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Synthesis, Characterization and Antibacterial Screening of Silver Nanoparticles Using *Cymbopogon citratus* (Lemongrass) Leaves Extract

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ABSTRACT

Lemongrass leaves extract was used to synthesize stable silver nanoparticles (Ag-NPs) by green context. UV–vis spectrophotometry, X-ray diffraction (XRD), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) spectroscopy techniques were used to characterize the synthesized nanoparticles. The antibacterial activity of the synthesized Ag-NPs was investigated by disc diffusion test method. The results indicated that the silver nanoparticles were successfully synthesized, and the particle size distribution ranged from 15–25 nm while maintaining a specific pH. The zone of inhibition (ZOI) was shown at 2 mm by *Escherichia coli* (*E. coli*) and 1.9 mm by *Staphylococcus aureus* (*S. aureus*) against leaves extract of lemongrass. MIC and MBC values of *E. coli*, and *S. aureus* are 7.7, 7.8 µg/mL and 7.6, 7.7 µg/mL respectively. This study revealed that Ag-NPs possessed a strong antimicrobial activity and can be developed as a new type of antimicrobial agent for the treatment of bacterial infections.

Keywords: Silver nanoparticles, green synthesis, antibacterial, lemongrass.

INTRODUCTION

Nanotechnology has a significant impact on the environment, human health, and society. It is a dynamic innovation, and rapidly growing multidisciplinary area that works at a scale of 1-100 nm. When compared to their equivalent materials, nanomaterials (whether natural, engineered, or incidental), exhibit dimensions of below 100 nm along with unique physical, chemical and biological properties such as high surface-to-volume ratio. They are highly appealing tools due to their potential applications in a diversity of industries, such as pharmaceutical, biotechnology, nanosciences and agriculture [1]. Nanoparticles have attracted much attention due to their different properties, including size, shape, optical, magnetic, and electrical properties [2]. They can be used

to fuse fibers, biosensors, and electromechanics while their anticancer properties have pharmaceutical potential, as such it necessitates the manufacture of nanoparticles using physiologically friendly processes [3].

Nanomaterials synthesis has recently become one of the most attractive technological fields of study [4]. Physical and chemical methods for synthesizing nanoparticles are superior to biological method but they are more expensive and produce toxic nanomaterials that limit their applications in the medical and pharmaceutical industries [5]. As a result, developing safe, clean, cost-effective, and biocompatible nanomaterials with environmentally friendly methods is critical and can be achieved by biosynthetic approaches using green chemistry context [6].

Silver nanoparticles (Ag-NPs) have gained significant attention in the research of metallic nanoparticle and these nanomaterials are used as antiseptics, health care products, medical device covers, optical devices, drug delivery, and as anti-cancer agents in manufacturing industries. Also, they are used in water treatment, agriculture, food packaging, and in the textile industry, wound-dressing, drug delivery, biosensors, orthopedics, and medical diagnostics [7]. Various biological activities of Ag-NPs, such as their anti-bacterial, antiviral, antifungal, antioxidant, anti-cancer, anti-mycobacterial, anti-platelet, and anti-diabetic activities, have been well-reported in several earlier studies [8]. Agricultural production is largely affected by different kinds of diseases due to pathogenic activities of microorganisms which leads to massive economic loss, using plant-mediated green synthesis of AgNPs. These economic losses can be managed and can also improve the agricultural production [9].

Green synthesis is the biological synthesis of nanoparticles through the utilization of plants or using bacteria, algae and fungi. The antioxidant or reducing properties of the biomolecules such as enzymes, proteins, flavonoids, sterols, terpenes, terpenoids, saponins and cofactors, act as both reducing and capping agents and are typically responsible for the reduction of metal compounds into their respective nanoparticles. Biological synthesis has numerous advantages, such as cost effective, environmental friendly, less amount of toxic by-products released and having a cleaner route of synthesis. Green methods have an advantage over physical methods of synthesizing NPs because it doesnot require extreme physical conditions like high pressure, high energy or temperature like physical methods. The green methods cause a favorable effect as it is endowed with natural capping agents [10].

