SEED EXTRACT MEDIATED GREEN SYNTHESIS OF CALCIUM OXIDE NANOPARTICLES FROM SNAKE GOURD (*Trichosanthes cucumerina)* FOR PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL INVESTIGATION

BY

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PSC2010327

DEPARTMENT OF CHEMISTRTY

FACULTY OF PHYSICAL SCIENCE

UNIVERSITY OF BENIN

UGBOWO, BENIN CITY

EDO STATE.

FEBRUARY, 2025

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) HONOURS DEGREE IN PURE CHEMISTRY

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CERTIFICATION

This is to certify that the project work and research was carried out and submitted by **BROWNSON YETUNDE CORDELIA** with the matriculation number **PSC2010327** under the supervision of **Prof. (Mrs.) E.U IKHUORIA** from the Department of Chemistry Faculty of Physical Science, University of Benin, Benin city.

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DEDICATION

This study is wholeheartedly dedicated to God for his faithfulness and the gift of life, mercy wisdom and grace, and to my beloved late mum Omodele I. Brownson whose unconditional love and support inspired me to do my best during the course of this study.

ACKNOWLEDGEMNET

I would like to express my profound gratitude and appreciation to all those who gave me the possibility and have helped me in making this project, without their active guidance, help, cooperation and encouragement, I would not have been able to complete this project work.

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I would also like to extend my heartfelt gratitude with much appreciation to the Head of Department, **Prof. E.E Irabor**, my course advisor, **Dr. Jacob** and other lecturers in the Department of Chemistry and most especially the crucial role **Mr. Clinton**, **Dr. Hilary** and my team members played at every stage of this project which made the completion of this research work very possible.

Special thanks to my siblings; **Seun, Susan and Segun**, my best friend, **Clara Chinaza Okpala**, Miss. Miracle Iyere (CEO Mimiushers), my school daughter; Mabel, classmates and project team; **Enioluwa** and **Jeffrey** for the support and encouragement during the course of this project.

I would like to acknowledge that this project was completed entirely by me and not by someone else.

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ABSTRACT

In this research study, the preparation of Calcium oxide (CaO) nanoparticles (NPs) from *Trichosanthes cucumerina* seeds was reported to be synthesized by precipitation method using $CaCl_2$ and NaOH as starting materials. The gradual change of the particles from pale yellow to a white precipitate indicated the formation of CaO nanoparticles. The synthesized nanoparticle was subjected to characterization using Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD) and Dynamic Light Scattering (DLS). The Phytochemical analysis of the CaO nanoparticles revealed that the aqueous extract was rich in phytochemicals as majority of the phytochemicals tested were positive which resulted in the rational support for the traditional use of *T. cucumerina* to cure wounds. *T. cucumerina* has antibacterial components that are effective against wound pathogens such as S. *aureus*, E. *coli*, B. cereus, K. *pneumoniae*, P. *aeroginosa*. The extracts displayed antibacterial activity against both gram (+) and gram (-) bacterial stain such as indicating the existence of a broad spectrum of antibacterial properties.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Nanoparticles are tiny particles that are typically between 1-100 nanometers (nm) in size and can exhibit significantly different physical and chemical properties based on their larger material counterparts. They occur widely in nature. Nanoparticles have unique properties that make them useful in a wide range of application including; medicine, electronics, energy, environmental remediation and cosmetics. An example of nanoparticle is Soot particles from forest fires can be as small as 10 nanometers in diameter.

Various methods have been used for the preparation of Calcium oxide nanoparticles and other nanomaterials (like NiO, FeO, ZnO, Ag and Au) such as Sol-gel, thermal decomposition, hydrothermal technique, combustion method, co-precipitation technique, biogenic method, precipitation method, two-step thermal decomposition technique, one-step multi-component synthesis and microwave synthesis (Gupta, *et al*., 2016).

Varying these methods, all physical and chemical properties of calcium oxide nanoparticles can be changed; morphology, specific surface area and capturing efficiency can be carefully controlled under specific synthesis conditions Kulkanrni & Wachs (2002). The main methods used in the synthesis of nanoparticles are Chemical approach and Green approach.

In nanoparticles synthesized by Chemical reduction, metal containing salts (denoted as precursors) are dissolved and chemically reduced in an appropriate solvent. The chemical approach involves electrochemical, microwave, solvothermal, micro-emulsion.

Green synthesis includes plant-based, fungi-based and bacteria-based synthesis. It involves using natural sources such as plants, microorganisms or bio molecules to synthesize nanoparticles in an environmental friendly and sustainable manner. Nanoparticles are synthesized using different plant parts such as root, fruit, stem, seed and leaf. The exact mechanism for synthesis of nanoparticles using plant remains to be elucidated.

The advantage Green synthesis have over chemical reduction are as follows; it offers a more sustainable, environmentally friendly, minimal pollution, energy efficient, less hazardous, safe product, lesser waste, improved safety and cost-effective alternative as well as competitive advantages rather than chemical synthesis.

Calcium oxide (CaO) also referred to as Lime is an important organic compound that can be used as a catalyst (Bharti *et al*., 2019), pellet for CO_2 capture and kinetic analysis (Zhang, *et al*., 2018), toxic waste remediation agent, or as an additive in refractory and paint industries (Arul *et al*., 2018), antimicrobial agent, a drug delivery agent as well as in various other biomedical applications. Among all the nanoparticles previously discussed in history, CaO nanoparticles have attracted numerous attractions.

Calcium oxide nanoparticles have been shown to exhibit unique catalytic and antibacterial properties against a range of bacteria. The mechanisms of bacterial activity include; cell membrane disruption, generation of reactive oxygen species, DNA damage and Protein denaturation. The factors affecting antibacterial activity include; particle size, concentration, pH, type of bacteria. The antibacterial functions of CaO nanoparticles can be applied in wound healing, waste treatment, food packaging and medical implants in order to prevent bacterial infections.

The primary motivation for synthesizing CaO NPs using a biological method is to suppress the usage of hazardous chemicals used in making its process, which are more cost-effective and ecologically profitable. However, due to the complexity of the biological extracts employed in chemical processes, large scale manufacturing of nanoparticles through green synthesis approach remains a significant problem. As a result of this problem, the production of CaO NPs using *Trichosanthes cucumerina* seed extract as an alternative biological agent for capping, stabilizing and reducing agents due to rich phytochemical parameters in the synthesis was inestigated in this study.

In recent research, *Triconsanthes cucumerina* aqueous seed extract have not been used to synthesize calcium oxide nanoparticles. *T. cucumerina* plant seeds extract was used in this study because previous studies recorded that it has a unique properties and presence of bio-reductive agents. The use of Snake gourd in the synthesis of CaO nanoparticles offers a green, cost effective and efficient approach. The bioactive compounds in *T. cucumerina* act as reducing and stabilizing agents, ensuring the formation of uniform stable CaO nanoparticles.

1.1.1 BACKGROUND OF STUDY

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential antimicrobial agents.

It has long been recognized that phytochemicals present in a plant material can function as a biological reduction on metal and metal oxide synthesis Nair & Jadhav, (2020) with Falvonoid compound being one of the most influential families of the secondary metabolites in plant tissues for metal ion reduction. The ability of Plant extracts to reduce metal ions has been known since 1900s, although the nature of the reducing agents involved was not well understood. In view of its simplicity, the use of plant extracts for reducing metal salts to nanoparticles has attracted considerable attention within the last 30 years (Ankamwar, 2010; Armendariz *et al*., 2004).

