Phytochemical Analysis, Antibacterial, Antioxidant Activity Evaluation of *Measa lanceolate* Forssk Leaf Extracts and its Mediated Zinc Oxide Nanoparticles

Irasa Ilala Ribo

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

June, 2023

Adama, Ethiopia

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Declaration

I hereby declare that this Master Thesis entitled**" Phytochemical Analysis, Antibacterial, Antioxidant Activity Evaluation of *Measa lanceolate* Forssk Leaf Extracts and its Mediated Zinc Oxide Nanoparticles"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other University. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Recommendation of Advisor

I, the advisor of this thesis, here by certify that I have read the revised version of the thesis entitled "**Phytochemical Analysis, Antibacterial, Antioxidant Activity Evaluation of *Measa lanceolate* Forssk Leaf Extracts and its Mediated Zinc Oxide Nanoparticles**" prepared under my guidance by Irasa Ilala submitted in partial fulfillment of the requirements for the degree of Master's of Science in Biotechnology. Therefore, I recommend the submission of a revised version of the thesis to the department following the applicable procedures.

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Major Advisor	Signature	Date

Approval Page

I the advisor of the thesis entitled **"Phytochemical Analysis, Antibacterial, Antioxidant Activity Evaluation of *Measa lanceolate* Forssk Leaf Extracts and its Mediated Zinc Oxide Nanoparticles"** and developed by Irasa Ilala, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major adviser	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Irasa Ilala have read and evaluated the thesis entitled "**Phytochemical Analysis, Antibacterial, Antioxidant Activity Evaluation of *Measa lanceolate* Forssk Leaf Extracts and its Mediated Zinc Oxide Nanoparticles**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biology (Biotechnology).

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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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LIST OF ACRONYMS AND ABBREVIATIONS

AAU	Addis Ababa University
DMSO	Dimethyl Sulfoxide
eV	electrovolt
FWHM	Full Width at Half Maximum
MBC	Minimal Bactericidal Concentration
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
nm	Nanometer
SD	Standard Deviation
SEM	Scanning Electron Microscope
SPSS	Statistical Package for Social Sciences
UV-Vis	Ultraviolet-Visible spectroscopy
XRD	X-ray Diffraction
ZnO NPs	Zinc Oxide Nanoparticles

ABSTRACT

Measa lanceolata (family Myrsinaceae) is a medicinal plant traditionally used for the treatment of bacillary dysentery, bacterial infections, cholera, dermatoses, hepatitis, impetigo, neuropathies, sore throat as well as for treatment of some types of tapeworms and viral infections against different infectious diseases. To perform phytochemical screening, evaluate the antibacterial and antioxidant activity of Measa lanceolata leaf extracts and its mediated ZnO Nps. The plant samples were collected, dried, and the extraction of phytochemicals were done using the maceration method with methanol, distilled water, chloroform, and n-hexane solvents. Zinc Oxide nanoparticles were synthesized by mixing Zinc nitrate hexahydrate and plant extract. The synthesized nanoparticles were characterized by Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscope, and Ultraviolet-Visible spectroscopy. The antibacterial activities of the leaf extract and synthesized nanoparticles were evaluated by the disc diffusion method against selected human pathogenic bacteria. The minimum inhibitory concentration and minimum bactericidal concentration was determined for extracts against the selected pathogens using the broth dilution method. The antioxidant activity of the leaf extract was determined by using DPPH radical scavenging activity. The crystalline size and corresponding peak absorbance of synthesized Zinc Oxide Nanoparticles were 24.3 nm and 373.97, respectively. The results of the study showed that the leaf extracts contain alkaloid, flavonoid, phenol, saponin, steroid and tannin. The methanol, distilled water, chloroform, and hexane extracts showed good antibacterial activity and the maximum zone of inhibition (16.3 ± 0.88 mm, 15.0 ± 0.58 mm, 14.00 ± 1.00 mm, 9.3 ± 0.33 mm) against S. aureus respectively compared to Ciprofloxacin 22.5±0.88 mm. The synthesized Zinc Oxide Nanoparticles showed the highest zone of inhibition against E.coli (17.7 ± 0.62 mm) compared to Ciprofloxacin (21.6 ± 1.2 mm). In the present study, the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration value obtained from distilled water extract has been found to be 12.5 mg/ml and 25 mg/ml against E.coli, respectively. The leaf extracts also exhibited high antioxidant activity with an IC_{50} value of 92.8 μg/ml compared to ascorbic acid 36.6 μg/ml. The results of this study therefore revealed that leaf extracts of Measa lanceolata posses antibacterial, antioxidant activity which justify use of this plant in the treatement of bacterial infections.

Keywords: antibacterial activity, antioxidant activity, leaf extracts, phytochemical constituent, *Measa lanceolata*

1. INTRODUCTION

1.1 Background of Study

Ethiopia is home to a large diversity of plant species, which contain many useful bioactive constituents (Tadeg *et al*., 2005). Traditionally in rural Ethiopia, *Measa lanceolata* is used to treat many kinds of diseases such as helminthes and bacterial infections (Dereje *et al*., 2009). Traditional healers around Malga district, sidama region, of Ethiopia chewing or sniffed the dry seed of *M. lanceolata* for the treatment of nasal problems, asthma, and Skin infection (Tamene, 2020). *M. lanceolata* belonging to the genus measa and family Myrsinaceae is known as locally in Ethiopia as "Gowacho" in Sidamic and "Yaregnd qolo" in the local name and is used to treat various diseases in the world. For, example in Kenya it is used as a traditional medicine in a mixture of seeds and ground fruit is applied as an anti-helminthic, seeds and dried fruit for loss of appetite, boiled water extracts of seeds and fruit for malaria, roots boiled in water for reduced overall body strengthen and as a nutrient, and seeds and ground or crushed fruit in water for sexually transmitted diseases such as syphilis and gonorrhea (Kiringe, 2006).

Plants are important to human beings beginning from prehistoric times and have been used for food, shelter, and medicine (Mekonnen *et al*., 2022). Plants that have active constituents of medicinal properties and are used to treat disease in different systems of medicine (Ssenku *et al*., 2022). Many people that are found particularly in developing countries are still using medicinal plants as a source for various life-threatening or deadest diseases. According to the World Health Organization, it estimated that around 80% of the population still uses herbal remedies to treat different ailments (WHO, 2004), because of their therapeutic benefits, easily available, cost-effective, and less or no side effects (Rajasree *et al*., 2016).

Nanoparticles (NPs) are zero-dimensional particles with all three dimensions in the range of 1 to 100 nm size. NPs can be used as a medium of transportation for drug delivery in cancer treatment due to its high bioavailability, efficiency, and improved permeability (Osonga *et al*., 2020). Nowadays, metal oxide nanomaterials were studied and found to be applicable in catalysis, and medical science including antibacterial, antiviral, and anti-carcinogenic (Ismail *et al*., 2019). Among different metal oxide nanomaterials, zinc oxide (ZnO) nanoparticle is one of the fore most considered metal oxide nanomaterials due to its distinct chemical, physical, biological, and optical properties (Vidya *et al*., 2013). ZnO NPs have many applications in different fields like optical, piezoelectric, mechanical,

biomedical, gas sensing, catalyst, drug delivery, antimicrobial, etc (Islam *et al*., 2022; Kebede *et al*., 2023).There are various methods of synthesizing ZnO NPs. Among these synthesis methods, green synthesis using plant extracts is the most favored approach. This is because of its eco-friendly, low-cost, non-toxic, and suitability for human therapeutic uses (Dönmez, 2020). Green synthesis of ZnO NPs has been achieved by crude compounds from various medicinal plants, such as *Cassia fistula*, *Melia Azadarch*, *Allium sativum*, *Zingiber officinale*, *Aloe vera,* and *Lippia adoensis* (Kebede *et al*., 2023).

Microorganisms cause different diseases in humans. Among these microorganisms, bacteria are the most and very dangerous microorganism and are prevalent in today's world. In Gram-positive bacteria *Staphylococcus aureus, is* mainly responsible for conditions like abscesses, endocarditis, food poisoning, osteomyelitis, pneumonia, scalded skin syndrome, septic arthritis, shock syndrome, skin infections (Tong *et al.*, 2015) and *Streptococcus pyogenes* can lead to causing benign human infections such as pharyngitis and impetigo (Walker *et al*., 2014). Additionally, Gram-negative bacterium like *Pseudomonas aeruginosa* is present in humans that causes cystic fibrosis, open wounds and post-surgery patients, urinary tract infection, and diabetic foot ulcers (Morin *et al*., 2021).

However, to the best of our knowledge, there has been not subjected to comprehensive phytochemical analysis and synthesis of ZnO NPs conducted on *M. lanceolata* so far and there is no published scientific report on the phytochemical analysis, synthesis of zinc oxide nanoparticles, antibacterial activities of the leave extracts of this plant in Ethiopia. With this background information, the current study aimed to explore the phytochemical analysis, synthesis of ZnO NPs, and antibacterial and antioxidant activity evaluation of *M. lanceolata* leaf extracts on selected pathogenic bacterial namely: *E. coli*, *P. aeruginosa*, *S. aureus and S. pyogenes.*

1.2 Statement of Problem

Diverse antibiotics target different human pathogenic microorganisms and their effectiveness has decreased due to the miss use of commercial antibacterial drugs commonly used to treat infectious diseases (Chanda and Rakholiya, 2011). Bacteria are becoming more resistant to antibiotics, making a significant problem in the treatment of many well-known illnesses. This condition has led scientists to search for new antibacterial substances from different sources as new antibacterial chemotherapeutic agents, but the

cost for the production of synthetic drugs is expensive and they produce adverse effects compared to plant-derived drugs (Abiramasundari *et al*., 2011).