Lemongrass (*Cymbopogon genus*) is a perennial grass in the Poaceae family that grows both tall and in clumps. It has more than 55 species, but the two most prevalent are *Cymbopogonflexuosus* and *Cymbopogoncitratus*. The genus is found in tropical and subtropical regions, South and North America, Africa, Australia, and Europe. Due to its antiseptic, antibacterial, antimicrobial, antifungal, and anti-inflammatory characteristics, this plant is widely used in pharmaceutical processes, as well as in agriculture and therapeutic uses [11].

Among several synthetic methods used for the synthesis of AgNPs, biologically-prepared AgNPs show high yield, solubility, and high stability when compared with conventional physical and chemical methods since it require many toxic components that are harmful to humans when consumed and it is very expensive. Biological methods are simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions. For translational research, green chemical approach for the synthesis of AgNPs shows much promise. After synthesis of precise particles, characterization is also necessary because the physicochemical properties of particles could have a significant impact on their biological properties. Therefore, to address the safety issue of any nano material for the purpose of human welfare, such as medicines, or in the health care industry, it is necessary to characterize the prepared nanoparticles before application.

The aim of the research is to synthesize silver nanoparticles using Lemongrass (*Cymbopogon genus*) leaves extract. The objective of this study is to find out the characteristics of the synthesized silver nanoperticles from Lemongrass (*Cymbopogon genus*) leaves extract as well as to bring to light the antibacterial property of the synthesized silver nanoperticles.

MATERIALS AND METHODS

Sampling and Extract Preparation

Lemongrass leaves were purchased from Katsina Biological Garden and authenticated by a Botanist at the Department of Biological Sciences, Umaru Musa Yar'adua University, Katsina, with a voucher number UMYUKSA012026.

Preparation of Plant Extract

Fresh lemongrass leaves (50 g) were separated, washed thoroughly, dried, and then crushed in 100 mL of double-distilled, sterilized water and allowed to boil for 30 minutes at 300°C. The

lemongrass extract cooled, and then filtered using No. 1 Whatman filter paper. The extract was then stored at 4°C for synthesis of Ag-NP.

Synthesis of Silver Nanoparticles

The plant extract was added dropwise to 100 mL of 1 mM $AgNO_3$ aqueous solution. The pH was checked at regular intervals with a digital pH meter model (CyberScan pH 610). When the pH reached 9, the beaker was heatedat 300°C for 30 min. The mixture was cooled in darkness and centrifuged at 10,000 rpm for 30 min. Then, the particles were kept at 4°C for subsequent analysis as shown in Figure 1 [12].

