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# Green synthesis and antibacterial activity of silver nanoparticles synthesized using *Ficus auriculata* fruit extracts

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**ABSTRACT**

Plant mediated synthesis of nanoparticles is a green chemistry approach that interlinks nanotechnology and plant biotechnology, since the biosynthesis of nanoparticles has been proposed as a cost effective and environmental/ecofriendly alternative to chemical and physical methods. In the present study silver nanoparticles (AgNPs) were synthesized from *Ficus auriculata* fruit extract, and were characterized by UV–Visible (UV-vis) spectroscopy, Fourier Transform Infra-red Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM) techniques. UV-Visible absorption spectra of the reaction medium containing AgNPs showed maximum absorbance at 435 nm. FTIR analysis confirmed the presence of various functional groups in synthesized AgNPs. The SEM analysis showed the synthesized AgNPs are irregular in structure having the size range of 5-40 nm. Green synthesized silver nanoparticles were checked for their bactericidal activity against *E. coli* DH5α strain with respect to plant extract and antibiotic kanamycin (25 μg/mL) as a control. We observed that the synthesized AgNPs have significantly higher antibacterial activity than kanamycin. Thus, the AgNPs synthesized could be put to use for checking and controlling bacterial growth and other therapeutic uses.

***Key words:*** *Silver nanoparticles, UV-vis spectroscopy, FTIR, SEM, Antibacterial activity, Ficus auriculata*

## Introduction

The term nano is derived from Greek word nanas which means "dwarf " or tiny particles which is $10^{-9}$ meters and ranges from 1 to 100 nm. Nanotechnology is the fast emerging area of science of the 21st century. It has ability to convert the nanoscience theory to useful applications (National Nanotechnology Initiative (NNI). Available online: www.nano.gov (accessed on 22 July 2019).) The field of nanotechnology is one of the most active areas of research in modern material science (Samer Bayda *et al.,* 2019). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Applications of nanoparticles and nanomaterials are constantly emerging. Among noble metal nanoparticles, AgNPs have a wide area as they have a large number of applications, such as in nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, and as antibacterial capacities. During last decades the applications of nanotechnology found place in many biology related areas such as diagnostics, drug delivery, and molecular imaging that are being intensively researched and offering excellent alternatives. AgNPs have properties that have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, photography, electronics, and in the food industry. During last decade principles of nanotechnology have also been applied to the field of molecular biology specially DNA nanotechnology and protein chemistry (Rothemund, 2006). In the field of nano-oncology amazing progress has been made by improving the efficacy of traditional chemotherapy drugs for a plethora of aggressive human cancers (Yuan *et al.,* 2019). These advances in nano oncology have been achieved by targeting the tumour sites with several functional molecules including nanoparticles, antibodies and cytotoxic agents. In this context, many studies have shown that nanomaterials can be employed to deliver therapeutic molecules to modulate essential biological processes, like autophagy, metabolism or oxidative stress, exerting anticancer activity (Cordani and Somoza, 2019). There are various medical applications of AgNPs like in dressings, silver coated medical devices such as nanolotions, nanogels, etc. (Ma *et al.,* 2010; Piccinno *et al.,* 2012). Different methods of preparation of AgNPs have been developed. These methods developed for AgNPs synthesis give preference to control their shape and size. There are several physical and chemical methods available to reduce $Ag^+$ to $Ag^0$ like irradiation of Ultraviolet rays (UV), heating and electrochemical reduction, hydrazine, sodium borohydride, polyethylene glycol etc. (Wiley *et al.,* 2005). Silver has been used since ages for its antimicrobial properties. The synthesis and use of silver nanoparticles has become crucial as many pathogenic bacteria have become antibiotic resistant. Three main steps involved in green synthesis of AgNPs, which is based on green chemistry approaches, includes selection of solvent medium, reducing agent, and nontoxic stabilizers. Plants are considered as chemical factories occurring in nature.They produce secondary metabolites which have reducing properties and these properties of plants are useful for the synthesis of metal nanoparticles. Since plants occur naturally, using them for synthesis of nanoparticles is cost effective, eco-friendly and requires low maintenance. Large amount of metabolites are found in plants. Thus, nanoparticles synthesized by their extracts are considered more stable and less time consuming as compared to other systems used in production of nanoparticles, such as microorganisms. It is easy and environmental/ecofriendly approach compared to complex process of maintaining cell cultures and handling chemicals. Thus, green synthesis approach of metals nanoparticle synthesis using plants or their parts was adopted to synthesize AgNPs using *Ficus auriculata* fruit extract.

