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Green Synthesis of Silver Nanoparticles from *Psidium guajava* Leaf Extract: Characterization and Evaluation of Antibacterial Activity Against Common Pathogens

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Abstract: The abundant bioresources found in nature, including plants are used in the green synthesis of Silver nanoparticles. It offers an improvement over chemical and physical processes since it is less expensive and more environmentally friendly. This study aimed to synthesize silver nanoparticle using green method, characterization of silver nanoparticle using analytical techniques and to evaluate the antibacterial activity of synthesized silver nanoparticle. *Psidium guajava* L. leaf was collected, authenticated and extracted by water. A 1mM solution of silver nitrate was prepared. Afterwards, separate quantities of 1, 2, 3, 4, and 5mL of the plant extract were added to 10mL of silver nitrate to facilitate the synthesis of silver nanoparticles (AgNPs). The synthesized AgNps were characterized using UV/VIS Spectrometry, SEM analysis and FTIR analysis, also the antibacterial activity of AgNP at different concentrations against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* was evaluated using well diffusion method. The synthesized Silver nanoparticle were detected by UV/ Vis spectrophotometer with absorbance peak at 459 nm. SEM analysis showed that silver nanoparticles were crystalline aggregate in nature and the size of AgNP was estimated between 1 - 200 nm. The reduction of silver nanoparticles and presence of functional groups of plant extract was confirmed by FTIR analysis of silver nanoparticles. AgNPs synthesized from *P. guajava* leaf revealed antimicrobial activity against all selected bacterial species. The use of nanotechnology in therapy development is an incredibly exciting and rapidly advancing field, the potential benefits of these emerging technologies make them a highly promising avenue for future medical research and innovation.

Keywords: Nanotechnology, Green Synthesis, Nanoparticles, Guava, Antibacterial Activity

1. Introduction

Nanotechnology is a rapidly evolving field that has the potential to revolutionize the way we develop new therapies for a variety of diseases and conditions. At its core, nanotechnology involves designing and manipulating materials at the nanoscale level, which is smaller than the size of cells and molecules. The development of experimental procedures for the synthesis of NPs with predetermined sizes, shapes, and tolerances on the nanometer scale, as well as their potential applications for enhancing human being-well, is the focus of nanotechnology [1]. Numerous protocols have been developed for the synthesis of NPs because of the wide range of applications that these particles can be used in. One of the key advantages of using nanotechnology for the development of new therapies is that it allows for highly targeted drug delivery [2]. In addition; nanotechnology also has significant potential for diagnostics and imaging. Nanoparticles can be engineered to be highly sensitive to specific diseases or conditions, allowing for earlier and more accurate diagnosis [3]. Biological, physical, and chemical processes can all be used to create NPs. Chemical processes have a number of drawbacks, such as the use of hazardous solvents and surfactant, higher cost, production of dangerous byproducts, and high energy consumption, all of which have the potential to be harmful to the environment and human health [2, 3]. The abundant bio-resources found in nature, including plants, bacteria, fungi, yeast, enzymes, and others, are used in the green and bio synthesis of AgNPs. It is an improvement over chemical and physical methods because it is less expensive, more environmentally friendly, and easier to scale up for large-scale synthesis. It also eliminates the need for toxic chemicals, high temperatures, and energy-intensive processes [2]. Numerous areas of research and daily life benefit greatly from the use of nanotechnology. This technology can be used to create drug delivery systems that significantly increase the therapeutic potential of a number of unstable and water-soluble medications [4]. Antibiotic resistance profiles raise concerns about the emergence and reemergence of pathogens and parasites that are multi-drug resistant. Multiple drug resistance is a widespread problem today, and significant resources are spent trying to solve it [5]. Recently, it has become known that metal nanoparticles are a promising antimicrobial agent that acts on a variety of target sites both extracellularly and intracellularly. Bacterial resistance to antibiotics has been caused by the widespread use of chemicals; in this situation, nano-antimicrobials offer some hope [6]. Guava, scientifically known as *P. guajava*, is a tropical tree belonging to the Myrtaceae family. It has medium-sized evergreen leaves and is indigenous to Sudan, but can also be found in South America, Europe, Africa, and Asia. Traditional medicinal practices have utilized guava leaves for treating different ailments, including infections. Recent ethno-pharmacological research has revealed that the leaves possess antibacterial properties, effectively combating both gram-positive and gram-negative bacteria [7], antipyretic, spasmolytic and central nervous system depressant activities [8]. The present study aimed to synthesize, characterize the silver nanoparticles from *P. guajava* leaves and study its antibacterial activity.