As a result, new antibacterial chemicals must be developed to combat these microbes (Sapna *et al*., 2016). Through various mechanisms of action, phytochemicals have demonstrated potential antibacterial activity against sensitive and resistant microorganisms (Rahman *et al*., 2011). So, it is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, and polyphenols are generally superior in their antibacterial activities (Patra *et al*., 2022). One possible solution is the innovative use of metal oxide as an antibacterial agent, combining advanced material science technologies. ZnO NPs have shown advantages in durability and chemical and physical stability over common organic and antibiotic compounds (Sirelkhatim *et al*., 2015).

Traditionally, Ethiopians have been using medicinal plants to treat different diseases and this has great contribution to primary healthcare systems. *M. lanceolata* is one of those ethnomedicinal plants that have been commonly visited by traditional healers in different parts of Ethiopia. The fruits, stems, leaves, and roots parts of the plant have been widely used by the local people for the treatment of different alignments.

1.3 Objectives of the Study

1.3.1 General Objective

To perform phytochemical screening, evaluate the antibacterial, antioxidant activity of *M. lanceolata* leaf extracts and its mediated ZnO NPs.

1.3.2 Specific Objectives

- To screen for the presence of various phytochemicals components in the leaf extracts of *M. lanceolata*.
- To synthesize ZnO NPs using *M. lanceolate* leaf extract.
- To characterize synthesized ZnO NPs by Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscope, and Ultraviolet-Visible spectroscopy.
- To evaluate the antibacterial activities of the leaf extracts and synthesized ZnO NPs against selected human pathogenic bacteria.

- To determine the minimum inhibitor concentration (MIC) of leaf extracts of *M. lanceolata*.
- To determine the minimum bactericidal concentration (MBC) of leaf extracts of *M. lanceolata.*
- To evaluate the antioxidant activities of leaf extracts of *M. lanceolata*.

1.4 Significance of the Study

The main significance of this study is to provide information on the phytochemical compositions, antibacterial and antioxidant activity of *M. lanceolata* leaf extracts, which are found in Ethiopia. Green synthesis shows better advantages over chemical and physical as it is lesser toxic, cost-effective, and environmentally friendly. Green chemistry reduces pollution risk at the source level and it enhanced the prevention of waste. Because of the effectiveness of green synthesis, we could synthesize ZnO NPs by using *M. lanceolata* leaf extracts and evaluate its antibacterial activity. Additionally, this research was expected to give a scientific explanation and provides baseline information for future pharmacological studies and the researchers who are interested to conduct further work on the area.

1.5 Scope of the Study

The study focused on leaf extracts of *M. lanceolata* and to conduct phytochemicals, synthesizing of ZnO NPs, and characterizing the ZnO NPs using techniques like FTIR, XRD, SEM, and UV-Vis spectroscopy. Additionally, it evaluated the antibacterial and antioxidant activities of plant leaf extracts and ZnO NPs against bacterial strains.

2. LITERATURE REVIEW

2.1 Traditional Medicinal plants

Medicinal plants are any plant that, in one or more of its organs, contains substances that can be used for healing purposes or which serve as a foundation for producing beneficial (Sofowora *et al*., 2013). According to the WHO, plant extracts or their active ingredients are used as medicine in traditional therapies by 80% of the world's population (Tugume and Nyakoojo, 2019). More than 50% of all modern clinical medicines come from natural products (Baker *et al*., 1995). The use of the whole plant part for treatment causes hazardous effects on humans and animals. Because the presence of different types of secondary metabolites found in medicinal plants changes the plant compounds in different climates, development of synergistic compounds, changes secondary metabolites, storages, and preparation of plant parts (Bourgaud *et al*., 2001).

Medicinal plants produce various ranges of secondary metabolites, such as polyphenols, tannins, vitamins, carotenoids, saponins, alkaloids, flavonoids, steroids, enzymes, and minerals (Madhuri and Pandey, 2009). These secondary metabolites can act as antibacterial, anti-carcinogenic, anti-inflammatory, anti-allergic, antioxidants, antipyretic activities, antiviral agents, cytotoxic, hypoglycemic, and other health benefits (Madhuri and Pandey, 2009; Eldahshan and Abdel, 2015; Bamola *et al*., 2018). These secondary metabolites protect the cells from the damage caused by unstable molecules known as free radicals (Harini & Nithyalakshmi, 2017). Utilizing natural antibacterial substances, particularly those derived from plants, for food preservation is gaining popularity. Therefore, it is necessary to look for plants with medicinal properties (Chavan, 2016).

Application of ethnobotany can principal to a strengthening of social variety conservation, better sustainability in the manipulation of plant resources, and the growth of novel plant products. And also, the application of ethnobotany can lead to rural developments by identifying and promoting useful plants resource for local use, in natural management and conservation identified, and promoting good conservation practices and biodiversity prospecting (Bussmann, 2002).

2.2 Distribution and Traditional Uses of *M. lanceolata*

M. lanceolata, also known as kalaba in local name, is a tree or shrub belonging to the Myrsinaceae family. This plant, which can grow between 2-20 meters in height, is

commonly found around the edges of evergreen forests, near riverbanks and streams, in open woodlands, and in valley areas. Its altitude range is between 1350-3000 meters and it is present across tropical Africa, extending to Ethiopia, South Africa, Madagascar, and Arabia (Farnsworth *et al.* 1985). Numerous rural communities in Ethiopia utilize this medicinal plant to treat both humans and their livestock (Mengiste *et al*. 2009). It thrives on stream banks and cliffs in central and coastal areas up to about 1500 m above sea level (Ngwenya *et al*., 2003). Leaves are simple, alternate, or in clusters at the ends of branches, with translucent glandular dots or streaks or glandular hairs (Burrows *et al*., 2018). Flowers are generally solitary discrete, bisexual or unisexual, actinomorphic 4 or 5 merely. Petals are usually white or pink, often with dark spots or stripes. Stamens are as many as opposite the petals. Fruit is often very small, round, white, and fleshy, crowned by remains of flowers (Burrows *et al*., 2018). It is growing in many African countries, including Central Africa, Ethiopia, and Kenya (Theunis *et al*., 2007).

Traditionally a leaf of *M. lanceolata* is used in traditional medicine against various diseases (Theunis *et al*., 2007). The fruit of this medicinal plant is widely used in East Africa to treat a variety of ailments, such as cholera, hepatitis, sore throat and tapeworms. In Central Africa, it is used against *Entamoeba histolytica* infections, while in Saudi Arabia a decoction of the heated fresh leaves is used to alleviate rheumatic arthritis. *M. lanceolata* is also reported to have antifungal, anticestodal and antischistosomal effects (Baisa *et al*., 2004; Bagalwa and Chifundera, 2007).

Its medicinal value derives from its use to treat pain in various diseases, including infectious hepatitis, bacillary dysentery, impetigo, bacterial infections in the small intestine, viral infections in the liver, throat and for the treatment of some types of dermatoses and neuropathies (Bayisa and Bultum, 2022). The presence of bioactive components such as polyphenols, tannins, and alkaloids in this plant makes it highly wanted due to its beneficial effects on the human body (Uoonlue and Muangrat, 2019). In some parts of Kenya, the plant is traditionally used for the treatment of helminthes and bacterial infections (Timothy *et al*., 2018). Among the Marakwet community, the stem bark is cut to pieces, boiled, and used to treat individuals with itchy skin rashes and dermatophytic conditions after bathing in stem bark decoctions (Kipkore *et al*., 2014). Various rural communities in Ethiopia use this medicinal plant to treat human beings as well as their livestock (Tolossa *et al*., 2013).

Table 1. Antimicrobial activity of *M. lanceolata*

Plant part	Solvent	Antimicrobial activity	References
Seed	n-Hexane	*Bacillus cereus, Staphylococcus aureus, Escherichia coli, Aspergillus Niger and Candida albicans*	Bayisa & Bultum, 2022
Roots, leaves, and stem bark	Distilled water, methanol, dichloro methane	*Candida glabrata, Crypto coccus, Aspergillus flavus, Microsporum gypseum &Trychophyton mentagrophytes*	Lizzy *et al*., 2018
Fruit	Acetone	*Staphylococcus aureus, Escherichia coli*	Mengiste *et al*., 2014

2.3 Phytochemical Constituents of *M. lanceolata*

Phytochemical studies of *M. lanceolata*, a plant with various medicinal properties, have revealed the presence of several bioactive compounds. According to research by Timothy *et al*., (2018), the plant contains alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. Phenolic compounds, particularly tannins, are the main constituents of *M. lanceolata*. The plant is known for its diverse range of phytochemicals, which include phenols, tannins, flavonoids, alkaloids, and anthraquinones (Timothy *et al*., 2018). The recently found flavonol glycosides and alkaloids in the plant's leaves, which further demonstrates the plant's rich phytochemical composition. Moreover, different parts of *M. lanceolata* plants contain different isomers of the same compounds, showing the plant's chemical complexity. These various compounds contribute to the therapeutic properties of *M. lanceolata*, making it a valuable resource for the development of plant-based medicines and treatments (Dagnaw and Mekonnen, 2017).

2.4 Nanoparticles (NPs)

Nanoparticles, in general, refers to atomic or molecular aggregates or clusters with size ranging from 1 and 100 nm, that can significantly alter their physicochemical properties compared with the bulk material (Joudeh and Linke, 2022). These particles are interesting, as they show unique and considerably different physical and chemical properties compared with their macro-scaled counterparts (Patra and Baek, 2014). Better properties, such as catalytic, magnetic, electro-mechanical, optical, chemical, and biological properties are displayed at nano scale. The higher strength, reactivity, mobility, enzymatic and chemical

activity, and dissolution properties are properties shown by NPs as a result of their high surface are (Sahoo *et al*., 2021).

