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Biological Synthesis of Silver Nanoparticles Using *Hibiscus Sabdariffa* Leaf Extract and It's Antibacterial Sensitivity Assay

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Abstract

In this study, $AgNO_3$ was effectively used to synthesize silver nanoparticles. Hibiscus sabdariffa leaf extract was used to promptly and easily synthesize silver nanoparticles. An UV-Visible spectrophotometer showed absorbance peak in range of 300-600 nm. The procedure was found to be easy to follow, quick, least step and safe for the environment. It is non-toxic, and a viable substitute for traditional physical or chemical procedures. At room temperature, silver ions were converted into silver nanoparticles in about 15 to 20 minutes without the need of any dangerous chemicals. The growing prevalence of antibiotic resistance in bacteria is becoming a significant issue for accurate Infectious illness diagnosis and treatment. Escherichia coli (E. coli), Enterococcus faecalis (E. faecalis), Pseudomonas aeruginosa (P. aeruginosa) and Klebsiella pneumoniae are microbes used for assessment and evaluation of antibacterial sensitivity test against Hibiscus sabdariffa based silver nanoparticles. It is found that gram positive and negative both strains of bacteria affected by the antibacterial qualities of Hibiscus sabdariffa based silver nanoparticles.

Keywords: *Hibiscus sabdariffa*, silver nanoparticles, antimicrobial sensitivity, Green synthesis, Plant extract, UV–Visible spectroscopy

INTRODUCTION

The advancement and revolution brought about by nanotechnology has significantly helped the technological and industrial sectors, including electrical, agricultural, medical, and electronic technologies, homeland security and food safety, transportation, and many more. [5] Presently a material with aggregates, filaments, or particles smaller than 100 nm is referred to as a nanomaterial. Particles having a very high surface-to-volume ratio are produced when materials are modified and fabricated at the nanoscale. The fundamental elements controlling the distinct qualities of nanomaterials are the size, distribution, and number of interfaces or grain boundaries as well as the chemical makeup of the constituent phases and their interactions. [3] When creating application-oriented NPs, choosing an appropriate synthesis strategy is essential. An enormous amount of work needs to go into creating sustainable and environmentally friendly processes. These nanoparticles can be synthesized using biological materials [4].

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Several different processes such as chemical, biological and physical have been utilized to produce nanoparticles. Biological approaches are becoming a good alternative to address the drawbacks of chemical methods. As an alternative to chemical and physical methods, the high yield manufacture of AgNPs of predetermined size utilizing a variety of biological systems, such as bacteria, fungus, and plant extracts, has received a

lot of interest. It has recently been demonstrated that the biologically-mediated production of nanoparticles is a simple, affordable, and ecologically safe method [8]. The primary source of antioxidant potential in roselle petale extract is anthocyanin. A study found that this plant's aqueous extract might suppress a number of pathogenic and infectious bacteria, including *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* [6]. Silver nanoparticles (AgNPs) are extensively utilized in consumer items, healthcare devices, and various other applications due to their potent antimicrobial properties [2].

AgNPs have the ability to physically engage with different bacteria's cell surfaces. This is especially crucial when it comes to Gram-negative bacteria; as multiple investigations have shown that AgNPs adhere to their surface and accumulate there. AgNPs have the ability to break down cell membranes, causing structural alterations that increase bacterial permeability. AgNPs buildup on the membrane cell compromises the integrity of the bilayer, making it more vulnerable to an increase in permeability and ultimately bacterial cell death [1].

MATERIALS AND METHODS:

Plant

The *Hibiscus sabdariffa* plants were purchased from Nagpur local market. Plucked any already-wilted or damaged leaves and then leaves were washed and kept in polyethylene bags at 5°C in refrigerator (Figure 1).

Preparation of Extracts

Silver nanoparticles were prepared based on the plant extract. *Hibiscus sabdariffa* leaf extract has the medicinal properties, cost-effectiveness, and ease of availability hence it is used for the green synthesis of silver nanoparticles. To get rid of organic matter, dirt, dust or grime, the Surface of Leaves of *Hibiscus sabdariffa* were cleaned with water. A beaker with 250 ml of distilled water and 50 g of finely chopped leaves has been heated for 30 minutes. The extract was filtered through Whatman filter paper after it cooled and stored at five degrees Celsius for future use.