*Ficus auriculata* is a small, perennial evergreen tree which is cultivated in South and Southeast Asia and Brazil for its edible fruits. The stem bark is used to treat diarrhea and dysentery (Manandhar, 1991). Its latex is applied on cuts and wounds as an antiseptic. It is also grown for ornamental purposes (Kunwar and Bussmann, 2006).

During last few decades it has been observed that both silver and gold nanoparticles are being synthesized using extract of various plants like *Chenopodium* (Dwivedi and Gopal, 2010), *Coleus amboinicus* Lour (Narayanan and Sakthivel, 2010), *Cinnamomum camphora* (Huang *et al.,* 2007), *Sorbus aucuparia* (Dubey, 2010), *Hibiscus rosa sinensis* (Philip, 2009), *Ocimum sanctum* (Philip *et al.,* 2011). There are reports on green synthesis of nanoparticles using extracts of fruits like Papaya (Jain *et al.,* 2009), Tansy (Dubey *et al.,* 2010), Pear (Ghodake *et al.,* 2010), lemon (Prathna *et al.,* 2011) and Gooseberry (Krishnaraj *et al.,* 2010). Although there were many reports on biosynthesis of nanoparticles using various other plant extracts, no report is published on the synthesis of AgNP's from fruits of *Ficus auriculata*. Therefore, the present study was undertaken to synthesize AgNPs using *Ficus auriculata* fruits extract and subsequently checked for its potential antibacterial activity against *E.coli*.

## Materials and Methods

### Materials

Fruits of *Ficus auriculata* were collected from a village of Khatima town, Uttarakhand, India. Silver Nitrate ($AgNO_3$) was procured from Sigma-Aldrich, USA. Nutrient Agar media was sourced from Hi-Media, Mumbai, India. *E. coli* DH5α culture was maintained on Nutrient Agar media. All the chemicals used in this study were analytical grade. Standard culture of *E. coli* DH5α was obtained from our Departmental laboratory culture stocks.

### Biosynthesis of Silver nanoparticles

About 11.9 g fruits of *Ficus auriculata* were thoroughly washed with tap water followed by de-ionized water to remove dust particles and other contaminants. Fruits were sliced into small pieces with sterile surgical blade and then crushed in sterile mortar and pestle by adding de-ionized water to prepare aqueous extract. The aqueous extract was centrifuged at 12000 rpm for 30 minutes at room temperature, supernatant was collected and stored at 4 °C for further use. Aqueous extract and 1.0 mM $AgNO_3$ were mixed in ratio of 1:2 for the Green synthesis of silver nanoparticles and incubated at room temperature to allow it to undergo a color change. Experiment was performed in triplicate to check the accuracy and repeatability of the results. To remove any kind of impurity, reaction mixture was washed by repeated centrifugation (3x) at 15,000 rpm for 20 minutes and supernatant was replaced by de-ionized water each time. Furthermore, purified nanoparticles were freeze dried (lyophilized) to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR and SEM and checked for their antibacterial activity.

**Characterization of Synthesized AgNP's**

The formation of AgNP's was confirmed by recording the spectral scan using UV-visible spectrophotometer (UV-10 Thermo Fischer) periodically at different time intervals. 1.5 mL of the diluted supernatant of the samples was placed in a quartz cuvette with a resolution of 1 nm and path length of 1 cm, placed in a UV- Vis spectrophotometer in the wavelength range of 300-700 nm to obtain the UV-Visible spectra of the samples. The pH was adjusted as required before scanning in quartz cuvettes with deionized water as reference. Fourier transforms infrared (FT-IR) analysis was carried out on Perkin Elmer Infra-red spectrophotometer to detect the chemical functional groups present in the samples. The transmittance of the sample was measured at the wavelength of FTIR spectra using KBr pellet of synthesized AgNPs. Spectra were recorded between 4000 and 400 cm"1.

