

Synthesis and Characterization of MnO₂/NiO
Nanocomposite Using Onion *Allium cepa* L.(Liliaceae) Peels
Extract and Evaluation of Their Antimicrobial and
Antioxidant Activities



Hirut Hailemariam Weldemedkin

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 Biotechnology

Office of Graduate Studies

Adama Science and Technology University

Jun 2024

Adama, Ethiopia

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DECLARATION

I declare that this Master Thesis entitled “**Synthesis and Characterization of MnO₂/NiO Nanocomposite Using Onion *Allium cepa* L. (Liliaceae) Peels Extract and Evaluation of Their Antimicrobial and Antioxidant Activities**” is my original thesis 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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We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled “**Synthesis and Characterization of MnO₂/NiO Nanocomposite Using Onion *Allium cepa* L. (Liliaceae) Peels Extract and Evaluation of Their Antimicrobial and Antioxidant Activities**” prepared under our guidance by Hirut Hailemariam submitted in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology. Therefore, we recommend the submission of a revised version of the thesis to the Department of Applied Biology following the applicable procedures.

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We, the advisors of the thesis entitled “**Synthesis and Characterization of MnO₂/NiO Nanocomposite Using Onion *Allium cepa* L. (Liliaceae) Peels Extract and Evaluation of Their Antimicrobial and Antioxidant Activities**” and developed by Hirut Hailemariam hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the last version of the thesis.

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We, the undersigned, members of the Board of Examiners of the thesis by Hirut Hailemariam have read and evaluated the thesis entitled “**Synthesis and Characterization of MnO₂/NiO Nanocomposite Using Onion *Allium cepa* L. (Liliaceae) Peels Extract and Evaluation of Their Antimicrobial and Antioxidant Activities**” 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 Biotechnology.

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LIST ABBREVIATIONS AND ACRONYMS

CFU	Colony Forming Unit
DTA	Differential Thermal Analysis
DMOS	Dimethyl Sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full width at half maximum
JPCDS	Diffraction Standard
MHA	Mueller Hinton agar
NC	Nanocomposite
NP	Nanoparticle
PL	Photoluminescence
ROS	Reactive Oxygen Species
RSA	Radical-Scavenging Assay
SEM	Scanning electron microscopy
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SPSS	Statistical Package for the Social Sciences
TGA	Thermo gravimetric analysis
UV DRS	UV–Vis diffuse reflectance spectroscopy
XRD	X-ray diffraction

ABSTRACT

*The most recent and advanced method for producing more nanoparticles which has antimicrobial effect is nanotechnology. The aim of this work was to synthesis MnO₂/NiO nanocomposite (NCs) and MnO₂ and NiO nanoparticles (NPs) using onion peel extract and to evaluate antimicrobial and antioxidant properties. Green synthesis method was used to synthesized the NPs and NCs. The onion peel extract served the dual purpose of reducing and capping agent in the synthesis process. The synthesized materials underwent characterization through various techniques including TGA, FTIR, UV-DRS spectroscopy, XRD, PL, and SEM. The antimicrobial activity of the biosynthesized nanocomposites (NCs) was assessed using the disc diffusion method against fungal (*Candida albicans*) and two strains of Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*), as well as two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Additionally, the antioxidant capacity of the samples was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays in vitro. The XRD patterns revealed distinct peaks confirming the formation of MnO₂ and NiO nanoparticles (NPs) as well as MnO₂/NiO nanocomposites (NCs). The average crystallite sizes were determined to be 14nm, 7.19nm, 6.27nm, 3.95nm, and 6.69nm for MnO₂, NiO NPs, and MnO₂/NiO NCs in three ratios (1:1, 1:2, and 2:1) respectively. FTIR analysis indicates the presence of hydroxyl, carbonyl, and alkyl functional in onion peel powder. SEM analysis verified the non-uniform with irregular-shaped aggregated structure for biosynthesized NCs. The results showed maximum zone inhibition values of 27.1mm and 26mm at a dosage of 75µg/mL towards Gram-negative and Gram-positive bacteria, respectively. MnO₂/NiO NCs, especially the 1:2 ratios, demonstrated superior antibacterial activity (21.2-27.16mm) at a concentration of 75µg/mL. The best antifungal activity was displayed by *Candida albicans* at MnO₂/NiO NCs in 1:2 (16.12mm) at a concentration of 75µg/mL. At 1000µg/mL, the DPPH radical scavenging activity showed inhibition for the 1:1, 1:2 2:1 MnO₂/NiO NCs volume ratios, respectively, of 71%, 71.28%, and 71%. Generally, among various ratios tested, the 1:2 ratios of MnO₂/NiO NCs demonstrated superior antimicrobial and antioxidative properties.*

Keywords: Antibacterial activity, Nanoparticle, Nanocomposite, Onion peel, Green Synthesis

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the Study

Innovations in Nano biotechnology have led to fascinating discoveries in materials science. "Nanotechnology" encompasses research and technology development conducted at atomic, molecular, and macromolecular scales, enabling controlled manipulation and exploration of structures and devices spanning from 1 to 100 nanometers in length (McNeil, 2005). This technology has been applied across various fields including cosmetics, environmental safety, defense, food, agriculture, and health. It enables the biosynthesis of economically and environmentally beneficial metal nanoparticles (Nahar *et al.*, 2020). Due to their small dimensions NPs exhibit remarkable and unique interactions with numerous biomolecules both within human cells and on their surfaces (Ajdari *et al.*, 2016; Patra *et al.*, 2010). The physical and chemical attributes of nanoparticles (NPs), encompassing biological, catalytic, electronic, magnetic, mechanical, and optical properties, hold significant potential for diverse medical applications like drug targeting, delivery, and formulation. Consequently, various sectors worldwide have increasingly focused on nanoparticles (Ajdari *et al.*, 2016; Gurunathan *et al.*, 2015).

A diverse ways of NPs can now be synthesized through a wide spectrum of techniques including physical, chemical, and biological methods. (Shiny *et al.*, 2019). Nevertheless, the majorities of chemical and physical techniques are expensive and require to apply hazardous reagents which cause a number of biological hazards. As a result, cheaper, more environmentally friendly, and less hazardous alternatives are being developed (Jagpreet, 2018). An emerging advantage of nanotechnology that offers a safe, economical, and ecofriendly synthesis method is the synthesis of nanomaterials using biological systems assisted by certain biotechnological tools. Conversely, green synthesis techniques have been acknowledged as a potentially effective means of overcoming the drawbacks associated with the aforementioned approaches. Because plant extracts are readily available, inexpensive, and biocompatible, great efforts have been made recently

to apply them to the creation of various metal oxides and metallic nanoparticles (Ahmed *et al.*, 2016).

The Alliacea family are among the earliest vegetables to be cultivated in recorded history (Hannan *et al.*, 2010). Onions belonging to this family, are among the earliest cultivated plants used for food and medicine, and folk healers have long used them to prevent infection (Tunncliffe *et al.*, 2011). The onion plant, or *Allium cepa* L., is a significant food which contains phytomolecules including anthocyanin, phenolic acid, flavonoids, copanenes, thiosulfate, and organosulfur compounds (Rodrigues *et al.*, 2017). These compounds have been shown to an effective properties fight against a variety of degenerative pathologies, including cancer, neurological and cardiovascular disorders, and other an normality because of oxidative stress (Slimestad *et al.*, 2007).

Nanoparticles generated using onion peel extract have demonstrated supporting antimicrobial properties against a variety of pathogens, such as fungus, bacteria, and viruses. Although the exact mechanism behind these nanoparticles' antimicrobial activity is unknown, it is thought that it comes from their low size, high surface area, and distinct physicochemical characteristics. Patra *et al.*, 2016b reported that significant antibacterial activity was demonstrated by silver NPs fabricating using onion peel extract as copping and reducing agents against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Ipek *et al.*, 2023 reported gold nanoparticles made with onion peel extract exhibited antifungal activity against the common fungal pathogen *Candida albicans*.

Due to the influence of multi-drug resistant pathogens, which have decreased the effectiveness of many currently prescribed antibiotics and increased healthcare costs, complications, and mortality, the accessibility and affordability of these medications have been compromised globally. Antibiotic resistance is thought to have a yearly direct health care and productivity cost of between \$20 billion and \$35 billion, according to a number of reports (Kebede *et al.*, 2021). This situation is made more challenging in low-income or developing nations by the lack of laboratory diagnostics, efficient surveillance systems, and access to the right antimicrobials. In the absence of alternative measures to acquire novel

pharmaceuticals, global mortality rates are projected to reach 10,000,000 by 2050, with costs potentially reaching up to \$100 trillion (Bakal *et al.*, 2017). The greatest alternatives to address these problems are antibiotics made from naturally occurring bioactive compounds (Equar *et al.*, 2019). The search for novel antimicrobial agents is heavily focused on medicinal plants and nanomaterials (Sisay *et al.*, 2019). According to a number of studies, medicinal plants contain bioactive substances that act as catalysts for the synthesis of antibiotics, including coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes (Sisay *et al.*, 2019).

Unpaired electrons in unstable atoms or molecules are known as free radicals, and they are created during regular biological processes. By pairing with other biomolecules found in human cells, such as proteins, carbohydrates, DNA, and lipids, these unpaired electrons have the ability to become stable and cause damage to macromolecules (Islam *et al.*, 2013). According to Phaniendra *et al.* (2015), this harm causes oxidative stress to rise in people with a number of illnesses, including diabetes, cancer, neurodegenerative diseases, the cardiovascular system, and ageing. It is well recognized that eating foods high in antioxidants lowers the chance of developing illnesses brought on by these free radicals. Antioxidants are substances that have the ability to scavenge free radicals by donating electrons, thereby preventing their entry into our bodies. Nanoparticles have been found to possess antioxidant properties, which can help protect cells from oxidative stress and damage caused by free radicals. These antioxidant properties can be attributed to the ability of nanoparticles to scavenge free radicals, mimic the activity of antioxidant enzymes, chelate metal ions, and exhibit antioxidant activity (Khalil *et al.*, 2020).

The objective of this study was to synthesize MnO₂, NiO NPs, and MnO₂/NiO NCs using onion peels. Subsequently, these materials were comprehensively characterized and analyzed for their antimicrobial and antioxidant properties. The characterization XRD, TGA/DTA, FTIR, SEM, UV-Vis DRS, and PL. Finally, the antimicrobial and antioxidant activities of the synthesized MnO₂, NiO NPs, and MnO₂/NiO NCs was evaluated.

1.2 Statement of the Problem

Infections because of pathogenic microbes are global problems and a constant health risk in all countries. Today, the burden of infectious diseases on health, economy, and other social dimensions is so complex that global costs cannot be estimated (Dye, 2014). Current antimicrobial therapies are declining in efficacy due to their limited variety, antagonistic interactions, and, above all, the impact of unfinished antibiotic therapy lead to the development of microbial resistance is a pressing issue globally. Multi-drug resistant microbes represent one of the most severe public health challenges faced by the world. Emergence of resistant microbes often reduces the effectiveness of current treatments and poses a serious public health risk (Versporten *et al.*, 2018). Strategies for maintaining antibiotic efficacy have been recommended by various scientists. One alternative to combating multi drug resistant microbes are the application of nanomaterials with antimicrobial properties. In addition, existing scientific obstacles and a lack of funding have hindered the production of new antibiotics. Therefore, careful consideration in nanomaterials synthesis with an antibacterial effect and their cost-effectiveness has led to the creation of MnO₂, NiO nanoparticles, and so many others. Many scientists have produced MnO₂, NiO nanoparticles, but a green synthesis of MnO₂/NiO nanocomposite using onion peel extract for antimicrobial and antioxidant is not well studied. The majority of chemical methods used to produce nanoparticles and nanocomposite were costly, and used hazardous chemicals that presented a variety of biological risks, and limited the uses of chemically synthesized nanoparticles, particularly in the medical field. Despite this, the uses of chemically synthesized nanoparticles to treat different diseases have been widely employed, which has resulted in environmental pollution and the emergence of resistant strains. In addition to requiring expensive equipment and high temperatures and pressures, physical synthesis methods were energy-intensive (Shiny *et al.*, 2019) . Utilizing biological methods for large-scale nanoparticle production offers an eco-friendly approach as it avoids the use of toxic chemicals. Additionally, it reduces the overall cost of synthesis by eliminating the requirement for external experimental conditions like high pressure and temperature (Singh *et al.*, 2018a). Consequently, biological approaches have emerged as promising alternatives to physical and chemical methods for

nanoparticle manufacturing. This has led to the increasing interest in the biological synthesis of NPs and NCs by provides a feasible alternative to existing techniques of eradicating dangerous germs. This study simple used magnetic stirrer and stirring the sample at room temperature to produce MnO₂, NiO NPs, and MnO₂/NiO NCs utilizing a green synthesis approach.

1.3. Objectives of the Study

1.3.1. General Objective

To synthesize and characterize MnO₂, NiO NPs, and MnO₂/NiO NCs using onion peel extract and evaluate their antimicrobial and antioxidant potential applications.

1.3.2 Specific Objectives

- To synthesize MnO₂, NiO NPs and MnO₂/NiO NCs using onion peel extract.
- To characterize the synthesized nanoparticles and nanocomposite using various techniques such as XRD, TGA, FTIR, SEM, UV-Vis DRS and PL analysis.
- To evaluate the antibacterial activity of the synthesized NPs and NCs against *Escherichia coli* and *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*
- To evaluate the antifungal activity of the synthesized NPs and NCs against *Candida albicans*.
- To investigate the antioxidant potential of the synthesized NPs and NCs using DPPH assay.

1.4 Scope of the Study

This study was focused on the synthesis of MnO₂, NiO NPs, and MnO₂/NiO NCs using onion peel extract. Additionally, the study involved the comprehensive characterization of the synthesized nanoparticles and nanocomposites using techniques such as XRD, TGA, UV-Vis DRS, PL, FTIR, and SEM. Furthermore, the antibacterial efficacy of the synthesized nanoparticles and nanocomposites was evaluated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes* bacterial strains. Moreover, the ability of the synthesized nanoparticles and nanocomposites to inhibit the growth of *Candida albicans* fungal strain was assessed using disc diffusion methods. Lastly, the antioxidant potential of the synthesized materials

was investigated through DPPH assays to determine their ability to scavenge free radicals or mitigate oxidative stress.

1.5 Significance of the Study

First applying onion peel extract as a green and sustainable source for the synthesis of nanocomposites was environmentally friendly. By utilizing waste materials like onion peels, the study contributes to reducing waste and create ways to reuse bio resources. Second, the development of NCs with antimicrobial properties is of huge importance in healthcare areas. With the rise of antimicrobial resistance and the limited effectiveness of conventional antibiotics, alternative solutions are urgently needed. The synthesis of MnO_2/NiO NCs offers a promising avenue to find new antibiotics. Also, this study's focus on antioxidant applications of the synthesized NPs and NCs. Antioxidants play a pivotal role in neutralizing detrimental free radicals and minimizing oxidative harm. By investigating the potential antioxidant properties of the MnO_2/NiO NCs, this study endeavors to contribute to the advancement of natural and efficient antioxidants for diverse applications.

This have significant implications for multiple sectors such as healthcare, food, pharmaceuticals, and environmental protection. Firstly, the healthcare industry can benefit from the antimicrobial properties of these nanocomposites. This can lead to the development of antimicrobial coatings, wound dressings, and medical devices, ultimately improving patient outcomes and reducing the risk of infections. Second, the food industry can utilize the antimicrobial and antioxidant properties of the nanocomposites to extend the shelf life of perishable products and enhance food safety. This can help prevent the growth of pathogenic microorganisms and reduce the risk of foodborne illnesses. The pharmaceutical industry can also benefit from the antioxidant properties of the nanocomposites. By incorporating these nanocomposites into drug delivery systems, pharmaceutical formulations can be protected from oxidative degradation, enhancing their therapeutic efficacy. Lastly, the study serves as a valuable resource for researchers and scientists in the field of nanotechnology. It provides insights into the synthesis and characterization techniques for these nanocomposites, opening up possibilities for further research and exploration of their applications in various industries.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Nanoparticles

Nanotechnology is a technology related to design, formation, and usage of materials with nano sized components and dimensions of these components (Tatli Seven *et al.*, 2018). It is the processes carried out by controlling the matter at the molecular level. Medicine, devices and systems that are formed and used due to their small dimensions have different properties or functions. The nanotechnology term was first used by Feynman in 1959 which is considered as the mark of beginning the of modern nanotechnology. It is possible to use nanotechnology in various fields such as medicine, pharmacy, food industry, veterinary medicine, and textile. Nanotechnology is thought to be originally designed for vaccines and cancer drugs (Sanginario *et al.*, 2017).

Greek word "Nano," which meaning "dwarf" or "very small," is used to signify one thousand millionth of a meter (10^{-9} meters). It is critical to distinguish between nanotechnology and nano science. According to Mansoori and Soelaiman, (2005) nanotechnology is the application of nano science to real-life uses such as devices. Nano science, also known is the scientific investigation nanostructured materials sizes (1 to 100 nm). Nanotechnology is one of the twenty-first century's most fascinating new technologies. It is a capacity to observe, measure, manipulate, construct, control, and fabricate materials at the nanoscale in order to put nano science theory into practice (Bayda *et al.*, 2020).

Small particles with a size in at least one dimension ranging from 1 to 100 nanometers are called nanoparticles. According to an analysis by Nanotechproject.com, there are currently more than 1,317 consumer products based on nanotechnology (Weiner *et al.*, 2016). Manufactured nanomaterial offer superior variable optical properties, higher reactivity, and better adjustable electronic characteristics relative to their macroscopic form or attributes in contrast to their bulk state counterparts (Ferrari, 2019). The dimensions, morphology, and composition of the nanoparticles exhibit diversity. They range in size from 1 nm to 100 nm and can take on shapes such as spherical, cylindrical, tubular, conical,

hollow, spiral, planar, etc. The surface may display texture disparities or maintain consistent smoothness. According to Ealias and Saravanakumar (2017), Specific nanoparticles exhibit crystallinity or morph and contain single or multiple crystal structures that are either dispersed and aggregated.

