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Phytoremediation of dyes using *Lagerstroemia speciosa* mediated silver nanoparticles and its biofilm activity against clinical strains *Pseudomonas aeruginosa*

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ABSTRACT

The aim of this study was to prepare silver nanoparticles by a green method using the aqueous leaves extract of *Lagerstroemia speciosa*. The prepared silver nanoparticles were characterized, studied for its photocatalytic and biofilm inhibition studies. The maximum absorbance peak was found at 427 nm and thus confirming the formation of silver nanoparticles. The average size of silver nanoparticles synthesized was found to be 12 nm using XRD and it was spherical in shape. The nanoparticles synthesized was investigated for photocatalytic activity for to two different dye molecules, methyl orange and methylene blue showing 310 and 290 min degradation time respectively. The silver nanoparticles biofilm inhibition assay against clinical strains of *Pseudomonas aeruginosa* showed lowest accumulation at a lower concentration. The biofilm inhibition was also studied by visual interpretation through Scanning Electron Microscopy states that 50 μg mL^{-1} exerts the highest inhibition compared against the control. This evident helps to analysis the silver nanoparticles for various applications in future.

1. Introduction

Human activities such as pollution are causing major challenge to the environment [1–3]. Among the pollution, the organic dye pollutant from textile, paper, medicine, plastic industries is now considered to be a major threat to the biodiversity. The usage of these dyes from the industries causes toxic. Several methods were being proposed to remove dyes from the textiles viz. physical, chemical and biological methods. Flocculation, membrane filtration, electrochemical technique, electrocoagulation, adsorption, ozonation, redox treatments and biological discoloration were considered to be the best techniques for abating dyes [4]. But these dyes cause eutrophication upon the accumulation in the water bodies, and reduce the oxygen level and cause damage to the aquatic ecosystem [5].

Noble metal nanoparticles are being used in a diverse field like sensors, diagnostic agent, bio-imaging, and in the biomedical field. In recent times, metal nanoparticle degrades the dyes as an effective photocatalyst at ambient temperature with visible light illumination [6]. The rate of absorption of nanoparticles will be increased in the presence of local electric field [7]. Hence various biological agents like microorganisms, plants, enzymes were being used. But the major advantage of green synthesis of metal nanoparticles mediated via plants is to reduce the by–products, which are hazardous in nature. Hence plants were been selected due to varied phytochemical, which act as the reducing agent and stabilizing agent naturally [8–11].

Thinking about the environmental pollution, plants were been selected for the present study, which explains the green synthesis of silver nanoparticles. Hence leaf extract of *Lagerstroemia speciosa* (commonly called as Banaba) belongs to Lythraceae used for the bioconversion of silver ions into silver nanoparticles. Banaba is used to treat hyperglycaemia condition in the Philippines and also used as an ornamental plant [12]. *Lagerstroemia speciosa* contains a wide range of biologically active compounds such as being rich in alkaloids, glycosides, flavonoids, tannins, terpenoids, phenols, saponins, alkaloids and vitamins [13]. *Lagerstroemia speciosa* was chosen because of its functional properties like anti-arthritic [14], anti-diabetic, anti-obesity [15], anti-bacterial [16], anti-inflammatory, Free radical scavenging, antioxidant [17,18], Analgesic and anti-diarrhoeal [19].

The present study reports on the biosynthesis of silver nanoparticles using *L. speciosa* aqueous leaf extract by green methodology. It was Analysed by UV- VIS absorption spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), particle size analysis and Zeta potential. The

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prepared silver nanoparticles were studied for its dye degradation under sunlight irradiation and biofilm inhibition against clinical strains of *Pseudomonas aeruginosa* mutans.

2. Materials and Methods

2.1. Reagents

Silver nitrate (99.99%), methyl orange, methylene blue reagents were procured from Merck Inc. (Mumbai, India). The glassware used in this experiment was cleaned with aqua regia and rinsed with double distilled water twice.

2.2. Collection and Processing of Plant Material

The leaves of *L. speciosa* (Fig. 1) was collected during March- Aug, from VIT University, garden, Vellore, INDIA. The plant leaves were authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Chennai, India. The collected leaves were cleaned with distilled water and then air shade dried in order to protect the volatile organic principles. It was blended into a fine powder with the help of a mechanical blender. It was stored in well air tight container.

