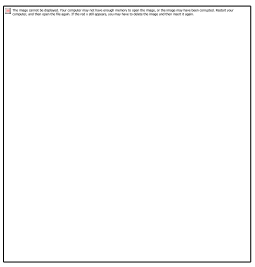
INVESTIGATION OF PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES OF MANGANESE OXIDE NANOPARTICLES. USING AFRICAN BUSH PEAR(Dacryodes erulis) SEED EXTRACT.



BY

EZEIBE CHINEMEREM BLESSING

PSC2010350

DEPARTMENT OF CHEMISTRY

FACULTY OF PHYSICAL SCIENCE

UNIVERSITY OF BENIN

BENIN CITY,

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CERTIFICATION

This is to certify that this project report was written by Chinemerem Blessing Ezeibe with Mat no PSC2010350 of Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City.

_____ _____

PROF. Emmanuel E.E.I IRABOR DATE

(Head of Department)

_____ _____

PROF. MRS E. U. IKHUORIA DATE

(Project Supervisor)

_____ _____

CHINEMEREM BLESSING EZEIBE DATE

(Student)

DEDICATION

I dedicate this work to the Almighty God who through His infinite mercies granted me wisdom, knowledge and understanding to complete this project successfully. I also dedicate this work to my family for their immense love, care, support and encouragement all through my academic programme.

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I acknowledge GOD almighty who gave me sufficient grace throughout my journey into the B.Sc. program and for keeping me in good health and vitality, from beginning till completion.

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ABSTRACT.

The synthesis of Manganese Oxide (MnO) nanoparticles using plant extracts has acquired attention as a sustainable and eco-friendly alternative to traditional chemical methods. This study explores the synthesis of Manganese Oxide nanoparticles using *Dacryodes erulis* seeds extract, investigating their antioxidant and phytochemical properties. The Phytochemical composition of *Dacryodes erulis* seeds, along with the reduction and stabilization properties of plant-based compounds, played a crucial role in the synthesis of nanoparticles. The synthesized Manganese Oxide Nanoparticles were characterized using various techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Dynamic Light Scattering (DLS). X-ray diffraction analysis revealed that the MnONPs were crystalline in nature. Dynamic Light Scattering (DLS) indicated its polydispersity with a PDI value of 0.250 and an average particle size of 48.78nm. Fourier Transform Infrared Spectroscopy (FTIR) indicated the presence of a hydroxyl (-OH), Carbonyl (C=O), and carboxyl (-COOH) functional group. Antioxidant activities of the Manganese Oxide Nanoparticles were assessed using DPPH, while phytochemical properties were evaluated through quantitative analysis. The results suggested that the synthesized Manganese Oxide Nanoparticles exhibited significant antioxidant properties making them promising candidates for applications in medicine, environmental cleanup, and energy production. Overall, this study demonstrated the potential of using African bush pear seed extract for the green synthesis of MnONPs.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Nanoparticles are small particles measuring less than 100 nanometers in size. A nanometer is one billionth of a meter. Nanoparticles have unique properties that make them useful in a variety of fields, including catalysis, medicine, and electronics. One of the most fascinating aspects of nanoparticles is their unique optical properties. Nanoparticle synthesis is becoming more popular due to its superior performance in terms of surface area increase. Metal oxide nanoparticles can be used for a variety of purposes. They are thought to be active catalysts in a variety of applications (Gerko *et al.*, 2006).

Manganese nanoparticles have been synthesized successfully using "Gamma radiolysis, due to its deep penetration, ability to uniformly irradiate large volumes, and capability to produce high-purity, well-controlled nanoparticles (Pillai *et al*., 2007, Prakash *et al*., 2013). The uniform energy distribution provided by gamma rays ensures consistent nanoparticle formation throughout the solution, making it particularly suitable for bulk synthesis (Srinivasan, 2014)." Plant-based synthesis of nanoparticles offers a sustainable and cost-effective alternative to conventional methods. Utilizing natural plant extracts as reducing and stabilizing agents enables the synthesis of stable nanoparticles with tailored properties. Among the various nanometal oxides, Manganese oxides are largely studied because they are abundant in nature and are widely used as green catalysts in the field of catalysis.

In recent years manganese dioxide nanoparticles achieved a high rate of interest in the research field of material science due to their extensive applications in various fields like catalysis,

lithium-ion batteries, biological fields, medicinal drug delivery, sensor and imaging techniques, etc. Due to its non-toxic behavior, it is safe to use in laboratory reactions. Manganese products are also non-hazardous (Vermon, 1983).

The plant of African bush pear (*Dacryodes edulis*) especially the seed has produced a lot of waste in the ecosystem at large in the sense that the avocado seeds can be harmful to the environment when regarded as waste and not disposed of properly (United States Environment Protection Agency, 2020). This research project investigates the usefulness of the seed extract of African bush pear by carrying out photochemical analysis. It also investigates its antioxidant properties using the potential of Manganese oxide nanoparticles (MnO NPs).

1.1.1 Background of Study

African bush pear (*Dacryodes edulis*) is known as "ube" among the Ibo-speaking people of South-eastern Nigeria. It belongs to the family Burseraceae, it is an evergreen tropical fruit tree that grows in the humid and sub-humid climate of the West African countries. They are consumed during the months of April to September. The African black pear (Dacryodes edulis) is also known as African plum or bush butter. It is an indigenous fruit tree of tropical Africa. When in season, the fruit pulp or flesh constitutes a unique and a much cherished local delicacy. It is consumed by savouring the fresh fruit or flesh after undergoing some processes; either by dipping it in hot water and adding a pinch of salt. It can also be consumed or eaten with corn. The seeds of African bush pear are usually discarded after consumption of the edible fruit pulp. The seeds of Dacryodes edulis have been reported to possess considerable nutritional value, devoid of toxins, and have been suggested as a valuable supplement in animal feed (Adejumo *et al*., 2015).

In recent course of research there is an interest in tapping valuable potentials from plant seeds and therefore, many plant materials have become more useful to man as a result. However, there are many plant materials which have not been adequately researched. The seeds of African bush pear (*D. edulis*) represent a common example. It is against this background that this study was designed or initiated, with the aim of determining the effect of manganese oxide nanoparticles on the Phytochemical and antioxidant properties of African bush pear seed extract. The African bush pear (*Dacryodes edulis*) seed contains a wide range of bioactive compounds, including flavonoids, tannins, alkaloids, phenolic compounds, and saponins. They are all known for their antioxidant, antimicrobial, and medicinal properties (Okwu & Nnamdi, 2008). Manganese oxide (MnO) nanoparticles have garnered significant attention from recent years due to their unique properties and potential applications. These unique properties include a high surface area, and biocompatibility. MnO nanoparticles are also commonly used in biomedical and environmental applications as they are safe and effective (Singh *et al*., 2018). The antioxidant properties of the seed extract is connected to its high phenolic and flavonoid content, which are crucial in scavenging free radicals and reducing oxidative stress. These properties are essential for protecting the body against oxidative damage, which is associated with chronic diseases such as cancer and cardiovascular disorders (Kalpana & Rajeswari, 2018).

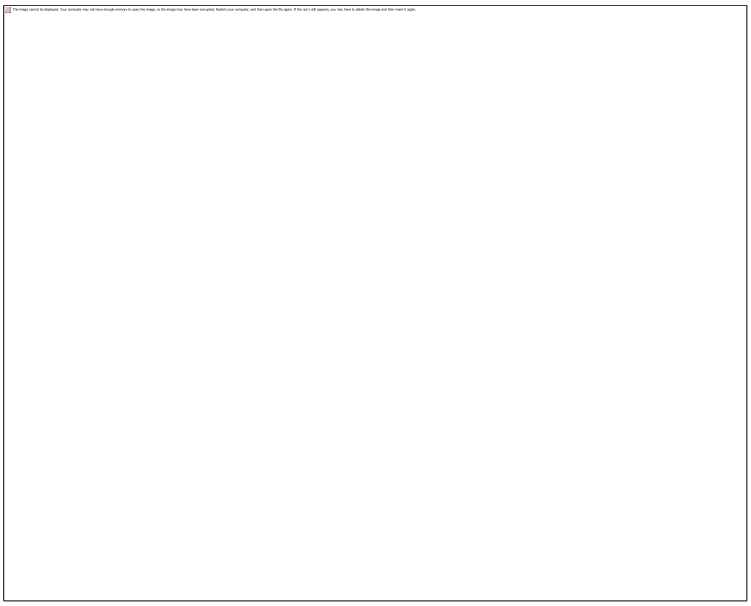

Plate 1.1: African Bush Pear (Wikipedia, 2024).

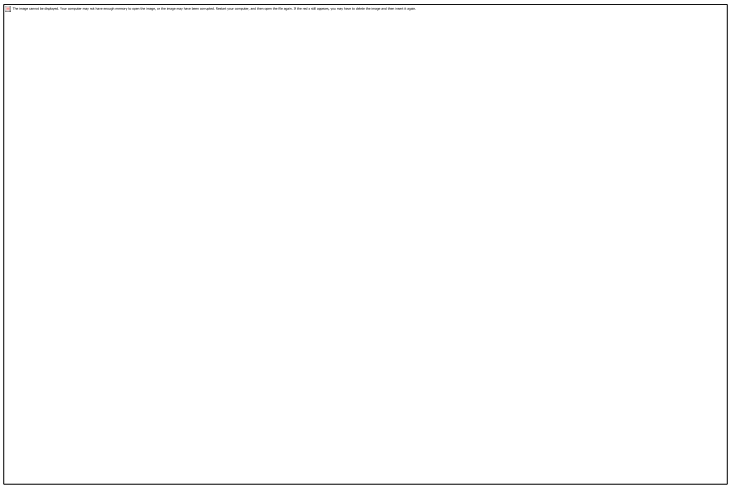

Plate 1.2: African Bush Pear Bisection Image (Wikipedia, 2024).