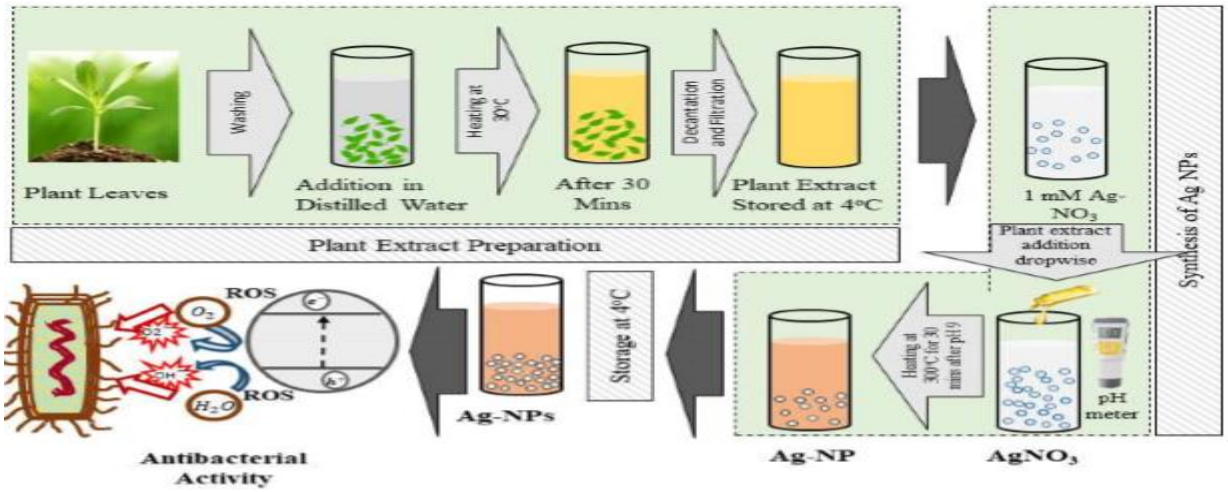

Figure 1: Schematic illustration of Ag-NPs which was synthesized through plant extract.

Characterizations of Silver Nanoparticles

The synthesized nanoparticles were characterized using scanning electron microscopy on MIRA TESKAN at 10 kV for the morphology investigation and estimation of particle size of the synthesized Ag-NPs. Electronic Dispersion X-ray was performed for the elemental analysis in order to authenticate the formation of Ag-NPs produced using lemongrass leaves extracts. XRD was performed and used to find the crystallographic structure of the Ag-NPs. Thermogravimetric analysis was performed on Mettler Toledo (TGA/SDTA851) in order to determine purity of the synthesized Ag-NPs, and was performed in the air from 30 to 800°C at a rate of 10°C per min.

Antibacterial Screening of Synthesized Silver Nanoparticles

The disc diffusion method was used for antibacterial screening of the synthesized Ag-NPs and $AgNO_3$ solution against *E. coli and Staphylococcus aureus* which were investigated. The

antibacterial activity of Ag-NPs against the selected gram-negative and gram-positive microorganisms was carried out using the Kirby–Bauer disk diffusion susceptibility test method [12]. The bacterial strains were spread on Mueller–Hinton agar (MHA) using sterile cotton swabs. For blank test, a sterile blank antimicrobial susceptibility disk was used. Samples were then poured into the wells in which one well was used as control, and then phosphate buffer saline (PBS) was poured as a control solution. The plant extract was poured into the well of each Petridish. The disks were then placed on the agar plate and incubated at average normal body temperature (37°C) for 24 hours. Zones of inhibition were observed after 24 hours of incubation.

RESULTS AND DISCUSSION

UV–Visible Spectroscopy

The generated solutions were measured for absorbance at different wavelengths in order to verify the synthesis of Ag-NPs that exhibits reddish brown colors, which arise due to the excitation of surface Plasmon resonance (SPR) in the Ag-NPs. It was observed that as the reaction proceeded and the pH of the solution increased, a band at 390–470 nm for the leaves extracts decreased in intensity which indicated the presence of nanoparticles in the solution.

Scanning Electron Microscopy

The synthesized Ag-NPs were confirmed using SEM together with EDX, as shown in Figure 2. SEM result reveals that the synthesized Ag-NPs are round in shape. The SEM image of synthesized Ag-NPs using lemongrass leaves extracts shows that the morphology of NPs and the reduction in the relative size of NPs is visible. The SEM result presented the occurrence of Ag-NP synthesis. The SEM micrographs showed aggregates of synthesized Ag-NPs, and the particles size were calculated using Schrrer's formula and found within the range of 20–25 nm that are within the aggregates which indicate the stability of NPs.