Scanning Electron Microscope (SEM) (ZEISS EVO-40) imaging and analysis were performed to characterize size and shape of the synthesized AgNPs. Purified nanoparticles were centrifuged at 15,000 rpm for 15 minutes and lyophilized to make a fine powder, a drop of this solution were mounted on the copper grid. The small drop of solution was allowed to dry for about 5 mint before visualizing and then the extra samples around SEM grid was removed with a blotting paper. The SEM images were recorded at 40,000x magnifications.

**Antibacterial activity**

The antibacterial activity of synthesized AgNPs against *E. coli* DH5á was evaluated using well diffusion method according to the procedure described by Perez *et al.*, 1990.The bacterial culture was inoculated in 100 mL nutrient broth and overnight incubated in incubator shaker. 100 mL of nutrient agar is prepared and poured in three petri plates (30 mL in each plate) and spread with the help of sterile spreader. With the help of sterile cork borer, three agar wells of 6 mm diameter eachwere punched in all the nutrient agar plates. The wells were marked as 1, 2 and 3. The agar well 1 and 2 loaded with 20 µL of silver nanoparticles and fruit extract, respectively. The well 3 loaded with standard antibiotic kanamycin (25ug/mL) as a positive control. Plates were subsequently incubated at 37 °C for 24 h and the zone of inhibition around the wells was measured in millimeter.

## Results and Discussion

The biosynthesis of the AgNPs in aqueous supernatant was observed by noting the absorption spectra at wavelength ranges from 300-700 nm (Fig.1.A). Color change was observed in the reaction mixture from colorless to golden brown within 30 minutes. It was observed that solution of silver nitrate turned golden brown on addition of fruits extract and after 1 h incubation at room temperature the color of the solution turned dark brown; it indicated the formation of AgNPs, while no color change was observed in the absence of plant extract (Fig.1.A). UV-Vis spectra were used to confirm the formation of AgNPs in the colloidal solution. In the UV-vis spectrum; a single, strong and broad surface plasmon resonance (SPR) peak was observed at 450 nm that confirmed the synthesis of AgNPs, which increased with the incubation time period 1 h 2 h and 48 h. (Fig.1.A). Several previous studies have noted that SPR peak located between 410 to 450 nm has been observed of AgNPs and might be attributed to special nanoparticles.

The FTIR analysis was carried out on Perkin Elmer spectrum two spectrophotometer using KBr pellet in the ratio of 1:300 mg. The transmittance of the sample was measured at the wavelength of 4000-600 $cm^{-1}$ at 4 $cm^{-1}$ resolution. It was analyzed for the identification of functional groups. These functional groups reflect the presence of chemical compounds which are responsible for the reduction of $Ag^+$ ions to $Ag^0$ and capping of the AgNPs (Fig. 1.B). The C–C stretch (in-ring) at 1600-1585 $cm^{-1}$, C–O stretch at 1260-1050 $cm^{-1}$ and O–H stretch hydrogen bonded at 3500-3200 $cm^{-1}$ were observed in plant extract while, C–C stretch (in-ring) at 1600-1585 $cm^{-1}$, and O –H stretch hydrogen bonded at 3500-3200 $cm^{-1}$ were observed in synthesized AgNP's. (Fig. 1.C). These data indicate that; the larger size of nanoparticles might be due to the presence of these capping molecules.