2.3. Preparation of Phyto-reducing Agent

Weight approximately 10 g of air dried leaves into the clean beaker and it was boiled with 100 mL double distilled water for half an hour at 60 °C to get decoction extract. The extract turns the colour from colourless to yellow brown colour indicates the extraction is complete. The hot extract was filtered through Whatman No. 1 filter paper and the filtrate was collected and stored at 4 °C for further biosynthesis of nanoparticles.

2.4. Phytochemical Analysis of Leaves Extracts of L. speciosa

Preliminary phytochemical analysis is performed to identify the chemical groups and used to determine the quality of the drug present in the leaves extract of *L. speciosa* [20–22].

2.4.1. Test for Alkaloids

2.4.1.1. Hager's Reagent Test. Few drops of saturated picric acid solution (Hager's reagent) is added to the aqueous extract (2 mL) of Leaves of *L. speciosa*. Bright yellow precipitate indicates the presence of alkaloids.

2.4.1.2. Mayer's Reagent Test. Few drops of Potassium mercuric iodide solution (Mayer's reagent) (2 mL) in dilute HCl was added to 2 mL of *L. speciosa* extract and boiled for about 5 min. The yellow colour precipitate obtained concludes the presence of alkaloids.

Fig. 1. Leaves of *Lagerstroemia speciosa.*

2.4.1.3. Dragendroff's Reagent Test. Bismuth nitrate (0.1 g) and Potassium Iodide (0.5 g) in 10 mL of HCl were mixed with 2 mL of *L. speciosa* extract and boiled for 2 min. The dark orange solution shows the presences of alkaloids.

2.4.2. Test for Carbohydrates

2.4.2.1. Molisch's Reagent Test. To 2 mL of leaf extract of *L. speciosa*, 2 mL of Molisch's reagent is added. The solution is boiled and cooled. Violet ring formation indicates the presence of carbohydrates.

2.4.3. Test for Glycosides

2.4.3.1. Keller Killani Test. The leaves of *L. speciosa* extract (1 mL) was added to glacial acetic acid (1 mL) containing a $FeCl_3$ solution and then 5 mL of Con. H_2SO_4 was added. No colour change is observed shows the absence of glycosides.

2.4.3.2. Legal Test. 2 mL of sodium nitroprusside and 2 mL of sodium hydroxide was added to 2 mL of leaf extract. No colour change is observed shows the absence of glycosides.

2.4.4. Test for Terpenoids and Phytosterols

2.4.4.1. Libermann-Burchard Test. To 1 mL of the leaf extract, 2 mL acetic anhydride is added and heated. A brown ring is developed slowly at the junction of two layers on adding few drops of Con. H_2SO_4 along the sides of the test tube and upper layer turns green shows the presence of steroids. Deep red colour is observed in the lower layer conforms the presence of terpenoids.

2.4.5. Test for Tannins

2.4.5.1. $FeCl_3$ Test. To 1 mL of leaf extract 2 mL of $FeCl_3$ solution is added. Violet colour shows the presence of tannins.

2.4.5.2. Polyphenolic Test. 1 mL of *L. speciosa* extract was introduced to few drops of ferric chloride solution, and it yielded in the formation of intense bluish green colour which projects the presence of the phenolic group.

2.4.6. Test for Flavonoids

2.4.6.1. Alkaline Reagent Test. To 1 mL of leaf extract of *L. speciosa*, add sodium hydroxide solution (1 mL). The intense yellow colour is developed and on further addition of dil. HCl it becomes colourless thereby indicating the presence of flavonoids.

2.4.6.2. Shinoda Test. This test is also called as Magnesium Hydrochloride reduction test. To 1 mL of the leaf extract of *L. speciosa*, magnesium ribbon, and add hydrochloric acid drop wise, crimson red appears in few minutes' shows the presence of flavonoids.

2.4.7. Test for Proteins and Amino Acids

2.4.7.1. Ninhydrin Test. The leaf extract of *L. speciosa* was heated with 0.2% solution of Ninhydrin solution, no violet colour indicating the absence of proteins and amino acids.

2.4.7.2. Million's Test. Million's reagent (1 mL) was added to 1 mL of leaf extract of *L. speciosa*, no white precipitate was formed, upon boiling indicates the absence of amino acids and proteins.