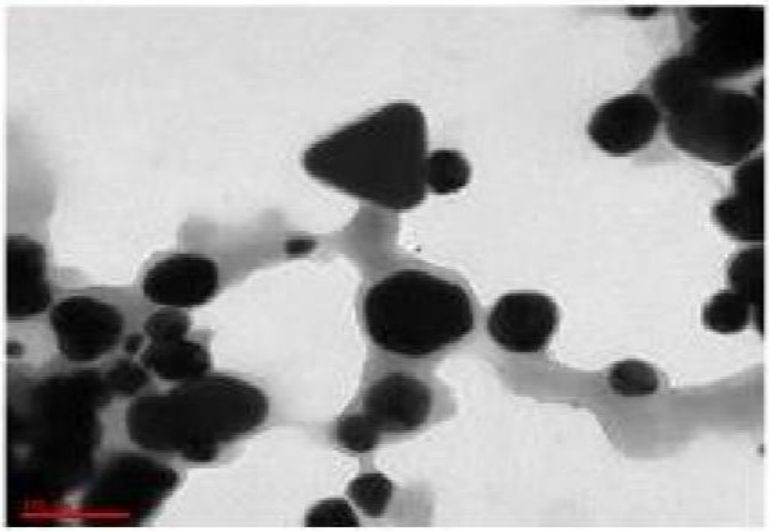

Figure 2: SEM of green synthesized Ag-NPs using lemongrass leaves extract

Energy-Dispersive X-ray Spectroscopy

EDX analysis is used to identify and quantify the elemental composition of materials. The existence of silver nanoparticles using lemongrass leaves extract in larger concentrations is confirmed by EDX spectroscopy, as shown in Figure 3.

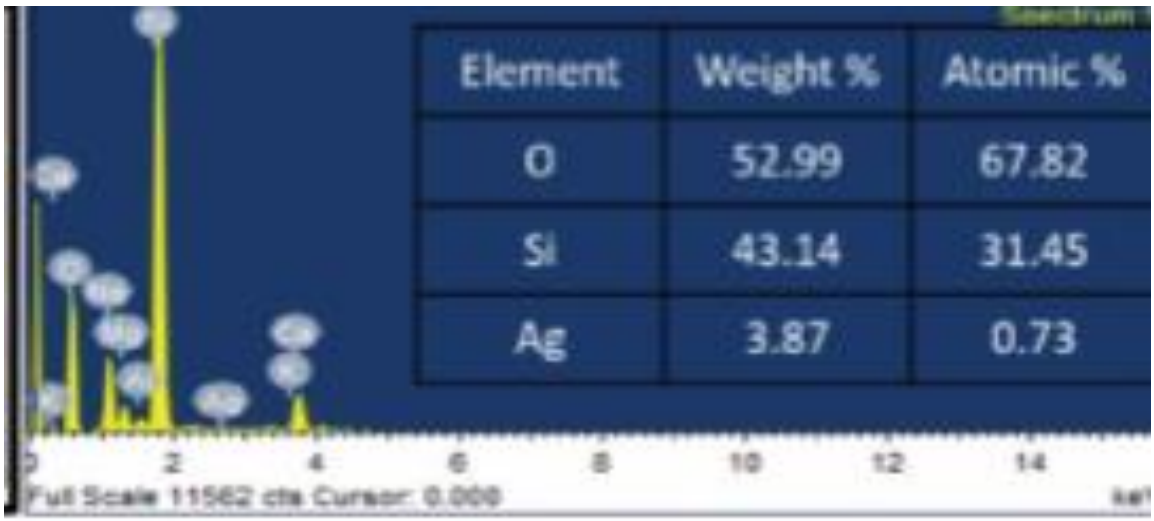

Figure 3: EDX analysis of synthesized Ag-NPs using lemongrass leaves extracts.

Thermogravimetric Analysis

TGA was done for the synthesized Ag-NPs using lemongrass leaves extract and shows the presence of pure silver nanoparticles as only one transition with a small increase in weight which may be due to oxidation of Ag in presence of air as can be seen in Figure 4.

