SYNTHESIS AND CHARACTERIZATION OF COPPER OXIDE NANOPARTICLES USING THEOBROMA CACAO FOR THE ADSORPTION OF METHYLENE BLUE DYE FROM SYTHENTIC WASTE WATER

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CERTIFICATION

This is to certify that the research project titled **"SYNTHESIS AND CHARACTERIZATION OF COPPER OXIDE NANOPARTICLES USING THEOBROMA CACAO FOR THE ADSORPTION OF METHYLENE BLUE DYE FROM SYTHENTIC WASTE WATER"** was duly carried out by **OLUWOLE, GREATNESS ADEOLA** (Matriculation No: 210531049) of the **Department of Chemistry, Faculty of Science, Lagos State University, Ojo, Lagos State.**

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DEDICATION

I dedicate this research work to God Almighty, my Lord Jesus Christ, and the Holy Spirit, whose guidance, grace, wisdom, and strength have brought me this far.

I also dedicate it to my beloved parents and my family for their love, encouragement, and unwavering support throughout this journey.

ABSTRACT

Nanaomaterials can be synthesized using several conventional techniques such as co-precipitation, micro-emulsion, sol–gel method, electrospray synthesis, laser ablation, and hydrothermal processing. Though, these approaches are often energy-demanding, environmentally hazardous, and produce cytotoxic byproducts. Green nanotechnology, on the other hand, offers a sustainable alternative by utilizing plant extracts rich in phytochemicals as natural reducing and stabilizing agents. In the present study, copper oxide nanoparticles (CuO NPs) were synthesized using *Theobroma cacao* leaf extract, which acted as both a reducing and stabilizing agent. The synthesized CuO NPs were characterized using UV–Vis spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy coupled with energy-dispersive X-ray analysis (SEM–EDX), and Fourier-transform infrared spectroscopy (FTIR). UV–Vis spectra displayed a broad absorption band at 405 nm, confirming nanoparticle formation. XRD analysis revealed the crystalline structure of CuO with high purity, showing diffraction peaks at $2\theta = 7.8°$, 20.1°, 38.1°, 44.9°, 57.9°, and 68.0°, corresponding to the (110), (111), (200), (202), (113), and (311) planes of monoclinic CuO, respectively**.** FTIR spectra showed characteristic O–H and C=O stretching vibrations, attributed to phytochemicals from the cacao extract, which were responsible for nanoparticle stabilization. SEM analysis revealed spherical morphology with moderate aggregation and EDX analysis confirmed copper and oxygen as the dominant elements. The application of the synthesized CuO NPs for adsorption of methylene blue dye is ongoing.

Keywords: *Theobroma cacao,* Copper Oxide nanoparticles, Green Synthesis.

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND THEIR MEANING

% - (PERCENTAGE)

℃ - (DEGREE CELSIUS)

Θ - (THETA)

º - (DEGREE)

~ - (TILDE)

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND TO THE STUDY

Over the past few decades, nanotechnology has grown significantly, establishing itself as a prominent and emerging field of research. Rooted in its interdisciplinary nature, nanomaterial-based technologies draw upon various scientific domains, including physics, chemistry, biology, and engineering. These fields leverage the distinctive properties of nanomaterials to promote innovation in both theoretical investigations and real-world applications. (Deng *et al*., 2021). Nanotechnology is the science of manipulating and using matter on a microscopic scale. It allows for the production of new materials, particularly those for medical uses, where older methods may be restrictive. Nanotechnology should not be regarded as a single approach with limited application (Galatage *et al*.2020).

Over the past few decades, significant advancements have been made in the synthesis and characterization of nanoparticles, driven by their broad applications in catalysis, biomedicine, optics, and energy (Rasheed, 2017). While chemical methods are commonly used for large-scale nanoparticle production due to their efficiency, they often pose environmental concerns such as high energy demands and the generation of hazardous waste. This has led to growing interest in developing more sustainable and eco-friendly synthesis approaches (Masakke, 2015). Nanotechnology, a modern scientific discipline, focuses on the synthesis and application of nanoparticles (NPs), which typically range in size from 1 to 100 nanometers. These nanoparticles have garnered considerable attention due to their unique physicochemical properties, including antibacterial activity, catalytic performance, optical behavior, electronic features, and magnetic characteristics (Murugan & Shanmugasundaram, 2014).

Biogenic synthesis, also known as green chemistry, offers an environmentally friendly and contaminant-free approach, making it especially suitable for medical applications where high purity is essential (Sharma *et al*., 2019). Plant extracts are rich in biomolecules and phytochemicals such as proteins, polysaccharides, amino acids, organic acids, vitamins, polyphenols, flavonoids, terpenoids, alkaloids, tannins, and alcohol-based compounds that act as natural reducing and stabilizing agents in nanoparticle synthesis (Patel & Desai, 2020). Furthermore, the use of plant-based synthesis methods is not only cost-effective but also enables biocompatible and scalable nanoparticle production (Kumar *et al*., 2018). Consequently, a growing number of researchers have focused on synthesizing nanoparticles using plant extracts (Ahmed *et al*., 2016).

Water pollution has become one of the most critical environmental issues requiring urgent attention from both scientists and policymakers (Félicité *et al*., 2021). Surface and groundwater are increasingly contaminated with inorganic and organic pollutants, with synthetic dyes representing a major concern due to their widespread use and environmental persistence (Félicité *et al*., 2021; Yusri *et al*., 2018). Synthetic dyes, unlike natural dyes, are extensively utilized in industries such as textiles, leather, food, pharmaceuticals, cosmetics, and printing (Félicité *et al*., 2021; Ahmad *et al*., 2019; Sari *et al*., 2022). Among these, the textile industry is recognized as a leading contributor to dye pollution due to the large volume of colored wastewater it generates (Félicité *et al*., 2021).

These dyes are chemically stable and resistant to biodegradation, making them persistent in aquatic environments (Ahmad *et al*., 2019). Even at low concentrations, synthetic dyes can block sunlight penetration in water bodies, which inhibits photosynthesis, lowers dissolved oxygen levels, and disrupts aquatic ecosystems (Félicité *et al*., 2021; Yusri *et al*., 2018; Sari *et al*., 2022). Long-term exposure to these dyes poses significant health risks to both humans and animals, including mutagenic and carcinogenic effects (Ahmad *et al*., 2019; Sari *et al*., 2022).

In particular, this study focuses on methylene blue (systematic name: 3,7-bis(dimethylamino)phenothiazin-5-ium chloride), a basic heterocyclic dye that is highly soluble in water and commonly used as a model pollutant in adsorption studies (Félicité *et al*., 2021). Additionally, the rapid expansion of Indonesia's textile industry growing by 10.45%—has presented significant challenges in managing industrial wastewater, which contains harmful substances such as acids, alkalis, dyes, hydrogen peroxide, starch, surfactants, dispersants, and metal-based soaps (Yusri *et al*., 2018). Many of these compounds, especially dyes like reactive, direct, and process dyes, are toxic, carcinogenic, and resistant to microbial degradation (Sari *et al*., 2022; Ahmad *et al*., 2019).

Copper is an essential trace element in the human body, involved in various enzymatic reactions, redox processes, and the proper functioning of the nervous and immune systems (Ahmed *et al*., 2019). Beyond its biological role, copper and its oxides have attracted attention in nanotechnology due to their unique physicochemical properties. Among them, Copper Oxide nanoparticles (CuO-NPs) have gained significant interest for their diverse applications in medicine, catalysis, sensing, and environmental remediation (Roy *et al*., 2020; Ikram *et al*., 2021).

Roy *et al*. (2020) reported that CuO-NPs exhibit remarkable antimicrobial activity against a wide range of pathogens, including *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. This activity is primarily attributed to the generation of reactive oxygen species (ROS) such as hydroxyl radicals (•OH), superoxide anions ($•O_2^-$), and hydrogen peroxide (H_2O_2), which disrupt microbial cell membranes and induce oxidative stress. Ikram *et al*. (2021) further explained that the nanoscale size of CuO particles enables strong interactions with bacterial membranes, resulting in cell wall rupture, leakage of intracellular contents, and eventual microbial death.

Additionally, the high surface area-to-volume ratio of CuO-NPs enhances their reactivity and promotes strong contact with microbial and pollutant molecules (Zhou *et al*., 2022). Their semiconducting nature (p-type, band gap ~1.2–1.9 eV) also makes them effective photocatalysts, capable of generating electron–hole pairs under visible light irradiation. This photocatalytic property has been widely exploited in the degradation of organic dyes and other pollutants in wastewater (Singh *et al*., 2023). Therefore, CuO-NPs are considered promising multifunctional nanomaterials with broad applications in both biomedical and environmental fields.

DEFINITION OF TERMS

Absorption: This refers to the process by which one substance becomes incorporated into another, such as the uptake of dye molecules (e.g., methylene blue) by a solid adsorbent like nanoparticles through physical or chemical interaction.

Analysis: This is the systematic investigation of a sample to identify its components and understand its essential features and structure.

Characterization: The process of measuring the physical, structural, and chemical properties of a material to gain insights into its composition, morphology, and behavior using techniques such as XRD, SEM, FTIR, and BET.

Extraction: A separation technique used to isolate desired components from a mixture, often involving solvents to remove phytochemicals from plant material.

