UNIVERSITY OF EDUCATION, WINNEBA

SYNTHESIS OF SILVER NANOPARTICLE STABILISED WITH *PENNISETUM PURPUREUM* LEAVE EXTRACT AND DETERMINATION OF ITS ANTIMICROBIAL ACTIVITIES

DOREEN ATTAH

MASTER OF PHILOSOPHY

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A thesis in the Department of Chemistry Education Faculty of Science Education, submitted to the School of Graduate Studies in partial fulfillment

of the requirements for the award of the degree of Master of Philosophy (Chemistry Education) in the University of Education, Winneba

APRIL, 2022

DECLARATION

I, Doreen Attah, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work and it has not been submitted, either in part or whole, for another degree elsewhere.

…… ……

Doreen Attah Date

Supervisors' Declaration

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis as laid down by the University of Education, Winneba.

…… …..……

Doctor. B. Ankubze Date

DEDICATION

Dedicated to family and husband, Mr. Isaac K. Mensah for being supportive of me throughout the course of this programme.

ACKNOWLEDGEMENTS

I wish to express my heartfelt appreciation to my principal supervisor, Dr B. Ankudze, a lecturer in the Department of Science Education, University of Education, Winneba, for his technical guidance and encouragement throughout the supervisory work on the thesis.

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I wish to extend my appreciation to Pastor David A. Dadebo and Mrs. Augustina F. Dadebo.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Silver nanoparticles (AgNPs) have attracted great attention due to their outstanding electrical, optical, magnetic, catalytic, and antimicrobial properties. However, there is a need for alternative production methods that use fewer toxic precursors and reduce their undesirable by-products. Plant extracts from the leaves of *Pennisetum Purpureum* plants can be used as reducing agents as well as enhance AgNPs antimicrobial activity. In the present study, silver nanoparticles (AgNPs) were synthesized using aqueous leaf extracts of *Pennisetum Purpureum*. The obtained AgNPs were studied for their optical, structural, surface morphological and antimicrobial properties. The prepared AgNPs were characterized by using UV-Visible spectra, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy dispersive X-ray (EDX) and Powder X-ray diffractometer (PXRD). The synthesized nanoparticles were quasi spherically shaped and well-dispersed with average sizes ranging from 29.70 ± 9.71nm from SEM image. The AgNPs derived from *Pennisetum Purpureum* exhibited antibacterial activity against seven bacterial (Methicillin resistant *Staphylococcus aureus* (NCTC 12493), *E. coli* (NCTC 12241), *S. mutants* (ATCC 700610), *P. aeruginosa* (ATCC 4853), *S. typhi* (ATCC 14028), *K. pneumonia (NCTC13440)* and, *B. subtilis* (ATCC 10004)) and one fungal (*Candida albicans* (ATCC 90028)) pathogens of the human compared to the crude extracts. This indicates that the biomolecules covering the nanoparticles may enhance the biological activity of metal nanoparticles. Hence, our results support that synthesis of AgNPs using *Pennisetum Purpureum* leaf extract constitutes a potential area of interest for the therapeutic management of microbial diseases. These observation on nanoparticle production using plant-mediated synthesis and performance offers insights into the potential for scaling up this production process for economic commercial implementation.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter is about the introduction of the study it focuses on the background, statement, purpose, objectives, significance and the delimitation of the study.

1.1 Background to the Study

Nanotechnology refers to the manufacture, portrayal, manipulation, and application of structures by controlling shape and size at nanoscale (Devi Bala et al., 2020). Nanoparticles can be categorized into inorganic and organic. Inorganic nanoparticles include zinc oxides, zinc sulphides, cadmium sulphides, gold, silver, copper and aluminum. Organic-based nanoparticles include molecules such as fullerenes, quantum dots and carbon nanotubes. Nanoparticles can be produced by physical, chemical, or biological method (Keshari, et al., 2020). Biological approach involves using micro-organisms or plants for the production of nanoparticles.

From ancient times, silver has been used as an anti-microbial agent and silver-based compounds are much cheaper than gold based (Srikar et al., 2016). Further, silver nanoparticles are non-toxic to eukaryotic cells including humans, but it has high toxicity against prokaryotic cells such as micro-organisms like bacteria, viruses, and fungi (Hussain et al., 2016; Keshari, et al., 2020). The silver nanoparticles have unique chemical, optical, electrical, magnetic, and mechanical properties. These unique properties of silver nanoparticles have made it useful in applications such as anti-microbial (Singh et al., 2015), targeted drug delivery (Parveen et al, 2012), sensing and imaging (Lee & El-Sayed, 2006), anti-cancerous (Castro-Aceituro et al., 2016) and wound healing (Rigo et al., 2013). The advancement in the synthesis of

silver nanoparticles has strongly impact many scientific areas. Due to low yield and the use of toxic compounds, physical and chemical methods are not suitable for the synthesis of silver nanoparticles. Micro-organism based AgNPs synthesis is also not preferred because most of the microbes are pathogenic (Bindhani & Panigrahi, 2015). Several works already reported that plants extract used for the synthesis of silver nanoparticles such as Aloe vera (Zhang et al., 2016) and tea leaf (Loo et al., 2012) contains bioactive molecules such as alkaloids, flavanols, glycosides, steroidal saponins, phenols, fatty acids and essential oil (Patil et al., 2011). The extract of *Cestrum nocturnum* has been used in burn and swelling, analgesic and bactericidal activity, local anesthetic effect, inhibitory effect on the central nervous system, cardiac arrhythmic, tumour inhibition and antioxidant activity (Khan, et al., 2011). In view of the above said reasons, this study was designed to explore the use of elephant grass extract for the synthesis of silver nanoparticles and determine its antibacterial properties against bacterial strains.

1.2 Statement of the Problem

The presence of bioactive chemicals on the surface of green produced silver nanoparticles shows considerable antibacterial activity. Most studies have focused on the use of edible plants and superfoods, which are always expensive and in high demand for green synthesis of antimicrobial sensitive silver nanoparticles. This study seeks to synthesize silver nanoparticles using easily accessible and low-cost grass extracts.

1.3 Purpose of the Study

The purpose of this study is to synthesize silver nanoparticle stabilized by leaf extract of elephant grass and determine its antibacterial properties.

1.4 Objectives of the Study

The objective of the study is to;

1. synthesize silver nanoparticles stabilized with *Pennisetum Purpureum* leaf extract.
2. determine antimicrobial properties of the synthesized silver nanoparticles.
3. determine the antioxidant activity of the synthesized silver nanoparticles.

1.5 Significance of the Study

Silver nanoparticles synthesized with readily available and inexpensive leaf extract will economize production and provide an alternative source of stabilizers for manufacturers. This study will also serve as the bases for further research.

1.6 Delimitation of the Study

This study will be focused on synthesizing silver nanoparticle stabilized by leaf extract and determine its antibacterial properties.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter reviewed works and contributions of that have been done by some researchers on nanoparticles and the various types of silver nanoparticles, various approaches of synthesis, techniques of characterization, purification processes, antimicrobial activities of silver nanoparticles and technique of determining antimicrobial properties.

2.1 Nanoparticles

The concept of a ‘‘nanometer’’ was first proposed by Richard Zsigmondy, the 1925 Nobel Prize Laureate in chemistry. He coined the term nanometer explicitly for characterizing particle size and he was the first to measure the size of particles such as gold colloids using a microscope (Hulla et al., 2015). Nanotechnology emerged as a novel and powerful tool to manipulate matters at a “nano-scale” and enabled the evolution of many aspects, including biotechnology, medicine, pharmaceuticals, agriculture, food, cosmetics, environment protection, electronics, information technology, construction, military, energy industry, space industry, and consumer products among others (Lee & Moon, 2020). According to Hulla et al., (2015), modern nanotechnology was the brain child of Richard Feynman, the 1965 Nobel Prize Laureate in physics. Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties (Farhang, 2009). Nanoparticle is defined as particles with a size between one to hundred nanometres. They are intermediate between macroscopic solid, atomic, and molecular systems (Ariga et al., 2016). Smaller size particles behave differently compared to their bulky forms. When

particles become smaller in size, their surface area increases. This causes an increase in electrical and thermal conductivity, lowered melting points, stronger magnetism, and optical activity (Nagarajan, 2008). Nanoparticles show different mechanical properties relative to microparticles and bulk materials, providing more effective options for the surface modification of many devices in the mechanical strength, or to improve the quality of nanomanufacturing/nanofabrication, etc. To be more specific, on the one hand, the mechanical effects of nanoparticles can affect the tribological properties of lubricants with nanoparticles as well as reinforce composite coat (Guo et al., 2013)

2.2 Types of Nanoparticles

Nanoparticles exist in different forms and are classified according to size, structural appearance, physical and chemical properties (Nagarajan & Hatton, 2008). Some categories of nanoparticles based on physical and chemical characteristics are shown in Fig. 1

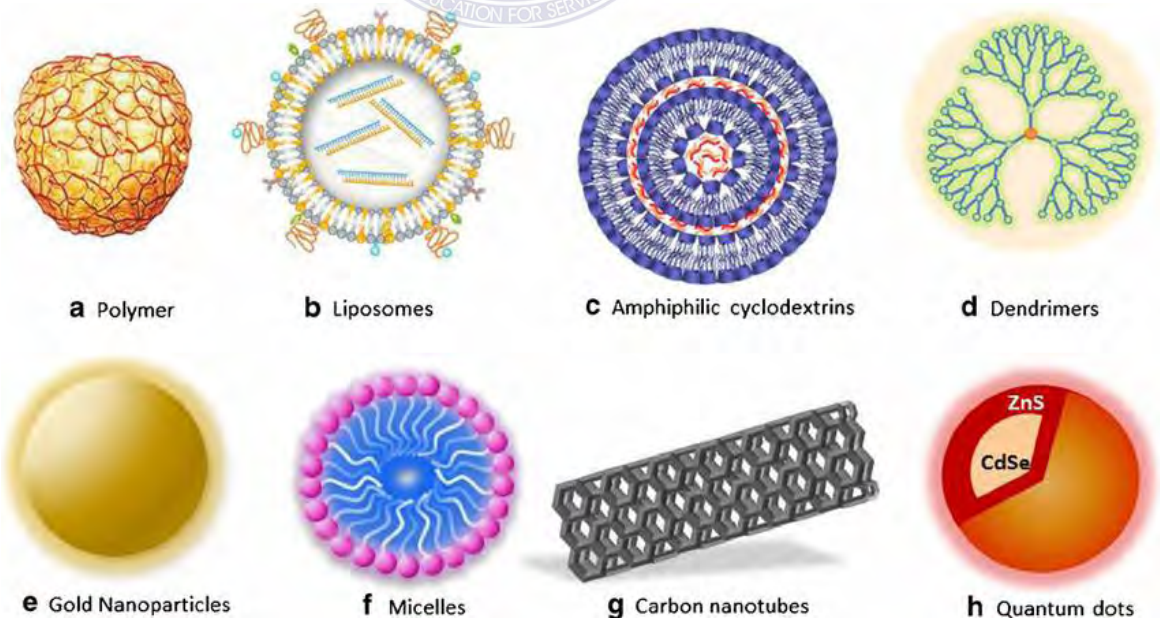

Figure 1.

Figure 1: Different types of nanoparticles (McCarthy et al., 2014).

2.3 Non-Metal Based Nanoparticles.

2.3.1 Ceramic Nanoparticles

Ceramic nanoparticles are inorganic solids composed of oxides, carbides, carbonates, and phosphates. These nanoparticles have high heat resistance and chemical inertness. They have applications in photocatalysis, photodegradation of dyes, drug delivery, and imaging (Nagarajan & Hatton, 2008).

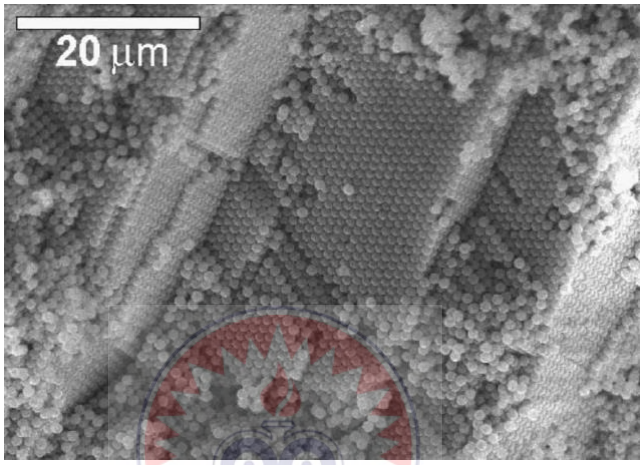

Figure 2: A cross-sectional image of ceramic nanoparticles of silica (Meseguer et al., 2002).

2.3.2. Carbon-based nanoparticles

Carbon-based nanoparticles are made up of two major components, according to Cheraghian, (2021). These are carbon nanotubes and fullerenes. Carbon nanotubes are graphene sheets that have been wrapped into a tube. Because they are hundreds of times stronger than steel, these materials are primarily employed for structural reinforcement. Carbon nanotubes carry heat along their length but are non-conductive across their width. Fullerenes are carbon allotropes with a hollow cage structure of sixty or more carbon atoms. They have a high electrical conductivity, a high strength, and an attraction for electrons.

2.4 Semi-metal Nanoparticles

Semi-metal nanoparticles exhibit properties that are like metals and nonmetals. These particles have very large bandgaps. They're employed in photocatalysis, electronics, photo-optics, and water splitting, among other things. Semi-metal nanoparticles include silicon and germanium (Ray, 2018; Cheraghian, 2021).

2.5 Polymeric Nanoparticles

Polymeric nanoparticles are nanoparticles made of organic materials. They can be formed into nano capsules or nanospheres. A matrix-like structure characterizes a nanosphere particle, whereas a core-shell morphology characterizes a nano capsular particle. Controlled release, drug molecule protection, the potential to combine therapy and imaging, and targeted targeting are some of the benefits of polymeric nanoparticles. They can be used in medicine delivery and diagnostics. Polymeric nanoparticles are biodegradable and biocompatible for drug delivery (Ray, 2018).

2.6 Lipid-Based Nanoparticles

Lipid nanoparticles have a spherical form and a diameter ranging from ten to hundred nanometers. It is made up of a lipid-based solid core and a matrix containing soluble lipophilic molecules. Surfactants and emulsifiers stabilize the exterior core of these nanoparticles. These nanoparticles have medicinal applications as drug carriers and delivery systems, as well as RNA release in cancer therapy (Ray, 2018; Cheraghian, 2021).

