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DEPARTMENT OF CHEMISTRY

Musa acuminate PEEL EXTRACT MEDIATED SYNTHESIS OF NiO AND Ag-NiO NANOMATERIALS AND EVALUATION OF THEIR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

BY

WONDIMAGEGN GETAHUN HABTEGEBRIEL

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CO-ADVISOR: BIRKINESH GIRMA (MSc.)

DECEMBER, 2023
JIMMA, ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY (INORGANIC CHEMISTRY)

DECEMBER, 2023

JIMMA, ETHIOPIA

DECLARATION

I declare hereby that this thesis entitled “***Musa acuminate*** **peel extract mediated synthesis of NiO and Ag-NiO NMs for their antibacterial and antioxidant applications**” is the true reflection of my student’s research and accepted in partial fulfillment of the requirement for the award of the Degree of Master of Science in Inorganic Chemistry by the School of Graduate Studies, Jimma University through College of Natural and Computational sciences. This research was done by **Wondimagegn Getahun** under my supervision and guidance.

Guta Gonfa (PhD) ------------------------ ---------------------

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APPROVAL SHEET FOR SUBMITTING FINAL THESIS

As members of the board of examining of the final m.sc. thesis open defense, we certify that we have read and evaluated the thesis prepared by **Wondimagegn Getahun** under the **title** “***Musa acuminate* peel extract mediated synthesis of NiO and Ag-NiO NMs for their antibacterial and antioxidant applications**” and recommend that the thesis be accepted as fulfilling the thesis requirement for the **Degree of Master of chemistry**.

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Final Approval and Acceptance

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Certification of the final Thesis

I here by certify that all the correction and recommendation suggested by the board of examiners are incorporated into the final thesis entitled ***Musa acuminate* peel extract mediated synthesis of NiO and Ag-NiO NMs for their antibacterial and antioxidant applications** By Wondimagegn Getahun.

__________________ _______________ ______________

Dean of SGS Signature Date

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LIST OF ABBREVIATIONS AND ACRONMYS

AA	Ascorbic Acid
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	1, 1-diphenyl-2-picrylhydrazyl
eV	Electron Volt
FT-IR	Fourier Transformation Infrared Spectroscopy
IC_{50}	Half- Maximal Inhibitory Concentration
MAPE	Musa Acuminate Peel Extract
MDR	Multi-Drug Resistances
MNMs	Metal Nanomaterials
MRI	Magnetic Resonance Imaging
NMs	Nanomaterials
SEM	Scanning Electron Microscope
SPR	Surface Plasmon Resonance
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
UV-Vis	Ultra Violate -Visible Spectroscopy
XRD	X-ray Diffraction
ZOI	Zone of Inhibition

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ABSTRACT

Microbial infections are considered as a serious issue in human health worldwide. On the other hand, Free radicals can cause damage to parts of cells such as proteins, DNA, and cell membranes. The advancement of nanotechnology based on bio-synthesis of medicinal plant supported nanoparticles has improved therapeutic capacity of NiO and Ag-NiO nanomaterials. The aim of this study was to synthesize NiO and Ag-NiO nanomaterials using *Musa acuminate* extract for their antibacterial and antioxidant activities. The nanomaterials were synthesized by reducing $Ni(NO_3)_2.6H_2O$ and $AgNO_3$ solution with *Musa acuminate* peel extract. The study was developed by optimizing parameters like concentration of precursor, amount of *Musa acuminate* peel extract, temperature, time, and pH. The synthesized nanomaterials were characterized by UV-Vis, FT-IR spectroscopic techniques, Powder XRD, and SEM analysis. The UV-Vis analysis showed a red shift as silver doped indicating a decrease in the optical bandgap values. FT-IR characterization confirms the presence of various functional groups present in the samples. From the XRD data, crystalline sizes of the formed NiO and Ag-NiO nanomaterials were obtained and to be 11.89 and 13.27 nm respectively. SEM showed that most of the particles were spherical in shape. The synthesized NiO and Ag-NiO nanomaterials were tested for antibacterial activity against Gram-positive and Gram-negative bacterial pathogen. Antioxidant activity was tested using DPPH assays at different concentrations of test samples and ascorbic acid was used as standard. NiO and Ag-NiO nanomaterials showed highested antibacterial activities against *Bacillus cereus* and *Staphylococcus aureus* than *Salmonella typhi*, and *Escherichia coli*. From antioxidant test, NiO and Ag-NiO nanomaterials prevented the oxidation of 50% of the DPPH at a concentration of 21.50 μg/mL and 19.74 μg/mL respectively. Generally, the synthesized nanomaterials showed good antibacterial and antioxidant activities.

Keywords: Antibacterial activity, Antioxidant activity, *Musa acuminate* peel, Nickel oxide nanomaterial, Silver-dope nickel oxide nanomaterial.

1. INTRODUCTION

1.1. Background of the studies

The emergence of drug-resistant bacteria remains a critical public-health challenge because it is associated with high mortality, morbidity, and treatment cost [1]. Infectious diseases caused by bacteria, fungi, viruses, and parasites are still a major threat to public health. Their impact is particularly great in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. Fungal infections can affect anyone, and they can appear on several parts of the body, such as a baby with thrush, yeast infection and fungal infections of the nails are just a few examples (Figure 1) and it can be contagious [2]. Similarly, most bacteria cause different health problems on living things. Therefore, in order to overcome this effect of bacteria, antibacterial agents must be required. In this case, nanomaterials were demonstrated to be a promising application to overcome the problem of microbial [3].

Figure 1. Example of bacterial and fungal diseases [4].

Free radicals are molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaves as oxidants or reducants [5]. Excessive amounts of these free radicals results high oxidative stress, which lead to cell damage and contribute to different diseases. In healthy organisms, protection against the harmful effects of reactive oxygen species is achieved by maintaining a delicate balance between oxidants and antioxidants. Antioxidants are molecules that can safely interact with free radicals, terminate the chain reaction, and convert them to a harmless molecule by donating an electron. Then, nanomaterials and nanocomposites employed as antibacterial and antioxidant agents. Also, they have the potential to overcome the effect of bacteria and oxidative stress [6].

Nowadays, nanoscale antibacterial and antioxidant agents have been developed and they have proven their effectiveness for treating infectious diseases [2]. Nanomaterials are of great interest due to their extremely small size and large surface to volume ratio. Nanomaterials are materials in the range of 1–100 nm in size and have unusual physical, chemical and photoelectric properties compared to bulk materials. Their properties are determined by their higher surface-area-to-volume ratios, higher surface energy and geometrical constraints [7]. The field of nanotechnology is expanding very rapidly, creating an incredible impact on human life including pharmaceuticals [2], health [5], electronics [8], and environmental sciences [9]. Metal oxides such as CuO, TiO_2, ZnO and NiO NMs have shown effective applications on antimicrobial [10]. Among metal oxide NMs, NiO NMs and their derivatives are widely used in many applications due to fair stability, cost-effectiveness and readily available [11]. However, Ag-NiO NMs are capable to scavenge the Reactive oxygen species with high capability. NiO NMs synthesized by facile green combustion method using *limonia acidissima* natural fruit juice shows a significant hydroxyl radical scavenging activity [12]. NiO/Ag-NiO NMs are known for its antibacterial and antioxidant activities against free radicals and various bacterial pathogens [13].

There are various physical and chemical techniques used for the synthesis of metal oxide nanomaterials. However, these methods have many disadvantages, as they require more energy, time, temperature and toxic on the surface, thus altering the medical applications. Current research results reveal that green synthesis is more advantageous than chemical and physical synthesis [14]. It eliminates the use of harsh, toxic, and expensive chemicals, and instead it utilizes biological entities like microorganisms, plant extracts; like *Musa acuminate* peel extract and others; due to stability and biocompatibility [15] . Bioactive compounds such as phenols, alkaloids, flavonoids, quinine, tannins present in biological entities act as good reducing agents in the synthesis of NMs [14].

Musa acuminate is an important crop worldwide; plantation generates tonnes of residues after each harvest season and during processing to obtain *Musa acuminate* pulps. The depositions may include leaves, pseudo stem, stalk and inflorescence, but 35%–50% of the total mass fruit represents the *Musa acuminate* peel [15]. *Musa acuminate* peels have been found to contain bioactive compounds such as flavonoids, tannins, phlobatannins, alkaloids [16], glycosides, anthocyanins [17], and terpenoids which affect various biological and pharmacological (antibacterial, antihypertensive, antidiabetic, and antiinflammatory) function [18]. Phenolic compounds found in the *Musa acuminate* peel (*Musa acuminate* Colla) range from 0.9 to 3.0 g/100 g dry weight [19]. To get enhanced antibacterial activity, a reduction in recombination rate must be required. Doping in NiO has been significantly investigated since their properties can be simply controlled by suitable dopants. A small number of dopant atoms can change the ability of NMs [20]. Ag was chosen to dope with NiO NMs because Ag has gained particular attention because of its excellent oxidation stability.