1.1.2 Statement of the Problem

Despite the rich phytochemical composition and traditional medicinal use of the African bush pear (*Dacryodes edulis*), there is limited scientific exploration of its seed, particularly regarding its bioactive compounds and potential health benefits (Okwu & Nnamdi, 2008). While recent research has shown the fruit of this plant to contain numerous beneficial phytochemicals, the seed remains underutilized and understudied. The antioxidant properties of the seed, which could play a vital role in preventing oxidative stress-related diseases, have not been extensively investigated (Kalpana & Rajeswari, 2018). Additionally, conventional methods of synthesizing nanoparticles often involve the use of harmful chemicals, raising environmental and health concerns. There is a growing need for eco-friendly and sustainable methods for nanoparticle synthesis (Iravani, 2011). The application of green synthesis methods, particularly using plant extracts such as the African bush pear seed, presents a promising alternative. However, the interaction between the seed extract and manganese oxide nanoparticles (MnO NPs), and how this combination enhances antioxidant and antimicrobial properties, has not been fully explored (Singh *et al.*, 2018).

Thus, the scope of this study seeks to address the following problems: The lack of comprehensive phytochemical profiling of African bush pear seed extract and its potential applications in medicine and industry (Okwu & Nnamdi, 2008). The need for environmentally sustainable methods for synthesizing nanoparticles, with a focus on using the seed extract of African bush pear in the green synthesis of Manganese oxide nanoparticles (Iravani, 2011). The limited understanding of how Manganese oxide nanoparticles is synthesized using African bush pear seed extract can enhance antioxidant and antimicrobial activities, which could have

significant applications in pharmaceuticals, food preservation, and environmental remediation (Singh *et al*., 2018).

1.1.3 Relevance of Research

Nanotechnology is emerging as a promising technology, showcasing impressive advances across diverse domains, including in the area of Phytochemical analysis and antioxidant properties in plants. Research has revealed the effectiveness of nanomaterials in African bush pear which is rich in nutrients, including proteins, fibers, vitamins, and minerals. Its seeds contain bioactive compounds with antioxidant, anti-inflammatory and antimicrobial properties (Mbah, *et al*., 2017). This demonstrates the potential of nanotechnology to revolutionize the Phytochemical and antioxidant properties wastewater treatment by offering innovative solutions to address pollution challenges (Kumar *et al.*, 2014).

The study of MnO nanoparticles (MnO NPs) in this case is for the determination of the antioxidant properties present in the seed extract of African bush pear (*D.edulis*) which has important implications for sustainable industrial practices. This study addresses critical issues in the environment, thereby paving way for a more utilization of the seed of African bush pear (*D.edulis*).

Overall, manganese itself plays a crucial biological role in mammals as an enzyme co-factor; therefore, its levels are maintained in fragile homeostasis, where both deficiency and excess of this element may be dangerous for mammals by causing neurological disturbances. Manganese oxide with their unique physicochemical properties, have garnered significant attention for potential medical applications. However, their varying oxidation states necessitate a

comprehensive toxicological evaluation to ensure safe and effective use. Manganese nanoparticles show their potential in another field of treating cancer. Overall, research on MnO-NPs for antioxidant properties in African bush pear seed extract has significant implications for food security, nutrition, medicine, sustainable agriculture, and environmental sustainability (United Nations, 2020; World wildlife fund, 2022).

1.1.4 Aim and Objectives

The aim of this project work is to synthesize manganese oxide nanoparticles using the African bush pear seed extract (*D.edulis*). The phytochemical and antioxidant properties of African bush pear seed extract using manganese oxide nanoparticles will also be investigated. These will be achieved by the following objectives:

1. Collection and preparation of the African bush pear seed extract.
2. Conducting of photochemical analysis of the seed extract.
3. Synthesis of manganese oxide nanoparticles.
4. Characterization of the synthesized manganese oxide nanoparticles.
5. Evaluation of the antioxidant properties of the synthesized nanoparticles in the presence of African bush (*D.edulis*) pear seed extract.
6. Application of the resulting composite material in fields such as medicine, food preservation and environmental protection.

1.1.5 Scope of Study

This study looks into the efficacy of MnO nanoparticles for the photochemical analysis of the African bush pear seed extract. The study's scope includes these keys areas:

1. Development of a sustainable and cost-effective method for MnO nanoparticles synthesis using African bush pear seed extract.
2. Characterization of the synthesized MnO nanoparticles using Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD).
3. The photochemical analysis of *D.edulis* seed extract.
4. The synthesis of manganese oxide nanoparticles, and the examination of their interaction.
5. Evaluation of the efficacy of the synthesized MnO NPs for the testing of photochemical analysis and antioxidant properties of the resulting composite material of the seed extract of *D.edulis.*

1.2 LITERATURE REVIEW

Manganese Oxide Nanoparticles (MnO_2 NPs) : Manganese oxide nanoparticles are nanostructured forms of MnO_2 that exhibit excellent chemical stability, electrical conductivity, and catalytic activity. These nanoparticles exist in different crystalline phases, including α, β, γ, and δ, each with distinct properties that influence their applications. Synthesis of MnO_2 Nanoparticles: There are several conventional methods for synthesizing MnO_2 nanoparticles they are; Chemical synthesis (e.g., precipitation, hydrothermal, and sol-gel methods) Physical synthesis (e.g., laser ablation, sputtering), Biological synthesis (using microorganisms or plant extracts). The chemical and physical methods often involve

toxic reagents and high energy consumption, making them less environmentally friendly. In contrast, green synthesis using plant extracts has gained popularity as a safer and more sustainable alternative.

Applications of MnO_2 NPs : MnO_2 nanoparticles have a wide range of applications, including: Biomedical applications (antioxidants, drug delivery, biosensors), Environmental applications (water purification, catalytic degradation of pollutants), Energy storage (supercapacitors, lithium-ion batteries).(Wikipedia, 2024).

Green Synthesis of MnO_2 Nanoparticles Using Plant Extracts:

Concept of Green Synthesis: Green synthesis refers to the use of biological systems such as plant extracts, fungi, or bacteria to produce nanoparticles in an environmentally friendly manner. This method eliminates the need for toxic chemicals and provides a sustainable alternative for nanoparticle synthesis. Role of Plant Extracts in MnO_2 NP Synthesis: Plant extracts contain phytochemicals such as flavonoids, tannins, alkaloids, and polyphenols, which act as both reducing and stabilizing agents during nanoparticle synthesis. These compounds help in the conversion of Mn^{2+} ions into MnO_2 nanoparticles without the use of synthetic reducing agents. Previous Studies on Green Synthesis of MnO_2 NPs; Several studies have successfully synthesized MnO_2 nanoparticles using plant extracts, including:Green tea extract(Camelliasinensis)Neem extract (Azadirachta indica), Aloe vera extract. These studies demonstrated that phytochemical-rich plant extracts could enhance the stability and biological activity of MnO_2 nanoparticles. African Bush Pear (Dacryodes edulis) and Its Phytochemical Composition. **Botanical Description:** The African bush pear, Dacryodes edulis, is a tropical fruit tree native to West and Central Africa. The fruit and seeds are commonly used for nutritional and medicinal purposes. Phytochemical Constituents; The seed extract of Dacryodes edulis is rich in bioactive compounds such as: **Flavonoids** – Known for their antioxidant and anti-inflammatory properties**.Tannins** – Have antimicrobial and antioxidant effects. **Saponins–** Possess anti-cancer and cholesterol-lowering

properties. **Alkaloids** – Exhibit antibacterial and antifungal activities. **Phenolic compounds** – Play a major role in free radical scavenging. The African bush pear has been used in traditional medicine for treating infections, inflammation, and digestive disorders. Due to its rich phytochemical profile, it is also explored for cosmetic and pharmaceutical applications.(Elechi and Owhoeke, 2020). **Antioxidant Properties of MnO_2 Nanoparticles:** Antioxidants are molecules that neutralize free radicals, preventing oxidative damage to cells. Oxidative stress is linked to several diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases. MnO_2 nanoparticles exhibit antioxidant activity through mechanisms such as: Catalytic decomposition of reactive oxygen species (ROS), Electron transfer reactions that neutralize free radicals, and Enhancement of enzymatic antioxidant activity. **Factors Influencing Antioxidant Efficiency:** The antioxidant potential of MnO_2 NPs depends on: Particle size and surface area, Morphology and crystalline structure, and Presence of phytochemicals in green-synthesized MnO_2 NPs. **Phytochemical and Antioxidant Properties of MnO_2 NPs Synthesized Using African Bush Pear Seed Extract:** Interaction of Phytochemicals with MnO_2 NPs: When MnO_2 nanoparticles are synthesized using Dacryodes edulis seed extract, the phytochemicals interact with manganese ions, facilitating nanoparticle formation and improving their stability. These phytochemicals may also enhance the antioxidant properties of the nanoparticles. The antioxidant properties of the synthesized MnO_2 nanoparticles can be assessed using: DPPH (2,2-diphenyl-1-picrylhydrazyl) assay – Measures free radical scavenging activity, FRAP (Ferric Reducing Antioxidant Power) assay – Evaluates the reducing potential, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay – Determines total antioxidant capacity**. Comparative Analysis:** The antioxidant activity of MnO_2 NPs synthesized with Dacryodes edulis extract can be compared to: Chemically synthesized MnO_2 nanoparticles, Standard antioxidants (e.g., ascorbic acid, quercetin). **Applications and Future Perspectives:**

Potential Applications: Biomedical – Antioxidant and anti-inflammatory agents in drug development. Food Industry – Natural preservative to prevent oxidation in food products.Cosmetics – Used in skincare products for anti-aging effects. They are various challenges in Green Synthesis including: Variability in plant extract composition, Difficulty in large-scale production, and Stability and reproducibility of synthesized nanoparticles. Further research is needed to optimize the synthesis conditions, explore potential toxicity, and investigate new applications of MnO_2 nanoparticles synthesized using African bush pear seed extract. Conclusion: This review highlights the potential of Dacryodes edulis seed extract in the green synthesis of MnO_2 nanoparticles and its ability to enhance their antioxidant properties. While promising, further studies are needed to explore its full potential in nanomedicine and industrial applications.

1.2.2 Scientific Classification of African bush pear

Kingdom	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Burseraceae
Genus:	Dacryodes

1.2.3 Phytochemical Analysis

Phytochemicals are bioactive compounds produced by plants to ensure their survival and development in challenging environments (Vishnu *et al.*, 2019). These chemicals protect plants from harsh UV radiation, herbivores, and competing plants, among other functions.