AgNPs (Green) Synthesis

AgNO3 with a concentration of 1 Mm was made. To prepare 1mM concentration of the $AgNO_3$ solution, 0.017mg of $AgNO_3$ was dissolved in 100ml of distilled water and plant extracts were mixed aseptically in the 9:1 ratio ($AgNO_3$: leaf extract). Incubation of setup in a dark chamber was necessary because of the photo-activation of silver nitrate at room temperature. At zero and 24 hours the color change of $AgNP_S$ were observed. Reduction of Ag^+ to Ag^0 was confirmed by the colour change of solution and the absorption spectra were measured at the wavelength range from 300-600 nm with the help of UV-visible spectrophotometer at1,12 and 24 hours of preparation.

UV–Visible Spectroscopy

The UV–Vis spectrum was analyzed. A UV-visible absorption spectrophotometer was employed, which has a resolution of 1 nm in the 300–600 nm range. The material was pipetted into a cuvette containing one milliliter, and it was then examined at room temperature.

Antibacterial Sensitivity Assay

In order to assess the antimicrobial properties of Silver Nanoparticles, agar well diffusion technique was used. To test for sensitivity, 1 milliliter of an overnight culture of every bacterial isolate was seeded onto nutrient agar plates that were kept at 45°C. After allowing the seeded plates to settle, equal-distance wells were made in the agar's surface using a sterile cork borer with an 8 mm diameter. The three wells were filled with 25µl, 50µl, and 100µl solution of AgNPs. The diameter of the inhibitory zones was determined after the plates were incubated for 24 hours at 37°C.The antibacterial assays were done on human pathogenic *Escherichia coli , Enterococcus faecalis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by using well diffusion method. In this study EMB(Hi media -M317), UTI(Hi media-

M1353), and Cetrimide (Hi media -MH024) medium was used to sub culture bacteria and were incubated at 37 °C for 24 h, Fresh overnight cultures of bacteria were obtained and spread out on nutrient agar (Hi medium M001-500G) plates. The area of inhibition around the disc containing the silver nanoparticles was utilized to assess the antibacterial properties.

RESULTS AND DISCUSSION

Plants Extract Preparation

Figure 1. *Hibiscus Sabdariffa* leaves

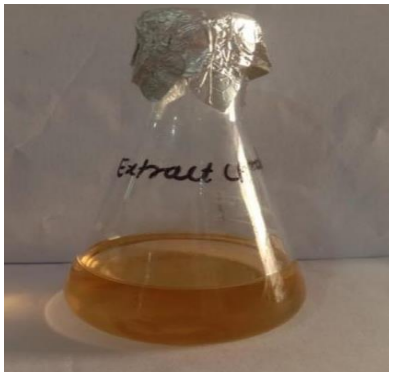


Figure 2. *Hibiscus Sabdariffa* leaf extract

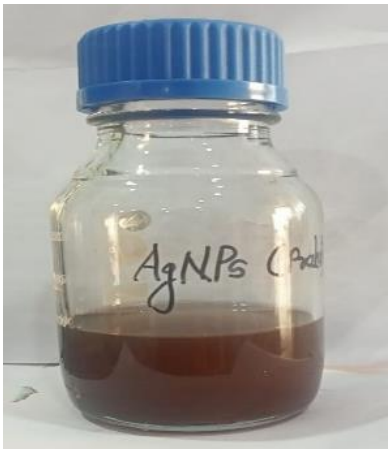


Figure 3. AgNPs.

Visual Observation and UV–Vis Spectroscopy

In this study, the colour of the solution changed to reddish brown as a result of adding plant extract from Hibiscus sabdariffa into silver nitrate (shown in Figures 2 and 3). There was a UV-visible absorption spectrophotometer that appeared with a resolution of 1 nm between 300 and 600 nm.

The UV–Visible spectra recorded after different time intervals of 1 hour, 12 hour and 24h from the initiation of reaction (Figure 4) size and shape-controlled nanoparticles in aqueous solutions examined using UV–Vis spectroscopy.