Phytochemicals are compounds found in plants that are required for normal functioning of the body (Leonard, 2005) and plays an active role in the amelioration of disease. It helps to reveal the constituents of the plant extracts (seeds and leaves) as well as the one that predominates over the others. It is also helpful in searching for bioactive agents that can be used as dietary supplement. It has been illustrated that secondary metabolites are responsible for the synthesis of various types of nanoparticles.

Present day pharmaceutical companies are in search of plants which have diverse secondary metabolites such as tannin, flavonoids, saponins and others of which, at least 12,000 have been isolated and this can estimate to about 10% of the total secondary metabolite (Mallikharjuna *et al*., 2007), these secondary metabolites are also used commercially as flavoring and coloring agents (Vishnu *et al*., 2019).

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. This study focused on the use of Antibacterial testing method for the in vitro investigation of plant extracts and pure drugs as potential antimicrobial agents. Antibacterial activity is described as the action of eliminating or preventing bacteria proliferation. It is also defined as a result of microorganisms contact surface area (Yetisgin *et al*., 2020). A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound.

The most common and basic methods are the Disk diffusion and broth or Agar dilution methods, to further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometry methods are recommended which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic), (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism.

According to existing research, the major processes underlying the antibacterial effects of NPs are disruption of the bacterial cell membranes, generation of ROS, penetration of the bacterial cell membrane, induction of intracellular antibacterial effects including interactions with DNA and proteins.

Research on *Trichosanthes cucumerina* (snake gourd) indicates its potentials as a medicinal plant and food source, with studies exploring its anti-diabetic, anti-inflammatory and nutritional properties (Arawwawala *et al*., 2009; Nadeeshani *et al*., 2016).

The aqueous extract of the seed is used for treatment of soft tissue wounds and fungi attach in children so there is need to know the chemical information about the seeds of *T. cucumerina* to improve our knowledge about the antibacterial component.

1.1.2 STATEMENT OF PROBLEM

Snake tomato is a neglected and underutilized crop because its cultivation is used as an alternative to the regular tomatoes. The pulp is known to provide protection against harmful free radicals (Uwummarongie *et al*., 2013). The scarcity of this plant made it difficult for the collection of the fruits. Conventional nanoparticle synthesis methods involve harsh conditions, high cost and environmental pollution.

T. cucumerina in Nigeria is fast going into extinction because most crop scientist stopped cultivating the crop and they are rarely found in home garden. In spite of its serpentine name in local Yoruba parlance, snake tomato emerges as a beacon of nutritional abundance and economic opportunity waiting to be rediscovered and embraced by farmers and consumers.

Despite the enormous benefits of snake tomato, its production is hindered by decline in soil fertility, inadequate knowledge on its nutritional potentials, pests and agronomic practices. Crop management practices especially soil fertility management sustains the yield and quality of any crop (Ani and Baiyeri, 2008).

1.1.3 RELEVANCE OF STUDY

This significance of this research focuses on the phytochemical screening of Calcium oxide nanoparticles of T. cucumerina aqueous seeds extract and its study regarding the antibacterial activity of the nanoparticles because there is lack of scientific reports on using the extract of snake tomato seeds as a biologically reductive and capping agent on the biosynthesis of CaO nanoparticles.

In recent years, the development of efficient green methods for the synthesis of metal nanoparticles has become a major focus. One of the most considered methods is the production of metal nanoparticles using plants (Ankanna *et al.,* 2010).

Even though several studies were concerned with the synthesis of Ca/CaO nanoparticles using biological routes in micro-organisms only (Long *et al*., 2009). The green synthesis of CaO nanoparticles by plant material is not carried so far. Hence, the present study was an attempt to synthesize the nanoparticles from seed extract of *Trichosanthes cucumerina* Linn. and test for the presence of phytochemicals to investigate antibacterial properties.

1.1.4 SCOPE OF STUDY

This study seeks to extract phytochemicals from *Trichosanthes cucumerina* seeds and use the phytochemicals to synthesize Calcium oxide nanoparticles and characterize the nanoparticles then apply it towards the application of antibacterial activity. The synthesized nanoparticles were characterized using techniques such as XRD, FTIR, SEM, DLS and TEM.

(a). X-Ray Diffraction is a technique used to determine the crystal structure of a material. The principle is based on the diffraction by the regular arrangement of atoms in a crystal lattice and it provides information about their crystalline structure, phase identification, crystallite size and lattice strain. (Banfield *et al*., 2001)

(b). Dynamic Light Scattering is a technique used to measure the size of distribution of particles in a suspension. It determines the hydrodynamic size distribution of nanoparticles in a solution revealing the particle size, colloidal stability and size distribution. The principle is based on Brownian motion and scattering of light by particles (Kumari *et al*., 2010).

(c). Fourier Transform Infrared Spectroscopy provides information on the chemical structure and functional groups found on nanoparticle surfaces which aids in the characterization of surface modifications and coatings (Yang *et al*., 2011).

(d). Scanning Electron Microscopy is a powerful technique used to characterize nanoparticles by providing detailed information about the size, shape, morphology, surface features (de la Rica 2010), allowing researchers to visualize and analyze the structure of nanoparticles on a microscopic scale, particularly their surface topography, with high resolution imaging capabilities often combined with Energy Dispersed X-ray spectroscopy (EDS) to identify elemental composition within the nanoparticles.

(e). Transmission Electron Microscopy is a technique used to characterize nanoparticles by imaging them with an electron beam. It produces high resolution images of nanoparticles like quantum wells, nanowires and quantum dots, allowing for visualization of their size, shape and morphology (Hirsch *et al*., 2003).

1.1.5 AIM

This study was therefore designed to investigate the phytochemical used to synthesize Calcium oxide nanoparticles and test for antibacterial activity.

1.1.6 OBJECTIVES

To achieve the above aim, the following objectives were considered;

- Preparation of the plant extract
- Phytochemicals screening of the extract
- Synthesizing the calcium oxide nanoparticles using the plant extract
- Characterization of the synthesized Calcium oxide nanoparticles
- Antibacterial application of the nanoparticles

1.2 LITERATURE REVIEW

The plant, *Trichosanthes cucumerina Linn*. is commonly called Snake tomato, snake gourd, viper gourd or long tomato. The common name "snake tomato" is derived from the *snake-like* shape of the fruit. They belong to the family Cucurbitaceae (Adebooye, 2008; Ojiako and Igwe, 2008, Sandyha *et al*., 2010). Robinson and Decker-Walters (1997) documented that the family Cucurbitaceae contains about 70 genera and over 700 species worldwide. The genus, Trichosanthes comprises about 100 species with only few domesticated in Asia and snake tomatoes being the prominent one.

T. cucumerina is a herbaceous annual climber that can grow to 5 meters (Brickell, 1990) with perennial root stock, it is a hairy vine bearing distinct fringed white flowers that opens at night. They are grown mainly as ornamentals in relation to other edible Cucurbits like pumpkin, melon and cucumber. The fruits are up to 150cm long (about 59 inches in length), very slender and curved. The roots are somewhat tuberous and whitish while the leaves are alternate, simple with no stipules.

Trichosanthes cucumerina is found wild across much of South and Southeast Asia (Echo, 2006) including India, Bangladesh, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar(Burma) and southern China (Guangxi and Yunnan). It is also regarded as native in northern Australia and naturalized in Florida, parts of Africa and on various islands in the Indian and Pacific Oceans. It was probably domesticated in ancient times in India (Echo, 2006).

In most western Africa, the young unripe fruits are eaten as vegetables (Echo, 2000) while the mature ripe fruits are blended into paste and used as substituent for tomato puree (Enwere, 1998; Badejo *et al*., 2016) to make stew and broths after the removal of the seeds. The ripe endocarp pulps are usually deep red with sweet aromatic taste (Adebooye *et al*., 2007; Deepa, 2017). Extracted juice from the leaves stimulate vomiting in case of consumption of toxic substances (Devi, 2017).