2. Methodology

2.1. Preparation and Extraction of Plant Material

The Guava leaves were obtained directly from a field in Khartoum state and were confirmed to be *P. guajava* Linn. at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The extraction process involved using *P. guajava* leaf extract to create silver nanoparticles. The guava leaves were washed in distilled water and boiled for 40 minutes in 100 ml of distilled water. After cooling, the extract was filtered through a Whatman No. 1 filter paper and stored at 4°C [9].

2.2. Microorganisms

The effectiveness of silver nanoparticles in combating various types of bacteria and fungi was examined. *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* were the microorganisms that were tested. These microorganisms were obtained from the Medicinal and Aromatic Plant and Traditional Medicine Research Institute, Khartoum, Sudan.

2.3. Synthesis of Silver Nanoparticle

A solution of silver nitrate at a concentration of 1mM was made in a flask. Afterwards, 1mL, 2mL, 3mL, 4mL, and 5mL of plant extract were individually added to 10mL of the silver nitrate solution. The resulting mixtures were then stored in a dark place for approximately 40 minutes, during which any color changes were observed as an indication of silver nanoparticle synthesis [9].

2.4. Characterization of Silver Nanoparticle

2.4.1. UV/VIS Spectral Analysis

After diluting a small portion of the sample in distilled water, the UV-Vis spectrum of the silver nanoparticle solution was measured to track the reduction of silver ions. Between 300 and 700 nm, a UV-Visible absorption spectrophotometer with a resolution of 1 nm was used [10].

2.4.2. Scanning Electron Microscope Analysis [SEM]

SEM analysis was recorded using Tescan VEGA3 scanning electron microscope. A very small amount of sample was dropped on a copper gird that had been coated with carbon to create thin films.

2.4.3. Fourier Transforms Infrared Spectroscopy [FTIR]

A sample with a known weight was placed in a mortar and pestle along with a small quantity of dry potassium bromide. The obtained powder was placed in an FFTIR device and the sample was scanned in the range of 400-4000 cm^{-1}. The obtained spectrum was compared with a reference chart to determine the functional groups present in the sample.

2.4.4. Antibacterial Assay

Well diffusion method

Bacterial culture has been adjusted to a suitable agar plate evenly using sterile swab. The plates were used for the sensitivity test after drying for 15 minutes. The well was impregnated with guava extract and silver nanoparticles; the experiment also included negative and positive controls. The diameter of the zone was inhibited from growing after 24 hours of incubation was measured [11].

3. Result and Discussion

3.1. Synthesis and Characterization of Silver Nanoparticles

3.1.1. Colour Change

The color of particles changes when they are in nanoscale compared to their larger form. Silver nanoparticles appear black or brown, as shown in Figure 1. The color of a solution changes from being colorless to reddish brown when different concentrations of guava leaf extract are added. This change in color is similar to what was observed in a previous study [9] when leaf extracts were mixed with a solution containing silver ions. The resulting reddish brown color indicated the formation of silver nanoparticles due to the stimulation of surface Plasmon vibrations.

Figure 1. *Colour change in five concentrations of P. guajva with silver solution. (a): Initially after adding extract to silver solution; (b): After 45 minutes from adding extract to silver solution.*

3.1.2. UV/VIS Spectral Analysis

The UV/Vis spectrophotometer was used to detect the synthesized Silver nanoparticles made from extracts of *P. guajava* (Figure 2). The UV-Vis spectrum of the colloidal solution containing Silver Nanoparticles showed a peak absorbance at 459 nm, confirming the successful synthesis of the silver nanoparticles in the solution.