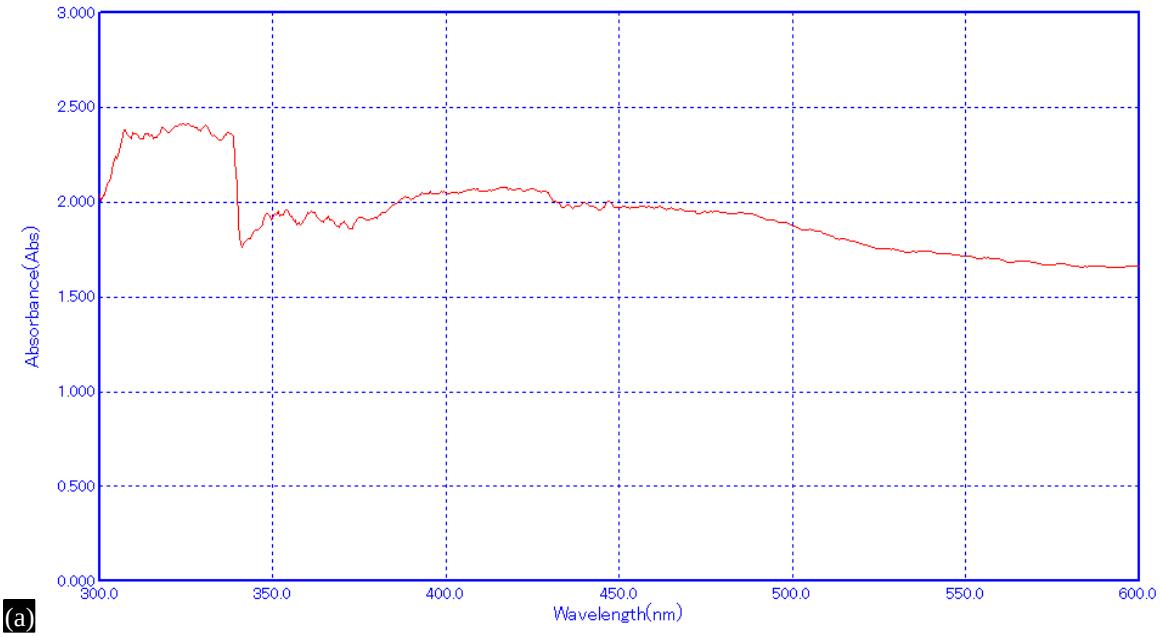
Antimicrobial activity

Due to their broad-spectrum effectiveness and profitable economic potential, silver nanoparticles have found use in appliances and consumer goods as well as medical equipment [7]. Silver has been used for centuries to prevent and treat a variety of illnesses because of its well-established antibacterial properties. Nanotechnology and nanomaterials are now fully integrated into everyday applications and objects. In addition, the strong antibacterial activity of silver nanoparticles is generating a lot of interest [1].