This decrease in size brings about significant changes in their physical properties to those observed in bulk materials. The size reduction contributes to a higher surface area to volume ratio, which leads to the appearance of surface effects related to the high number of surface atoms, as well as to a high specific area (Navya and Daima, 2016). Nanoparticles can be made of materials of diverse chemical nature, the most common being biomolecules, carbon, metals, metal oxides, NPs, non-oxide ceramics, silicates, organics, and polymers exist in several different morphologies such as spheres, cylinders, platelets, tubes, etc (Panigraahi., 2013).

2.5 Metallic Oxide NPS

Metal oxides are an interesting category of inorganic materials, which have been extensively explored and studied due to their diverse structures and properties. These materials exhibit a more complex character than pure metals, with metal oxide bonding varying from nearly ionic to highly covalent and even metallic in nature (Nikolova and Chavali, 2020). Metal oxides exist in a variety of different forms, each with their unique compositions, morphologies, structures, and physiochemical properties. Specifically, metal oxide nanoparticles are of particular interest due to their unique and phenomenal optical, electronic, and magnetic properties. These distinct properties render metal oxide nanoparticles valuable for various industrial applications such as catalytic processes, electronics, sensors, magnetic storage media, and solar energy conversion (Fawcett *et al*., 2017). Aspects such as structural characteristics (including lattice symmetry and cell parameters) and the influence of size are related to the electronic properties of the oxide. Both structural and electronic properties play a significant role in determining the chemical properties of the solid and can be classified based on size (Subbenaik, 2016).

Owing to their small size and high density of corner or edge surface sites, metal oxide nanoparticles can display unique chemical properties. Particle size is anticipated to impact essential property groups in any material. Changes in cell parameters due to the size-related structural alterations have been observed, for example, in metal oxide nanoparticles of CuO, ZnO, SnO_2, Al_2O_3, MgO, ZrO_2, AgO, TiO_2, Ceo, etc. As the size increases in nanoparticles, decreasing the number of surface and interface atoms generate strain or stress and adjoining structural perturbations (Kebede *et al*., 2023). The specific

size of the nanoparticles can alter magnetic, conducting, chemical, and electronic properties (Issa *et al*., 2013). Magnetic, electronic, and chemical properties of the nanoparticles can be dependent on a specific size of the nanoparticle materials (Joudeh and Linke, 2022).

There are different types of metal oxide synthesized from different plant species, zinc oxide nanoparticles (ZnO NPs), stannic oxide ($SnO_{2)}$, Zirconium dioxide ($ZrO_{2)}$, Magnesium oxide (MgO), etc. Among all metal oxide nanoparticles, ZnO NPs have gained considerable attention for the fabrication of efficient, high isoelectric biosensors due to their unique features that include high chemical stability, high electrochemical coupling coefficient, nontoxicity, biocompatibility, good stability, inflammatory activity, anticancer and antibacterial properties (Sauvik and Ahmaruzzaman, 2022).

2.6 Synthesis of ZnO NPs

ZnO NPs can be synthesized by various techniques such as physical, chemical, and biological methods. Physical methods like electro-spinning, laser ablation, photo irradiation, radiolysis, spray pyrolysis, ultrasonication, etc, have been employed for the synthesis of ZnO NPs. However, chemical methods are more popular due to better control over the size and shape of the nanoparticles (Nagarajan and Kuppusamy, 2013). Chemical methods mainly include a sol-gel method, solvothermal, co-precipitation, and template-based methods (Khandel and Shahi, 2016). Moreover, the biological synthesis of nanoparticles using actinomycetes, algae, bacteria, fungi and plants has gained attention as they are eco-friendly, simple, and efficient. Among these plant extract-based synthesis has been greatly acknowledged and explored with many plant species (Manokari and Mahipal, 2017).

2.7 General Feature of Test Microorganisms

The human pathogenic microorganisms used in the study were *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa.*

S. aureus belongs to the *Staphylococcus* genus (staphylococci) and is a Gram-positive, non-spore forming spherical bacterium that typically forms grape-like clusters (Lowy, 1998). *S. aureus* is a type of facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7.5. Staphylococci are non-motile and do not form spores (Carroll *et al*., 2007). *S. aureus* is commonly found in the environment, including soil,

water, and air as well as in the nose and on the skin of humans. Its growth and survival rely on different environmental factors such as temperature, water activity, pH levels, oxygen availability, and the type of food source (ICMSF, 1996).

S. aureus is one of the most common bacterial infections in humans and is the causative agent of several human infections, including bacteria, gastroenteritis, infective endocarditis, lung infections (eg pneumonia and empyema), meningitis, skin and soft tissues, osteomyelitis, toxic shock syndrome and urinary tract infections (Patrick, 2006; Tong *et al*., 2015). *S. aureus* remains a versatile and dangerous pathogen in humans. The frequencies of both community-acquired and hospital-acquired staphylococcal infections have increased steadily. Treatment of these infections has become more difficult because of the emergence of multidrug-resistant strains (Rasigade and Vandenesch, 2014).

S. pyogenes commonly known as Group A Streptococcus (GAS), is a Gram-positive bacterium that can cause a diverse range of illnesses in humans. These illnesses can range from mild infections like pharyngitis and skin infections to severe, localized infections such as pharyngitis, life-threatening conditions such as toxic shock-like syndrome, invasive infections like pneumonia, bacterium and necrotizing fasciitis (a rapidly progressing skin and muscle infection, sometimes referred to as the flesh-eating disease (Carapetis *et al*., 2005). Among its various consequences, *S. pyogenes* infections can lead to acute rheumatic fever, a multisystem inflammatory disorder that can affect the heart, joints, skin, and brain. If not treated promptly and effectively, acute rheumatic fever can result in permanent heart damage, known as rheumatic heart disease, which can lead to heart failure and other serious complications (Karthikeyan and Guilherme, 2018).

The pathogenesis of *S. pyogenes* is complex and highly adapted to human hosts, allowing the bacteria to evade the immune system and cause a wide range of clinical manifestations. Treatment for *S. pyogenes* infections typically involves antibiotics, such as penicillin, and supportive care measures, depending on the severity and type of infection. Early diagnosis and proper treatment are crucial to prevent complications and long-term sequelae of *S. pyogenes* infections. Moreover, preventive measures, such as good hygiene practices, can help limit the spread of this bacterium and reduce the risk of infection (Brouwer *et al*., 2023).

Escherichia coli is a gram-negative, facultatively anaerobic, rod-shaped, coli-form bacterium of the genus *Escherichia* that is commonly found in the lower intestine of

warm-blooded organisms, Gram-negative bacterium, usually motile by peritrichous flagella. Most strains of *E. coli* are not harmful but they are part of the healthful bacterial flora in the human gut (Lim *et al*., 2010). However, some types can cause illness in humans, including abdominal pain, diarrhea, fever, and sometimes vomiting. *E.coli* is, produces a trusted source of a toxin known as Shiga. It is one of the most power full trusted Source toxins, and it can cause an intestinal infection (Andersen, 2005). *E. coli* is the most common cause of acute urinary tract infections as well as urinary tract sepsis (Steven *et al*., 2014). *E.coli* may also cause acute enteritis in humans as well as animals and is a general cause of traveler's diarrhea, a dysentery-like disease affecting humans, and hemorrhagic colitis often referred to as 'bloody diarrhea'(Evan, 1996).

P. aeruginosa is a common opportunist pathogen belonging to the genus Pseudomonas (Mackie & McCartney, 1989). *P. aeruginosa* is an obligate aerobe, motile, rod-shaped, and measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains. *P. aeruginosa* grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other Pseudomonas species. It does not ferment carbohydrates, but many strains oxidize glucose (Brooks *et al*., 2007). *P. aeruginosa* commonly lives in soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They commonly inhabit hospital food, sinks, taps, mops, and respiratory equipment.

P. aeruginosa causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are frequently associated with this bacterium: urinary tract infections, ventilator-associated pneumonia, surgical site infections, ear infections (external otitis, malignant external otitis), Skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis. Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of getting an infection and developing complications (Trautmann *et al*., 2008). *P. aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to amikacin, colistin, gentamicin, and tobramycin-resistant forms have developed, making susceptibility testing essential (Yayan *et al*., 2015).

2.8 Antibacterial Activities of Plants

Traditional medicinal plants possess various chemical substances that support certain physiological and biochemical activities in the human body and they are known as phytochemicals. These chemicals are non-nutritive substances used to heal various infectious diseases, as well as provide disease-preventive properties. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial (Dalal, 2016). Interestingly, there is a worldwide concern about the use of antibiotics to treat bacterial infections may result in the increase and spread of organisms resistant to broad-spectrum antibiotics opening ways to use plants as natural sources for novel antibacterial agents with identical activity (Carocho and Ferreira, 2013).

Plant secondary metabolites are commonly accountable for their antibacterial properties. These metabolites offer clues to manufacture new structural types of antibacterial chemicals that are comparatively safe for humans (Pandian *et al*., 2006). Plant antibacterial secondary metabolites are generally categorized into three broad classes, namely phenolic compounds, terpenes, and alkaloids (Ganesan *et al*., 2017). These compounds have numerous mechanisms that underlie antibacterial activity, e.g., disturbing microbial membranes, weakening cellular metabolism, controlling biofilm formation, inhibiting bacterial capsule production, attenuating bacterial virulence by controlling quorum-sensing, and reducing microbial toxin production (Ganesan *et al*., 2017).

Natural medicinal plants, such as *M. lanceolata*, are rich sources of bioactive compounds. Thus, the biological properties of leave extracts are documented, specifically their antimicrobial, anti-fungi, and antioxidant effect. Mengiste *et al.* (2014) studied the antibacterial activity of *M. lanceolata* and found that the methanol extracts of fruit of the plant *exhibit* good antibacterial potential against *S. aureus* and *E. coli*. While the lowest zone of inhibition (6.0 ± 0.5mm) was recorded in the same extract in strain *Escherichia coli*. At the same concentration, the highest mean inhibition zone is (10.8 ± 0.1 mm) for aqueous extract against *S. aureus*. In general, water & methanol extracts of *M. lanceolata* showed higher antibacterial activity, while ethyl acetate & chloroform extracts showed weaker antibacterial activity. The methanolic and aqueous extracts of the present study showed a higher zone of inhibition than that of *M. lanceolata* studied by (Mengiste *et al*., 2014).