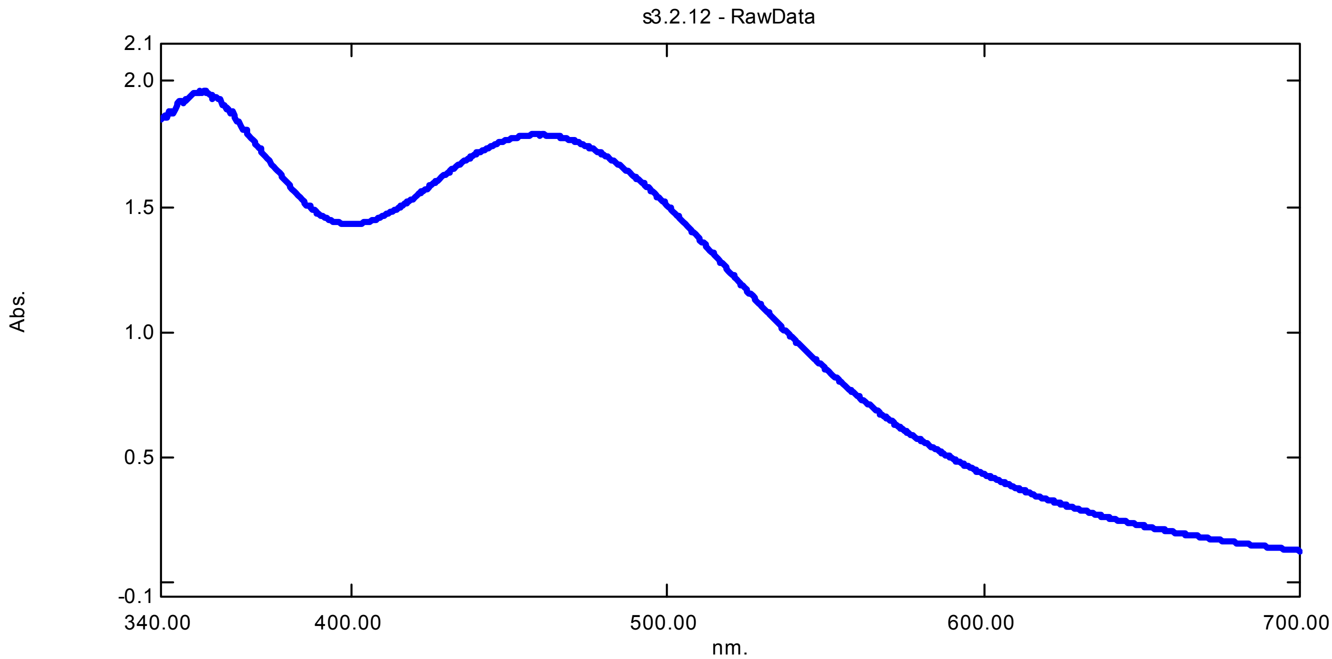


Figure 2. *UV/VIS SPEC Absorption Spectrum of Silver Nanoparticles Synthesized by treating 1mM Aqueous $AgNO_3$ Solution with P. guajava Extract after 24 hours.*

3.1.3. Scanning Electron Microscope Analysis [SEM]

The size, shape, and structure of silver nanoparticles were analyzed using Scanning Electron Microscopy (SEM). The images of the synthesized silver nanoparticles can be observed in Figure 3. In this study size of silver nanoparticle was found to be between 1-200 nm with different magnifications while the actual size of silver nanoparticle between 1-100 nm [12]. The SEM analysis confirms the formation of silver nanoparticle, which in form of crystalline aggregate.