Onagoruwa (2002) established that the pulp does not turn sour easily compared to the solanaceous tomato (*Lycopersicon esculentum L.*), the fruit pulp have been reported to be a very good source of ascorbic acid (Vitamin C) with low oxalate content (Adebooye and Oloyede, 2005) essential amino acids, calcium among other nutrients than most regular tomatoes.

The immature fruit has distinct broad intense green and narrow pale stripes while the mature fruit is fibrous and can be sweet. Usually, only the immature fruits are used for culinary purposes. Along with other plants components, the aerial parts of T. *cucumerina* are used to treat indigestion, bilious fever, boils, sores, and skin eruptions like eczema, dermatitis, psoriasis, inflammation, ulcers and diabetes.

Onagoruwa (2002) noted that there are two types of snake gourd in Nigeria. Both types have long fruits but they differ in color, one is deep green while the other is light green. The snake gourd has slightly twining stem (Grey-Wilson and Mathews, 1983). The stems are always slender, green, hairy and four angled with unpleasant odor (Sandhya *et al*., 2010).

Table.1 Nutritional facts of Snake Gourd Fruit

S/N	Food Property	Composition
1.	Calories	86.2g
2.	Total Fat	3.9g
3.	Saturate Fat	0.5g
4.	Polyunsaturated Fat	1.6g
5.	Monounsaturated Fat	1.6g
6.	Total Carbohydrate	12.5g
7.	Dietary Fibre	0.6g

Source: DrHealthBenefits.com

Plate.1 Unripe T. *cucumerina* Fruit

Snake tomato is propagated by seed and it produces tendril bearing vines that will sprawl if not supported. The seeds are thick, brownish and sculptured with sizes ranging from 1-1.5cm long. They comprise profuse amounts of the antioxidants lycopene and bioflavonoid glycosides which significantly improve insulin activity, synthesis in the pancreatic beta cells, used as purgative, astringent and treatment of diabetes (Song *et al*., 2012; Adebooye, 2008).

The seeds, when dried are used in alternative medicine for treating anthelminthic and diarrhea. It has been reported that the seeds contain antibacterial (Yusuf *et al*., 2007) which may make it a potential insecticide. **Table. 1** represent the reported proximate composition of the seed. Idowu (2015) reported 45% oil content for the seed. The oil content of the seed compared favorably with that of most seed oil, they noted that this anti-nutritional oxalate is low and safe for humans.

Skumarlabot (2007) noted that other parts of the plant such as shoots, tendrils and leaves are eaten as vegetables. However, it was recommended that these parts should be boiled before eaten to remove some unpleasant odors (Echo, 2006).

Plate.2 Dried seeds of snake gourd

Recently, there has been a lot of attention focused on producing medicines and products that are natural to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms. The world health organization[2] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents.

The presence of phytochemical constituents in medicinal plants have impressive pharmaceutical properties such as analgesics, aesthetic, antibiotics, anti-parasitic, anti-inflammatory, oral contraceptive, hormones and ulcer therapeutic laxative which made them useful for healing as well as for curing of human diseases.

Table.2 Nutrient Composition of *T.cucumerina* seeds

Nutrients	**Composition**
Crude protein	26.2-26g/100g
Fat	44.6-57.2g/100g
Phosphorus	78.0-81.5mg/100g
Calcium	41.0-46.7mg/100g

Source: Oloyede and Adebooye (2005)

1.1.1 CLASSIFICATION OF *Tricosanthes cucumerina*

Habitat: Cultivated farmland but also grow on fences

Description of plant: Herb, climbing by means of tendrils

English name: Snake gourd

Regional name: Chichinga/Chichinge (Bengali), Potlakaaya (Telugu), Chachinda (Hindu).

Common name: Snake tomato, long tomato, viper gourd (Ojiako and Igwe, 2008)

Local name: Tomato Uduk Ikot (Ibibio), Tomato elejo (Yoruba), Otutu ala (Igbo)

Table. 3 Botanical classification of Snake Gourd

Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	*Trichosanthes*
Species:	***T. cucumerina***
Binomial name	
Trichosanthes cucumerina	

Calcium oxide (CaO) nanoparticles display a remarkable structural and optical effect so they can be used in several applications. Calcium oxide nanoparticles offer several benefits including antimicrobial activity, potential drug delivery and applications (through their ability to generate reactive oxygen species ROS which ultimately leads to the death of the microbes) in environmental remediation and agriculture making a promising material for various fields. The following are some benefits of calcium oxide nanoparticles;

- Antimicrobial Activity – It was said to be due to the release of active oxygen and the alkaline pH when CaO NPs dissolve in water, leading to damage of bacterial cel membranes.

Broad-spectrum activity: Cao NPs exhibit activity against various bacteria, fungi, and even endotoxins.

Anti-biofilm agent: They can effectively disrupt and prevent the formation of biofilms, which are bacterial communities that can be difficult to treat.

- Biomedical Applications

Drug delivery: Cao NPs can be used as a potential drug delivery agent due to their biocompatibility and ability to encapsulate and release drugs.

Wound healing: The potential of CaO NPs in wound healing applications has been investigated (Nandhini *et al*., 2024).

Dental applications

Photodynamic and Photo-thermal therapy: The unique structural and optical properties make them suitable for use in photodynamic therapy (PDT) and photo-thermal therapy (PTT).

- Environmental Remediation

Water purification: CaO NPs can be used for the adsorption of heavy metals and other pollutants from water

Soil remediation: They can help mitigate heavy metal toxicity in plants and soils, improving plant growth and food security

Arsenic toxicity: CaO NPs have been shown to alleviate arsenic toxicity in barley, a common problem in some regions

- Other Applications

Catalysis

Gas adsorption

Food preservation

Agriculture

Industrial applications

1.2.2 PHYTOCHEMICAL SCREENING

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract such as alkaloids, flavonoids, tannins, saponins, sterols, terpenes, phenols, terpeoids, cardiac, glycosides, protein, carbohydrates and fats. Plants exhibit a wide range of structural and biological diversity they contain phytochemicals which have a significant inhibitory effect on the growth of pathogens.

Phytochemicals based on their metabolism activity are primarily categorized into two groups (Krishnaiah 2007).

- Primary metabolites – They are essential and needed for daily growth and maintenance and are found in every cells such as sugars (like fructose, glucose, maltose, lactose), amino acids, nucleic acids and chlorophyll.
- Secondary metabolites – These are small molecules which are not needed for the growth and maturity of the plant but they are crucial for plant survival and have significance characteristics like color, aroma and taste. They consist of alkaloids, flavonoids, saponins, tannins, phenolic compounds and many more. The secondary metabolites will be studied in this work

Alkaloids – They are one of the main and largest components produced by plants and they are metabolic byproducts that are derived from the amino acids (Naseem 2014).

Flavonoids – They are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Dixon *et al*., 1983). Available report tends to show that secondary metabolites of a phenolic nature including flavonoids are responsible for the variety of pharmacological activities (Mahomoodally *et al*., 2005; Pandey 2007).

Tannins – They are widely applied to a complex large biomolecule of polyphenol nature, having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Navarrete 2013).

Saponins – They are an important group of plant secondary metabolites that are widelyspread throughout the plant kingdom. They are basically phytochemicals that are found in most vegetables, beans and herbs (Francis *et al*., 2002; Haralampidis *et al*. 2002).