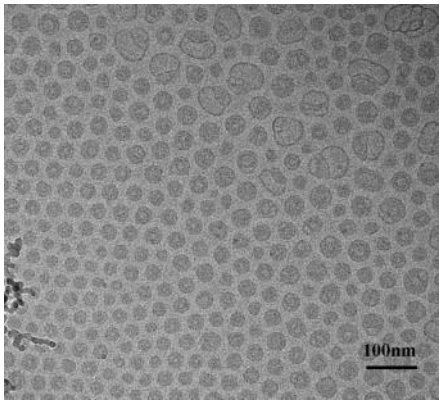

Figure 3: TEM image of lipid nanoparticles.

2.7 Metal Nanoparticles

Metal precursors are used to synthesize metal nanoparticles. Chemical, electrochemical, or photochemical processes can be used to create these nanoparticles. Metal nanoparticles are obtained chemically by reducing metal-ion precursors in solution with chemical reducing agents. These agents can adsorb tiny molecules and have a high surface energy (Ray, 2018).

2.7.1 Noble metal nanoparticles

Noble metal nanoparticles have been useful in the field of biomedicine over the past decades due to their importance in personalized healthcare and diagnostics. In particular, platinum, gold and silver nanoparticles have achieved the most dominant industrial applications and biomedicals. In biomedicine they are used as antimicrobial and antiviral agents, diagnostic tools, drug carriers and imaging probes. They have high resistance to corrosion and oxidation (Habibullah et al., 2021). For instance, gold nanoparticles are used to coat sample before analysing in scanning electron microscope. This is usually done to enhance the electronic stream, to get high quality images.

2.7.2 Synthesis of silver nanoparticles using plant extract

Development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. The bio reduction behaviour of various plant leaf extracts such as Helianthus annus (Asteraceae), Basella alba (Basellaceae), Oryza sativa, Saccharum officinarum, Sorghum bicolour and Zea mays (Poaceae) in the synthesis of silver nanoparticles was investigated employing UV/Visible spectrophotometry, XRD (X-ray diffraction) and SEM (Scanning Electron Microscopy). H. annus was found to exhibit strong potential

for rapid reduction of silver ions. It was observed that there is no correlation always between the colour development and the increase in absorbance exhibited by the nanometal synthesised. The work adds to the confirmation of previous reports on biosynthesis of nanometals using plant leaf extracts (Leela & Vivekanandan, 2008). Plant based synthesis has been the most reported synthesis methodology (Siddiqi et al., 2018). The advantage of this method is easy product recovery and the ability of the extract to serve as both the reducing and stabilizing agent. Plant based synthesis uses a plant component such as the leaves, stems, roots, shoots, flowers, barks, and seeds extract for the synthesis of nanoparticles. (Balashanmugam et al., 2015).

One of the first approaches of using plants as a source for the synthesis of metallic nanoparticles was with alfalfa sprouts, which was the first report on the formation of silver nanoparticles using a living plant system (Gardea-Torresdey et al., 2003). Green synthesis using plants appears to be faster. Shankar et al., (2003) showed that using Geranium leaf takes around nine hours. It has been demonstrated that the production of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time (Mittal et al., 2013). The advantages of employing plants for nanoparticle production are that they are readily available and safe to handle, and they contain a wide range of active compounds that can facilitate the reduction and stabilization of silver ions. Main compounds which affect the reduction, and the stabilization of the nanoparticles are phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, chlorophyll pigments, caffeine, ascorbic acid, and other vitamins have also been reported (Sharma et al.,

2009). The non-toxic phytochemicals such as Phenolic compounds possess hydroxyl and carboxyl groups, which are able to bind to metals (Ahmad, 2010). In this research, extract from elephant grass was prepared and used as both reducing and stabilizing agent in synthesizing silver nanoparticles.

2.7.3 Silver nanoparticles and their properties

Silver nanoparticles have been of interest since the six century AD owing to their dichroic character when integrated into glass (Rauwel et al., 2015). Because of their unique physical and chemical properties, silver nanoparticles are used in a variety of disciplines, including medicine, food, health care, consumer goods, and industry. Among these are optical, electrical, thermal, high electrical conductivity, and biocidal characteristics (Zhang et al., 2016). Owing to their biocidal properties, silver nanoparticles have been integrated into applications, such as wound treatment, sterilization, food processing, water purification, antibacterial agents, healthcare-related products, consumer products, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopaedics, drug delivery, as anticancer agents, (Chernousova et al., 2013). Plasmonic properties of silver nanoparticles have make them useful as plasmonic light traps in solar cell, and in applications such as inks, microelectronics, medical imaging, medical device coatings, optical sensors, and sanitation management (Rauwel et al., 2015).

Figure 4: Images of silver nanoparticles present at different concentration. (Peng et al., 2011)

2.7.4 Preparation of silver nanoparticles

Generally, silver nanomaterials can be prepared by two broad methods, classified as top-down and bottom-up methods (Yadav et al., 2011).

Top-down processes involve breaking bulk materials into smaller particles of nano-dimensions using various physical and chemical methods. It involves cutting, milling, and shaping the materials into the desired order. Physical methods include pyrolysis (Jain et al., 2008), nanolithography (Lusker & Garno, 2011), thermolysis (Davies et al., 2017), and radiation-induced methods (Iravani et al., 2014). The advantages of top-down methods are speed, used of radiation as a reducing agent, and the absence of involvement of hazardous chemicals. But it has its disadvantages such as low yield, high energy consumption, solvent contamination, lack of uniform distribution of particles, and imperfect surface structure of the resulting nanoparticles, which affects their physical and chemical properties (Shameli et al., 2012).

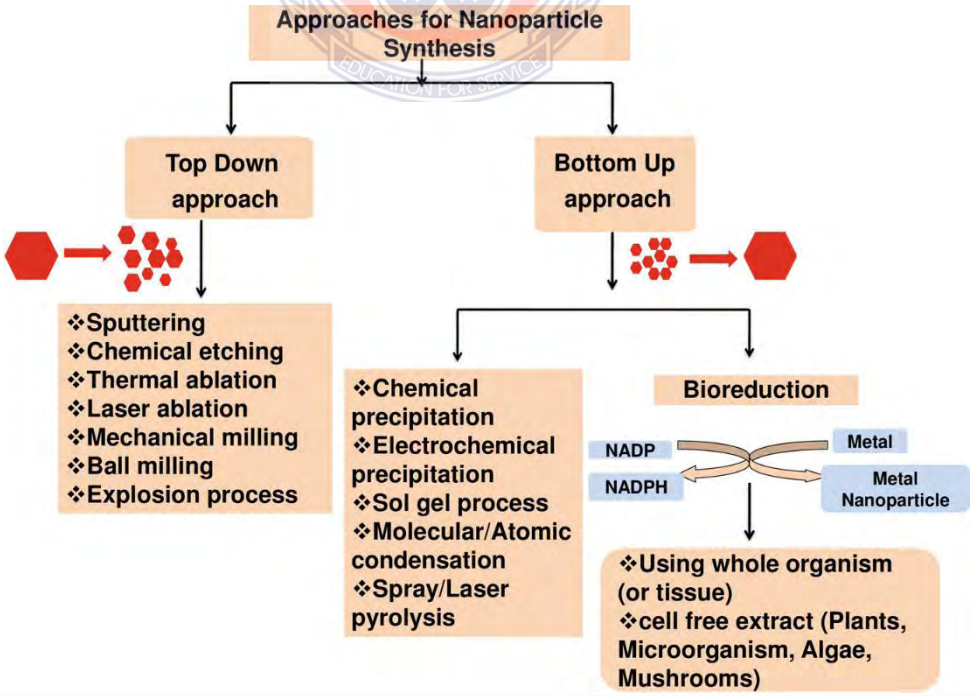

Figure 5: General methods for the synthesis of silver nanoparticles (Reshma et al., 2019)

The bottom-up approach is usually a wet-chemical synthesis procedure, it includes chemical reduction (Sun, 2006; Hasan, 2015), electrochemical methods (Khaydarov et al., 2009), and sono-decomposition and green synthesis (Sharma et al., 2015; Ahmad et al., 2019). Chemical methods use water or organic solvents to prepare the silver nanoparticles (Wiley et al., 2005). Metal precursors, reducing agents, and stabilizing or capping agents are the most common components used in this process. Stabilizers such as donor ligands, polymers, and surfactants are used to prevent agglomeration of the predominantly produced nanoclusters. The benefits of chemical synthesis of nanoparticles include ease of production, low cost, and high yield; however, the use of chemical reducing agents is harmful to living organisms (Gurunathan et al., 2015), making the synthesized nanoparticles unsafe for biomedical applications aside from green synthesis.

2.8 Green synthesis of silver nanoparticles

The excessive use of chemicals in chemical synthesis has made it dangerous for the future biological applications of nanoparticles (Siddiqi et al., 2018). This has resulted in the exploration of other, ecological methods with a minimal use of chemicals. Many approaches have been investigated, and microorganisms such as bacteria, yeasts, fungi, and algae have been used in the biosynthesis of metal nanoparticles (Rauwel et al., 2015). Recently, green synthetic methods employing plant extracts has proven to be the most useful and eco-friendly method. (Siddiqi et al., 2018). Green synthesis of silver nanoparticles shows high yield, solubility, and high stability (Gurunathan et al., 2015). They are simple, rapid, non-toxic, dependable, and can produce well-defined size and morphology under optimized conditions. The primary focus of this study is the green synthesis of silver nanoparticles using plant extract from elephant grass.

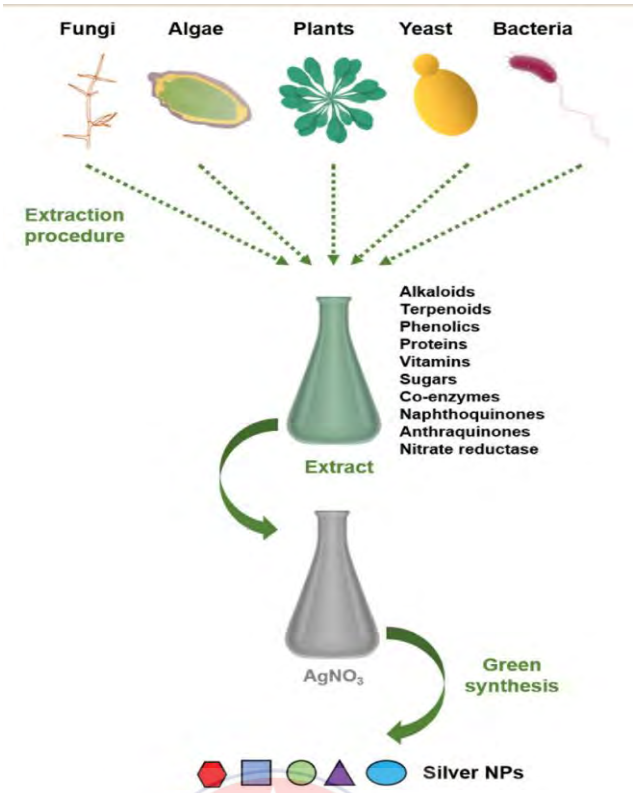

Figure 6: Schematic illustration of green synthesis of silver nanoparticles (Anupam et al., 2019).

2.8.1 Synthesis of silver nanoparticles using plant extract

Plant based synthesis has been the most reported synthesis methodology (Siddiqi, et al., 2018). The advantage of this method is easy product recovery and the ability of the extract to serve as both the reducing and stabilizing agent. Plant based synthesis uses a plant component such as the leaves, stems, roots, shoots, flowers, barks, and seeds extract for the synthesis of nanoparticles. (Balashanmugam & Kalaichelvan, 2015).

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of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time (Mittal et al., 2013). The advantages of employing plants for nanoparticle production are that they are readily available and safe to handle, and they contain a wide range of active compounds that can facilitate the reduction and stabilization of silver ions. Main compounds which affect the reduction, and the stabilization of the nanoparticles are phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, chlorophyll pigments, caffeine, ascorbic acid, and other vitamins have also been reported (Sharma et al., 2009). The non-toxic phytochemicals such as Phenolic compounds possess hydroxyl and carboxyl groups, which are able to bind to metals (Ahmad et al., 2010). In this research, extract from elephant grass was prepared and used as both reducing and stabilizing agent in synthesizing silver nanoparticles.

2.8.2 X-ray diffraction

X-ray diffraction is used for the analysis of both molecular and crystal structures (Das et al., 2009), qualitative identification of various compounds, and particle sizes (Ivanisevic, 2010). X-ray diffraction has contributed more to mineralogical characterization of clay fractions of soils than has any other single method of analysis. X-ray photons may be considered as "packets" of monochromatic electromagnetic waves, generated as random events, emanating outward in a spherical array from their point source. Commercial X-ray diffraction equipment includes a high-voltage generator, an X-ray source, an X-ray beam collimating system, and a detecting and recording system. This technique involves a precise and systematic analysis of X-ray

scattering curves (Chung, 1974). Crystal monochrometers for selective discrimination of diffracted X-rays from nondirectional X-ray scatter are available for use with recent X-ray spectrometers. Qualitative interpretation of diffraction patterns involves identification of crystalline species from the array of diffraction maxima obtained from a sample. For the most reliable and accurate estimation, the use of X-ray diffraction analysis in conjunction with other methods such as differential-thermal, integral-thermal, infrared, surface-area, elemental analysis, and other species-specific chemical methods is advisable (Whittig & Allardice, 1986). A modified Warren-Averbach method is proposed for the analysis of the X-ray diffraction line profile based on the approximation by the Voigt function, which yields stable solutions, and the efficiency of the method is shown. The analysis within the frame-work of the Warren-Averbach method makes it possible to restore the distribution function of nanoparticles (crystallites) over true diameters, which satisfactorily correlates with electron microscopy data (Dorofeev et al., 2012). When X-ray light reflects on any crystal, it leads to the formation of many diffraction patterns, and the patterns reflect the physico-chemical characteristics of the crystal structures. Thus, X-ray diffraction can analyse the structural features of a wide range of materials including biomolecules, and polymers (Zhang et al., 2016). The production of diffraction patterns is required for the analysis of these materials. Every material has a unique diffraction beam that can be characterized and identified by comparing diffracted beams to the Joint Committee on Powder Diffraction Standards library's reference database. The diffracted patterns also reveal whether the sample materials are pure or contaminated. This method has been used to measure phase identification, determination of structural imperfections in samples from various disciplines and characterization of various nanomaterials and their properties (Das, 2014). The

working principle of X-ray diffraction have been employed in this study to determine the face centered cubic nature of the silver nanoparticles synthesized using elephant grass.