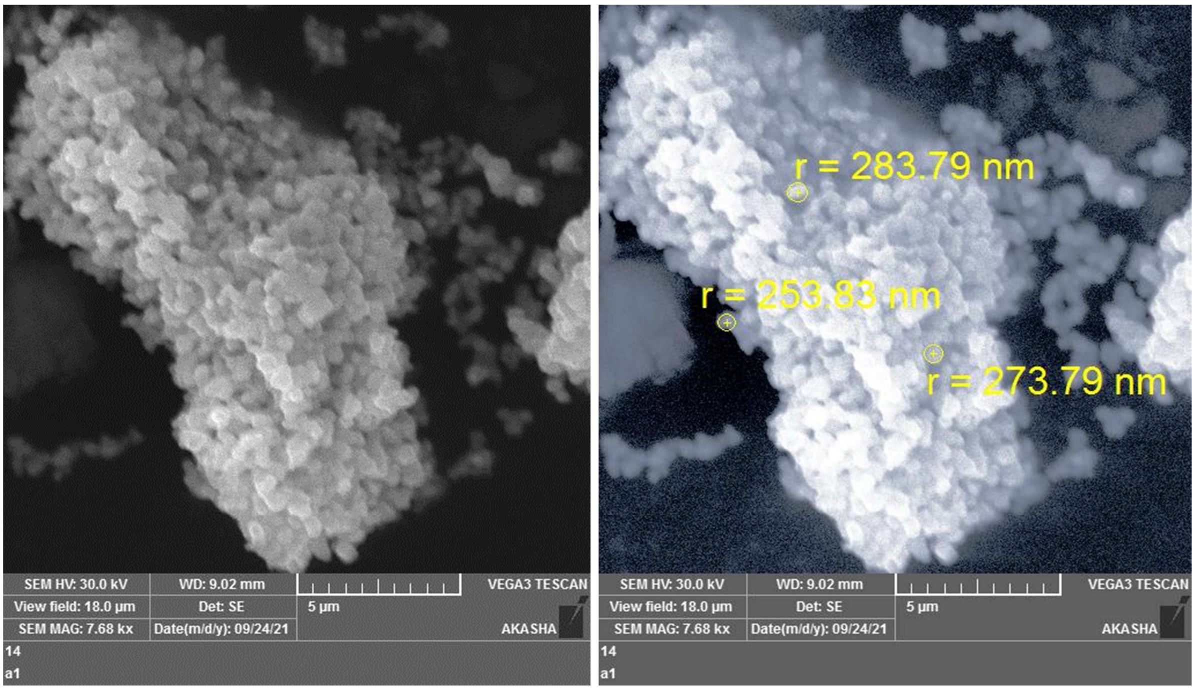


Figure 3. *Scanning Electron Microscope Analysis [SEM] image of synthesized silver nanoparticle from P. guajava leaf.*

3.1.4. Fourier Transforms Infrared Spectroscopy [FTIR]

The FTIR analysis of silver nanoparticles confirmed that the plant extract served as both a capping and reducing agent, as well as containing functional groups. This was evidenced by the spectrum's broadband, which indicated vibrational stretches at 1384 cm^{-1} caused by O-H stretching. Additionally, a peak at 1616 cm^{-1} was observed due to -C=O stretching, and a peak at 601 cm^{-1} was observed due to C-X stretching. Furthermore, a peak at 1037 cm^{-1} was present due to C-N stretching. The FTIR results provided support for the idea that certain organic compounds from the plant extract acted as both reducing agents and caps on the nanoparticles. Other studies also found similar results, confirming that the plant extract facilitated the reduction of Ag+ to Ag [9]. the result shown in Figure 4.

