INTRODUCTION

Much excitement has been created among researchers at the birth of matter at the nanoscale which has promised to metamorphose many aspects of material sciences. The field of nanotechnology is related with the evolution of exploratory processes for the production of nanoparticles of various sizes, controlled disparity, and shapes.[1] Silver being important metal, silver nanoparticles (AgNPs) have a number of applications from electronics to medical diagnosis.[2] The various techniques available for the preparation of AgNPs are electrochemical method,[1] chemical reduction method,[4] and microbial synthesis method.[5] Methods other than biological one involve the use of chemicals and high energy requirements which are rather difficult.[6] Hence, it is necessary to synthesize AgNPs by a route which is cost effective and eco-friendly.

The AgNPs can be synthesized using microorganisms, plants, and viruses or their by-product such as proteins and lipids. The synthesis rate of AgNPs is much faster in plant-mediated synthesis rather than using microorganisms.[8] With the huge plant diversity, much more plants are still not explored for the synthesis of AgNPs. The extracts of *Tabernaemontana divaricata*,[7] *Chrysanthemum indicum*,[8] *Azadirachta indica*,[9] *Calliandra haematocephala*,[9] and *Pleurotus citrinopileatus*[10] plants reported as an alternate source for the synthesis of AgNPs.

In Ayurveda, *Berberis aristata* (*B. aristata*) is a versatile medicinal plant used singly or in combination with other medicinal plants for treating a variety of ailments such as enlargement of spleen, leprosy, rheumatism, fever, morning or evening sickness, and snakebite.[11-14] Stem extract of *B. aristata* (*Daru Haldi or Daruharidra*) found in the Himalayas in India till date has not been used for nanoparticle synthesis. Daruharidra is used for the treatment of skin disorders, internal medication as well as external applications.[13] It is used in inflammation, wound healing, skin diseases, and jaundice.[16] Therefore, the plant is used as reducing agent for converting silver ions into AgNPs. AgNPs have been extensively used as antibacterial agents in the health industry, food storage,[17] nanoscale sensors,[18] larvicidal activity,[19] and anticancer activity.[8,20]
MATERIALS AND METHODS

Preparation of Plant Extract

The stem of Daruharidra was brought from Wagh Brother’s Agency, Itwari region, Nagpur. These parts of plants were washed with distilled water to remove debris and other contamination and dried in hot air oven at 60°C for 24 h. Dried parts of plant were then crushed into fine powder using domestic blender. Then, 10 g of plant powder was weighed individually, kept in a beaker containing 100 ml of distilled water, and boiled at 60°C for 20 min. The extracts were filtered using Whatman filter paper no.1 and stored in a refrigerator for further use.

Synthesis of AgNPs

Aqueous solution of 1 mM of AgNO$_3$ was prepared. 5 ml of prepared extract Daruharidra was added in flask containing 30 ml of 1 mM AgNO$_3$ solution. The solution was then kept in a light as well as dark condition for 24 h incubation. The color change was observed from light yellow to dark brown after incubation, which indicates the bioreduction of Ag$^+$ ions.

Characterization of Synthesized AgNPs

Ultraviolet-visible (UV-VIS) spectrometry

Optical property of AgNPs was determined by UV-VIS spectrophotometer. The reduction of Ag$^+$ ions was observed by measuring spectrum in the range of 200–800 nm by comparing with control (distilled water + plant extract) as blank.

Fourier transform infrared (FTIR) analysis

The chemical composition of the synthesized AgNPs was studied using FTIR spectrometer. It is used to analyze possible biomolecules and also bonding interactions. The absorbance was measured in the range of 400–4000 nm. Sample was placed into one of the KBr plates, and the plates were kept into the sample holder.

Scanning electron microscopy (SEM)

SEM is the type of electron microscope that produces an image of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in a sample, producing various signals that contain information about the sample’s surface topography and composition. SEM can achieve resolution better than 1 nm. Specimens can be observed in a high vacuum, wet condition, and a wide range of cryogenic temperatures. Samples for SEM can be solid, bulk specimens of any size that will fit the specimen chamber.

Transmission electron microscopy (TEM)

TEM is a microscopy technique in which a beam of electrons is transmitted through an ultrathin specimen, interacting with the specimen as it passes through it. A thin specimen is exposed to high-energy electron beam. It gives microstructure and nanostructure size and morphology.