Based on the previous literature reports, NiO NMs were synthesized from various plants extract such as *Clitoria ternatea* flower extract [21], *Pergularia tomentosa* [22], *Muntingia calabura* [23], etc. Only sparse information is available on the biosynthesis of metal oxide NMs using *Musa acuminate* peel such as CuO NMs [24], Au NMs [25], ZnO, NMs [26], and TiO_2 NMs [27]. There are no previous studies on *Musa acuminate* peel extract synthesis of Ag-doped NiO NMs for antioxidant activity. In this work, the synthesis of NiO and Ag-doped NiO NMs was reported using peel extract of *Musa acuminate* as a reducing and stabilizing agent. The effects of various reaction parameters affecting the synthesis of NMs were also studied. The physicochemical characteristics were investigated through XRD, SEM, UV-Vis, and FTIR spectroscopy. Moreover, the antibacterial and antioxidant activities of synthesized samples were investigated.

1.2. Statement of the problems

Infectious diseases caused by bacteria, fungi and parasites have become a major issue in health care. During the last decade, a rapidly increment in the development of new antibiotics materials has been observed as a consequence of the spread of antibiotic resistant. Some antibiotic agents are extremely irritant and toxic. On the other hand, Free radicals can cause damage to parts of cells such as proteins, DNA and cell membranes [2,5].

Many researchers reported that new multi-drug resistance strains of bacteria and free radicals have yet developed and become a serious problem in public health. To fill this gap, there is much interest in finding way to formulate new type of safe, effective economically feasible, and broadly applicable new drugs. A possible alternative is the synthesis of new metal or metal oxide nanomaterials with antimicrobial, and antioxidant properties. Metal oxide nanomaterials synthesised impose limited environmental hazards and are relatively biocompatible and environmentally eco-friendly.

The conventional methods of nanomaterial synthesis, via physical and chemical methods have been remained expensive, use of toxic, hazardous and non-ecofriendly chemicals, use of high pressure, temperature and energy, required complication operative conditions [9,15]. Therefore the great need for efficient to overcome health problems and ecofriendly method attracted the attention of researchers to develop the biological methods using plant extracts. Metallic/oxide nanomaterials and nanocomposites are thoroughly being explored and extensively investigated as potential antibiotics. A number of plant extract mediated synthesis of NiO and Ag-NiO nanomaterials and their wide application as antibacterial agent have been reported in the various literature. However, synthesis of NiO and Ag-NiO nanomaterials using *Musa acuminate* peel extract has not been reported so far. Thus the present study may intended to evaluate the antibacterial and antioxidant application of NiO and Ag-NiO NMs supported by *Musa acuminate* peel extract.

Generally, this research was designed to answer the following questions:

1. Does *Musa acuminate* peel rich in phytochemicals that are responsible for NiO and Ag-NiO NMs synthesis formation?

2. Do the synthesized NiO and Ag-NiO NMs have efficient potential to antibacterial and antioxidant activities?

1.3. Objectives

1.3.1. General Objective

To evaluate the effect of doping Ag into NiO NPs on its antibacterial and antioxidant activity.

1.3.2. Specific Objectives

- To prepare *Musa acuminate* peel extract.
- To conduct a phytochemical screening test.
- To synthesize NiO and Ag-doped NiO NMs using *Musa acuminate* peel extract as a stabilizing and capping agent.
- To characterize the synthesized NiO and Ag-doped NiO NMs using UV-Vis, FT-IR, SEM, and XRD spectroscopic techniques.
- To evaluate the antibacterial and antioxidant activities of the synthesized NiO and Ag-doped NiO NMs using disc diffusion method.

1.4. Significance of the Study

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens. This study provides important information on synthesis of NiO and Ag-NiO NMs using biological alternative methods than conventional synthesis methods. Therefore, most effective, cheap, and simplest technique of nanomaterials synthesis become much interesting area of research and essential methods.

In general, the findings of this study have the following significances.

- This study would serve as baseline information for other researcher whom wants to work in this area.
- Upgrading the knowledge about the NiO and Ag-NiO NMs regarding its antibacterial and antioxidant activities.
- The outcome of NiO and Ag-NiO NMs may provide potential input for developing a drug in the future.

2. REVIEW OF RELATED LITERATURE

2.1. Human Pathogenic Disease and Oxidative stress

Despite tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, viruses, and parasites are still a major threat to public health. Their impact is particularly great in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. Pathogens affect all living organisms and cause illness to humans in a variety of different ways. Bacteria are the most numerous human pathogens and have an impact on human health. Some serious bacterial diseases include cholera, diphtheria, bacterial meningitis, tetanus, Lyme disease, gonorrhoea, and syphilis [2]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents mainly among plant extracts with the goal of discovering new chemical structures, which overcome the above disadvantages. Current research on natural molecules and products primarily focuses on plants, since they can be sourced easily and be selected based on their ethno-medicinal uses [28].

More than 600 fungal species are associated with humans, either as commensals and members of our microbiome or as pathogen. That causes some of the most lethal infectious diseases. Fungal infections can affect anyone, and they can appear on several parts of the body, such as: a baby with thrush, yeast infection and fungal infections of the nails are just a few examples. Fungal infections can be contagious. They can spread from one person to another [29].

Oxidative stress is described as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. It is caused by free radicals in human cells. Free radicals are unstable molecules with unpaired electrons and are generally produced as a result of the influence of external and internal factors. Primary oxygen free radicals are superoxide and hydroxyl radicals. They are derived from molecular oxygen under chemical reduction conditions. Excessive amounts of these free radicals can lead to cell damage and contribute to many diseases. Antioxidants play a vital role in these defense mechanisms. In healthy organisms, protection against the harmful effects of reactive oxygen species is achieved by maintaining a delicate balance between oxidants and antioxidants [30]. Therefore, Metal/metal oxide nanomaterials, including silver, nickel, and gold nanomaterials, exhibit potential for the treatment and prevention of illnesses resulting from the overproduction of ROS. The creation of nanomaterials, also known as antioxidants, has been greatly improved through the integration of nanotechnology and nanoscience [31].

2.2. Nanomaterials, Nanotechnology, and Nanoscience

The term “nano” is derived from a Greek language meaning dwarf or extremely small, ranging in dimension from 1−100 nm. NMs have a very high surface-to-volume ratio because of their very small size [32]. Among the various types of NMs, nanostructured transition metal oxides deserve special consideration for their outstanding properties and technological applications [15]. These materials are synthesized and characterized by various methods [19]. Nanomaterials can be classified based on their origin, Size, and Chemical composition [20].

Nanotechnology is the application of science and technology to control matter at the molecular level, which is also referred to as the ability for designing, producing, characterizing, and applying to structure, devices, and systems by controlling shape and size at the nanometer scale [33]. Nanotechnology emerges from the physical, chemical, biological, and engineering science where novel techniques are being developed to probe and manipulate single atoms and molecules. Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nanosize objects [34].

2.2.1. Metal oxide Nanomaterials

Nickel and silver have outstanding physical and chemical properties, which makes their essential in many products such as electronic materials, electrodes, and medicinal applications [30].

Metal oxide nanomaterials have received specific research attention from researchers owing to their different properties such as catalytic, magnetic, optical, biological, and medicinal properties. Nanostructured metal oxides have been extensively discovered for various fundamental scientific and technological interests and to access new classes of functional materials with unique properties and noteworthy applications. In recent years, there has been an increasing interest in the synthesis of nanosized crystalline metal oxides because of their large surface areas, unusual adsorptive properties, surface defects, and fast diffusivities, conductivity, ionic structure, freezing, melting, and colour, within the nano range [21].

2.2.2. Nickel Oxide Nanomaterials and its Ag doping

In recent research, NiO NMs have drawn a greater interest, because of their unique properties. It belongs to a wide band gap (3.3 – 3.7 eV) p-type semiconductor. NiO with a cubic structure is well known because of its chemical stability and electrical properties [35]. NiO NMs possess a wide range of applications in lithium ion batteries, electrochromic test devices, super-capacitors, smart windows, water remediation through photocatalysis, electrochemical sensing, and catalysis of chemical processes [36]. It is also used as ant ferromagnetic layers, in lightweight structural components in the aerospace, inactive optical filters, cathode materials for alkaline batteries, and materials for gas or temperature sensors, such as Carbon monoxide sensors, Hydrogen sensors, and formaldehyde sensors [25].

NiO NMs include high chemical stability, low dielectric constant, high catalytic activity, and intelligent drugs, as well as being applicable as energy storage in supercapacitors. This product is also capable of exhibiting antibacterial, cytotoxic, and superparamagnetic qualities that can be exerted throughout the preparation of Magnetic resonance imaging images [11]. NiO NMs also have applications in the adsorption of toxic environmental pollutants and dyes [37].

Many transition metals have been used for doping, Ag-doping of pure NiO NMs to change their physical, chemical, and mechanical properties. When a narrow band gap semiconductor metal oxide nanomaterials are mixed with a broadband gap semiconductor material, the doping nanomaterial exhibits enhanced physicochemical properties compared with every single material [38]. Dope nanomaterials have enhanced chemical, physical, and mechanical properties, which allow their application for biological uses, and nanomedicine. NiO doped with silver (Ag) has antibacterial and antioxidant properties which are acceptable for biomedicines [11].

2.3. Strategies for Nanomaterial Synthesis

Principally there are two approaches used to synthesize NMs including the top-down approach and the bottom-up approach. The top down approach is making use of the starting bulk materials of the same materials that are going to be synthesized and applying energy to break down the large materials into Nano sized structures or particles (Figure 2) [38]. However, there is a disadvantage associated with top down Approach (physical) method whereby this creates particles with a wide size distribution and does not provide full control on particle size. Such imperfections would have a significant impact on the physical and chemical properties of nanostructures and nanomaterials. The bottom-up (chemical and biological) synthesis mostly relies on chemical and biological methods of production. Also, this approach is more accepted in synthesis of nanomaterials due to many merits such as, more homogenous chemical composition and better ordering [39].