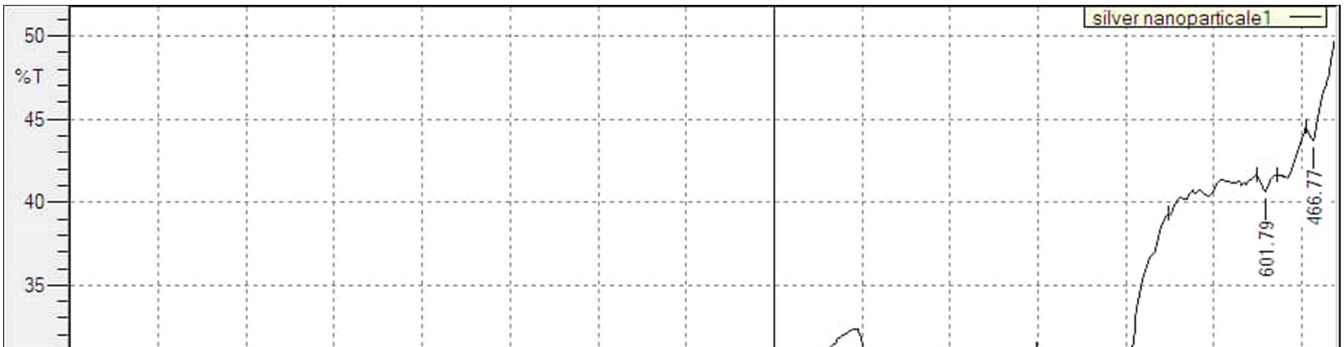


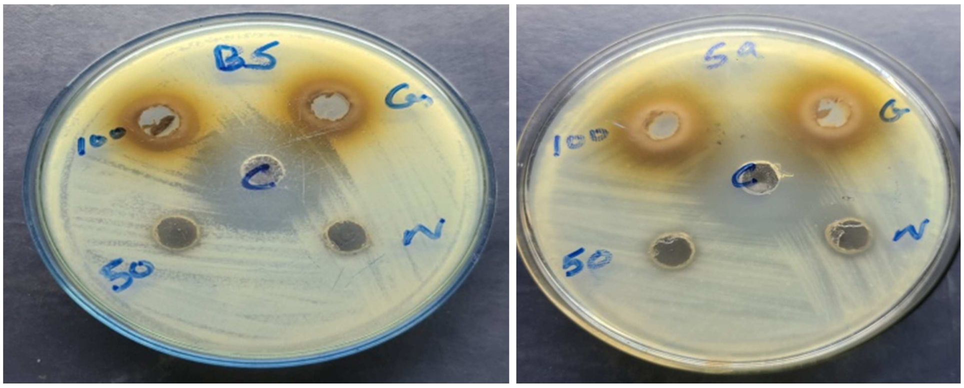
Figure 4. *FTIR Spectrum of Silver Nanoparticles.*

3.2. Antibacterial Activity

The antibacterial activity of the synthesized Silver nanoparticles has been investigated against: *E. coli*, *Ps. aeruginosa, B. subtillis,* and *S. aureus*. The activity of synthesized AgNPs was found to be concentration dependant showing high activity at low concentration. It show highest activity against *Ps. aeruginosa* with inhibitory zone 28.5 mm at low concentration [25mg/ml], followed by *B. subtillis* with inhibitory zone 20 mm at 25mg/ml, and finally *E. coli* with inhibition zone 13.5 mm at 25mg/ml, this result was higher when compared to the inhibition activity of high concentration of *Psidium guajava* leaf extract [100 mg/ml]. The AgNPs at higher concentration 50mg/ml showed no activity against *Ps. aeruginosa* and *B. subtillis*, while it showed low activity against *E. coli* and *S. aureus* with inhibition zone of 11.5mm. Ciprofloxacin was used in this study as positive control antibiotic. This result mentioned in Table 1; Figures 5&6.

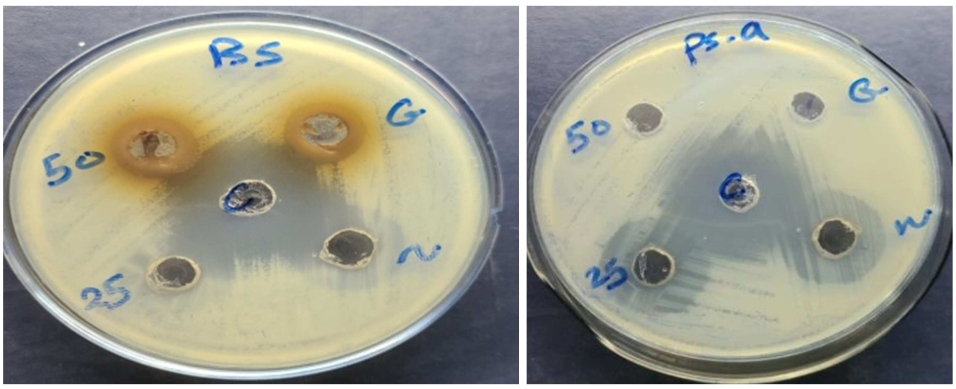
Table 1. *Antibacterial activity of synthesized silver nanoparticle against four bacteria.*