Synthesis: The formation of complex chemical compounds from simpler ones, particularly the creation of nanoparticles from precursor materials through chemical, physical, or biological methods.

Phytochemicals: Naturally occurring compounds in plants that possess biological activity, such as flavonoids, alkaloids, tannins, and terpenoids, which often act as reducing and stabilizing agents in nanoparticle synthesis.

Adsorption: A surface phenomenon where molecules of a substance, such as dye, adhere to the surface of a solid or liquid adsorbent material. It is critical in wastewater treatment processes.

Nanoparticles (NPs): Ultrafine particles ranging from 1 to 100 nanometers in size with unique physicochemical properties due to their high surface area and reactivity.

Green Synthesis: An eco-friendly method of producing nanoparticles using biological agents like plant extracts, which minimizes toxic byproducts and promotes sustainable development.

PROPERTIES OF CuO-NPS

Copper oxide nanoparticles (CuO-NPs) exhibit distinctive structural, optical, surface, and biological properties that make them highly attractive for applications in environmental remediation, catalysis, sensing, and biomedicine. Structurally, CuO-NPs generally crystallize in a monoclinic phase, with particle sizes typically ranging from 10 to 50 nm, as confirmed by X-ray diffraction (XRD) and electron microscopy techniques (Khine *et al.*, 2022; Ukpe, 2023). Their nanoscale dimensions confer a high surface area-to-volume ratio, which enhances surface reactivity and interactions with pollutants and biological systems.

Optically, CuO-NPs are p-type semiconductors with a narrow band gap of approximately 1.2–1.9 eV, enabling absorption in the visible region (Singh *et al.,* 2023). This makes them highly effective in photocatalytic degradation of dyes and organic pollutants under solar or artificial light irradiation. UV–Vis spectroscopy often reveals strong absorption in the visible range, supporting their role as photocatalysts and photosensitizers in wastewater treatment.

Surface morphology studies using atomic force microscopy (AFM) and scanning electron microscopy (SEM) show that CuO-NPs possess irregular, quasi-spherical shapes with a rough but stable surface texture, which improves their adsorption efficiency and reactivity (Abbas & Aadim, 2022). Their surface chemistry also allows interactions with dissolved oxygen and water, which promotes the generation of reactive oxygen species (ROS) during photocatalytic and antimicrobial processes.

Biologically, CuO-NPs demonstrate strong antibacterial and antifungal activities, particularly against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. The antimicrobial action is attributed to their ability to generate ROS, disrupt microbial membranes, and release Cu^{2+} ions that interfere with cellular metabolism (Ikram *et al*., 2021; Zhou *et al*., 2022). Their antimicrobial activity, combined with their biocompatibility when synthesized via green methods, supports their potential in medical and environmental applications.

In environmental applications, CuO-NPs exhibit excellent adsorption and catalytic properties, making them highly effective for removing organic dyes (e.g., methylene blue, methyl orange) and heavy metals from aqueous solutions (Singh *et al*., 2023). Their high porosity, reactive surface sites, and visible-light-driven photocatalytic activity enable degradation efficiencies exceeding 90% under optimized conditions.

SYNTHESIS APPROACHES

Several methods have been employed to synthesize CuO nanoparticles, including:

1. Physical Methods: Techniques such as laser ablation, thermal evaporation, and sputtering, which require sophisticated equipment and controlled conditions.
2. Chemical Methods: Approaches like sol-gel synthesis, co-precipitation, hydrothermal processing, and thermal decomposition, which allow precise control of nanoparticle size and morphology but often involve toxic reagents.
3. Biological Methods: Also known as biogenic synthesis, these use microorganisms, plant extracts, or other biological systems as reducing and stabilizing agents.
4. Green Synthesis: A sustainable approach that overlaps with biological methods, emphasizing eco-friendly, cost-effective, and non-toxic nanoparticle production using plant-derived phytochemicals.

Among these, the green synthesis method has gained increasing attention due to its advantages, including:

1. High surface area and stability
2. Strong biocompatibility
3. Safety and environmental friendliness
4. Use of bioactive phytochemicals as reducing and capping agents
5. Low toxicity and cost-effectiveness
6. Applicability in biomedical and wastewater treatment fields

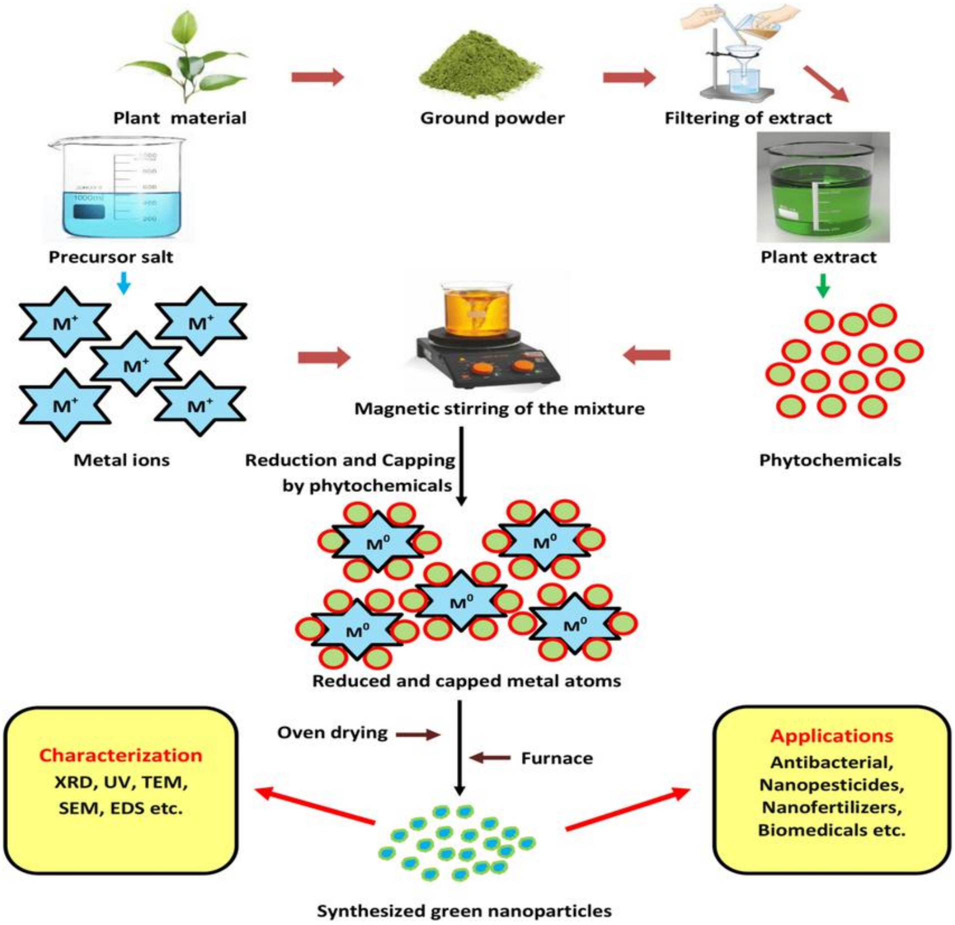

FIG 1: DIAGRAM SHOWING THE PRODUCTION FLOW NANOPARTICLES. (Shiraz *et al*., 2023)

1.2. THE PROBLEM/GAP IDENTIFIED

This study aims to advance the field of nanotechnology by developing a sustainable and environmentally friendly method for synthesizing copper oxide (CuO) nanoparticles using *Theobroma cacao* plant extract. It involves the green synthesis and comprehensive characterization of CuO-NPs, with a focus on their potential application in the removal of methylene blue dye from wastewater offering a promising approach to address environmental pollution through eco-conscious nanomaterial innovation.

1.3. SCOPE OF THE STUDY

This study investigates the green synthesis of copper oxide nanoparticles (CuO-NPs) using *Theobroma cacao* extract as a reducing and stabilizing agent. The nanoparticles will be characterized using standard analytical techniques to confirm their structure and properties. Their adsorption capacity for methylene blue dye will be evaluated through batch experiments, considering parameters such as contact time, pH, initial concentration, and temperature. The work is conducted on a laboratory scale with synthetic wastewater. The aim is to advance eco-friendly nanomaterial-based adsorption methods for wastewater treatment.

1.4. AIM AND OBJECTIVES

AIM OF THE STUDY

The aim of this research is to synthesize copper oxide nanoparticles (CuO-NPs) using *Theobroma cacao* extract, and to evaluate their efficiency in removing methylene blue dye from aqueous solutions.

OBJECTIVES

1. To synthesize copper oxide nanoparticles (CuO-NPs) using *Theobroma cacao* extract through a plant-mediated green synthesis method.
2. To characterize the synthesized CuO-NPs using a range of analytical techniques including Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Ultraviolet–Visible Spectroscopy (UV-Vis), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Spectroscopy (EDX),

3. To assess the adsorption performance of the synthesized CuO-NPs in the removal of methylene blue dye from synthetic wastewater.

