

Green Synthesis of Silver Nanoparticles from Flower Extract of *Hibiscus rosa-sinensis* and Its Antibacterial Activity

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ABSTRACT: Silver nanoparticles (SNPs) exhibit magnificent applications in medicine as antimicrobial agent. The use of different plant parts for the synthesis of nanoparticles is considered as a green technology. This technology is one of the best eco-friendly method and does not gives any harmful effect. The present study deals with the flower extract of medicinal plant *Hibiscus rosa-sinensis* and to evaluate its anti-bacterial properties. The synthesis and characterization of silver nanoparticles was confirmed by UV-Visible spectrophotometer, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM). Agar well diffusion method was used to confirm the antibacterial activity of silver nanoparticles. UV-Visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 422 nm. FTIR analysis confirmed reduction of Ag⁺ ions to Ag⁰ ions in synthesized silver nanoparticles. The SEM analysis showed the particle size between 5-40 nm and spherical in structure. The silver nanoparticles have shown bactericidal effects against *Aeromonas hydrophila* infected fish *Catla catla*. This study revealed that *H.rosa-sinensis* synthesized silver nanoparticles exhibit good and potent antimicrobial activity against fish pathogen.

KEYWORDS: *Hibiscus rosa-sinensis*, Silver nanoparticles, UV, SEM, FTIR, Antimicrobial activity.

I. INTRODUCTION

Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometres. Nanotechnology is one of the fastest developing science over the last few years. This is an interdisciplinary science that connects knowledge of biology, chemistry, physics, engineering and material science [1]. The major challenge, the world facing today is the mode of treatment of pathogenic bacteria which have become resistant to the existing antibiotics. Day by day, the resistance to existing antibiotics or drugs is increasing for one or more reasons. This increasing incidence of antibiotic resistance among the microbial organisms necessitates an alternate therapy to curb the resistant infectious microorganisms [2]. A new approach to prevent or combat microbial pathogens is by the use of silver nanoparticles especially synthesized with the help of natural medicinal plants. Medicinal plants are already known for many therapeutic values and have been used since ages for curing many diseases and disorders including infectious diseases. This is because of the phytoconstituents present in them [3]. The use of plants for fabrication of AgNPs has drawn the attention of researchers as it is rapid, low-cost and single step method for the biosynthesis process. The rate of reduction of metal ions using plants has been found to be much faster as compared to microorganisms and in stable formation of metal nanoparticles [4]. The shape and size of nanoparticles synthesized from plants can be controlled and modulated by changing the pH. The biosynthetic method employing plant extracts has received attention as being simple and viable compared to chemical and physical methods for synthesizing metal nanoparticles [5]. The plant *Hibiscus rosa-sinensis* belongs to the family Malvaceae. With attractive and colourful flowers, plants of *Hibiscus* are widely planted as ornamentals and are used in traditional medicine. The plant species have been used as a folk remedy for the treatment of skin diseases, as an anti fertility agent, antiseptic and carminative and the flower possesses anti-spermatogenic, androgenic, anti-tumor and anticonvulsant properties [6, 7]. Hence the

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present study focused on the “Green synthesis of silver nanoparticles from flower extract of *Hibiscus rosa-sinensis* and its antibacterial activity”.

II. MATERIALS AND METHODS

2.1 Plant collection and authentication

The plant *H.rosa-sinensis* (L.) was collected from Saliyamangalam, Thanjavur, Tamil Nadu, India. It was taxonomically identified and authenticated by Rev. Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The voucher specimen was deposited at the Rapinat herbarium and the voucher number is **SS001**.

2.2 Synthesis of silver nanoparticles

About 150g of fresh flower of *H.rosa-sinensis* was weighed and washed with de-ionized water before use. The crushed flower were mixed with 1000 ml of de-ionized water and allowed to stand for 6 hrs at room temperature. The mixture was filtered. 150 ml of collected filtrate was mixed with 600 ml of 3 mM silver nitrate solution. The colour of the solution gets changed from red to dark brown which indicates the formation of silver nanoparticles. The reduced solution was centrifuged at 7000 rpm for 15 minutes. The centrifugation process was repeated for two to three times to remove any impurities adsorbed on the surface of silver nanoparticles. The dried powder was used for the experimental work [8].

2.3 Characterization Techniques

2.3.1 UV-Vis spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV - Vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

2.3.2 SEM analysis

The morphological characterization of the samples was done using JEOL Jsm- 6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis.

2.3.3 FT-IR analysis

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm^{-1} range at a resolution of 4 cm^{-1} . The sample was prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard in analysis of the samples.