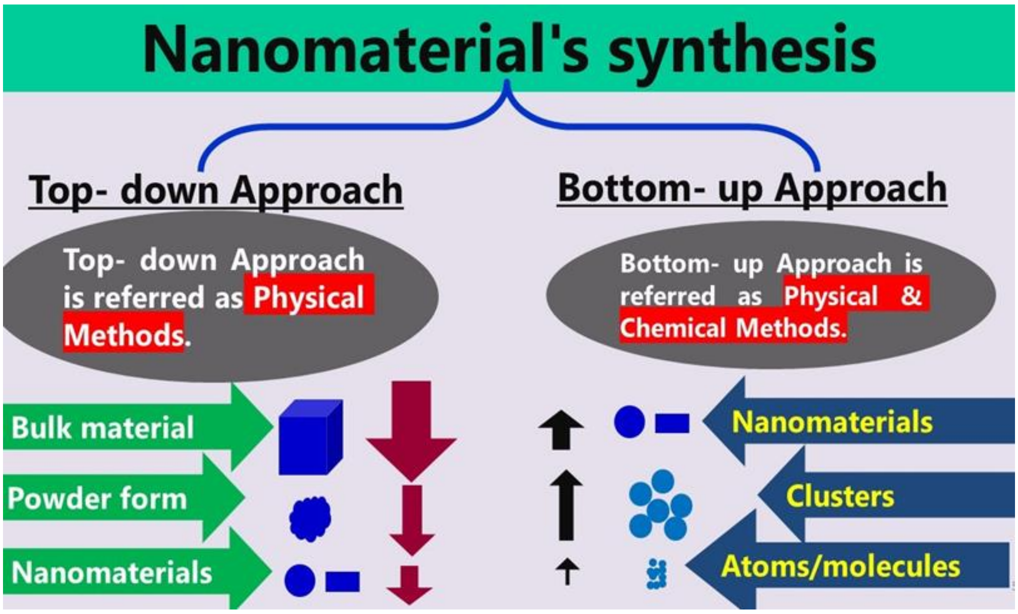

Figure 2. Representation of top-down‘ and 'bottom-up‘ approaches for synthesis of nanoscale materials [40].

Physical synthesis follows the top-down approach to synthesize nanomaterials from bulk materials by methods such as pulsed laser ablation. Chemical synthesis follows the bottom-up approach to synthesize nanomaterials from atomic-level materials by methods such as chemical precipitation, sol-gel process, hydrothermal synthesis, thermal decomposition, and microwave-assisted synthesis [41]. On the other hand, many plant extracts, dead biomass, and bacteria are used in green synthesis. Biological method to synthesize MNMs including NiO NMs (Figure 3) have been emerged as cost effective option in the field of “green chemistry” [37].

Biomolecules such as alkaloids, terpenoids, flavonoids, and phenols have been reported as good reducing and stabilizing agents for NiO NMs synthesis. This approach provides a safer alternative to producing NMs with desired physical and chemical properties [21]. Green synthesis of NiO NMs is a growing interest in the synthesis of NiO NMs for different biological applications from different medicinal plants [42]. The nanomaterials that are formed can be toxic in terms of shape, size and surface chemistry. To avoid these problems nowadays the nanomaterials are formed with the help of plant extract [38] .

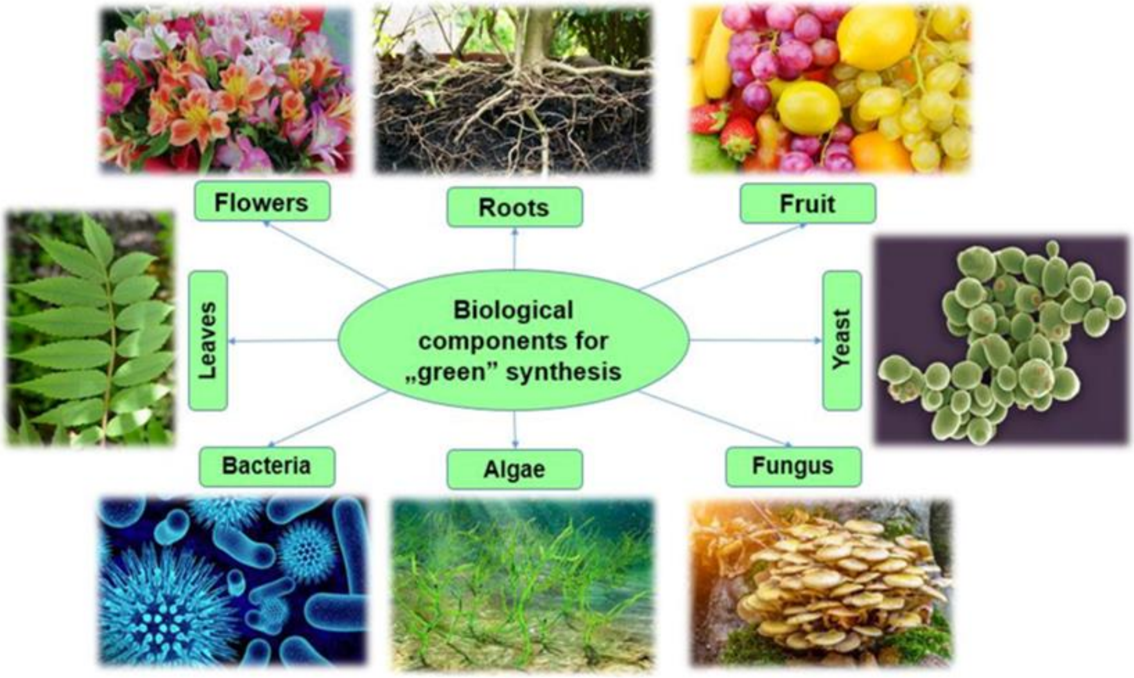

Figure 3. Different types of biological route used for the preparation of metal oxide nanomaterials [43].

2.3.1. Mechanism of plant extract mediated Synthesis

The secondary metabolites of plants and microbial enzymes are responsible for providing electrons to metal ions, thus reducing them from metal ions into metal atoms /elemental metal. The metabolites present in plant extracts have potential to reduce metal ions in a much shorter time as compared to fungi and bacteria, which demand longer incubation time. Several studies have revealed that the phytochemicals in the biological entities first form complexes with the nickel salts and then reduce the ions to form NiO NMs [44].

In order to avoid further growth and maintain the particle in the Nano-range, a substance called capping agent is added. Biomolecules may act as the reducing agent, or the same molecules may function as both the reducing agent and the capping agent [45]. The probable mechanism involved in the synthesis of NiO NMs is represented as follow (Figure 4). The mechanism indicate the metallic ions have been reduced to the zero-valent sate by phytochemicals from plant extract, the zero-valent reacted with the molecular oxygen dissolved in the reaction mixture, leading to the formation of metal oxide. As nucleation and particle growth kinetics are controllable, the formation of particles that fit into the nanoscale can be achieved.

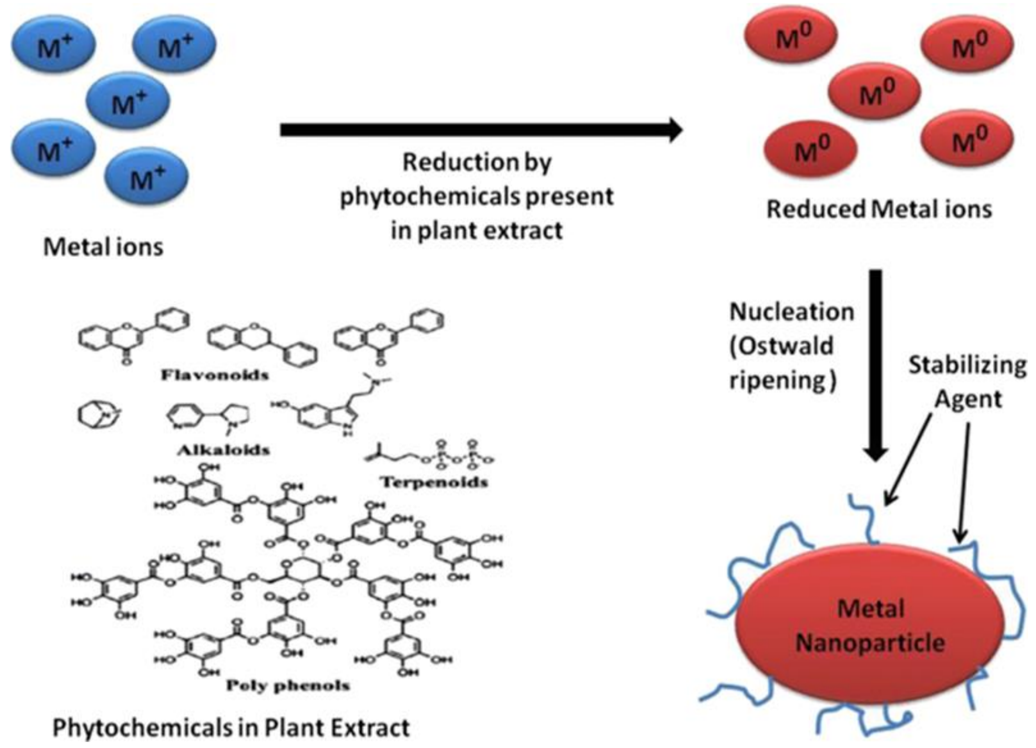

Figure 4. mechanism of plant extract mediated synthesis of nanomaterials [46].