2.9 Antioxidant Activities of Plants

DPPH radical scavenging assay is the most common method used in the study of the antioxidant activity of plant extracts. It results in the formation of stable free radical which can be detected by common spectrophotometric techniques. The decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. This assay is more indirect type as it measures the inhibition of reactive species (free radicals) generated in the reaction mixture and its results depend on the type of reactive species used (Padamanabhan *et al*., 2014). The concentration of the plant extracts required to scavenge DPPH showed a dose-dependent response.

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay experiment was used to evaluate the antioxidant activity of the methanol, water, ethyl acetate, and chloroform extract fractions of *M. lanceolata*. The methanol of the leaf extract was observed to have higher activity than the ethyl acetate and chloroform extracts of the *M. lanceolata* leaf at a concentration of 1.0 mg/ml. The purification activity of the methanol extract reached 92.2 ± 2.4%. The extracts from water, ethyl acetate and chloroform reached 88.2 ± 1.4, 77.0 ± 2 and 69.3 ± 1.8%, respectively. The EC_{50} was used to study the antioxidant potency of the samples. The lower the EC50 value, the higher the wobble potential. IC_{50} values ranged from 76.7 ± 7.3 μg/ml for the methanol extract to 282 ± 50 μg/ml for the chloroform extract. The strongest removal activity (lowest IC_{50} value) was recorded for the methanol extract, which appeared to be more than three times stronger than the chloroform extracts and twice as strong as the ethyl acetate, and twice as strong as the water extracts (Ismael *et al*., 2021). Whereas the DPPH scavenging activity of methanol extracts was not significantly different from that of BHT. But this value is weaker than the DPPH scavenging activity of *M. Lanceolata* reported from South Africa (Perera *et al*., 2015).

3. MATERIALS AND METHODS

3.1 Description of Study Area

Leaves samples of *M. lanceolata* (yarengd qolo) were collected from Wonsho Woreda, Racco Kebele, Sidama regional state, Ethiopia which is 329 kilometers far from Addis Ababa. Geographically, Wonsho Wereda is located between 06˚39'30" to 06˚46'30" North latitude and 38˚20'30" to 38˚34'30" East longitude with an approximate altitudinal range from 1978 m (West end, lower) to 2149 m (East or upper end) above sea level. The study area is characterized by mean annual rainfall and mean annual temperature ranging from 832 mm to 1658 mm and 18˚C - 21˚C, respectively. The pattern of rainfall distribution is bimodal. The short rainy season lasts from mid-November to February, while the long rainy season is from March to May and it extends from August to October. According to the Sidama Zone Finance and Economic Development Sector office, Wonsho Woreda is divided into four climatic zones based on altitude and annual rainfall variations. These zones are Wet Dega, Moist Woyna Dega, Wet Woyna Dega, and Wet Kola. The study site is classified as "Moist Woyna Dega." (Alemu, 2019).

3.2 Plant Samples Collection, Identification and Authentication

Two kilograms of fresh and healthy leaves a part of *M. lanceolata* were collected in January, 2023 from Racco Kebele, Wonsho woreda, Sidama regional state, Ethiopia. The collected plant material was identified, and authenticated and voucher specimen number (IE001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

Figure 1. Picture of *M. lanceolata* (Picture taken by Irasa Ilala on 22/01/2023)

3.3 Research Design

The study involved a laboratory-based experimental design. The research was focused on the screening for phytochemical content of leaves extracts, synthesis of and characterization of ZnO Nps, antibacterial and antioxidant activity evaluation of leaf extracts of *M. lanceolata* on selected pathogenic Gram-positive (*S. aureus*, *S. pyogenes*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*). The phytochemicals were extracted by solvents leaf extracts following standard protocol and each extract applied on four human pathogenic microorganisms in three replications to test their antibacterial activities. ZnO Nps were synthesized using the distilled water plant extract and synthesized ZnO Nps were characterized by FTIR, SEM, UV-vis and XRD. The synthesized ZnO Nps against human pathogens was determined using disk diffusion methods. The MIC and MBC were determined for leaf extracts against the selected pathogens using the broth dilution method. Ciprofloxacin was used as a positive control for antibacterial activity tests against bacterial pathogens, whereas DMSO was used as a negative control for bacterial test pathogens. The study was conducted in the Applied Biology laboratory of Adama Science and Technology University, Ethiopia.

3.4. Sample preparation and extraction

3.4.1 Sample preparation

The leaf samples were washed thoroughly with distilled water to remove dust and dirt from the plant part. Then, the leaves were chopped into small pieces using a sharp scalpel blade and shade dried in the dark for two weeks by protecting them from direct exposure to sunlight to prevent the degradation of active ingredients. The resulting dry plant material was ground coarsely into fine powder form using an electric grinding machine and then sieved (0.25 mm) to obtain uniform particle size.

3.4.2 Methanol, Chloroform and n-Hexane extractions

Fifty grams of the plant powder were added into three separate 1000 ml volume conical flasks labeled with methanol, chloroform, and n-hexane. A 500 ml of each of methanol (97%), chloroform (99%), and n-hexane (99%) were poured into the different labeled flasks and extracted in (ratio 1:10 w/v). The flasks were wrapped by aluminum foil and placed on a rotary shaker to mix well the plant powder at 120 rpm for 2 days at room temperature. Each extract was filtered with the aid of Whatman No. 1 filter paper (Buckinghamshire, UK) and were concentrated by evaporating the solvent in a Rotary

evaporator (model DW-RE-3000, Chongqing, China) at 45 ºC and concentrated extracts were placed in large surface Petri dishes. Finally, the extracts yields were measured using an electronic balance and kept in a refrigerator at 4 °C until use for antibacterial testing (Wado *et al*., 2021).

3.4.3 Distilled water extraction

Extraction using distilled water was performed by soaking fifty grams of the dry powdered plant materials with 250 ml of distilled water in (ratio 1:5 v/w) in 1000 ml of a conical flask and shaking the mixture for 2 days using a rotary shaker. The mixture was filtered through Whitman no. 1 filter paper. The filtrate was then kept on a hot plate at 80 ºC until the water was evaporated. The extract yield was measured and calculated as described by Messina *et al*. (2019) using the following formula.

Extract yield (%) = Weight of dried extract (g)/Weight of leave material (g)*100 1

3.5 Preliminary Phytochemical Screening

The preliminary screening of phytochemical constituents of the extracts was done for the investigation of secondary metabolites compounds such as alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, and steroids by using standard procedures.

Test for alkaloids

One or two drops of Mayer's reagent were mixed with the acidified leaf extract. The appearance of a white or yellow precipitate indicates the presence of alkaloids (Kancherla *et al*., 2019).

Test for Saponins

When a few drops of distilled water was added to 1 ml of leaf extract and the mixture was violently agitated, the presence of saponins was determined by the existence of persistent foam (Onuminya *et al*., 2017).

Test for Tannins

The leaf extract was boiled in 20 ml of distilled water in test tube and filtrated. To the filtrate, few drops of 0.1% Iron III chloride was added to gave a brownish green color which confirms the presence of tannins (Auwal *et al*., 2014).

Test for Flavonoids

A 3 ml of leaf extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids (Edema and Alaga, 2012).

Test for Terpenoids

A 5 mL of the leaf extract was mixed with 2 ml of chloroform. Then 3ml of concentrated sulphuric acid was added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids (Samkeliso *et al*., 2018).

Test for Phenols

The *M. lanceolata* leaf extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols (Kiliobas *et al.,* 2019).

Test for Steroids

Test for steroids was performed following the methods of Asmari, *et al*., (2017). The extract (1 ml) dissolved in 10 ml of chloroform and equal volume of concentrated H_2SO_4 was added. The upper layer turns red and the H_2SO_4 layer was shown yellow with green fluorescence color indicates the presence of steroids.

3.6 Bio Synthesis of ZnO NPs

3.6.1 Preparation of leaf extract.

A 10 grams of the powder sample was boiled for 30 min in 100 ml of distilled water. Finally, the extract was filtered with the Whatman No. 1 filter paper and stored at 4 ^oC for further use (Javed *et al*., 2022).

3.6.2 Preparation of Zinc nitrate hexahydrate solution

Commercially purchased Zinc nitrate hexahydrate (molecular formula $(Zn(NO_3)_2.6H_2O)$ (molecular weight 297.49) was used to prepare a 0.2 M concentration of Zinc nitrate hexahydrate solution. A 0.2 M Zinc nitrate hexahydrate solution was prepared by dissolving 2.93 g of Zinc nitrate hexahydrate in 50 ml distilled water using a 250 ml volumetric flask.

3.6.3 Synthesis of ZnO NPs

For the synthesis of zinc oxide nanoparticles, 50ml of the leaf extract was mixed with 50 ml of Zinc nitrate hexahydrate solution in the same ratio. 1M of NaOH was added drop by drop to the solution to control the pH of the solution at 12 (Shaheen *et al*., 2022). The

mixture was heated and stirred on a magnetic stirrer for 2 h at 50 ºC until the white precipitate was observed. Then, the pellet was centrifuged three times for 10 min intervals at 5,000 rpm and dried in an oven overnight. Then, the dried sample was calcined in a muffle furnace at 450 °C for 2 h and the powder was collected for characterization.