Phytochemical analysis is a scientific process that involves analyzing, extracting, and experimenting to identify various phytoconstituents present in different plant parts (Tinky *et al*., 2020). This process is crucial for drug discovery, as it enables researchers to identify active components that can be further investigated. Phytochemicals are categorized into primary and secondary metabolites. Primary metabolites including sugars, amino acids, proteins, nucleic acids, and chlorophyll, contribute to plant growth and development. Secondary metabolites such as flavonoids, tannins, saponins, alkaloids, steroids, and polyterpenes, play a vital role in plant survival under stress conditions. These compounds impart characteristics like aroma, colour, and taste to plants.

Phytochemicals can be extracted from plant materials using a variety of extraction techniques. Traditional methods like maceration, percolation, infusion, digestion, decoction, and hot continuous extraction (soxhlet extraction) have been widely used, recently environmental friendly method such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), and Accelerated Solvent Extraction (ASE) have been introduced (Azwinda, 2015; Dhanani *et al.*, 2017). Solvents such as water, acetone, hexane, benzene, ether and chloroform are used in extraction of phytochemicals (Tiwari *et al.* 2011). Extraction of phytochemicals depends on both the pre-extraction factors such as (drying method, moisture content, part of the plant used and others) and also the extraction factor such as (solvent used, temperature, pH and others) (Azwinda, 2015; Tiwari *et al.* 2011).

Previous research done on the seed extract of African bush pear using ethanol and aqueous solutions shows the presence of phytochemicals such as Tannins, Flavonoids, Saponins, Alkaloids and phenols (Udinyiwe and Aghedo, 2022).

1.2.4 Alkaloids

Alkaloids are a group of naturally existing chemical compounds, usually characterized by the presence of basic nitrogen atoms, and occasionally including neutral or weakly acidic compounds (Manske and Holmes, 2014). Some synthetic compounds are also characterized as alkaloids (Kumar *et al*., 2020). In addition to carbon, nitrogen, or hydrogen, alkaloids can also contain sulfur and infrequently, bromine, phosphorus, or chlorine (Zhang *et al*., 2022). Alkaloids are seen to be produced by a large variety of organisms which includes fungi, bacteria, plants and animals. The biological precursors of the majority of alkaloids are amino acids like tyrosine, histidine, aspartic acid and others. Many of these compounds are seen to have a low solubility in water but are readily dissolved in organic solvents if consumed (Andreas, 2009). Alkaloids are secondary metabolite hence they play a crucial role in plant protection and its survival against microorganism, exhibiting antibacterial and antifungal activities (Ahmed *et al*., 2022).

An example of well-known alkaloid includes morphine, strychnine, quinine, ephedrine, and nicotine. Alkaloids which are contained in a ring system are known as “indole”. Alkaloids exhibit a wide array of medicinal properties. Morphine, a potent narcotic, is utilized for pain relief, but its addictive nature restricts its practicality. On the other hand, codine, which is a methyl ether derivative from opium poppy, serves as an effective analgesic with relatively lower addictive potential.

1.2.5 Tannins

Tannins are large polyphenolic compounds that are soluble in water and contain an ample number of hydroxyl and other appropriate groups, such as carboxyls, enabling them to form strong complexes with protein and other macromolecules (Praveen and Kumud, 2012). Tannin are present in high concentration in nearly every part of a plant such as the bark, leaves, fruits,

roots, and seeds, an increase in the production of tannin in plant usually arise as a result of sickness, therefore it is assumed that tannin is involved in the biological role of the plant to fight against infection, insects or animal herbivory (Michel *et al.,* 2011). Tannis are typically found in form of light white amorphous powders or shiny, nearly colorless loose masses, characterized by a distinctive odor and an astringent taste (Frutos *et al.,* 2004., Takuo and Hideyuki, 2011).

These compounds have wide-ranging applications, from ancient tanning practices (dating back to ca. 1500BC in the Mediterranean) to medical and food industry uses. In medicine, particularly in traditional Asian healing practice such as Japanese and chines natural remedies, plant extract containing tannins are utilized as astringents for addressing diarrhea (Zhang *et al.,* 2019), as diuretic (Wang *et al.,*2020; Li *et al.,*2020), against stomach and duodenal tumors (Kumar *et al.,*2018), as well as in the production of anti-inflammatory, antiseptic, and hemostatic pharmaceuticals (Liu *et al.,* 2020; Singh *et al*., 2019).

1.2.6 Phenolic compounds

Phenolic phytochemicals represent the largest and most prevalent category of phytochemicals found within the plant kingdom. Among dietary phenolics, the three most significant groups are flavonoids, phenolic acids, and polyphenols. Flavonoids stands as the most extensive group of plant phenols and have been the subject of extensive study. Phenolic acids constitute a diverse group, encompassing widely distributed hydroxybenzoic and hydroxycinnamic acids (Zhang *et al*., 2020).

Natural phenolic compounds are crucially important as they play a vital role in cancer prevention and treatment (Huang *et al.,* 2010). The diverse bioactivities exhibited by phenolic compounds account for their chemopreventive properties, including antioxidant, anti-carcinogenic, anti-mutagenic, and anti-inflammatory effects. Additionally, these compounds contribute to inducing

apoptosis by halting the cell cycle, regulating carcinogen metabolism and oncogenesis expression, inhibiting DNA binding and cell adhesion, as well as impeding migration, proliferation, or differentiation, and blocking signaling pathway (Huang *et al.*, 2010).

1.2.7 Flavonoids

Flavonoids, as secondary metabolites, primarily consist of a benzopyrone ring that carries phenolic or polyphenolic groups at various positions (Cavalcente *et al.*, 2018). Over 10,000 flavonoid compound has been isolated and identified as at recent (Aleksandra, 2014). Most flavonoids are widely accepted as therapeutic agents (Shkondrov *et al.*, 2017). Flavonoids have various range of application as it is used in the production of natural dye (Villela *et al.*, 2019; Paramita *et al.*, 2018), in the production of skin care products (Lanzerendofer and Stab, 1996; Danihelova *et al.*, 2012) and also in the production of anti-wrinkle skin agent (Chuarienthong *et al.*, 2010). Flavonoid has also been seen to be used extensively as anticancer, (Zhao *et al.*, 2019) antimicrobial, antiviral, antiangiogentic (Zhao *et al.*, 2019; Camero *et al.*, 2018), antimalarial, antioxidant, antitumor, and anti-poliferative agents (Patel *et al.*, 2018).

Flavonoids possess significant antioxidant capabilities, providing protection to the human body against free radicals and reactive oxygen species (Praveen and Kumud, 2012). The arrangement of hydroxyl groups and other attributes within the chemical structure of flavonoids plays a crucial role in determining their antioxidant and free radical-scavenging actions.

1.2.8 Saponins

Saponins are glycosides of triterpenes and steroids, they are important bioactive component which plays an important role in plant survival and also serve as a vital resource in pharmaceuticals as its underexplored biodiversity could lead to new drug discovery (Thomas and Michel, 2012). A characteristic feature of saponins is their ability to foam when extract using an aqueous solution (Hostettmann and Marston 1995). As at recent plant derived saponins are used in the treatment of a range of diseases in convectional and traditional medicine (Harada 2005; Cinatl *et al.,* 2003; Jayatilake *et al.,* 2003; Germonperz *et al.,* 2004).

Saponins are known to have a wide range of importance, example include: anti-bacterial, antiviral, anti-inflammatory, anti-fungal, anti-cancer, and anti-parasitic (Sprag *et al.,* 2004; Podolak *et al.,* 2010). Additionally, some saponins are also known to be used as flavoring due to their intense sweetness or bitterness (Price *et al.,* 1987; Heng *et al.,* 2006; Kitagawa, 2002).

1.2.9 Terpenoid

Terpenoids, also known as isoprenoids or terpenes, are a substantial class of natural compounds present in most living organisms (Oldfield and Lin, 2012). They are widely distributed in nature, particularly in plants, where they serve as components of essential oils. Their fundamental unit is hydrocarbon isoprene $CH_2=C(CH_3)-CH=CH_2$. Many plants produces volatile terpenes either to attract insects for pollination or to deter certain animals that consume them (Dengenhardt *et al.,* 2003).

Additionally, terpenoids plays a significant role in the cosmetic and flavor industries due to their volatile nature and the diverse array of scent and flavors they offer (Schmidt, 2010). Beyond

their aromatic and flavor uses, terpenes also exhibit medicinal properties such as anti-carcinogenic, anti-malaria, anti-ulcer, anti-microbial and others (Dudareva *et al.*, 2004).

1.2.10 Nanoscience and Nanotechnology

Nanotechnology has created a large impact in the world at large. It is the manipulation of matter with at least one dimension sized from 1 to 100 nanometers. It has helped in the areas of production and designs; controlling sizes and shapes at the nanoscale (Bhushan, 2017). It is also utilized in the areas of medicine, energy, electronics and environmental protection (Roco *et al*., 2011). Besides, the environmental and health impacts of nano materials themselves are still being studied (Maynard *et al*., 2006). Nanotechnology is also increasing the amount of wild-generated power (that is, in terms of solar energy, wind energy and hydro energy) which is another advantage. It also has a large impact in the aspect of computing. With it computers are able to boot rapidly by using magnetic random access memory (MRAM) due to its nanometer-scale magnetic tunnel junctions, which stores data fast and effectively during a system shutdown (Wang, 2018).

The advent of nanoscience and nanotechnology has resulted to important changes in the life of human. Therefore, important and necessary steps must be established for assessing the harmful effects of nanoparticulate chemicals in relation to the environment as well as the human health.

1.2.11 Classification of Nanomaterials

There are different types of nano materials, and different ways to which it can be classified.

Natural nano materials: According to the name 'natural', it occurs naturally in the world. These are particles which make up to volcanic ash, smoke, and even some molecules in our bodies, such as the haemoglobin in our blood. The brilliant colours of a peacock’s feathers are also as a

result of spacing between the nanometer-scale structures on their surfaces (Kinoshita *et al*., 2008).

Artificial nano materials: They occur from objects or processes created by individuals. For example the exhaust from the fossil fuel burning engines and some forms of pollution like air pollution, water pollution, and soil pollution (Kumar *et al*., 2023).