Steroids – They are a large group of chemical substances classified by a specific carbon structure. They include drugs used to relieve swelling, inflammation such as cortisone; vitamin D and some sex hormones such testosterone and estradiol (Hill *et al*., 2007).

Terpenoids – They are small molecular products synthesized by plants and are probably the most widespread group of natural products. They show significant pharmacological activities such as antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Boroushaki *et al*., 2016).

Phenolic compounds – They are secondary metabolites which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization (Derong Lin *et al*., 2016).

Phytochemicals can be extracted from plant materials using different extraction techniques. Traditional methods such as decoction, maceration, percolation, infusion, digestion, soxhlet extraction have been widely used. Recently, environmental friendly methods 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, ethanol, hexane, acetone, benzene, ether and chloroform are used in the extraction of phytochemicals (Tiwari *et al*., 2011). The extraction of phytochemical depends on both the pre-extraction process such as (drying method, moisture content, part of plant used) and also the extraction factor such as (solvents, temperature, PH).

(a) Decoction

This is a process that involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded and powdered plant material is placed in a clean container, water was added and stirred continuously. The ratio of crude plant part to solvent is usually 1: 4 or 1: 16 Heat is then applied throughout the process to hasten the extraction process.

(b) Maceration

This is an extraction procedure in which coarsely powdered plant part either leaves, steam, seeds, fruits, bark is placed in a container; the menstruum is poured on top until it completely covers the powder. The container is then closed and kept for at least three days. The content is stirred periodically to ensure complete extraction. After extraction, the micelle is separated from the marc by filtration or decantation.

(c) Digestion

This is an extraction method that involves the use of moderate heat. Heat should be applied throughout the extraction process to decrease the viscosity of extraction solvent and enhance the removal of secondary metabolites.

(d) Infusion

Fresh infusions are prepared by macerating the crude plant part for a short period of time with cold or hot water. These are dilute solutions of the readily soluble constituents of crude plant parts.

(e) Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. An apparatus called *Percolator*, a narrow cone-shaped glass vessel with opening at both ends is generally used for the extraction.

1.2.3 ANTIBACTERIAL INVESTIGATION

Antibacterial activity investigates the ability of a substance, typically a plant extract, chemical compound or natural product to inhibit or kill bacteria usually by evaluating its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against various bacterial strains using methods like agar diffusion or broth dilution assays, and often aiming to identify potential new antimicrobial agents to combat antibiotic resistance.

Antibacterial agents can target the cell wall, cell membranes and other parts of the bacterial cell, they also affect DNA replication and protein synthesis. The antibacterial activity was screened against Gram positive (such as *Staphylococcus aureus* and *Bacillus cereus*) and Gram negative (such as *Escherichia coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa*).

Antibacterial agents can be classified into two categories; Bactericidal substances which kill microbes and bactericidal substances which prevents or stop bacteria from growing and reproducing (Zhou *et al*., 2022). The MBC is the least amount at which an antibacterial substance kills bacteria while MIC is the minimum amount at which an antibacterial compound prevents the growth of bacteria (Ahmad *et al*., 2022; Skrzypczak and Przybylski, 2022).

S. *aureus* is a spherically-shaped bacterium usually found in the upper respiratory tract and on the skin and can be treated with antibiotics such as ciprofloxacin, vancomycin.

B. *cereus* is a rod-shaped bacterium commonly found in soil, food and marine sponges, the specific name "cereus" means waxy in Latin which refers to the appearance of colonies grown on blood agar and can be treated using antibiotics such as vancomycin, levofloxacin.

E. *coli* are germs called bacteria found in the environment, food, water and the intestines of people and animals. It can be treated with antibiotics such as ciprofloxacin, trimethoprim/sulfamethoxazole (TMP/SMX).

K. pneumonia is a rod-shaped, non-motile, encapsulated, lactose-fermenting, facultative anaerobic bacterium. It appears as a mucoid lactose fermenter on MacConkey agar with thick capsules which makes it difficult to treat.

P. aeruginosa is a rod-shaped bacterium that can cause disease in plants and animals including humans. Antibiotics such as ciprofloxacin and levofloxacin remain currently the only oral treatment options.

Bacterial resistance to antibiotics has increased due to several factors including the overuse and misuse of antibiotics, when antibiotics are used too frequently or inappropriately, bacteria develop resistance to the antibiotics and become more difficult to treat (Xu *et al*., 2022; Aminian *et al*., 2022).

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Apparatus used

Plant seed sample (reducing or stabilizing agent)

2.1.2 Reagents used

Calcium chloride salt (precursor), Sodium hydroxide, Distilled water, Hager's reagent, Picric acid, Dil. HCL, Conc. Sulfuric acid, 5% KOH, 10% Ferric Chloride, 10% Lead Acetate, Acetic Anhydride, Glacial Acetic acid, Fehling solution A and B, Chloroform, Ethanol, 1% Barium Chloride, Tween 80, Ciprofloxacin. *Staphylococcus aureus* and *Bacillus cereus, Escherichia coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa.*

All chemicals used were of analytical grade

2.2 METHODOLOGY

The Preliminary Qualitative Phytochemical screening of the aqueous extract were done according to standard methods described by Trease & Evans, 1989.The characterization techniques employed during this research study were previously reported methods by (Ahmad *et al*., 2022; Khine *et al*., 2022; Marquis *et al*., 2016). The antibacterial activity was carried out according to standard procedures published by the Clinical and Laboratory Standard Institute (CLSI).

2.2.1 Collection and Identification of Plant

The fruits of *Trichosanthes cucumerina* plant were collected from the University of Benin Demonstration Secondary School (UPSS) school garden, University of Benin, Ugbowo campus, Benin city, Nigeria. Identification and Authentication of the plant was done by Prof. Akinnibosun Henry Adewale (FLS, MRSB; London), Herbarium unit in the Department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Edo state with the following Voucher specimen number: **UBH-T244**.

2.2.2 Test Organism

Five(5) bacterial isolates recovered from University Of Benin Teaching Hospital, Benin city namely; *Pseudomonas aeruginosa, Klebsiella pnenoneae, Escherichiaa coli, Staphylococcus aureus, Bacillus cereus* were obtained from the department of Microbiology laboratory of University of Benin, Ugbowo, Benin city. The bacteria were characterized to species level by using different laboratory procedures including; Gram's stain, cultural characterization and biochemical tests such as (Indole, Methyl red, Vougues Proskeaur, Catalase, Citrate Utilization and Coagulase tests) as described by Holt *et al*., 1994.

The isolates were maintained on nutrient agar slants at $4°C$

2.2.3 Washing and Drying

Fresh and disease-free seeds of T. cucumerina were washed several times with distilled water to remove the associated dirt and dust. The seeds as seen in plate.2 were then air-dried under close watch for 3 weeks before ground into powder form. The powdered seed was then allowed to pass through sieve to recover the crude extract.

2.2.4 Extraction

The method used for obtaining the aqueous extract is Decoction which is a traditional technique used to extract bioactive compounds, flavors and nutrients from plant materials. The process used involves;

10g of crude plant was mixed with 100ml of distilled water in a 250ml Erlenmeyer flask and allowed to sit for 15 minutes. The mixture was heated while stirring using magnetic stirrer till the temperature increased to 60℃, the heat was the removed and the mixture was left to continue stirring at 200rpm for one hour.

After one hour, the heated mixture was allowed to cool for about 45mins and it was then filtered to obtain a fine solution. The filtrate was collected, labeled and stored in the fridge for further analyses.