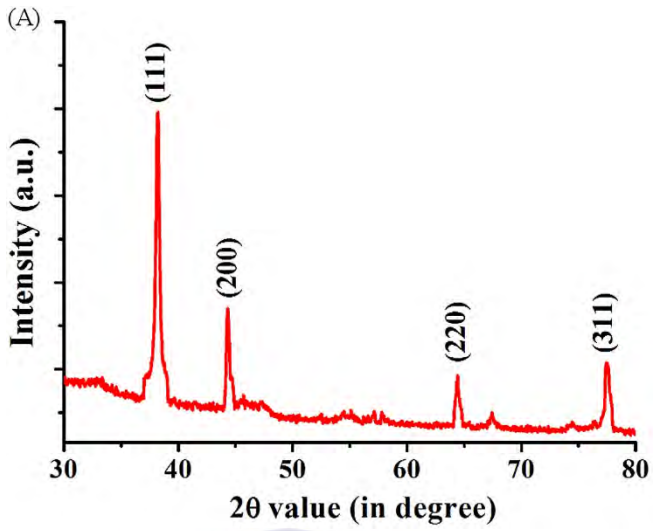

Figure 7: XRD spectrum of silver nanoparticles showing the various diffraction planes.

2.8.3 Powder X-ray Diffraction

X-ray diffraction has contributed more to mineralogical characterization of clay fractions of soils than has any other single method of analysis. X-ray photons may be considered as "packets" of monochromatic electromagnetic waves, generated as random events, emanating outward in a spherical array from their point source. Commercial x-ray diffraction equipment includes a high-voltage generator, an x-ray source, an x-ray beam collimating system, and a detecting and recording system. Crystal monochrometers for selective discrimination of diffracted x-rays from nondirectional x-ray scatter are available for use with recent x-ray spectrometers. Qualitative interpretation of diffraction patterns involves identification of crystalline species from the array of diffraction maxima obtained from a sample. For the most

reliable and accurate estimation, the use of x-ray diffraction analysis in conjunction with other methods such as differential-thermal, integral-thermal, infrared, surface-area, elemental analysis, and other species-specific chemical methods is advisable (Whittig & Allardice, 1986). The accuracy and reliability of the PXRD quantification method depend on the methods of sample preparation and handling (Padrela et al., 2012). Many crystalline solids cannot be prepared in the form of single crystals of sufficient size and/or quality for investigation using single-crystal X-ray diffraction techniques, and the opportunity to carry out structure determination using powder diffraction data is therefore essential to understand the structural properties of such materials (Harris & Kariuki, 2001). Although single-crystal X-ray diffraction (XRD) is the most powerful and routine technique for determining the structural properties of crystalline solids, the requirement for a single-crystal specimen of appropriate size and quality imposes a limitation on the scope of this technique. Indeed, many crystalline solids exist only as microcrystalline powders and are therefore not suitable for investigation by single-crystal XRD. To establish the structural properties of such materials, the most direct approach is to use powder XRD, although it is important to emphasize that carrying out structure determination from powder XRD data is significantly more challenging than from single-crystal XRD data (Dudenko et al., 2013). Over the past decade, structure determination from powder diffraction data (SDPD) has matured into a technique that, although not completely routine, is widely and successfully used in the context of organic, inorganic and organometallic compounds (David et al., 2002; David & Shankland, 2008). Although the refinement stage of the structure determination process can be carried out fairly routinely from powder diffraction data using the Rietveld profile refinement technique, solving crystal structures directly from powder data is associated with several intrinsic

difficulties. Nevertheless, substantial progress has been made in recent years in the scope and potential of techniques in this field. A brief survey of the underlying methodologies is given, with some emphasis on recently developed techniques for carrying out the structure-solution stage of the structure-determination process (Harris & Kariuki, 2001).

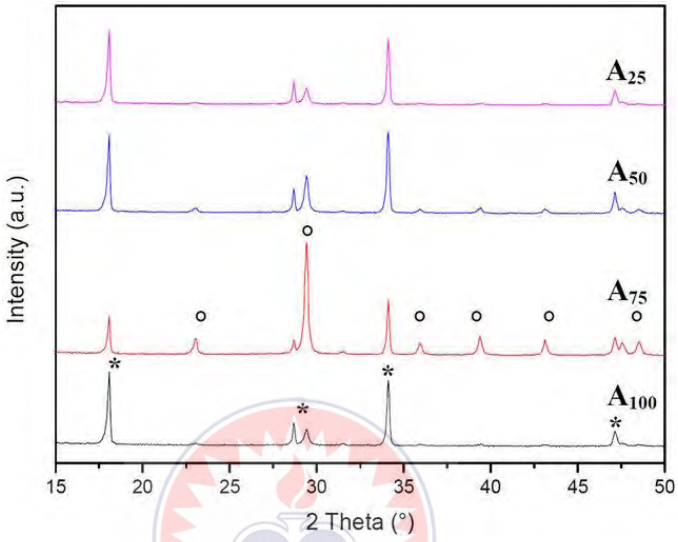

Figure 8: XRD patterns of the aqueous nanolime suspensions after 30 minutes of air exposition time; Sample A $Ca(OH)_2$ (Taglieri et al., 2013)

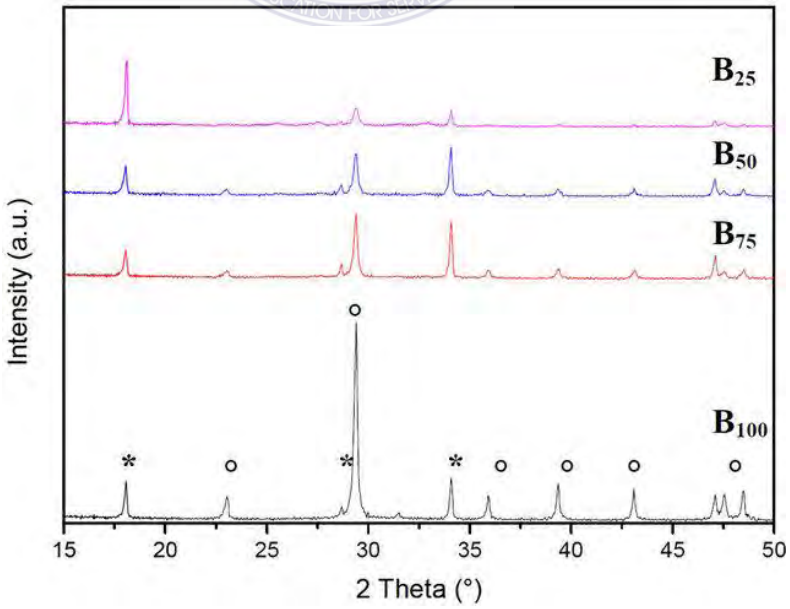

Figure 9: XRD patterns of the aqueous nanolime suspensions after 30 minutes of air exposition time: sample B $CaCO_3$ (Taglieri et al., 2013)

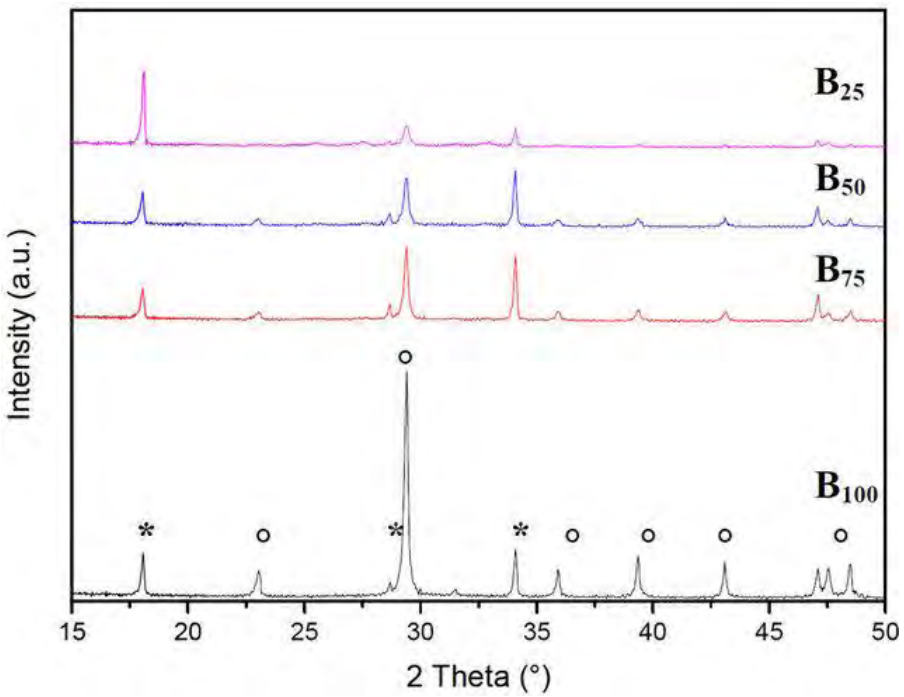

Figure 10: XRD patterns of the aqueous nanolime suspensions after 30 minutes of air exposition time sample $CaCO_3$ from (Taglieri et al 2013)

2.9 Characterization of Nanoparticles

Nanoparticles' physicochemical qualities are critical for their behaviour, bio-distribution, safety, and efficacy (Zhang et al., 2016). Before applying nanomaterials for human welfare, nanomedicines, or the healthcare industry, it is important to characterize the generated nanoparticles (Lin et al., 2014). To evaluate the synthesized nanomaterials, many analytical techniques have been used, however this study focused on ultraviolet visible spectroscopy, X-ray diffractometry, and scanning electron microscopy.

2.9.1 Ultraviolet visible spectroscopy

Initially, preliminary confirmation and stability of nanoparticles is carried out by Ultraviolet visible spectroscopic technique (Sastry et al.,1998; Baker et al., 2013). Ultraviolet visible spectroscopy can be used to obtain the size, aggregation state, and population of nanoparticles of a particular size (Zewde et al., 2016). It is fast, easy,

simple, sensitive, selective for different types of nanoparticles. (Tomaszewska et al. 2013). Silver nanoparticles have distinct optical characteristics that allow them to interact with certain wavelengths of light (NanoComposix, 2012). The conduction and valence bands in silver nanoparticles are relatively near to each other, and electrons travel easily inside this band. These free electrons cause a surface plasmon resonance absorption band to form as a result of the collective oscillation of electrons of silver nano particles in resonance with the light wave (Das et al., 2009). The position of silver nanoparticles' plasmonic peak in the UV visible spectrum is affected by particle size, dielectric medium, and chemical environment (Zewde et al., 2016). The observation of this peak, which is assigned to a surface plasmon, has been well reported for a variety of metal nanoparticles ranging in size from two to hundred nanometers (Sastry, et al., 1998). In practice, as the particle size increases the absorbance peak increases, and broadens in width. The surface plasmon resonance peaks at around 435 nm are usually taken to confirm the reduction of silver nitrate into silver nanoparticles (Vanlalveni, et al., 2021). In general, spherical nanoparticles exhibit only a single surface plasmon resonance band in the absorbance spectra, whereas two or more surface plasmon resonance bands are observed for anisotropic particles depending on the shape (Durán, et al., 2007). The absence of peak in the region 335 and 560 nm in Ultraviolet Visible spectra are sometime used as an indication of the absence of aggregation in nanoparticles (Kora et al., 2010). In this research, the surface plasmon resonance absorption peak for silver nanoparticles prepared from elephant grass extract was observed using ultraviolet visible spectroscopy. This was done to establish nanoparticle formation and stability.

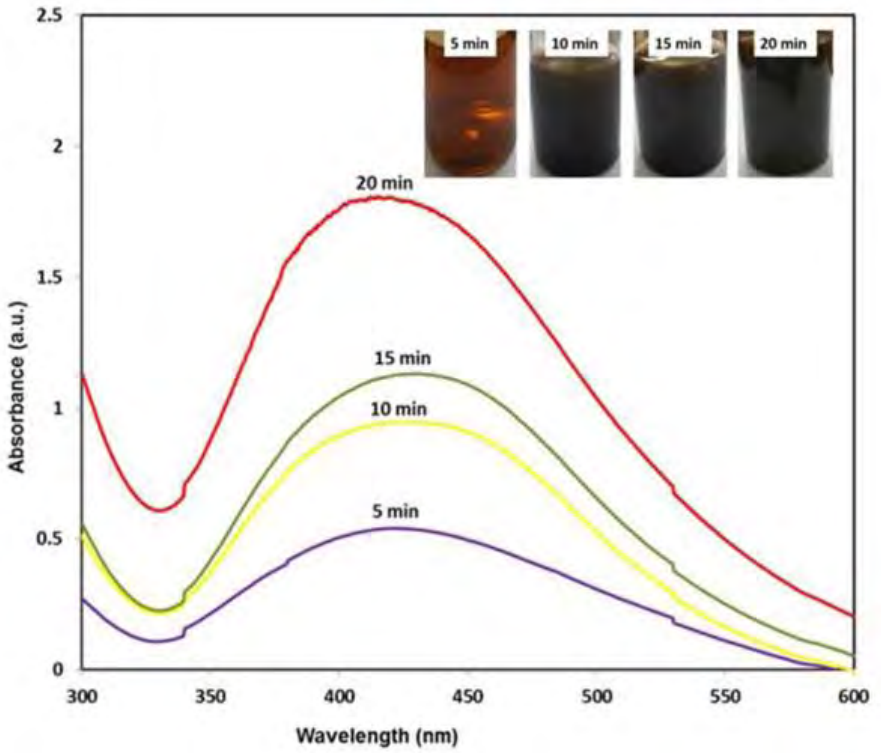

Figure 11: UV-Vis spectra of silver nanoparticles prepared with plant extract obtained at different time intervals.

2.9.2 Scanning electron microscopy

SEM is a surface imaging technique capable of resolving diverse particle sizes, size distributions, nanomaterial forms, and surface morphology of manufactured particles at the micro and nanoscales. We can use scanning electron microscopy to investigate the morphology of particles and generate a histogram from the images by either manually measuring and counting the particles or by utilizing specialized software (Fissan et al., 2014). The limitation of scanning electron microscopy is that it cannot resolve interior structure, although it can provide useful information about particle cleanliness and aggregation. Modern high-resolution scanning electron microscopy can discern the shape of nanoparticles as small as 10 nm. In this work, scanning electron microscope was used to determine the size and morphology of plant extract based synthesized silver nanoparticles.

2.10 Stabilization of silver nanoparticles

Stability refers to the condition in which the colloidal particles do not aggregate at a significant rate. The DLVO theory (Chin et al., 2016) suggests that the stability of a colloidal suspension is determined by the sum of the van der Waals attraction and repulsion between colloidal particles as they approach each other driven by the Brownian motion. When the van der Waals attraction is stronger than the repulsion, colloidal particles will aggregate, and the state of the suspension is unstable. If the repulsion is sufficiently high to overcome the van der Waals force, the system will achieve stability. Thus, in order to enhance the stability of a colloidal suspension, the repulsion between the particles needs to be strengthened. According to the types of repulsion, the typical fundamental mechanisms of colloidal stability are divided into two kinds: steric repulsion, and electrostatic repulsion. In practice, the most popular way to achieve this is to add an additional component like a surfactant or polymer that adsorbs on the colloidal particles and changes their surface properties (Yu & Xie, 2012).