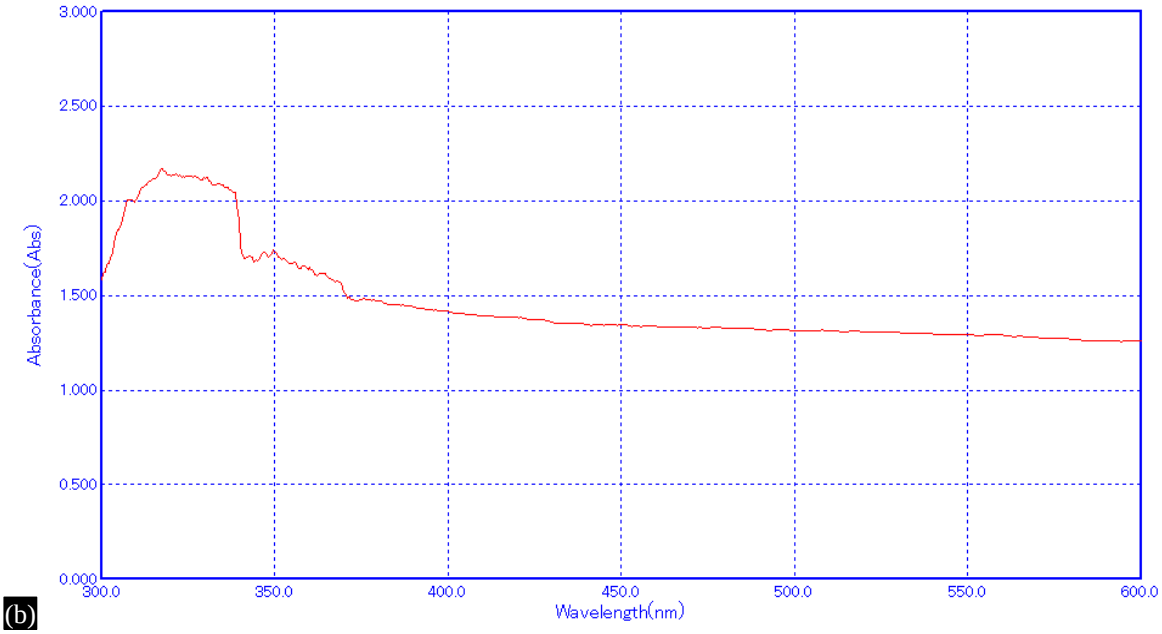
We studied silver nanoparticles produced by *Hibiscus sabdariffa* extract as potential antibacterial agents. Silver nanoparticles have been tested for antibacterial activity against Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa as well as Klebsiella pneumoniae confirming the zones of inhibition (Table 1). Silver nanoparticles that have been synthesized show potent antibacterial effects against the microorganisms used, as evidenced by the inhibition zone observed. Figure 5 displays the antibacterial activity findings of produced silver nanoparticles as determined by the well diffusion technique. The increased surface area of the silver nanoparticles allowed for greater interaction with the cell walls of bacteria, demonstrating their effective antimicrobial properties [9, 10].

Table 1. Zone of inhibition in mm.

Organisms	Isolate	25µl	50µl	100µl
	Zone of inhibition in mm			
Klebsiella pneumoniae	AgNPs	14	14	14
Escherichia coli	AgNPs	13	12	14
Pseudomonas aeruginosa	AgNPs	15	14	15
Enterococcus faecalis	AgNPs	12	13	13



(a)



(b)