2.2.5 How to prepare standard solution

Standard solution is a solution containing an accurately known concentration. They are generally prepared by dissolving a solute to a specific volume or by diluting a solution of known concentration with more solvents.

$$\text{Molarity} = \frac{\text{no. of moles (mol)}}{\text{Volume (L)}}$$

2.0M Calcium chloride salt was prepared by weighing 22.2g of the precursor salt in 100ml of distilled water in a volumetric flask and stirred until the solution becomes limpid with a PH of 9.3

2.0M Sodium hydroxide was prepared by weighing 8g of the pellets into a 100ml of distilled water and stirred until the crystals dissolved completely with a PH of 14.

(Both preparation processes were exothermic).

2.2.6 Synthesis

The synthesis of CaO NPs was carried out by a previously reported method of (Ramli *et al.*, 2019) through a green approach, a strong base such as Sodium borohydride or Sodium hydroxide is usually used as a reducing agent.

The prepared salt solution was heated and stirred at 60℃ for 30minutes then 20ml of the aqueous extract was added and allowed to stir vigorously for another 30 minutes, on addition of the aqueous extract, the color was observed to be yellowish brown and the PH was measured to be 5.5. About 30ml of 2.0M aqueous NaOH was added to the vigorous stirring of the mixture in drops to rise the PH to 11, the color was seen to be more yellowish, the mixture was agitated for 30mins until a white milky precipitate of $Ca(OH)_2$ was visible.

$$CaCl_2 + 2NaOH_{(aq)} \longrightarrow Ca(OH)_{2(s)} + 2NaCl_{2(aq)}$$

The mixture was centrifuged at 10,000rpm for 10 minutes, the clear layer was poured out and the suspension was washed repeatedly with distilled water then centrifuged again to remove NaCl from the suspension. The duration of each filtering process was 2.5h. The precipitate thereafter was transferred into a clean crucible and oven-dried at a temperature of 100℃ for 2 hours, the particles were granulated into fine and smooth texture. After drying, the fine particles were calcined at 700° C for 3 hours to obtain a white powdered CaO NPs. As a result, about 7g of calcium oxide nanoparticles were obtained by calcination.

(This process was carried out repeatedly for a greater yield of the nanoparticles).

These works were done from the Central Research Laboratory (CRL) of the University of Benin.

Precautions taken

i. The decoction method employed was started with cold water rather than placing the crude directly in boiling water.

ii. All glassware was cleaned and rinsed with distilled water before used

iii. The PH meter was calibrated alongside the centrifuge

2.2.7 Characterization Techniques

Calcium oxide (Cao) has an individual catalytic and optical property which can be synthesized successfully by green synthesis. To characterize CaONPs, previously reported methods by (Ahmad *et al*, 2022; Khine *et al*, 2022; and Marquis *et al*, 2016) were followed.

- XRD Analysis: The morphology of the calcium oxide nanoparticles was studied through the X-ray diffraction to show the polycrystalline nature, phase composition of the nanoparticles using a diffractometer. XRD analysis was performed using a Bruker D8 Advance diffractometer with Cu-Kα radiation ($\lambda = 1.54Å$) and a step size of 0.02° over the range from 10-80° at a scan rate of about 4°/min.
- DLS: The particle size distribution of the calcium oxide nanoparticles was carried out using Diffraction Light Scattering
- FTIR: The functional groups of the synthesized nanoparticles were analyzed using Fourier Transform Infrared Spectroscopy. The bond vibrations were in range of 650-4000nm^{-1} using Perkin Elmer spectrum 400. (These techniques were conducted by Mr. Isa Yakubu, Zaria, Nigeria).

2.2.8 Reagent preparation for phytochemical screening

1. **Hager's reagents** – Saturated aqueous solution of Picric acid.
2. **Fehling's Solutions** – Solution A: 34.66gm Copper sulphate + distilled water to make a final volume of 100ml.

Solution B: 173gm Potassium sodium tartarate + 50gm NaOH + distilled water to make a final volume of 100ml.

2.2.9 Qualitative analysis of secondary metabolites

The phytochemical analysis was carried using Trease & Evans, (1989) methods

Detection of alkaloids -- The test is known as Hager's test, in a typical test, few drops of saturated Picric acid solution was added to 2ml of the extract. A bright yellow precipitate formation indicates the presence of alkaloids.

Detection of Flavonoids -- A few drops of 10% lead acetate solution was added to the extract and the formation of a yellow color precipitate indicates the presence of flavonoids. This test is known as Lead Acetate test.

Detection of Steroids -- A few drops of acetic anhydride are added to the extract and the formation of violet to blue to green in some part of the sample indicates the presence of steroids

Detection of Terpenoids – 5ml of the extract was mixed with 2ml of chloroform and 3ml of concentrated sulfuric acid was added carefully to form layer. A reddish brown colored ring at the interface indicates the presence of terpenoids, the test is also referred to as Salkowski's test

Detection of Saponins -- 5ml of the extract was mixed vigorously with 5ml of distilled water. The formation of frothing indicates the presence of saponins.

Detection of Phenolic compounds -- To detect a phenolic group in a compound, the most common test is the ferric chloride test as described by (Harith *et al*., 2018) where adding a neutral ferric chloride solution to the compound will produce a colored complex, typically appearing as blue, violet, purple, green, or red-brown, indicating the presence of a phenol group.

Detection of Tannins -- A few ml of the aqueous extract was mixed with a few ml of water and allowed to heat in a water bath, the mixture was filtered then Ferric chloride was added to the filtrate. A dark green color indicates the presence of tannins.

Detection of Glycosides – 5ml of extract was added to glacial acetic acid, a drop of 5% ferric chloride was added with a little amount of conc. sulfuric acid. The formation of a brown ring shows a positive test for glycosides.

Detection of Reducing sugars --1ml each of Fehling's solution A & B was added to 1ml of extract and allowed to boil in a water bath. The appearance of a brick-red shows the presence of reducing sugar.

2.3 Test for Antibacterial activity – AGAR WELL DIFFUSION METHOD

The sensitivity of each extracts was determined using the agar well diffusion method as described by (Ahmed, *et al*., 2010) with modifications.

Varying concentrations of the nano extract ranging from 62.5-7.813mg/ml were prepared.

11.5g of Mueller Hinton Agar was prepared and sterilized at 121^{o}C for 15mins using autoclave. Upon sterilization, the molten agar was allowed to cool and 30ml was dispensed into five different petri dishes, then it was allowed to cool and solidify.

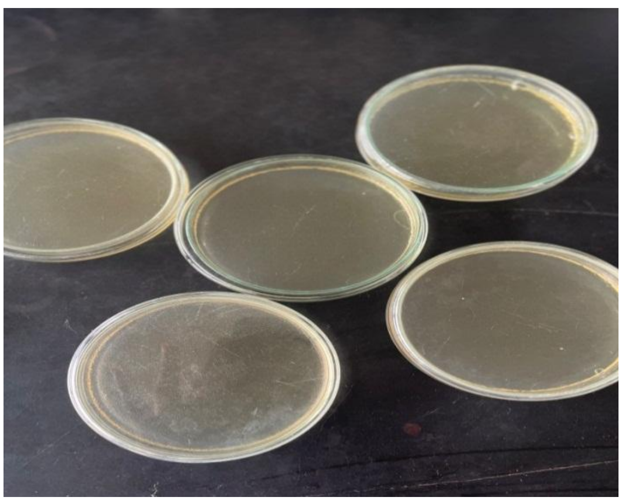


Plate.3 Solidified Agar

While the solidification period was on, the agar plates were allowed to dry to remove excess moisture from the surface using hot air oven at 50^{o}C for 10mins. After drying the plates, the organisms were standardized by preparing a suspension of the organisms in 1ml of normal saline or sterile water and the turbidity of the suspension of the organism were compared with 0.5McFarland turbidity or standard (1.5x10^{6} CFU), the entire surface of the agar was seeded with the organisms.