2.3.2. Botanical information of *Musa acuminate* (Banana)

Medicinal plants are the cheap and safe alternative sources for the prevention against antimicrobial infections. Among those plants, *Musa acuminate* in English, it is called "Banana", (Figure 5) which belongs to the family, Flowering plants. About 91 known species are found [47]. The traditional uses of *Musa acuminate* are controlling many clinical problems. It is used to cool compress for burns or wounds [48]. Banana is known to have antioxidant that can help reduce inflammation in the skin, natural moisturizer that can help keep your skin hydrated and supple, and fights acne; which can help regulate the hormones that cause acne [49]. It is thus vital to find applications for the peels as they can add to real environmental complications. Banana peels contains large amounts of phenols which assists in creation of metallic nanoparticles [48]. This study was intended to evaluate the banana peel extracts supported NiO and Ag-NiO NMs as antibacterial antioxidant activities.

Figure 5. Banana plants (A), banana fruits (B) and banana peels (C) [48].

2.3.3. Factors Affecting the Plant extract mediated Synthesis of Nanomaterials

There are several factors that affect the synthesis, characterization, and application of nanomaterials. Important factors that affect the synthesis of nanomaterials, the nature and concentration of the plant extract, pH, temperature, metal salt concentration, and contact time are known to affect the rate of nanomaterials synthesis [50].

Temperature has been considered as one of the crucial parameters that influences the synthesis of metallic oxide NMs. The incubation time of nanoparticles synthesized using plant extract has been examined to influence the morphological properties and qualities of nanomaterials. Other factors such as storage conditions and exposure to light also affect the reaction time of NiO/Ag-NiO NMs. A long incubation period has been stated to cause aggregation and shrinkage or growth of particles [51]. Precursor Salts for Synthesis of NiO/Ag-NiO NMs Various nickel salts such as chloride, nitrate, and acetate were used to prepare NiO/Ag-NiO NMs however, nitrate precursor for synthesizing NiO/Ag-NiO nanomaterials are the base agent which provides hydroxyl ion to react with nickel salt and gives the $Ni(OH)_2$ precipitation. The pH of reaction plays a pivotal role in the formation of NiO/Ag-NiO NMs. It regulates the formation of nucleation centres [38]. In most cases, the optimum pH for the biogenic synthesis of nanomaterials from plant extract is 8.5- 10 [52].

2.4. Applications of Nanomaterials

2.4.1. Antibacterial Activity

Different types of nanomaterials and nanocomposites have different mechanisms for combating antibiotic resistant bacteria. Cell wall disruption and membrane damage occurs when nanomaterials bind electrostatically to the bacterial cell wall and membranes, leading to alteration of membrane potential, membrane depolarization, and loss of integrity, which results in an interruption of electron transport, impaired respiration, interruption of energy transduction or cell lysis, and eventually cell death (Figure 6). At high concentrations, the ROS leads to cell death and at low doses it causes severe DNA damage and mutations [53].

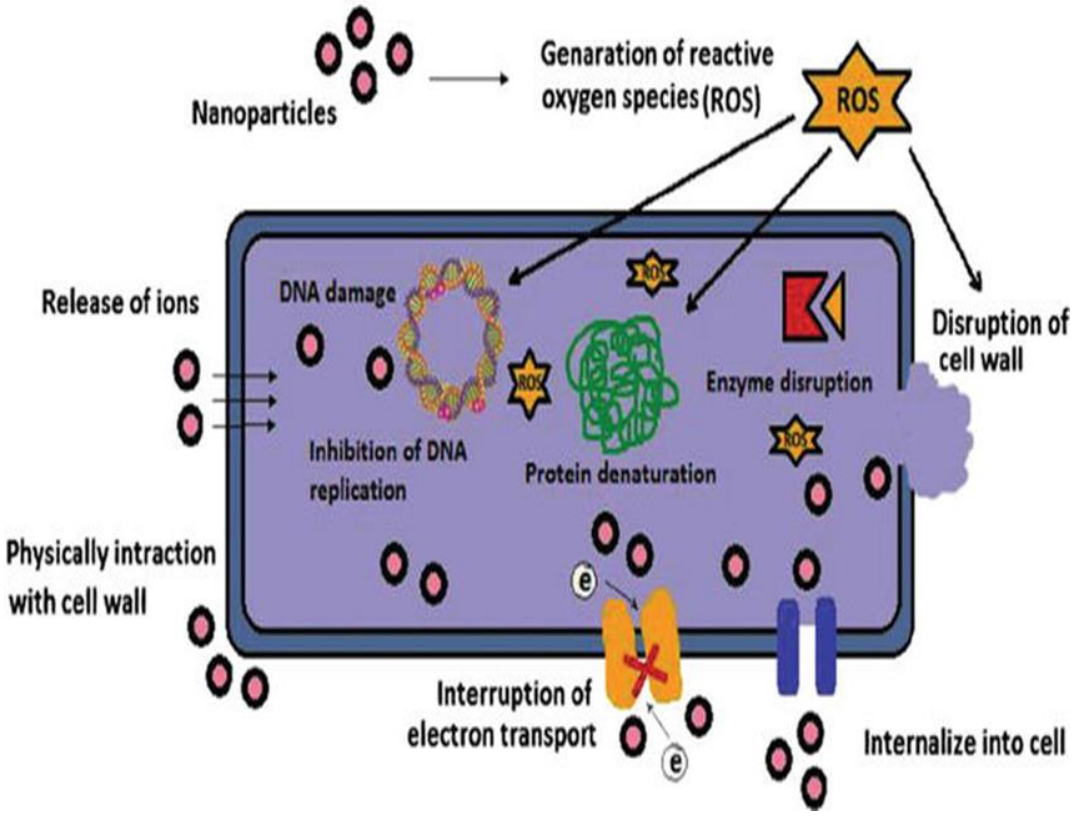

Figure 6. probable mechanism of metal oxide NMs toxicity against microbial [54].

2.4.2. Antioxidant Activity

The DPPH method is a common method for assessing the antioxidant activity of plant species and metallic nanomaterials. To select concentrations of aqueous plant extract and the synthesized NMs solutions, an equal volume of methanolic DPPH solution was added and incubated at room temperature for 30 minutes. Then the radical scavenging activity of the NMs and plant extract was monitored at 517 nm using a UV-vis spectrophotometer. The absorbance obtained at 517 nm for plant extract and NMs was used for calculating the percentage DPPH inhibition and 50 percent inhibitory concentrations (IC_{50}) separately [55].

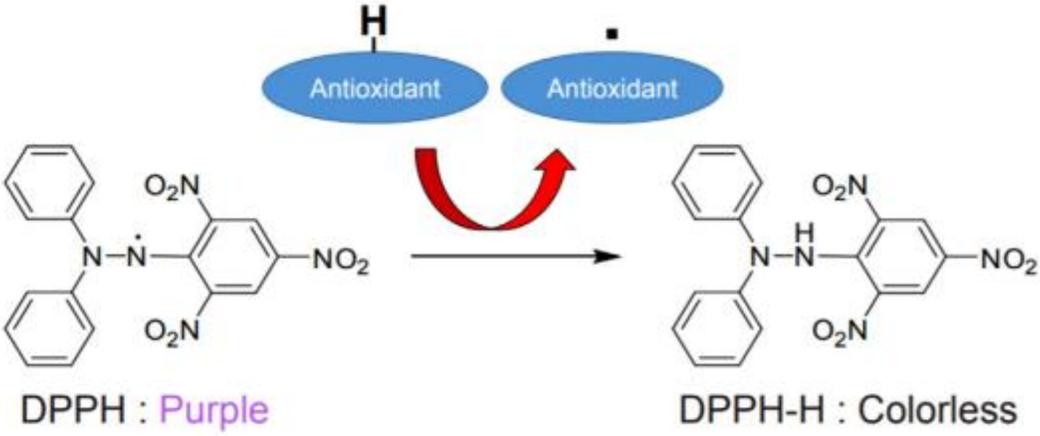

Figure 7. Reaction between DPPH• and antioxidant to form DPPH [56].

DPPH radical is a stable free radical due to its unpaired electron delocalization over the whole molecules. The donation of a proton (H+) to this radical causes a colour change. The colour change results from the odd electron of a nitrogen atom in DPPH being reduced by receiving a hydrogen atom from antioxidants [56].

2.5. Characterizations of Nanomaterials

Characterization of a nanomaterials are physical and chemical properties, is important for ensuring the reproducibility of toxicology studies, and is also vital for studying how the physical and chemical properties of nanomaterials determine their biological effects. Important characterization of nanomaterials are; Size, and shape. The size and shape of nanomaterials are crucial characteristics that determine their physicochemical properties, which include their catalytic activity and bioactivity, their optical, magnetic, and mechanical properties, and so on [57].