Intentionally produced nano materials: They occur when a group of individuals like a scientists come together to share ideas on the production of some nano materials. There are four main types of the intentionally produced nano materials (Kumar *et al*., 2022). They include:

1. carbon-based nanoparticles
2. metal based nanoparticles
3. dendrimers
4. nano composites

Carbon-Based Nano materials: They are a class of nano materials that are composed primarily of carbon atoms. These materials have unique properties that make them useful for a wide range of applications (Geim and Novoselov, 2007). They include:

a) Carbon Nanotubes (CNTs)

b) Graphene

c) Fullerenes

Metal-Based Nano materials: They are a class of nano materials that are composed primarily of metal atoms. They have unique properties that make them useful for a wide range of applications (Wang, 2009). They include:

a) Gold Nanoparticles (AuNPs)

b) Silver Nanoparticles (AgNPs)

c) Iron Oxide Nanoparticles (Fe3O4 NPs)

Dendrimers: They are complex nanoparticles which are built from linked or branched units. Each of the dendrimers has three sections comprising the core, an inner shell, and an outer shell. Each dendrimers also has a branched ends (Tomalia *et al.,* 2020).

Nanocomposites: A nanocomposite is a material that combines nanoparticles with other materials to create a composite with enhanced properties. There are three main types of nano composites (Omidi *et al*., 2022). They include:

a) nano ceramic matrix composites (NCMCs)

b) Metal matrix composites(MMCs)

c) Polymer matrix composites(PMCs)

1.2.11 Nanoparticles

Nanoparticles are defined as particles with dimensions ranging from 1–100 nanometers. Nanoparticles include carbon, like the fullerenes as well as some type of nanometer-scale of many elements, such as gold etc. The properties of nano particles have created a large impact in the study of nano medicine; the development of nano medicine is the usage of gold nanoparticles

which has help to fight lymphoma which is a type of cancer that attacks the cholesterol cells (Wang *et al*., 2022).

1.2.13 Classification of Nanoparticles

Nanoparticles can be classified into different ways or types. They are:

1. Carbon-Based Nanoparticles: They are tiny particles made of carbon, typically between 1-100nm in size (Liu, Y., *et al*. 2023). They include:

    a) Carbon Nanotubes (CNTs)

    b) Graphene

    c) Fullerenes

2. Metal-Based Nanoparticles: They are tiny particles made of metals, typically between 1-100nm in size. They exhibit unique physical, chemical, and biological properties due to their small size and high surface-to-volume ratio (Kumar *et al*., 2023). They include:

    a) Gold Nanoparticles (AuNPs)

    b) Silver Nanoparticles (AgNPs)

    c) Iron Oxide Nanoparticles (Fe_3O_4 NPs)

3. Semiconductor Nanoparticles: They are tiny particles made of semiconductor materials, typically 1-100nm in size. They exhibit unique and electronic and optical properties due to their small size and quantum confinement effects (Kim *et al*., 2022). They include:

a) Quantum Dots (QDs)

b) Nanowires

c) Nanoporous Materials

4. Ceramic-Based Nanoparticles: They include:

a) Titanium Dioxide Nanoparticles (TiO2 NPs)

b) Zinc Oxide Nanoparticles (ZnO NPs)

c) Silicon Carbide Nanoparticles (SiC NPs)

5. Polymeric Nanoparticles: They include:

a. Polymer Nanoparticles

b. Dendrimers

c. Polymer Nanocomposites

1.2.14 Manganese oxide Nanoparticles

The chemical formula for manganese oxide is MnO_2 (Greenwood and Earnshaw, 1997). It has a molecular weight of 86.9368g/mol. It appears as a black or brown solid powder that is odourless. Among all elements, manganese is the 10th most abundant on the Earth's crust. It is second only to iron as the most common naturally occurring heavy metal on the planet, and which also has a density approximately 5.03g/cm³. It is only soluble in acids, but in water and most organic solvents it is insoluble. Manganese oxide naturally occurs as the mineral pyrolusite, which is a common and important ore of Mn. It can also be found in other minerals such as manganite, hausmannite and birnessite. In modern times, MnO is now the subject of ongoing research,

especially in the aspect of nanotechnology and materials science. MnO nanoparticles are being studied for their enhanced catalytic, magnetic and electronic properties, making them suitable for applications in areas like drug delivery, environmental remediation and energy storage (Ding and Shen, 2013).

1.2.15 SYNTHESIS OF MANGANESE OXIDE NANOPARTICLE

Nano-sized manganese oxide can be synthesized through many processes such as sol-gel method, green synthesis, microwave, electrochemical, hydrothermal method, ultrasound, chemical vapor deposition and co-precipitation method, and micro emulsion method,

SOL-GEL METHOD

This approach involves five key stages: precursor selection, hydrolysis and condensation, polymerization, particle development, and gel formation. It is a method used for synthesizing metal oxide nanoparticles at low temperatures ranging from 200 to 600°C. It includes using specific chemicals as precursors for the gel. The sol-gel synthesis technique offers several benefits: a straightforward procedure (Al Abdullah *et al.*, 2017), the creation of a smooth powdery structure for MnO nanoparticles, the ability to shape materials into intricate geometries (Kumar *et al.*, 2015), high nanoparticle purity, and the ability to control the stability and phase formation of the precursors (Mustapha *et al.*, 2020). However, this method has drawbacks, such as potential breakage during drying, challenges in porosity control, the use of toxic agents to reduce nanoparticle sizes, and fragile bonding (Carter and Norton, 2013).

LITERATURE STUDIES

Srisvastava *et al.*, (2013) conducted the synthesis of nano-sized MnO using the sol-gel method with the addition of Manganese chloride ($MnCl_2$) and NaOH in a drop-wise manner. The mixture

underwent stirring with a magnetic stirrer at room temperature, followed by filtration, washing with distilled water, and drying in hot air at 100°C. The resulting precipitate was crushed to achieve consistent particle size, sieved, and then calcined in a muffle furnace at 600°C for 2 hours.

1.2.17 GREEN SYNTHESIS METHOD

This emerging field focuses on utilizing eco-friendly materials that are non-toxic and safe for biological systems as an alternative to traditional chemical and physical methods for synthesizing nanomaterials in the realm of bionanotechnology. Green synthesis offers advantages such as the abundance of raw materials, ease of synthesis, and environmental friendliness (Balogun *et al.*, 2020). However, a drawback is the risk of direct contact with human tissues, potentially leading to malfunctions (Cruz *et al*., 2020).

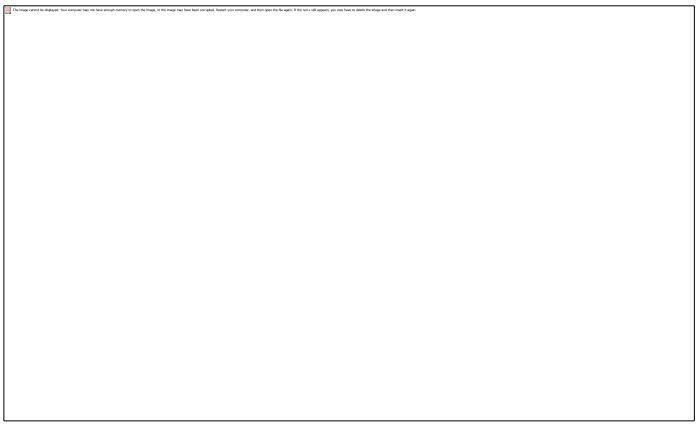

Plate 1.3: The key merits of Green Synthesis (Kumar *et al*., 2018).

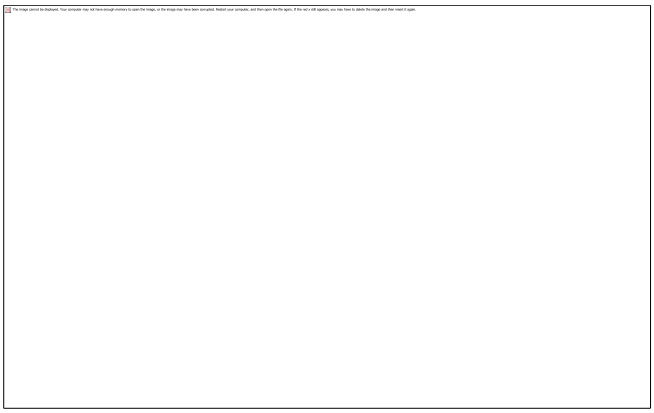

Plate 1.4 Schematic exemplification of green chemistry combination in metal nano materials cloning (Wikipedia, 2024).

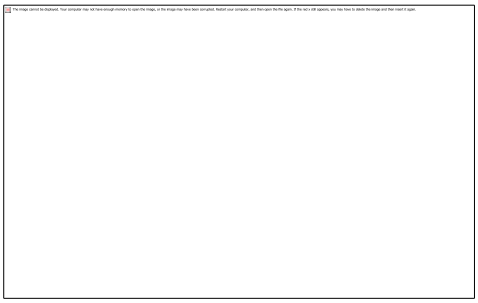

Plate 1.5: Schematic illustrations of plant as a source for the green synthesis of Nanoparticles and the properties and biomedical implantation of nanoparticles (Nath and Banerjee, 2013).

1.2.18 CHARACTERIZATION OF NANOPARTICLES

Following the synthesis of nanoparticles (NPs), various methodologies are employed to assess their conformational characteristics such as size, shape, uniformity, dispersity, and morphology. Commonly utilized techniques for NP characterization include Dynamic Light Scattering (DLS),

UV-Vis Absorption Spectroscopy, X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Analysis (EDAX), and Scanning Electron Microscopy (SEM).

SEM (Scanning Electron microscope): SEM is a technique used to produce high-resolution images of the surface morphology of materials. SEM uses a focused beam of high-energy electrons to scan the surface of a sample. The electrons interact with the sample, producing signals that are detected and used to create an image. SEM helps to characterized nanoparticles and nanostructures (Goldstein *et al*., 2017).

FTIR is a technique that is used to analyze the vibrational modes of molecules. It measures the absorption of infrared radiation by molecules. The absorption spectrum is related to the vibrational modes of the molecules. FTIR helps in analyzing the composition and structure of materials (Smith, 2011).