1.5 SIGNIFICANCE OF THE STUDY

The contamination of water bodies with synthetic dyes like methylene blue poses major environmental and health risks, while conventional removal methods are costly and generate secondary pollutants. This study presents a green, economical route for synthesizing CuO nanoparticles (CuO-NPs) using *Theobroma cacao* extract, a renewable plant source rich in phytochemicals. The biogenic synthesis avoids hazardous chemicals and yields stable, functional nanoparticles with high surface area and potential antimicrobial and photocatalytic properties. The research emphasizes the application of CuO-NPs in dye adsorption, linking waste valorization with sustainable nanotechnology. The findings aim to support industries and communities seeking eco-friendly, cost-effective water purification strategies.

CHAPTER TWO

LITERATURE REVIEW

2.1. THEORETICAL REVIEW

In recent years, cacao has gained significant traction in both domestic and global markets, driven by evolving consumer preferences (Cruz and Cañas, 2018). Belonging to the Malvaceae family (Alverson *et al.*, 1999), *Theobroma cacao L.* thrives as a shade-loving tropical crop and serves as the backbone of the chocolate industry (Ramírez-Guillermo *et al.*, 2018). It is primarily cultivated in tropical zones located between 10° north and 10° south of the equator (Arvelo *et al.*, 2017).

Cacao is grown in warm and humid climates across more than 50 countries spanning Africa, the Americas, Asia, and Oceania. In the American continent alone, 23 nations produce cacao commercially, highlighting its economic and social relevance to the regions where it is cultivated (Arvelo *et al.*, 2017). Among the top producers in the Americas are Bolivia, Brazil, Peru, Colombia, and Ecuador, recognized for their substantial output levels (Batista, 2009).

The cocoa bean, a globally significant raw material, plays a central role in the production of chocolate and various cosmetic products (Araújo *et al.*, 2020; García-Ávila *et al.*, 2021). Originally native to the Amazon region (Motamayor *et al.*, 2002), cocoa has gradually adapted to a wide range of environments over the centuries. This adaptability is regulated by several endogenous factors, including plant physiology, hormone signaling, energy metabolism, nutrient uptake, cell division and expansion, and genetic mechanisms (Araújo *et al.*, 2020; Santana *et al.*, 2022). As a result of domestication and adaptation to distinct climatic conditions, numerous cocoa varieties have emerged (C ilas *et al.*, 2011). This process highlights the plant's genetic diversity and its capacity to respond or adapt to microclimatic fluctuations (Clément *et al.*, 2011; Lachenaud *et al.*, 2007; Pokou *et al.*, 2009; Schnell *et al.*, 2005; Turnbull & Hadley, 2018).

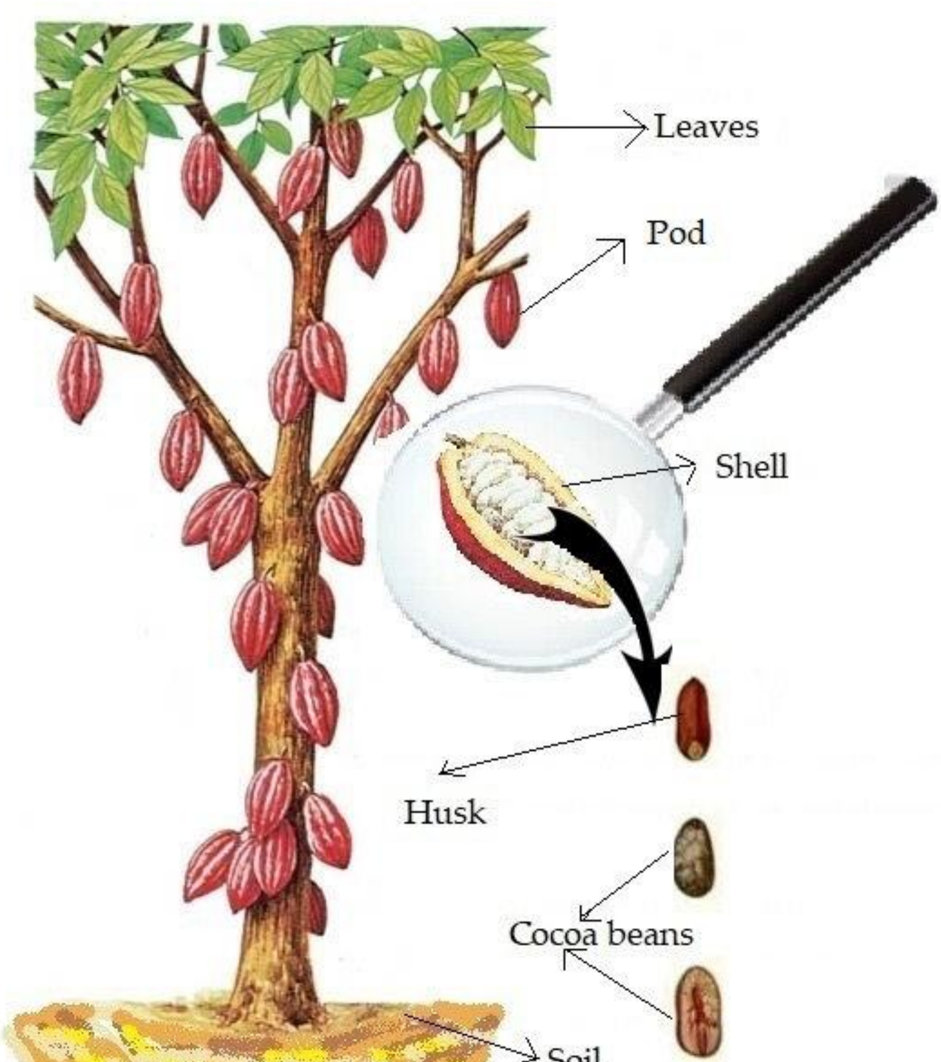

FIG 2: A PICTORIAL REPRESENTATION OF A WELL LABELLED THEBROMA CACAO TREE (Ramos Mejía *et al.*, 2022)

The cacao fruit, scientifically known as *Theobroma cacao*, is a tropical drupe of high agricultural and economic importance, primarily used in chocolate and cosmetic industries.

The fruit is an oblong to ellipsoidal pod, typically measuring between 10 to 32 cm in length and 6 to 15 cm in diameter. Its external surface has ten distinct longitudinal ridges and varies in texture from smooth to warty. As it matures, the color of the pod shifts from green to shades of yellow, orange, red, or purple, depending on the variety and stage of ripeness (Afoakwa, 2014; García-Ávila *et al*., 2021).

Internally, each pod contains 20 to 60 seeds (commonly referred to as cocoa beans), aligned in five rows corresponding to the ovary's compartments. These seeds are embedded in a white, mucilaginous pulp with a pleasant, sweet-sour flavor reminiscent of tropical fruits like lychee and melon. This pulp plays a vital role during fermentation, influencing the flavor profile of the final cocoa product (Motamayor *et al*., 2002; Santana *et al*., 2022).

Anatomically, the pod consists of three major layers: the epicarp (outer skin), mesocarp (fleshy middle layer), and endocarp (inner layer). The epicarp is relatively thin and degrades as the fruit ripens. The mesocarp contains large parenchyma cells rich in water and polyphenols, while the endocarp forms the mucilage surrounding the seeds. Histological analyses reveal that this mucilage is rich in pectins, proteins, and polysaccharides, facilitating microbial activity during fermentation (Araújo *et al*., 2020; Lanaud *et al*., 2017).

Cacao seeds themselves are ovoid to ellipsoid in shape, measuring 2–4 cm in length. Each seed is composed of an outer shell and inner cotyledons that serve as storage tissues during germination. The cotyledons are rich in lipids, proteins, and polyphenols, which later contribute to the sensory qualities of chocolate (Clément *et al*., 2011; Schnell *et al*., 2005).

Chemically, each fruit layer contains distinct compositions: the epicarp is high in hemicellulose and ash; the mesocarp is fiber- and cellulose-rich; and the endocarp contains proteins and fats. The mucilaginous pulp is also high in sugars, organic acids, and pectins, essential in fermentation (Afoakwa, 2014).

Native to the Amazon region, cacao has adapted to various tropical environments through centuries of domestication. This adaptation involves physiological, hormonal, and genetic mechanisms that have led to a wide diversity of cultivars suited to specific microclimates. Such diversity highlights cacao's evolutionary response to climatic variations and its potential resilience in the face of climate change (Motamayor *et al*., 2002; Turnbull & Hadley, 2018).