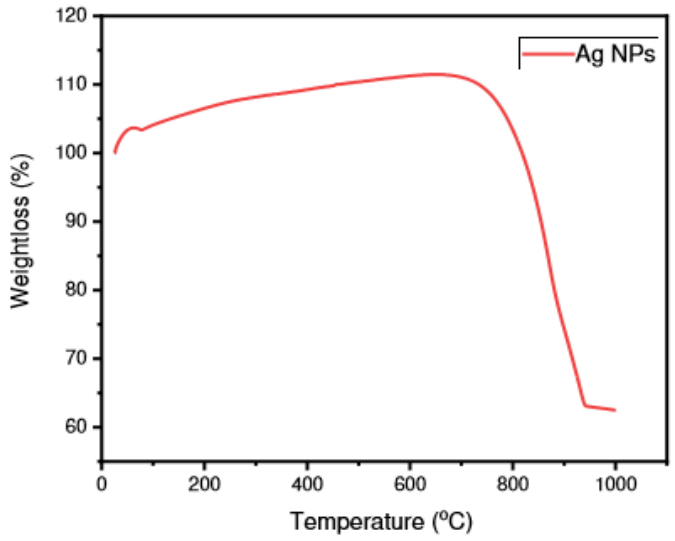

Figure 4: Thermogravimetric analysis curve of silver nanoparticles.

X-Ray Diffraction Analysis

This analysis is used to analyze the crystallographic structure of the synthesized silver AgNPs. Figure 5 shows an XRD graph indicating the prominent peaks at 38°, 44.5°, 64.5° and 77.5° respectively which correspond to (111), (200), (220) and (311) planes. The peaks obtained are attributed to the silver with a crystal lattice structure. similar patterns for AgNPs was reported by [12].

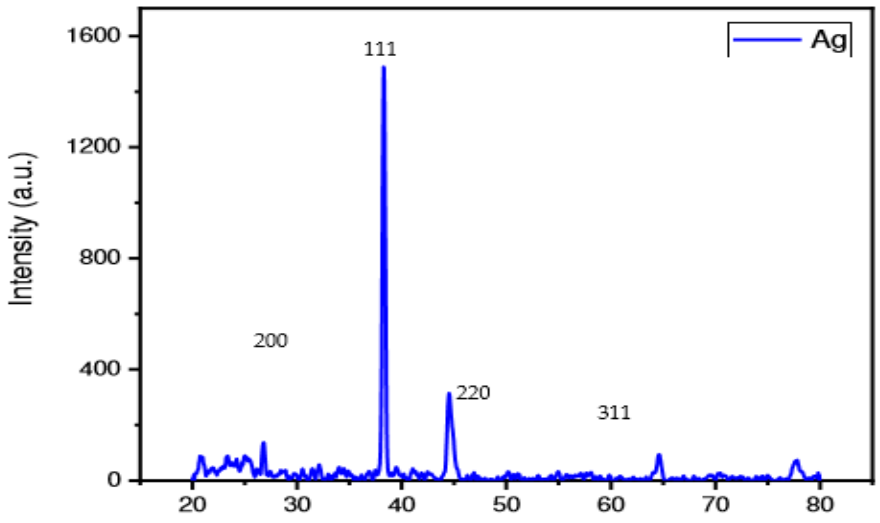

Figure 5: XRD analysis of synthesized Ag-NPs using lemongrass leaves extract.

Antibacterial Property of Synthesized Silver Nanoparticles.

Silver has been found to be one of the best antimicrobial substances. It has been used to cure bacterial infections even before the use of advanced antibiotics, and used as a food and water preservative. At present, Ag -NPs are used in the medical industry as topical ointments to prevent infection against burns and open wounds [13]. It was discovered that silver reacts with moisture in the skin and the fluid of the wound and gets ionized. This ionized silver is highly reactive, binds with tissue proteins and bacterial DNA, RNA, bringing structural changes in the bacterial cell wall and nuclear membrane leading to cell distortion and death or inhibits bacterial replication [14]. In addition, Ag NPs have been used as linings or as layers in many manufactured goods such as air conditioners, washing machines, refrigerators, sportswear, toys and filters because of its outstanding antimicrobial activity. Ag-NPs accumulate within the cell membrane of bacteria, which results in the permeability of the cell membrane, causing cell death [15].