A sterile cork borer of 8mm or 10mm in diameter was used to cut a well on the agar plates and the base was sealed with molten Mueller Hinton Agar then 1g of the Cao NP was measured using analytical weighing balance, it was ground to powder in a crucible, 2ml of Tween 20 stock solution was added to make it a solution, (the reaction was exothermic).

0.2ml of the Cao NP extract concentration was introduced into the wells using a calibrated micropipette with a rubber teat. The plates were allowed to stand for 30mins and incubated right upside down for 24hrs at 37^{o}C. After the 24hrs incubation, the inhibition zone diameter of the nano extracts was measured using a meter rule and a pair of divider. The results were recorded.

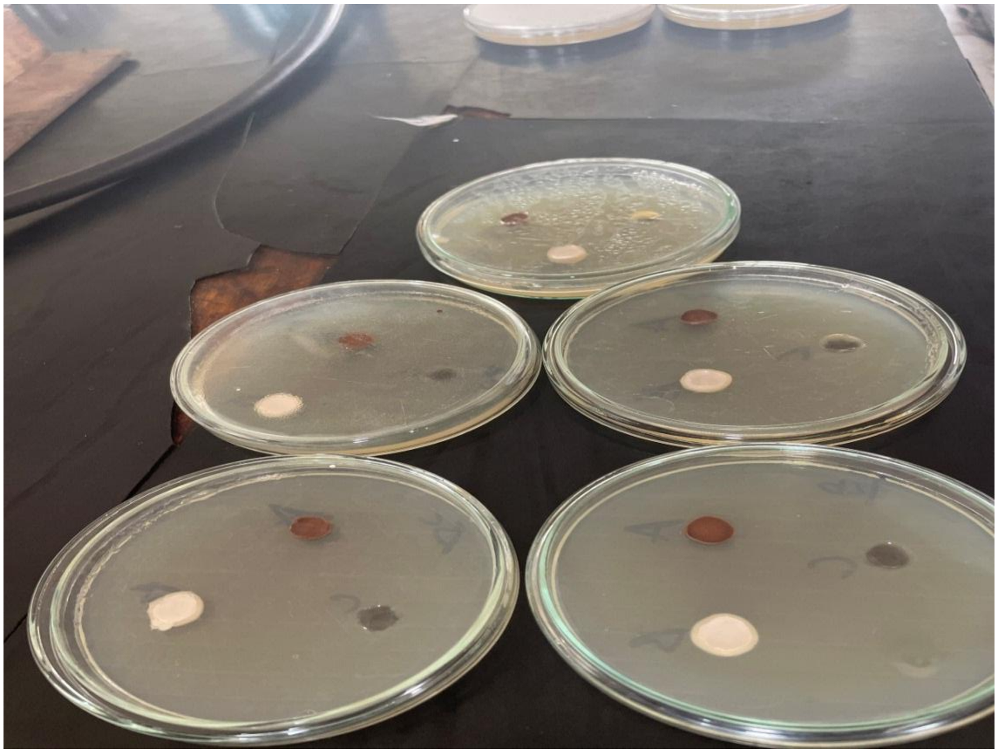


Plate.4 Nano extract in the well of the agar

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 QUALITATIVE ANALYSIS

Phytochemical investigation from the Cao NPs of *Trichosanthes cucumerina* seeds extract shows a large number of organic complex and biologically active compounds. The results of the qualitative phytochemical analysis as shown in **table 3** indicates the presence of alkaloids, tannin, euglenol, terpenoid, saponin, glycoside, phenols, reducing sugar while flavonoids and steroids were not detected.

Table.4 Phytochemical constituents of the extract

Phytochemicals	Test	Observation	Inference
Alkaloids	Wagner's test	Reaction was Exothermic with a red precipitate	+
Tannins	Ferric chloride	Dark blue color	+
Saponnins	Foam test	Frothing (presence of emulsion)	+
Terpenoids	Salkowaski's test	The reaction was exothermic with a cloudy lower layer and a brownish red at the interface	+
Flavonoids	Lead acetate test	A white milky precipitate was observed	+
Glycosides	General test	Oily on addition of glacial acetic acid forming a brown ring the reaction was exothermic	+
Phenolic compounds	Ethanol/$FeCl_3$	The reaction was exothermic	-
Euglenols	KOH/HCL	A pale yellow color was observed	+
Steroids	Acetic acid/H_2SO_4	No reaction	-
Reducing sugar	Fehling's test	Appearance of a brick-red precipitate	+

Key: +, presence of phytochemical; -, absence of phytochemical

3.2.1 VIBRATIONAL PROPERTIES

The FTIR spectroscopy as shown in fig 1 indicate that the strong IR band located at 678cm^{-1}and 711cm^{-1} is attributed to the fundamental vibrations of CaO, confirming the successful formation of Calcium oxide nanoparticles. The broad peak observed at region 3640-3400cm^{-1} typically corresponds to the O-H stretching vibrations of the adsorbed water or hydroxyl groups, indicating possible moisture retention in the sample confirming the presence of an aromatic group

The peaks in the region of 1600-1400cm^{-1} corresponds to the bending vibrations of H-O-H, water molecules confirming the presence of surface hydroxyl groups. At region 2100cm^{-1}, the peaks could be associated with C≡C stretching or weak vibrational modes of impurities. The wave number within the range of 2500-2300cm^{-1} may be due to the presence of CO_2 adsorption, suggesting atmospheric carbon dioxide interaction with the sample.

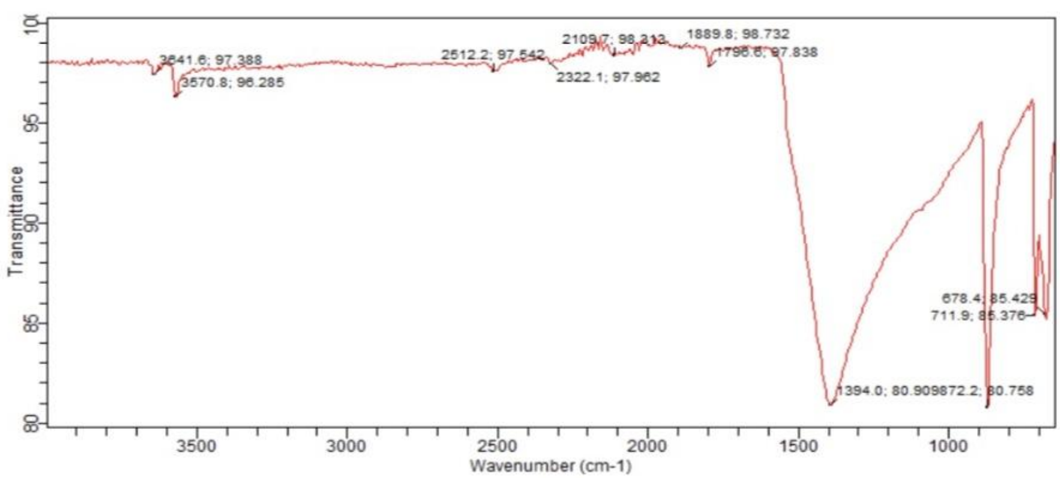


Fig.1 Fourier Transform InfraRed Spectroscopy of CaO NPs using *Trichosanthes cucumerina*

3.2.2 PARTICLE SIZE ANALYSIS

The Dynamic Light Scattering study shown in **Fig.2** revealed that the calcium oxide nanoparticles possess an average particle diameter of 47.63nm which confirms that the particle exist in form of nanoparticles. The polydispersity index (P.dI) of the CaO NPs obtained by DLS analysis is 0.237 indicating that the nanoparticles are mono-dispersed and are uniformly distributed.

