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Silver Nanoparticles Biosynthesized Using *Ficus hispida* Aqueous Extract And Their Antibacterial Studies

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Abstract: *In recent years, the green synthesis of silver nanoparticles using various plant extracts has attracted great attention. This is because, these methods are simple, inexpensive and, eco-friendly. In this study, it was observed that silver ions were reduced by Ficus hispida leaf extract after 5 min, leading to the formation of crystalline silver nanoparticles. The silver nanoparticles produced by the Ficus hispida extract were characterized by UV–vis spectrophotometry, In addition, AgNps synthesized by Ficus hispida extarct was tested for anti microbial activity by agar well diffusion method against the pathogenic bacteria Escherichia coli, Klebsiella pneumoniae (Gram negative) Staphylococcus aureus and Bacillus subtilis (Gram positive). The important outcome of the study will be the development of value added products from medicinal plants* Ficus hispida *for biomedical and nanotechnology based industries.*
Keywords*: biosynthesis; silver nanoparticles; plant extracts; antibacterial activity*

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I. Introduction:

The history of herbal medicine is as previous as human civilization. India has Associate in nursing ancient heritage of ancient system of drugs together with written material, Siddha, Unani and medical aid. The Ayurvedic drugs system and Indian pharmacological medicine give an excellent deal of knowledge on traditional knowledge practices and ancient aspects of therapeutically necessary herbs with the rising worldwide interest in adopting and learning ancient system of drugs and exploiting their potential supported totally different health care systems, the analysis of wealthy heritage of therapeutically active herbs is primarily administrated on the idea of morphological, phytochemical medicine and numerous instrumental techniques like natural process, etc. The employment of foods in solidification numerous ailments and up health is almost as previous as humanity. Among such foods, none is also older than the fig, recent investigations of that have indicated that it's been cultivated for over eleven. *Ficus hispida* is a coarsely hairy shrub or medium sized tree, up to 10 m tall, grows in secondary forests, open lands and river banks, up to 1200 m in altitude (Nadkarn et al., 1976). The development of reliable, eco-friendly processes for the synthesis of nano materials is an important aspect of nanotechnology. Nanotechnology also requires the synthesis of nano materials of different chemical compositions, sizes and morphology with an excellent control over these characteristics nanoparticles such as: short time stability and safety problem can be understandable by the use of other biologically synthesized methods such as the use of microbes. Many organisms can produce either extracellular or intracellular inorganic substances (Mann,s 1995). Silver is well-known for its antimicrobial property since time immemorial and ancient Greeks and Romans took advantage of silver's antimicrobial effects and used silver particles to fight against infections. Silver nanoparticles (AgNPs) have gained significant attention in recent years. There are several physical and chemical methods for synthesis of metallic nanoparticles, to reach the target of achieving simple and eco-friendly technology researchers in this field have turned to biological systems. However, not all biological entities can synthesize nanoparticles due to their enzyme activities and intrinsic metabolic processes, hence; selection of the biological medium entails versatile screening procedures and careful selection of an appropriate biological entity that can produce nanoparticles with well-defined properties, such as size and morphology (Baldi et al., 2016; Southam, and Beveridge 1994; Singh et al., 2011)

In this paper, we report a low-cost convenient green synthesis approach to obtain large quantities of silver nano particles by reduction of silver ions with using *Ficus hispida*.

II. Materials and Methods:

Reagents and chemicals: Silver nitrate and all analytical grade chemicals were purchased from Merck Chemicals, Mumbai. Freshly prepared triple distilled water was used throughout the experiment.

Preparation of leaf extract by boiling method

Ficus hispida, Indian medicinal plant, was collected from Vana Vignan, (17^0 59'34''N 79^0 33'32''E) Warangal, Telangana India **(Figure 1),** on the basis of cost-effectiveness, ease of availability and medicinal property. Healthy and fresh leaves were collected locally and washed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. 10 g of these fresh leaves of each plant type were weighed separately and transferred into 250 mL beakers containing 100 mL distilled water and boiled for 5 min. By using Whatman No. 1 filter paper the extracts were then filtered thrice through to remove particulate matter and to get clear solutions. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Plant-Mediated Synthesis of Ag NPs