2.10.1 Electrostatic stabilization

One way to counterbalance the van der Waals attraction between colloidal particles in polar liquids is to shell the particles with a Coulombic repulsion (He, 2014). In liquid dispersion media, ionic groups can adsorb to the surface of a colloidal particle to form a charge layer (He, 2014). To maintain electro-neutrality, an equal number of counterions with the opposite charge will surround the colloidal particles and give rise to overall charge-neutral double layers. In electrostatic stabilization, it is the mutual repulsion of these double layers surrounding particles that provides stability (He, 2014). Thus, if the electric potential associated with the double layer is sufficiently high, the electrostatic repulsion between the particles prevents their aggregation. An

example is gold sols prepared by the reduction of [$AuCl_4^-$] using sodium citrate. (Turkevich, 1985). One great disadvantage of electrostatic stabilization of particles is its great sensitivity to the ionic strength of the dispersion medium. In addition, it only works in polar liquids which can dissolve electrolytes. However, because this mechanism is simple and cost effective, electrostatic stabilization is still used in stabilizing dispersions in aqueous media (He, 2014).

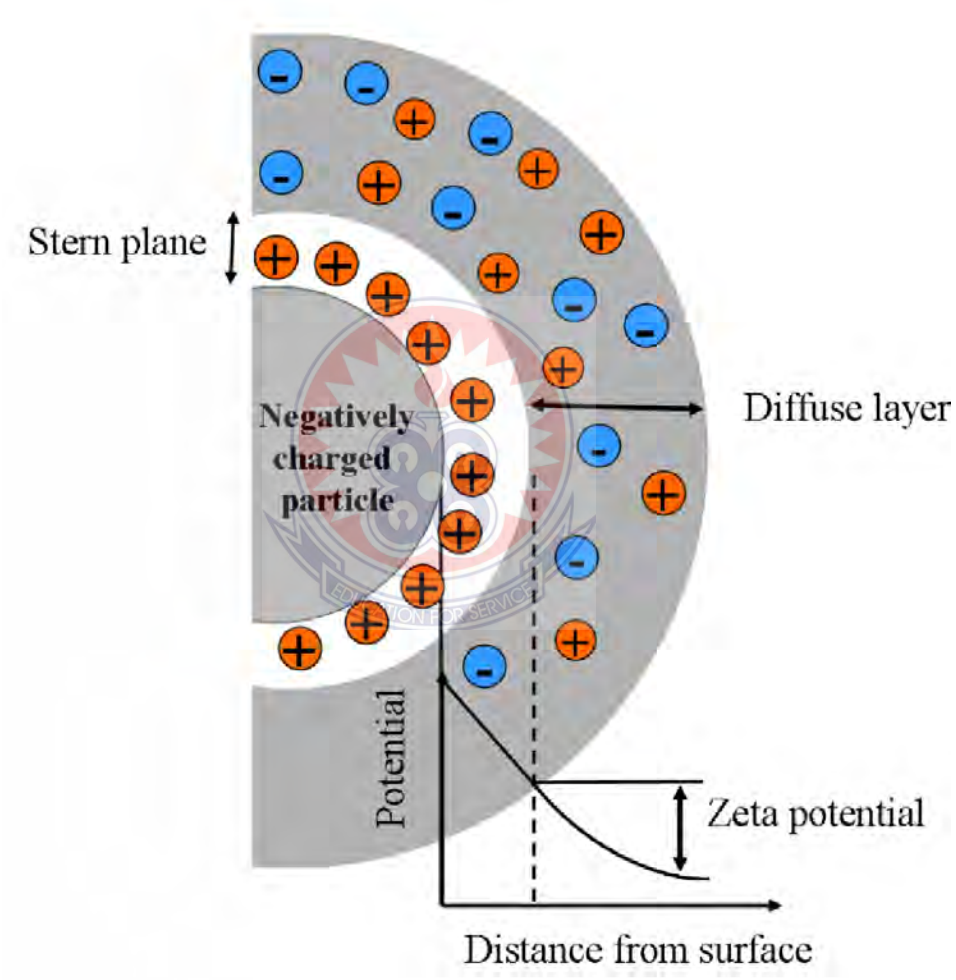

Figure 12: Electrical double layer illustrating the electrostatic stabilization of nanoparticles (Kovtun et al., 2009).

2.10.2 Steric stabilization

Steric stabilization provides an alternate route of controlling colloidal stability that can be used in aqueous and non-aqueous systems (He, 2014). In this approach,

stabilization is achieved by the coordination of sterically bulky organic molecules that act as protective shields on the metallic surface. The nanometallic cores are thus separated from each other, preventing coagulation. The organic molecules create steric repulsion (He, 2014). When two particles with adsorbed polymer layers approach each other at a distance of less than twice the thickness of the adsorbed layer, an interaction between the two layers takes place. The degree of stabilization can be defined quantitatively in terms of the energy change (ΔH) occurring upon the interaction of the adsorbed layers (He, 2014). The Gibbs free energy change (ΔG) of the overlap interaction of the adsorbed layers is expressed as ΔG = enthalpy change(ΔH) –[temperature(T)x entropy change (ΔS)]. If ΔG is negative upon the overlap of the adsorbed layers, flocculation or coagulation will result, and if ΔG is positive, stabilization will result. Under isothermal conditions, the stability is then a function of the enthalpy change (ΔH) and the entropy change (ΔS) (Israelachvili, 1991). Steric stabilization has several distinct advantages over electrostatic stabilization in that it has relative insensitivity to the presence of electrolytes and has equal efficacy in both aqueous and nonaqueous dispersion media. Steric stabilization is effective in both nonaqueous media and aqueous media. This explains why steric stabilization is usually preferred for non-aqueous dispersion media (He, 2014).

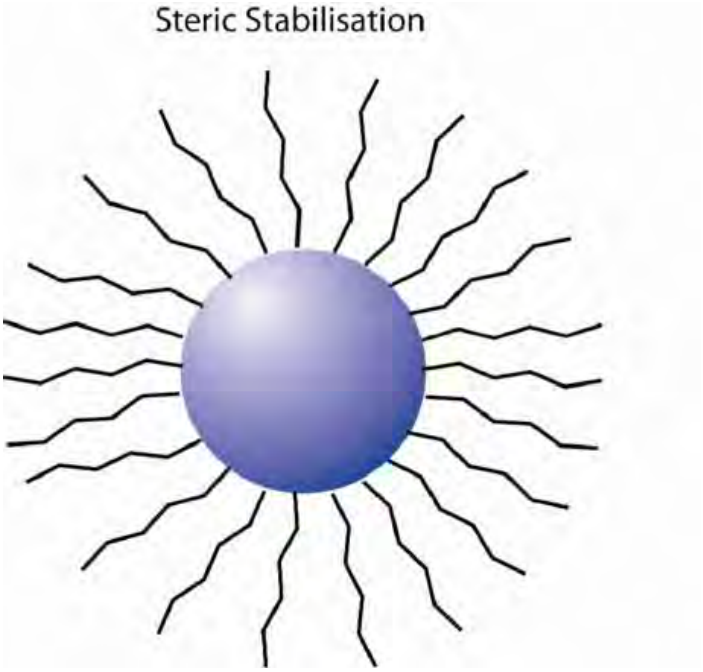

Figure 13: Illustration of steric stabilization of nanoparticles (Hagendorfer et al., 2011).

2.11 Purification of Silver Nanoparticles

Purification of nanoparticles is carried out by an array of techniques which forms a base for nanoparticle separation (Baker et al., 2013). Following bio-reduction (Kalishwaralal et al., 2009), purification is necessary to separate the nanometal-bio complex from unconverted -biomolecules. While certain nanoparticles, such as those that are magnetic, provide properties that make purification simple by using permanent magnets (Medley et al., 2011), most rely on several utilized methods to separate the unbound biomolecule from the nanometal-bio complexes.

2.11.1 Centrifugation Method

Usually, centrifugation techniques are used to separate the nanoparticles based on the size and shape (Baker et al., 2013). Centrifugation can be used to purify functionalized metal nanoparticles-bioconjugates from unconjugated biomolecule.

Depending on the density, size, and structure of the metal nanoparticles and biomolecules, centrifugation can be used to separate functionalized metal nanoparticles from the reaction mixture and then either removing the soluble bioconjugate or, alternatively, resuspending the bioconjugate precipitate in the buffer of choice. For example, such a method has been used to purify layered double hydroxide nanoparticles intercalated with the acetyl choline esterase inhibitor enalaprilats from free drug (Ribeiro et al., 2009).

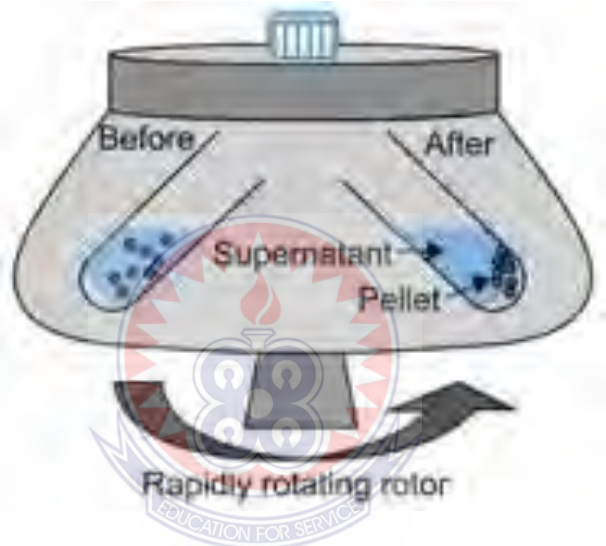

Figure 14: A simple centrifugation scheme to illustrate the purification of silver nanoparticles.

2.11.2 Dialysis method

Dialysis method is cheap, commercially available, and simple to use. it uses some form of permeable or semipermeable membrane with a given size or molecular weight cut-off value to separate the biomolecule from the metal nanoparticle-bioconjugate. In dialysis, the membrane containing the sample to be purified is immersed in a large excess volume of liquid and species of molecular weight less than membrane

molecular weight cut-off of the biomolecule flow in the direction of high to low concentration (Tiwari et al., 2009).

2.12 Application of Silver Nanoparticles

2.12.1 Medical application

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications (Sukumaran et al., 2012). Nanoparticle in medicine is used for therapeutic, imaging, and diagnostics of cancer.

2.12.2 Characteristics of silver nanoparticles which make them useful in the medical field

Generally, the small size of nanoparticles provides a larger surface area for the particle and hence increases their effect and penetration potential. Based on the size factor alone, nanoparticles could penetrate the circulatory system and translocate even the blood–brain barrier in the human system (Sukumaran et al., 2012).

The antimicrobial nature of silver nanoparticles is the most exploited in the medical field. studies have shown that, the acceleration of wound healing in the presence of nanoparticles is due to the reduction of local matrix metalloproteinase activity and the increase in neutrophil apoptosis within the wound. It has been suggested that the matrix metalloproteinase can induce inflammation and hence cause non-healing wounds (Kirsner, 2001). Silver nanoparticles can inhibit the activities of interferon gamma and tumour necrosis factor alpha which are involved in inflammation (Shin et al., 2007). The anti-inflammatory effects induced by nano silver make it useful anti-inflammatory agents for various therapies.

Silver nanoparticles are used in bone cements that are used as artificial joint replacements. Polymethyl methacrylate loaded with nano silver is being considered as bone cement as the nano silver can induce antimicrobial activity (Alt et al., 2004).

The current methods use to prevent surgical infection include antibiotics and antiseptics. Surgical meshes are used to bridge large wounds and for tissue repairs. Though these meshes are effective, they are susceptible to microbial infections. Silver nanoparticle-coated polypropylene mesh is said to have good antimicrobial activity and can be used for surgical meshes (Cohen et al., 2007). The antimicrobial property of silver nanoparticles has been in medical treatments such as intravenous catheters, endotracheal tubes, wound dressings, bone cements, and dental fillings prevent microbial infections.

Nano silver also has the capacity to be used in biosensing. The plasmonic properties of nano silver are exploited for biosensing. Nano silver biosensors can detect a large number of proteins that normal biosensors find hard to detect. This unique advantage can be utilized for detecting various abnormalities and diseases in the human body including cancer (Zhou et al., 2011). The plasmonic properties of nano silver also make it useful for bioimaging as they do not undergo photobleaching, hence can be used to monitor dynamic events over an extended period of time (Lee & E-Sayed, 2006). The plasmonic nature of nano silver can also be used to destroy unwanted cells. The cells can be conjugated to the target cells and then be used to absorb light and convert it to thermal energy; the thermal energy can lead to thermal ablation of the target cells (Loo et al., 2005).