2.5.1. Ultraviolet-Visible (UV–Vis) Spectroscopy

UV-Visible spectroscopy is relatively facile and low-cost characterization method that is often used for the study of nanomaterials in aqueous solution by analysing the unique optical properties and optical band gap which depends on the size of the nanomaterials between 200 nm to 800 nm. It measures the intensity of light reflected from a sample and compares it to the intensity of light reflected from a reference material [58].

2.5.2. Fourier Transformed Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy is used to identify the possible functional groups of the active components present in the solution or on the surface of NMs that are responsible for reducing, capping, and stabilizing of synthesized nanomaterials. The possible functional groups are identified in the ranges of 4000-400 cm^{-1} [59].

2.5.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is extremely useful for the determination of morphology information of materials. The electrons interact with the atoms that make up the samples producing signals that contain information of the sample's [60]. SEM images have a characteristic of three-dimensional form and are useful for judging the surface morphology of the sample [53]. This technique provides information regarding the species present at different depths of the sample. SEM may be used to determine the crystallography of crystalline samples and individual crystallite orientations, as well as crystallographic parameters of the sample [60].

2.5.4. Powder X-ray Diffraction (XRD)

In a variety of X-ray spectroscopic modalities, the diffraction of X-ray can be simply described as the reflection of a collimated beam of X-rays incident on the crystalline planes of an examined specimen [61]. X-ray diffraction (XRD) technique is used to realize structural properties of materials and give information like crystal structure/phase, lattice parameters, crystallite size, orientation of single and poly crystals, defects, strains, and so on [62].

3. MATERIALS AND METHODS

3.1. Chemicals and Materials

3.1.1. Chemicals

Nickel (II) nitrate hexahydrate ($Ni(NO_3)_2•6H_2O$), ascorbic acid ($C_6H_8O_6$, ~99%), Sodium hydroxide (NaOH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Silver nitrate ($AgNO_3$), Ethanol (C_2H_5OH ~99.9%) and distilled water which was used as solvent throughout the experiment. All chemicals were analytical grade and used without further purification.

3.1.2. Apparatus

Oven, Furnace, Digital balance, Magnetic stirrer with hot plate, Mortar, and Pestle, Refrigerator, Crucible, Centrifuge, Volumetric flask, Beakers, Conical flask, Test tubes, Droppers, Spatula, Aluminium foil, and Filter paper were used.

3.1.3. Scientific Instruments

UV-Vis (Ultraviolet–Vis Spectroscopy) SPECORD 200 PLUS - 223E1128F, FT-IR (Fourier transform infrared spectrometry) Shimadzu IR-470 spectrometer (Shimadzu, Japan), SEM (Scanning electron microscopy) JCM-6000Plus, and XRD (X-ray diffraction) XRD-700 and pH meter, were used for present study. Absorption spectra were recorded with the UV-Vis spectrophotometer. The FT-IR spectrometer was used to examine the functional groups. The morphology of the sample was studied by Scanning Electron Microscopy. The Crystalline nature and size were identified with the aid of the X-ray diffractometer]63].

3.2. Methods

3.2.1. *Musa acuminate* peel Collection and Extract Preparation

Musa acuminate (Muuzii in Afan Oromo, Muz in Amharic) peel was collected from Eatfruit in the month of March, 2023 from Jimma town, South West Ethiopia. It is located 346 km from Addis Ababa. The collected *Musa acuminate* peel was washed thoroughly with distilled water to remove the dust and organic impurities present in it. Then, the peels were air-dried on paper toweling. The dried peels were crushed and stored for further use. Extracts were prepared using reported standard procedure [63]. In typical extraction procedure, 5 g of peel was taken into a 250 mL beaker containing 100 mL distilled water and were heated on a hot plate at 70 ℃ for 30 min. After the formation of the peel extract, the mixture was allowed to cool down before being filtered. Then it was filtered using Whatman No. 1 filter paper and kept at 4 °C in the refrigerator for further use [63].

3.2.2. Phytochemical Screening of *Musa acuminate* peel extract

Phytochemical screening of freshly prepared *Musa acuminate* peel extract was carried out using simple chemical tests to identify the presence of phenols, flavonoids, tannins, and saponins in the sample [64,65].

1. Test for phenolic compounds

To 2 mL of *Musa acuminate* peel extract 6 drops of 5% ferric chloride ($FeCl_3$) solution was added and the colour formed was observed.

2. Test for Flavonoids (Alkaline Reagent Test)

2 mL of *Musa acuminate* peel extracts were treated with 10 drops of a 0.1 M sodium hydroxide solution and the colour change was observed.

3. Test for Tannins

2 mL of *Musa acuminate* peel extracts was treated with 8 drops of a 1% lead acetate solution and the colour change was observed.

4. Test for Saponins (Foam Test)

2 mL of *Musa acuminate* peel extracts were diluted with 1 mL of distilled water and shaken in a graduated cylinder for 15 min and the change was observed.

3.2.3. Optimization of Synthesis Parameters

In this experiment, the effect of the concentration of precursor solution, pH, temperature, amount of peel extract, and time were investigated by varying one parameter and keep the other constant at a time to obtain the optimum values.

3.2.3.1. Optimization of Precursor

The concentration of nickel nitrate was optimized with the increase in the concentration of nickel nitrate solution (0.05, 0.1, 0.15, and 0.2 M) in a volume of *Musa acuminate* peel extract (10 mL) and pH 10. The reaction mixture was incubated for 2:30 h and the absorbance of the resulting solution was measured by UV-Vis spectrometer to check for sharpest peak as optimum value [66].

3.2.3.2. Optimization of pH

The pH of the reaction was optimized by increasing the pH value was 8, 9, 10, and 11 in a 10 mL of peel extract and the concentration of precursor was constant and was adjusted using 0.1 M NaOH. After 2:30 h measured the absorbance of the mixture using the UV-Vis spectrometer to check for sharpest peak as optimum value [66,67].

3.2.3.3. Optimization of Temperature

The temperature of the reaction was optimized by increasing the temperature (60, 65, 70, and 75 ℃) and measured the absorbance of the mixture after 2:30 h. The effect of these parameters on the synthesis of NiO NMs was monitored by using the UV-Vis spectrometer to check for sharpest peak as optimum value to check for sharpest peak as optimum value [66,68].

3.2.3.4. Optimization of Plant Extract

Musa acuminate peel extract was optimized with the increase in the volume of *Musa acuminate* peel extract (2, 5, 10, 20 mL) in 50 mL of 0.15 M nickel nitrate solutions (ratio 1:25, 1:10, 1:5, 2:5). After 2:30 h of incubation, the absorbance of the resulting solution was measured by UV-Vis spectrometer to check for sharpest peak as optimum value [66].

3.2.3.5. Optimization of Time

The reaction time was optimized changing the time (90, 120, 150, and 180 min) in a constant concentration of precursor, pH, Temperature, and plant extract. After each time of incubation, the absorbance of the resulting solution was measured by UV-Vis spectrometer to check for sharpest peak as optimum value [66,68].

3.2.4. Synthesis of nickel oxide nanomaterials

To synthesize NiO NMs using *Musa acuminate* peel extract was conducted according to the reported literature with slight modification [69]. Accordingly, (0.15 M, 2.18 g) nickel (II) nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$) was dissolved in 50 mL of distilled water , and 5 mL of *Musa acuminate* peel extract was added to the solution. The mixture was progressively stirred for 30 min at room temperature. To adjust pH 9, 0.1 M NaOH was added drop by drop. The resulted product was stirred at 70 ℃ for 2 h. Subsequently, the obtained solid was centrifuged and washed several times with distilled water and ethanol to remove impurities. The obtained nanomaterials were dried at 75 ℃ for 1 h in the oven and kept for further characterization by UV-Vis, FTIR, XRD, and SEM and for evaluations of their antibacterial and antioxidant activities [66,69].

3.2.5. Optimization and Synthesis of silver doped nickel oxide nanomaterials

3.2.5.1. Optimization of dopant

The amount of dopant was optimized with the increase of different volume (1% to 10%) of dopant was added to the precursor by keeping volume of *Musa acuminate* peel extract, Temperature, time, and pH constant and the absorbance of the resulting solution was measured by UV-Vis spectrometer to check for sharpest peak as optimum value [70].

3.2.5.2. Synthesis of silver doped nickel oxide nanomaterials

To synthesize Ag-NiO NMs, 46.5 mL of (0.15 M, 2.18 g) Nickel (II) nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$) was dissolved in 50 mL of distilled water), and then 3.5 mL of (0.15 M, 1.27 g) silver nitrate ($AgNO_3$); was added drop wise followed by a drop-wise addition of 5 mL of *Musa acuminate* peel extract. The mixture was progressively stirred for 30 min at room temperature. To adjust the pH 9 of the solution 0.1 M NaOH was added drop by drop. The resulted product was stirred at 70 ℃ for 2 h. Subsequently, the obtained solid was centrifuged and washed several times with distilled water and ethanol to remove the impurities. Ag-doped NiO nanomaterials was dried at 75 ℃ for 1 h in the oven and kept for further characterization by UV-Vis, FTIR, XRD, and SEM and for evaluation of its antibacterial and antioxidant activities [69,71].