2.4 Antibacterial Assay

The antibacterial activities of silver nanoparticle synthesized extract were carried out by agar well diffusion method. The antibacterial activities of the silver nanoparticle synthesized extract were tested against the fish pathogen *A.hydrophila* strains. The petriplates were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium was poured into each sterile petriplate and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swabs, 24 hours bacterial culture with known population count was spread over the plate by spread plate technique.

Three wells of 5 mm size each made into the agar plates with the help of sterile cork borer. The wells were loaded with 50 μ l of normal flower extract and silver nanoparticle synthesized extract. All the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well

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indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

III. RESULTS AND DISCUSSION

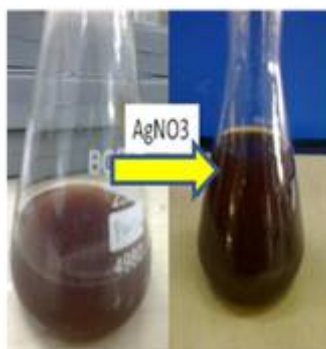
3.1 Phytochemical analysis of *H.rosa-sinensis*

Phytochemical screening was performed to test the presence of different phytochemicals from flower extract of *H.rosa-sinensis*. The qualitative phytochemical analysis of flower extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and anthraquinones.



3.2 Biosynthesis of silver nanoparticles

H.rosa-sinensis extract is used to produce silver nanoparticles in this experiment. Ag^+ ions were reduced to Ag nanoparticles when plant extract is mixed with AgNO_3 solution in 1:8 ratio reduction is followed by an immediate change in light colour to dark brown colour in the aqueous solution of the plant extract due to excitation of surface plasmon vibration in silver nanoparticle. Further formation of AgNPs in aqueous extract can be monitored by colour change. The appearance of a dark brown color in the reaction vessel indicates the formation of silver nanoparticles.



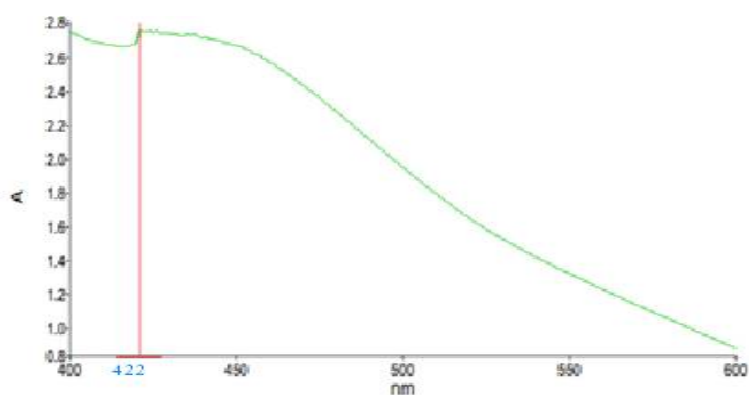
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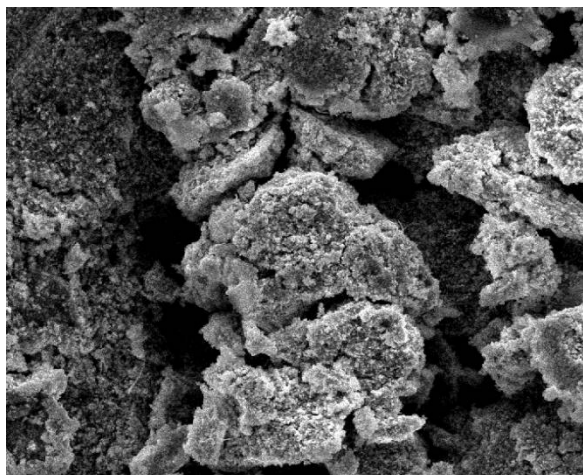
3.3 UV-Vis Spectra analysis

The reduction of silver is confirmed in the samples by visual observation. The sample exhibited dark brown. This colour variation may be attributed to excitation of surface plasmon vibration in silver nanoparticles. After 24 hrs incubation in dark room condition, the light coloured reaction mixtures turned into dark brown indicating silver nanoparticle formation. In the present result, the Surface Plasmon Resonance (SPR) of AgNPs produced a peak at 422nm, which suggests the dispersal of silver nanoparticles.



3.4 SEM analysis of silver nanoparticles

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells. SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which were irregular in shape. The size of the silver nanoparticles was found to be 5-50 nm, with an average size 15 nm. The larger silver particles may be due to the aggregation of the smaller ones.