2.12.3 Electronics

Silver nanoparticles are considered to apply a silver paste for electrode because of their high conductivity (Chen et al., 2009) Silver nanoparticle is employed in the field of microelectronic materials. The melting point of smaller sized silver nanoparticle reduces and with increased surface energy. This property of silver nanoparticle is useful in electronics and used as conductive fillers in electronically conductive adhesives (Umadevia, et al, 2013). The electrical conductors fabricated with a thick film of silver nanoparticle reduces the electrical loss. The lower electrical loss at higher frequency is attributed to the lower surface roughness that give better packing and used to fabricate antennas (Chen, et al 2009). Silver nanoparticle is successfully employed in the field of microelectronic materials. The melting point of smaller sized silver nanoparticle drastically reduced and with increased surface energy. This property of silver nanoparticle is useful in electronics and used as conductive fillers in electronically conductive adhesives (ECAs). The electrical conductors fabricated with a thick film of silver nanoparticle reduces the electrical loss. The lower electrical losses at higher frequency are attributed to the lower surface roughness that give better packing and used to fabricate antennas. The electro reflectance (ER) effect of silver nanoparticle is important in the field of electro –optical devices and sensors. In this case, the change in the electronic charge which stored on the particles that alters the absorption spectrum of the particle ensemble. The effect is typically 100 times stronger than for a bulk metal surface, and is readily discernible to the unaided eye. The ER effect makes it possible to directly monitor the changes in the electrostatic charge stored on small metal particles using absorption spectroscopy (Thamilselvi & Radha, 2017)

2.13 Anti-Bacterial Activity of Silver Nanoparticles

Silver nanoparticles have piqued the interest of researchers in the medical area due to their appealing and distinctive nano-related qualities, such as their high inherent antibacterial effectiveness and non-toxicity. Some critical features of silver nanoparticles' (AgNPs) particular antibacterial activities imply their inherent physical and chemical properties, which include preserving AgNPs' nanoscale size, enhancing their dispersion and stability, and avoiding aggregation (Guan et al, 2019). Silver metal has been recognized as a very potent antibacterial agent, which is able to kill numerous types of microorganisms that cause various infectious diseases (Chernousova & Epple, 2013). In 2015, Vance and co-workers redeveloped the nanomaterials consumer products inventory. They observed that about 42% of the total products intended for health and fitness applications silver nanoparticles were the most frequent used nanomaterial due to their antimicrobial properties (Vance et al., 2015). The clinical use of silver is limited due to its numerous adverse effects and high toxicity (Burd et al., 2007; Poon & Burd, 2004). Many investigations have been conducted to demonstrate that the anti-pathogenic action of AgNPs is superior to that of silver ions (Li et al, cited by Burdusel et al,2018). The worrying and rising phenomenon of pathogenic drug resistance is a huge concern for the global healthcare system. As a result, AgNPs are promising candidates for the nanotechnology-derived development of unique and effective biocompatible nanostructured materials for novel antibacterial applications (Premkumar et al, 2018) Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Organic compounds used for disinfection have some disadvantages, including toxicity to the human body, therefore, the interest in inorganic disinfectants such as metal oxide nanoparticles (NPs) are increasing (Hajipour et al., 2012). Among the several

possible uses of AgNPs in this area, focus and on their promising implications in wound dressing, tissue scaffold, and protective clothing applications (Gudikandula et al, 2017 cited by Burdusel et al, 2018). In this context, nanomaterials are emerging tools for controlling infections without development of any resistance. Several metal and metal oxide-based nanomaterials have been used as antimicrobial agents that efficiently control the infection against pathogens (Omar et al., 2019). Silver nanoparticles possess a broad spectrum of antibacterial, antifungal and antiviral properties. Silver nanoparticles can penetrate bacterial cell walls, changing the structure of cell membranes and even resulting in cell death. Their efficacy is due not only to their nanoscale size but also to their large ratio of surface area to volume. They can increase the permeability of cell membranes, produce reactive oxygen species, and interrupt replication of deoxyribonucleic acid by releasing silver ions. (Yin, et al., 2020). AgNPs are one of the most commonly employed metallic nanoparticles in modern antimicrobial applications due to their inherent broad bactericidal activities against both Gram-negative and Gram-positive bacteria, as well as their physicochemical features (Shao et al, 2018). According to many studies, AgNPs interact with the bacterial membrane and penetrate the cell, causing significant disruptions in cell function, structural damage, and cell death (Yan et al, cited by Burdusel et al, 2018).

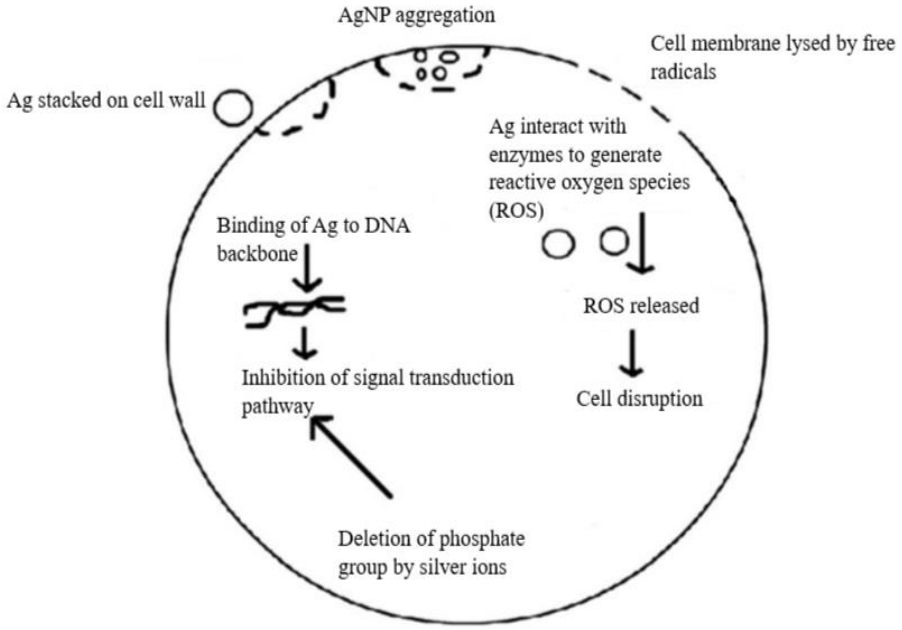

Figure 15: Proposed mechanism describing various modes of action of silver nanoparticles (AgNP on bacterial cell adapted from Burdusel et al, 2018).

Due to the intrinsic antibacterial and anti-inflammatory properties of single metallic nanoparticles, it has been demonstrated that AgNPs can be successfully used to design and produce superior wound and burn dressings and because of the mutation-resistant antimicrobial activity of nano silver-based biomaterials, AgNPs are used in a variety of pharmaceutical formulations such as burn ointments, antibacterial garments, and medical device coatings (Bhat et al, 2011; Lopez et al, 2016). In the modern attempt to reduce or even eradicate microbial contamination and colonisation processes, the current methodologies rely on the advantageous combination of antimicrobial silver nanoparticles with natural or synthetic polymers (Schneider, 2017). The major advantage of nano silver-based biomaterials designed for unconventional antibacterial applications is related to their intrinsic anti-pathogenic effects exhibited against both pathogenic and biofilm-organized microorganisms. The bactericidal activity of AgNPs is attributed to silver cations, which possess the ability to specifically bind to thiol groups of bacterial proteins, disrupting their physiological activity and leading to

cell death. Silver nanoparticles exert their bactericidal activity through initial binding to the cell surface leading to permeability alteration and cellular respiration impairment, followed by cell-barrier penetration and intracellular metallic silver ion release (Burdusel et al., 2018).

It is critical to understand the action of nano silver-based systems on bacterial cells and bacterial biofilms in order to successfully deploy them as effective antibacterial agents (Radzig et al., 2013). AgNPs have bacteriostatic or bactericide activity against biofilm-organized bacteria which may be due to intrinsic activity against isolated or blocked cells, disruption of external polymer substances within the extracellular biofilm matrix, or interference with bacterial signalling molecules (Piewngam et al., 2018).

2.13.1 Synergistic effect of silver nanoparticles

Silver nanoparticles (Ag NP-s) represent an important nanomedicine-based advance in the fight against polyresistent bacteria. In this study, the fungus Trichoderma viride was utilized for extracellular biosynthesis of extremely stable AgNps (Fayaz et al., 2010). Silver nanoparticles form promising template for designing antimicrobial agents against drug resistant pathogenic microorganisms (Thomas et al., 2014). This is due to the wide range of antimicrobial properties of silver nanoparticles (Franci et al., 2015) and human pathogens having developed resistance against most of the antibiotics resulting in their decreasing efficacy. Synergistic effects of AgNPs with various antibiotics were evaluated against targeted microbes (fungal and bacterial), human pathogens. The results showed that AgNPs in combination with sampled antibiotics have better antimicrobial effect as compared with AgNPs alone and hence can be used in the treatment of infectious diseases caused by microbes (Jyoti et al., 2016). Therefore, it may be used to augment the activities of antibiotics.

To find out the solution of the problem of pathogens developing resistance to most antibiotics which is a challenge in medical science, therefore, we need to find environmentally benign biomaterial/bioresources in the synthesis of silver nanoparticles and their synergistic role with antibiotics (Saratale et al., 2017). The antibacterial potential of biosynthesized silver nanoparticles, standard antibiotics, and their conjugates were evaluated against multidrug-resistant biofilm-forming coagulase-negative *S. epidermidis* strains, *S. aureus*, *Salmonella Typhi*, *Salmonella Paratyphi*, and *V. cholerae* (Thomas et al., 2014).

2.13.2 Methods to determine anti-bacterial activities of silver nanoparticles

Antimicrobial activity of silver nanoparticles is evaluated through several clinical microbiological methods, where the disk diffusion and the broth or agar dilution methods are the main techniques used (Correa et al., 2020).

The agar disk diffusion method is used to analyse the growth of common microorganisms, such as bacteria, fungi, and yeast, in a rapid manner. In this method, a standardized concentration of the microorganism is inoculated onto a Petri dish containing the growth culture medium, and filter paper disks with the antimicrobial agents are placed on the agar surface. The Petri dishes are incubated under appropriate growth conditions. The antimicrobial agent is spread on the agar plate, and it inhibits the microorganism growth by forming disks corresponding to inhibition zones (NCCLS, 2015). In order to obtain reliable results, A standardized methodology is used to the measures the correlation between inhibition zone diameter and the minimum inhibitory concentrations of the antimicrobial agents. Thus, specific culture media, various incubation conditions, and interpretive criteria for the inhibition zones are used. As a result, approximate minimum inhibitory concentration values can be

obtained; however, this method cannot distinguish between bactericidal or bacteriostatic effects (Balouiri et al., 2016).

The agar (or broth) dilution method. This method consists of preparing a series of plates (or tubes) containing a standardized suspension of the microorganism to be tested into agar or broth medium, containing various concentrations of the antimicrobial agents. After incubation under the appropriate conditions, they can be determined and the results can be analysed using approved cut-off points. When using this technique, the experimental conditions must be carefully controlled in order to achieve reproducible results (NCCLS, 2011). This technique is usually combined with the dynamic contact methodology (ASTM E2149-10 directive) in which different nanoparticles concentrations are put into contact for a given time period with a solution containing a known concentration of microorganisms. Therefore, after the nanoparticles perform their antimicrobial activity in the liquid culture medium, it can be further inoculated onto the Petri dish with agar and incubated at the specific growth conditions, according to the target microorganisms (NCCLS, 2015, NCCLS, 2011).

2.14 Elephant Grass

Elephant grass (Pennisetum purpureum), also known as Morrone Cenchrus purpureus, Napier grass, grass or Uganda grass, is a species of perennial tropical grass native to the African grasslands (Negawo et al., 2017). It has low water and nutrient requirements, and therefore can make use of otherwise uncultivated lands (Stephenson et al., 2010). Historically, this wild species has been used primarily for grazing. Recently, however, it has been used as part of a push–pull agricultural pest management strategy (Khan et al., 2014). Napier grasses improve soil fertility, and protect arid land from soil erosion. It is also utilized for firebreaks, windbreaks, in

paper pulp production and most recently to produce bio-oil, biogas and charcoal (Iwai et al., 2015).

Zain et al., (2013) reported that Pennisetum purpureum extracts can be used as natural herbicide for weed management. *Pennisetum purpureum* has high phenolic content and has been used as healthy food materials, livestock feed, biofuels and more. *P. purpureum* extract has the potential of being disinfectant to limit the spread of coronaviruses (CoVs) and enteroviruses (EVs) because the extract can inhibit the infection of EV71, feline coronavirus (FCoV), and pig epidemic diarrhea disease virus (PEDV) in cells, and significantly reduce the severity of symptoms caused by infectious bronchitis virus (IBV) in chicken embryos (Chen et al., 2022

CHAPTER THREE

METHODOLOGY

3.0 Overview

This chapter discusses activities and the methods employed in carrying out the activities. The materials used, source of sample, treatment of sample, synthesis of the silver nanoparticles. It also talks about the activities employed in characterizing the synthesized particles.

3.1 Study area

This study was carried out in Winneba in the Effutu Municipality in Central Region of Ghana.

3.2 Sample Collection

Elephant grass (*Pennisetum purpureum*) leaves were used for this study. The leaves were collected by cutting it off the stem of the grass adjacent the Presby Hostel in Winneba, located in the Effutu Municipality in the central region of Ghana. The leaves were placed in a transparent polythene and sent to the laboratory on the same day.

3.3 Reagents

Silver nitrate ($AgNO_3$) was used as the precursor for the preparation of silver nanoparticles. Leaf extract from *Pennisetum purpureum* was used to reduce silver ions to form nanoparticles. Hypochlorite solution was used to disinfect the harvested *Pennisetum purpureum* leaf before use. Methanol (Sigma Aldrich, analytical grade), DPPH (Sigma Aldrich, analytical grade), ABTS (Sigma Aldrich, analytical grade), DMSO (Sigma Aldrich, analytical grade) were used in the MIC, antioxidant and synergistic effect studies of the prepared silver nanoparticles.

3.4 Preparation of Silver Nanoparticles Using Pennisetum Purpureum Leaves Extract

3.4.1 Sample drying

The harvested *Pennisetum purpureum* leaves were first dried overnight at temperature range of 60ºC - 80ºC in an oven.

3.4.2 Preparation of the *Pennisetum purpureum* leaf extracts

2.5g of the dried leaves were first washed with distilled water. The leaves were further disinfected with hypochlorite solution and washed severally with distilled water. The disinfected leaves were placed in 40 mL of water and incubated for three (3) days. After the incubation, the extract solution (pale yellow colour) was decanted through a Whatman filter paper and kept for further use.

3.4.3 Green synthesis of silver nanoparticles

Silver nitrate salt (0.42 g) was dissolved in distilled water to prepare 0.01moldm^3 of $AgNO_3$ solution. The prepared solution was used as source of silver ions for the preparation of the nanoparticles. About 1.0 mL of aqueous 0.01mol/dm^3 silver nitrate ($AgNO_3$) solution was added to 4.0 mL of leaf extract for analysis. The solutions were kept at room temperature for two to three days and the formation of silver nanoparticles were observed as the development of colour change from pale yellow to brown solution.

3.5 Characterization of Prepared Silver Nanoparticles

3.5.1 Centrifugation

The prepared Perp-AgNPs was purified by subjecting it to centrifuge and was spun at 3000 rpm (rotation per minute) for 5 – 10 minutes. This ensured that the excess

Pennisetum Purpureum was removed. A pipette dropper was used to draw excess solution out.

The purified AgNPs was transferred onto a petri dish and dried in an oven at temperature range of 80ºC to 100ºC for about an hour.

3.5.2 Ultraviolet-visible spectroscopic analysis

For further confirmation of silver nanoparticles formation, ultraviolet visible spectral analysis was carried out to measure the plasmonic absorption of the silver nanoparticles. About 1 mL of the sample was diluted with 2 mL of distilled water and placed in a cuvette; then, the UV-Vis spectrum of solution was measured between wavelength 270 to 800 nm in a spectrophotometer with a resolution of 1 nm. The absorbance of the sample was measured using 1 mL of distilled water as a blank control.