XRD (X-Ray Diffraction): XRD is a non-destructive analytical technique used to identify crystalline materials and also analyze material properties (Cullity and Stock, 2001). X-ray diffraction can measure through the thickness of a specimen with metallic inclusions to a depth of 0.3–0.5mm (Parleuliet *et al*., 2006). The principles of XRD are; X-ray scattering, diffraction (Cullity & Stock, 2001), and Bragg's law: The diffraction pattern is related to the crystal structure of the material (Bragg, 1913).

DLS is a technique used to analyze nanoparticles and colloidal dispersions by measuring the scattering of light due to Brownian motion (Berne & Pecora, 2000). DLS key functions are; Particle Size Measurement: It determines the hydrodynamic diameter of nanoparticles in solution. **Polydispersity Index (PDI) Analysis**: It indicates the uniformity of particle size distribution (low PDI = more uniform).

EDAX (or EDS): Energy Dispersive Spectroscopy) is an elemental analysis technique used in conjunction with Scanning Electron Microscopy (SEM) to determine the elemental composition of a sample. Its functions are: **Elemental Composition Analysis:** It identifies and quantifies elements present in the sample, **Material Characterization:** Helps confirm the presence of expected elements in synthesized nanoparticles.

1.2.19 Spectrophotometry

Spectrophotometry is an analytical method which measures how much a chemical substance absorbs light by measuring the intensity of light as it passes through the sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. A spectrophotometer is a device which measures the amount of photons (the intensity of light) absorbed after it passes through a sample solution. The concentration of a known analyte can easily be determined by a spectrophotometer by measuring the intensity of light detected. They are various type of spectrophotometer and each depends on the range of wavelength of the source.

In UV-Vis absorption spectra are utilized to analyze the aqueous suspension of NPs regarding their size and shape (Perelshtein *et al.*, 2008). Typically, wavelengths ranging from 300 to 800 nm are employed to identify NPs within the size range of approximately 2 to 100 nm (Tran *et al*., 2010).

1.2.20a ANTIOXIDATIVE PROPERTIES

An antioxidant is a stable molecule which helps the body system to fight off harmful free radicals, which has been linked to health condition like diabetes and cancer (Arnarson, 2023). These compounds function to delay or hinder (inhibit) cellular damages mainly by scavenging

and neutralizing free radicals (Lobo *et al.,* 2010). Antioxidant can be divided into three main groups depending on their mechanism: primary antioxidant, secondary antioxidant and tertiary antioxidant. Primary antioxidant act as a free radical scavengers, secondary antioxidant hinders chain reaction and the last one which is tertiary antioxidant helps in the repair of damaged biomolecules (Daramola and Adegoke, 2021). Antioxidant can further be classified into two main groups which are Natural antioxidant and synthetic antioxidant. Natural antioxidant are taken in as supplement from natural sources or they are synthesized by the body, example of natural antioxidants include: flavonoids, vitamin (A, C and E) and others while synthetic antioxidants are artificially produced or synthesized using various techniques example include: BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene) and others (Mamta *et al.*, 2014). The assays for measuring antioxidative properties are; DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay: It measures the ability of antioxidants to neutralize DPPH radicals. The DPPH assay typically changes from deep purple to light yellow or pale violet, depending on the strength of the antioxidant. In methanol or ethanol solution, DPPH appears deep purple due to its stable In methanol or ethanol solution, DPPH appears deep purple due to its stable free radical form. When reduced by an antioxidant (electron or hydrogen donor), the color fades to pale yellow or light violet as the radical is neutralized. The extent of this color change is measured spectrophotometrically at 517 nm to quantify antioxidant activity Agbafor and Nwachukwu, 2011). Therefore, DPPH measures the free radical scavenging activity, ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Assay: It uses ABTS radicals, which turn colorless when reduced by antioxidants, Oxygen Radical Absorbance Capacity (ORAC) Assay: It measures the ability of antioxidants to neutralize peroxyl radicals, based on fluorescence decay. Ferric Reducing Antioxidant Power (FRAP) Assay: It measures the reduction of Fe^{3+} (ferric) to Fe^{2+} (ferrous) by antioxidants, forming a blue-colored complex.

1.2.20b Importance of Antioxidant and phytochemical analysis

Antioxidants play important role in the body's defense against oxidative stress. Oxidative stress can lead to a variety of chronic diseases, including the rate of cancer, heart disease and neurodegenerative disorders. Phytochemical analysis on the other hand, is the study of the chemicals in plants, also known as Phytochemicals, using analytical methods. It can be used to identify, quantify, and extract Phytochemicals from plants with properties that have been linked to health benefits, such as anti-inflammatory and antibacterial properties, as well as anti-cancer properties (Reddy *et al*., 2018).

CHAPTER TWO

2. 1 MATERIALS AND REAGENT USED

The following materials and reagents were used for the synthesis of manganese oxide nanoparticles, phytochemical analysis and for the Antioxidant properties. They include:

African bush pear seed, distilled water, manganese chloride Tetrahydrate ($MnCl_2 \cdot 4H_2O$), sodium hydroxide (NaOH), ethanol (C_2H_5OH), DPPH, test tubes, beakers, what man filter paper, 10ml measuring cylinder, UV-30 spectrophotometer, sample container, conical flask, crucible, oven, magnetic stirrer and hotplate, centrifuge, mortar and pestle, weigh balance, thermometer, pH meter, mechanical grinder, and stop watch or timer.

2.2 METHODOLOGY

2.2.1 COLLECTION OF AFRICAN BUSH PEAR (Dacryodis erudis), PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES OF SEED EXTRACT

African bush pears were obtained from New Benin Market, Benin City, Edo state, Nigeria. The phytochemical analysis and the antioxidant property tests were carried on the seed extract at the Chemistry Annex in the Department of Chemistry, University of Benin, Benin-City, Nigeria.

2.2.2 PREPARATION OF AFRICAN BUSH PEAR SEED EXTRACTION

The seed of the African bush pear were removed and washed with distilled water to remove impurities. It was then subjected to air-drying (in order for it to be grinded effortlessly into a powdered form). A MACERATION PROCESS was employed to extract the bioactive compounds from the African bush pear seed. Specifically, 300ml of distilled water was heated to 60ºC using a hotplate and stirrer (MSH–30A). Then, 30g of the dried extract was added to the heated water and allowed to steep for 40 minutes, enabling the solvent (water) to extract the desired compounds from the plant materials, (Journal of pharmaceutical research, 2017).

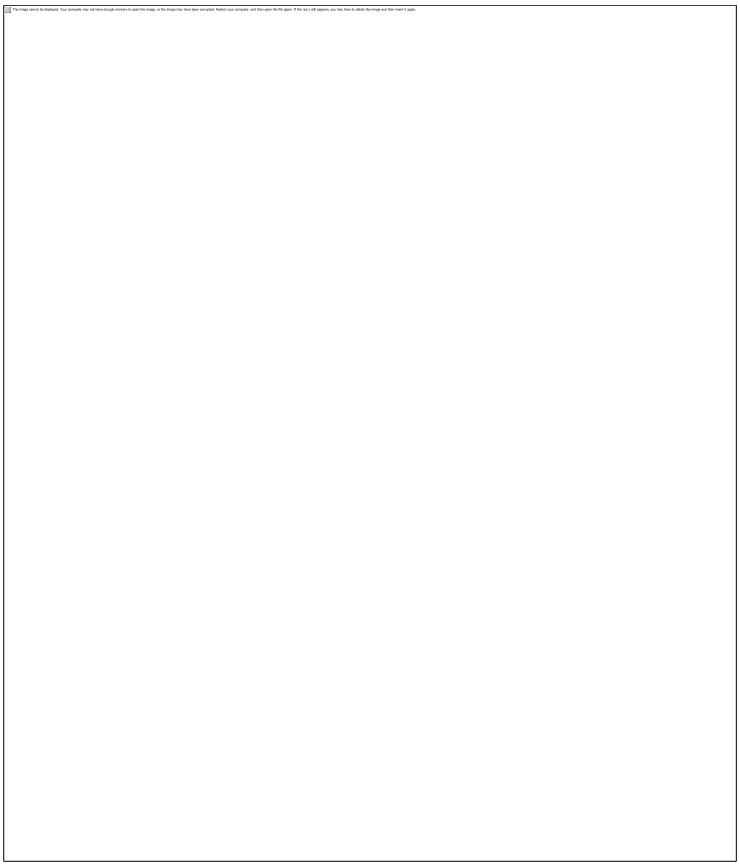

Plate 2.1: Filtration of Aqueous Extract Of African bush pear seed.

The mixture was then subjected to a two-step filtration process, using a whatman filter paper to separate the African bush pear seed extract from the residual solids. The resulting extract was collected, stored in a refrigerator to preserve its bioactive compounds, and subsequently used for further analysis and experimentation**.**

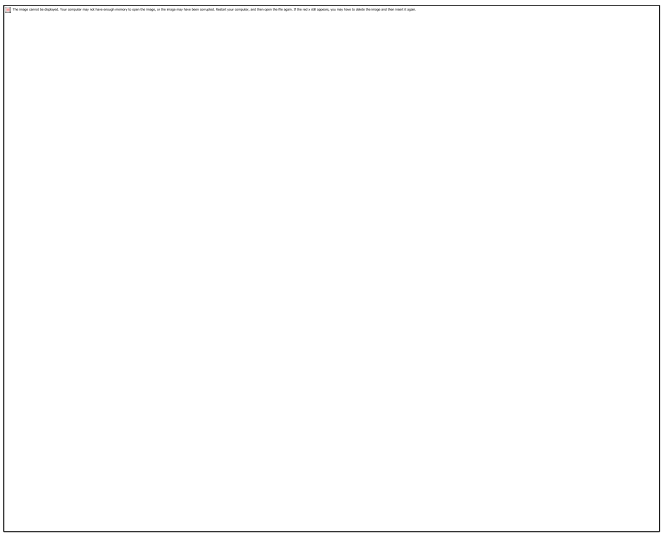

Plate 2.2: SYNTHESIS OF MANGANESE OXIDE NANOPARTICLES (MnONPs) - (a)

Plate 2.3: SYNTHESIS OF MANGANESE OXIDE NANOPARTICLES (MnONPs) – (b)

The MnONPs were prepared using the African bush seed extract. A solution of 39.6g of Manganese Chloride was prepared by dissolving 39.6g of Manganese chloride in 200ml of

distilled water. The solution was then heated using a hotplate stirrer for approximately 40 minutes to 1 hour, until the manganese chloride was fully dissolved. Next, 10ml of the extract was added to the manganese chloride solution, and the pH of the solution was adjusted accordingly (Journal of nanotechnology, 2018). The pH of the solution was adjusted using NaOH, and then the solution was stirred on a magnetic stirrer and hotplate at 60ºC. This step is crucial for the synthesis of manganese oxide nanoparticles, as it allows for the precipitation of manganese oxide from the solution (Journal of materials science, 2019).