Nanoparticles can be categorized as either organic (including polymers and lipids) or inorganic (comprising metals, metal oxides, metalloids, and metalloid oxides), or as a hybrid of both. combination of both, determines their chemical and physical characteristics in addition to their size (Cupaioli *et al.*, 2014). Furthermore, nanoparticles' physical characteristics are influenced by their shape. The surface area of nanoparticles is influenced by their size and morphology, as well as their composition and surface characteristics (Kettler *et al.*, 2014). NPs possess a higher surface area-to-volume ratio compared to larger particles. Therefore, the main benefit of nanomaterials is their ability to offer an extremely large surface area using only a small quantity of material.

Because of the special chemical and physical characteristics of nanomaterial, interest in them has grown significantly in recent years (Awwad *et al.*, 2015). It is also noticeable that distinctive chemical and physical properties of nanomaterial set them apart. set them apart from those found in bulk in terms of Nanomaterial can be identified by their diffusion, electrical resistance, conductivity to electricity, strength, hardness, chemical reactivity, and a wide range of biological activities. have contributed to an exponential increase in research interest in these materials (Sahooli *et al.*, 2012). Because based on particular features involving size, shape, distribution, ionic strength, capping agent, and morphology, they show radically new or enhanced qualities. The scientific investigation of nanoparticles is quite important. They serve as a link between the bulk material and the atomic/molecular structure (Kurkure *et al.*, 2016a). Additionally, due to their potential uses, which are greatly impacted by their morphology, size, and structure (Gopalakrishnan *et al.*, 2012).

2.2. Nanoparticles of metal oxides

Developing regulated formation of nanoparticles metal with precise dimensions, shapes, and compositions is basic challenge. Within the context of nanotechnology

in contrast to bulk metals, metal nanoparticles have special optical, magnetic, electronic, and catalytic properties. This might lead to exciting new applications in fields like optics and electronics as well as the biomedical sciences (Gopalakrishnan *et al.*, 2012). Specifically, metal oxide NPs are potential asset for biomedical applications because of their unique features. These benefits include stability, efficient production, customizable dimensions, consistent swelling, integration into hydrophobic and hydrophilic systems, and straightforward functionalization due to the surface's negative charge, accommodating various molecules (Nikolova and Chavali, 2020). Since metal oxides, such as NiO, ZnO, MgO, and CuO stable under extreme conditions and are considered as safe, they are frequently utilized in the production of nanomaterial's safe (Kumar *et al.*, 2021). Various metal nanoparticles or metal oxides have been used in medicine, biology, and pharmacy in recent years. These include copper, gold (Nadeem *et al.*, 2017), and NiO (Singh *et al.*, 2018b). The antibacterial activity of nanoparticles against various bacteria is what makes them useful in biology (Khan *et al.*, 2021b). Previous research indicates that the antibacterial properties of nanoparticles can cause modifications to their size, shape, morphology, and functional group (Narayanan and Sakthivel, 2011). Rather, because of their safety and stability when paired with organic nanoparticles, inorganic nanoparticles can be used in multiple applications than one (Taghavi *et al.*, 2013). Among the fundamental characteristics of metal oxide nanoparticles are their antibacterial activity, which results in the inactivation bacteria which have both gram-positive and gram-negative (Khan *et al.*, 2021a; Khatami *et al.*, 2018).

2.2.1 Manganese Oxide Nanoparticles (MnO₂ NPs)

Pharmaceutical, catalysis, ion-exchange, adsorption, sensor, and energy industries have all used MnO₂NPs (Moon *et al.*, 2015). MnO₂ and NiO nanoparticles have also not had their green synthesis applications thoroughly investigated, nor has the application of MnO₂ nanoparticles emerged from green synthesis. Antimicrobial agents, these nanoparticles are non-toxic. According to Ganeshan *et al.*, (2017); Zhang *et al.*, (2014), MnO₂ is regarded as one of the best catalysts because of its inexpensive, non-toxic also compatibility with the environment. Manganese oxide exists in several forms: greenish manganese(II) oxide (MnO), dark brown manganese(III) oxide (Mn₂O₃), black manganese dioxide (MnO₂), and volatile

liquid manganese(VII) oxide (Mn_2O_7). Each form has distinct chemical compositions and properties. Because of its special qualities, MnO_2 is one of the most appealing oxides. According to Ganeshan *et al.* (2017), MnO_2 is a semiconductor with a high visual constant coupled with a small band gap that possesses catalytic and ferroelectric qualities. It has the same basic structure of MnO_6 but it is linked differently. It can exist in a variety of crystal structures, consisting hollandite (a), pyroclastite (b), nsutite (c), birnessite (d), and akhtenskite (k), etc (Najafpour and Allakhverdiev, 2012).

Manganese based nanoparticles are environmentally friendly, cost-effective, Its high capacity for adsorption and biocompatibility enable them perfect for a range of biological applications, included but not limited to medication delivery, photo thermal therapy, antioxidants, anticancer, and antibacterial bio sensing (Cai, 2018). Manganese nanoparticles have been shown to have high antimicrobial characteristics (antibacterial and antifungal), hence they are being intensively researched and investigated (Kamran *et al.*, 2019). Antibacterial characteristics nanocomposite tested against two types of bacteria: Either gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Staphylococcus bacillus*) bacteria can be found. Results were contrasted with samples that received no treatment with curcumin and a chloramphenicol positive control experiment.

Furthermore, the four fungal strains (*Aspergillus niger*, *Candida albicans*, *Trichophyton simii*, and *Curvularia lunata*) that the nanoparticles proved to have antifungal efficacy against were using the medication fluconazole as the positive control. Thus, it is evident from the data that the biosynthesized Mn nanoparticles have antibacterial and antifungal properties. Several nanoparticles, including manganese nano forms, have antioxidant-like capabilities for scavenging due to their many oxidation states. Reactive oxygen species (Mahlangeni *et al.*, 2020; Pardhiya *et al.*, 2020) demonstrated the *in vitro* antioxidant activity of Bovine serum albumin template MnO_2 kidney tissue of human embryos with nanoparticles. It was discovered that these nanoparticles increased energy of the kidney cells and functioned comparable to the antioxidant activity of catalase, superoxide dismutase, and peroxidase. Nano manganese oxide exhibits unique

thermal, optical, mechanical, electrical, chemical, and magnetic abilities due to its unique structure. (Ramanujam and Sundrarajan, 2014).

Manganese oxide, one of the many metal oxides, is an interesting kind of inorganic material because of its amazing chemical and physical characteristics. Applications for manganese oxide nanoparticles include ion exchange, catalysis, biosensors, energy storage, and adsorption (Gnanam and Rajendran, 2011). Although there are many possible manganese oxides, MnO_2 , Mn_2O , and Mn_3O_4 are the most well-known and stable oxides (Gnanam and Rajendran, 2013). Because manganese oxides are plentiful, environmentally benign, and utilized as green catalysts in the catalysis industry, they are also extensively researched. Joshi *et al.* (2020) report the antibacterial the procedure of well diffusion was employed to examine MnO_2NPs ' activity against *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Streptococcus mutans*, and *Staphylococcus aureus*.

2.2.2 Nickel oxide (NiO) Nanoparticle

Phase stability, a bandgap of 3.6 eV, a large electrical conductivity, high magnetic permeability, and low eddy current loss set apart NiO, an instance of transition metal oxide that is ideal for sensing and photo catalysis at a low cost of fabrication. (Mezher *et al.*, 2020). Nano crystalline NiO's particular interest is its surface effect attraction properties and size, as well as its chemical stability and technical uses. have increased interest in it (Boudiaf *et al.*, 2021). Semiconductors, dye-sensitized solar cells, magnet materials, electrochromic films, clear p-type conducting films, anti-ferrous layer gas capsules, heterosensitive catalytic materials, and alkaline battery cathodes, are just a few of the uses for NiO (Khodair *et al.*, 2020, 2019). Because biosynthesis a combination of chemical and physical approaches, is more cost-effective and ecologically friendly since it requires less energy and is free of toxic substances or high temperatures. They also improve the control of biosynthesis and crystal development. NPs offer a wide range of practical uses. (Anand *et al.*, 2020).

In recent times, reports of the green synthesis of NiO NPs for a variety of applications have been made. NiO NPs were created in a study employing plant extract from *Moringa oleifera* by (Pandey *et al.*, 2013). They were found to contain a particularly powerful cytotoxic action against HT-29 cancer cells and the

best antibacterial qualities against bacterial infections. Additionally, *Coriandrum sativum* L. worked by Kaviyarasu *et al* (2017) to pair up nickel oxide using a microwave-assisted leaf extraction method. The results demonstrated that the nano crystalline NiO produced was appropriate for possible catalytic applications. The competent The generated NiO NPs' antimicrobial properties makes them suitable for use in the therapeutic field. The synthesis of nickel oxide nanoparticles was reported by (Gonzalez, 2006; Iqbal *et al.*, 2019), who used *Rhamnus virgata* as an agent for the interaction, stability, and lowering. They reached find that *Rhamnus virgata*'s NiO NPs, which have minimal cytotoxicity towards normal cells, may have important biomedical uses. Furthermore Uddin *et al.*, (2021) synthesized nickel oxide nanoparticles using a *Berberis Balochistanica* leaf extract. Further more profound antibacterial and antifungal qualities have been documented against numerous bacteria and fungus.

2.2.3 Nanocomposite

In recent times, a term nano-composite material has evolved to encompass a broad category of systems, encompassing materials that are one, two, and three dimensions. As well as amorphous materials that are mixed at the nanoscale and composed of distinctly different components (Weiner *et al.*, 2016). "Materials with a nanoscale structure that improve the macroscopic properties of products" is what Okpala (2013) defines as nanocomposites. He noted that the common building blocks of nanocomposites are nanoparticles and polymer, clay, carbon, or a combination of these. Since the beginning of time, the basic culture of humanity has been the creation of new material. Therefore, two or more materials can be combined to create a new material known as composite material, which has unique properties. Solids composed of two or more distinct elements or phases on a scale bigger than an atom are called composite materials. The development of composite materials dates to the dawn of civilization when mud bricks and concrete were created. Composites are made of two different kinds of materials: the matrix, also known as the binder, which binds and encloses pieces of the other material, known as the reinforcement. A composite is referred to as a nanocomposite if one of its constituent materials has a nanometer-scale dimension (Kurkure *et al.*, 2016b).

2.2.3.1 MnO₂/NiO Nanocomposites

MnO₂/NiO nanocomposites prepared by chemical method have been thoroughly investigated for a range of uses (Obodo *et al.*, 2022). MnO₂/acid-treated carbon nanotube nanocomposites were synthesized by (Xu *et al.*, 2014), and their improved electron transfer efficiency and high electrical conductivity made them a promising candidate for rechargeable zinc ion batteries. MnO₂/NiO nanocomposites were synthesized by (Gupta *et al.*, 2017) for photo catalysis, showcasing their potential to degrade the antibiotic tetracycline under UV light. The catalytic oxidation activity of MnO₂-nanowire@NiO-nanosheet core-shell hybrid nanostructures was reported by Deng *et al.* (2018). These nanostructures performed significantly better than pure MnO₂. (Balaraju *et al.*, 2018) investigated the magnetic and structural characteristics of NiO-MnO₂ nanocomposites and discovered that the magnetization strength rose as the Mn concentration rose. The combined findings of these investigations demonstrate the potential of MnO₂/NiO nanocomposites for several uses, such as photo catalysis, catalytic oxidation, and energy storage.

2.3 Techniques for Nanoparticle synthesis

There are various methods for synthesizing nanoparticles, this includes physical, chemical, biological, and combinations of these; the end features of the products, like their ability to fully satisfy requirements, are essential. According to Salleh *et al.* (2020), The top-bottom procedure and bottom-up strategy are the two primary approaches used in the production of nanoparticles. According to Landage *et al.* (2014), the basic idea behind In top-bottom synthesis, a bulk material initially is altered into the required nanostructure, and then colloidal safeguards are added to stabilize the outcome: nano-sized metal oxide nanoparticles. This process starts with larger structures and progresses to smaller ones until the target size is reached. The process of creating a structure cluster by cluster, molecule by molecule, or atom by atom is called "bottom-up synthesis." The term "building up approach" refers to this process, which is applied in reverse for NPs derived from simpler chemicals (Khan and Khan, 2023).

2.3.1 Physical Method

Physical methods involve the use of heat, electrical, mechanical, or high-energy radiation to transform materials into nanoparticles (NPs) through processes such as scratching, vaporizing, evaporating, or condensing them. The benefit of these methods, which are essentially top-down in nature, is that they generate uniform, monodisperse NPs without polluting the solvent. However, because so much waste is produced during the synthesis, physical procedures are less effective. High energy ball milling, laser ablation, electro spraying, inert gas condensation, physical vapor deposition, laser pyrolysis, and flash spray pyrolysis are a few of the most used physical methods to create NPs., and melt mixing (Park *et al.*, 2004). The use of high temperatures or pressures for chemical reduction has certain disadvantages, such as the high energy costs involved in increasing the temperature of the surrounding environment. The proximity of the source material and the amount of time required to attain thermal stability (Scandorieiro *et al.*, 2016).

2.3.2 Chemical Process

The process uses hazardous chemicals that can be harmful to the environment and the person using them (Carson, 2002). Because of a combination of the significant surface area and surface charge, harsh chemicals might bond to them and generate harsh and hazardous byproducts that might harm human health and the environment. Organisms that belong to distinct trophic levels, including humans, plants, invertebrates, and bacteria, may suffer negative consequences if these chemicals are released into the environment (Alaee and Ilani, 2017). Various chemical methods can be used, including chemical vapor deposition, pyrolysis, homogeneous, direct, solvothermal, and hydrothermal methods; reverse micelles processes (Kolekar *et al.*, 2013).

2.3.3. Green Synthesis of Metal Oxide Nanoparticle

Kuppusamy *et al.* (2016), report that a safe, clean approach for producing nontoxic, biodegradable nanoparticles is biosynthesis. Biosynthesis uses bacteria, plant extracts, fungi, etc. together with the precursors to make nanoparticles instead of applying typical chemicals for bio reduction and capping. Because of their special and improved qualities, biosynthesized nanoparticles are used in biomedical applications (Saba, 2014). Furthermore, high temperatures or pressures are not

necessary for this method. Because it's more environmentally friendly than other methods, microorganisms are used more often to generate numerous types of nanoparticles. There are several advantage to creating zinc nanoparticles applying eco-friendly materials like plant leaf extract, bacteria, fungus, and enzymes, including compatibility with the environment and adaptability for pharmaceutical and various other biomedical uses. (Moritz and Geszke-Moritz, 2013).

Physical and chemical techniques are used to create nanoparticles and Nanocomposite materials. Physical synthesis techniques have the drawbacks of being costly and energy intensive. The use of hazardous chemicals chemical synthesis is restricting uses chemically synthesized NPs, particularly in the medical field, and makes them less environmentally friendly. Developing of a technique for producing nanoparticles using biological synthesis and Nanocomposite was prompted by research into biocompatible, nontoxic, and environmentally friendly methods of producing nanoparticles and nanocomposite. The process of creating nanoparticles and nanocomposites by biological means uses biomaterials like plant extracts, bacteria, fungi, yeast, viruses, and macro and micro algae. Because of Due to these biomaterials' and plant extracts' reach, renewable nature, accessibility, affordability, rate efficiency, and stability of the produced nanoparticles and nanocomposite, it is thought that they are more suitable for the synthesis of nanoparticles and MnO₂/NiO nanocomposite. Many organic molecules, including proteins, carbohydrates, vitamins, alkaloids, and enzymes, are found in plant extracts. These molecules are not only environmentally friendly and inexpensive, but they can also potential decrease materials that settle during synthesis nanoparticles. plant portions that possess the ability to produce metal nanoparticles should be chosen (Shiny *et al.*, 2019).

2.4. Factors Influencing Green Synthesis of NPs

Numerous factors influence the nucleation and formation of stabilized nanoparticles. There have been several claims made regarding the characteristics of crystalline nanoparticles (NPs) with different forms and controlled sizes, including antimicrobial, antioxidant, larvicidal, and anti-biofilm properties. The shape and magnitude of these properties are primarily determined by the processing limitations of the plant extract, in addition to a metallic salt retort, pH,

reaction time, temperature, and the proportion of plant extract to metal salts (Drummer et al., 2021). In the sections that follow, each of these components is briefly discussed in relation to the organism's growth phase.

2.4.1. Effect of pH on Synthesis of NPs

The production of nanoparticles using green technology is influenced by the pH of the solution. The size and texture of the synthesized nanoparticle are determined by the pH of the solution medium, according to the researchers (Armendariz *et al.*, 2004). Thus, by varying the pH of the solution media, the size of NPs can be regulated. The impact of pH on the size and shape of the synthesized silver nanoparticle was shown by Patra and Baek. (2015). The stability of nanoparticles can also be impacted by the pH of the surroundings. When introduced to a different pH environment, NPs synthesized at a particular pH may experience changes in stability. For instance, neutral pH environments may allow gold NPs produced at pH 7 to be more stable than acidic or basic pH environments (Levard *et al.*, 2012).

2.4.2. Effect of Concentration

It has been noted that the morphology of NPs is influenced by the amount of plant extract. Huang *et al.* (2007) reported that the morphology of developed gold and silver nanoparticles (NPs) was affected by varying the concentration of camphor leaf extract. For example, NPs' shape varied when the extract amount exceeded that of chloroauric acid. Similarly, the shape of Au NPs varied when the concentration of *Aloe Vera* was changed in a reaction solution containing similar ions.