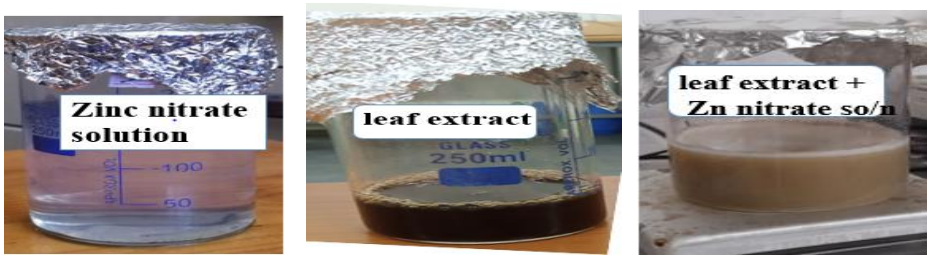

Figure 2. Synthesized zinc oxide nanoparticles

3.6.4 Characterization of Synthesized ZnO NPs

UV–Visible spectroscopy

The optical characterization of ZnO NPs was carried out using UV-Vis Spectroscopy (UV-Vis spectrophotometer, T80 instrument) to measure energy band gap of the particles in the wave length region of 200 nm - 800 nm. First a DMSO solvent was inserted in a 1 cm cuvette as a reference and ZnO NPs was placed in the second cuvette compartment for UV – Vis absorbance measurement.

X-ray diffraction (XRD) Analysis

The crystalline structure of the synthesized ZnO NPs were investigated by the X-ray diffraction using X-ray diffractometer (Bruker D8 Advance Diffractometer). XRD spectrum was recorded from 10˚ to 80˚ with 2θ angles using Cu Kα (λ =1.54 Å) radiation operated at 40 kV and 30 mA. Debye Scherrer's equation indicated in equation 2, was used to estimate the crystalline size

$$D = \frac{0.9\lambda}{\beta \cos\theta} \qquad (2)$$

Where, D is the Crystallite size, λ is the X – ray source wavelength, β is the Full Width at Half Maximum (FWHM) of a peak, and Θ is the Bragg diffraction angle.

Scanning Electron Microscope (SEM) Analysis

Scanning electron microscope (SEM) (JSM-6480 LV) was used to study the morphology of synthesized nanoparticles. The surface structure of ZnO nanoparticles were examined using scanning electron microscope images. The particle size distribution of the

nanoparticles were estimated from the Image J software by treating the nanoparticles as spheres and thus calculating the particle size distribution from the deduced area.

Fourier transform infrared (FTIR) Analysis

FTIR (Perklin-elminer, CT, USA) measurements were carried out to identify the possible bio molecules (functional group) responsible for the reduction of zinc oxide nanoparticles. FTIR was used to characterize the nanoparticles using the powdered nanoparticles samples by KBr pellet method. The absorbance maxima were scanned by FTIR at the wavelength of 400- 4000 cm-1

3.7 Culture and Maintenance of Bacterial Strains

Pure cultures of the test bacterial pathogens were obtained from Oromia Regional Laboratory, Adama. These included, two Gram-positive *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 11778), and two Gram-negative *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27823) were used in study. The bacterial pathogens were cultured on Mueller Hinton Agar (beef extract, casein hydrolysate, starch and agar) and incubated at 37 ^{o}C for 24h. Well separated bacterial colonies were then used for the development of the respective inocula.

3.8 Preparation of Leaf Extracts and ZnO Nps Standard Concentrations

One gram of each methanol, distilled water, chloroform, and hexane extract were taken separately and dissolved in 10 ml of Dimethyl Sulfoxide (DMSO) to prepare 100 mg/ml stock solution, which was a standard concentration. From this stock solution, different concentrations were prepared to obtain (50, 25 and 12.5 mg/ml).

For the ZnO NPs, 0.1 grams was dissolved in 10 ml of DMSO to prepare a 10 mg/ml standard stock solution. Subsequently, different concentrations were prepared from the stock solution, including 5, 2.5 and 1.25 mg/ml.

3.9 Evaluation of Antibacterial Activities of Leaf Extracts and ZnO Nps

In *vitro,* antibacterial activities of all solvent leaf extracts and synthesized ZnO NPs were examined using the disc diffusion method against four pathogenic bacteria. Diffusion discs of approximately 6 mm diameter was prepared from Whatman No. 1 filter paper by using a puncher and placed in a beaker and sterilized by oven for 30 min. Then the required number of sterile discs were impregnated on slides using forceps aseptically. A 20 µl of leaf extracts and synthesized ZnO NPs were taken from the prepared concentrations and

impregnated on a sterile 6 mm disc using sterile micropipette tips followed by air drying for 3-5 min. The medium was then poured into Petri dishes and allowed to solidify. The inoculum (100 µl) was spread evenly onto 20 ml Mueller-Hinton agar set in Petri dishes using a sterile cotton swab. This was followed by placing each piece of sterile assay disc impregnated with each leaf extracts and synthesized ZnO NPs on the surface of MHA petri plates using sterile forceps. Paper discs that were similarly handled but without leaf extract (DMSO) served as negative controls and paper discs impregnated with Ciprofloxacin (at the concentration of 10 µl /ml) were used as positive controls. The Petri dishes were then left at room temperature for 30 min for proper diffusion and incubated at 37 ^{o}C for 24 h for each bacterium. Petri dishes were observed for inhibition zones (zones showing no microbial growth) around the discs after the incubation period and then the resulting diameters of zones of inhibition were measured using a ruler. The results were indicated by the zone of inhibition including the diameter (6 mm) of the paper disk. All the tests were conducted in triplicates to minimize test error and the average of the three measurements was used to represent the results.

3.10 Determination of Minimum Inhibitory Concentration (MIC)

The leaf extracts that showed significant antibacterial activity was selected for the determination of MIC. The MIC of the leaf extracts was determined by the broth dilution method. The initial concentration of the leaf extract (100 mg/ml) was diluted using two fold serial dilution by transferring 1ml of the sterile leaf extract (stock solution) into 1ml of sterile nutrient broth. Then mixed it into a vial and then serially diluted it into 5 vials then mixing well using vortex shaker the serial dilution gave concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml (Eve *et al.,* 2020). Each concentration was inoculated with 0.02 ml of the standardized bacterial cell suspension and incubated at 37 ^{o}C for 24 h. The turbidity or cloudiness of the broth was an indicator of bacteria growth in the broth (Abou *et al*., 2010). The lowest concentration, at which there has no turbidity (determined by the naked eye), has considered the MIC value of the leaf extracts (Radojević *et al.*, 2012).

3.11 Determination of Minimum Bactericidal Concentrations (MBC)

The MBC was recorded as the lowest extract concentration killing 99.9% of the bacterial inocula to prevent the growth of an organism after subculture onto media after 24 h incubation at 37 °C (Parvekar *et al*., 2020). The broth dilution method was used to determine the leaf extract's minimum bactericidal concentration. A fresh nutrient broth

medium was used to transfer samples from the MIC determination set. A loop full of all the tubes used in the MIC, which did not show bacteria growth inoculated and sub-cultured onto sterile nutrient agar media streak plating by a sterile wire loop. 1 ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and sub-cultured onto nutrient agar and incubated at 37 ^{o}C for 24 h. After incubation the concentration at which there has no single colony of bacteria was taken as MBC.

3.12 Antioxidant Assay against Leaf Extracts

The antioxidant activity of the leaf extracts was measured based on their free radical scavenging activity, which was determined by the DPPH method. As a result of the color changing from purple to yellow, the absorbance was decreased when the DPPH radical is scavenged by an antioxidant through the donation of hydrogen to form a stable DPPH-H molecule (Matkowski and Piotrowska, 2006). The leaf extracts were dissolved in three methanol containing vials to gave concentrations of 1000, 500, 250 and 125 µg/ml. Ascorbic acid used as a positive control, while a sample free DPPH solution in methanol was as a negative control.

The solution of DPPH (0.04 mg/ml) in methanol was prepared, and 3 ml of the solution was mixed with 1 ml of extract solution at various concentrations immediately and then incubated for 30 min at room temperature to complete the reaction. The absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage (IP) of free radical and calculated using the following formula:

$$\textbf{DPPH Scavenged activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100 \quad (2)$$

$A_{control}$ and A_{sample} are the absorbance values for the control and sample respectively (Olugbami *et al*., 2014).

3.13 Data Analysis

The data experimental were analyzed using Statistical Package for Social Sciences (SPSS) (version 22) software. The statistical difference (Mean value and SD) along with comparison of the mean zone of inhibition of the extracts for individual bacterium was carried out by employing a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p<0.05$).

4. RESULTS AND DISCUSSION

4.1. Plant Extracts Yield

The distilled water, methanol, chloroform, and hexane solvents gave different extraction yields. The highest percentage yield was observed in distilled water solvent (8.6%, 4.3g) followed by methanol (6.8%, 3.4g), chloroform (5.8%, 2.9g), and the lowest yield was obtained by hexane (2.4%, 1.2g).

4.2. Phytochemical Screening

The result of this study confirmed the presence of various phytochemicals in leaf extracts of the study plant. The phytochemicals present in the leaf extracts included alkaloid, flavonoid, phenol, saponin, steroid, tannin and terpenoid. The results revealed that alkaloid, saponin and flavonoid were present in all three solvent extracts. Tannin was detected in distilled water and methanol extracts, while terpenoid was only found in the distilled water extract. Phenol was present in both distilled water and methanol extracts, but not in the chloroform extract. Steroid was detected in distilled water and methanol extracts as shown in (Table 2). But, phytochemical analysis was not performed hexane extract.

Table 2: Phytochemical constituents of the leaf extracts

Phytochemicals	Extracts		
	Distilled water	Methanol	Chloroform
Alkaloid	+	+	+
Saponin	+	+	+
Tannin	+	_	_
Phenol	+	+	_
Flavonoid	+	+	+
Terpenoid	+	_	_
Steroid	+	+	_

Key: (+) presences (-) absence

Figure 3. Phytochemical screening of different solvent extracts.