Silver nitrate solution with the concentration of 1 mM was prepared freshly under dark conditions to prevent its decomposition. During continuous shaking, 90 mL of $AgNO_3$ solution was heated up to 80 °C and 10 mL of *Ficus hispida* extract was slowly added to the hot liquid solution and incubated in the temperature range 75–85 °C using a water bath for 15 min. The formation of Ag NPs was observed by transformation from the light brownish yellow to the dark brownish red color monitored by UV-Vis spectroscopy. (Balaz et al., 2017)

UV-Vis spectra Analysis

The localized surface plasmon resonance of silver nanoparticleswas characterized by using UV–Vis spectrophotometer (ELICO SL-159 Spectrophotometer in the range 350 - 470 nm).

Antibacterial activity

AgNps synthesized by *Ficus hispida extarct* was tested for anti microbial activity by agar well diffusion method against the pathogenic bacteria *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative) *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive). Twenty ml of nutrient agar medium was spewed into sterilized petri dishes. The pathogenic bacterial strains were grown in nutrient broth for 24 h. To prepare the bacterial lawns 100 µl nutrient broth culture of each bacterial organism (1×10^5 CFU/ml) was used. Sizes of 8 mm diameter agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells were filled with 60 µl of Ag nanoparticles solution synthesized from *Ficus hispida* and 60 µl of 1 mM silver nitrate, along with 60 µl of 30 µg/ml of streptomycin served as a positive control (Krishna and Charya et al., 2016). The plates were incubated at a temperature of 37oC for 24 h and then were examined for the existence of zones of inhibition. The diameter of such zones of inhibition was measured and the mean value for each organism was recorded and expressed in millimeter unit.

III. Result and Discussion

The use of plants for nanoparticle synthesis offers a wide range of benefits over other biological synthesis methods because it does not require the maintenance of cell cultures and incorporates support for the large-scale synthesis of nanoparticles (Shanker et al., 2005) In recent days plant mediated synthesis of nanoparticles is a common practice. There are many reports of biosynthesis of silver nanoparticles using plants. However, after extensive literature survey and to the best of our knowledge, we report the biosynthesis of silver nanoparticles by *Ficus hispida***.** Extract of *Ficus hispida* is used to produce silver nanoparticles in this experiment Ag+ ions were reduced to Ag nanoparticles when the extract is mixed with $AgNO_3$ solution in 1:8 ratio reduction is followed by on immediate change in yellowish to brown color in the aqueous solution of the plant extract due to excitation of surface Plasmon vibration in silver nanoparticle.(Praba et al., 2015) Further formation of AgNPs in aqueous extract can be monitored by color change. **(Figure. 2).** Shows the color changes when the aqueous extract of *Ficus hispida* plant was mixed with an $AgNO_3$ solution. The mixture was kept at room temperature for 24 hours. The appearance of a yellowish-brown color in the reaction vessel indicated formation of AgNPs. AgNPs exhibit this yellowish-brown color in aqueous solution due to excitation of surface plasmon resonance in the AgNPs **(Figure 3).** Banerjee, Satapathy, Mukhopahayay, and Das, 2014; Tripathy et al., 2010, reported the appearance of the brown colour was due to the excitation of the Surface Plasmon Resonance (SPR), typical of silver nanoparticles having absorbance values in the visible range of 446-448 nm. The antimicrobial effects of silver nano particles against four pathogenic microorganisms were evaluated by agar well diffusion method. The pathogenic bacteria, *E.coli, K. pneumoniae* (Gram negative) *S.aureaus* and *B.*

subtilis (Gram positive) were effectively controlled by silver nano particles. The inhibitory effect of Ag nanoparticles was more in S. *aureus* and *E.coli* as compared with other bacteria **(Table 1)** and these results suggest that the antimicrobial effects of Ag nanoparticles are bacterial specific. Okafor et al., 2013 reported that the plant extract-produced AgNPs exhibited high inhibitory effects against *E. coli* and *Salmonella* while moderate activity was observed for Pseudomonas *aeruginosa*, *Bacillus subtilis* and *Kocura rhizophila*. Allafchian et al., 2016 reported the antibacterial activity of biosynthesized silver nanoparticles, was studied against Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative (*S. typhimurium* and *E. coli*) bacteria using the agar well diffusion assay, and the zone of inhibition. The synthesized AgNPs displayed efficient antibacterial activity against both Gram-negative and Gram-positive bacteria.