2.4.3. Effect of Temperature

Another crucial factor that influences the synthesis of nanoparticles using all three techniques is temperature. While chemical methods require a temperature lower than 350°C, physical methods require the highest temperature (>350°C). Temperatures below 100°C or room temperature are typically required for the synthesis of nanoparticles using green technology. The type of nanoparticle that forms is determined by the temperature of the reaction medium (Rai *et al.*, 2006)

2.4.4. Effect of Time

The duration of incubation of the reaction medium has a significant impact on the type and quality of nanoparticles produced with green technology (Darroudi *et al.*, 2011). Similar to this, the synthesis process, exposure to light, storage conditions, and other factors all had a significant impact on the properties of the synthesized nanoparticles, which changed over time (Kuchibhatla *et al.*, 2012). Variations in time can arise from a variety of factors, including the aggregation of particles caused by prolonged storage, the growth or shrinkage of particles during long storage, the presence of a shelf life, and other factors that impact their potential (Baer, 2011)

2.5. Applications of MnO₂/NiO Nanocomposites

Due to the ineffectiveness of current antibiotic therapies against multidrug-resistant bacterial pathogens, hospital and community-acquired infections are becoming an increasingly common global health concern (Franci *et al.*, 2015). The most common bacterial diseases are causing an increasing number of illnesses and deaths, making antibacterial confrontation one of the biggest threats to human health (Walker *et al.*, 2009). It has been noted recently that the overuse and misuse of antibiotics is giving rise to resistance genes (Yousaf *et al.*, 2020). Therefore, cutting back on antibiotic use is necessary as the first step. Due to this, it is essential to develop new military strategies combine bacteria resistant multiple vaccine and to lessen side effects chemical drug consumption to combat microbial infections. The medications made from plants or plant products are less hazardous, less expensive, and have fewer side effects. According to (Rai *et al.*, 2012), plant extract-derived nanoparticles are currently being considered as a potential antibiotic replacement.

2.5.1. Antibacterial Application of MnO₂/NiO NPs and NC

The environmentally beneficial properties of biogenic synthesis of nanoparticles (NPs) make it a desirable process that is quick, easy, and sustainable. They possess antibacterial, antifungal, cytotoxic, anticancer, antioxidant, remedial, and enzyme inhibitory characteristics. (Sana *et al.*, 2021). According to Rehman *et al.* (2021) these NPs widely utilized in photo-electronic devices, gas detectors,

electrochemical films, and smart windows anti-microbial activity, catalysis, and photo catalysis. NiO nanoparticles are excellent photo catalysts for the degradation of several organic contaminants because of their photo-stability, ease of availability, and low toxicity. Numerous researchers have tampered with NiO NPs to cause dye degradation and antibacterial effects (Ezhilarasi *et al.*, 2018; Hameed *et al.*, 2009). The size, stability, and concentration of the NPs determine their antibacterial activity, and they give the bacteria more time to be retained during the antibacterial activity (Anand *et al.*, 2020).

The environmentally friendly pair NiO Nanoparticles accomplished recently, interest in synthesis NiO NPs from various other medicinal plants for various biological applications is growing. Plants such as *Moringa oleifera* (Lam.) (Nasseri *et al.*, 2016), *Agathosma betulina* (Berg.), Thovhogi *et al.* (2015), *Callistemon viminalis* (Sims.) (Sone *et al.*, 2016), *Tamarix serotina* (Bunge. ex Boiss.) (Yuvakkumar *et al.*, 2014), and *Nephelium lappaceum* (L.) have all been reported to synthesise NiO NPs through plant-mediated means. (Ovais *et al.*, 2018) .

Human health is greatly at risk from bacterial pollution. Using nanotechnology, new inorganic antibacterial agents can be created. There's a chance that nano-inorganic metal oxide will lessen bacterial contamination. According to Hoseinpour and Ghaemi (2018), form highly oxygen species deemed reactive ($\text{OH}^\cdot$, H_2O_2 , and O_2^{2-}) on their surface, which cause fatal damage to bacteria and fungi, is how their antifungal and antibacterial properties are understood. Usually antibacterial qualities of Mn NPs investigated; instead, most studies focus on the use of Mn NPs in electrical characteristics and catalytic activities. The Mn NPs exhibited approximately the same antibacterial activity against *E. coli* and more powerful activity against *S. aureus* when compared to chloramphenicol (Jayandran *et al.*, 2015).

2.5.2. Antifungal Applications of MnO₂/NiO NPs and NC

Fungal diseases are quite uncommon; however, less attention has been on resistance to antifungal drugs. These illnesses have extremely high rates of morbidity and mortality, ranging from superficial infections (oral or genitourinary tract thrushes) to possibly fatal systemic infections. It has been estimated that 1.5 million people worldwide perish from fungal infections, which affect

approximately 1.7 billion people worldwide (Delarze and Sanglard, 2015). Even though most pathogenic fungi are opportunistic pathogens, individuals with compromised immune systems are susceptible to their deadly effects. According to Ashley *et al.* (2006), The increasing popularity of immunosuppressive treatments for organ transplants and cancer has led to an increase in the frequency of aggressive fungal illnesses. Research has shown that the environmentally friendly produce of MnO₂ and NiO nanoparticles has strong antifungal activity. *Blumea lacera* leaf extract used to synthesize MnO₂ NPs which Morbale and Patil (2022), showed to have antifungal actions. Similarly, (Ahmad *et al.*, 2022) discovered that NiO nanoparticles with strong antimicrobial qualities were produced through the solution combustion method. These results are in line with a larger body of research on the antimicrobial and antifungal characteristics of metal oxide nanoparticles, such as bio functionalized MONPs (Haneefa, 2017) and MgO NPs Farani *et al.*, (2023). Additionally, in the same reports of, four fungus strains: *Aspergillus nagger*, *Candida albicans*, *Trichophyton simii*, and *Curvularia lunata* used to test the antifungal properties of Mn NPs and *curcumin* using the diffusion method. The outcomes were contrasted. with fluconazole, a standard medication (Jayandran *et al.*, 2015).

2.5.3. Antioxidant Application of MnO₂/NiO NPs and NC

The activity of antioxidants is the process of restricting or preventing biomolecules' oxidation, particularly that of lipids and proteins, and oxidative chain reactions. (Pansambal *et al.*, 2023). By reacting with free radicals, antioxidants reduce oxidative stress by blocking the chain of events that would otherwise produce damaging byproducts. Both synthetic and natural antioxidants, however, show poor absorption, limited cell permeability, and biodegradation until they reach the site of activity. In this case, metal oxide nanoparticles, which contain antioxidants in addition to metals, function as a substitute for natural and artificial antioxidant supplements (Khalil *et al.*, 2020). Antioxidants can be chemically synthesized is naturally present in microbes, plants, and animals. Higher plants and their elements are abundant in natural antioxidants such as tocopherols and polyphenols, which occur in excess in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas, and oils. (Shahidi and Zhong, 2015).

2.5.3.1. Antioxidant Capacity Evaluation

Antioxidant capacity is the overall ability of an organism or food to catch free radicals and prevent their harmful effects. In order to determine the antioxidant capabilities of various plant parts and their derivatives, a variety of tests have been routinely used. These are DPPH (2,2 diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), ORAS (oxygen radical absorption capacity), and ABTS. Among them, DPPH is discussed below.

2.5.3.2. DPPH free Radical Scavenging Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl is a stable free radical with purple color, which shows hydrogen acceptor ability towards antioxidants. Hence, it is commonly used in DPPH assay for measuring the antioxidant activity of different natural samples such as wine, fruits, herbal tea, etc. It is involving the use of free radical DPPH, which is widely used to test the ability of compounds to act as free radical scavenger's/ hydrogen donors and to evaluate the antioxidant activity (Gülçin, 2012). The reduced DPPH-H is formed when a free radical scavenger pairs the odd DPPH radical electron with hydrogen. Up on reacting with antioxidants, produces corresponding hydrazine DPPH-H and it becomes pale yellow color. A strong absorption spectrum is caused by the DPPH free radical's odd electron at 517nm.

2.6. Botanical Descriptions of Onion Plant Peel

Scientific Classification of onion

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Asparagales
Family	Alliaceae
Genus	Allium
Species	<i>Allium cepa</i>

Onion (*Allium cepa* L.) is cultivated globally for its nutritional value and recognized functional and therapeutic properties (Aslam *et al.*, 2022). Due to behavioral changes that caused a rise in the desire for freshly cut and prepared onions, the production of onions has increased globally by 25%. Consequently, more waste has been produced. The primary waste products from onion processing

are the skin (peel) (Alok and Sunil, 2020), It is created up of the distorted bulbs, undersized roots, and the two outermost leathery layers. More than 500,000 tons of peeled onions are produced annually in Europe, compared to 300–500 kg produced daily in India. This peel releases an odor and pollutes the environment as it breaks down in the soil. Even though it has the potential to contain biologically active substances like flavonoids and phenols, is still a product that is underutilized. Peel also has a wide range of therapeutic benefits, including antibacterial, ant mutagenic, and antioxidant (Kumar *et al.*, 2022).

Due to its inedibility, the onion skin is immediately removed prior to processing and selling. But because of its makeup and odor, it can't be disposed of in a landfill or used as fodder, making it the primary waste product of onion processing (Celano *et al.*, 2021b). Consequently, to produce allium sustainably, onion skin must be valorized by converting it into a "food by-product." The food, cosmetic, and pharmaceutical industries can use valuable molecules found in food processing by-products as functional ingredients (Gontard *et al.*, 2018). In this sense, developing the most effective and efficient resource recovery systems requires an understanding of the biological activities and chemical characterization of onion skin.

2.6.1. Utilizing Onion Peel Extract as a Green Synthesis Method

Due to the high concentration of flavonoids in onions (*Allium cepa L.*), one of the mostpopular vegetables, there are numerous health advantages. With their hypoch olesterolemia,thrombolytic, and antioxidant properties, onion extracts can be powerful cardiovascular and anticancer agents (Dillon *et al.*, 2003). The antioxidative properties and health benefits of onions have been linked to flavanols, which are mainly found in the form of glycosides of quercetin and kaempferol (Gennaro *et al.*, 2002). The peel of an orange onion contains various phenolic compounds, including kaempferol, gallic acid, quercetin, and ferulic acid (Albishi *et al.*, 2013). Organic solvent-based conventional extraction methods have been used in the past to extract natural antioxidants (Kumar *et al.*, 2011). Applying clean, eco-friendly technology to create high-quality, highly active extracts without posing a risk of solvent toxicity has increased recently. Organic compound extraction, hydrolysis, and wet oxidation are just a few of the many uses for the

environmentally benign subcritical water extraction technique (Kruse and Dinjus, 2007).

Numerous studies have investigated the use of onion peel extract as a green synthesis technique. The extract was utilized to create silver nanoparticles, which Yap *et al.*, (2020) demonstrated could be used as a green catalyst in the acetylation reaction. According to the study, the produced nanoparticles had exceptional catalytic activity and could be recycled several times. Similarly, the extract was used by (Patra *et al.*, 2016a) to create gold nanoparticles that had potent antioxidant, antibacterial, and anticandidal qualities. These investigations demonstrate the potential of onion peel extract as a green synthesis technique for a range of uses.

2.6.2. Bioactivities of Onion Peel

One of the primary causes of these illnesses is oxidative stress, which also includes a poor diet, an increase in processed food intake, inactivity, and exposure to contaminated environments. These changes dietary habits have led to an increase in the incidence of disorders like obesity, diabetes, cardiovascular disorders, and cancer (Sharifi-Rad *et al.*, 2020). Onions' outer layer includes phytonutrients that could be used biologically active ingredient development, intelligent packaging, and sustainable "green" agricultural pest control. Consequently, the various uses for onion scraps may aid in the development of low-cost, long-term, and effective methods to reduce agricultural food waste (Celano *et al.*, 2021a). Two characteristics of onion peel extracts, such as their antioxidant and antimicrobial properties, are covered in detail in this section.

Onion peel is an excellent supply of bioactive substances that have several health advantages. Its high concentrations of quercetin, total flavonoids, and phenolic have been related to antimicrobial, antidiabetic, cardio protective, neuroprotective, and anti-obesity effects (Kumar *et al.*, 2022). After optimizing the extraction of quercetin from onion peel, the resulting extracts demonstrated anti- α -amylase and antioxidant properties, suggesting that they could be used as potential anti-diabetes agents (Silva *et al.*, 2020). Moreover, substantial antioxidant activity has been shown by onion peel extracts, adding credence to their possible health advantages

(Joung and Jung, 2014). These results demonstrate onion peel's potential as an important agricultural byproduct with a variety of bioactivities.

2.6.3. The Role of the Phytochemicals in the Plant Extract for Nanoparticle Synthesis

Because of the phytochemicals contained in the extracts, which are essential to the synthesis of these particles, a number of plant parts, such as leaves, peels, roots, seeds, barks, fruits, and gums, are utilized in the creation of metal and metal oxide nanoparticles (Din *et al.*, 2020). In biosynthesis, the phytochemicals function as lowering and capping materials. The plant-derived biomolecules' functional groups, which are mostly polar groups like amine, hydroxyl, carboxylic acids, thiol, and carbonyl groups, are reduced by extract-derived electrons that migrate to the metal. This process initiates nucleation (Ahmed and Mustafa, 2020). In order to keep the NPs from clumping together, they may include high quantities of reducing, capping, or stabilizing chemicals because of their enormous surface area. Flavonoids, amine groups of proteins, hydroxyl groups of polysaccharides, polyphenols, carboxylic groups, and carbonyl groups, among other groups, include a potential chelating site to generate stable metal cations with Cu(II), Zn(II), Fe(II)/Fe(III), Pb(II), and other groups during synthesis. (Marslin *et al.*, 2018).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of Sampling Area

Experiment of the study was carried out in Adama Science and Technology University, Applied Biology and Applied Chemistry Laboratories. The onion plant sample was collected from Adama city, Oromia local markets. The botanical identification of onion plant was conducted at the National Herbarium, Addis Ababa University with local name Keyi shinkurete, code HH001.

3.2. Microbial Samples and Reagents

Four pathogenic bacterial strains two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and two Gram-positive bacteria (*S. aureus* and *S. pyogenes*) and *Candida albicans* fungi strains were obtained from Applied Biology Department laboratory of Adama Science and Technology University, Adama, Ethiopia. All aqueous solutions in the experiment were prepared with deionized water from ASTU Applied Chemistry and Applied Biology laboratories class were used. Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 98% Lpba chemie), potassium permanganate (KMnO_4 , alpha chemika, India), distilled water, Sodium hydroxide (NaOH 99%, Sigma Aldrich, India), ethanol ($\text{C}_2\text{H}_5\text{OH}$ 99.8%, Wasse PHARMA PLC, Ethiopia), methanol (CH_3OH 99.8%, Sigma Aldrich, India), hydrochloric acid (HCl , Riedel dehaen, Germany, 37% HCl), sulfuric acid (H_2SO_4 98%, Sigma aldria), benedict's solution, ascorbic acid (98%, Sigma Aldrich, Germany), DPPH (97%, Sigma Aldrich), dimethyl sulfoxide (DMSO 99.5%, SRL, Japan), mueller hinton agar (Sigma Aldrich, India), , sabouraud dextrose agar (Sigma Aldrich, India), ferric chloride solution (FeCl_3 99%, Sigma aldrich), , iodine (I 98.5%, Loba Chemie) potassium iodide (KI 99.8%, Samir tech-chem), and chloroform (CHCl_3 99.8%, Blulux) were used in the experiment.

3.3. Sample Preparation and Extraction

3.3.1. Preparation of Onion Peels

The collected onion peels were washed via distilled water. The clean peels were completely dried at room temperature in open air and under shadow for about 2

weeks (15 days) to remove any remaining wetness content from surfaces of peels. As shown Figure in 1, after that, dried peels were ground by electrical grinding machine to get a fine powder followed by packing it within baker.

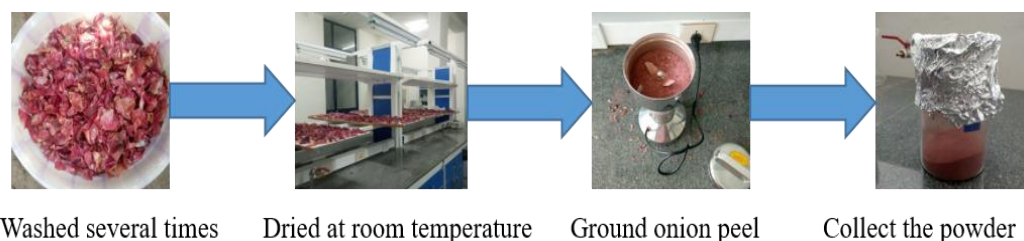


Figure 1. The general process of collection and sample preparation of onion peel

3.3.2. Preparation of Onion Peels Extract

The extraction process done by adding 40g of powdered onion peel to a flask containing 800mL of distilled water. To protect the mixture from light, the flask was covered with aluminum foil. Subsequently, the mixture allowed stirring using a magnetic stirrer and was heated to 80°C for 1hr on a magnetic stirrer with a hot plate. Following this, the heater was deactivated, allowing the mixture to gradually cool to room temperature and settle, as showed in Figure 2. Once settled, the resulting extract underwent filtration using Whatman No. 1 filter paper to yield a clear solution. Finally, the filtrate was stored within a refrigerator at 4°C using brown bottle to prevent the effect of light for further green synthesis application. (Abdullah *et al.*, 2021).

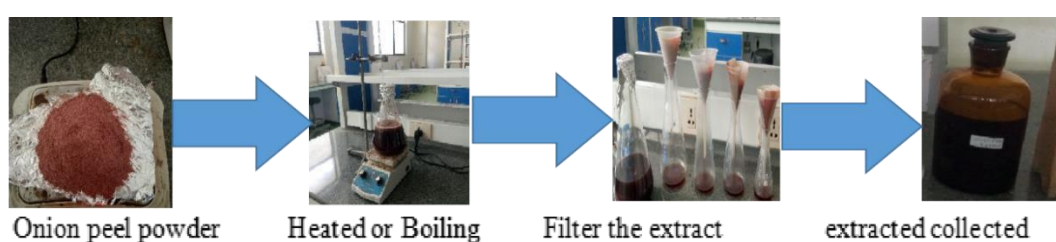


Figure 2 Preparation of onion peel extract

3.4. Phytochemical Test of onion peels Extracts

Alkaloids Test (Wagner's test)

The presence of alkaloids in onion peel extract checked through Wagner's test by using the 2mL onion peel extracts solution were added in Wagner's reagent (Iodine in Potassium Iodide) in the test tube (Nisha and Suja, 2018).