3.2.6. Characterization of NiO and Ag-doped NiO NMs

3.2.6.1. UV-Vis Analysis

The electronic spectra of the NiO and Ag-NiO NMs in solution were recorded in the range of 250-800 nm on a SPECORD 200 PLUS - 223E1128F UV/Vis spectrophotometer (Analytik Jena AG). Generally, surface Plasmon resonance of synthesized nanoparticles and energy band gap of synthesized samples were analysed by UV-Vis spectroscopy.

3.2.6.2. FT-IR Analysis

Fourier Transform Infrared (FT-IR) spectra recorded in 4000-400 cm^{-1} were used to identify the presence of functional groups and to receive valuable information regarding the presence of ligand in the metal NMs as KBr discs pest on a Perkin Elmer.

3.2.6.3. XRD Analysis

The crystallinity and crystalline phase of synthesized samples were determined through X-ray diffraction (XRD) profiles (XRD-700 using 2CuKα radiation) in 2θ range of 10^o to 80^o.

3.2.6.4. SEM Analysis

The morphological features of the prepared NiO and Ag-NiO NMs nanostructures were studied using scanning electron microscopy (JCM-6000Plus). Sample simply deposited onto the top of an adhesive fastened to an aluminum stub/holder. Computer software was used to analyze the compositional characterization of materials.

3.2.7. Antibacterial Activity

For antibacterial study, pure bacterial pathogen cultures were obtained from the Biology Department, Jimma University. Antibacterial activity was done according to the standard procedures reported in the literature [72]. In this study, two types of bacteria were obtained from the microbiology laboratory, biology department, Jimma University. The antibacterial assays were done for Gram-positive bacteria; *S. aureus* and *B. cereus* and Gram-negative bacteria; *E. coli* and *S. typhi* tested by the agar disc diffusion method. An aqueous banana peel extract and NiO and Ag-NiO nanomaterials were added on to the well of 6 mm diameter filter paper disks on agar. The plates were incubated at 37 ºC for 24 h. Clear zones of inhibition around the wells were measured and was compared with the standard drug (Gentamycin). 1% DMSO was used as a negative control and Gentamycin was used as a standard drug. All the above procedures were repeated three times and mean standard deviation of a zone of inhibition was taken.

3.2.8. Antioxidant Assay

DPPH Radical Scavenging Assay

DPPH radical scavenging assay of the synthesized NMs was performed according to the method of Akintola [73]. 2 mL of different concentrations of the synthesized nanomaterials and ascorbic acid (2-64 µg /mL) were mixed with DPPH (0.1 mM) solution. This reaction mixture was kept in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517 nm against a blank solution. Ascorbic acid (2 mL) was used as standard. The % RSA was calculated by using the Equation:1

$$\% \text{ scavenging activity} = [(A_0 - A_1)/A_0] \times 100 \text{ [73]} \ldots\ldots\ldots\ldots\ldots\ldots\text{(1)}$$

Where: A_0 = Absorbance of control, A_1 = Absorbance of the sample

3.2.9. Methods of Data Analysis

Origin 2016 software was used to analyze the data collected from UV-Visible, XRD, and FT-IR spectroscopy.

4. RESULTS AND DISCUSSION

4.1. Phytochemical screening of *Musa acuminate* peel extract

The phytochemical screening was conducted for justification of different types of bioactive compounds that appear in the *Musa acuminate* peel extract by using a different type of chemical. Based on the results the naturally existing phytochemicals in the *Musa acuminate* peel extract were used as reducing and capping agent during NiO and Ag-NiO NMs synthesis. The results of phytochemical analysis of *Musa acuminate* peel extract are shown in Figure 8 and Table 1 which revealed the presence of secondary metabolites such as polyphenols, flavonoids, tannins, and Saponins.

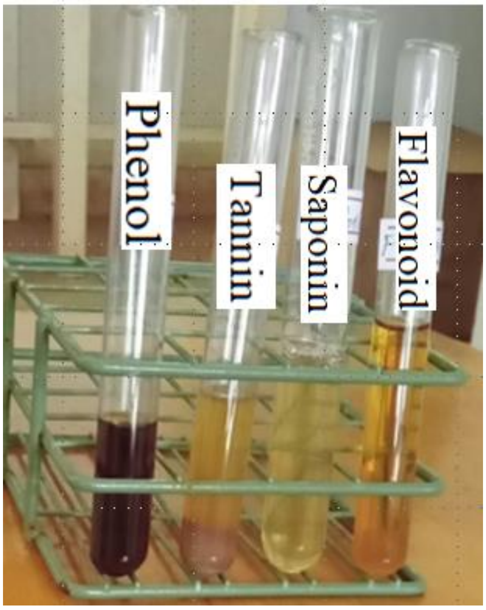

Figure 8. Phytochemical Test of *Musa acuminate* peel extract

The colour change was observed when the peel extract was tested for the presence of polyphenols, flavonoids, tannins, and Saponins. This result has a close agreement with the findings of literature report [73].

Table 1.Phytochemical analysis of *Musa acuminate* peel extract

S/No	Phytochemicals	Chemical tests	Colour observation	Inference
1	Phenols	2 mL of *Musa acuminate* peel extract and 6 drops of 5% ferric chloride	Blue black	++
2	Flavonoids	2 mL of *Musa acuminate* peel extract and 10 drops of a 0.1 M NaOH	Intense yellow	+
3	Tannins	8 drops of a 1% lead acetate	Yellowish precipitate	+
4	Saponins	diluted with 1 mL of distilled water and was shaken	Foam formed	+

Key; + = indicates the presence of phytochemicals ++ = the presence of the highest phytochemicals

During phytochemical screening of *Musa acuminate* peel extract it was confirmed that the presence of the highest phenol content which is observed by changing the colour of peel extract to blue black in presence of ferric chloride reagent. In Testing of flavonoid contents Sodium hydroxide was used as testing agent and formation of yellow colour, confirm the presence flavonoid. Based on this result presence of phenol and flavonoids in *Musa acuminate* peel was very important for bio reduction of metal oxide NMs synthesis.

4.2. Optimization of different parameters

4.2.1. Optimization of precursor concentration of $Ni(NO_3)_2.6H_2O$

The ratio of the concentration of precursor must correspond to the volume of plant extract used [74]. The formation of nickel oxide nanomaterials was studied by the variation of nickel nitrate concentration from 0.05 to 0.2 M. The SPR absorption was increased due to increasing the concentration of nickel ions from 0.05 to 0.2 M (**Appendix**, figure A_2), and 0.15 M was optimum because it gave a sharp peak which shows the formation of nanoparticles since the intensity of the surface Plasmon peak has direct proportionality with the concentrations of synthesized nanomaterials in the solution [75].

4.2.2. Optimization of pH effect of NiO NMs

The pH was one of the factors that influenced the size, shape, and composition of nanomaterials [76]. The effect of pH on the formation of NiO NMs has been evaluated by UV-Vis spectroscopic studies. pH in aqueous media can highly influence the progress of the metal ion reduction reaction [77]. The role of pH on NMs synthesis could be seen in its effect on the capping and stabilizing abilities, and consequently the growth of the nanomaterials. The presence of OH ion in an alkaline pH environment might enhance the reducing and stabilizing capabilities of the biomolecules in the plant extract [78]. The difference in the intensity of the SPR peak ranged between 250-600 nm as shown in (**Appendix**, figure A_3) the pH of obtained NiO NMs was found to be four variations in pH (8, 9, 10, and 11) [79]. Absorption in UV-Vis spectra was sharp at pH 9. Increase in pH from 9 to 11 decreases the sharpness. In acidic, nanoparticles aggregated out of the nucleation, while, at alkaline, great number of nuclei formed, instead of aggregation [80]. So pH 9 was found to be optimum to synthesize nanomaterials, and has sharp peak at 302 nm wavelength, which was matched the values reported in the literature [81].

4.2.3. Optimization of Temperature effect on synthesis of NiO NMs

Temperature is the most important factor in disturbing the dimension and form of the nanomaterials and their level of synthesis. As the temperature level increases, the reaction response rate is also strengthening the formation of nucleation centers [82]. During the plant extract mediated synthesis of nanomaterials, the reaction temperature is a factor that plays the most important function in influencing the yield of synthesized nanomaterials [83].

The role of temperature in the synthesized nickel oxide nanomaterials was carried out by varying the temperature of a reaction system 60, 65. 70, and 75 ℃ [84]. The difference in the sharpnes of the SPR peak ranged between 250-600 nm as shown in (**Appendix**, figure A_4). Absorption in UV-Vis spectra was sharp at temperature 70 ℃. However, increase temperatures from 70 ℃ to 75 ℃ or above affect the properties of NMs, probably due to the degradation of the phytochemicals involved in the synthesis process. This results the NMs would form aggregates, an enhanced surface activity of the formed nuclei and an inefficient capping process and cause the growth rate of NMs [85]. So, 70 ℃ was found to be optimum to plant extract phytochemical mediated synthesis of nanomaterials, which was in agreement with the values reported in the literature [84].

4.2.4. Optimization of Plant extract

The synthesis of NMs using plants extract is mainly influenced by the volume of plant extracts used. The volume of plant extracts used in the synthesis of nanomaterials plays a significant role in the reduction of metal ions to reduced metal in their oxidation number [86].