IV. Conclusion

In conclusion substantial amounts of silver nanoparticles (AgNPs) were produced and this was verified using UV-visible Spectroscopy. Appearance of a Surface Plasmon Resonance (SPR) peak, which corresponds to the silver nanoparticles. In conclusion based on our results, silver nanoparticles synthesized by plant extract showing a great promising antimicrobial activity. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as antimicrobial systems and pharmaceutical applications.

Figure 1. Picture of *Ficus hispida* plant

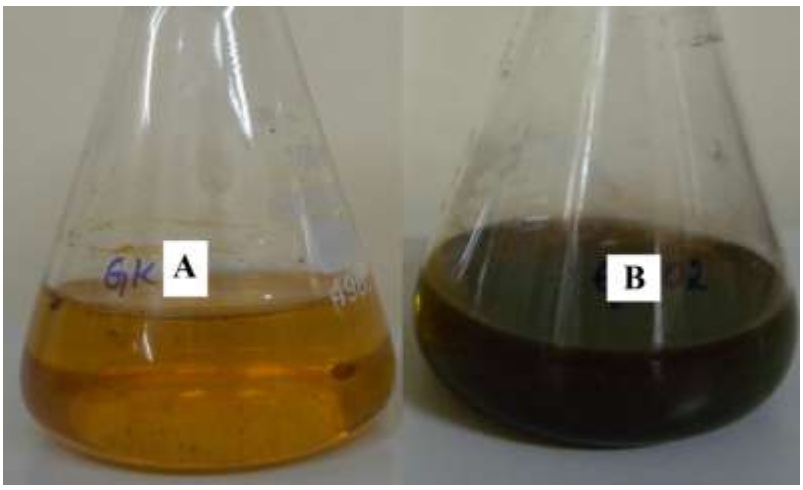

Figure 2. Formation of silver nanoparticles and it is identification

by the colour change. (A) plant extract without silver nitrate, (B) plant extract with silver nitrate solution

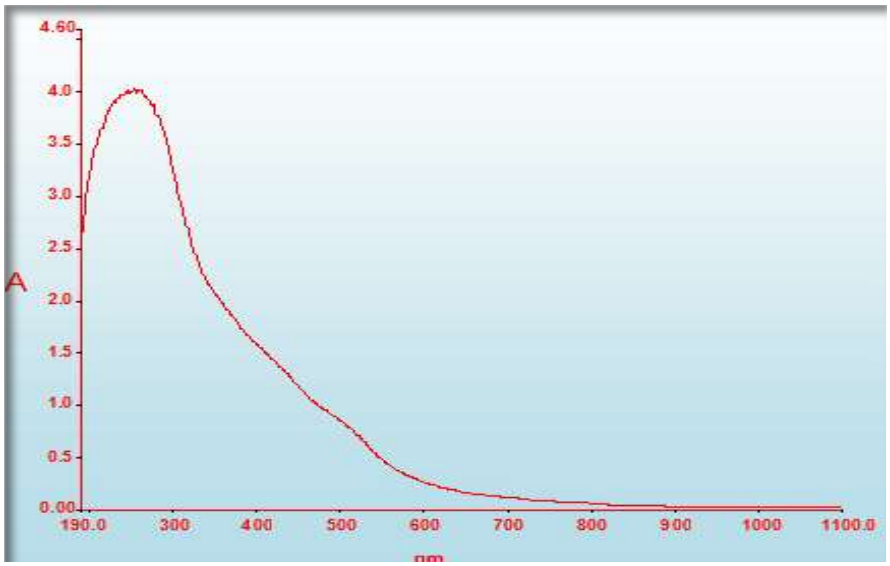

Figure 3. UV-Vis Absorption Spectra of *Ficus hispida* leaf extract

Table1: Antibacterial activity of silver nanoparticles produced *Ficus hispida*

Organisms	Silver nitrate	Silver nano particle solution	Streptomycin
Bacillus subtilus	18	24	28
Klebsilla pneumonia	15	21	26
Staphylococcus aureus	15	26	29
E. coli	16	26	30

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