Test for Flavonoids

To check flavonoids in the onion peel extract prepare alkaline reagent test by using 5mL of aqueous onion peel extract mixed with a three drops of NaOH solution (Garg *et al.*, 2019).

Test for Tannins (Ferric Chloride Test)

To confirm the presence of tannins, 5mL of aqueous onion peel extract was diluted with 5mL of distilled water. Following dilution, 3 drops of a 1% ferric chloride solution were added. This addition was made to verify the presence of tannins in the extract (Shaikh and Patil, 2020).

Terpenoids Test (Salkowski test)

To assess the presence of terpenoids in the onion peel extract, 4mL of the extract was utilized, followed by the addition of 3mL of chloroform. Subsequently, 3mL of concentrated sulfuric acid (H_2SO_4) was cautiously introduced to create a distinct layer. This process was employed to detect the presence of terpenoids in the extract (Chiew *et al.*, 2014).

Test for Steroids

The presence of steroids in onion peel extract was checked through salkowski test by using the 5mL aqueous onion plant peel extract was combined with 2mL of chloroform, followed by the addition of concentrated H_2SO_4 (Yaseen *et al.*, 2017).

Test for Glycoside

The presence of glycoside in onion peel extract was checked through salkowski test by using 2mL of concentrated H_2SO_4 was added directly to the aqueous plant extract (Nisha and Suja, 2018).

Test for Benedict

The identify reducing sugar in the onion peel extract had checked through the benedict test by using 3mL of aqueous extract had taken into a test tube then benedict reagent was added drop by drop and heated for 2min (Egbuna *et al.*, 2019).

Test for Phenols

To test phenolic functional group in onion peel extract was checked by using 5mL of aqueous extract taken into a test tube and 5 drop of 3% of FeCl_3 was added in to an extract drop by drop wisely (Yaseen *et al.*, 2017).

Test for Saponins

To ascertain the presence of saponins in the peel extract, a frothing test was conducted. This involved dissolving 3mL of the aqueous onion plant peel extract in 5mL of distilled water, creating a solution. The mixture was then vigorously shaken to observe if froth or foam formed, indicating the presence of saponins (Bano and Deora, 2019).

3.5. Synthesis of Nanomaterials

3.5.1. MnO_2 NPs Synthesis

MnO_2 NPs synthesis was done by following the previous literature with little modifications (Dessie *et al.*, 2021a). MnO_2 NPs was synthesized using its precursor salt and the peel extract in equal volume ratio in a beaker. Initially, 0.5M of KMnO_4 solution was prepared using volumetric flask and then 100mL from the stock solution was taken and poured into the beaker as shown as Figure 3. After that ,100mL of onion peel extract was added dropwise to the 0.5M of 100mL of the KMnO_4 salt solution. Then mixed component was stirred on a hot plate for 4hrs at room temperature. The pH of the formed suspension was then adjusted at 12 using 2M of NaOH using pH meter. Furth more, 20min stirring was done to confirm the homogenization of the added base. The formed suspension was then stayed overnight within a refrigerator. The washing procedure was performed by centrifugation three times using distilled water and ethanol for 10min in 1000rpm. Precipitate then being collected using ceramic crucible dish and oven dried at 80°C for 6hrs. The thermal stability was then confirmed using TGA/DTA and then based on this result it was calcined at 400°C using furnaces.

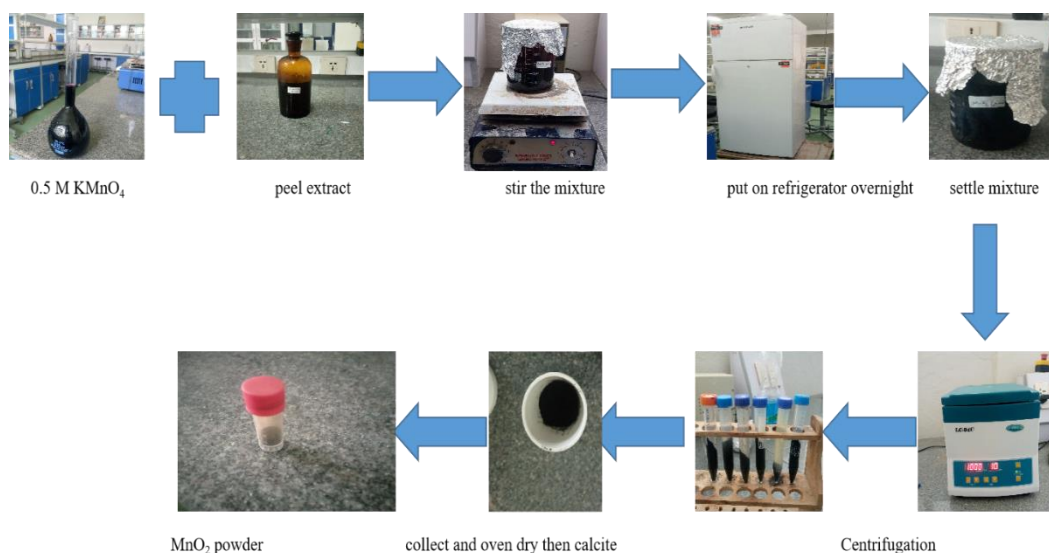


Figure 3. Biosynthesis process of Manganese dioxide nanoparticles

3.5.2. NiO NPs Synthesis

NiO NPs synthesis were performed following the previous literature with little modifications (Dessie *et al.*, 2021a). In Figure 4 NiO NPs was synthesized using volume ratios of its $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ salt and the onion peel extract in equal volume ratio in a beaker. Initially, 0.5 M of $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution was prepared using flask and then 100mL from the stock solution was taken and poured into the beaker. After that 100mL onion peel extract was added dropwise to the 0.5M of 100mL of the $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ salt solution. Then mixed component was stirred on a hot plate for 4hrs at room temperature. The pH of the formed suspension was then adjusted using 2M of NaOH using pH meter. Furth more, 20min stirring was done to confirm the homogenization of the added base. The formed suspension was then stayed overnight within a refrigerator. The washing procedure was performed by centrifugation three times using distilled water and ethanol for 10min at 1000rpm. Precipitate was collected using ceramic crucible dish and oven dried at 80°C for 6hrs. The thermal stability was then confirmed using TGA/DTA and then based on this result it was calcined at 400°C using furnaces.

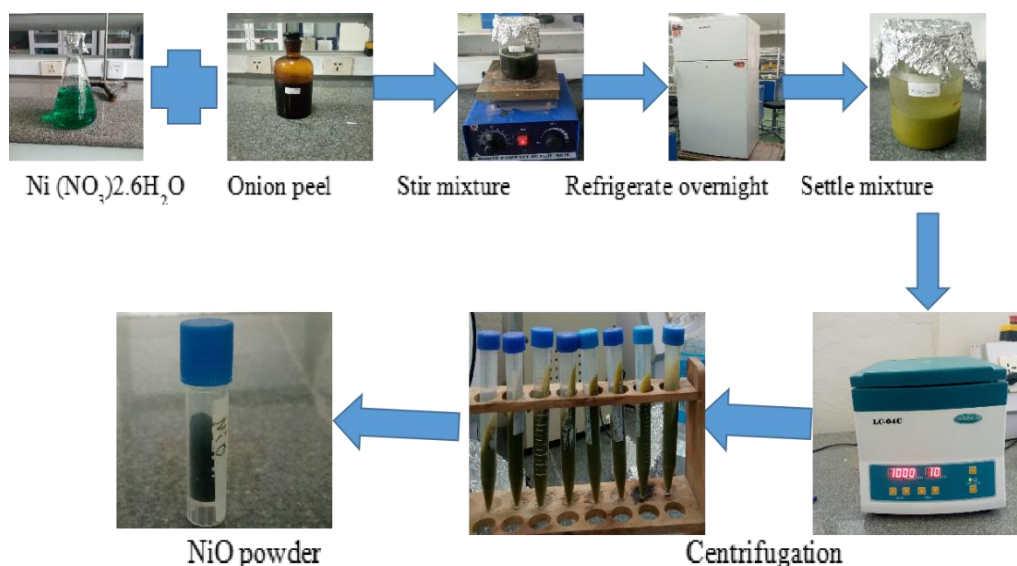


Figure 4. Biosynthesis process of Nickel oxide nanoparticles

3.5.3. MnO₂/NiO Nanocomposites

The synthesis of NCs was done by following the previous literature with little modifications (Dessie *et al.*, 2021a) in three ratios (1:1, 1:2, and 2:1). In detail, the typical biosynthesis process of nanocomposites was first various molar (0.5, 0.25, and 1M) aqueous solutions of KMnO₄ and 0.5M of Ni(NO₃)₂.6H₂O which was constant for all three ratios NCs was dissolved in 100mL distilled water separately. Then 100mL of 0.5M for 1:1 ratio of NC, 100mL of 0.25M for 1:2 ratio of NC, and 100 mL of 1M for 2:1 ratio of NC of KMnO₄ were stirred for 4hrs separately by mixing mix with 50mL onion peel extract Figure 5. Then the pH of KMnO₄ solution and onion peel extract was adjusted at 12 using 2M NaOH 20min stirring was done to confirm the homogenization of the added base. Then 100mL of 0.5M Ni(NO₃)₂.6H₂O and 50mL of onion peel extract were added on each and stir 4 hrs. After that the pH was adjusted at 12 using 2M NaOH. Furth more, 20min stirring was done to confirm the homogenization of the added base. The formed suspension was then stayed overnight within a refrigerator. The washing procedure was performed by centrifugation three times using distilled water and ethanol for 10min at 1000rpm. Precipitate then collected using ceramic crucible dish and oven dried at 80°C for 6hrs. The thermal stability was then confirmed using TGA/DTA and then based on this result it was calcined at 500⁰C using furnaces.

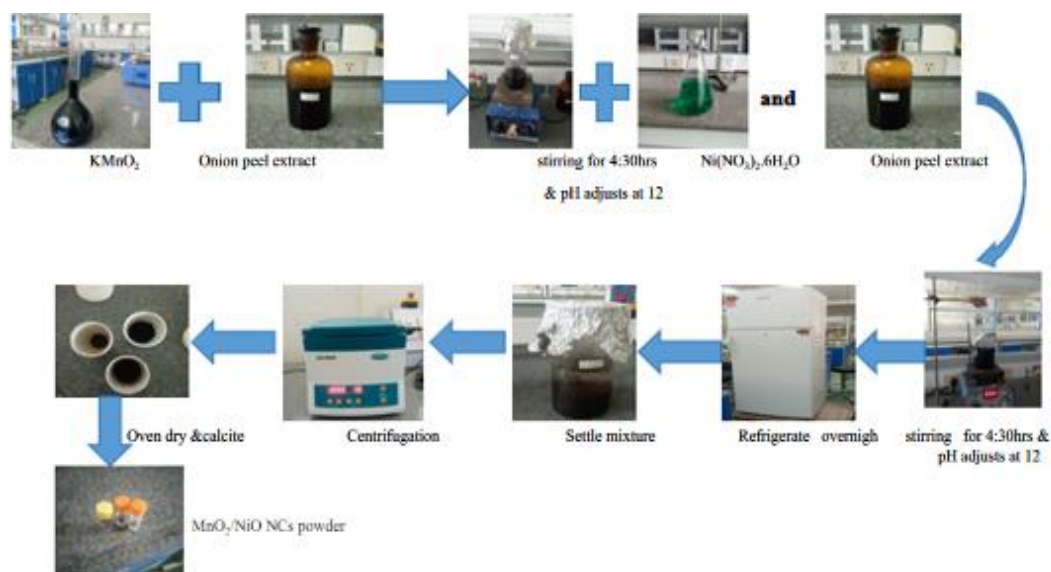


Figure 5. Biosynthesis process of MnO₂/NiO nanocomposite

3.6. Characterization Techniques of MnO₂/NiO NCs and NPs

The green synthesized MnO₂, NiO NPs and MnO₂/NiO NCs were subjected to characterization for the determination of temperature stability, crystalline or amorphous natures, evaluation of size and morphology, band gap, and determine functional groups used in the reducing agent in green synthesis of NPs.

3.6.1. Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA)

Thermogravimetric Analysis (TGA) is a technique where the mass of a material, including nanomaterials, is monitored as a function of temperature or time. This monitoring occurs while the sample is subjected to a controlled temperature program in a controlled atmosphere. Differential Thermal Analysis (DTA) use controlled temperature programmed, the sample was subjected to measures the temperature difference between them as a function of temperature (Sherif, 2022). To estimate the calcination temperature of synthesized MnO₂, NiO NPs and MnO₂/NiO NC, Thermogravimetric Analysis (TGA) was conducted using a DTG-60H detector manufactured by (Shimadzu, Japan). The analysis was performed at a sample flow rate of 50mL/min. This setup allowed for precise monitoring of the mass changes of the sample as a function of temperature or time under controlled conditions.

3.6.2. Analysis of X-ray Diffraction (XRD)

The synthesized MnO₂ and NiO nanoparticles were analyzed and their crystalline size was estimated using an X-ray diffractometer (DW-XRD-Y7000, DRAWELL, China) for MnO₂/NiO. Using Origin Pro software, the XRD data was plotted from 10° to 80° in terms of diffraction intensity versus 2θ. In addition, the diffraction pattern revealed details about the electron density inside the unit cell as well as the size and shape of the cell based on peak positions. The Scherrer formula was also used to estimate the size of the crystallite (Ghaffar *et al.*, 2022).

$$D = K\lambda/(\beta\cos\theta) \quad (1)$$

In this case, λ is the wavelength of the X-ray source, D is the crystallite size, K is the Scherrer constant, β is the Full Width at Half Maximum (FWHM) of a peak, and θ is the Bragg diffraction angle.

3.6.3. Scanning Electron Microscopy (SEM)

According to Akhtar (2018), surface phenomena, topography, morphology, composition, and shape data can all be determined using SEM. The size and morphology of synthesized MnO₂, NiO nanoparticles MnO₂/NiO NC were determined by scanning electron microscope (JCM-6000plus, JEOL/EO, Japan).

3.6.4 Fourier Transform Infrared Spectroscopy (FTIR)

Measurements using Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer, CT, USA) were performed in order to determine which potential biomolecules (functional group) were in charge of the reduction of MnO₂/NiO nanoparticles. Using the powdered nanoparticle samples obtained from the KBr pellet method, FTIR was employed to characterise the nanoparticles. FTIR was used to scan the absorbance maxima between 500 and 4000 cm⁻¹. Version 8.5 of Origin Pro was used to record and analyses the FTIR spectral data.

3.6.5 UV-Vis Diffuse Reflectance Spectroscopy (DRS) Analysis

The optical properties of the green-synthesized MnO₂, NiO nanoparticles (NPs), and MnO₂/NiO nanocomposites (NCs) in various ratios (1:1, 1:2, 2:1) were analyzed using a UV-vis spectrophotometer (Carry 100 UV-Vis, Agilent Technologies, USA). To determine the band gap of NPs and NCs energy Kubelka Munk function plots were employed (Zhang *et al.*, 2019)

3.6.6 Photoluminescence (PL) Spectroscopy

The emission of light from the green synthesized NPs and NCs was measured under optical excitation by using a photoluminescence instrument. For this analysis, about 5 mg of calcined MnO₂, NiO NPs and MnO₂/ NiO NCs in various ratio (1:1, 1:2, 2:1) nanoparticles were suspended in DMSO in separate vials and mix well. The prepared NPs and NCs suspension was added to the cuvette and subjected to PL scanning and data was recorded. Obtained data were analyzed as graphs by using Origin Pro (8.5 version) software.

3.7 Method of Antimicrobial and Antioxidant Activities Evaluation

The bacteria and fungi are used for inhibitory zone tests to investigate the antimicrobial properties of MnO₂, NiO NPs and MnO₂/ NiO NCs in various ratio (1:1, 1:2, 2:1) using the agar well diffusion method. The bacterial studies were evaluated and carried out in 4 different bacterial strains; which are two Gram-negative (*Escherichia coli*, and *Pseudomonas aeruginosa*), and two Gram-positive (*Staphylococcus aureus*, and *Streptococcus Pyogenes*) which were obtained from the Applied Biology Department laboratory of Adama Science and Technology University and one fungal strain; *Candida albicans* was also taken from laboratory of Applied Biology Department in Adama Science

3.7.1. Preparation of Bacteria Inoculums

On Muller Hinton agar, each species of bacteria was sub cultured and incubated for 24 hrs at 37°C. Using a sterile inoculating loop, multiple colonies (three to five) of a fresh culture of Muller Hinton agar (MHA) were selected to create the inoculum suspension. The colonies were then transferred into a test tube with five milliliters of MHB and left overnight to incubate. After that, sterile saline solution was added to dilute the fresh cultures, and they were thoroughly vortexed. This process was repeated until the turbidity 0.5 McFarland standard was met, which is equivalent to 1.5×10^8 CFU/mL for the suspension of bacterial inoculum (Wiegand *et al.*, 2008)

3.7.2. Method of Antibacterial Activity Evolution

To find out how effective synthetic NPs and NC were against bacteria, the disc diffusion assay method was employed. To obtain fresh culture, the bacterial

inoculum was sub cultured into the MHBs and incubated for an additional night. Subsequently, the aforementioned 0.5 McFarland standard was used to adjust the inoculums. Sterile cotton swabs were used to collect bacterial inoculum suspensions, which were then applied to sterile MHA plates for the strains of (*Staphylococcus aureus* ATCC25923 and *Streptococcus pyogenes* ATCC13311) and two gram-negative bacteria (*Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922), respectively. Green synthesized MnO₂, NiO NPs, and MnO₂/NiO NC in different ratios (1:1, 1:2, 2:1) were prepared at concentrations of 75µg/mL, 50µg/mL, and 25µg/mL stock solution of MnO₂, NiO NPs, and MnO₂/NiO NC in different ratios (1:1, 1:2, 2:1) for NCs in order to determine their antibacterial activity.