The formation of nickel oxide nanomaterials depends on the ratio of plant extract to $Ni(NO_3)_2$ (v/v). To identify the optimum volumes of plant extracts, different volumes (2, 5, 10, and 20 mL) were reacted at the fixed volume of precursor (50 mL). The UV-Vis spectrum was recorded for the sharpness of absorption peaks position with variation in the amount of plant extract to precursor salt as shown in (**Appendix**, figure A_5). The sharp peak was observed at 5 mL, but increasing the concentration of plant extract causes the agglomeration of nickel oxide NMs, thus larger structures are formed because the reducing agent would normally accelerate the growth of particles [85]. The 5 mL was found to be typical to plant extract phytochemical mediated synthesis of nanomaterials it showed sharp peak at 302 nm [86].

4.2.5. Optimization of reaction Time

The reaction time is the most important aspect that controls the structural morphology of nanomaterials along with temperature and pH [86]. The effect of reaction time was evaluated during the green synthesis of nanomaterials. Different reaction time 90 to 180 min by a difference of 30 min and 120 min was optimum as shown in (**Appendix**, figure A_6). If the reaction time is exceeded, the NMs start to aggregate and form larger structures [87]. With this min intensity of UV-Vis peak increased which shows all precursors were reacted with peel extract and reduction in metal ions was completed [87].

4.2.6. Optimization concentration of dopant

The absorption peak of Ag-NiO NMs was also affected by the concentration of dopant [79]. To see the effect of concentration of dopant (Ag) on the synthesis of Ag-NiO NMs; the different concentration (1% to 10%) of dopant was added to get the optimum value of the reaction. The concentration of dopant was increased from 1% to 10% the absorption peak was varied (**Appendix**, figure A_7-A_9) which resulted in a change in the optical band gap value. Finally, the obtained 7% silver doping was optimum since it gave UV-Vis absorbance value at the largest wave length (red shift). The result was in agreement with the reported literature [88].

4.3. Synthesis of NiO and Ag-NiO Nanomaterials

During the synthesis of NiO NMs, the colour of the mixtures of nickel nitrate hexahydrate and *Musa acuminate* peel extract was green at the beginning. After heating 2 h with continuous stirring at 70 ℃, a light green colour was observed which indicates the formation of NiO NMs (**Appendix**, figure A_1). Ag-NiO NMs were synthesised in a similar way to NiO NMs but, with the addition of Ag as dopant and a light black colour was observed that indicates the formation of Ag-NiO NMs. The nickel and silver ions were reduced to their corresponding NiO and Ag-NiO NMs, and the synthesised nanomaterials were capped through phytochemicals present in plant extract [87].

4.4. Characterization of NiO and Ag-NiO nanomaterials

4.4.1. UV-Vis Spectroscopy analysis of NiO and Ag-NiO NMs

UV-Vis spectroscopy is one of the most widely used simple and sensitive techniques for the identification of nanomaterials formation. Absorption spectra of NiO and Ag-NiO nanomaterials were taken using a UV-Vis Spectrophotometer. Initially, the synthesized NiO and Ag-NiO NMs were analysed with a UV-Vis Spectrophotometer between 250-800 nm wavelength ranges. These NMs showed a sharp peak at the wavelength of NiO and Ag-NiO nanomaterials are 302 and 309 nm respectively (Figure 9), corresponding to nickel oxide and silver doped nickel oxide nanomaterials, and this peaks were consistent with a recently reported study [87,88].

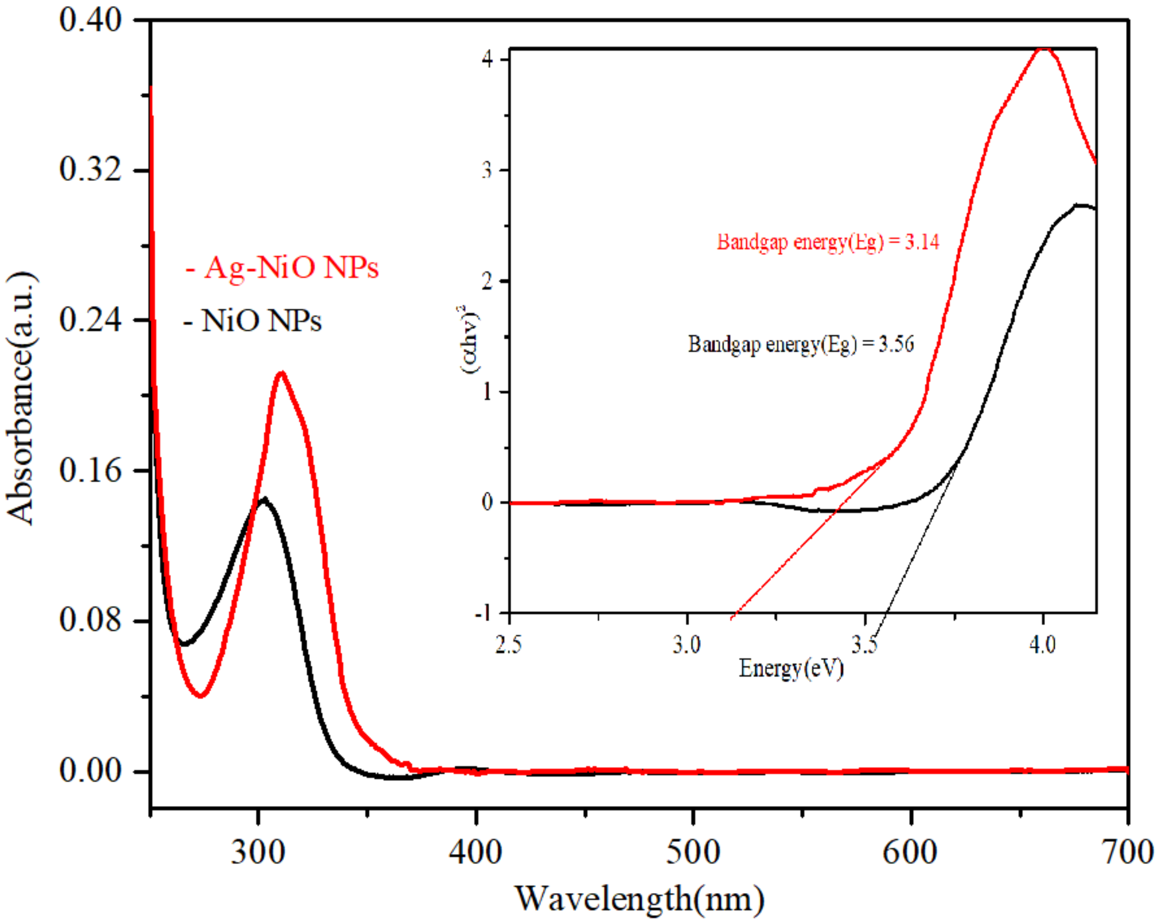

Figure 9. UV-Vis spectra of NiO, Ag-NiO NMs and inset energy band gap

The band gap for the absorption peak is obtained by extrapolating the linear portion of $(\alpha h\nu)^2$ versus Energy. Eg was found to be 3.56 eV and 3.14 eV for NiO and Ag-NiO NMs respectively. It has been observed that the band gap decreases with silver doping with the decrease in particle size. The bandgap energy of nanomaterials is decreased with Ag doping, which causes the enhancement of antibacterial [88].

4.4.2. FTIR analysis of NiO and Ag-doped NiO NMs

The dual role of the plant extract, as a reducing as well as capping agent, and the presence of some functional groups in both the *Musa acuminate* peel extract and the synthesized NiO and Ag-NiO NMs were investigated by FTIR analysis. FTIR analysis was used to identify and get an approximate idea of the possible bio-molecule that are responsible for capping and stabilization of the NiO and Ag-NiO NMs with the peel extract of *Musa acuminate*.