Particles are said to be mono-dispersed when they are <0.5mm and they are most preferable because their uniform size and properties enable predictable and reproducible in various applications such as drug delivery, catalysis and sensing leading to efficiency and accuracy. When particles are >0.5mm, they are said to be poly-dispersed because the particles are not uniformly arranged.

Due to the smaller size, significantly larger surface area to volume ratio of the calcium oxide nanoparticles tends to have great impact on biological systems often leading to increased interaction with cell membranes enhancing easy penetration into the bacteria cell tissues, and potentially higher toxicity depending on the material and application. Smaller nanoparticles can be more readily absorbed by the body, potentially causing greater cellular damage or enabling targeted drug delivery to specific sites within the body.

Results

			Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm):	47.63	Peak 1:	2.811	0.1	0.5856
PdI:	0.237	Peak 2:	9.194	89.3	3.212
Intercept:	0.757	Peak 3:	118.5	8.0	76.07
Result quality	Good				

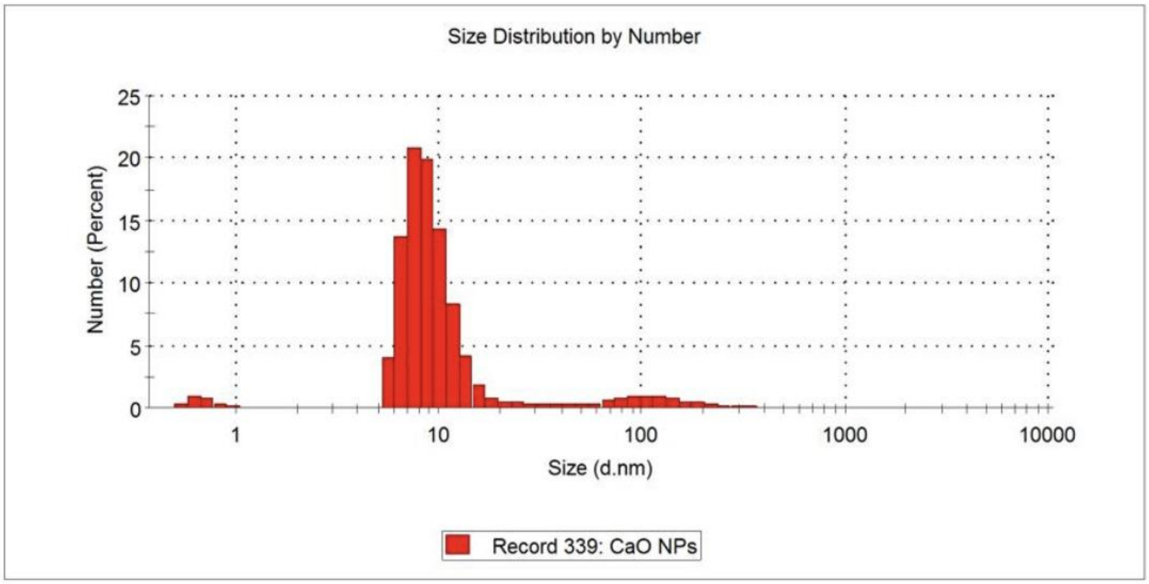


Fig.2 DLS size distribution by number of T. cucumerina nanoparticles

3.2.3 QUANTITATIVE X-Ray DIFFRACTION

The X-Ray Diffraction of Calcium oxide nanoparticles as shown in Fig.5 shows sharp peaks that illustrated the crystalline nature of the nanoparticle in good agreement with the standard JCPDS No. 77-2376 confirming the successful synthesis of CaO nanoparticles. (Ulakpa *et al.*, 2024). The 2θ diffractive peaks were observed at angles of 32^0, 37^0 and 54^0 corresponding to the (111), (200) and (022). All recorded peak intensities were characteristics of the cubic structure of the nanoparticles.

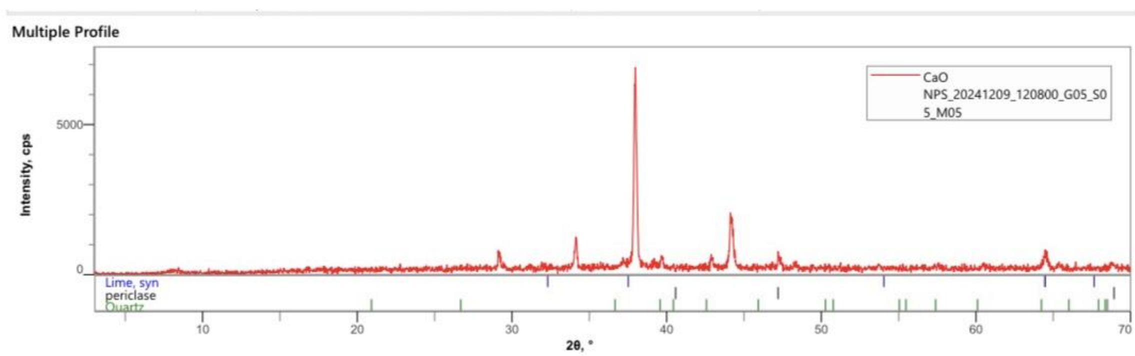
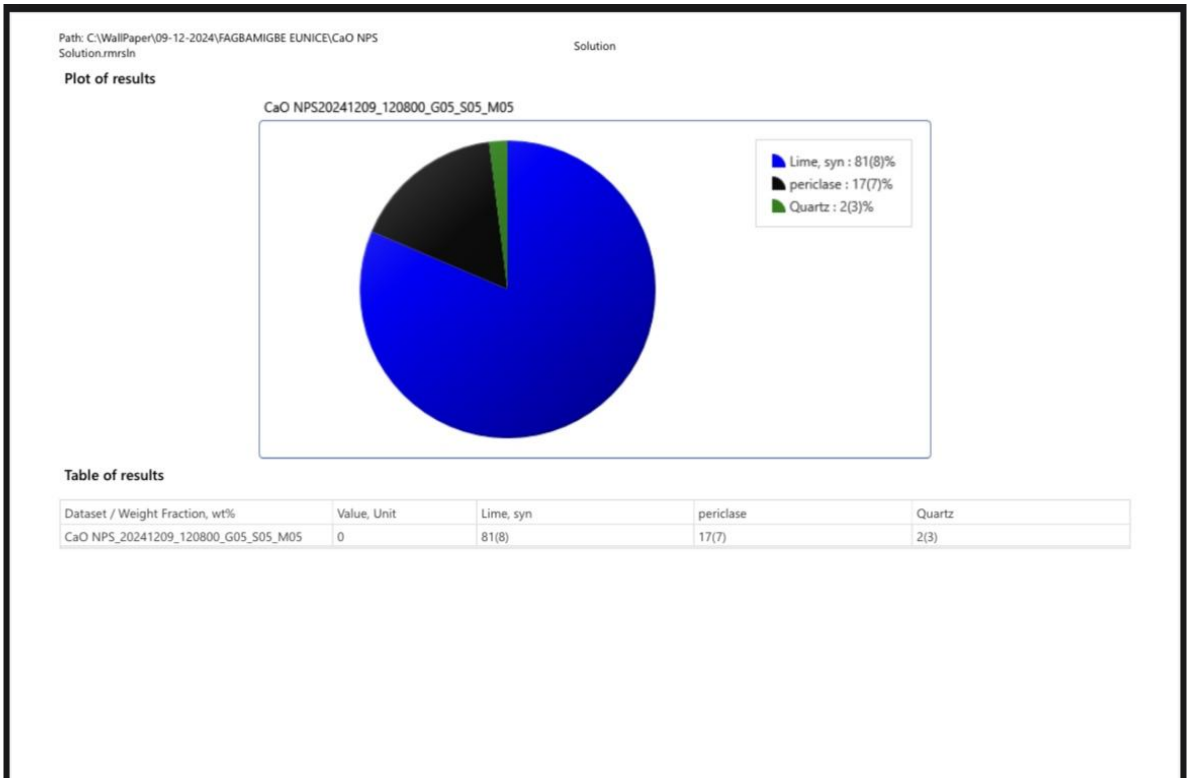