Ciprofloxacin was used as a positive control, because of its broad-spectrum action against both Gram-positive and Gram-negative bacteria, the fluoroquinolone antibiotic ciprofloxacin is frequently utilized in antibacterial activity. Ciprofloxacin inhibits the DNA gyrase of bacteria, thereby inhibiting DNA replication. So, ciprofloxacin is a well-characterized and widely-used antibiotic that is often used as a positive control in antibacterial tests due to its stability, ease of use, and known mechanism of action. DMSO was used as a negative control in antibiotics assays, it's important to distinguish between the effects of the test substance and any effects that might be brought on by the solvent or other assay components (Prasetya *et al.*, 2019). Since DMSO is frequently employed in antibacterial assays as a solvent for test compounds, using it as a negative control can help ensure that any antibacterial activity seen is brought on by the test drug and not the solvent respectively.

For this test, roughly 6mm diameter Whatman No. 1 filter sheets cut with a puncher and autoclave-sterilized. For 20min, these sterile 6mm discs were immersed in every concentration of green synthesized MnO₂, NiO NPs, and MnO₂/NiO NC. Positive controls were sterile paper discs soaked in a standard antibiotic solution containing 30µg/mL of ciprofloxacin. On the other hand, DMSO-impregnated discs served as the negative control. The discs were then all placed on sterile agar plates (MHA) labelled with their labelled location in the laminar airflow cabinet

using sterile forceps. The plates were then left in the hood for 30min to allow the antimicrobial agent to diffuse from the disc (Muthuvel *et al.*, 2020).

3.7.3 Preparation Fungi Inoculums

On Saboured Dextrose agar, the fungi were subculture and incubated for 48hrs at 27°C. Using a sterile inoculating loop, multiple colonies (three to five) of a fresh culture of Saboured Dextrose agar (SDA) were selected, and the resulting inoculum suspension was then transferred into a test tube with 5mL of SDB and incubated for a full night. After the fresh culture with saline solution, it was thoroughly vortexed. Next, the turbidity of the fungal isolate was adjusted to 0.5 McFarland standard, which is equivalent to 1×10^6 cells/spores for fungal species. (Wiegand *et al.*, 2008)

3.7.4 Method to Evaluate Antifungal Activity

A similar method of determining antibacterial activity evaluation was used to test antifungal activities against *Candida albinos* ATCC9955. The preparation of Sabouraud Dextrose Agar (SDA) involved dissolving it in distilled water. To obtain a boiled and homogenous solution, the mixture was then heated on the hot plate and autoclaved for 15min at 121°C. After that, the autoclaved medium was put into different petri dishes and given time to solidify. A sterile cotton swap was dipped into the modified fungal strain suspension and rotated several times while exerting firm pressure on the interior wall of the tube above the fluid level, following a 15min modification of the turbidity of the inoculum suspension. The purpose of this was to remove surplus fluid from the swap. Next, to ensure a uniform dispersion of inoculums, the dried surface of the SAD plate was inoculated by streaking the swap across the whole surface while rotating the plates at a speed of about 60°. After that, the plate was kept open in the hood for three to five minutes to let any extra surface moisture absorb. Sterile filter paper was used to create a disk with a 6 mm diameter, which was then cut into small, uniformly sized circles. After that, 0.01mL of the prepared test sample was impregnated into each of them. Using sterile forceps, the MnO₂, NiO NPs, and MnO₂/NiO (1:1, 1:2, 2:1) NCs impregnated disc was placed on the surface of the inoculation agar. To guarantee that every disc made full contact with the agar surface, each was pressed down. Ketoconazole (20µg/mL) was utilized as the positive control in the well. It served

as a control for the test solution by measuring the diameter of the inhibition zone that developed. DMSO was employed as a negative control group. After that, the plate was covered with Parafilm and left to incubate for 48hrs at 27°C to look for fungal infections. Using a transparent ruler, the diameters of the zone of inhibition surrounding each disk were measured after incubation, and the mean of the readings for each pathogen at different ratios for all synthesized nanomaterials was recorded. (Bhoye *et al.*, 2023).

3.7.5 Method to Evaluate Antioxidant Activity

The DPPH assay, also known as the radical-scavenging assay (RSA), was used to assess the ability of the biosynthesized MnO₂, NiO NPs and MnO₂/NiO NCs (1:1, 1:2, and 2:1 ratio) within three different volumes of ratios. Using the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method, the free radical scavenging activity of the biosynthesized MnO₂, NiO NPs, and MnO₂/NiO NCs (1:1, 1:2, and 2:1 ratio) was determined by (Foti, 2015). With this by measuring the drop in DPPH absorbance at 517nm, an antioxidant's capacity to scavenge radicals was ascertained. Stock solution was prepared by taking 8mg of each biosynthesized nanomaterial and added to 5mL of methanol to dissolve it. The serial solution was done to give 62.5, 125, 250, 500, and 1000µg/mL in five vials. The 1mL of each biosynthesized MnO₂, NiO NPs MnO₂/NiO NC (1:1, 1:2, and 2:1 ratio) following the addition of 4mL DPPH (made in methanol by dissolving 4mg DPPH in 100mL methanol) at various concentrations (62.5, 125, 250, 500, and 1000µg/mL), the mixture was vigorously shaken. The resulting solution was then left in a dark area for 30min before being measured at 517nm using a UV-Vis spectrophotometer. To achieve the same concentration, ascorbic acid was dissolved in methanol as a reference standard. In the control experiment, DPPH was added to methanol. Using the following formula, the radical scavenging activity was determined and expressed as a percentage of the control (free radical solution minus plant extract) (Obeng *et al.*, 2020):

$$\% \text{ Scavenging [DPPH]} = [(A_0 - A_1) / A_0] \times 100$$

(2)

where A₀ represented the blank's absorbance (the same volume of methanol was used in place of the extract) and A₁ represented the absorbance when the extract

or standard was present. The results were plotted in the graph as a percentage of radical scavenging vs. concentrations of MnO₂, NiO NPs MnO₂/NiO (1:1, 1:2, and 2:1 ratio) NCs for each nanomaterial.

3.8 Data Analysis

The mean $\pm$ standard deviation of three replicates used to convey the experimental results of the antimicrobial tests. In order to analyze the experiment results, the data was submitted to the statistical package of social science (SPSS 22.0), and the results were displayed in tables. The least significant difference test and one-way ANOVA were used to assess the differences in means. At $p \leq 0.05$, the threshold for statistical significance was established. All of the instrumental analyses were analyzed and graphed using the OriginPro8 programmer. Following the organized data entry, the line and stack lines selected by the Y offset graph line were taken into consideration.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Preliminary phytochemical Analysis of Onion Peel Extract

The results of the qualitative phytochemical analysis of the onion peel extract are shown below in Table 1 and Figure 6. According to the results gathered, the main components of onion peels are alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides, Benedicts, phenol, and saponins. These compounds were what helped in facilitate the biosynthesis of nanoparticles and nanocomposite. Five of the phytochemicals that were tested for presence tannins, alkaloids, terpenoid, benedicts, phenol, and saponins were confirmed based on the anticipated color change according to a prior report (Verma *et al.*, 2018). The presence of these phytochemicals in this plant is the reason to have significant antimicrobial and antioxidant activity. But in the case of glycoside, flavonoids, and steroids, no reaction and color formation were observed which is in agreement with previous reports and considered as absent for current study (Chiew *et al.*, 2014). The difference could be due to environmental factors such as mineral insufficiency, drought, cold, and disease (Szakiel *et al.*, 2011). According to our qualitative result, we assumed that the phytochemicals which contributed for synthesis of metal oxide NPs were those indicated in plus sign.

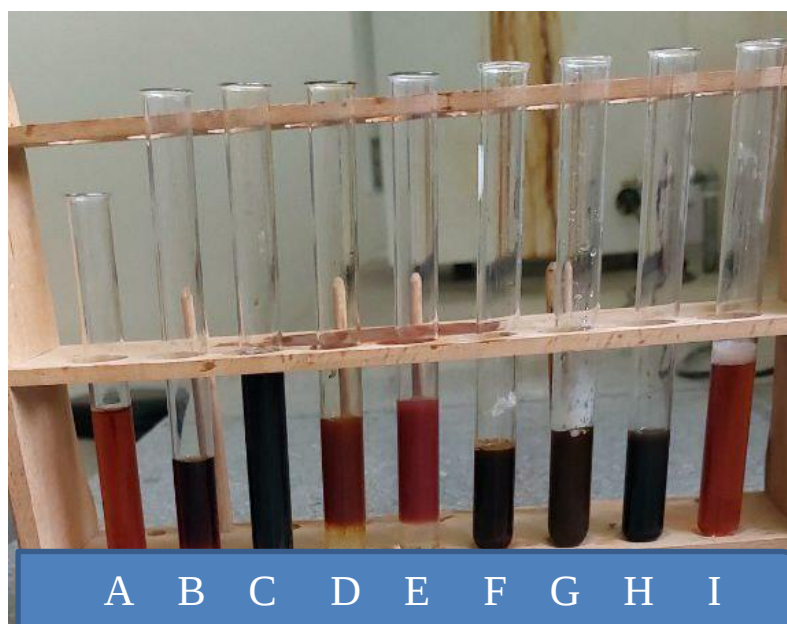


Figure 6 Phytochemical test of onion peel extract

Table 1. The qualitative analysis of Phytochemicals in the onion peel extract.

Code	Phytoconstituents	Chemical test/reagent	Results	Colors observed
A	Alkaloids	Wagner's test	+	Reddish brown
B	Flavonoids	Alkaline Reagent Test	-	Dark-reddish
C	Tannins	Ferric Chloride Test	+	Dark
D	Terpenoids	Salkowski test	+	Reddish-brown
E	Steroids	salkowski test	-	Red
F	Glycoside	Salkowski test	-	Brown
G	Benedict	Benedict test	+	Green
H	Phenols	Ferric Chloride test	+	Dark green
I	Saponins	Frothing test	+	Formation of froth

Plus (+) = presence and Minus (-) = absent

4.2. Green synthesis of MnO₂ and NiO NPs MnO₂/NiO NC

After the nitrate and potassium permanganate solution and onion peel extract were mixed, the color change was observed in the period of time which is slightly green for nickel oxide and purple color for manganese oxide NPs and for MnO₂/NiO NC. As the reaction time of the mixture increase or stirring the mixture on the hot plate, a gradual color change were there but after the pH is adjusted at 12 using 2M of NaOH the color change is totally observed. NiO NPs had a green color and MnO₂ NPs and MnO₂/NiO NC had a dark brown color. The formation of this color confirmed the synthesis of NiO and MnO₂ NPs MnO₂/NiO NC respectively

4.3. TGA/DTA Analysis Result

Figure 7 showed the green synthesized material's TGA/DTA thermal stability analysis result MnO₂ and NiO NPs MnO₂/NiO NC respectively. Figure 7 (a) DTA of the prepared sample of MnO₂ found at 83.51°C (-20.27uV) indicates exothermic

peak because physically adsorbed water has been lost. At about 218.64 °C (7.57 uV) indicated a strong endothermic peak due to the simultaneous breaking of bond from the absorbed water plus organic compound decomposition with a weight loss of 2.303 mg (21.84%) from total 10.546 mg and appearing of pure MnO₂ near to 400 °C. Above 400 °C, a continuous plateau or subsequently, no depletion in the TGA curve was observed, indicating that the sample size does not change when the temperature increases (Dessie *et al.*, 2021b). The TGA graph shows the three phases of weight loss that seem connected with two exothermic peak and two endothermic peaks in the NiO DTA graph. Figure 7(b) an intense exothermic peak was found at 68 °C (-16.24 uV) in DTA graph of NiO observed as a result of the physically adsorbed water being lost.

The breakdown of nickel complexes, the elimination of nitrates, and the breakdown of organic compounds were all suggested as the causes of the exothermic peaks at 297 °C caused by losses (Sabouri *et al.*, 2021). One sharp endothermic peaks observed at 328 °C (9.1 uV) could potentially be associated with the removal of water during Ni(OH)₂ crystallization and thermal oxidative degradation to form NiO. respectively (Rakshit *et al.*, 2013). The TGA graph show at 118 °C, the first weight loss was noted and was attributed to the vaporization of water molecules. The second weight loss observed at 302 °C was most likely caused by the disintegration of chemically bounded groups. The third weight reduction that was found to exist between 400 °C was directly related to the disintegration of the carbon-based compound and the creation of NiO phases. In addition, a gradual weight loss was observed in the TGA curve between 400 and 735 °C, which is indicative of a tiny hydroxyl group volume loss during the induced changes in the NiO phase from total weight of 8.499 mg the weight loss was 2.176 mg (25.6%).

Similarly, the MnO₂/NiO NC TGA/DTA graph showed in figure 7 (c). DTA curve indicates that three endothermic and three exothermic peaks were found. The first exothermic peak was at 64 °C (-5.45 uV), because of loss absorbed physically water. Next exothermic peak was at 287 °C this was due to decomposition of organic materials. The last exothermic peak was at 482 °C which was connected to the crystallization formation and decomposition of some organic materials which were residual from before decomposition. The first endothermic peak indicates that the

crystallization formation of MnO_2 at peak 206°C and also at 341°C second endothermic peak found which was because of crystallinity formation of NiO . At the 521°C (-1.37uV) there was another endothermic peak. These peaks could be the result of a change in crystallinity or could also be caused by a phase transition in the NPs to MnO_2/NiO NC. The TGA curve indicate that the initial reduction in weight occurred at 370°C . This reduction in weight is the result of physically molecules of adsorbed water disappearing from the surface of the nanocomposites. Above 370°C subsequently, no depletion in the TGA curve was observed, indicating that the sample size does not change when the temperature increases. From total sample weight of 7.094mg weight loss was 1.464mg (20.6%). (Worku *et al.*, 2021)

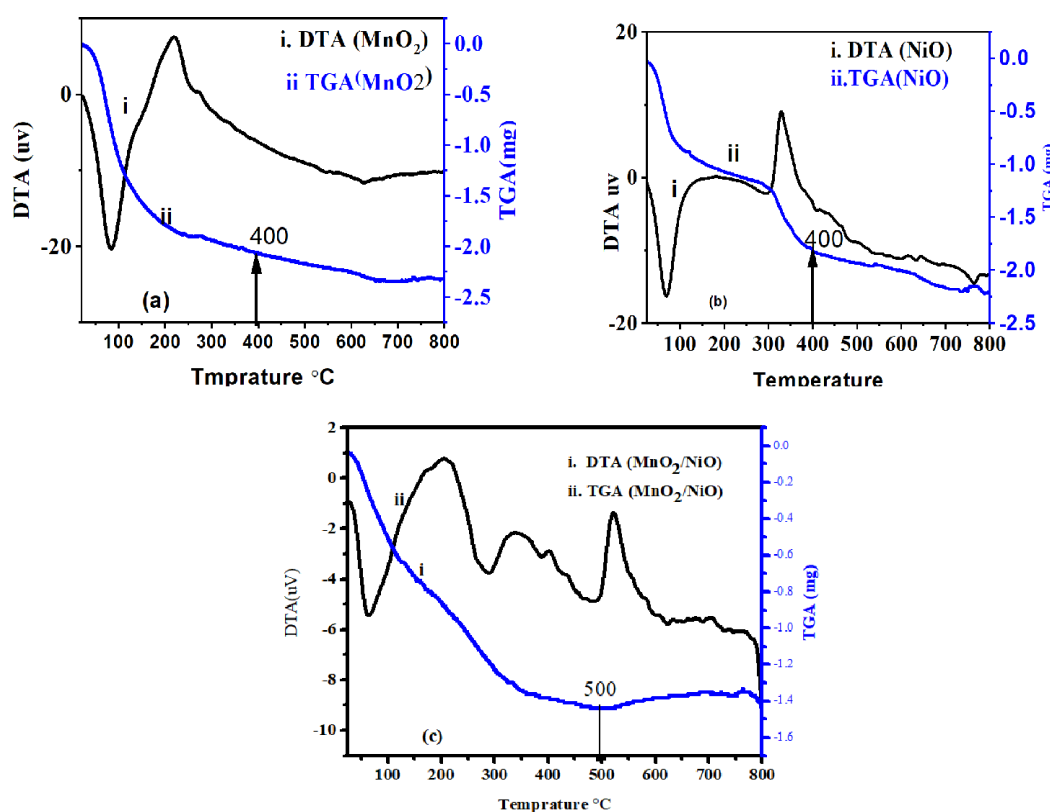


Figure 7. TGA/DTA curve of MnO_2 NPs, NiO NPs and MnO_2/NiO NC (a, b, c) respectively

4.4. XRD Analysis Result

XRD patterns of $\alpha\text{-MnO}_2/\text{NiO}$ nanocomposites in three ratios (1:1, 1:2, and 2:1) as well as pure $\alpha\text{-MnO}_2$ and NiO were showed in (Figure 8). From Figure 8(a), XRD pattern is used to analyses the structural details and crystallinity of NiO

nanoparticles made using onion peel extract. It was visible from the sharp, strong, and intense NiO peaks that the final products are quite crystalline. The presence of NiO accounts there are diffraction peaks at $2\theta = 37.25^\circ$, 43.26° , 63.11° , 75.46° , and 79.45° that are associated with the crystal planes (222), (111), (200), (220), and (311) (JCPDS file no. 047-1049) (Ezhilarasi *et al.*, 2016). The purity of the NiO phase was confirmed by the XRD pattern that was obtained, which unmistakably demonstrated the formation of NiO and revealed no extra diffraction peaks. α -MnO₂ spectrum, as shown in Figure 8(b), exhibits three strong features at $2\theta = 12.479^\circ$, 25.228° , and 37.218° . The diffraction planes at (110), (220), and (211) are identified as these. The official α -MnO₂ XRD pattern Crystallography Open Database, Entry # 053-0633) was utilized to index the planes (Dessie *et al.*, 2021a; Ezhilarasi *et al.*, 2018).