3.5.3 Infra-red spectroscopy

Infrared Spectroscopy technique measures the absorption of infra-red radiation by a sample resulting from the vibrational stretching and bending modes within the molecule (Khan et al., 2018). In particular, Fourier Transform infra-red spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles (Lin et al., 2014). FTIR has also been extended to the study of nano materials, such as confirmation of functional groups (molecules) covalently bound to silver, carbon nanotubes, graphene, and gold nanoparticles through the appearance of characteristic spectral bands (Gurunathan et al., 2014). Infrared spectroscopic analysis was used to characterize the synthesized silver nanoparticles to identify plant compounds bound to its surface. Sample of the prepared silver nanoparticles was dried and measured directly with FTIR. The transmittance spectrum of the diluted

sample was then recorded. The FTIR measurements were carried in the diffuse reflectance mode at 4 cm^{-1} resolutions using the Perkin Elmer FT-IR spectrometer.

3.5.4 Scanning electron microscopic analysis of silver nanoparticles

The Scanning electron microscope analysis was used to ascertain the morphology and size of the synthesized silver nanoparticles. A drop 10µL of silver nanoparticles suspension was put onto grids of 150 mesh with a carbon support film and then the sample dried at room temperature. Extra solution was removed using a blotting paper. The dried sample was rinsed with ethanol and dried again. Then fixed on scanning electron microscope holder for measurement.

3.6 Antibacterial Studies of the Prepared Silver Nanoparticles

3.6.1 Test organisms

The bacteria and fungi strains were obtained from the Microbiology unit of the School of Basic and Biomedical Sciences, University of Health and Allied Science, Ho. The microbial strains include: Methicillin resistant *Staphylococcus aureus* (NCTC 12493), *E. coli* (NCTC 12241), S. mutants (ATCC 700610), *P. aeroginosa* (ATCC 4853), S. typhii (ATCC 14 028), K. pneumonia (NCTC13440) and Candida albicans (ATCC 90028), *B. subtilis* (ATCC 10004).

3.6.2 Minimum inhibitory and bactericidal concentrations (MIC And MBC) determination

The MIC of the silver nanoparticles was determined by the micro broth dilution method using protocol adopted by Mogana (2020), and the Clinical and Laboratory Standards Institute (CLSI) guidelines with slight modification. A 10 mg/ml stock solution was prepared by weighing 10 mg and then dissolving in 1ml of DMSO. A two-fold serial dilution was prepared until 10 different concentrations were obtained.

An aliquot of 100µl of double strength Mueller Hinton broth was dispensed into each well and mixed with 100 uL of the silver nanoparticles to prepare well concentrations ranging from 10 – 0.002 mg/mL. Wells 11 and 12 served as both positive control (Broth + organism only) and negative control (Broth with no organism only) respectively for each microorganism on each column. This process was likewise done for the antibiotics, Ciprofloxacin, Tetracycline, Fluconazole, Nystatin and Ketoconazole at concentrations ranging from 256- 0.125 ug/mL in separate plates against all the test bacteria and fungi accordingly. This was followed by the addition of 100 ul of each of the 0.5 McFarland standardized, test organisms after which the plates were subjected to incubation at 37 C for 24/48 h for bacterial and fungal strains respectively. This experiment was performed in triplicate with the MICs recorded.

3.6.3 Determination of minimum bactericidal (MBC) and fungicidal concentration (MFC)

In order to confirm if the prepared silver nanoparticles would be able to kill the microbial cells (bacteri-/fungi-cidal effect), the MBC and MFC were determined. Aliquots from each well from susceptibility testing assays were transferred to plates containing Nutrient agar and then incubated 24/48 h at 37°C. The plates were then Results were then checked for the presence or absence of growth in the Nutrient agar or SDA.

3.6.4 Determination of synergistic effect of Perp-AgNPs with selected antimicrobial agents

In-vitro analysis of the interaction between the silver nanoparticles and the antibiotics (tetracycline and ciprofloxacin) against the bacterial strains and the antifungals (fluconazole, ketoconazole and nystatin) against *C.* albicans strains, were evaluated by adopting checkerboard microdilution assay as described previously by Segatore et

al., (2012) and Bellio et al., (2021), with slight modifications with test concentration concentrations for each antibiotic and the silver nanoparticles ranging from 1/32MIC to 2xMIC. The effect of the interactions was measured by computing the fractional inhibitory concentration index (FICI) from the relation:

$$\text{FICI} = \frac{\text{Ac}}{Aa} + \frac{Sc}{Sa}$$

where Ac is the MIC of antibiotic/antifungal in combination, Sc is the MIC of each test silver nanoparticles, Aa is the MIC of each antibiotic/antifungal alone and Sa is the MIC of the silver nanoparticles alone. The interaction was considered synergistic if the FICI was ≤ 0.5, partial synergistic if FICI was > 0.5 and < 1, additive if FICI was =1, no difference if the FICI was >1 and ≤ 4, and antagonistic if the SFICI was >4.0. The interaction was considered synergistic if the FICI was ≤ 0.5, partial synergistic if FICI was > 0.5 and < 1, additive if FICI was =1, no difference if the FICI was >1 and ≤ 4, and antagonistic if the FICI was >4.0 (Loho, et al., 2018).

3.7 ABTS scavenging activity of the silver nanoparticles

The antioxidant activity (free radical scavenging activity) of the silver nanoparticles was determined against ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) by mixing 10 ml each of ABTS and 2.4 mM potassium persulphate in order to generate the ABTS free radical. The mixture was further diluted in 50 ml of methanol forming the working solution. A 150 µl of the solution was then added to 50 µl of the prepared compound concentrations (1.25, 1.25, 1.25, 0.625, 0.625, 0.313, 0.313, and 0.313 mg/ml), vortexed and incubated at 30ºC for 10 min. The absorbance of each concentration was then recorded at 734 nm for control ABTS (C) and the test samples (T) and as well as Ascorbic acid as positive control. The free radical scavenging

activities for the silver nanoparticles and the referenced Ascorbic acid against ABTS was therefore evaluated by inputting data into the relation:

$$\%\ ABTS\ scavenging\ activity = [\frac{(C)-(T)}{(C)}] \times 100\%$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

This section describes the results and the data obtained from the synthesis process, characterization instruments and probable implications.

4.1 Extraction of Potent Reducing Agent from the Leaves of *Pennisetum Purpureum*

Several approaches were employed to arrive at a simple approach to attaining reducing agent from the *Pennisetum purpureum* leaves capable of reducing silver ions to nanoparticles. The various preliminary approaches are summarized below.

In the first attempt, fresh *Pennisetum purpureum* leaves were grinded and then filtered to obtained an extract solution as shown in Fig. 17. However, this extract solution could not lead to any significant colour change after addition of silver ions and observed after few days of incubation.

(a) (b)

Figure 16: (a) Image of grinded *Pennisetum purpureum* leaves, (b) Image of grinded *Pennisetum purpureum* leaves under filtration.

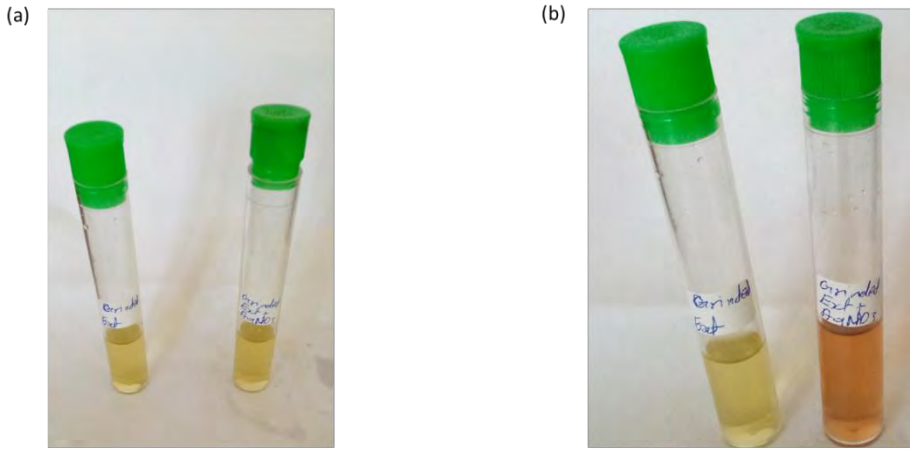

Figure 17 **: (a) Extract solution of grinded *Pennisetum purpureum* leaves with (right) and without (left) $AgNO_3$, immediately after addition, (b) extract solution of *Pennisetum purpureum* leaves without $AgNO_3$ (left) and with $AgNO_3$ (right) after several minutes of addition.**

In the second attempt to come up with an approach to attain *Pennisetum purpureum* leaves extract for reducing silver ions, the fresh leaves, were soaked in deionized water without grinding for three days. The extract solution was obtained by decanting off the water soaking the leaves through a filter paper, the filtrate served as the reductant solution. Silver nitrate (0.01M) was added to the extract solution and kept for up to three days. No visible characteristic colour change signifying the reduction of the silver ions was observed as shown in Fig. 19.

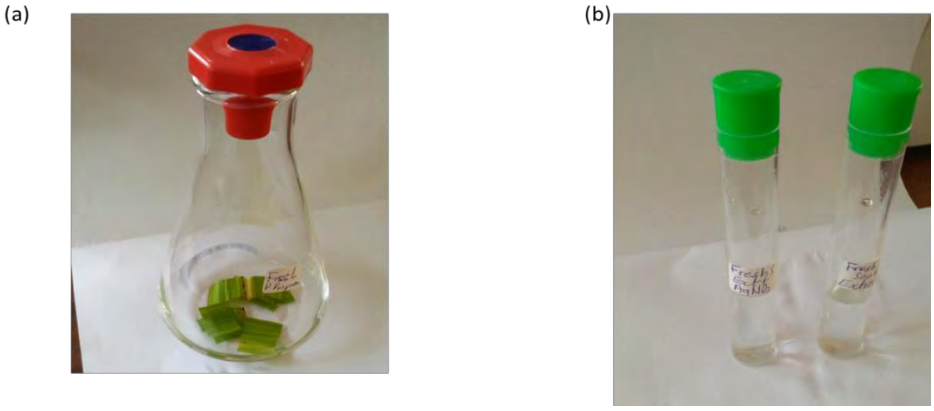

Figure 18: Images of (a) fresh *Pennisetum purpureum* leaves incubated in deionized water (b) fresh *Pennisetum purpureum* leaves extract with (left) and without (right) 0.01M $AgNO_3$, and kept for three days.

In the third approach, the *Pennisetum purpureum* leaves were first dried in an oven. The dried leaves were then washed several times with deionized water and then soaked in deionized water for 3 days. Fig. 20. shows the image of the dried leaves as well as the extract solution before and after the addition of silver nitrate. Extract of *Pennisetum purpureum* obtained by soaking the dried leaves in deionized water was capable of producing characteristic colour change for silver nanoparticles and thus was used in the subsequent processes to produce silver nanoparticles for this study.

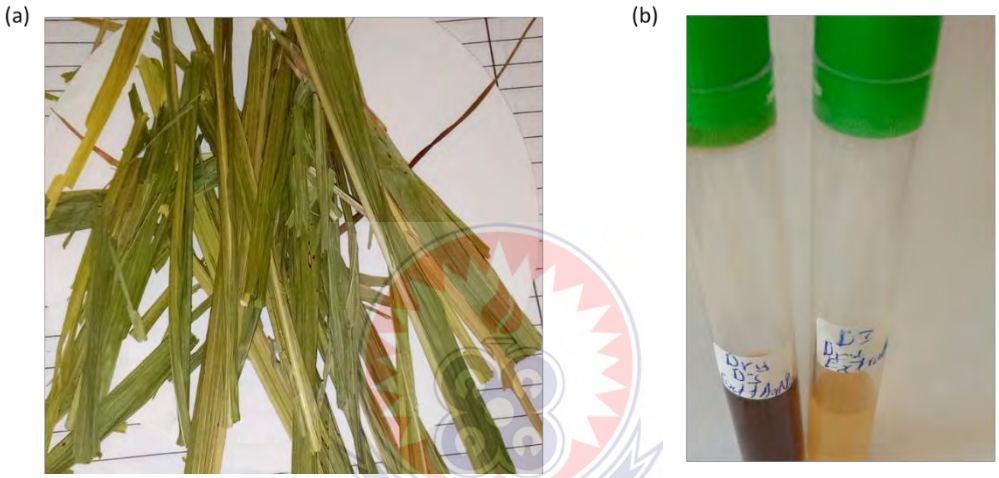

Figure 19 **: (a) Dried *Pennisetum purpureum* leaves, (b) *Pennisetum purpureum* leaves extract before (right) and after (left) addition of silver nitrate (0.01M)**

4.2 Preparation of Silver Nanoparticles with Leave Extract of *Pennisetum Purpureum* (*Perp-AgNPs*)

The *Pennisetum purpureum* extract solution obtained by soaking the dried leaves was used to synthesize silver nanoparticles, *Perp-AgNPs*. On addition of the $AgNO_3$ solution to the extract solution, the colour of the solution changes from pale yellow to ruby red after about 5 minutes. The solution further changes its colour from ruby red to dark brown on standing for about 30 minutes and becomes cloudy as shown in Fig. 21.

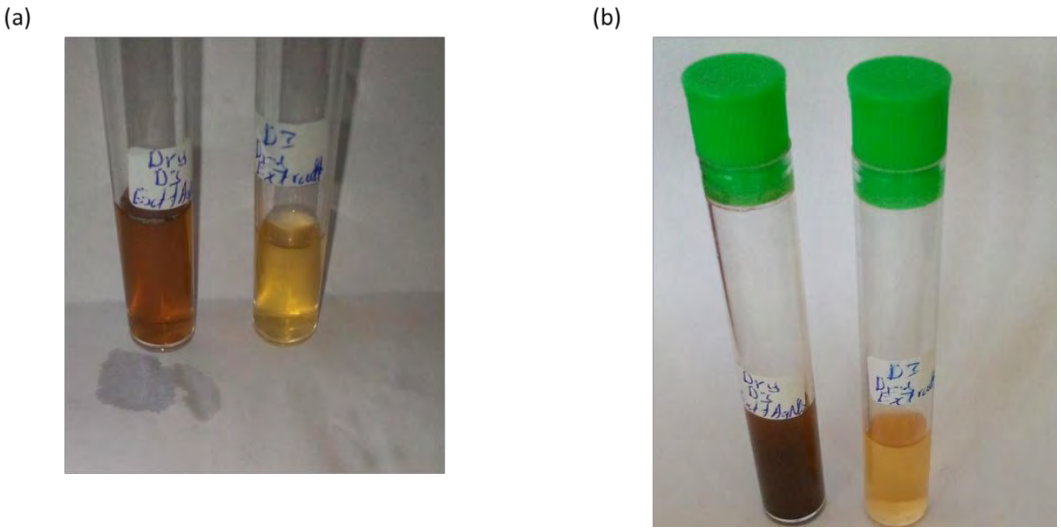

Figure 20: (a) Image of colour change of solution on addition of $AgNO_3$ (a) to ruby-red after about 5 minutes and (b) to dark brown after about 30 minutes.