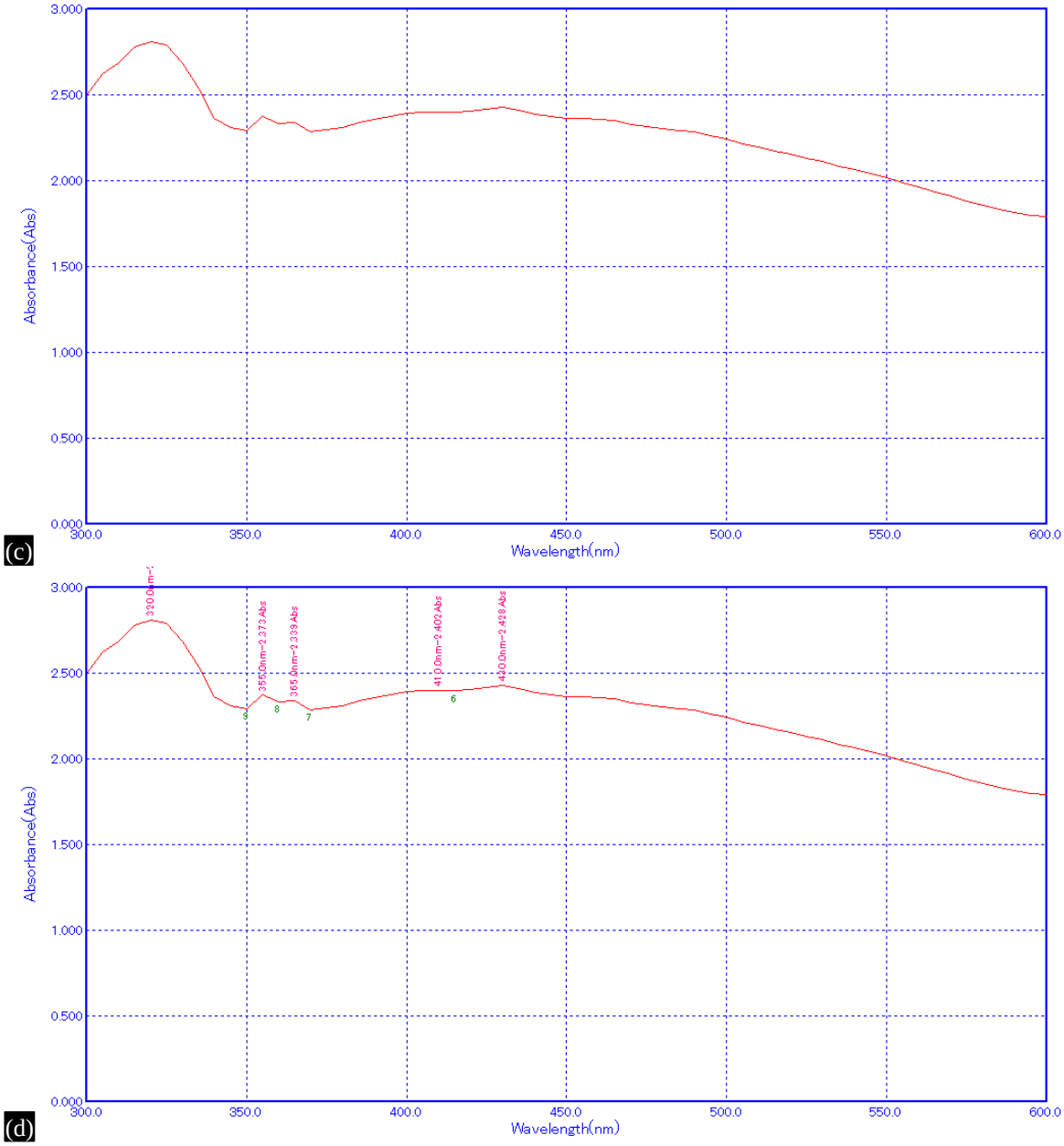
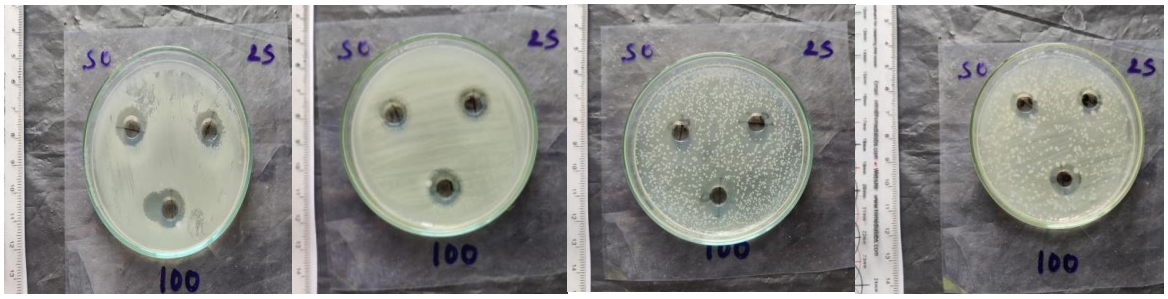


Figure 4. UV–Vis spectra showing absorbance at different time interval. (a) 1h AgNPs, (b) 12 hours AgNPs, (c) 24 hours AgNPs, (d) 24 hours AgNPs (peak).



Klebsiella pneumoniae (AgNPs) | *Escherichia coli* (AgNPs) | *Pseudomonas aeruginosa* (AgNPs) | *Enterococcus faecalis* (AgNPs)

Figure 5. Antibacterial activities of prepared silver nanoparticles.

CONCLUSION

This study reported an easy green production of stable silver nanoparticles using *Hibiscus Sabdariffa* leaf extract at room temperature. It was discovered that the synthesis was effective in terms of reaction time and the stability of the produced nanoparticles. It turns out to be a quick, green, and environmentally friendly method of producing silver nanoparticles that is both economical and effective. As a result, this reaction pathway meets every requirement for being a completely green chemical process. The produced silver nanoparticles showed effective antibacterial properties against each of *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis* bacteria. Utilizing plant extract for synthesis has several advantages, including reduced waste and safer goods, cost and energy savings, as well as environmental and human health protection. This environmentally friendly technique has the potential to be applied in biomedical applications as a competitive substitute for the traditional physical and chemical methods used to synthesize silver nanoparticles.

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