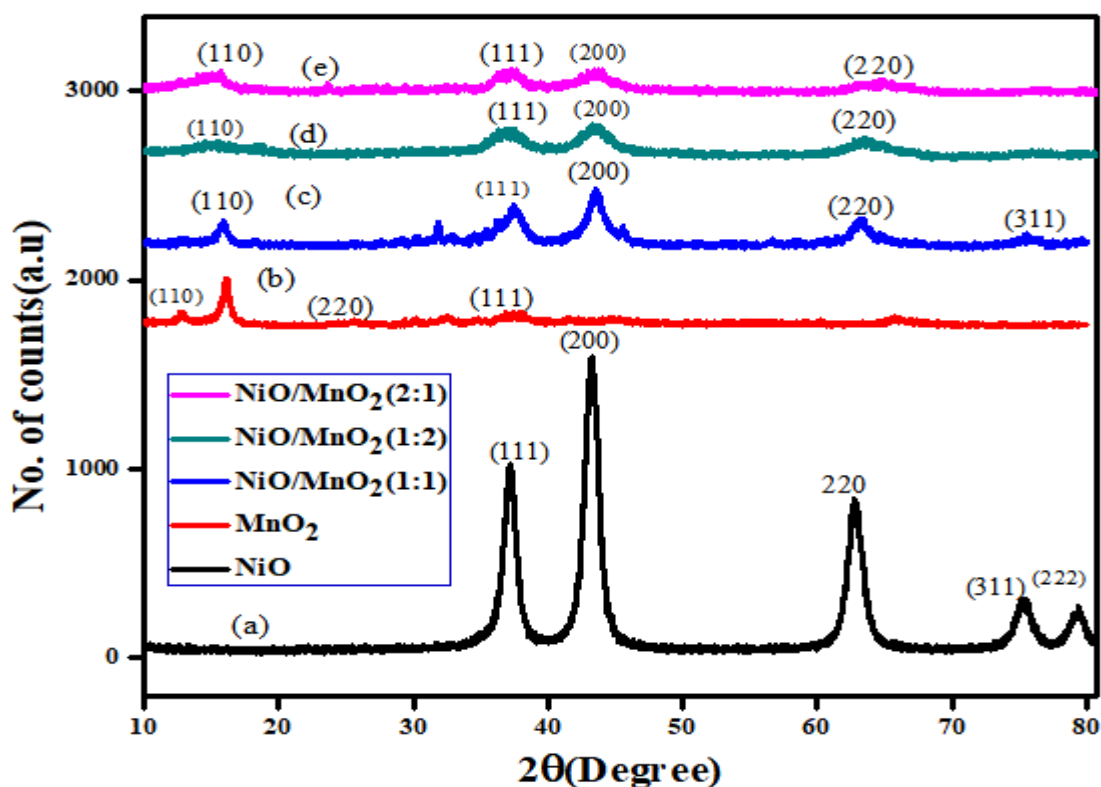


Figure 8. X-ray diffraction patterns NiO, MnO₂ NPs and MnO₂/NiO NCs biosynthesized under different volume ratios of KMnO₄ salt 1:1, 1:2, and 2:1.

The XRD pattern of the 1:1, 1:2, and 2:1 ratios of α -MnO₂/NiO nanocomposite is displayed in Figure 8(c), (d), and (e) respectively. The nanocomposite is represented by the peaks at 28.42° , 36.25° , and 40.62° (COD, Entry # 049-1295).

Because of their distinct mixtures, it was obvious that the diffraction peaks of these NCs are confirmed by the typical XRD patterns of NiO and α -MnO₂. This demonstrated that the α -MnO₂-NiO NCs had been effectively created using an assisted technique with onion peel extract. Table 2 displays the crystallite size of each nanomaterial sample's produced particles. The Debye-Scherrer formula was utilized to determine the crystallite size (D) using equations (1).

Table 2. XRD data used for calculating the crystallite size of biosynthesized nanoparticles

NPs and NCs	Peaks (2 theta)	FWHM	Crystallite size D (nm)
MnO₂	16.1433	0.6629	11.96956622
	15.4936	0.48	16.51745428
	12.8601	0.585	13.51410545
	Average= 14		
NiO	43.2345	1.2245	6.901078633
	37.1698	1.1231	7.379779304
	62.7873	1.2588	7.311172818
	Average= 7.2		
1:2 NC	43.5366	2.25	3.75966117
	36.9262	2.58	3.210204931
	63.2633	2.33	3.959988375
	Average =3.6		
1 :1 NC	43.5616	1.38	6.130416571
	37.4258	1.54	5.386026912
	63.1599	1.47	6.273232111
	Average= 5.9		
2:1 NC	36.5765	1.4134	5.853924529
	43.5216	1.94	4.360203889
	37.4957	1.24	6.690481599
	Average= 5.6		

FWHM: full width at half maximum, it is the width values of XRD peaks at which the half of maximum amplitude reached. According to the Scherrer's calculation,

the 1:2 NC is smaller than the other composites (3.64 nm) and has a larger surface area. 1:1 NC ratio and 2:1 NC ratio has crystallite size 5.92nm and 5.63nm respectively. The ratio of precursor salts used in the synthesis process can affect the size of the crystallites in a nanocomposite. It is the various growth mechanism factors that are responsible for this change in particle size. For instance, the concentration of reactants can affect the rate at which nanoparticles nucleate and grow. Smaller crystallite sizes may arise from a higher rate of nucleation brought on by higher concentrations. The 1:2 ratio exhibits a smaller crystallite size compared to 1:1 and 2:1, possibly due to the higher concentration of nickel nitrate than potassium permanganate. Lower concentrations, on the other hand, may cause larger crystallite sizes and a slower rate of nucleation. In the 2:1 ratio NC, the size of the crystallites is larger and the concentration of nickel nitrate is lower than that of potassium permanganate (Harish *et al.*, 2022). Particle aggregation may also be influenced by the potassium permanganate salt ratio. Greater particle aggregation can result in larger crystalline sizes when potassium permanganate salt, which was found in 2:1 and 1:1 concentration, is present in higher concentrations. On the other hand, a lower potassium permanganate salt concentration (1:2) may result in less particle aggregation and smaller crystalline sizes (Kotoulas *et al.*, 2022). Reduced crystallite size can result in a higher surface area, which can enhance the nanocomposite interaction with microorganisms and boost its antimicrobial activity. ROS which can harm microorganisms' cell membranes and enhance the antimicrobial activity, can also be produced at a higher rate on surfaces with increased surface area when illuminated (Ohira and Yamamoto, 2012).

4.5. UV DRS Analysis Result

UV DRS spectroscopy is a preliminary and high performance approach to examining the bio synthesis of NPs. Figure 9(a) shows that the estimated band gap of NiO and MnO₂ was 0.74, and 0.84eV. The band gap results of green synthesized nanocomposite 1:1, 1:2, and 2:1 was recorded 1.34, 1.45, and 1.37eV in Figure 9(b). The band gap results of NPs are less than the NCs. The emergence of new energy levels as a result of chemical flaws or vacancies in the intergranular areas may have an impact on this outcome (Das and Golder, 2018). The dissolved KMnO₄ salt component in the produced nanocomposite is most likely what is causing the variation in eV values. The different ratios of KMnO₄ salt in the NCs

have effect on dimension and shape of the NPs. Because of the overlapping numerous of atomic orbitals caused by the greater crystallite size, there are more molecular orbitals, which results in a drop in eV. Consequently, depending on the shape and particle size, eV values change (Firooz *et al.*, 2018). The graph's % reflectance gradually dropped as wavelength increased, indicating that the electronic transition happened in UV range.

In Figure 9 the graph percent of reflectance verses wave length the biosynthesized NCs showed a broad peak than NPs. This indicate that the NCs had more reflected light and it was more modified than NPs in their optical properties. Table 2 as particle size decreases, band gap energy rises because of quantum confinement effects. When photons interact with a semiconductor material at an energy level that is just enough to promote an electron from the valence band to the conduction band, or when the energy of the photons matches the energy gap of the material, the photons was be absorbed. The produced nanoparticles have a non-vanishing energy spacing at the molecular level, which results in a highly confined particle and a more structured absorption spectrum. Therefore, for the material to electronically move more energy is needed to move from the valence band to the conduction band (Koshy and George, 2015). So 1:2 MnO₂/NiO NC has small crystallite size with high band gap. A higher surface area resulting from a smaller crystalline size can lead to a narrower band gap and a higher rate of direct electron transition. Because more electrons can move from the valence band to the conduction band and generate reactive oxygen species (ROS), which can damage the cell membranes of microorganisms. This can enhance the green synthesized nanocomposite's antimicrobial activity (Silva *et al.*, 2019).

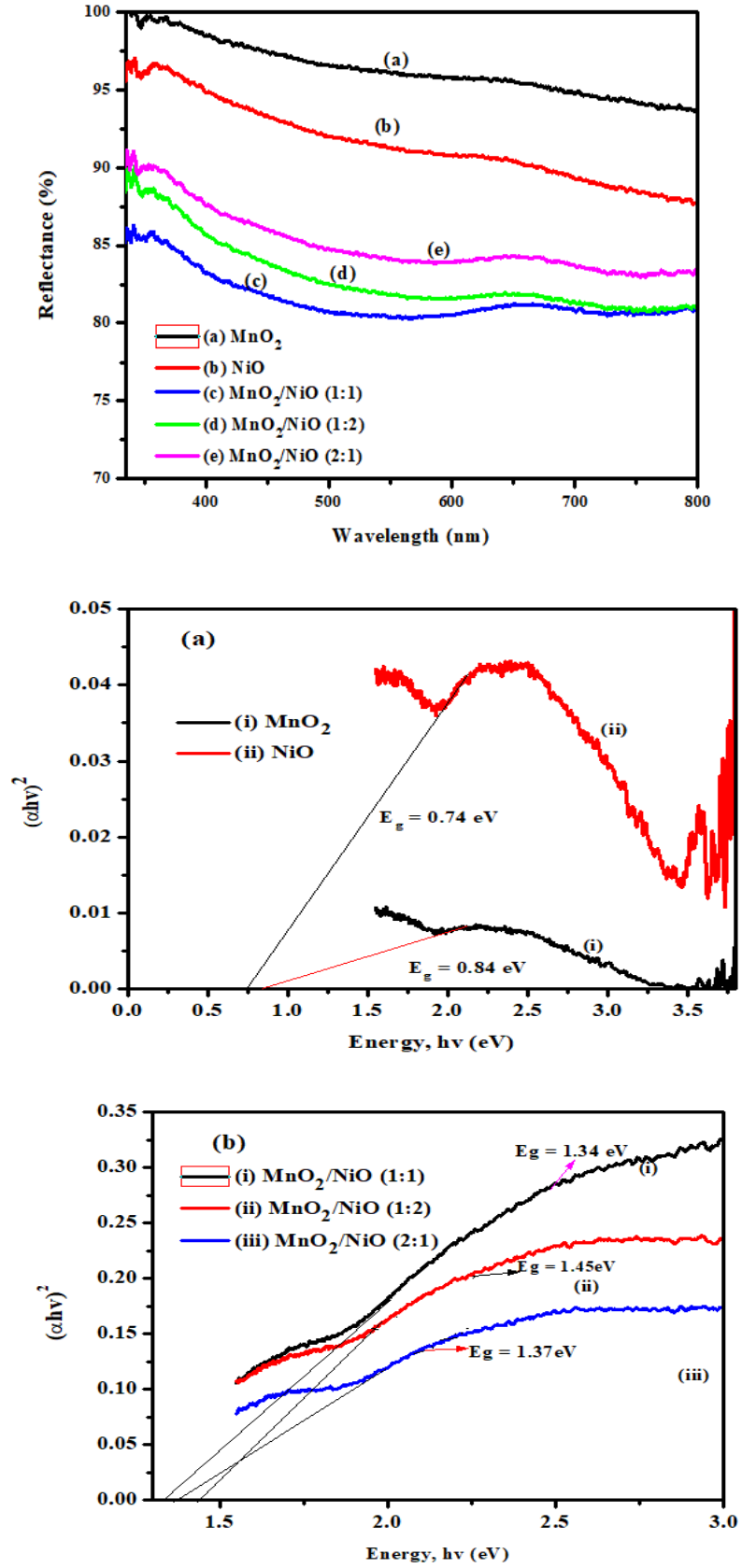


Figure 9. Optical properties study using the DRS-UV-vis spectroscopic technique

4.6 FT-IR Analysis Result

FTIR analyses were performed to determine the possible functional groups of the biomolecules contained within onion peel powder. Biosynthesized nanomaterials that are involved in capping, reduction of the potassium permanganate and nickel nitrate ions synthesize MnO_2 and NiO NPs and MnO_2/NiO NCs, and proficient stabilization of the synthesized MnO_2 and NiO NPs and MnO_2/NiO NCs. The FTIR spectroscopy helped reveal the bonding features of the onion peel powder and MnO_2 and NiO NPs MnO_2/NiO NCs. Figure 10 shows the FTIR spectra of biosynthesized MnO_2 and NiO NPs MnO_2/NiO NCs at a 1:1 volume ratio and the extracts of onion peel powder the measurements were captured within the spectrum ranging from 4000 to 500cm^{-1} . The FTIR analysis of onion peel extract reveals the extract contains a variety of functional groups. Spectrum in black color in figure 10 typically shows onion peel there is a broad peak observed at approximately 3273cm^{-1} , indicating the stretching vibration of O-H bonds of alcohols and phenols. Another peak around $2853\text{--}2919\text{cm}^{-1}$ is attributed to the C-H stretching vibration of alkanes. The peak around 1726cm^{-1} is because of formation of Carbonyl group, aldehyde group. The peak at around 1604 cm^{-1} is due to the C=C stretching vibration of alkenes, while the peak at around 1421cm^{-1} and 1508cm^{-1} indicate that Organic sulphates group. The peak around 1012cm^{-1} is associated with the peaks corresponding to the C-O stretching vibration of alcohols and ethers suggest the existence of diverse bioactive compounds within the onion peel extract, such as flavonoids, phenolic acids, and terpenoids (Ateeq *et al.*, 2023). It is common for a nanoparticle to lose its functional groups after calcination. The process of calcination usually entails raising the material's temperature to a high point, frequently with the presence of oxygen or air. Organic elements that might have been present in the nanoparticle prior to calcination, such as functional groups, may be eliminated as a result of this process. As a result, the nanoparticle may mostly consist of an inorganic substance with few or no functional groups, such as metal or metal oxide (Abdullah *et al.*, 2023).

The blue-colored spectra depicted in Figure 10 indicate characteristic peaks observed at 1400cm^{-1} , which are associated with surface water stretching (-OH), bending of H-O-H, stretching collisions of O-Mn-O, and vibrations of Mn-O bonds, respectively (Dessie *et al.*, 2020). The bands observed between 600cm^{-1} and

475cm⁻¹, notably at approximately 596cm⁻¹ and 495cm⁻¹, are associated with the stretching collision of O-Mn-O bonds. Meanwhile, the bands spanning from 870cm⁻¹ to 450cm⁻¹ exhibit N-H stretching, aromatic C-H stretching, quinoid ring stretching vibrations, and benzenoid ring vibrations (Moon *et al.*, 2015). Additionally, the presence of Mn-O bond stretching vibrations distinctly indicates the formation of MnO₂ nanoparticles (Kareem Jassem *et al.*, 2019).

As showed in Figure 10's red spectra, exposure of the samples to the atmosphere leads to the appearance of a broad absorption peak at 3389cm⁻¹, attributed to the stretching vibrations of O-H groups and the H-O-H bending vibrations of surface-adsorbed water on the hybrid metal oxide nanoparticles. The nanoparticles' capacity to adsorb water molecules demonstrates their large surface area. Weak band stretching vibrations were observed at 1636 and 1449cm⁻¹. Another peak, indicating C-O symmetrical stretching vibrations, is located at 1365cm⁻¹. The formation of metal-oxygen (Ni-O) bonds gives rise to a notable absorption peak at 930cm⁻¹ (Karthik *et al.*, 2019).

Figure 10 below displays the green color spectrum, which confirms The formation of the MnO₂/NiO nanocomposite is indicated by two prominent bands at 1434 and 983cm⁻¹. These bands signify the division of O-Mn-O or Ni-O bonds within the vibrational band. Stretching vibrations of the O-H adsorbed nanocomposite surfaces of NiO and MnO₂/NiO contribute to the peak observed at 2954cm⁻¹. The peaks around 1638cm⁻¹ are linked to chelate vibrations in both symmetric and asymmetric COO formats. (Racik *et al.*, 2019). Hence, the FT-IR spectrum results align with the XRD data, thereby confirming the formation of highly pure single NiO and MnO₂ NPs, as well as MnO₂/NiO NCs.

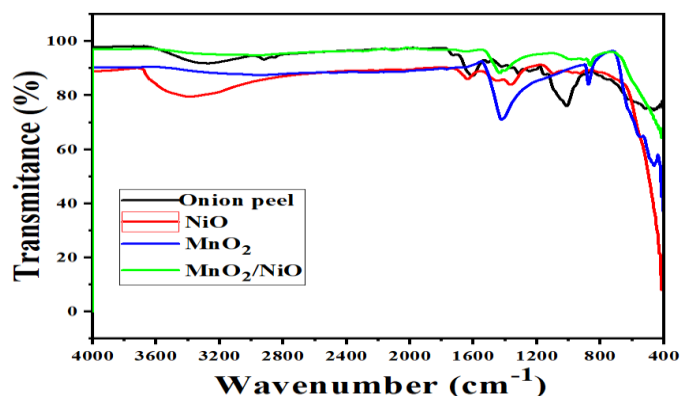


Figure 10. FT-IR spectra of biosynthesized MnO₂, NiO NPs MnO₂/NiO NC and onion peel extract

4.7. SEM Analysis Result

Surface morphological characteristics were investigated using scanning electron microscopy (SEM) synthesized nanoparticles and nanocomposite samples; magnifications of 6000, 3000, and 1500 were recorded in the images. The pure NiO as prepared Figure 11 shows that the grain growth is nearly uniform structure. It displays the agglomeration of nanoparticles with asymmetrical shapes. The agglomeration is due to aggregating or overlapping of smaller particles there are some larger particle. The structures that have been formed are intricately linked to one another. The SEM micrograph of low aggregated pure α -MnO₂ NPs with a rough and randomly oriented structure as shown in Figure 12 (Dessie *et al.*, 2020). The formation of a non-uniform with irregular-shaped aggregated structure with slightly aggregated morphology is clearly suggested by Figure 13a and b for 1:1, c and d for 2:1, e and f for 3:2 NCs ratios. The aggregation of nanoparticles likely occurred due to factors such as polarity, electrostatic attraction, and high surface energy, common during nanoparticle synthesis in water. This aggregation may result from prolonged redox reactions between precursor salt aqueous solutions and plant extracts (Adeyemi *et al.*, 2022). To create α -MnO₂/NiO structures with highly integrated characteristics and distinct structural arrangements compared to pure nanoparticles, utilizing onion peel extract as a reducing agent could be beneficial. The varying ratios of manganese permanganate mixed with nickel nitrate salts lead to noticeable differences in the morphology and shapes of the synthesized nanocomposites.