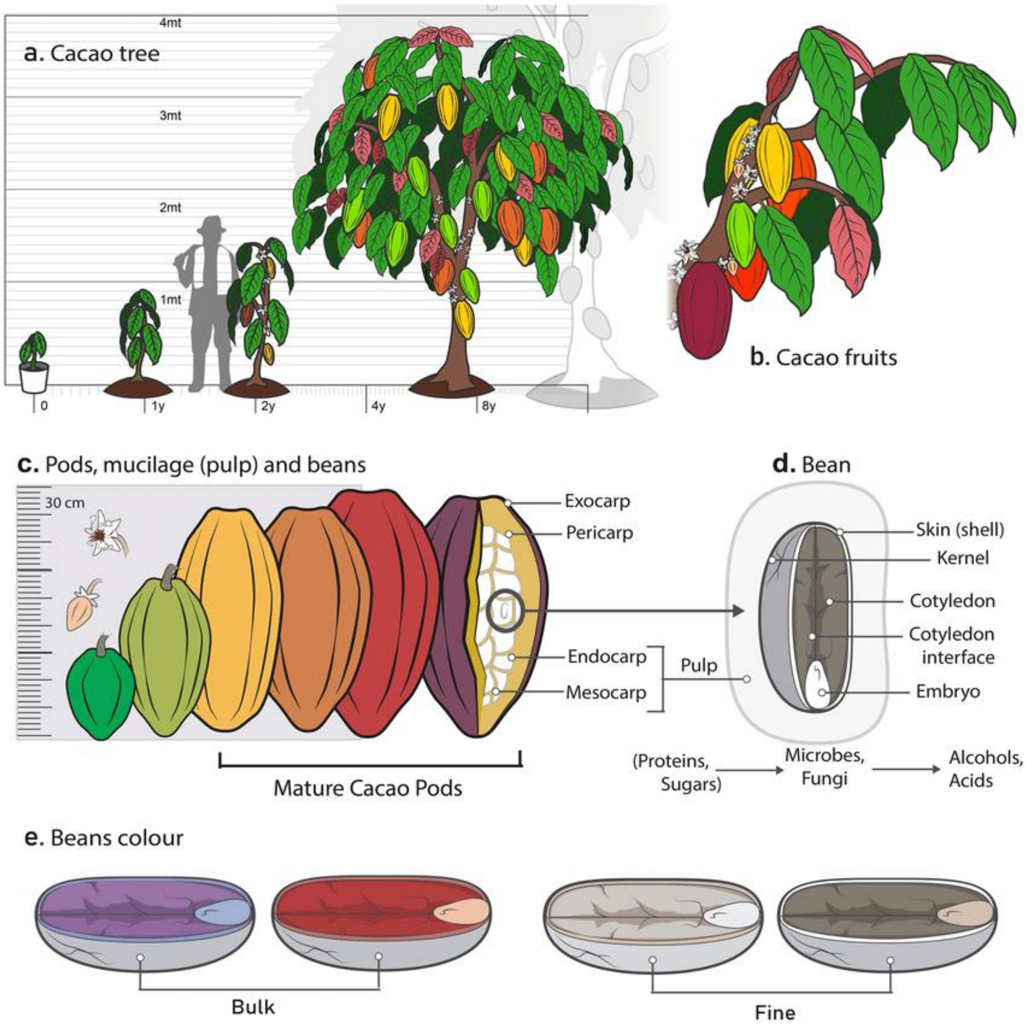

FIG 3: A PICTORIAL REPRESENTATION OF A WELL LABELLED CACAO (Rojas *et al.* (2022)

TABLE 1: TAXONOMY OF CACAO

RANK	**SCIENTIFIC NAME AND COMMON NAME**
Kingdom	Plantae – Plants
Clade	Tracheophytes – Vascular Plants
Clade	Angiosperms – Flowering Plants

Clade	Eudicots
Clade	Rosids
Order	Malvales
Family	Malvaceae – Mallow family
Sub-family	Byttnerioideae
Tribe	Theobromateae
Genus	*Theobroma*
Species	*Theobroma cacao* – Cacao tree
Binomial name	*Theobroma cacao* L.

(Schoch, C. L., *et al*. (2020)

2.2. PREVIOUS WORK ON THE STUDY REVIEW

GREEN SYNTHESIS OF METAL AND METAL OXIDE NANOPARTICLES

The synthesis of metal and metal oxide nanoparticles typically involves the use of a capping or stabilizing agent following the reduction of metal ions from salt solutions by strong bases such as sodium borohydride or sodium hydroxide. This bottom-up approach often relies on chemical reducing agents like sodium borohydride and hydrazine, along with capping agents, and sometimes employs volatile organic solvents such as toluene or chloroform. These solvents dissolve the stabilizers and reducing reagents, many of which are toxic and can leave harmful residues in the final nanosystem. Although these conventional methods may produce pure nanoparticles, they pose significant environmental and material costs during synthesis, prompting the need for safer and more sustainable alternatives (Ahmad *et al.*, 2003; Singh *et al.*, 2010).

Green synthesis has emerged as an eco-friendly alternative grounded in green chemistry principles, utilizing biological systems—including prokaryotic and eukaryotic organisms such as microbes, plants, and animals—to facilitate nanoparticle formation. This biological route can occur extracellularly or intracellularly, where primary and secondary metabolites from plants reduce metal ions, leading to the generation of metal and metal oxide nanoparticles. These metabolites also form a natural stabilizing coating on the nanoparticle surface, which prevents uncontrolled aggregation during synthesis (Bharde *et al.*, 2005; Dameron *et al*., 1989; Lee *et al*., 2011).

The properties and synthesis efficiency of these nanoparticles can be optimized by controlling factors such as temperature, pH, and reagent concentration. Various plant parts—leaves, seeds, bark, roots, and fruits—have been explored as bioreactors for nanoparticle synthesis, each with a unique phytochemical profile that affects the type and quality of the produced nanoparticles. The distinct biochemical composition of each plant tissue is influenced by its physiological role and the biotic and abiotic stresses experienced, which researchers must consider when selecting appropriate biological sources (Gopinath *et al.*, 2014; Joglekar *et al*., 2011; Vigneshwaran *et al*., 2006).

Nanoparticles can be synthesized through two main approaches: the "Top-Down" and "Bottom-Up" methods. The "Top-Down" approach involves breaking down bulk materials into nanosized particles through physical or chemical means, often leading to defects. Conversely, the "Bottom-Up" method builds nanoparticles from atoms or molecules via reduction/oxidation reactions, typically resulting in nanoparticles with fewer defects and more uniform chemical composition. Biological synthesis, a subset of the "Bottom-Up" approach, extensively employs microorganisms and plant extracts due to their intrinsic biochemical pathways and enzyme activities, which facilitate nanoparticle formation under optimized growth and reaction conditions (Singh *et al.*, 2010; Dameron *et al.*, 1989; Sweeney *et al.*, 2004).

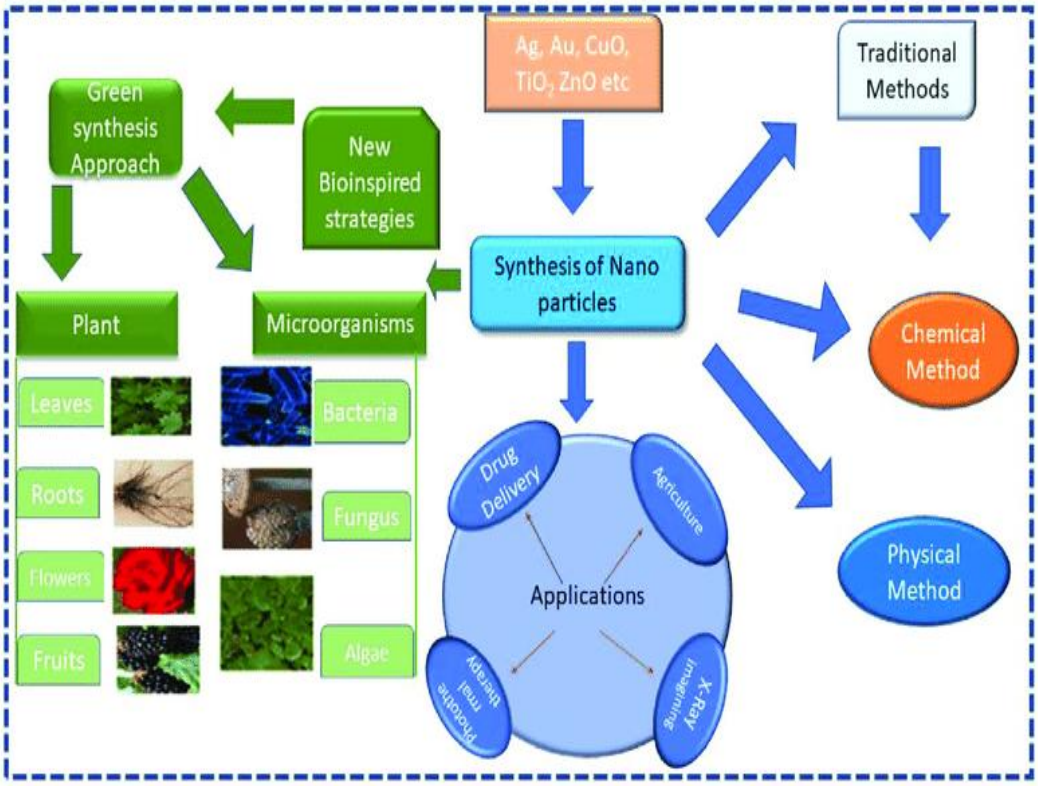

FIG 4. A SCHEMATIC DIAGRAM SHOWING GREEN SYNTHESIS, IT'S SOURCE, METHODS OF SYNTHESIS AND VARIOUS APPLICATIONS. (Singh *et al*. (2023)

Bacteria are often favored for nanoparticle (NP) synthesis over other microorganisms due to their ease of cultivation under controlled laboratory conditions and their relatively rapid growth rates. These microbes can tolerate high concentrations of metal ions and reduce inorganic substances to nanoparticles via extracellular or intracellular pathways. They absorb metal ions from their surroundings and enzymatically reduce these ions to their elemental forms. Using bacterial cells as bio-nanofactories, a variety of metal nanoparticles such as silver (Ag), gold (Au), magnesium (Mg), copper (Cu), selenium (Se), and iron (Fe), as well as metal oxide nanoparticles like silver oxide (Ag_2O), copper oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO_2), manganese oxide (MnO_2), magnesium oxide (MgO), and iron oxide (Fe_2O_3), have been synthesized. These biologically produced nanoparticles find applications in numerous areas, including the fabrication of third-generation biosensors, biofilm formation, biolabeling, cellular imaging, sensor devices, and diagnostic tools (Bharde *et al*., 2005; Singh *et al*., 2010).

