhibited 60% inhibition while all other fungi were inhibited to 10-20% respectively. The present study demonstrates the MIC_{50} for *C.lunata* was 300 µg/ml. The other most inhibited fungi *A.fumigatus* had its MIC_{50} between 750-1000 µg/ml. It is clearly evident that *Curvularia lunata* was inhibited effectively by the silver nanopraticles.

Fig 1: Green synthesis of silver nanoparticles using *Plumbago capensis* extracts. R-Root S-Stem F-Flower extracts. Note the reddish orange colour in root and stem and chocolate brown in flower extract.

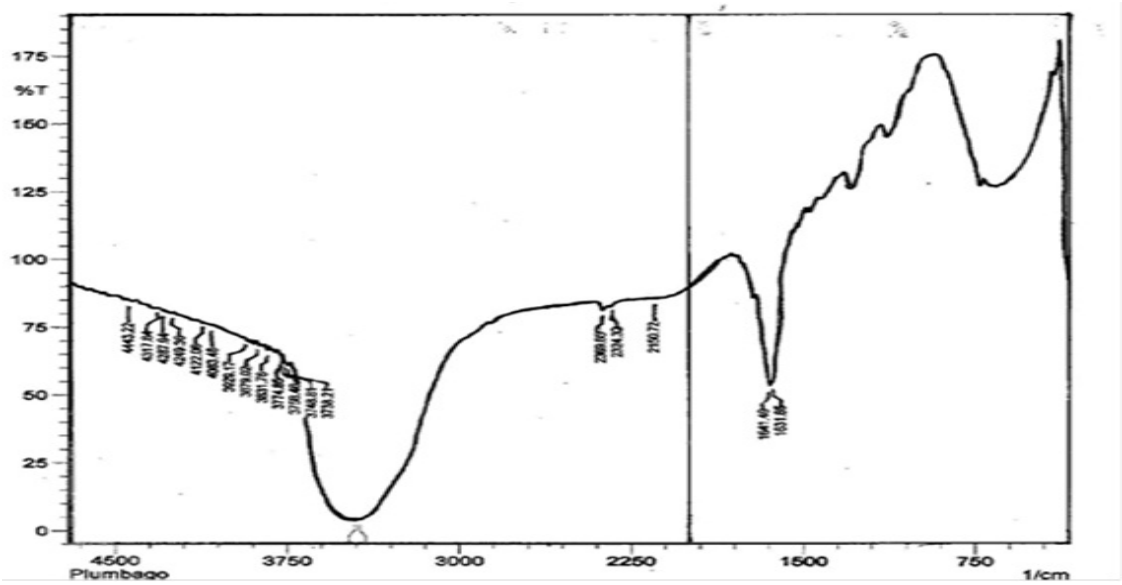

Fig 2: FT-IR Spectrum of silver nanoparticles synthesized using *Plumbago capensis* root extract.

Table 1: Minimum concentration of P*lumbago capensis* for the synthesis of silver nanoparticles

Plumbago Capensis extract	**100 µl**	**200 µl**	**300 µl**	**400 µl**	**500 µl**	**600 µl**	**700 µl**	**800 µl**	**900 µl**
Root	+	+	+	+	+	+	+	+	+
Stem	-	-	-	-	-	-	-	+	+
Flower	+	+	+	+	+	+	+	+	+

Note- + = positive synthesis, - = Negative synthesis

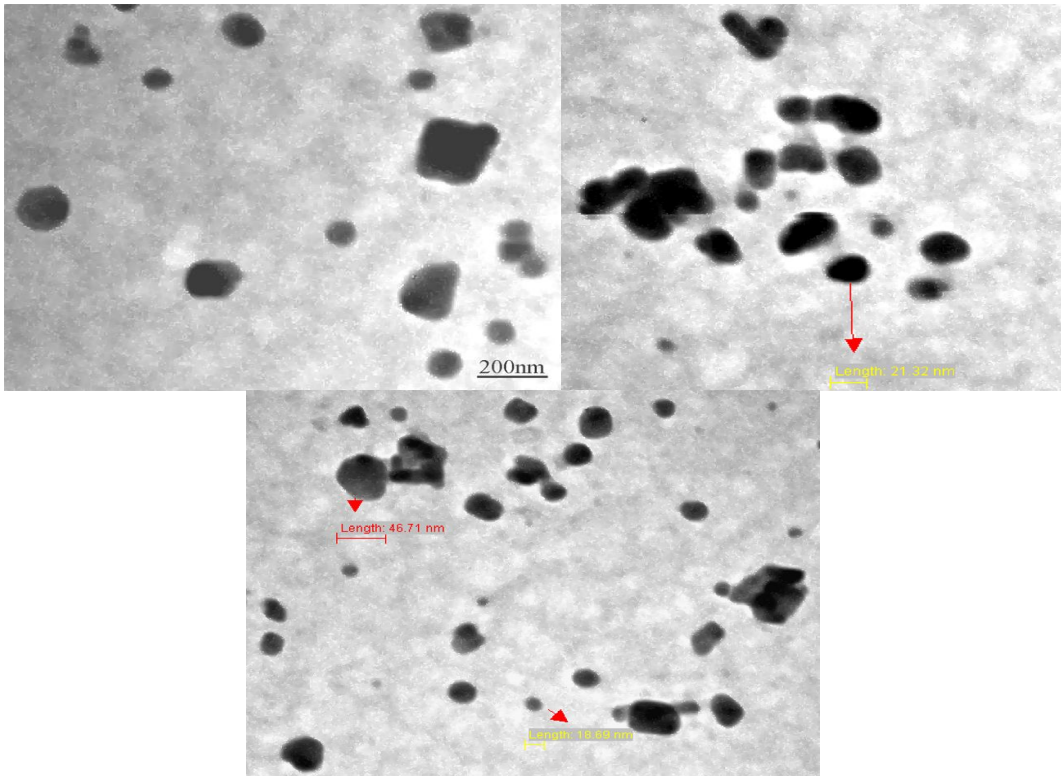

Fig 3: Transmission electron microscopy of silver nanoparticles. All micrographs 200nm–bar scale.