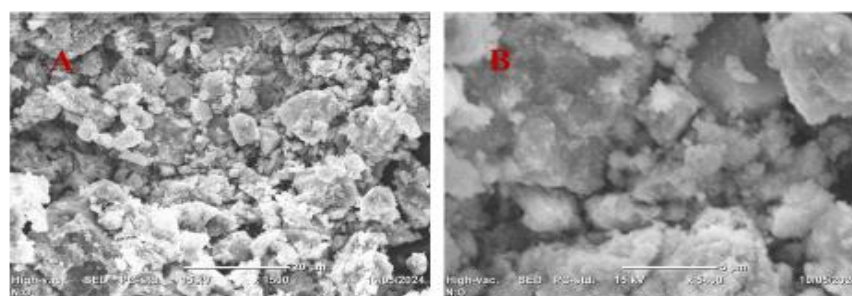


Figure 11. SEM images of NiO NPs two different magnifications (20 and 5 μ m respectively)

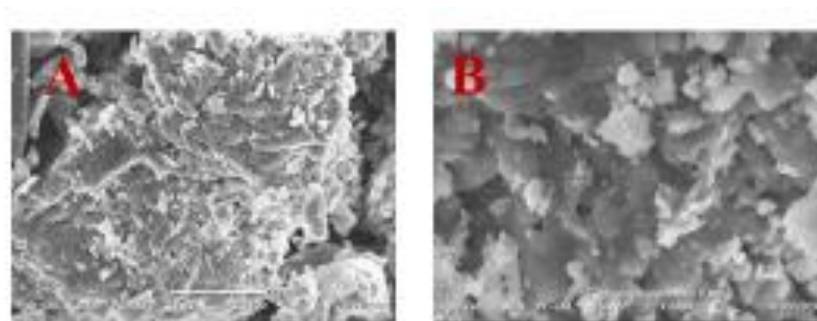


Figure 12. SEM images of MnO₂ NPs two different magnifications (20 and 5 μ m respectively)

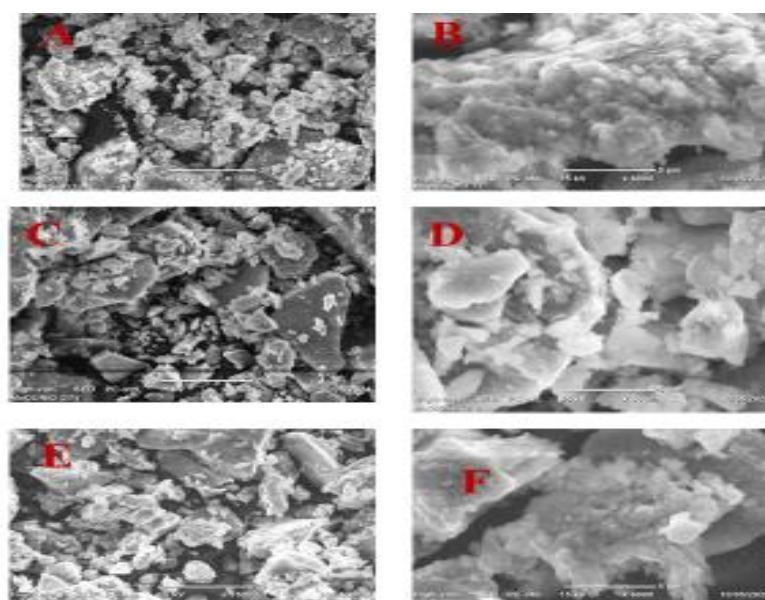


Figure 13. SEM image MnO₂/NiO NC (A and B) 1:1, (C and D) 1:2, and (E and F) 2:1 ratio, with two different magnifications (20 and 5 μ m respectively)

4.8. PL Analysis Result

Figure 14 the PL of NiO spectra exhibit intense emission peaks at 340nm and a broad emission around 412nm. The UV emission at 340nm confirms the radiative recombination of excited electrons with holes in the valence band. The emission peak at 412nm, attributed to the presence of defects, including (in) defects, confirms the existence of intrinsic defects. Additionally, the presence of a shoulder peak near 340nm corresponds to the surface defects (Ezhilarasi *et al.*, 2018). Hence the onion peel The extract utilized in this study successfully synthesized NiO nanomaterials containing intrinsic defects, oxygen vacancies, and surface defects, known to enhance photocatalytic and biological activity. Consequently, it is hypothesized that the synthesized NiO nanomaterial, characterized by reduced particle size, high surface defects, and a large surface area, possesses enhanced activity. Gaussian curve fitting of the PL spectra of MnO₂ reveals three distinct emission peaks at 343, 414, and 433nm. The photoluminescence (PL) spectra exhibit an ultraviolet emission band at 343 nm, which is attributed to the band edge emission signals of MnO₂. (Mohammad Toufiq *et al.*, 2014). A highly intense blue emission peak at 414nm arises from oxygen vacancy-related defects, while a broad, weaker emission in the green region is also evident around 433nm, likely attributed to surface defects or surface dangling bonds. MnO₂/NiO NCs ratios (1:1, 1:2, and 2:1) show an intense peak at 412nm. Ratio 1:1 also show shoulder peak at 433nm. MnO₂/NiO exhibited all of the emissions seen in the MnO₂ and NiO spectrum, indicating the presence of MnO₂ and NiO. This is significant because it enhances the charge transfer features, this phenomenon subsequently enhances the production of reactive oxygen species (ROS) through electron-hole reactions with water and oxygen, respectively.

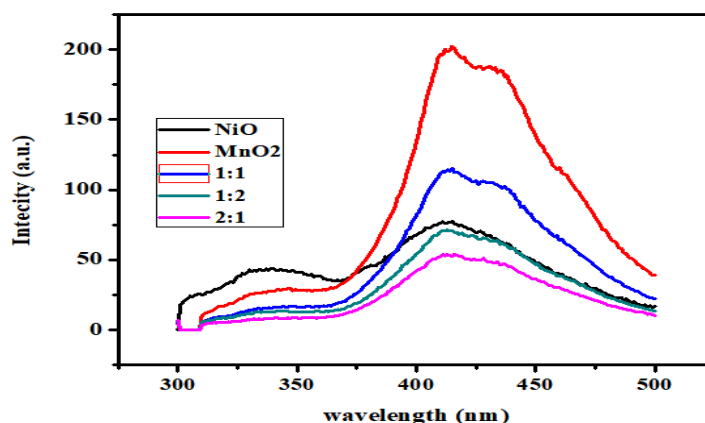


Figure 14. photoluminescence (PL) intensity versus wavelength for MnO₂, NiO, MnO₂/NiO in three ratio

4.9. Antibacterial Activity of Biosynthesized NPs and NC

Figures 15 show photographs depicting antibacterial test results at concentrations of 25 µg/mL, 50 µg/mL, and 75 µg/mL for each synthesized NPs and NCs, with detailed summaries provided in Table 3. The presence of an inhibition zone indicates the antibacterial effect observed in the biosynthesized samples. Overall, both MnO₂ and NiO NPs, as well as MnO₂/NiO NCs, demonstrated dose-dependent antibacterial activity against both gram-positive and gram-negative bacteria tested. From three ratios of biosynthesized NCs 1:2 ratio of MnO₂/NiO NC showed better performance on the antibacterial activity as compared to the rest two MnO₂/NiO NCs ratios (1:1 and 2:1) and also that of MnO₂ and NiO NPs. In a 1:2 ratios, the range of zone of inhibition was between 21.2-27.16 mm at the concentration of 75 µg/mL (Table 3). This possible due to smaller average crystalline size which is suitable to penetrate bacterial membranes and harm bacteria only on a single cell level (Modi *et al.*, 2023). As previously suggested reports in different nanomaterial can prevent the growth of bacteria by interfering with the uptake of nutrients and DNA replication, these MnO₂/NiO NCs may also interfere with biological functions and harm DNA. The PL and DRS results (Figures 14 and 9, respectively) corroborate this result and demonstrate high surface defects, indicative of roughness, can generate reactive oxygen species (ROS), potentially causing damage to the bacteria's cells (Dayem *et al.*, 2017).

2:1 ratio of MnO₂/NiO NC showed the second strongest activity with a maximum zone of inhibition of 23.13, 19.1, 14.1, and 19.1 mm at the concentration of

25µg/mL. 25.2, 20.2, 15.13, and 20.2mm at the concentration of 50µg/ml and 26.1, 21.2, 16.1, and, 15.16mm at the concentration of 75µg/mL against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, respectively (appendix 2). 1:1 MnO₂/NiO NCs also showed good performance followed by a 2:1 volume ratio that exists the bioactive compound within a different ratio of metal salt and interacts with the surface of the bacteria cell wall and to penetrate it. In a 1:1 volume ratio, the range of zone of inhibition was between 15.1-22.1 mm at the concentration of 75µg/mL. At a concentration of 75µg/mL, a maximum zone of inhibition of 22.1mm was observed against *S. aureus*. Conversely, the smallest zone of inhibition was noted in *Pseudomonas aeruginosa* at 25µg/mL. This might be due to the larger average crystalline nature of the formed MnO₂/NiO NCs in its XRD spectrum as compared to the 1:2 and 2:1 NCs ratios.

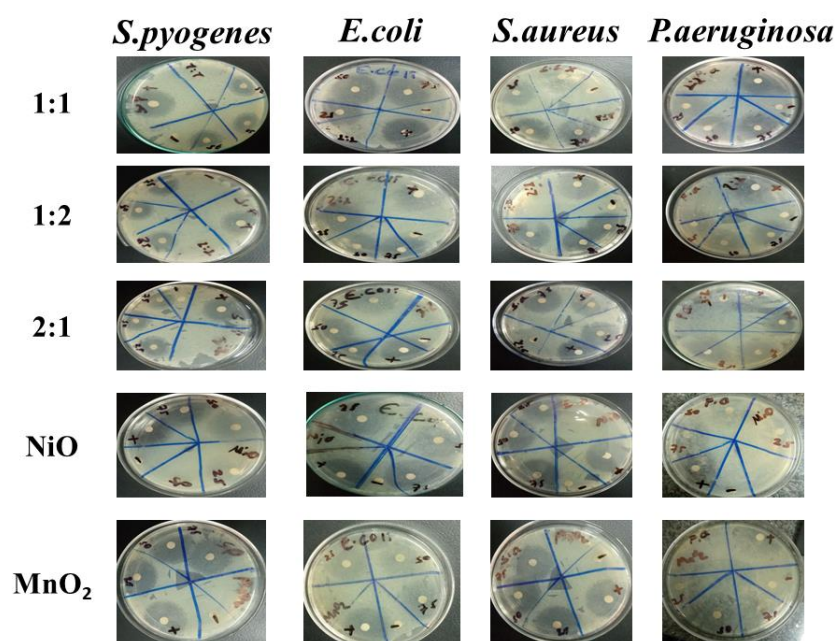


Figure 15. Disc diffusion antibacterial activity evaluation of MnO₂, NiO NPs, and MnO₂/NiO NCs ratios against two gram-positive (*E. coli* and *P. aeruginosa*) and two gram-positive (*S. aureus* and *S. pyogy*)

Compare to the MnO₂/NiO NCs MnO₂ and NiO NPs showed smaller antibacterial activity. This could be due to the larger average crystalline which found in XRD results table 2. In the MnO₂ NPs, and NiO showed the range of inhibition zone between 15.2 -21mm and 16.1-20.2mm respectively. A maximum zone of inhibition of 21mm was produced against *S. aureus* for MnO₂ and, 20.2 mm also

for NiO against *S. aureus* at the concentration of 75 µg/mL. The least zone of inhibition was observed in *Pseudomonas aeruginosa* at 25 µg/mL for MnO₂ and *S. pyogenes* at 50µg/mL for NiO. The two nanoparticles showed the least performance on the antibacterial activity as compared nanocomposite volume ratios because of their large surface area, which allows for better contact with the cell wall of bacteria. Depending on our result, the antibacterial activity of biosynthesized NCs in three volume ratios were found to by varying KMnO₄ salts shows promising antibacterial activities. The statistical analysis showed that all concentrations of the tested NPs and NCs significantly inhibited all bacterial strain as compared to the ciprofloxacin ($P < 0.05$). However, there was significant difference ($P < 0.05$) among 2:1NC and other nanomaterials against bacterial strain (appendix1).

When the concentration of nanocomposite is 25µg/mL, 50µg/mL, and 75µg/mL increase the zone of inhibition also increases within the three-volume ratios. The antibacterial activity of biosynthesized increased when the concentration of NPs and NCs is 25µg/mL, 50µg/mL, and 75µg/mL increase the zone of inhibition also increases (appendix 3). This is because the higher concentration of NPs and NCs enhances their ability to interact with bacterial cells, leading to improved antibacterial activity. In terms of the mechanisms involved, the increased concentration of NPs and NCs can lead to; increased production of reactive oxygen species (ROS), which can damage bacterial cells. Enhanced NPs and NCs disruption of bacterial cell membranes, leading to cell lysis and death (Dayem *et al.*, 2017). The order of inhibition was as follows 1:2 ratio NC > 2:1 ratio NC >1:1 ratio NC >NiO NPs > MnO₂ NPs. The antibacterial result indicated that the synthesized MnO₂/NiO NCs shows good inhibition zone in both grams positive and gram-negative bacterial strains

Table 3. Antibacterial activity (zone of inhibition) of MnO₂, NiO NPs, and MnO₂/NiO NCs

Bacterial strains and Zone of Inhibition 1:1NC in mm± SD				
Conc. (µg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
25	15.1±0.1	13±0.1154	20.1±0.1	14±0.115
50	17.1±0.152	14±0.0577	21±0.0577	17±0.1
75	19.1±0.152	15.1±0.115	22.1±0.1	19.1±0.1
Ciprofloxacin(30µg/mL)	28.1±0.1	27.1±0.1	28.1±0.057	27.1±0.1

Bacterial strains and Zone of Inhibition 1:2 NC in mm ± SD				
Conc. (µg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
25	22.1±0.1	25±0.1	24.1±0.1	13.5±0.15
50	25.1±0.1	26.1±0.1	25.1±0.1	13.2±0.11
75	27.16±0.15	26.16±0.15	26±0.005	21.2±0.15
Ciprofloxacin(30µg/mL)	30.1±0.1	30.2±0.173	30.1±0.1	26.1±0.1

Bacterial strains and Zone of Inhibition 2:1 NC in mm ± SD				
Conc. (µg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
25	23.13±0.15	19.1±0.1	14.1±0.1	19.1±0.1

50		20.2±0.05	15.13±0.05	20.2±0.115
	25.2±0.2	77	77	9
75		21.2±1.67		15.16±0.15
	26.1±0.1	43	16.1±0.1	27
Ciprofloxacin(30µg/ mL)	30±0.1154	29±0.0577	29.1±0.1	30.1±0.1

Bacterial strains and Zone of Inhibition MnO₂ NPs in mm ± SD

Conc. (µg/mL)	<i>E. coli</i>	<i>P. aeruginos</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
		<i>a</i>		
25		11.1±0.115		
	15.1±0.1	4	16.1±0.1	13±0.1154
50	16.3±0.152		18.3±0.152	
	7	13.1±0.1	7	15.1±0.1527
75				17.16±0.152
	19.1±0.1	15.2±0.1	21±0.0577	7
Ciprofloxacin(30µg/m L)	28.3±0.1	28.1±0.1	29.1±0.1	28.1±0.1

Bacterial strains and Zone of Inhibition NiO NPs in mm ± SD

Conc. (µg/mL)	<i>E. coli</i>	<i>P. aeruginos</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
		<i>a</i>		
25	23.17±0.15		18.13±0.11	15.17±0.15
	27	16.1±0.1	54	27
50	16.1±0.1	18.1±0.1	20.1±0.1	15±0.1154
75	18.1±0.1	20.1±0.1	20.2±0.1527	16.1±0.1
Ciprofloxacin(30µg/m L)	27±0.1154	27.1±0.1	28.1±0.1	28.16±0.15

4.10. Antifungal Activity Analysis of Biosynthesized NPs and NC

Table 4 and Figure 16 display the inhibition zone of synthesized NPs and NCs. By comparison, the compounds and concentrations of NPs and NCs at 25µg/mL and 50µg/mL demonstrated weaker antifungal activity than at 75µg/mL, indicating that the activity is dose dependent. In the inhibition zone, synthesized NPs and NCs compare in decreasing order to 1:2 > 1:1 > 2:1 > MnO₂ > NiO, respectively. The MnO₂/NiO NCs increased antifungal activity attributed that beneficial interaction between MnO₂ and NiO, thereby increasing the generation of ROS and small crystallite size (Oussou-Azo *et al.*, 2020). Reactive oxygen species (ROS) can induce oxidative damage to proteins, lipids, and nucleic acids within cells, resulting in cell death and a higher degree of cell membrane damage.