DWE-distilled water extract, ME-methanol extract, CHE-chloroform extract.A, H and O, alkaloid; B, I and P, saponin; E, L and S, flavonoid; C, tannin; D, and K, phenol; G, and N, steroid; F, terpenoid showed positive results.J and Q, tannin; R, phenol; N and T, terpenoid; U, steroid showed negative results.

4.3 Characterization of ZnO Nps

4.3.1 UV-Vis Absorbance Analysis

The formation of ZnO NPs using leaf extract was confirmed by measuring the UV-visible spectrum of the reaction mixture at wavelengths ranging from 200 to 800 nm. The synthesized ZnO NPs had an optical band gap energy of 3.31 eV at a maximum wavelength of 373.97 nm indicated the presence of ZnO NPs (Figure 4).

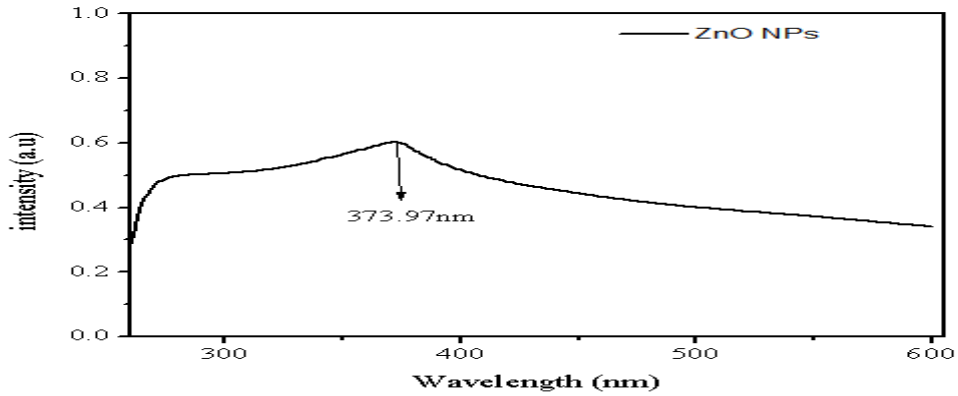

Figure 4: UV–vis absorbance spectra of ZnO NPs.

4.3.2 X-ray Diffraction (XRD) Analysis

Analysis of the crystalline size of the synthesized ZnO NPs were carried out by XRD. The XRD analysis of synthesized ZnO NPs from leaf extract showed distinctive Bragg diffraction at 2θ values of 31.79, 34.45, 36.27, 47.57, 56.62, 62.89, 66.40, 67.97, 69.10 and 77.00. It was indexed to the plane (100), (002), (101), (102), (110), (103), (200), (112), and (201). The strongest peaks are 2θ = 36.27, 31.79, and 34.45. According to

Debye–Scherrer's equation the average crystallite size for ZnO NPs was 24.3 nm, which were derived from the FWHM of the more intense peak (β =0.360, 0.337 and 0.351) corresponding to the (101, 100 and 002) plane located at 2θ = 36.27°, 31.79° and 34.45°, respectively.

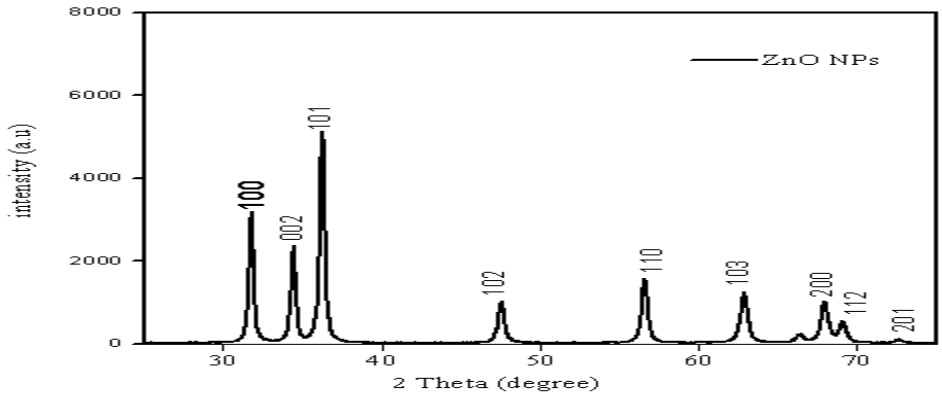

Figure 5. XRD patterns of synthesized ZnO NPs

4.3.3 Scanning Electron Microscope (SEM) Analysis

SEM images in Figure 6 revealed the shape of ZnO NPs was an agglomeration of particles with sizes ranging from 24 to 51 nm.

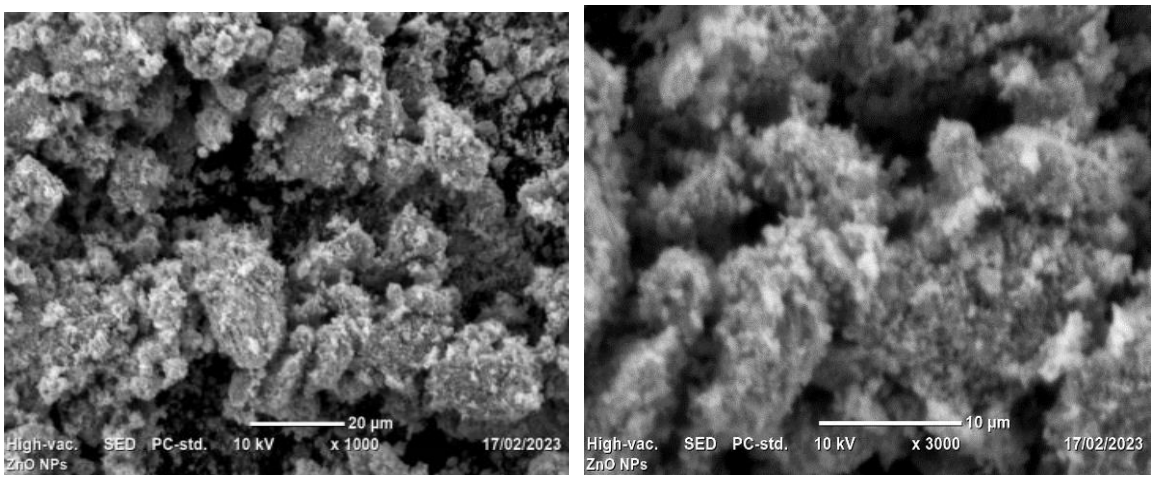

Figure 6. SEM image of synthesized ZnO NPs

4.3.4 Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy was used to identify the types of chemical bonds or the functional groups associated with the formation of ZnO NPs. ZnO NPs FTIR spectrum was recorded in the range of 4000–500 cm^{-1}, observation (figure 7) shows that the bands of ZnO NPs

occur at 2924.14 cm^{-1}, 2854.69 cm^{-1}, 2725.67 cm^{-1}, 1460.48 cm^{-1}, 1377.36 cm^{-1}, 1041.78 cm^{-1} and 722.62 cm^{-1}.

Table 4. Functional groups involved in the synthesis of ZnO Nps

Type of chemical bond	Absorption frequency (cm-1)	Functional group
CH Alkanes	2924.14	C-H stretching
CH Alkanes	2854.69	C-H stretching
Methylene group	1460.48	C-H bending
OH group	1377.36	OH stretching
CH Alkanes	1041.78	C–H rock
CH Alkyne	722.62	C-H bend

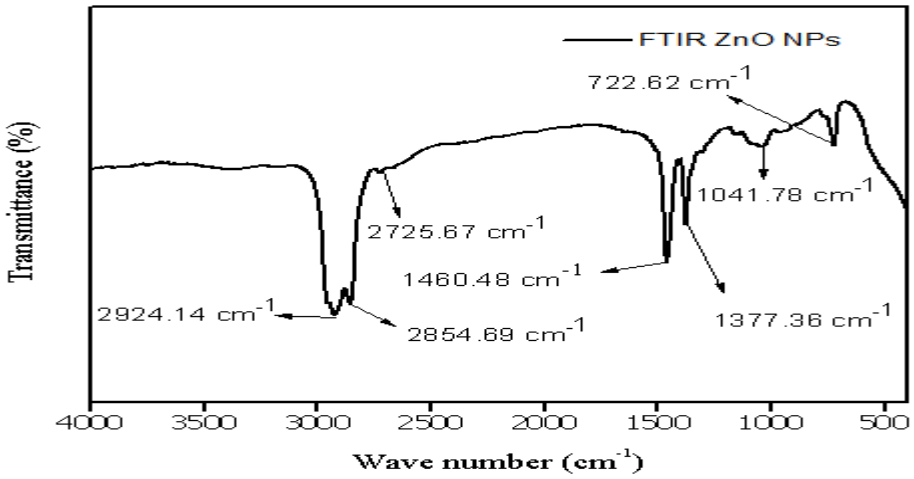

Figure 7. FTIR spectra of ZnO NPs

4.4 Evaluation of Antibacterial Activity of Leaf Extracts and ZnO Nps.

4.4.1 Antibacterial Activity of Leaf Extracts

The results of the evaluation of the antibacterial activities of leaf extracts showed that different solvent extracts have different antibacterial activities. The results of the antibacterial activity test given in Table 5. The mean zone of inhibition values indicated that all leaf extracts exhibited antibacterial activities ranging from 6.3±0.8 mm to 16.3 ±0.88 mm. The methanol, distilled water, chloroform, and hexane extracts showed different activity against the *S. aureus* (ATCC 25923) and *S. pyogenes* (ATCC 11778). The methanol,

chloroform, and hexane extracts did not show any activity against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27823) at all concentrations except the distilled water extract which showed activity against *E. coli* (10.3 ± 1.15mm). Upon the antibacterial activities against *P. aeruginosa,* methanol, and distilled water solvent extracts showed antibacterial activity with a zone of inhibition ranging from (11.0±1.00 to 6.3±0.88, 8.3±0.3to 6. 3±0.4 mm) at 100 mg/ml to 25 mg/ml respectively. But in both extracts were no activity observed at the lowest concentration (12.5 mg/ml). The methanol extract shows the highest zone of inhibition ($p<0.05$) the zone of inhibition ranges from 16.3 ± 0.88 mm to 9.3 ± 0.57 mm at 100 mg/ml and 12.5 mg/ml, respectively against *S. aureus.* The methanol, distilled water, chloroform, and hexane extracts were slightly more active against *S. aureus* (16.3 ± 0.88, 15.0 ± 0.58, 14.00 ± 1.00 and 9.3 ± 0.33 mm respectively) at 100 mg/ml. Similarly, methanol, distilled water, chloroform, and hexane extracts were active against *S. pyogenes (*14.0 ± 1.00, 14.3 ± 0.70, 12.7 ± 0.33 and 9.0 ± 1.20 mm respectively) at 100 mg/ml. Distilled water extract inhibited the growth of *E.coli* (10.3 ± 1.15 mm) at 100 mg/ml. Compared to positive control (18.7 ± 0.80 - 25.3 ± 1.00 mm) for ciprofloxacin. The negative control (DMSO) showed no inhibition against all bacterial pathogens.