Note: During the addition of NaOH, a distinct colour change was observed, where the solution transformed from dark brown to light brown. This colour change indicates a change in the oxidation state of the manganese ions, which is a crucial step in the synthesis of manganese oxide nanoparticles (Journal of Nanoparticles, 2015).

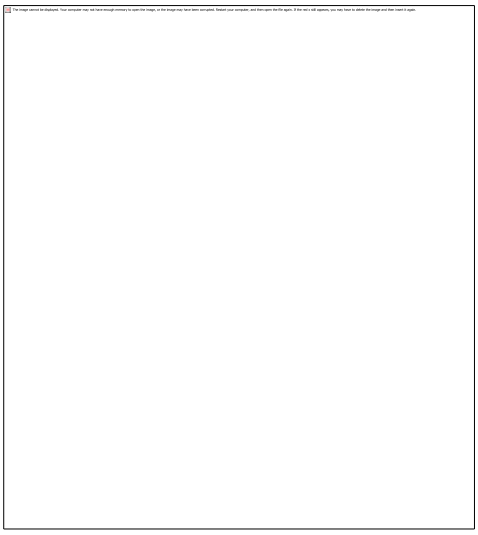

Plate 2.4: Mixture of manganese Chloride and African bush pear

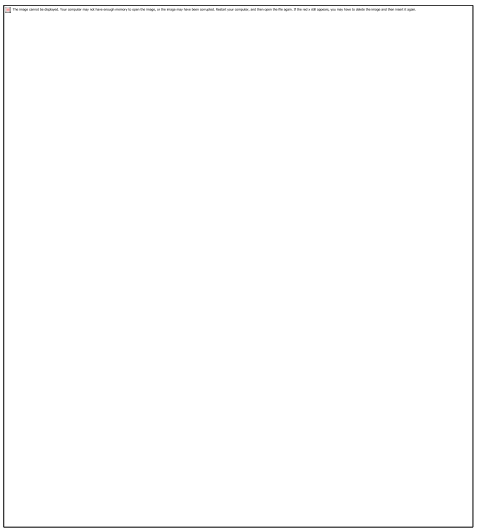

Plate 2.5: After addition of NaOH

After 24hrs, the particles were separated by centrifugation at 10,000 rpm or 10mins using a centrifuge of model (BKC-TH18II), the supernatant was decanted while ethanol was added to remove impurities and centrifuged again under the same condition. The particles were obtained in a clean crucible, dried in the oven for 1hr at 100 degrees Celsius and thereafter calcinated at 600 degrees Celsius for 2hrs then powdered using a mortar and pestle to obtain the sample labelled as MnONPs with slight modification (Journal of Nanoparticles, 2015).

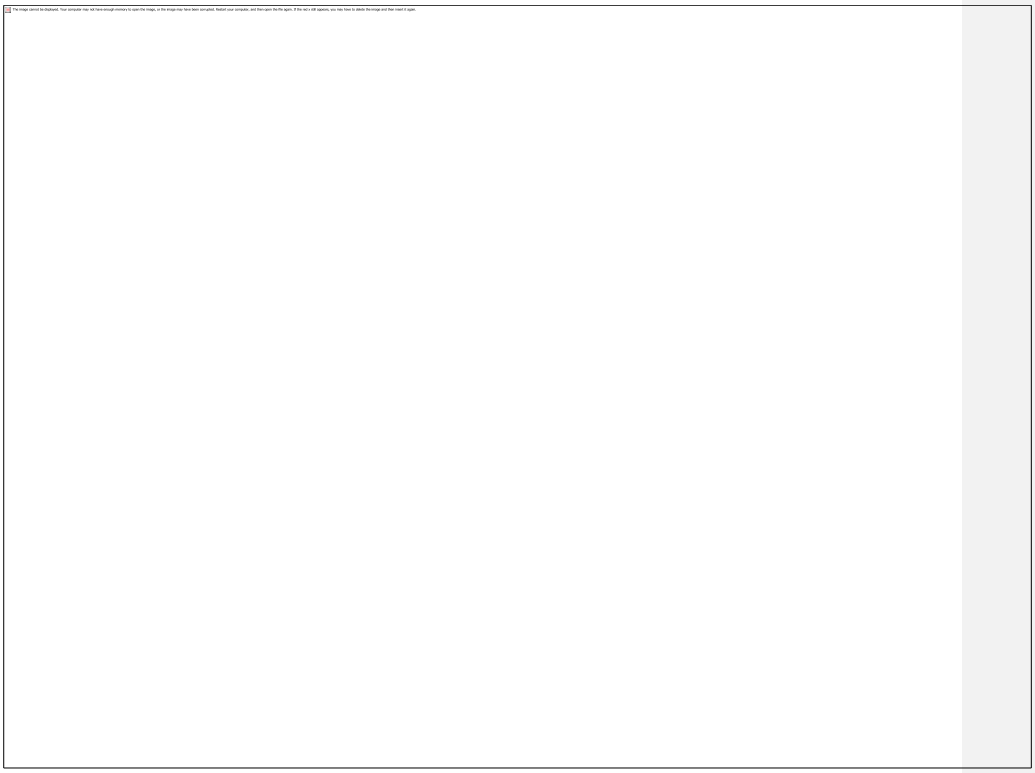

Plate 2.6: MnO nanoparticles after calcination and powdering

2.2.3 CHARACTERIZATION OF THE SYNTHESIZED MANGANESE OXIDE NANOPARTICLE

A sample of the synthesised manganese oxide nanoparticles was sent out to Zaria for characterization. The characterization techniques used were:

1. Dynamic Light Scattering (DLS)
2. Fourier Transform Infrared Spectroscopy (FTIR) and

3. X-Ray Diffraction (XRD).

(Journal of Nano materials, 2019).

XRD provides data on the symmetry, sizes, and metallic NP detection state (Feldheim and Foss, 2002). X-rays can penetrate nanomaterials, and the resulting diffraction pattern collected is correlated with structural characteristics. For example, XRD peaks (2θ) observed at angles of 28.51°, 33.06°, and 47.42° corresponding to 111, 200, and 220 planes, respectively, along with normal separation peaks, indicate the cubic structure of CeO_2 NPs (Sangeetha *et al.*, 2011). XRD analysis further confirms the crystalline patterns of PbNPs and provides information on their average particle size using the Scherer equation (Elango and Roopan, 2015).

FTIR Spectroscopy is employed to identify various functional groups or metabolites that contribute to the reduction and stabilization of nanoparticles (NPs) on their surface (Lloyd *et al.*, 2011). The observed functional group bands at 3450, 3266, and 2932 cm^{-1} correspond to amine stretching frequencies, alcohol O-H stretching, and alkane C-H extension, respectively, in NPs synthesized using Aloe Vera extracts. Peaks in the region of 600-400 cm^{-1} are attributed to ZnO (Schaffer *et al.*, 2009). Additionally, carboxyl ion peaks at 1648, 1535, 1450, and 1019 cm^{-1}, along with a 1450 cm^{-1} peak, were reported to stabilize Ag NPs synthesized using Solanum torvum leaf extract (Ali *et al.*, 2016).

DLS and EDAX are utilized to analyze the size distribution of liquids and essential components of NPs, respectively.

2.2.4 Phytochemical Analysis of Seed Extract

The quantitative phytochemical Analysis of the seed extract was carried out as described by Elechi and Owhoeke *et al.* (2020).

Qualitative phytochemical screening was conducted on the African Bush Pear seed extract to identify the presence of key secondary metabolites, including tannins, flavonoids, saponins, and alkaloids. To test for tannins, a few drops of 1% ferric chloride ($FeCl_3$) solution were added to 2 mL of the seed extract, and the presence of tannins was indicated by a blue-black or greenish-black coloration **Elechi and Owhoeke *et al.* (2020)** For flavonoids, a few drops of 1% ammonia solution were added to 2 mL of the extract, followed by concentrated sulfuric acid, with the appearance of a yellow color that turned orange upon standing confirming their presence **Elechi and Owhoeke *et al.* (2020)**. Saponins were tested by adding a few milliliters of distilled water to 1 mL of the extract and shaking the mixture vigorously, where the formation of a stable froth persisting for several minutes indicated their presence **Elechi and Owhoeke *et al.* (2020)** . Alkaloids were detected by adding a few drops of Dragendorff's reagent (bismuth nitrate solution) to 2 mL of the extract, with the formation of an orange precipitate confirming their presence **Elechi and Owhoeke *et al.* (2020)** Each of these tests was performed in triplicates, and the results were recorded for comparison with positive and negative controls.

2.2.5 Anti-oxidant Activity of MnO Nanoparticles

The antioxidant activity of the synthesized MnO nanoparticles was evaluated using various in-vitro assays. These assays measure the ability of the nanoparticles to scavenge free radicals or reduce oxidants, which is important for their potential therapeutic applications in oxidative stress-related conditions (Journal of Nanoparticle Research, 2018).

2.2.5.1 In-vitro Antioxidant Assays

Determination of total antioxidant capacity (DPPH radical scavenging ability)

DPPH Radical Scavenging Assay: The DPPH radical scavenging assay was used to assess the antioxidant capacity of the synthesized MnO nanoparticles. In this assay, DPPH is a stable free radical that exhibits a purple color in solution. The presence of an antioxidant leads to the reduction of DPPH, resulting in a decrease in absorbance. The protocol involves preparing a series of concentrations of MnO nanoparticles and incubating them with a constant concentration of DPPH. The decrease in absorbance is measured at 517 nm using a UV-Vis spectrophotometer (Clinton, 2024).

PROCEDURE:

The free radical scavenging abilities of the dilute pear samples were measured using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) technique as described by Baliyan et al. (2022) with slight modifications.