Biological Applications of Green-Synthesized Metal and Metal Oxide Nanoparticles

Green-synthesized metal and metal oxide nanoparticles have shown significant promise in biomedical fields such as diagnostics, wound healing, tissue engineering, immunotherapy, regenerative medicine, dentistry, and biosensing technologies. Their biotoxicological profiles, particularly their antimicrobial, antifungal, and antiviral properties, have been widely explored. Additionally, these nanoparticles demonstrate potential as anticancer agents, antimicrobials, antioxidants, anticoagulants, as well as inhibitors of cell migration and proliferation (Gopinath *et al*., 2014; Singh *et al*., 2010).

1. Anticancer Agents: Metal oxide nanoparticles selectively target tumor cells, inducing cytotoxicity through mechanisms like reactive oxygen species (ROS) generation, apoptosis, necrosis, and modulation of signaling pathways such as p53 and related genes.
2. Antimicrobial Activity: Metal oxide nanoparticles have gained attention over the last two decades as effective alternatives against antibiotic-resistant microbes. Their unique physicochemical properties allow them to combat pathogens through multiple mechanisms, while also serving as drug delivery carriers, reducing the likelihood of microbial resistance development.
3. Antioxidant Agents: Despite concerns that metallic and metal oxide nanoparticles may induce oxidative stress, certain nanoparticles such as TiO_2, cerium oxide, and Fe_3O_4 exhibit

strong antioxidant activity. TiO_2, in particular, is widely used due to its low toxicity and protective role in cellular antioxidant defense systems.

4. Antiproliferative Agents: Metal oxide nanoparticles, including those of zinc, copper, iron, and cobalt, are known to selectively induce cytotoxic effects in various cancer cell types, either independently or synergistically with other anticancer treatments.

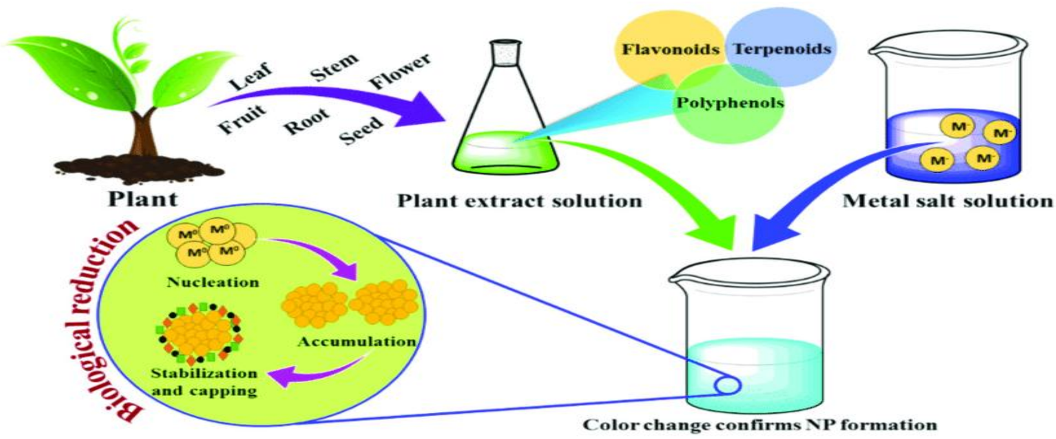

FIG 5: A SCHEMATIC DIAGRAM OF PLANT EXTRACT-MEDIATED SYNTHESIS (Singh *et al*., 2021)

Recent studies have demonstrated the potential of green synthesis of copper oxide nanoparticles (CuO-NPs) using various plant extracts as bio-templates and reducing agents. For example, *Suaeda maritima* extract was successfully employed to produce CuO-NPs with an average size of 34 nm, showing efficient methylene blue degradation due to the phytochemical-mediated stabilization of the nanoparticles (Sankar et al., 2022). Similarly, CuO-NPs synthesized using *Bergenia ciliata* rhizome extract exhibited nanoscale dimensions (~20 nm), spherical morphology, and significant photocatalytic and antibacterial activities, with characterization confirmed by UV–Vis, FTIR, XRD, SEM, and EDX analyses (Naseer et al., 2021). Other reports, such as the work of Khan et al.

(2020), have shown that porous CuO nanosheets with crystallite sizes around 12 nm and a high surface area (~30.9 m^2/g) achieved rapid dye degradation efficiency of up to 97% within minutes, highlighting their superior photocatalytic performance. Furthermore, the green synthesis of CuO-NPs using durian (*Durio zibethinus*) husk extract revealed strong optical absorption around 389 nm and a band gap suitable for visible-light photocatalysis, underlining the versatility of plant-mediated synthesis for environmental applications (Haq et al., 2023). Collectively, these findings confirm that CuO-NPs produced via biogenic routes possess high surface reactivity, semiconducting properties, and the ability to generate reactive oxygen species, making them highly effective for dye removal and sustainable wastewater treatment.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1. MATERIALS AND REAGENTS

In this study, the reagents used include commercially available methylene blue dye (the target pollutant), analytical grade Copper (II) Sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) as precursor for CuO nanoparticles, distilled water for synthesis, the fresh plant material (*Theobroma cacao* leaves), and sodium hydroxide (NaOH) for experimental procedures.

The materials used include beakers, magnetic stirrer, magnetic bar, conical flask, flat bottom flask, and burette.

3.2. METHODS

Preparation of *Theobroma cacao* Extract

Fresh *Theobroma cacao* leaves were collected from **BASIRI QUARTERS, ONDO ROAD, AKURE, ONDO STATE.** The leaves were thoroughly washed with distilled water to remove dust and impurities, and air-dried at room temperature under shade. The dried leaves were pulverized into fine powder using a clean mortar and pestle.

Approximately 25 g of the powdered sample was placed in a clean container with 400 mL of distilled water and allowed to macerate at room temperature for 48 hours with occasional stirring. After the maceration period, the mixture was filtered using Whatman No. 1 filter paper to obtain a clear aqueous extract. This extract served as the reducing and stabilizing agent in nanoparticle synthesis.

SYNTHESIS OF CUO NANOPARTICLES

0.01 M of Copper (II) Sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) was prepared by dissolving 1.249 g in 500 mL of distilled water. A ratio of 2:3 of plant extract and the precursor salt solution was poured into a 500 mL Erlenmeyer flask, and two drops of sodium hydroxide were added to adjust the mixture to an alkaline medium.

The mixture was heated on a magnetic stirrer for 30 minutes at 60 °C to ensure that the metabolites present in the plant extract reacted with the metal ions in the precursor solution. A light brown coloration was noticed after 10 minutes, confirming the formation of CuO nanoparticles. The resultant mixture was cooled and centrifuged at 400 rpm for 15 minutes to ensure separation and to increase yield and efficiency. After centrifugation, the CuO nanoparticles were washed with acetone to remove residual reactants and ensure uniform dispersion. The CuO nanoparticles were then dried and stored in a sample bottle for further characterization.

CHARACTERIZATION OF CUO NANOPARTICLES

X-ray Diffraction (XRD) Analysis

X-ray Diffraction (XRD) is a technique used to analyze the crystalline structure, phase composition, and mineral content of materials. It provides detailed information about the arrangement of atoms within a crystalline sample. XRD is commonly used to determine crystallinity, identify phases, and assess the mineral composition of materials, including nanocomposites.

The principle of XRD involves directing X-rays at the sample and measuring the angles and intensities of the diffracted rays. According to Bragg's Law, the pattern of diffraction peaks is indicative of the crystal structure and lattice parameters of the material. By analyzing these diffraction patterns, researchers can determine the degree of crystallinity, phase purity, and the presence of different minerals or phases.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is an advanced imaging technique designed to analyze the surface structure and morphology of samples with high magnification. Unlike light microscopy, which relies on visible light, SEM uses a focused beam of electrons to scan the sample, producing detailed, high-resolution images. This technique is crucial for examining the surface features and topography of materials, allowing researchers to visualize fine structural details that are otherwise invisible.

The principle behind SEM involves the interaction of a focused electron beam with the sample surface. When the electron beam strikes the sample, it generates various signals, including secondary electrons, backscattered electrons, and characteristic X-rays. These emitted signals are collected by detectors to create a detailed image of the sample's surface. The resulting images reveal intricate surface textures, pores, and structural features at the nanoscale.

Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared (FTIR) Spectroscopy is a technique used to identify and quantify functional groups in a sample by measuring its infrared absorption spectrum. This method provides insights into the molecular vibrations and chemical bonds present in the material. FTIR is particularly useful for characterizing organic and inorganic compounds, helping researchers understand the chemical composition and functional groups of a sample.