Table 5: Antibacterial activity of leaf extracts at various concentrations (Zone of inhibition (mm in diameter) (M ± SD) (n=3)

Extracts	Conc. (mg/ml)	Name of bacteria			
		S. aureus	*E.coli*	*S. pyogenes*	*P. aeruginosa*
Methanol	100	16.3 ±0.88	-	14.0±1.00	11.0±1.00
	50	14.7±0.57	-	13.0±0.57	9.0±1.00
	25	12.0±1.00	_	10.3±1.00	6.3±0.88
	12.5	9.3±0.57	_	6.7±0.33	_
Distilled water	100	15.0 ±0.58	10.3±1.15	14.3±0.70	8.3±0.3
	50	12.7±0.33	9.3±0.57	9.0±1.00	7.3±0.57
	25	10.3±0.33	8.3±0.58	6.7±0.33	6.3±0.4
	12.5	8.7±0.67	6.7±0.33	_	_
Chloroform	100	14.00±1.00	_	12.7±0.33	_
	50	13.0±0.58	_	7.3±0.57	_
	25	10.7±0.67	_	6.7±0.33	_
	12.5	8.0±0.5	_	_	_
Hexane	100	9.3 ±0.33		9.0±1.20	_
	50	7.6±0.57	_	9.0±0.00	_
	25	6.6±0.88	_	7.3±0.40	_
	12.5	_	_	_	_
Ciprofloxacin		25.3±1.00	18.7±0.80	21.5±0.89	23.0±1.15
DMSO		_	_	_	_

Conc-concentration, -ve (Negative), shows that the leaf extracts used could not inhibit the growth of the bacteria.

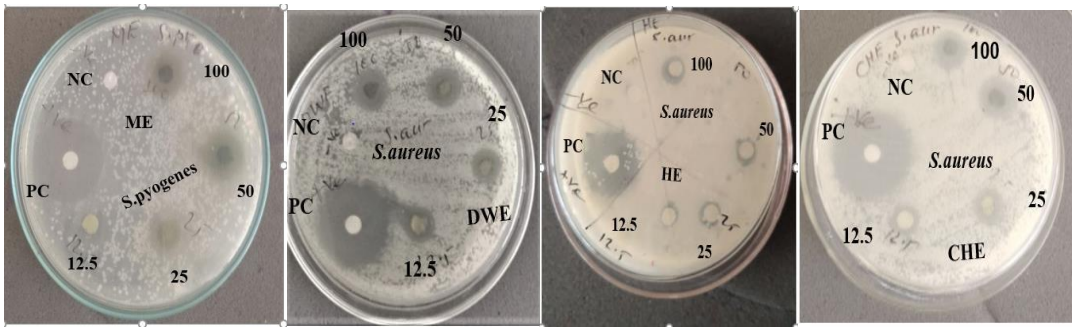

Figure 8: Antibacterial activities of leaf extracts.

DWE-distilled water extract, ME-methanol extract, HE-hexane extract, CHE-chloroform extract, and. NC-negative control, PC-positive control. The numbers 12.5, 25, 50, and 100 indicates the concentration of the extracts in mg/ml.

4.4.2 Antibacterial activity of ZnO Nps

The results of this study indicated that synthesized ZnO NPS have antibacterial activity on all the bacterial strains at all concentrations except *S.pyogenes*. At 10 mg/ml, the antibacterial activity is highest ($p<0.05$) against *E.coli* (17.7 ±0.62 mm), followed by *P. aeruginosa* (14.0 ±0.80 mm) ($p<0.05$), and *S. aureus* (9.7 ±0.57 mm). At 5 mg/ml, the antibacterial effects slightly decrease, with *E.coli* showing the highest activity (13.6 ±0.89 mm).

Table 6. Antibacterial activity of ZnO Nps (Zone of inhibition (mm in diameter) (M ± SD) (n=3)

Name of bacteria	Concentrations (mg/ml)					
	10	5	2.5	1.25	Ciprofloxacin	DMSO
S. aureus	9.7 ±0.57	9.0±0.00	7.6±0.33	7.0±0.00	25.0±1.00	-
E. coli	17.7±0.62	13.6±0.89	9.6±0.57	8.0±1.00	22.6±1.2	-
S. pyogenes	-	-	-	-	22.7±0.89	-
P. aeruginosa	14.0±0.80	9.0±1.00	8.6±1.00	7.0±0.00	22.6±1.52	-

-ve (Negative), shows that the leaf extracts used could not inhibit the growth of the bacteria.

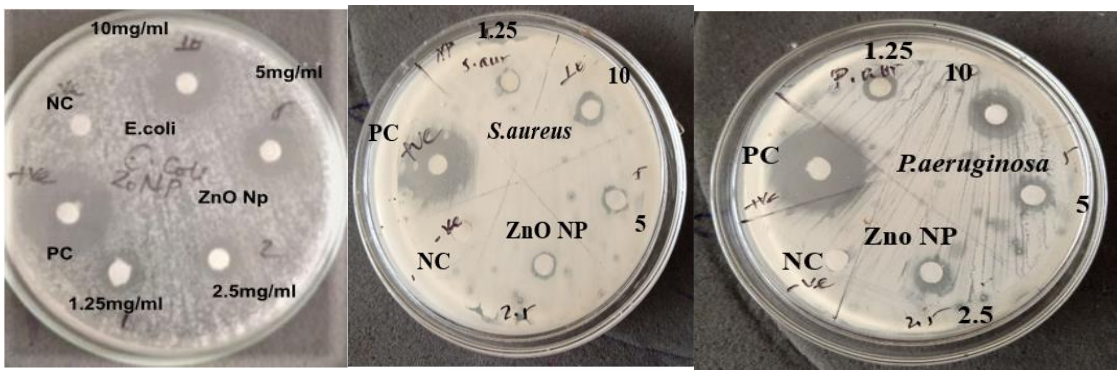

Figure 9: Antibacterial activities ZnO Nps.

ZnO NP- Zinc Oxide Nanoparticles, NC-negative control, PC-positive control, The numbers 12.5, 25, 50, and 100 indicates the concentration of the extracts in mg/ml.

4.5 Minimum inhibitory concentration and minimum bactericidal concentrations

In the present study, the MIC and MBC values obtained from methanol, distilled water, and chloroform extracts showed a difference in the inhibitory and bactericidal concentrations against standard pathogens. For *E. coli*, the MIC and MBC values of the distilled water extract was 12.5 and 25 mg/ml, respectively. The methanol and chloroform extract did not show any inhibition or bactericidal activity against *E. coli*. The MIC and MBC values was similar on all three extracts, with an MIC of 6.125 and MBC of 12.5 mg/ml against *S. aureus. P. aeruginosa* shows MIC at 25 and MBC at 50 mg/ml when treated with both methanol and distilled water extracts. But just like *E. coli*, the chloroform extract did not show any inhibitory or bactericidal activity against *P. aeruginosa*. Finally, for *S. pyogenes*, the MIC and MBC values were the same with methanol and chloroform extracts at 12.5 and 25 mg/ml, respectively, and both MIC and MBC values of the distilled water extracts were slightly higher at 25 and 50 mg/mL, respectively.

Table 7. The MIC and MBC value of the antibacterial activity of the leaf extracts.

Bacterial strains	MIC and MBC (mg/ml)					
	Methanol		Distilled water		Chloroform	
	MIC	MBC	MIC	MBC	MIC	MBC
E.coli	-	-	12.5	25	-	-
S. aureus	6.125	12.5	6.125	12.5	6.125	12.5
P. aeruginosa	25	50	25	50	-	-
S. pyogenes	12.5	25	25	50	25	50

4.6 Antioxidant activity of leaf extracts

The DPPH radical scavenging activities (%) of distilled water, methanol, and chloroform extracts were found to be 78, 79, and 45.5 at 1000 µg/ml, respectively. The observation showed that Ascorbic acid, when used as a positive control (µg/ml), demonstrated the greatest scavenging effect at both very low and high concentrations, with effectiveness rates of 93.2, 92.6, 92.1 and 90% at 1000, 500, 250 and 125 µg/ml, respectively. The

lowest IC50 value was shows by methanol extract (92.8 µg/mL), followed by distilled water extract (160.7 µg/ml) and chloroform extract (440 µg/ml), respectively.

The IC_{50} values of leaf extracts were calculated from a graph of percentage scavenging activity against concentrations of the extracts (Figure 10). The IC_{50} was used to examine the antioxidant effects of the samples. The lower the IC50 value, the higher the scavenging potential. The IC50 values ranged from 92.8 µg/ml for methanol extract to 440 µg/ml for chloroform extract.