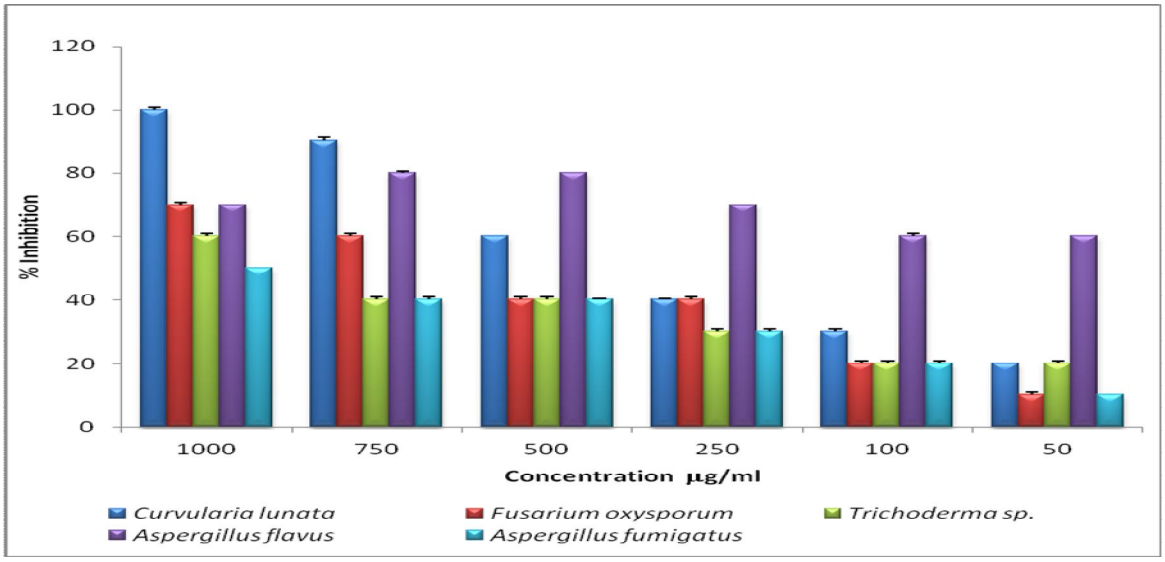

Fig 4: Antifungal activity of silver nanoparticles synthesised from *Plumbago capensis* root extract by spore germination assay.

All values in triplicate ± Standard deviation.

DISCUSSION

In green synthesis of silver nanoparticles, the reaction mixture when boiled with the plant extract gives range of colours. Vyom parashar et al., 2009 reported the formation of

nanoparticles with *Parthenium* leaf extract where the colour change was from water colour to yellowish brown. The colour change was evident due to the changes in its physical properties such as surface Plasmon phenomenon. Leaf extract of *Mentha piperta* in the aqueous solution of the silver ion solution, changed from pale water colour to reddish brown. Elumalai *et al* 2010 reported that Silver nanoparticles exhibit dark –brown colour in aqueous solution of *Euphorbia hirta.*

Each metallic nanoparticle has its characteristic absorption pattern. Vyom parashar *et al*., 2009 reported the formation of nanoparticles with *Parthenium* where maximum absorption peak is at 474nm. Elumalai *et al.,* 2010 reported the formation of nanoparticles with Euphorbia hirta where maximum absorption peak was at 430nm. Kavia *et al*., 2011 reported the formation of nanoparticles with *Polyalthia longifolia* at 451 and 435nm.

From the FT-IR analysis studies we confirmed that the carbonyl group from the aminoacid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form the metal nanoparticles (i.e capping of silver nanoparticles) thereby avoid agglomeration and stabilize the particles. Thus it is evident that the biomolecules present in the extract could perform dual functions of formation and stabilization.

Nanoparticles of varied shapes are reported earlier. Elumalai *et al.,* 2010 reported spherical shaped nanoparticle formed with diameter range of 40-50nm in *Euphorbia hirta* extract. Spherical particles of approximately 40nm were synthesized with *Lantana camera* leaf extract. The nanoparticles observed in the study were spherical in shape, but tend to get aggregated with raising temperature. This is due to the binding force between the AgNPs and the capping molecules that may get decreased with increasing temperature inspite of the fact that the size of the nanoparticles is reduced.

The efficacy of the particle reflect on its size. Particle ranging from 18- 47nm were effective against a larger spore such as *Curvularia lunata*, while it was ineffective in controlling smaller spores bearing fungi. Perhaps the mode of action may be by directly adhering and inhibiting the cell wall formation as reported by Anjana and anitha (2012) and thereby prevents the spore germination. However penetration of silver nanoparticles is not well documented. (Saxena et al., 2010).

Today the role of nanoparticles in the industries, pharmaceuticals and agriculture has been constantly increasing. The inhibitory effect of the nanoparticles on fungal plant pathogen can help to solve problems associated with chemical management of plant diseases since the nanoparticles work better before spores penetrate and colonize with in the plant tissue.

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