Fig. 3 XRD diffractogram of calcined CaO NPs using *T.cucumerina*



Dataset / Weight Fraction, wt%	Value, Unit	Lime, syn	periclase	Quartz
CaO NPS_20241209_120800_G05_S05_M05	0	81(8)	17(7)	2(3)

Fig. 4 Quantitative X-ray diffraction analysis

The above Pie chart and table provide the weight fraction of each detected phase:

- Lime (Cao): 81.8% - This confirms that the sample is primarily composed of Calcium oxide which is a high purity nanoparticle. The main peaks typically appear around 2θ = 32^0, 27^0, 54^0, 64^0
- Periclase (MgO): 17.8% - Indicates the presence of Magnesium oxide which might probably be mixed with the nanoparticles. The peaks appear around 2θ = 42^0, 62^0, 78^0
- Quartz (SiO_2): 2.0% - This shows that there is a small amount of silica in the nanoparticle likely as a result of contamination or as a residual impurity. The observed peaks appear around 2θ = 20^0, 26^0, 50^0

3.3 ANTIBACTERIAL TEST

Aqueous Cao nanoparticles of *Trichosanthes cucumerina* seeds were tested for its antibacterial activity. The result presented in Table 4 shows that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. The nanoparticles of CaO indicate good antibacterial properties because of large surface area, the reduced particle size increases the reactivity of CaO nanoparticles with gram positive and gram negative bacteria used during the investigation.

Depending on the size of the nanoparticles, smaller sized nanoparticles can easily enter into the bacterial cells, an inhibition mechanism proceeds inside the bacterial cell which will then lead to the distortion of the cell membranes that causes the death of the cells (Ramola *et al*., 2019). The Highest zone of inhibition was demonstrated by *S. aureus.* along with Ciprofloxacin 50mg/ml which was used as the positive control.

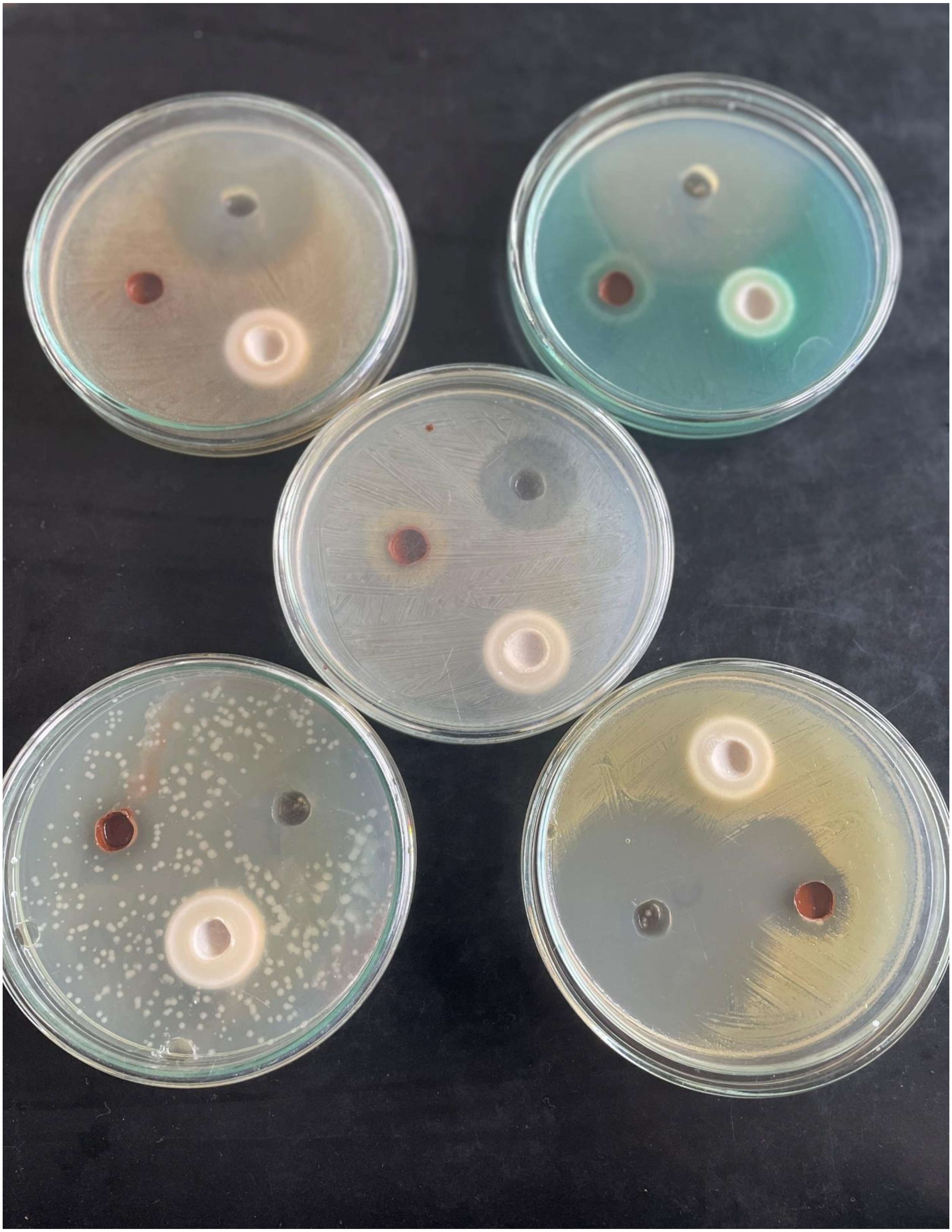


Plate.5 Bacterial Zone of Inhibition

S/N	ORGANISM	0.1mg/ml (Ciprofloxacin) CONTROL (mm)	24mg/ml CAO NP (mm)
1.	*Pseudomonas aeruginosa*	40mm	20mm
2.	*Escherichia coli*	28mm	22mm
3.	*Staphylococcus aureus*	24mm	20mm
4.	Klebsiella pneumoniae	40mm	18mm
5.	*Bacillus cereus*	45mm	22mm

Table.5 antibacterial activity of CaO nanoparticles of snake tomato seeds

CONCLUSION

The results of this study showed that, green synthesis method can be applied for rapid, cost effective, and eco-friendly way for the synthesis of calcium oxide nanoparticles which can be further used in various industrial, environmental, agricultural and medical applications. CaO-NPs were synthesized from *Trichosanthes cucumerina* L. seeds for phytochemical screening which detected the presence of alkaloids, tannins, saponins, terpenoids, euglenols and the absence of flavonoids and steroids.

The synthesized nanoparticles in this study showed a characteristic stretching of specific functional group in the FTIR spectrum (O-H, CO_2, carbon-carbon triple bond vibration, H-O-H and CaO). The FTIR spectrum confirmed the presence of Ca-O vibrational peaks while also indicating possible surface interactions with moisture and atmospheric gases. The DLS analysis proved that the synthesized nanoparticles are mono-dispersed. The antibacterial studies showed sensitivity to both gram negative and gram positive bacteria.

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