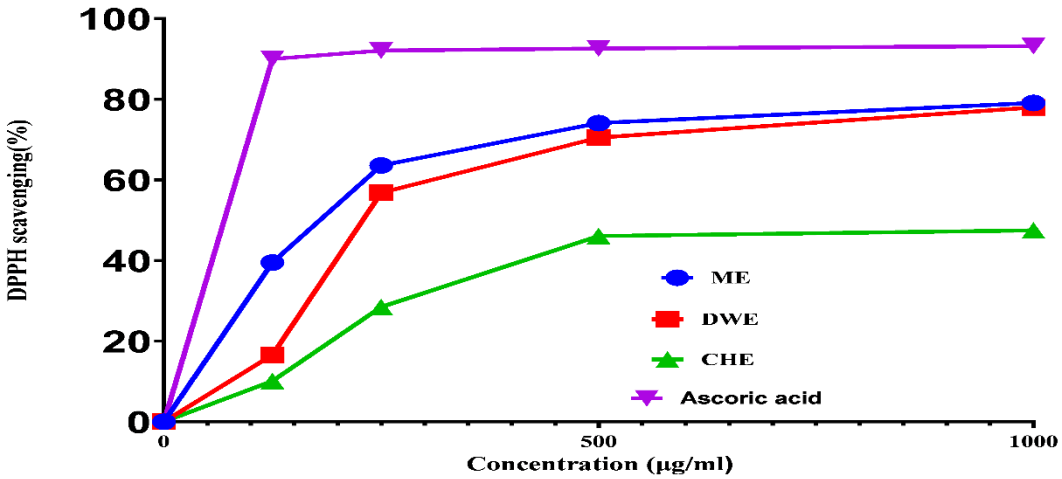

Figure 10. DPPH radical scavenging activity (%) of methanol, distilled water, and chloroform leaf extracts *M. lanceolata* and control (ascorbic acid).

4.7 Discussion

These findings show that the detected phytochemical substances of leaf extract bioactive elements, indicating that the *M. lanceolata* has a valuable reservoir of bioactive compounds with significant medical value. For instance, Timothy *et al*. (2018) reported that alkaloids, terpenoids, flavonoids, saponins, tannins, and phenols are the most common phytochemicals that are found in distilled water extract of *M. lanceolata*. Plant antibacterial properties are reported to be caused by phytochemicals such as tannins, flavonoids, saponins, and phenolic compounds (Jan *et al*., 2017). Flavonoids have been related to numerous biological activities, such as antibacterial, antioxidant, and anti-inflammatory effects. Pathogens are known to be inhibited by the combined action of tannins, flavonoids, alkaloids, and saponins (Othman *et al*., 2019).

The Uv-Vis absorption band of synthesized ZnO Nps were observed at 373.97 nm and had optical band gap energy of 3.31 eV. Some similar result of absorption peak was also

reported at 373 nm (Husain *et al*., 2022), and the energy value was closely related to the standard optical band gap energy of ZnO NPs. X-ray diffraction patterns of synthesized ZnO NPs peaks agreed with the ordinary data (JCPDS card no. 00-065-0725) in regard of intensities and positions. The average particle size of synthesized ZnO NPs obtained from XRD data (24.3 nm) shows good results compared to the previous report (Moharram *et al*., 2014). The SEM image revealed individual ZnO nanoparticles as well as numerous aggregates. This SEM result conciedes with results already reported, with shows the formation of spherical shaped nanoparticles and aggregated molecules in leaf extract of *Calotropis gigantea* (Chaudhuri and Malodia, 2017). The FTIR ZnO Nps spectra show the identity of C-H stretching of intermolecular bonded alkane at 2924.14 cm^{-1} and 2854.69 cm^{-1}. The peak at 1460.48 cm^{-1} indicated the occurrence of C-H bending (Bashir *et al*., 2020). The fingerprint region of the spectra accounts for the presence of O-H bending of alcohol, C-H rock, and C-H bending at 1377.36 cm^{-1}, 1041.78 cm^{-1}, and 722 cm^{-1} (Bayu *et al*., 2019), respectively.

All evaluated extracts showed varied inhibitory activity against all bacterial strains, *S. aureus* was the most sensitive against all solvent extracts, but *E. coli and P. aeruginosa* were the most resistant against chloroform and hexane tests. The chloroform test did not show antibacterial activity at all concentrations against *E. coli* and *P. aeruginosa*. Similar to the previous result chloroform extract has no antibacterial effect at all concentrations (Ismael *et al*., 2021). At 100 mg/ml, the highest inhibition zone (16.3 ± 0.88 mm) was recorded for methanol test against *S. aureus*. Whereas the lowest inhibition zone was recorded (8.7 ± 0.88 mm) in the same extract in *E.coli* strain.

The distilled water and methanol extracts of the present study showed higher inhibition zone than that of *M. lanceolata* studied by Ismael *et al*., (2021) and Timothy *et al*. (2018) but showed lower inhibition zones than *M. lanceolata* 80% methanol and acetone leaf extracts (Samuel, 2004). All solvent leaf extracts showed good antibacterial activities against *S. aureus* which is in agreement with a study done on extracts of *Ricinus communis* (Jeyaseelan and Jashothan, 2012). Most leaf extracts were resistant to Gram-negative bacteria, this could be due to several possible reasons. Among these a reason, Gram-negative bacteria have a double membrane structure. So, the presence of a double membrane in the Gram-negative bacteria may prevent some leaf extract from entering the cell, resulting in increased resistance compared to Gram-positive bacteria. Gram-positive

bacteria lack this important layer, which makes Gram-negative bacteria more resistant to antibiotics than Gram-positive ones (Breijyeh *et al*., 2020).

The antibacterial activity of synthesized ZnO NPs were examined by disc diffusion method. According to the evidence of zone of inhibition of the recorded data shown in Figure 9 and Table 6, we observed that ZnO NPs synthesized using the *M. lanceolata* extract, reported for the first time, exhibited high inhibition zone against Gram-negative bacteria *E.coli* (17.7 ± 0.62 mm) and *P. aeruginosa* (14.0 ± 0.80 mm) compared to gram-positive bacteria *S. aureus* (9.7 ± 0.57 mm) at 100 mg/ml. This may be ZnO NPs entered into the bacterial cell creates reactive oxygen species (ROS). These ROS then interact with cysteine-containing proteins and iron within the cells, ultimately leading to damage in both the DNA and the cell walls (Kebede *et al*., 2023). Besides, *M. lanceolata* leaf extract that was used as a reducing agent has phytochemicals that are toxic to bacteria.

In the present study, the MIC value obtained from distilled water extract was 12.5 mg/ml and 25 mg/ml against *E.coli* and *P. aeruginosa* respectively, which was a similar result with Ismael *et al*. (2021) report with MIC of 12.5 mg/ml for *E. coli* and 25 mg/ml for the *P. aeruginosa.* As the result showed (Figure 10) as the concentration of the samples in the assay increased, the DPPH scavenging activity also increased. It was observed that methanol of leaf extract had higher activity than water and chloroform extracts at a concentration of at all concentrations. The scavenging activity of methanol extract reached 79.1% at 1000 µg/ml. Distilled water and chloroform extracts reached 78 and 45.7 %, respectively at 1000 µg/ml. The lower the IC_{50} value, the higher the scavenging potential. The IC_{50} values ranged from 92.8 µg/ml for methanol extract to 440 µg/ml for chloroform extract. The strongest scavenging activity (lower IC_{50} value) was recorded for methanol extract which appeared more than four folds stronger than that of chloroform extracts and more than half times stronger than that of the distilled water extracts. In the present study, all three leaf extracts showed relatively low antioxidant activity than those reported data with Ismael *et al*. (2021). This difference in the results was probably due to the methods used in extraction, the plant variety, and the methodology for the evaluation of the antioxidant activity.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Medicinal plants have medicinally important compounds in their various parts. In this study, it investigated phytochemical constituents, and green synthesis ZnO NPS of using *M. lanceolata* leaf extract of medicinal plant. The synthesized ZnO NPS were characterized using UV-vis, XRD, SEM, and FTIR methods. The absorption spectra of synthesized ZnO NPs were found to be 373.97 nm with a band gap energy of 3.31 eV. The XRD examinations of synthesized ZnO NPs were the formation of 24.3 nm of particle size. The band at 2924.14 cm^{-1}, 2854.69 cm^{-1}, 2725.67 cm^{-1}, 1460.48 cm^{-1}, 1377.36 cm^{-1}, 1041.78 cm^{-1} and 722.62 cm^{-1} in FTIR spectrum confirms the formation of ZnO NPS. The leaf extracts and synthesized ZnO NPs exhibited antibacterial activity against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* strains. Generally, the result of the present study confirmed the traditional use of this plant against various bacterial infections. Therefore, *M. lanceolata* is a better source of antibacterial agents and hence can be of interest in the development of new chemotherapeutic drugs.

5.2 Recommendation

The *M. lanceolata* leaves suggest that it holds potential as an effective and economical antibacterial agent. However, to determine their effectiveness the *M. lanceolata* leaf should be tested against more resistant clinical isolate pathogenic bacteria, such as methicillin-resistant *S. aureus,* and also be explored to assess their possible antibacterial and antioxidant activities. Further research is needed to isolate the active constituents responsible for the antibacterial effects and to evaluate their potential for development into alternative therapeutic agents against resistant bacterial infections. We have synthesized ZnO Nps using the green synthesis method. Green synthesis using plant extracts is cost-effective, eco-friendly, and saves the nature of the resulting Nps. Because of these, green synthesis of ZnO NPs is recommended for antibacterial activities, biosensors, photocatalysis, cancer treatment, gas sensor, etc.

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