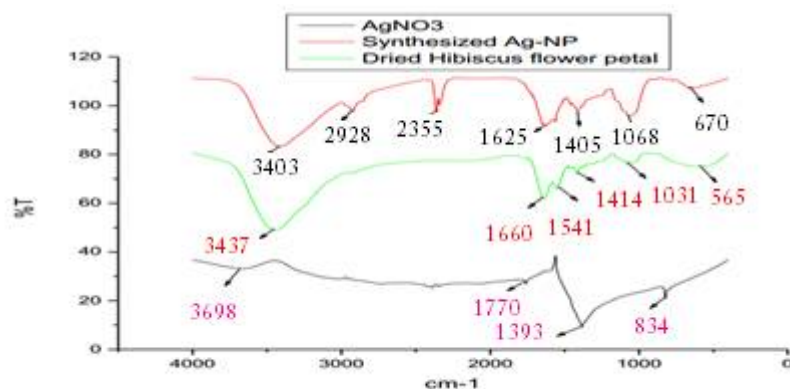
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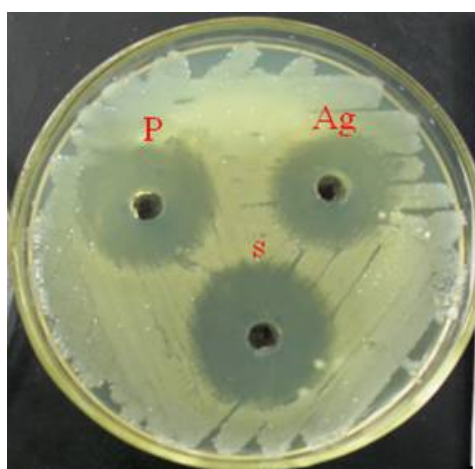
3.5 FTIR analysis

In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the silver nanoparticles given in the below figure exhibited peaks at 3403cm^{-1} , 2928cm^{-1} , 2355cm^{-1} and 670cm^{-1} which indicates the functional group of the plant component involved in the reduction and stabilization of AgNPs. The transmittance attributes O-H stretch, C=C bond and C-H which reveals that the water soluble heterocyclic components, polyols and certain proteins present in the extract which are involved in the reduction of silver nitrate.



3.6 Antibacterial activity of normal and silver nanoparticles synthesized flower extract

The antibacterial activities of normal and silver nanoparticle synthesized *H.rosa-sinensis* flower extract were carried out by agar well diffusion method. The maximum zone of inhibition by normal flower extract against *A.hydrophila* is 14mm. The synthesized silver nanoparticles exhibited maximum zone of inhibition against *A.hydrophila* is 16mm.



Ag – Silver

P - Plant Extract

S - Silver nanoparticles

Green synthesis of nanoparticles has been an emerging research area now a days. The advancement of green synthesis has an advantage over chemical and physical methods. The plant extract synthesized silver nanoparticles are environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles; furthermore there is

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no need to use high temperature, pressure, energy and toxic chemicals [9]. Chemical antimicrobial agents are increasingly becoming resistant to a wide spectrum of antibiotics. An alternative way to overcome the drug resistance of various microorganisms is therefore urgently needed. Ag ions and silver salts have been used for decades as antimicrobial agents in various fields due to their growth inhibitory abilities against microorganisms [10]. In general, silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death [11]. In the present study, antibacterial activities of normal and silver nanoparticles synthesized *H.rosa-sinensis* extract were carried out by agar well diffusion method. Synthesized silver nanoparticles were tested against selected fish bacterial pathogen *A.hydrophila*. The silver nanoparticles shows maximum zone of inhibition against bacterial fish pathogen when compared to normal flower extract. The results indicated that silver nanoparticles have good antibacterial activity against bacterial *A.hydrophila*. Similar report was reported that the biological synthesis of silver nanoparticles from *Dracocephalum moldavica* seed extract showed excellent antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Staphylococcus epidermidis* and *Bacillus subtilis* [12].

IV. CONCLUSION

This study concludes that *H. rosa-sinensis* has the capability to synthesize AgNPs and its medicinal activities. The confirmatory report of our studies indicates that herbal medicine can be used in fish health management and curing of diseases. Further studies can be carried out on valuable herbal resources and standardization of dosage. It is concluded from the obtained results that the herbal treatment for *Aeromonas hydrophila* infection in fish by *H.rosa-sinensis* are very effective and efficient. Improvement in herbal drugs can be tried in different plants and combination. This can also be tried in other fishes so that it will be a big boom to aqua culturists.

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