The formation of the Perp-AgNPs can be attributed to the reducing properties of the various elements such as aldehydes and other carbonyl compounds as well as nitrogen containing compounds present in the leave extract. These compounds are found capable of donating and releasing electrons to Ag^+ leading to its reduction to Ag^0. Few Ag^0 atoms come together to form seeds which serve as template for the growth of the Perp-AgNPs.

4.3 Purification by Centrifugation

The prepared Perp-AgNPs was purified by subjecting it to centrifuge and was spun at 3000 rpm (rotation per minute) for 5-10 minutes. This ensured that excess *Pennisetum Purpureum* was removed. The dropper pipette was used to draw excess solution out.

The purified Perp-AgNPs was transferred onto a petri dish and dried in an oven at temperature range of 80◦C to 100◦C for about an hour.

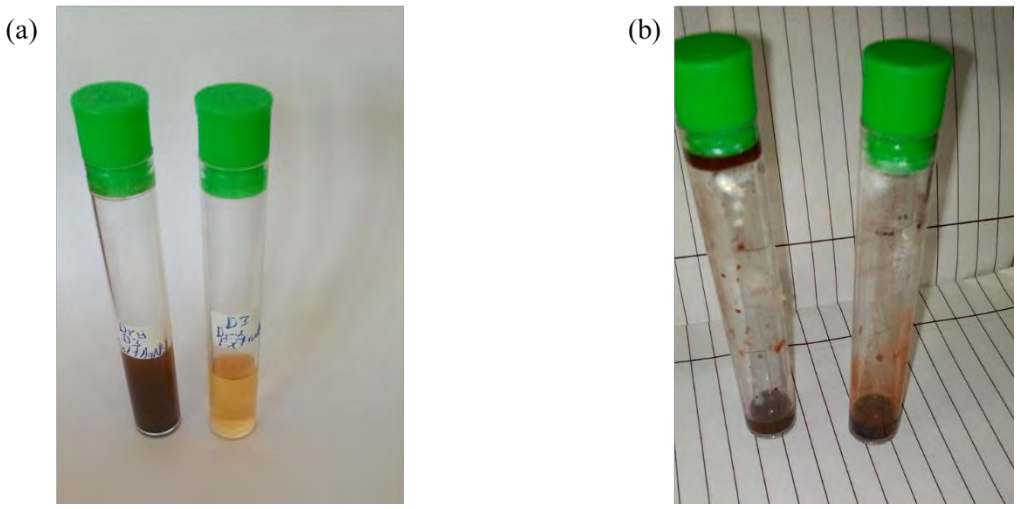

Figure 21: Image of Perp-AgNPs (a) before centrifugation and (b) after centrifugation

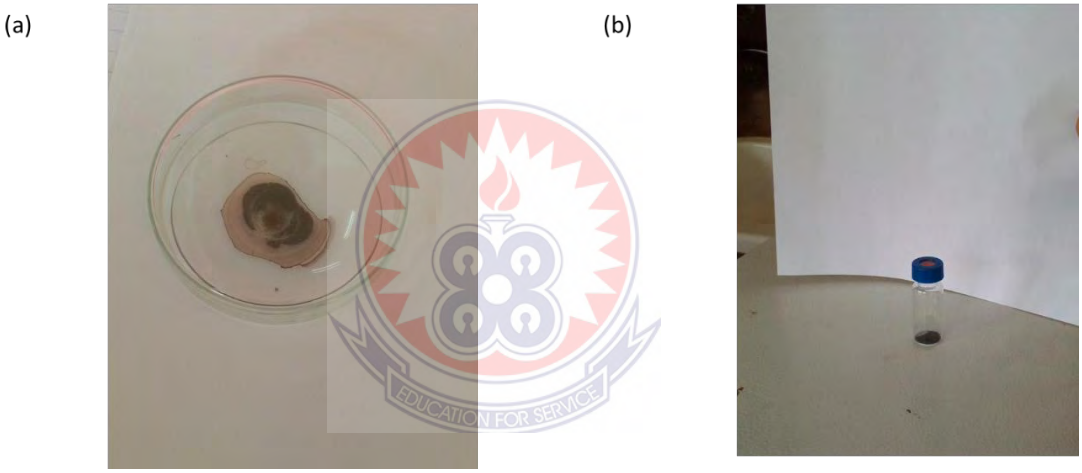

Figure 22: Image of Dried Perp-AgNPs (a) on the petri dish from oven and (b) scrapped dried Perp-AgNPs in a valve

4.4 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Perp-AgNPs

FTIR spectroscopy is a technique used to identify the characteristic functional groups from the spectral bands that allow us to know the conjugation between the nanomaterial and the adsorbed biomolecules (Kanchana et al., 2010).

FTIR identifies the presence of organic and inorganic compounds in the sample (Coury & Dillner, 2008). Depending on the infrared absorption frequency range 400–4000 cm^{-1}, the specific molecular groups prevailing in the sample will be determined

through spectrum data analysis (Smith et al., 2018). The molecules may be responsible for the stability of the Perp-AgNPs. From spectrum shown by Perp-AgNPs, prominent bands were observed at 543 cm^{-1}, 1000 cm^{-1}, 1234 cm^{-1}, 1383 cm^{-1}, 1593 cm^{-1} and 1740cm^{-1} (Figure 24). The absorption at 1740 cm^{-1} may indicate the presence of a− C=O stretching, the band at 1593 cm-1 may be assigned to C=C aromatic stretching. Bands around 1234 cm^{-1}, and 1383 cm^{-1} can be assigned to C-N amine stretch and C-C alkene stretch, respectively. These functional groups may have acted as electron donating group facilitating the reduction of Ag^{+} and stabilizers preventing the particle from aggregation.

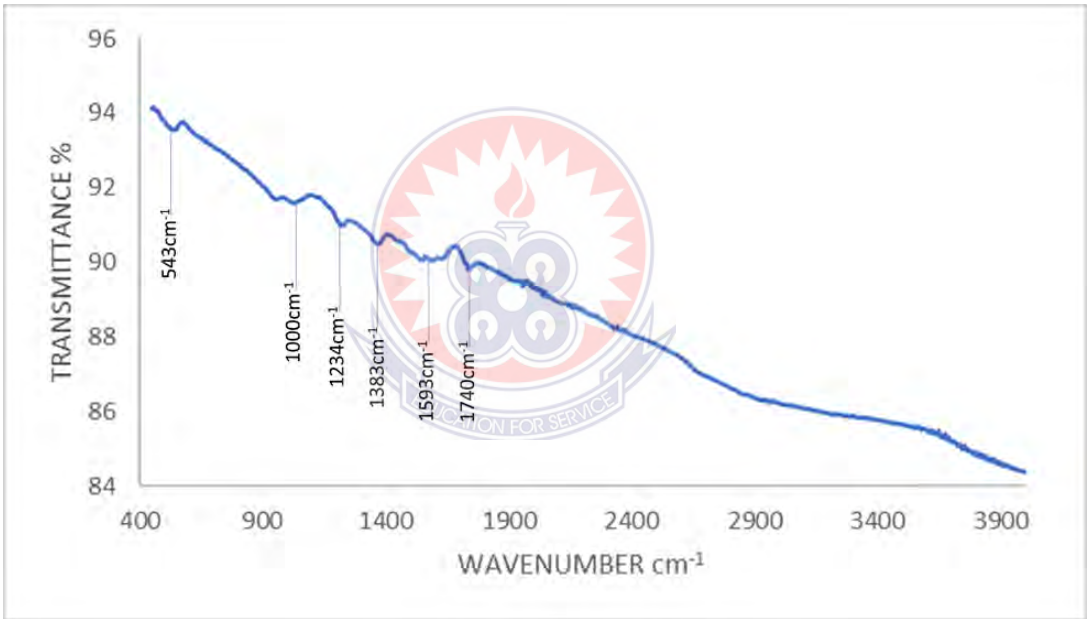

Figure 23: FTIR spectrum of the Perp-AgNPs.

4.5 UV-Vis Absorption of Perp-AgNPs

Silver nanoparticles owing to its size in the light range can excite surface plasmons (Temple et al., 2009). This is due to the coherent oscillation of electrons on the surface of the nanoparticle. This plasmon excitation can be indicative of the presence of metal nanoparticles (Yoon et at., 2010). The plasmonic absorption of the Perp-AgNPs was observed around 432 nm in the visible region of the electromagnetic

spectrum as shown in Figure 25. This plasmonic absorption value is consistent with those studies reported by (Ankanna et al., 2010).

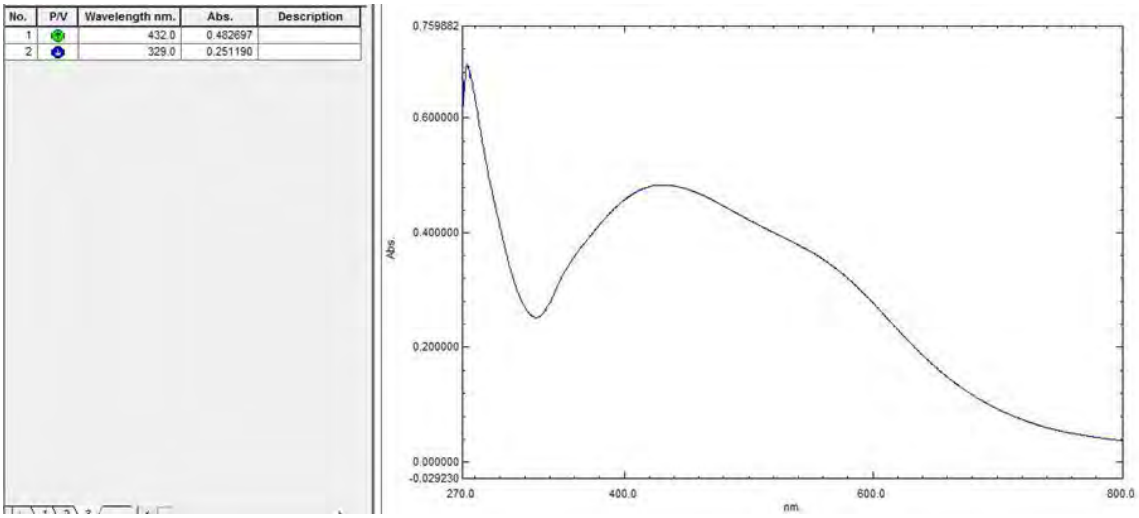

Figure 24: UV-Vis spectrum of Perp-AgNPs

4.6. Electron Microscope

The scanning electron microscope (SEM) is one of the most versatile instruments available for the examination and analysis of the microstructure morphology and chemical composition characterizations (Zhou & Wang, 2007). SEM provides information on surface topography, crystalline structure, chemical composition and electrical behaviour of specimen (Vernon-Parry, 2000).

The morphology of the silver nanoparticles (Perp-AgNPs) obtained through green synthesis using extract solution of *Pennisetum purpureum* was observed with Hitachi S-4800 FE-SEM (field emission scanning electron microscope). The Perp-AgNPs was observed to be quasi spherical in shape. The SEM image is shown in Figure 26. From the SEM images, the size of the particles was determined to be 25.70 ± 9.71 nm.

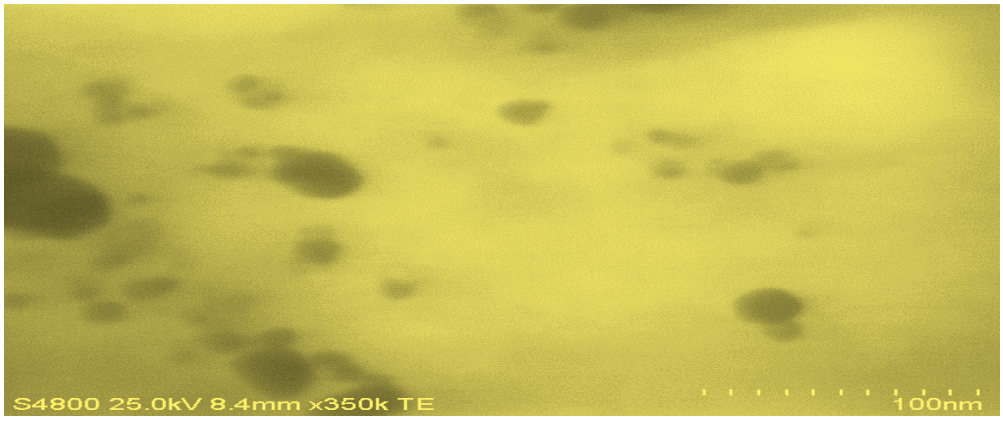

Figure 25: **SEM image of Perp-AgNPs**

4.7. Energy Dispersive X-Ray Analysis (EDX)

This is an x-ray technique used to identify the elemental composition of materials (Warren, et al., 2002). EDX systems are attachments to Electron Microscopy instruments (Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM)) instruments where the imaging capability of the microscope identifies the specimen of interest (Egerton, 2005). The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed (Mondal et at., 2021). The EDX spectrum of the Perp – AgNPs was recorded as presented in Figure 27. As shown in Fig. 27, the spectrum indicates silver in the synthesized Perp-AgNPs.

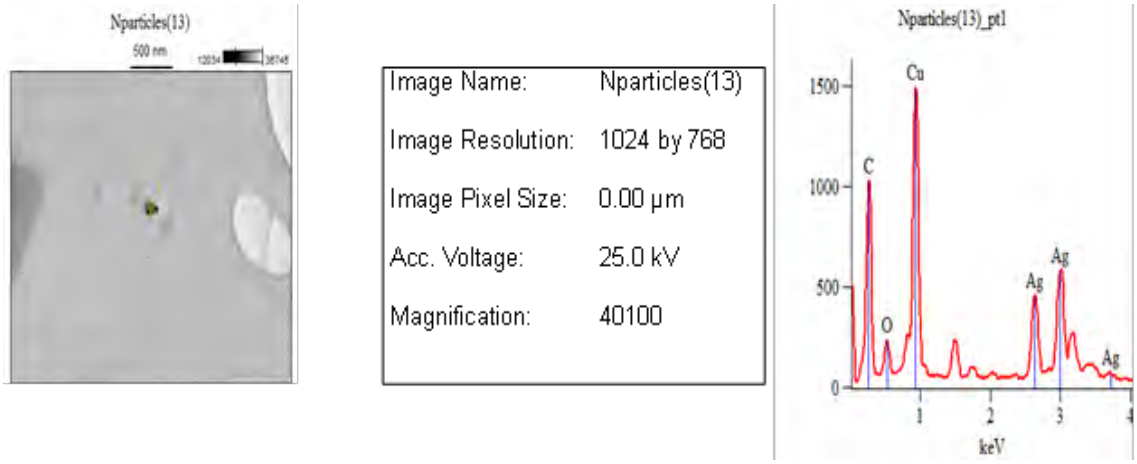

Figure 26: EDX spectrum of Perp-AgNPs. The intense peak for Cu is from the copper grid on which the Perp-AgNPs was deposited.