For SEM analysis the synthesized AgNP's were

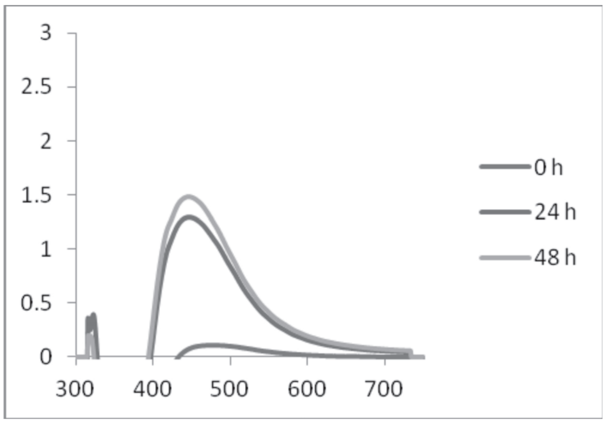


**Fig. 1A**

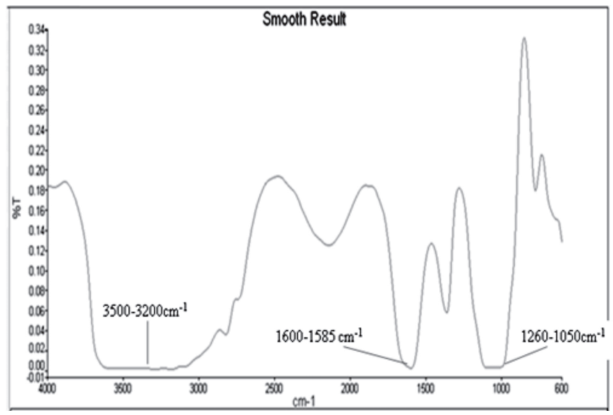


**Fig. 1B**

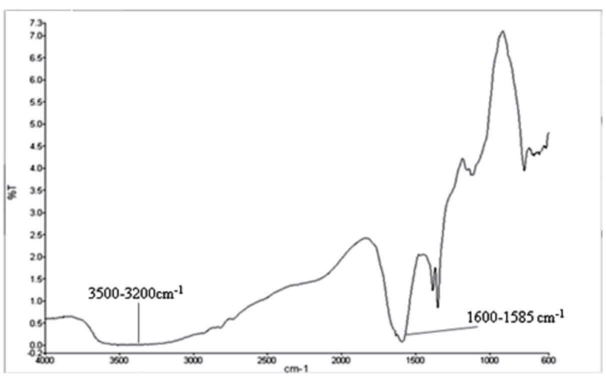


**Fig. 1C**

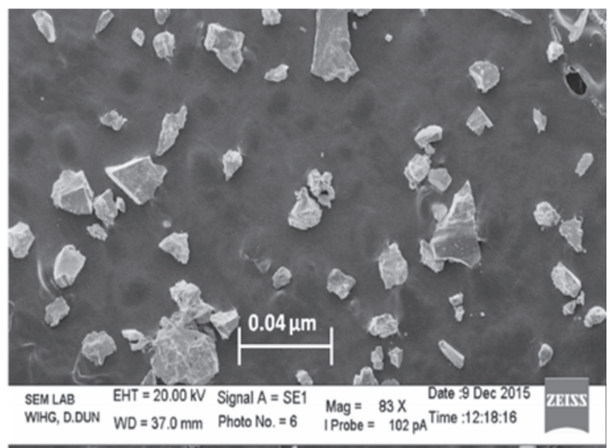


**Fig. 1D**

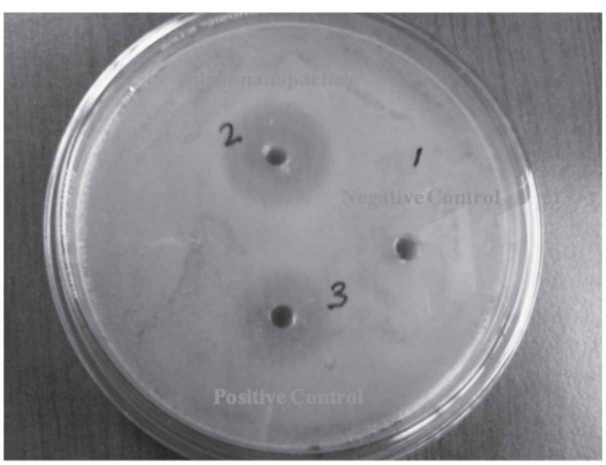


**Fig. 1E**

**Fig. 1. A.** UV-Visible spectra of synthesized AgNPs. **B.** FTIR spectra of aqueous extract of *F.auriculata* fruit. **C.** FTIR spectra of KBr pellet of synthesized AgNPs. **D.** SEM Image of synthesized AgNPs. **E.** Antibacterial activity of AgNPs. Was assessed by comparing (1) negative control, only extract. (2) synthesiged AgNPs with (3) the positive control, Kanamycin.

purified by washing using centrifugation at 15,000 rpm for 15 min thrice, after the complete washing AgNPs were lyophilized to make a fine powder. During SEM analysis a small amount of purified AgNP's were mounted on the copper grid and analyze on ZEISS EVO-40 SEM machine (Fig.1.D). The SEM analysis was performed at Wadiya Insititute of Himalayan Geology (WIHG) Dehradun, Uttarakhand. The SEM analysis showed the particle size between 5-40 nm, and irregular shaped structure.