FTIR operates by passing infrared light through the sample and measuring the absorption at various wavelengths. Different chemical bonds absorb infrared light at characteristic frequencies, leading to a spectrum that represents the molecular absorption and transmission properties of the sample. The resulting spectrum allows for the identification of functional groups based on their unique absorption peaks.

ADSORPTION EXPERIMENT

Adsorption experiments were carried out at room temperature. To examine the effect of contact time, a 1-gram mass of CuO nanoparticles (CaO-NPs) was added to 200 mL of a 10 ppm methylene blue dye solution, which was constantly stirred. At various intervals (20, 40, 60, 80, 100, and 120 minutes), 5 mL samples were collected.

Methylene blue dye isotherm experiments were performed by altering the initial concentrations from 10 to 60 ppm. A 0.6 gram mass of CaO-NPs was added to 25 mL of methylene blue solution and stirred for the equilibrium time.

The effect of pH was studied by keeping the methylene blue dye concentration and CaO-NPs mass at 10 ppm and 0.2 g, respectively, and adjusting the pH from 3 to 11. The mixtures were then filtered and stored in a cool, dry place.

At equilibrium, the concentration of the methylene blue dye solution, Ce, was quantitatively determined using a UV-visible spectrophotometer at a wavelength of 665 nm. The equilibrium biosorption capacity of the process was calculated using Equations (1) and (2), which provide the adsorption percentage and amount of methylene blue dye adsorbed at time t, respectively.

[illegible] (1)

Q_t [illegible] (2)

Where C_0 (ppm) and C_e (ppm) are the initial and equilibrium concentrations of methylene blue dye, respectively. C_t (ppm) represents the methylene blue dye concentration in the solution after a contact time *t* (min), V (L) is the volume of the solution, and m (g) is the mass of the CaO-NPs. At equilibrium, the amount of methylene blue dye adsorbed was calculated as follows:

Q_e [illegible] (3)

Where C_0 (ppm) and C_e (ppm) are the initial and equilibrium methylene blue dye concentrations, respectively; V (L) is the volume of the solution, and m (g) is the mass of the CaO-NPs.

ADSORPTION ISOTHERMS STUDIES

Langmuir, Freundlich, and Temkin models are widely recognized as the most frequently utilized isotherm models for activated carbon in water and wastewater treatment. Thus, this study focused on applying these models to the adsorption isotherm analysis. The quantity of the methylene blue dye adsorbed on the various CuO nanoparticle (CaO-NPs) adsorbents (Q_t) was calculated using Equation (4) as shown below:

$Q_t =$ [illegible] (4)

In Equation (4), the volume of adsorbate (V) is expressed in liters (L), while the mass of adsorbent (m) is given in grams (g). The initial concentration of methylene blue dye in the adsorbate (C_0) is stated in mg/L, and the concentration of methylene blue dye remaining in the adsorbate at a specific time (C_t) is also given in mg/L. The concentrations of adsorbate that remain constant over time in various adsorption processes are recorded as equilibrium concentrations (C_e).

To determine the adsorption isotherm governing the removal of methylene blue dye from the adsorbate by the adsorbent, graphs of log Q_t against log C_e (for the Freundlich model), C_e/Q_e against C_e (for the Langmuir model), and Q_e against ln C_e (for the Temkin model) were plotted. Thereafter, the best-fitted equations from the plots were compared to the linearized forms of the Langmuir, Freundlich, and Temkin isotherm models, which were used to determine the adsorption capacities. The model that provided higher values of the coefficient of determination (R^2) was considered the isotherm governing the adsorption process.

Freundlich isotherm (Equation 5):

Log Q_t = [illegible] (5)

where Q_t is the amount of dye ions adsorbed on adsorbent in mg/g, C_e is the equilibrium concentration (mg/l),K_F is the Freundlich constant indicating adsorption capacity (mg/g), and n is the adsorption intensity (g/L).

Langmuir isotherm (Equation 6):

[illegible] (6)

where Q_e is the adsorption capacity at equilibrium (mg/g), C_e is the equilibrium concentration (mg/L), Q_m is the maximum monolayer adsorption capacity (mg/g), and K_L is the Langmuir equilibrium constant (L/mg).

Temkin isotherm (Equation 7):

$$Q_e = \frac{\bullet\bullet}{\bullet\bullet}\bullet\bullet\ \bullet\bullet\bullet\ \frac{\bullet\bullet}{\bullet\bullet}\bullet\bullet\ \bullet\bullet \qquad (7)$$

where B_t is a constant related to the heat of adsorption and A_t is the equilibrium binding constant (L/g).

The essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor (R_L), defined as:

$$R_L \bullet \frac{\bullet}{\bullet\bullet\ \bullet\bullet\ \bullet\bullet} \qquad (8)$$

Where Co is the initial concentration (mg/L), K_L is the Langmuir equilibrium constant (L/mg).

The value of separation factor (R_L) provides important information about the nature of adsorption. The value of (R_L) indicates the type of Langmuir isotherm to be irreversible ($R_L = 0$), favourable($0<R_L<1$), linear ($R_L =1$) or unfavourable ($R_L >1$). Apparently when $K_L>1$), sorption is favorable

ADSORPTION KINETICS STUDIES

The indigo dye adsorbed at equilibrium (mg/g) at different contact time t were determined and recorded as Qe through Equation (9) stated below:

$$Q_e = \frac{(\bullet\bullet\bullet\bullet\bullet)}{\bullet}\bullet\ \bullet \qquad (9)$$

All symbols in Equation (4) remain the same as previously explained. Graphs were plotted based on the linearized forms of Pseudo 1st and 2nd-order kinetics. The rate constants of adsorption were determined by comparing the equation of the best-fitted plots with the linearized forms of Pseudo 1st and 2nd order kinetics given in Equations (10) and (11), respectively.

$$\text{Log}\ (Q_e\text{-}Q_t) = \text{Log}\ (Q_e) - (\text{[illegible]})t \qquad (10)$$

[illegible] (11)

Equations (10) and (11) indicate that K_1 and K_2 are the rate constants for Pseudo 1st and 2nd order adsorption, respectively, with units of min^{-1} and $g.mg^{-1}.min^{-1}$. The other symbols in the equations are the same as those previously explained. The order of the kinetics governing the adsorption process was determined by examining the coefficient of determination (R^2) values associated with the best-fitted plots for the equation.

THERMODYNAMIC STUDIES

Thermodynamic parameters are essential in adsorption studies because they reveal the feasibility and nature of the adsorption process. In particular, the Gibbs free energy change ($\Delta G°$) is a key indicator, as a negative value at a given temperature confirms that the adsorption occurs spontaneously. The Gibbs free energy change ($\Delta G°$) can be determined using Equation (9):

$$\Delta G° = -RT\ \ln K_a \qquad (12)$$

The change in enthalpy ($\Delta H°$) and change in entropy ($\Delta S°$) were determined using the Equation,

$$\text{Ln}K_L = \text{[illegible]} - \text{[illegible]} \qquad (13)$$

Where **R** is the gas constant (8.314 J/mol K), **T** is the temperature in Kelvin (K), and; $\mathbf{K_L}$ is the Langmuir constant.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1. CHARACTERIZATION OF GREEN-SYNTHESIZED CUO NANOPARTICLES

4.1.1. UV-VIS ABSORPTION SPECTROSCOPY ANALYSIS

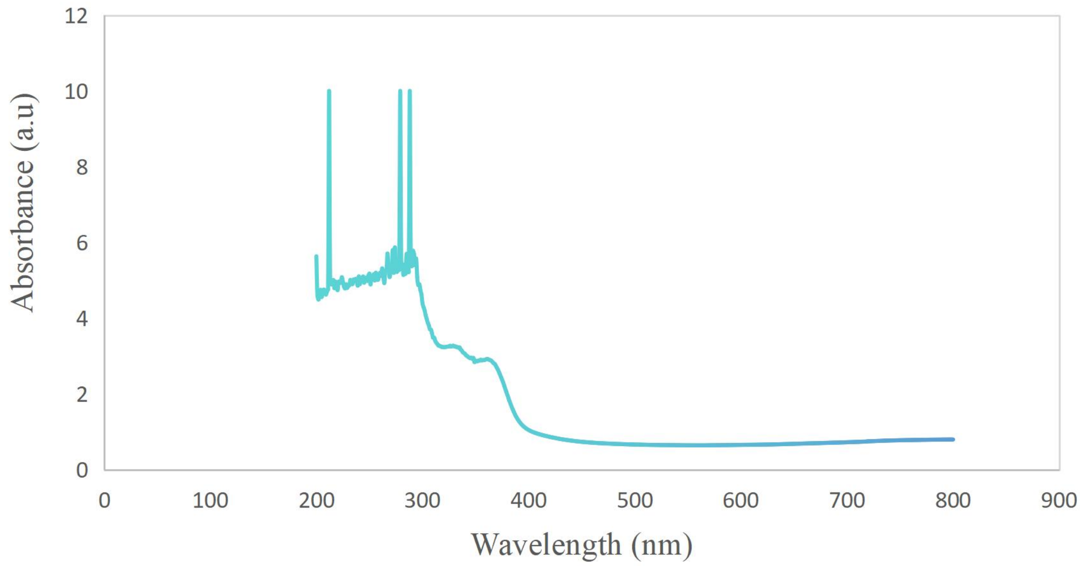

FIG 6: UV–Vis absorption spectrum of green-synthesized CuO nanoparticles using *Theobroma cacao* extract.