4.8 Powder X-Ray Diffraction (P-XRD)

P-XRD is a versatile non-destructive characterization tool that offers a detailed view of the chemical composition and crystallographic structure of the sample (Rajeswari et al., 2020). When X-rays are incident on crystalline solids, they are scattered in accordance with Bragg's law, which assumes the diffraction to take place only when the distance travelled by the rays reflected from successive planes of crystal differs by a complete number of wavelengths (Subramani et al., 2022).

Since silver nanoparticles crystallize in face-cantred cubic crystal structure (Awwad & Salem 2012), they produce characteristic diffraction planes. The P-XRD spectrum of the Perp-AgNPs is presented in Figure 28 The characteristic FCC diffraction planes of silver nanoparticles were observed at a 2θ vales of 38^{O}, 44^{O}, 64^{O} and 78^{O} representing (111), (200), (220) and (311) diffraction planes.

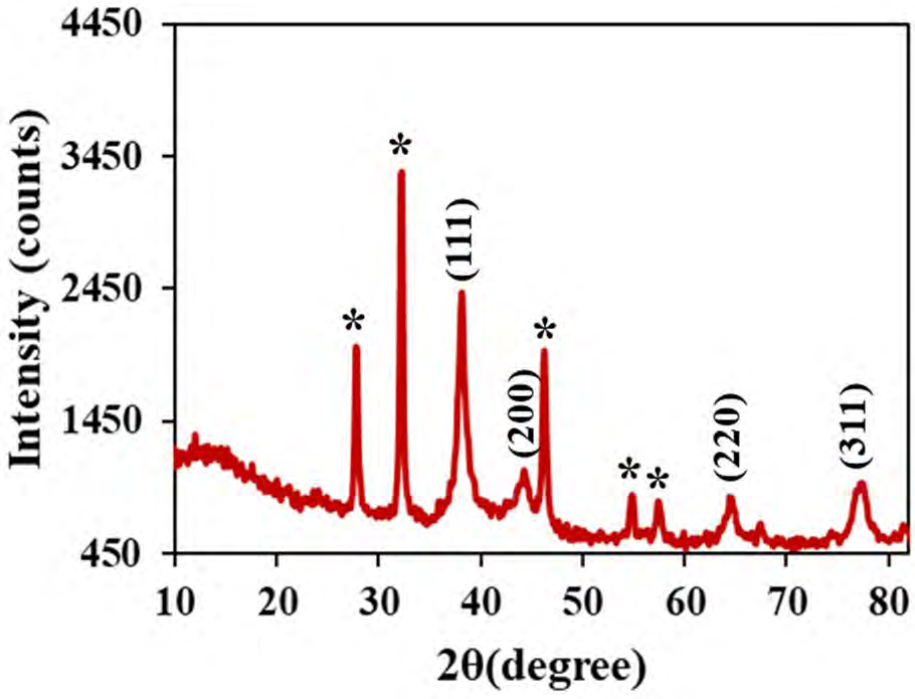

Figure 27: P-XRD pattern of Perp-AgNPs.

4.9. Antimicrobial Activities of Perp-AgNPs

The antibacterial properties of the Perp-AgNPs towards selected microbial cells was investigated. To get a deeper understanding of the potency of the prepared Perp-AgNPs, the antibacterial properties was compared to that of the extract solution used in preparing it. Emphasis was placed on MIC and MBC/MFC analysis of the prepared nanoparticles towards the bacterial or fungal cells. In addition, the synergistic effect of the Perp-AgNPs was also investigated.

4.9.1. Minimum inhibitory concentration (MIC) and Minimum bacteri-/fungi-cidal concentration (MBC/MFC) of Perp-AgNPs

To verify if the *Pennisetum purpureum* extract and silver nanoparticles were able to inhibit or kill the microbial cells (bacteri-/fungi-cidal effect) the plates were evaluated for MBC and MFC. Briefly, aliquots from each well from susceptibility testing assays were transferred to plates containing nutrient agar, which were incubated at 37°C for

24/48 h. Results were then evaluated by analyzing the presence or absence of growth in the nutrient agar or sabouraud dextrose agar (SDA) (Bagiu et al., 2012). The results of the antimicrobial activity of *Pennisetum purpureum* extract and Perp-AgNPs is presented in Table 1.

Table 1: Results of MBC and MIC of Pennisetum purpureum extract and Perp-AgNPs on human pathogenic microbes (mg/ml).

Pennisetum purpureum leave extract				Perp-AgNPs		
Organism	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
EC	>0.5	1	2^{bc}	1.25	1.25	1^{bc}
KP	0.5	0.5	1^{bc}	1.25	1.25	1^{bc}
MRSA	>0.5	1	2^{bc}	1.25	2.5	2^{bc}
PA	>0.5	1	2^{bc}	0.625	2.5	4^{bc}
ST	>0.5	1	2^{bc}	0.625	1.25	2^{bc}
SM	0.5	0.5	1^{bc}	0.313	0.625	2^{bc}
CA	>0.5	1	2^{bc}	0.313	0.313	1^{bc}
BS	>0.5	1	2^{bc}	0.313	2.5	8^{bs}

EC- E. coli, KP- Klebsillia pneumonia, MRSA- Methicilin resistant Staphyloccocus aureus, PA- Pseudomonas aeruginosa, ST- Samonella typhi, SM- Streptococcus mutans, CA- Candida albicans and BS- B. subtilis.

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of a drug that inhibits bacteria growth so that there is no turbidity in a culture media. But Minimum Bactericidal Concentration (MBC) is the lowest concentration of a drug that kills bacteria (Owuama, 2017). Usually, the concentration which is considered as MBC is higher than the concentration for MIC. Lower MIC and MBC values indicate higher efficacy (Hajlaoui et al., 2010).

From Table 1. above, the data reveals that the *Pennisetum purpureum* extract showed activity against *Klebsillia pneumonia* and *Streptococcus mutans* with MIC of

0.5mg/mL but MIC values above 0.5mg/mL against *E. coli,* Methicilin resistant *Staphyloccocus aureus, Pseudomonas aeruginosa, Samonella typhi, Candida albicans* and *B. Subtilis.*The Perp-AgNPs showed activity against *E. coli*, *Klebsillia pneumonia* and Methicilin resistant *Staphyloccocus aureus* with MIC of 1.25mg/mL, 0.625mg/mL against *Pseudomonas aeruginosa* and *Samonella typhi* and 0.313mg/mL against *Streptococcus mutans, Candida albicans* and *B. Subtilis*.

Comparatively, *Pennisetum purpureum* extract showed greater activity against *E. coli*, *Klebsillia pneumonia,* Methicilin resistant *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Samonella typhi* than Perp-AgNPs. For *Streptococcus mutans, Candida albicans* and *B. Subtilis*, Perp-AgNPs showed greater activity than *Pennisetum purpureum* extract.

4.9.2 Determination of antioxidant activities

The antioxidant activities of the Perp-AgNPs were tested against ABTS by measuring their scavenging potentials for the extracts. The ABTS scavenging are widely used methods for the determination of the antioxidant properties of compounds. The ABTS scavenging activity of Perp-AgNPs was determined by measuring the absorbance of the concentrations of samples ranging from 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078 mg/mL using ascorbic acid as the reference compound. The higher the percentage scavenging activities of the extracts, the greater the antioxidant potentials (Wu & Ng, 2008). The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples (Fu, et al., 2013). According to Phongpaichit, (2007) as cited by Jadid, et al., (2017), extracts which possess IC_{50} values ranging from 50 to 100 mg / mL is considered to exhibit intermediate antioxidant activity.

The percentage scavenging activities and the IC_{50} of the extracts, Perp-AgNPs and ascorbic acid at the various concentrations are displayed in Tables 2 to 4.

Table 2: Table of results for antioxidant activity of Pennisetum purpureum extract

Conc.	ABTS activity [IC_{50} = 4.484399 ± 0.00 mg/ml]		
	Exp1	Exp2	Mean ± SD
1	92.52	91.59	92.06 ± 0.66
0.5	85.98	83.18	84.58 ± 1.98
0.25	75.23	64.49	69.86 ± 7.60
0.125	75.23	27.10	51.17 ± 34.03
0.0625	72.43	16.82	44.63 ± 39.32
0.0313	69.16	6.54	37.85 ± 44.28
0.0156	32.71	1.87	17.29 ± 21.81
0.0078	0.93	0.93	0.93 ± 0.01

Table 3: Table of results for antioxidant activity of Perp-AgNPs

Conc.	ABTS activity [IC_{50} = 45.44 ± 0.00 mg/ml]		
	Exp1	Exp2	Mean ± SD
1	98.79	97.85	98.32 ± 0.66
0.5	97.90	96.92	97.41 ± 0.69
0.25	94.95	93.97	94.46 ± 0.69
0.125	94.67	93.74	94.21 ± 0.66
0.0625	93.74	92.66	93.20 ± 0.76
0.0313	92.90	91.64	92.27 ± 0.89
0.0156	92.34	90.65	91.50 ± 1.19
0.0078	91.31	90.14	90.72 ± 0.83

Table 4: Table of results for antioxidant activity of Ascorbic acid

Conc.	ABTS activity [IC_{50} = 29.69857 ± 0.00 mg/ml]		
	Exp1	Exp2	Mean ± SD
1	99.11	98.13	98.62 ± 0.69
0.5	98.55	97.52	98.04 ± 0.73
0.25	98.04	97.06	97.55 ± 0.69
0.125	97.57	96.68	97.13 ± 0 63
0.0625	97.38	96.36	96.87 ± 0.73
0.0313	96.50	95.51	96.00 ± 0.69
0.0156	92.06	91.50	91.78±0.40
0.0078	82.71	82.20	82.45 ± 0.36

Pennisetum purpureum extract exhibited antioxidant potency percentage scavenging activity ranging from 51.17% to 92.06% at concentrations ranging from 0.125mg/mL to 1.00mg/mL. Perp-AgNPs showed greater antioxidant potency with percentage free radical scavenging activity ranging of 90.65% to 98.32% at concentrations of 0.0156 mg/mL to 1.00mg/mL Ascorbic acid recorded percentage free radical scavenging activity ranging from 82.45% at the same concentrations. Ascorbic Acid is a natural water-soluble vitamin (Vitamin C). Ascorbic acid is a potent reducing and antioxidant agent that functions in fighting bacterial infections, in detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries (Null, 2011). Perp-AgNPs exhibited comparatively higher antioxidant scavenging activity than Ascorbic acid. Perp-AgNPs exhibited percentage scavenging activity of 90.72% at the least concentration of 0.0078mg/mL while Ascorbic acid recorded 82.45% at that same concentration.

4.9.3 Determination of synergistic effect of Perp-AgNPs

Synergistic effects are the combined effects of at least two medicines that have a bigger impact than any of them could have on its own. The interaction was considered synergistic if the FICI was ≤ 0.5, partial synergistic if FICI was > 0.5 and < 1, additive if FICI was =1, no difference if the FICI was >1 and ≤ 4, and antagonistic if the FICI was >4.0 (Loho, et al., 2018). The results of the synergistic activity of Perp-AgNPs with antibacterial/ antifungal agents against bacterial/fungal strains are presented in table 5 below.

Table 5: Synergistic activity of Perp-AgNPs with antibacterial/ antifungal agents against bacterial/fungal strains.

Strain	MIC(CIP/FLU) mg/mL	FIC (CIP+ Perp-AgNPs)	INT.	MIC(TET/KET) mg/mL	FIC (TET+ Perp-AgNPs)	INT.
EC	125	0.044	**S**	7.81	0.57	**PS**
KP	125	0.044	**S**	7.81	0.29	**S**
MRSA	7.81	0.5	**S**	7.81	0.072	**S**
PA	125	0.13	**S**	7.81	0.32	**S**
ST	3.9	0.76	**PS**	15.63	0.069	**S**
SM	250	0.005	**S**	7.81	0.14	**S**
CA	125	0.5	**S**	7.81	0.074	**S**
BS	64	0.749	**PS**	512	0.31	**S**

TET: Tetracycline; CIP: Ciprofloxacin; FLC: Fluconazole; KET: Ketoconazole; INT: Interpretation; FICI: Fractional Inhibitory Concentration Index, S: Synergism; PS: Partial Synergism.

The results above reveals that the Perp-AgNPs combined with Ciprofloxacin/ Fluconazole exhibited synergistic effect against all the test organisms except for *Samonella typhi* and *B. subtilis* of which it exhibited partial synergy. The combination of Perp-AgNPs with Tetracycline/ Ketoconazole exhibited partial synergy against only *E. coli* but synergistic against the other test organism. Comparatively, Perp-AgNPs exhibited greater synergism with Tetracycline/ Ketoconazole than with Ciprofloxacin/ Fluconazole.

CHAPTER FIVE

CONCLUSION

5.0 Overview

This is about the confirmation and otherwise of some expectation and inferences derived from analysis.

5.1 Conclusion

This study was intended to design a simple, economic and environmental-friendly approach to synthesize silver nanoparticles using green technology approach. *Pennisetum Purpureum* leaf was investigated for the first time as active reductant for silver ion in the preparation of silver nanoparticle. A simple approach which involves simple soaking of the dried leaves and using the extract solution as reducing agent was employed in the preparation of silver nanoparticles. In a typical approach, the reducing agents were obtained by simply soaking the leaves of *Pennisetum Purpureum* in deionized water for 72 hours and filtering the solution. The plasmonic absorption, energy dispersive X-ray spectrum, SEM image and FTIR spectrum of prepared silver nanoparticles were recorded and studied.

The prepared *Pennisetum purpureum* mediated silver nanoparticles (Perp-AgNPs) were investigated for antibacterial properties. Perp-AgNPs solution was exposed to eight different microbial cells and their behaviour recorded. The minimum inhibitory concentration (MIC), minimum bacteri/fungi-cidal concentration (MBC/MFC) of the Perp-AgNPs were studied. Analysis of data revealed that the Perp-AgNPs possess significant antimicrobial properties. In addition, the Perp-AgNPs exhibited high antioxidant and synergistic properties. This study thus provides an access to the synthesis of eco-friendly silver nanoparticles with antibacterial properties.

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