The antibacterial activities of synthesized Ag-NPs solution against *E. coli* and *S. aureus* were investigated using a disc diffusion method. Figure 6 and Table 1 show the Zone of Inhibition for *E. coli* (2 mm) and *Staphylococcus aureus* (1.9 mm) against lemongrass leaves extract.

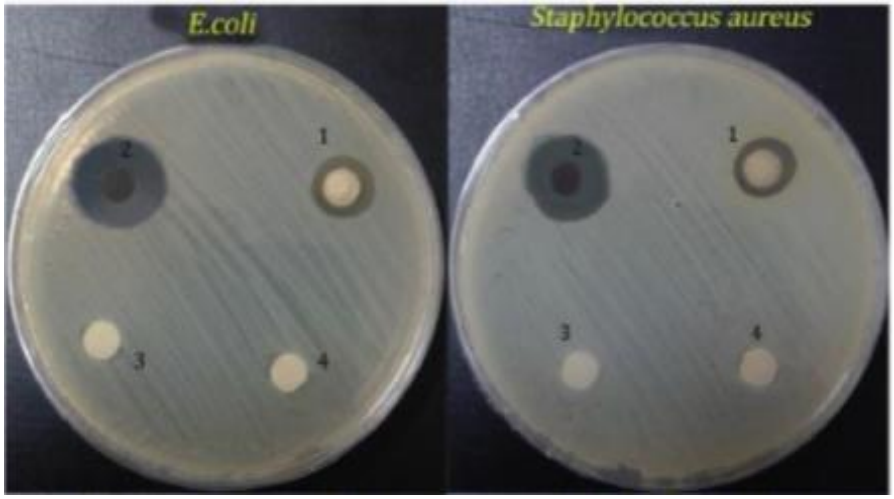

Figure 6: Antibacterial testing of Ag-NPs using Agar disc diffusion method (1) $AgNO_3$ (2) AgNPs, (3) Deionized water and (4) Lemongrass leaves extract.

Table 1: The diameter of ZOI (mm), MIC value (µg/mL), and MBC value (µg/mL).

Bacteria	Plants	Diameter of Inhibition Zone (mm)	MIC (µg/mL)	MBC (µg/mL)
E. coli	Lemongrass	2	7.7	7.8
	Control	0.0	-	-
S. aureus	Lemongrass	1.9	7.6	7.7
	Control	0.0	-	-

The MIC values of *E. coli* for lemongrass were found to be 7.7µg/mL while *Staphylococcus aureus* showed an MIC of 7.6 µg/mL. Similarly, the MBC values *of E. coli* and *Staphylococcus aureus* are 7.8 µg/mL and 7.7 µg/mL respectively. The MIC and MBC value of *E. coli* showed that *E. col*i was less susceptible to Ag-NPs.

Green nanotechnology is now used as a new dimension in tackling microbial attack through green synthesis. The silver nanoparticles which were synthesized with plant extracts have been reported to be used in drug delivery, proteomic studies, cancer therapy and medicine [16]. Ag-NPs are used in many different industries in present society, as their distribution and assimilation in to the ecosystem is inevitable. Therefore, understanding the transfer of Ag-NPs throughout the ecosystem and their impacts on plants is of crucial importance.

CONCLUSION

Lemongrass leaves extract was used to synthesize stable silver nanoparticles. The synthesized AgNPs was characterized using SEM, XRD as well as spectroscopy techniques. Antibacterial activity of the synthesized nanoparticles was screened using Agar Well Diffusion method. The colour change pattern of stem bark extracts of lemongrass leaves extract from yellow to brown showed the preliminary confirmation of synthesized silver nanoparticles from lemongrass leaves extract. The intense peak obtained at 470 nm using UV-visible spectroscopy also indicated the presence of silver nanoparticles in stem bark of lemongrass leaves extract. The results showed that Ag-NPs from lemongrass leaves extract have strong antibacterial activity. However, some studies have also shown harmful effects of Ag-NPs on plants in different aspects.

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