When considering MnO₂ or NiO alone, there might be less cell membrane damage than MnO₂/NiO NCs. A greater surface area for interaction with the fungal cell membrane may be provided by the MnO₂/NiO nanocomposite structure, resulting in a higher degree of damage and increased antifungal activity. The MnO₂/NiO NCs particular synthesis process and composition may also have an impact on its antifungal efficacy (Slavin and Bach, 2022). The MnO₂/NiO 1:2 NC inhibition zone measurements were 11.1, 15.1, 16.13mm at concentrations of 25, 50, and 75µg/mL, in that order. Inhibition zones of 10.1, 12.1, and 15.13mm were observed in the 1:1 NC of MnO₂/NiO at concentrations of 25, 50, and 75µg/mL, respectively. The antifungal activity of the MnO₂/NiO 2:1 NC was lower than that of the other NC ratios. At 25, 50, and 75µg/mL concentrations, it exhibited an 8.16, 10.1, and 12.13mm inhibition zone, respectively. When compared to the other two ratios (1:1 and 2:1), the biosynthesized MnO₂/NiO NC (1:2) ratio demonstrated superior antifungal activity performance. The average crystalline size may be smaller, which could explain this. Because smaller crystals have a larger surface area and can interact with fungal cells more, breaking down cell membranes and preventing growth, they may have higher antifungal activity (Rudramurthy *et al.*, 2016). Compared to MnO₂/NiO NCs, MnO₂ and NiO NPs displayed the least inhibition zone. This could be because MnO₂ and NiO NPs have larger average crystalline sizes. Reduce this can happen when crystalline size increases surface area.

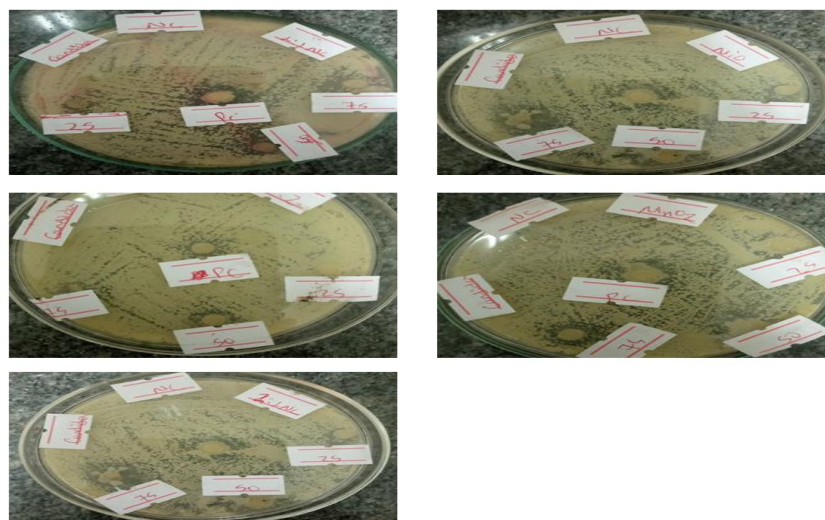


Figure 16. Disc diffusion antifungal activity evaluation of the biosynthesized MnO_2 , NiO NPs, and MnO_2/NiO NCs *Candida albicans*.

Table 4. Antifungal activity (zone of inhibition) of MnO₂, NiO NPs, and MnO₂/NiO NCs

Conc. (µg/mL)	<i>Candida albicans</i> fungal strains and zone of inhibition in mm ± SD	
1:1 NC	25	10.1±0.1
	50	12.1±0.1
	75	15.13±0.1527
	Ketoconazole(20µg/mL)	20.1±0.1
1:2NC	25	11.1±0.1
	50	15.1±0.1
	75	16.13±0.1527
	Ketoconazole(20µg/mL)	20.1±0.1527
2:1NC	25	8.16±0.1527
	50	10.1±0.1
	75	12.13±0.1527
	Ketoconazole(20µg/mL)	18.1±0.1
MnO₂NPs	25	8.1±0.1
	50	9.1±0.1
	75	11.16±0.1527
	Ketoconazole(20µg/mL)	19.1±0.1
NiO NPs	25	6.16±0.1527
	50	9.16±0.1527
	75	10.16±0.1527
	Ketoconazole(20µg/mL)	17.1±0.1

4.11. Antioxidant Activity Analysis of biosynthesized NPs and NC

The free radical scavenging capability of the biosynthesized silver nanoparticles was assessed using the DPPH assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay relies on the stable free radical's ability to lose color in the

presence of antioxidants. The absorbance at 517 nm and the distinct deep purple hue are due to the presence of an unpaired electron in the DPPH radical. A reduction in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the tested compound. Presence of antioxidant potential was demonstrated *in vitro* by an antioxidant assay using MnO₂/NiO NCs, and MnO₂, NiO NPs.

In every antioxidant method, the percentage of inhibition showed that, up to the specified concentration NCs and NPs scavenged free radicals in a concentration-dependent manner. The data is contrasted with results obtained using ascorbic acid as a reference (Foti, 2015). Table 5 illustrates the connection between various analogues of the biosynthesized NPs and NCs and their capacity to scavenge the DPPH radical. Figure 17 (A) showed that absorbance verses concentration. Absorbance is high when the concentration of nanomaterial is low. The absorbance value may be high for a variety of reasons when the concentration of the nanomaterial is low. For example, at low concentrations, the nanomaterial may not have sufficient scavenging capacity to effectively neutralize the free radicals, which would result in a higher absorbance value. Lower concentrations of nanomaterials might also have less surface area available to interact with free radicals, which would decrease their effectiveness as scavengers and raise absorbance values (Dai *et al.*, 2024).

All of the compounds under investigation exhibited moderate to high radical scavenging activities when measured against the control's absorbance (0.904). At 1000µg/mL, the DPPH radical-scavenging activities for the 1:1, 1:2 and 2:1 MnO₂/NiO NCs ratios were 71.09%, 71.28%, and 71%, respectively. Additionally, MnO₂ and NiO NPs revealed 70.24 and 70.98%, respectively. The decreasing order of inhibition was as follows: 1:2 ratio NC > 1:1 ratio NC > 2:1 NC ratio > MnO₂ NPs > NiO NPs. The 1:2 ratio of MnO₂/NiO NC exhibited greater percentage of inhibition, while the MnO₂/NiO NC ratio of 1:1 got the next highest percentage of inhibition Figure 17 (B). As a result, MnO₂/NiO NCs exhibit a higher percentage of inhibition than MnO₂ and NiO NPs, and the percentage of antioxidant potential increases along with concentration. The antioxidant potential was contrasted with ascorbic acid, the standard antioxidant.

Table 5. Antioxidant activity of MnO₂, NiO NPs, and MnO₂/NiO NCs

Con. (µg/mL)	Control	Biosynthesized NPs and NCs										Positive control	
		1:1		1:2		2:1		NiO		MnO ₂		AA	
	Abs	Abs	%RSA	Abs	%RSA	Abs	%RSA	Abs	%RSA	Abs	%RSA	Abs	%RSA
1000	0.904	0.2613	71	0.2596	71.28	0.262	71.0	0.269	70.2	0.2623	70.9	0.0716	92
500	0.904	0.3036	66.4	0.295	67.36	0.2883	68.1	0.309	65.8	0.2923	67.6	0.0746	91.7
250	0.904	0.3266	63.8	0.383	57.63	0.307	66.0	0.3593	60.2	0.3166	64.9	0.0843	90.6
125	0.904	0.357	60.5	0.6053	33.04	0.3683	59.2	0.512	43.3	0.384	57.5	0.0883	90.2
62.5	0.904	0.5316	41.1	0.822	9.07	0.4913	45.6	0.645	28.6	0.6273	30.6	0.101	88.8

Abs= Absorbance; Conc. = Concentration; %RSA= Radical scavenging activity.

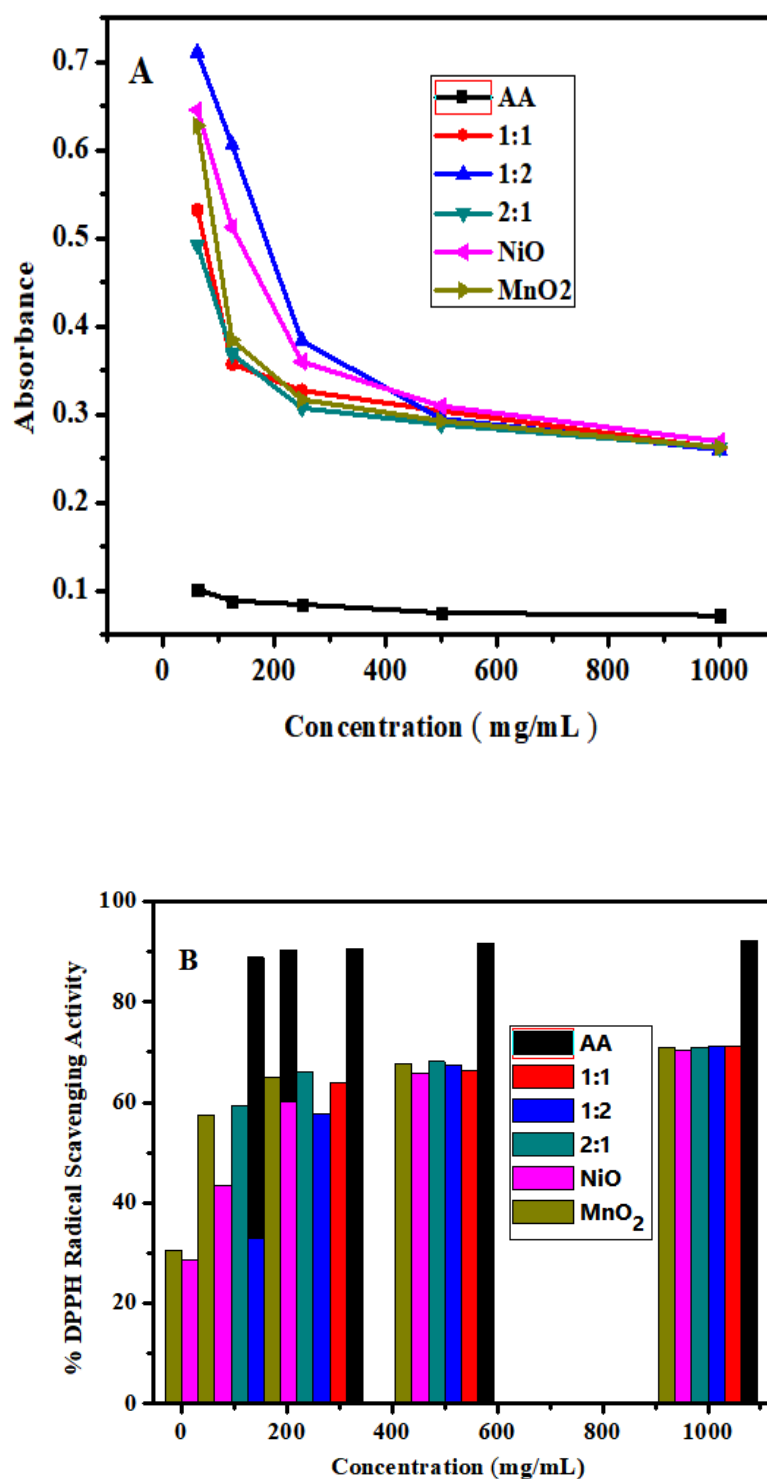


Figure 17. A) The DPPH assay of concentration versus absorbance, (B) Antioxidant potential biosynthesized NPs and NCs

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this work, nickel nitrate and potassium permanganate salts were used as precursors in the presence of onion peel extract. The biosynthesis of NiO, MnO₂ NPs and MnO₂/NiO NCs was successfully conducted. Consequently, the NiO, MnO₂ NPs, and MnO₂/NiO NC in various ratios of 1:1, 1:2, and 2:1 were characterized using FT-IR spectroscopy, TGA, XRD, UV-DRS, PL, and SEM. The best-performing MnO₂/NiO NC and a more crystalline nature were produced when the biosynthesis was carried out in a 1:2 ratios. According to crystalline analysis of XRD, the average crystalline size for 1:1, 1:2, and 2:1 was 6.27, 3.95, and 6.69nm, respectively. SEM micrographs verified the biosynthesized MnO₂/NiO NC's non-uniform with irregular-shaped aggregated structure. FT-IR analysis verified the existence of different functional group which used to stabilizing and capping agents. UV DRS characterization of biosynthesized NPs and NCs indicate that 1:2 ratio NC (1.45eV) has the highest band gap.

The research investigated the antibacterial effectiveness of MnO₂/NiO nanocomposites (NCs) and separately biosynthesized MnO₂ and NiO nanoparticles (NPs) *in vitro*. Among various ratios tested, the 1:2 ratios of MnO₂/NiO NCs demonstrated superior antimicrobial properties. This is attributed to its uniform spherical shape, large surface area, and small average crystalline size, resulting in electronic effects that enhance the interaction between the nanoparticles and bacterial cell membranes. Antifungal studies of biosynthesized MnO₂/NiO NCs conducted against *Candida albicans* revealed more efficiency for MnO₂/NiO NCs. In antimicrobial activity test 1:2 MnO₂/NiO NC showed better results. Among the different ratios of MnO₂/NiO NCs, (the 1:2 ratio) has also better performing the antioxidant activity.

5.2. Recommendations

In present study the green synthesis method was employed to produce the MnO_2/NiO nanocomposites (NCs), which is safe for the environment. Green synthesis of MnO_2/NiO NC is a rapidly expanding field of research with great potential. Given that the MnO_2/NiO NC exhibited strong antimicrobial and antioxidant activity, it is essential for assessing further activities, including anticancer, electrochemical, photocatalytic, and biomedical applications. The antimicrobial activity of green synthesized MnO_2/NiO NCs may be enhanced when exposed to UV light. This study also recommended, green synthesized MnO_2/NiO NCs should be optimized for various parameters and further characterized by other methods like BET and TEM etc. For practical application, researchers may include medical professionals from health centers, such as doctors and chemists.

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7. APPENDICES

Appendix 1 Significance of NPs and NCs in antibacterial activity

Multiple Comparisons

Tukey HSD

(I) Nano	(J) Nano	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:2	1:1	3.82778*	.81496	.000	1.4838	6.1718
	2:1	5.10556*	.81496	.000	2.7616	7.4496
	MnO ₂	6.51111*	.81496	.000	4.1671	8.8551
	NiO	4.75278*	.81496	.000	2.4088	7.0968
	Pc	-6.39722*	.81496	.000	-8.7412	-4.0532
1:1	1:2	-3.82778*	.81496	.000	-6.1718	-1.4838
	2:1	1.27778	.81496	.621	-1.0662	3.6218
	MnO ₂	2.68333*	.81496	.015	.3393	5.0273
	NiO	.92500	.81496	.866	-1.4190	3.2690
	Pc	-10.22500*	.81496	.000	-12.5690	-7.8810
2:1	1:1	-5.10556*	.81496	.000	-7.4496	-2.7616
	1:2	-1.27778	.81496	.621	-3.6218	1.0662
	MnO ₂	1.40556	.81496	.517	-.9384	3.7496
	NiO	-.35278	.81496	.998	-2.6968	1.9912
	Pc	-11.50278*	.81496	.000	-13.8468	-9.1588
MnO ₂	1:1	-6.51111*	.81496	.000	-8.8551	-4.1671
	1:2	-2.68333*	.81496	.015	-5.0273	-.3393
	2:1	-1.40556	.81496	.517	-3.7496	.9384
	NiO	-1.75833	.81496	.262	-4.1023	.5857
	Pc	-12.90833*	.81496	.000	-15.2523	-10.5643
NiO	1:1	-4.75278*	.81496	.000	-7.0968	-2.4088
	1:2	-.92500	.81496	.866	-3.2690	1.4190
	2:1	.35278	.81496	.998	-1.9912	2.6968

	MnO ₂	1.75833	.81496	.262	-.5857	4.1023
	1:2	-.92500	.81496	.866	-3.2690	1.4190
	Pc	-11.15000*	.81496	.000	-13.4940	-8.8060
Pc	1:1	6.39722*	.81496	.000	4.0532	8.7412
	1:2	10.22500*	.81496	.000	7.8810	12.5690
	2:1	11.50278*	.81496	.000	9.1588	13.8468
	MnO ₂	12.90833*	.81496	.000	10.5643	15.2523
	NiO	11.15000*	.81496	.000	8.8060	13.4940

The mean difference is significant at the 0.05 level.

Appendix 2 Significance of bacteria's

Multiple Comparisons

Tukey HSD

					95% Confidence Interval	
(I) Pathogens	(J) Pathogens	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
<i>E. coli</i>	<i>P. aeruginosa</i>	3.29074*	1.02875	.009	.6268	5.9547
	<i>S. aureus</i>	.63148	1.02875	.928	-2.0325	3.2955
	<i>S. pyogenes</i>	3.83704*	1.02875	.001	1.1731	6.5010
<i>P. aeruginosa</i>	<i>E. coli</i>	-3.29074*	1.02875	.009	-5.9547	-.6268
	<i>S. aureus</i>	-2.65926	1.02875	.051	-5.3232	.0047
	<i>S. pyogenes</i>	.54630	1.02875	.951	-2.1177	3.2103
<i>S. aureus</i>	<i>E. coli</i>	-.63148	1.02875	.928	-3.2955	2.0325
	<i>P. aeruginosa</i>	2.65926	1.02875	.051	-.0047	5.3232
	<i>S. pyogenes</i>	3.20556*	1.02875	.011	.5416	5.8695
<i>S. pyogenes</i>	<i>E. coli</i>	-3.83704*	1.02875	.001	-6.5010	-1.1731
	<i>P. aeruginosa</i>	-.54630	1.02875	.951	-3.2103	2.1177
	<i>S. aureus</i>	-3.20556*	1.02875	.011	-5.8695	-.5416

*. The mean difference is significant at the 0.05 level.

Appendix 3 Significance of concentrations in antibacterial activity

Multiple Comparisons

Tukey HSD

		Mean Differenc e			95% Confidence Interval	
(I) Concentratio n	(J) Concentratio n		Std. Error	Sig .	Low er Boun d	Upper Boun d
25mg/ml	50mg/ml	- 1.03889	.9224 2	.49 9	- 3.216 0	1.138 2
	75mg/ml	- 1.83611	.9224 2	.11 7	- 4.013 2	.3410
50mg/ml	25mg/ml	1.03889	.9224 2	.49 9	- 1.138 2	3.216 0
	75mg/ml	-.79722	.9224 2	.66 3	- 2.974 3	1.379 9
75mg/ml	25mg/ml	1.83611	.9224 2	.11 7	- .3410	4.013 2
	50mg/ml	.79722	.9224 2	.66 3	- 1.379 9	2.974 3