**Antibacterial activity of AgNPs**

Several pathogenic bacteria are already known to exhibit resistance against several available antibiotics, exploiting AgNPs to target resistant bacteria could be another alternative of limiting the propagation of these bacterial pathogens**.** The antibacterial activity of biosynthesized AgNPs was evaluated against *E. coli* using agar well diffusion method. Zone of inhibition around the well denoted as a function in bacterial growth inhibition. Results show that the biosynthesized AgNPs exhibited more pronounced antibacterial activity than standard antibiotic kanamycin and plant extract (Fig.1.E). Bactericidal effect may be attributed to Ag and antibacterial action to the binding potentiality of $Ag^{+}$ ions with various bacterial cell compartments as DNA molecules and cytoplasm which leak out from the injured cell wall. In this study, the appearance of clear inhibitory zones confirmed the antibacterial activity of newly synthesized AgNPs. These findings are in agreement with several previous studies that examined antibacterial activity of AgNPs.

## Conclusion and Recommendations

The study highlights the green synthesis of AgNPs facilitated by *Ficus auriculata* fruits. The synthesized AgNPs have been characterized by UV- Vis, FT-IR and SEM. The AgNPs have also been evaluated for its antibacterial activity.UV–vis spectra and SEM analysis confirmed the reduction of Ag+ to $Ag^{0}$ and finally the synthesis of AgNP's. The efficacy of synthesized AgNP's was measured by testing the antibacterial activity. The zones of inhibition formed in incubated bacterial culture indicated that, the AgNP's has efficient antibacterial activity against E. coli DH5α strain than plant extract and antibiotic like Kanamycin. Hence, the green synthesis of AgNPs using *Ficus auriculata* fruits was shown to be rapid, eco-friendly and produces nanoparticles are fairly uniform in size and shape. Green chemistry approach seems to be biological approach appears to be rapid, eco-friendly and easy, cost-efficient substitute of conventional physical and chemical methods of silver nanoparticles synthesis. On the other hand, the potential of AgNPs on human pathogens opens a door for a new range of antimicrobial activity. So, it can have summarized that,such a rout of green synthesis of AgNPs is economically efficient as well as ecofriendly in nature and also capable of rapidly synthesize the silver nanoparticles in ambient temperature and could be of massive use in medical sciences for their efficient antibacterial activity.

The silver nanoparticles produced by *F. auriculata* fruit extracts are economical, efficient and the process is eco-friendly. Nanoparticle nature of colloidal silver was confirmed by visual observation of colour change, UV–vis spectrophotometry and SEM techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zone of inhibition formed in the antimicrobial activity screening test indicated that the AgNPs synthesized by this process has greater antimicrobial activity against the tested strain of bacteria compared to the tested antibiotic. The biologically synthesized silver nanoparticles could be of immense use in medical field for their therapeutic potential as such or as nanoconjugates and nanocomposites for efficient anti-bacterial and antimicrobial function. Green chemistry approach is a biological approach which is rapid, eco-friendly and easy, cost-efficient, substitute of conventional physical and chemical methods of silver nanoparticles synthesis.

Nanotechnology is an emerging area of science that has potential to drive sustainable agriculture. Agriculture based production processes generates tremendous amount of waste that can be converted into a ver low cost raw material for production of nanoparticles. The green AgNPs synthesized by use of herbal or plant or plant part based extracts are easy to synthesize and can make use of various parts or byproducts of various agri-based processes and recycle material otherwise considered as waste to make cost effective and valuable metal nanoparticles and could bring higher returns to the farmers and could be a significant value addition and an important contribution to the rural economy. Besides the bactericidal nature of AgNPs tested in this study, AgNPs also have broad antimicrobial (anti-bacterial, anti-fungal) activity in addition to pesticidal activity. Therfore, AgNPs could be employed to cure plant and animal diseases by utilizing their cytotoxic properties.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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