The optical properties of the biosynthesized copper oxide (CuO) nanoparticles were investigated using UV–visible spectroscopy at ambient temperature. The absorption spectrum (Fig. 6) displays strong absorbance in the UV region and a distinct absorption edge at approximately 400 nm. This edge is associated with the intrinsic electronic transition between the valence and conduction bands of CuO and indicates the formation of nanoscale CuO particles. The observed blue shift of the absorption edge relative to bulk CuO is consistent with quantum-confinement effects expected for small nanoparticles. The broad nature of the UV–Vis band and the absence of additional broad visible peaks suggest reasonably well-dispersed nanoparticles with limited agglomeration. Phytochemicals in *Theobroma cacao* extract likely acted as reducing and capping agents during synthesis, stabilizing the nanoparticles and contributing to the observed optical behavior. These UV–Vis results therefore confirm the successful green synthesis of CuO nanoparticles and agree with literature reports on biosynthesized metal-oxide nanoparticles.

4.1.2. SCANNING ELECTRON MICROSCOPE-ELECTRON DISPERSION X-RAY (SEM-EDX) ANALYSIS

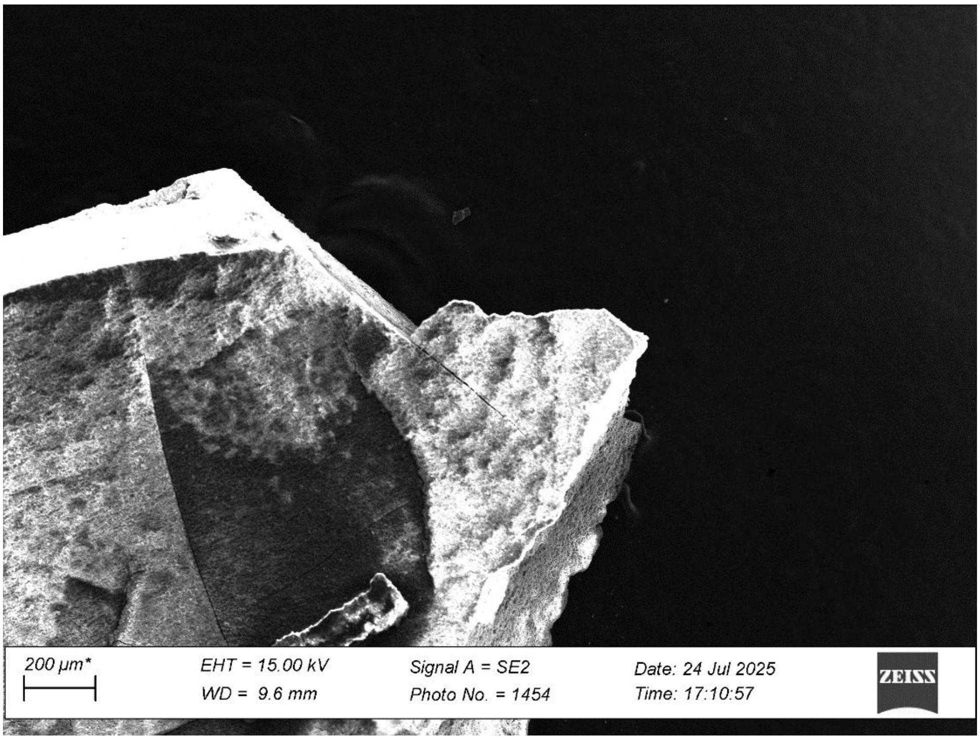

FIG 7a**:** SEM micrograph of biosynthesized CuO nanoparticles showing irregular plate-like morphology.

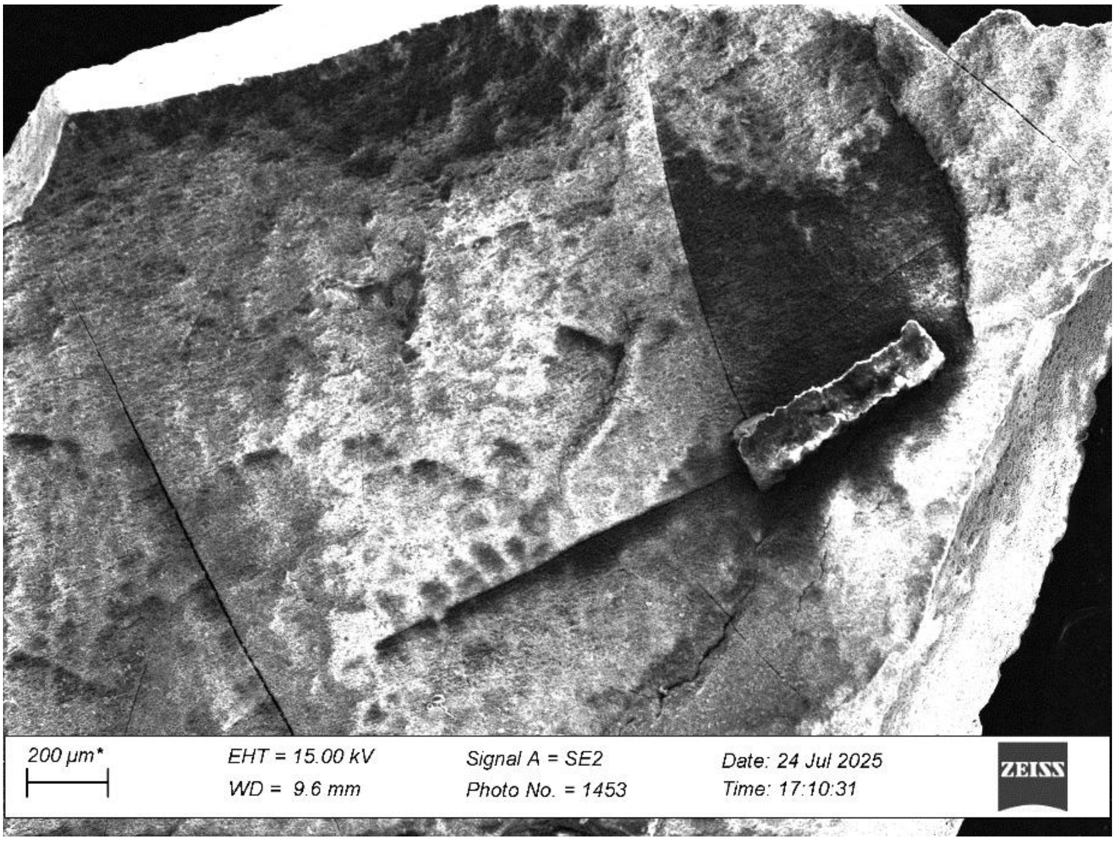

FIG 7b: SEM micrograph of CuO nanoparticles at higher magnification displaying aggregated structures.

EDX (ENERGY-DISPERSIVE X-RAY SPECTROSCOPY)

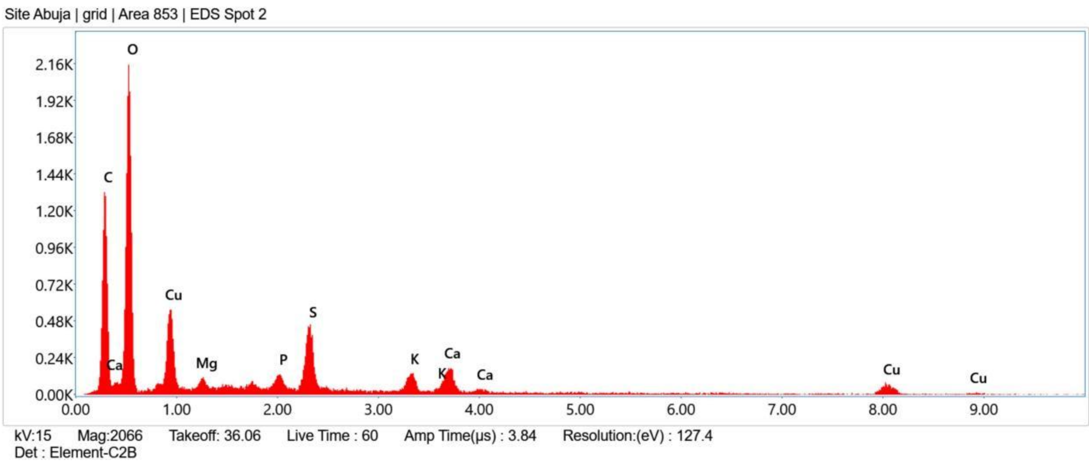

FIG 7c: EDX spectrum of bio synthesized CuO nanoparticles confirming Cu and O as major elements, along with a significant C peak from phytochemical residues and the carbon substrate.