Sample	Concentration	Diameter of Inhibition Zone [mm]			
		Ps. aeruginosa	*B. subtillis*	*E. coli*	*S. aureus*
AgNPs	50mg/ml	-	-	11.5	11.5
	25mg/ml	28.5	20	13.5	-
Guava leaf extract	100mg/ml	16.5	16.5	19	19.5
	50mg/ml	-	16	16	11.5
Control +ve (Ciprofloxacin)	10 mcg	37	30	27	25



N: Silver nanoparticle G: *Psidium guajava* leaf extract C: Control Positive [Ciprofloxacin].

Figure 5. *Inhibition Zone of synthesized Silver nanoparticle [50mg/ml] and P. guajava leaf extract [100 mg/ml] against B. subtillis and S. aureus..*



N: Silver nanoparticle G: *Psidium guajava* leaf extract C: Control Positive [Ciprofloxacin].

Figure 6. *Inhibition Zone of synthesized Silver nanoparticle [25mg/ml] and P. guajava leaf extract [50mg/ml] against B. subtillis and Ps. aeruginosa..*

The small size, high surface area to volume ratio, and ability to generate reactive oxygen species (ROS) of silver nanoparticles contribute to their strong antibacterial effects. These effects have been observed against a diverse range of bacterial strains, including both Gram-positive and Gram-negative bacteria [13]. The high antimicrobial activity of AgNPs is attributed to their large surface area to volume ratio and high percentage of surface atoms in comparison to bulk silver metal [14]. Furthermore, the compact nature of AgNPs enables easy passage through cell membranes, thereby influencing internal cellular processes [15]. Additionally, AgNPs possess remarkable antibacterial traits, which can be attributed to their meticulously constructed surface that allows for maximum interaction with the surroundings [16]. Previous studies reported that the Silver nanoparticles exhibit antimicrobial activity against bacteria through various mechanisms of action, such as Disruption of Cell Membrane; Silver nanoparticles penetrate bacterial cell membranes and disrupt their structure, leading to the leakage of cellular contents and eventual cell death [17]. Furthermore; the antimicrobial activity of silver nanoparticles against bacteria is based on their ability to disrupt several essential bacterial functions, which ultimately leads to bacterial death [18]. In 2017, a study was conducted to assess the antibacterial effects of *P. guajava* leaf. The study examined extracts of guava leaves and their impact on six bacterial strains, namely *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Klebsiella pneumonia*. The findings was complying with the present study, it revealed that the guava leaf extracts demonstrated a significant level of antibacterial activity against all tested bacterial strains, ranging from moderate to high [19]. The antibacterial activity of guava leaves has been attributed to the presence of various bioactive compounds such as tannins, flavonoids, and phenolic acids [20]. These compounds have been shown to have strong antimicrobial properties against a wide range of gram-positive and gram-negative bacteria. The development of novel drug compounds for the prevention, diagnosis, and treatment of various diseases is being significantly accelerated by nanotechnology [21]. Recent advancements in nanotechnology, biology, and medicine have opened up a new research area in the development of new drugs [22]. Currently, there is extensive work being done to create various nanoparticles and nanodevices in order to enhance the safety and efficacy of nanosystems and nanodevices. This progress will significantly benefit the healthcare system [23]. The main goals in this research include regulating particle size, surface characteristics, and the controlled release of therapeutic drugs for targeted drug action at an optimal rate and dosage [24].

4. Conclusion

Successful synthesis, characterization and evaluation of antimicrobial activity of silver nanoparticle were revealed. This study reported a cost-effective, energy-efficient, and eco-friendly method for synthesizing stable silver nanoparticles using *P. guajava* extract. The synthesized silver nanoparticles may has the potential to revolutionize drug discovery by facilitating the development of more efficient and effective therapies. Further investigations are recurred to understand the exact mechanism of antimicrobial activity of AgNPs.

Conflict of Interest

The authors declare that they do not have any conflicts of interest.

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