An ethanolic dilution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) of concentration (2×10^{-4} M) was prepared. 10ml of each sample of the extract was then collected at different concentrations which were 250, 200, 150, 100, and 50 µg/ml, 2ml of ethanolic dilution of DPPH was then added to each sample. The mix was then kept in a dark room at room temperature for 30 minutes and the absorbance of the control (Ac) which was prepared with the ethanolic dilution of DPPH and the absorbance of the test (At) were measured at 517nm using a UV-30 spectrophotometer. The percentage inhibition of the extract was then compared with a known standard of Ascorbic acid (vitamin C) Kumar et al., (2013).

The equation given below was used to calculate the% total antioxidant capacity:

(Ac–As) ÷ Ac ×100

Where: Ac—DPPH reagent absorbance; As—Testing sample absorbance.

DPPH Radical Scavenging Assay:

The **DPPH** radical scavenging assay was used to assess the antioxidant capacity of the synthesized MnO nanoparticles. In this assay, **DPPH** is a stable free radical that exhibits a purple color in solution. The presence of an antioxidant leads to the reduction of DPPH, resulting in a decrease in absorbance. The protocol involves preparing a series of concentrations of MnO nanoparticles and incubating them with a constant concentration of DPPH. The decrease in absorbance is measured at 517 nm using a UV-Vis spectrophotometer (Emeribe, 2024)

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CHAPTER THREE

RESULT AND DISCUSSION

3.1 MECHANISM OF GREEN SYNTHESIS OF MnO NANOPARTICLES USING AFRICAN BUSH PEAR SEED EXTRACT

The synthesis of MnO nanoparticles using African bush pear seed extract involves the **Formation of $Mn(OH)_2$:** Phytochemicals in the African bush pear extract, such as antioxidants, interacted with manganese ions (Mn^{2+}) from the manganese salt precursor. These phytochemicals acted as both reducing and stabilizing agents, converting the manganese ions into manganese hydroxide ($Mn(OH)_2$). The reduction potential of manganese ions was increased due to their attachment to chloride anions in the precursor salt, facilitating electron donation. The Mn^{2+} ions coordinated with the hydroxyl (OH) groups of the antioxidants, leading to the formation of $Mn(OH)_2$. **Calcination:** The $Mn(OH)_2$ precipitate was then subjected to calcination at 600°C. During calcination, the $Mn(OH)_2$ underwent a dehydration reaction, releasing water molecules and formed MnO nanoparticles. The overall mechanism can be summarized as follows: $\mathbf{Mn^{2+} + Phytochemicals\ (Antioxidants) \rightarrow Mn(OH)_2}$.

$\mathbf{Mn(OH)_2 \rightarrow MnO + H_2O}$ **(Calcination).** The composition of the African bush pear seed extract, including the specific phytochemicals and antioxidant properties present, can influence the size, shape, and properties of the synthesized MnO nanoparticles.

3.2 RESULTS OF THE CHARACTERIZATION OF THE MnO NANOPARTICLES

3.2.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier Transform Infrared (FTIR) Spectroscopy is used to identify functional groups in a sample by measuring how molecules absorb infrared light at different wavelengths. The resulting FTIR spectrum displays absorption peaks that correspond to specific molecular vibrations.

The FTIR spectrum in Fig. 3.1 showed the presence of an O-H stretching vibration at broad peak **3354.8cm^{-1}** which may have originated from the African bush pear used in the synthesis, a C=O stretching vibration in an unsaturated carbon to oxygen bond at peak **1848.6cm^{-1}** which could be also due to the presence of residual organic matter from the African bush pear and presence of a carboxylate functional group at peak **1703.4cm^{-1}** which may have been formed during the interaction between the manganese precursor and the photochemical present in the African bush pear. Accoding to this result, The sharp absorption band observed at 3354.8cm-1 can be attributed to the stretching vibrations of hydroxyl (O-H) groups. This band is a result of the formation of Manganese Hydroxide ($Ca(OH)_2$) through the hydration process of MnO . Furthermore, the peak a**t 1638.3cm^{-1}** indicates the presence of physically adsorbed moisture or water molecules attached to the nanoparticles, as it signifies the O-H bending, adsorbed water stretching vibrations. This can also be due to the hydroxyl groups from polyphenols, flavonoids or water molecules. A strong peak in the range of **1703.4–1800cm^{-1}** corresponds to C=O stretching vibrations, which are characteristic of carbonyl groups found in flavonoids, tannins, and other secondary metabolites. This suggests that these compounds play a role in stabilizing the Manganese Oxide nanoparticles. The peak observed **2109.7cm^{-1}** corresponds to carbonyl or Mn–C stretch which can be gotten from esters, alcohols, other bioactive molecules. Furthermore,

the peak observed at **976.8cm^{-1}** indicated Mn-O bond confirming the formation of Manganese Oxide nanoparticles. *(Ulakpa et al., 2024).*

The Mn–O peak is crucial as it confirms the presence of metal-oxygen bonding in the synthesized nanoparticles. If this peak is weak or absent, it may suggest incomplete conversion of precursors or interference from other compounds.

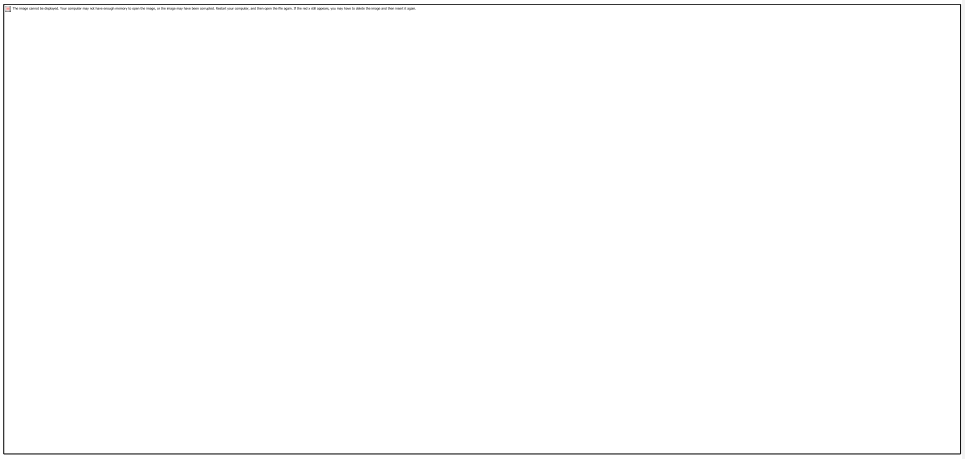

Fig. 3.1 FTIR spectrum of the synthesised MnO nanoparticles

3.2.2 X-RAY DIFFRACTION (XRD)

X-ray diffraction analysis (XRD) is a technique used for the determination of crystalline structure, phase composition and particles size of synthesized nanoparticles. As the samples were in the form crystalline powder; the XRD analysis focused on identifying specific lattice planes

that generated peaks at corresponding angular positions, known as 2θ, as determined by Bragg's law (16) *(*Ulakpa *et al.*, 2024). The 2θ peaks observed for MnO nanoparticles were found at angles of 18°, 36°, and 56°, corresponding to the (101), (211), and (400) crystallographic planes of cubic MnO, respectively. These peaks align with the standard JCPDS card (No. 01-072-1982), confirming the successful synthesis of pure MnO nanoparticles (Ulakpa *et al.*, 2024).

Fig. 3.2 XRD pattern of the synthesized manganese oxide nanoparticles

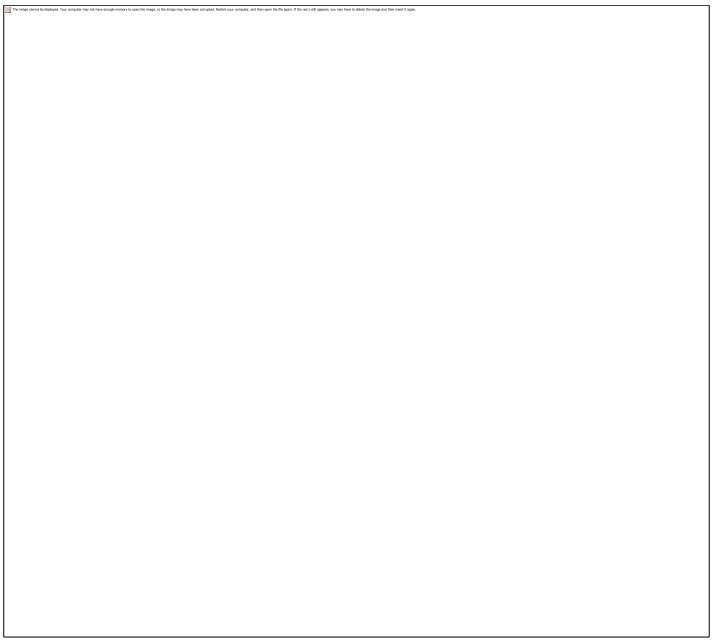

Fig. 3.3 Phase composition of the sample

3.3 DYNAMIC LIGHT SCATTERING (DLS)

The DLS results provided the size distribution of the particles in the sample. The results shown in Fig. 3.4 indicated that the sample (MnO nanoparticles) had an average particle size of 48.78nm which is in the range of the desired size and a PDI value of 0.250 which implied that it was monodisperse and the particle size distribution of the sample is relatively broad with a good result quality. The Polydisperity Index (PDI) shows how the nanoparticles are uniform in size. According to previous studies, if the polydisperity index is less than 0.5, then the particles can be said to be monodisperse to some certain degree but if the polydisperity index is greater 0.5 then the particles can be said to be polydispersed. According to this result, the polydisperity index was less than 0.5, therefore the nanoparticles were monodispersed.