Table 2: Elemental composition of biosynthesized CuO nanoparticles from EDS analysis

ELEMENT	**Weight %**
C K	46.4
O K	38.05
Mg K	0.26
P K	0.52
S K	2.89
K K	1.28
Ca k	2.39
Cu k	8.17

The surface morphology of the biosynthesized copper oxide (CuO) nanoparticles was examined using Scanning Electron Microscopy (SEM). The SEM micrographs (Figures 7a–b) reveal irregularly shaped, plate-like structures with rough and granular surfaces associated with pores that can enhance adsorption of pollutants. The particles appear aggregated, which can be attributed to interparticle interactions such as van der Waals forces and hydrogen bonding. Such agglomeration is commonly observed in green-synthesized nanoparticles due to the presence of phytochemicals from the plant extract, which function as both reducing and stabilizing agents. The observed morphology is consistent with the successful formation of nanoscale CuO, in agreement with previous reports on biosynthesized metal oxide nanoparticles (Nzilu et al., 2023; Gunalan et al., 2012).

Energy Dispersive X-ray (EDX) spectroscopy (Fig, 7c) was used to determine the elemental composition of the synthesized material. The EDX spectrum displays dominant peaks of copper (Cu) and oxygen (O), confirming the formation of CuO nanoparticles. A strong carbon (C) peak was also observed, which can be attributed to both the SEM sample holder/carbon tape and residual phytochemicals from the *Theobroma cacao* extract that capped and stabilized the nanoparticles. Additional elements, including magnesium (Mg), potassium (K), sulfur (S), calcium (Ca), and phosphorus (P), were present in smaller amounts, likely originating from natural biomolecules in the plant extract.

The high intensity of Cu and O peaks validates the formation of CuO nanoparticles, while the presence of carbon and other bio-elements further supports the involvement of *Theobroma cacao* phytochemicals in reduction and stabilization during synthesis. Collectively, the SEM–EDX results confirm the successful green synthesis of CuO nanoparticles using *Theobroma cacao* extract.

4.1.3 X-RAY DIFFRACTION ANALYSIS

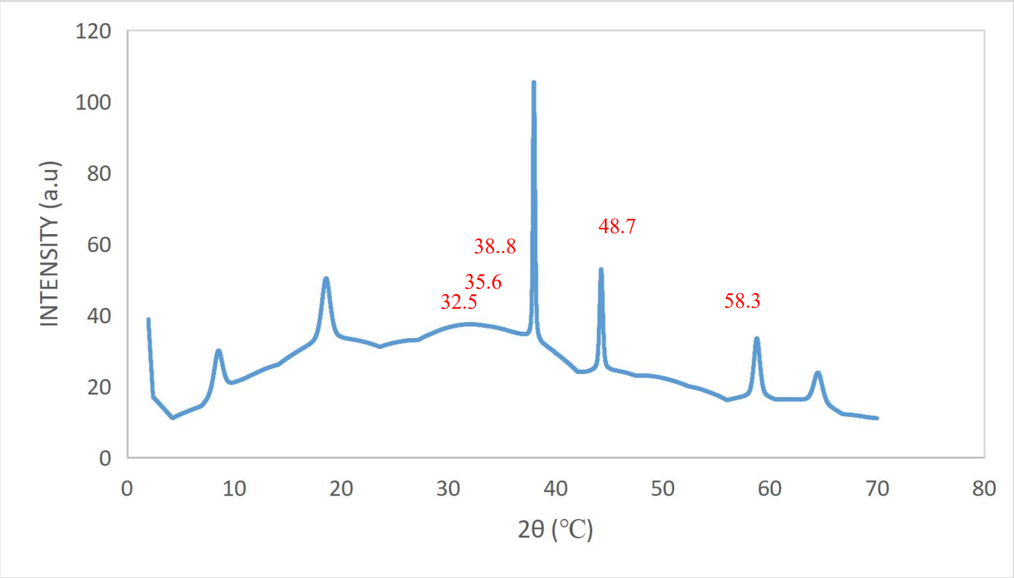

FIG 8: XRD pattern of green-synthesized CuO nanoparticles

The crystalline structure of the biosynthesized copper oxide (CuO) nanoparticles was examined using X-ray Diffraction (XRD). The diffraction pattern (Figure 8) displayed sharp and intense peaks, confirming the crystalline nature of the material. Prominent peaks were observed at 2θ ≈ 32.5°, 35.6°, 38.8°, 48.7°, and 58.3°, which correspond to the (110), (111), (200), (202), and (113) planes of monoclinic CuO, respectively, as indexed from the JCPDS card No. 48-1548. The absence of extraneous peaks indicates the high phase purity of the synthesized nanoparticles. The average crystallite size, estimated from the most intense diffraction peak at 35.6°, was approximately 20 nm, confirming the successful green synthesis of nanoscale CuO. These XRD results therefore validate the formation of pure, crystalline CuO nanoparticles and emphasize the role of *Theobroma cacao* phytochemicals in facilitating nanoparticle nucleation and stabilization.

4.1.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS.

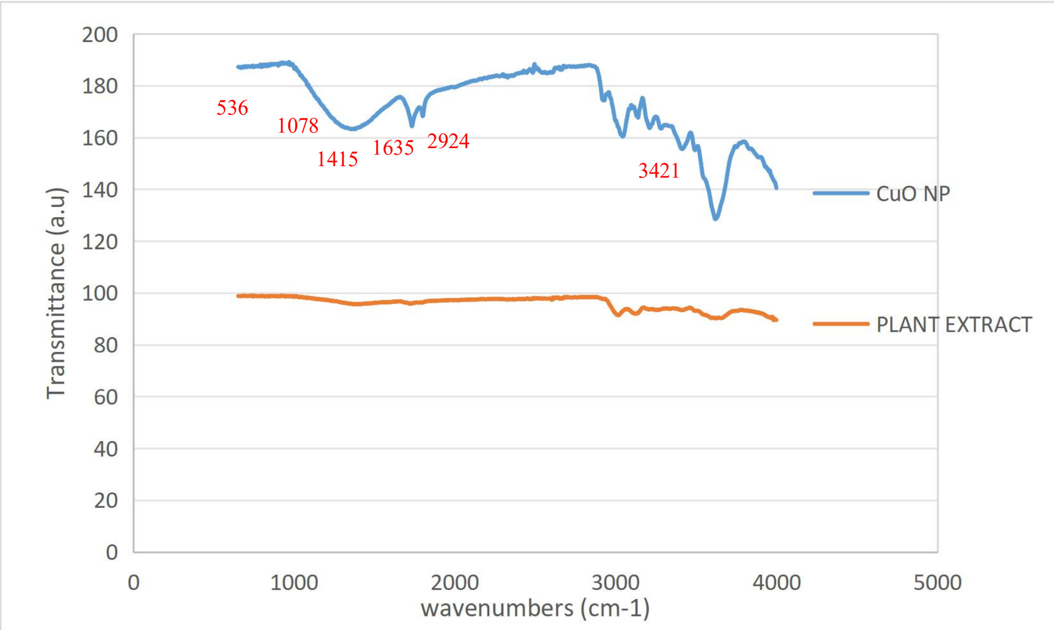

FIG 9: FTIR spectrum of bio-synthesized CuO nanoparticles showing characteristic functional groups from *Theobroma cacao* extract and the Cu–O vibration peak at 536 cm^{-1}.

The functional groups present on the surface of the biosynthesized copper oxide (CuO) nanoparticles were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR spectrum (Figure 9) revealed several distinct absorption bands attributed to phytochemicals from *Theobroma cacao* extract, which acted as reducing and stabilizing agents during synthesis. A broad absorption band observed at 3421 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups, indicating the presence of alcohols or phenolic compounds. The peak at 2924 cm^{-1} is assigned to aliphatic C–H stretching, while the band at 1635 cm^{-1} can be attributed to C=C stretching in aromatic compounds. The absorption at 1415 cm^{-1} represents O–H bending or C–N stretching vibrations, suggesting contributions from phenolic or amine groups. Furthermore, a peak at 1078 cm^{-1} corresponds to C–O stretching vibrations, characteristic of alcohols, ethers, esters, or polysaccharides present in the plant extract.

Most importantly, the distinct absorption band at 536 cm^{-1} is assigned to metal-oxygen bond which correspond to the Cu–O stretching vibration, which confirms the successful formation of CuO nanoparticles which in agreement with earlier report (Oyewole *et al.*2025) . These findings indicate that the phytochemicals in *Theobroma cacao* extract played a dual role as reducing and capping agents, facilitating the stabilization of the CuO nanoparticles.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

In this study, copper oxide nanoparticles (CuO-NPs) were successfully synthesized using *Theobroma cacao* extract through an eco-friendly green synthesis approach. Comprehensive characterization was carried out using FTIR, SEM, XRD, and UV analyses, which confirmed the structural, morphological, crystalline, and optical properties of the nanoparticles. The findings revealed that *Theobroma cacao* extract served as an effective reducing and stabilizing agent, enabling the formation of stable, environmentally sustainable, and biologically compatible CuO-NPs. These nanoparticles hold significant promise for applications in wastewater treatment and other environmental remediation processes.

5.2. RECOMMENDATION

Further research is recommended to optimize the synthesis conditions of CuO-NPs in order to enhance their efficiency, stability, and reproducibility. Future investigations should also:

1. Evaluate their effectiveness in treating real industrial wastewater under varying environmental conditions.
2. Explore the feasibility of scaling up production to meet practical environmental remediation needs.
3. Assess their potential biomedical applications, particularly in antimicrobial therapies and other therapeutic interventions.

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