Antimicrobial Assay

Antimicrobial analysis was done on the Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) by well-diffusion method using pour plate technique. Nutrient broth cultures were prepared using E. coli and P. aeruginosa and incubated at 37°C for 24 h (Orbital Shaking Incubator, BIO-TECHNICS INDIA). 1 ml of bacterial culture was inoculated in the nutrient agar medium, and the plates were made using pour plate method and allowed for solidification. After solidification, wells were punched using sterile tips. 200 µl of AgNPs were loaded and incubated at 37°C for 48 h (Cooling Incubator, REMI). Antimicrobial activity was measured based on the zone of inhibition obtained around the wells.

RESULTS AND DISCUSSION

UV-VIS Spectrometry

Reduction of silver ions into AgNPs during exposure to plant extracts was observed as a result of the change in color, which is the primary method to confirm the synthesis of AgNPs [Figure 1]. The sharp absorption of AgNPs was observed at 481 nm (1.9187 ABS) for B. aristata (Daruharidra) stem [Figure 2]. The peak was raised due to the effect of surface plasmon resonance surface plasmon resonance of electrons in the reaction mixture, which is also responsible for the brown color.

FTIR Analysis

The FTIR spectrum of synthesized AgNPs from Daruharidra is shown in Figure 3 with four main bands. The broadband appearing at 3450/cm is signed for O-H stretching vibration, indicating the presence of hydroxyl groups. The peaks at 1600/cm correspond to C-C and C-N stretch. The peaks at 1400/cm correspond to C=C and phenolic hydroxyl vibrations.

Figure 1: Synthesized silver nanoparticles from Daruharidra
The peaks at 1000/cm correspond to C-O vibrations. These functional groups have been reported to play a crucial role in stability, capping of AgNPs. From FTIR results, it can be concluded that some of the bioorganic compounds from *B. aristata* extract formed the strong coating or capping on the AgNPs.

**SEM Analysis**

The SEM images obtained at 10 µm, 5 µm, and 1 µm are shown in Figure 4. The SEM image showed the presence of high-density and compact agglomerates of silver particles. The nature of AgNPs was found to be highly granulated. The images also showed the presence of various shapes such as spherical, ellipsoidal, and few irregular fused agglomerates. Similar kind of result was also obtained with *Coffee arabica* seed.

**TEM Analysis**

TEM has been used to identify the size, shape, and morphology of nanoparticles. TEM micrograph of the synthesized AgNPs at 100, 50, and 20 nm scale is shown in Figure 5. It was found that AgNPs were spherical in shape and SAD pattern showed definite crystalline ring structure. Similar kind of result was reported with *Pleurotus*.

**Antimicrobial Activity**

The results of antimicrobial activity of synthesized AgNPs by well diffusion method are shown in Table 1. Silver is a naturally occurring element which is non-toxic and does not accumulate in the body to cause harm effects and is considered to be safe for the environment. Most manufactured goods such as washing machines, air conditioners, and refrigerators are using linings of AgNPs for their antimicrobial qualities. We observed that the synthesized AgNPs have antimicrobial activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* [Figure 6 and 7].

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**Figure 2:** Ultraviolet-visible spectra of silver nanoparticles synthesized from Daruharidra

**Figure 3:** Fourier transform infrared spectra of silver nanoparticles synthesized from Daruharidra

**Figure 4:** Scanning electron microscopy images of silver nanoparticles by Daruharidra (a) 10 µm, (b) 5 µm, and (c) 1 µm

**Figure 5:** Transmission electron microscopy images of silver nanoparticles from Daruharidra at (a) 100 nm, (b) 50 nm, (c) 20 nm, and (d) 10 nm
AgNPs were reported to be more efficient than silver ions in terms of its antimicrobial activity.\textsuperscript{[26]}

**CONCLUSION**

The present study is aimed to develop eco-friendly, fast, and cost-effective method for the biosynthesis of AgNPs from *B. aristata*. The silver ions are exposed to an aqueous solution of Daruharidra stem, and a rapid color change of plant extract signifies the biosynthesis of AgNPs, which was confirmed by UV spectroscopic method. This eco-friendly method could be competitive alternative to the conventional physical and chemical method used for the synthesis of silver nanoparticle and has a potential to use in biomedical applications. These green-synthesized AgNPs are polydispersed, spherical with agglomeration and have sizes ranging from 20 to 100 um which shows antimicrobial activity against Gram-negative bacteria *E. coli* and *P. aeruginosa* and acts as a potential antimicrobial agent. Moreover, high amount of small-sized nanoparticles can be produced with a little amount of plant extract and is beneficial for the society and environment. However, further investigation is needed to identify the scaling up of this extract of the biosynthesis of AgNPs for its medicinal use.

**REFERENCES**