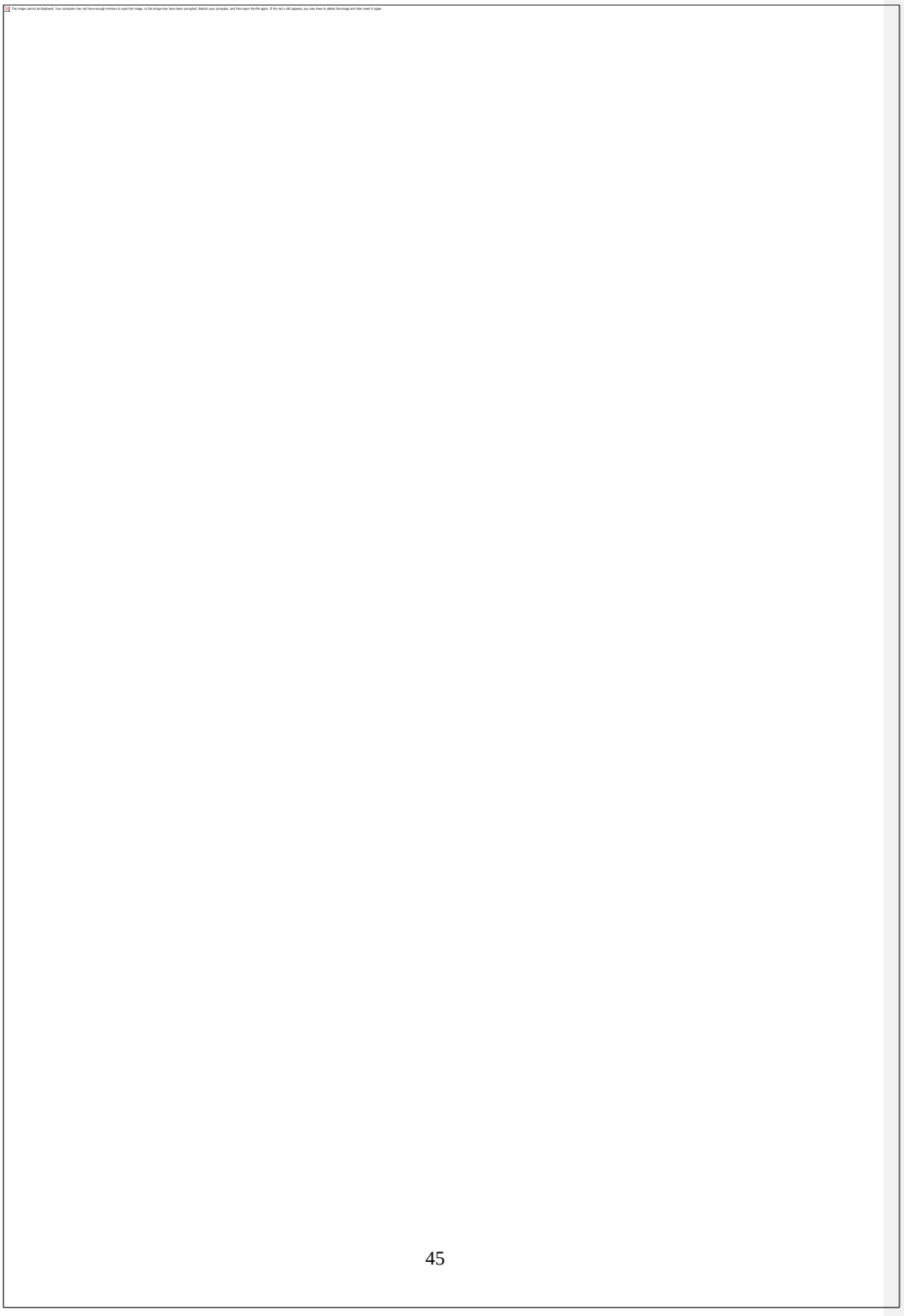

Fig. 3.4 Size distribution report by intensity

3.4 RESULTS OBTAINED AFTER QUALITATIVE ANALYSIS

Table 3.1: Result for phytochemical analysis of seed extract

+ = present ++ = strongly present

Table 3.1 Shows a significant presence of various phytochemicals in the seed extract of Dacryodes *edulis*. The analysis indicated that all tested phytochemicals were detected in the seed extract.

Phytochemicals	**TEST**	**Seed extract sample**
Alkaloid	Wagner's test	+
Tannin	Ferric chloride	–
Saponin	Frothing	+
Flavonoid	Lead acetate	+
Phenolic compounds	Lead acetate	–
Reducing Sugar	Fehling's test A&B	++

3.5 DETERMINATION OF ANTIOXIDANT PROPERTIES OF SEED NANOPARTICLES

The table below shows the concentration, absorbance value and the percentage inhibition of the seed extract with DPPH (2,2-Diphenyl-1-picrylhydrazyl) recorded at a wavelength of 517nm.

Table 3.2: Result for the concentration, absorbance value and the percentage inhibition of the seed extract with DPPH (2,2-Diphenyl-1-picrylhydrazyl)

		SEED EXTRACT	
S/N	CONC (µg/ml)	% IHI AA	% IHI SAM
1	250	0.903	0.133
2	200	0.421	0.094
3	150	0.255	0.083
4	100	0.134	0.078
5	50	0.080	0.058

The absorbance value of the control which is (DPPH and Ethanol) = 2.120

Table 3.2 revealed the antioxidant properties analysis carried out on seed extract, it is observed from the table that as the concentration of the samples decreases the ascorbic acid increase this is due to the presence of more DPPH radicals which are purple in color, as fewer molecules of the extract are present to donate hydrogen atom the radical, this thereby lead to an increase in

ascorbic acid. It is important to note from the table above that the ascorbic acid is inversely proportional to the antioxidant activity that is the (% Inhibition), the higher the absorbance (ascorbic acid) the lower the antioxidant activity being measured.

The graph below shows how the concentration of the extract varies with the percentage inhibition.

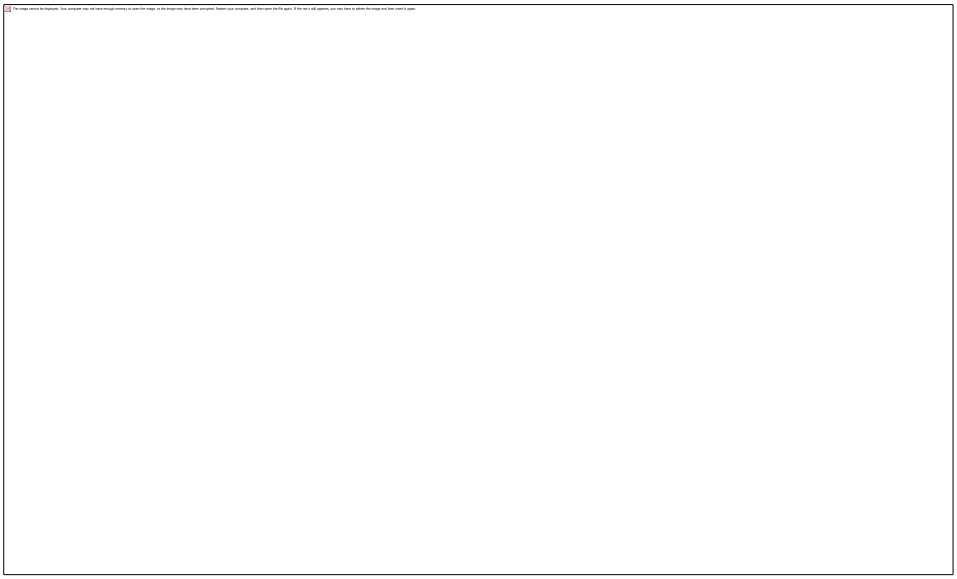

Fig. 3.6 A Graph of %Inhibition against Concentration

Phytochemicals and minerals are essential for tackling global health challenges, the bioactive compounds in plants plays a crucial role in a wide array of biological and chemical process.

However, prior to utilizing medicinal plant such as *Dacryodes edulis* (African bush pear); extensive research is imperative as the efficacy of treatment heavily depends on the quality of plant material. Exploring natural plant resources is valuable for uncovering active constituents to ascertain their actual medicinal benefits (Samatha *et al.,* 2012, Kumar *et al.,* 2018). A deep understanding of the diverse phyto-constituent present in plants hold significant importance, providing benefits in crafting intricate chemical compounds and assessing their biological impacts in screening protocols.

The research focused on the comparative study on the minerals, phytochemicals and antioxidant activity of *Dacryodes edulis* seed. From Table 3.1 it can be seen that seed sample are rich in the phytochemical as all the bioactive compound tested for are present, this is expected as *Dacryodes edulis* is a medicinal plant which has been used traditionally in the treatment of aliment such as pain, fever and so on.

3.6 CONCLUSION

The green synthesis of MnO NPs using African bush pear seed extract provided a sustainable and cost-effective approach to Phytochemical and antioxidant properties. This study highlighted the importance of exploring natural resources for the development of eco-friendly and efficient materials for environmental remediation (Journal of food science and technology, 2017).

Dacryodes edulis (African bush pear) is a medicinal plant recognized traditionally for its therapeutic abilities (Journal of pharmacy and pharmacology, 2017). This study showed that it was rich in phytochemicals and that this phytochemical distributed themselves in the varying amount across the leaves and seeds of the plant. The presence of phytochemicals in this plant

would help in the synthesis of new drugs which would help reduce global health challenges. Minerals such as Calcium, Magnesium, and Iron which were present in this plant would also help to serve as supplements and make the plant more nutritionally valuable.

3.7 RECOMMENDATIONS FOR FURTHER STUDIES

Based on the findings of this study, the following recommendations were made:

• **Scale-up of the process:** The green synthesis of ZnO NPs using plantain peels should be scaled up to a pilot or industrial level to demonstrate its feasibility for large-scale drug delivery.

• **Research of other plant extracts:** The search for other plant extracts with potential for synthesizing nanoparticles should be encouraged to expand the range of green synthesis methods.

• **Toxicity testing:** The toxicity of the MnO NPs should be thoroughly investigated to ensure their environmental safety.

• **Integration with other technologies:** The combination of MnO NPs with other plants, such as Cherry, Tumeric, and Ginger could enhance the overall treatment efficiency.

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APPENDIX

PREPARATION OF STANDARD SOLUTION OF SEED SAMPLE Seed sample

The standard solutions prepared were 250, 200, 150, 100 and 50 µg/ml. They were prepared using dilution formula after the concentrations of the extract were determined.

To prepare 250 µg/ml a 100ml volumetric flask was used;

Dilution formula used

$C_1V_1 = C_2V_2$

Initial concentration (C1) = 250µg/ml

Final concentration (C2) = 50µg/ml

Final volume (V2) = 100ml

$250 \times V_1 = 50 \times 100$

$V_1 = 2ml$

200 µg/ml, 150 µg/ml, 100 µg/ml, 50 µg/ml, were prepared using the same manner of approach.