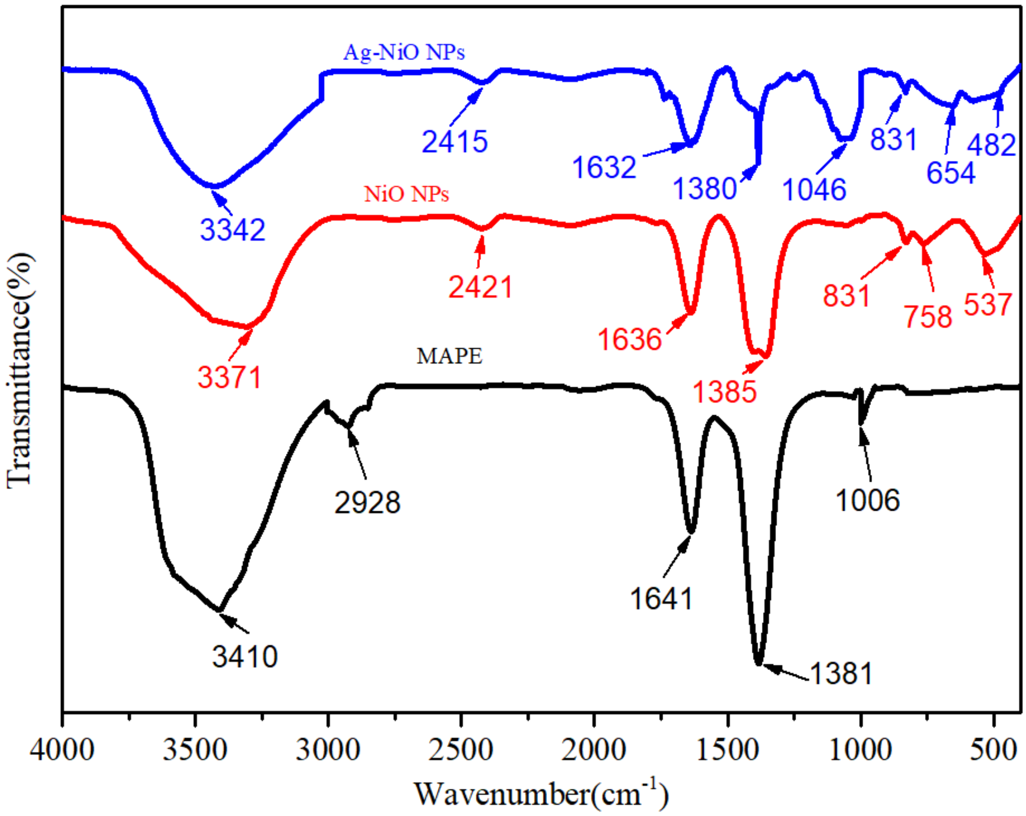

Figure 10.FTIR analysis of Musa acuminate peel extract, NiO, and Ag-NiO NMs

The FTIR spectrum of peel extract showed multiple peak values indicating the presence of phenols, alcohols, alkanes, aromatic compounds, and carboxylic acid (Figure 10). The major vibrational modes in the *Musa acuminate* peel extract are those located at 3410, 2928, 1641, 1381, and 1006 cm^{-1}. A strong and broad peak at 3410 cm^{-1} can be due to hydrogen-bonded O-H of alcohols. This agrees with the conclusion that *Musa acuminate* peel extract is composed of Polyphenols, flavonoids and other similar phytochemicals, which was confirmed by using qualitative phytochemical analysis [88]. The other peaks at 2928 cm^{-1} is assigned to C-H stretching in alkanes, and the peak at 1641, 1381, and 1006 cm^{-1} represents N-C=O stretching bond of amides, C-H bending vibration of CH_2 bond of alkanes and stretching vibrations of C-N functional groups of amines, respectively. The intensity of peaks related to the polarity of the bonds. This is in close agreement with the value reported in the literature [88,89].

As observed in (Figure 10) the vibrational modes of FTIR spectrum of NiO NMs are located at 3371, 2421, 1636, 1385, 831, 758, and 537 cm^{-1}.The absorption bands at 3371 cm^{-1} are responsible for O-H stretching, 2421 cm^{-1} C≡N bonding of nitrile, and 1636 cm^{-1} C=O bending of carbonyl groups. The band at 1385, and 831 cm^{-1} corresponded to C-H bond, vibration of Ni-OH [90,91]. The bands appeared at 758 and 537 cm^{-1} were the stretching and bending vibration of Ni-O; indicating the formation of NiO NMs [91]. It has been observed that the peaks shift to the lower wavenumber. This indicates that there is electrostatic attraction between electropositive and electron rich substances for the reduction processes [89] .

The FTIR spectra used to confirm the presence of functional group and plant metabolites were used for the reduction and stabilization of green synthesized NiO and Ag-NiO NMs. In the FTIR spectrum of Ag-NiO NMs, absorption bands at 3342, 2415, 1632, 1380, 1046, 831, 654, and 482 cm^{-1} were present. Absorption band at 482 and 654 cm^{-1} indicate Ni-O and Ag-O vibration respectively. The peaks at 1380, and 1632 cm^{-1} are due to C-N, and N-C=O stretching respectively whereas 831 cm^{-1} is related to the vibration of Ni-OH. Peak indicated at 3342 cm^{-1} is related to the O-H stretching [91].

4.4.3. XRD analysis of NiO and Ag-NiO NMs

The crystallinity and crystalline phase of synthesized samples were determined through X-ray diffraction (XRD) profiles in 2θ range of 10° to 80°. The XRD pattern of NiO NMs is shown in Figure 11. The Bragg peaks were obtained at 2θ values of 37.41, 43.45, 63.08, 75.59, and 79.23 degrees corresponding to (111), (200), (202), (311), and (222) respectively. We have not detected any other additional peaks throughout this diagram, which confirms the purity of obtained nanoparticles. The gathered data has been following the standard pattern (JCPDS # 96-101-0096). All the reflection can be indexed to spherical shaped and crystalline NiO, similarly with recently reported works of literature [91,92].

After doping NiO NMs with Ag (Figure 11), the formation of extra peaks was observed at 2θ: 35.73 and 67.51 degree related to the crystal planes (132) and (022) respectively. The additional peaks obtained at 35.73 and 67.51 degree correspond to (132) and (022) indicate silver. This is identical with reported literature [92,93].

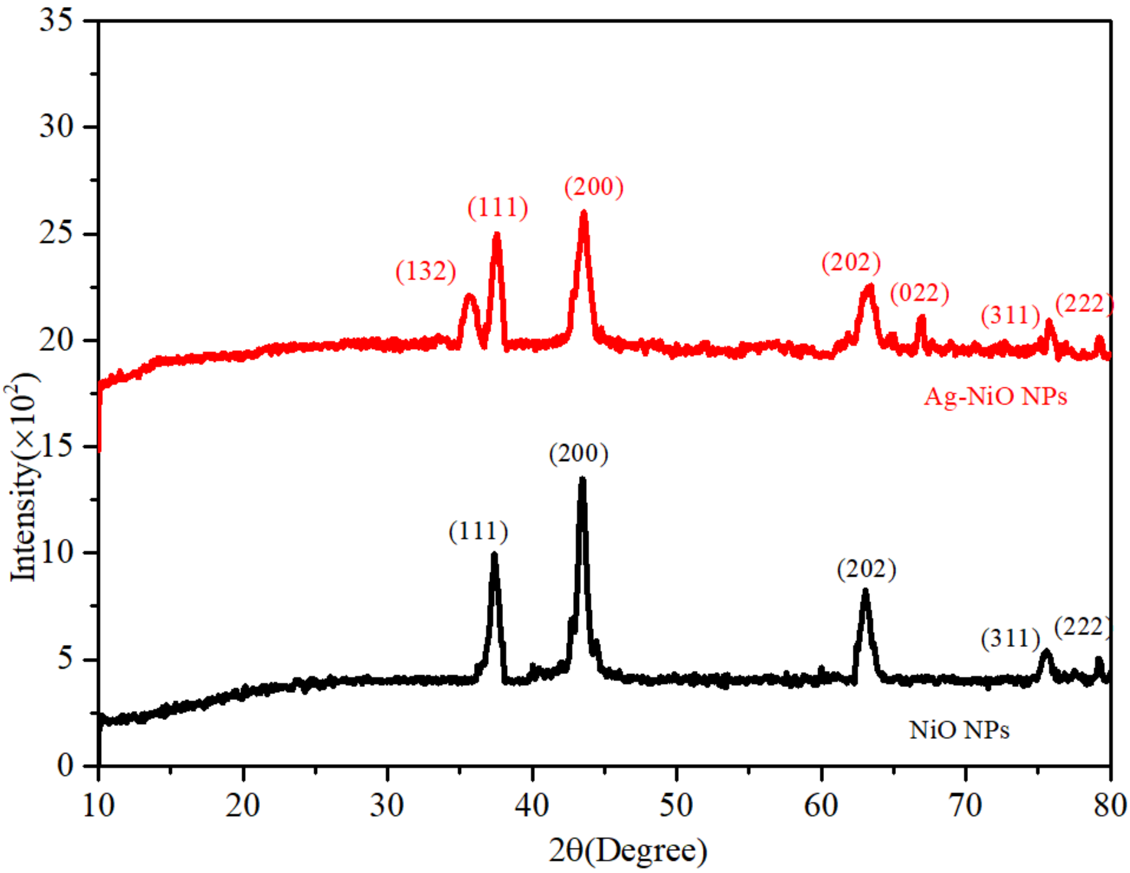

Figure 11. XRD diffractograms of NiO and Ag-NiO NMs

The average size of the synthesized NiO and Ag-NiO NMs was calculated by using Debye-Scherer formula

$$D= k\lambda/\beta\cos\theta$$ [92] ……………………................. (2)

where D is the average particle size/crystallite size of the nanoparticles, k denotes Debye diffraction constant which is taken as 0.9, λ is the wavelength of X-ray diffraction (0.15406 nm), β is the angular line full width at half maximum (FWHM) intensity in radians, θ the Bragg's angle. The crystallite size of NiO and Ag-NiO NMs prepared using *Musa acuminate* peel extract were 11.89 nm and 13.27 nm respectively. This result is in close agreement with the literature that reported the average size of NiO NMs was between 11.598 -15.527 nm in the range [94,95].

4.4.4. SEM analysis of NiO and Ag-NiO NMs

The surface morphological features of synthesized pure and doped NiO samples were studied by scanning electron microscope; the images were recorded with the magnification of 5000 (Figure 12). The image indicates that the NiO NMs show most synthesized nanomaterials are spherical. This result is in close relationship with recent findings literature that reported spherical NiO NMs [96]. In the case of Ag-doped NiO NMs, a spherical structures was observed. This is in good agreement with reported literature [94].

Some of the synthesized nanomaterials are found agglomerated due to aggregating or overlapping of smaller particles and they are essentially a cluster of nanomaterials, which is closely consistent with the result in literature [97]. It can be seen that pure NiO NMs reveal smaller-sized particles, while the doped sample shows also comparatively small-sized particles.

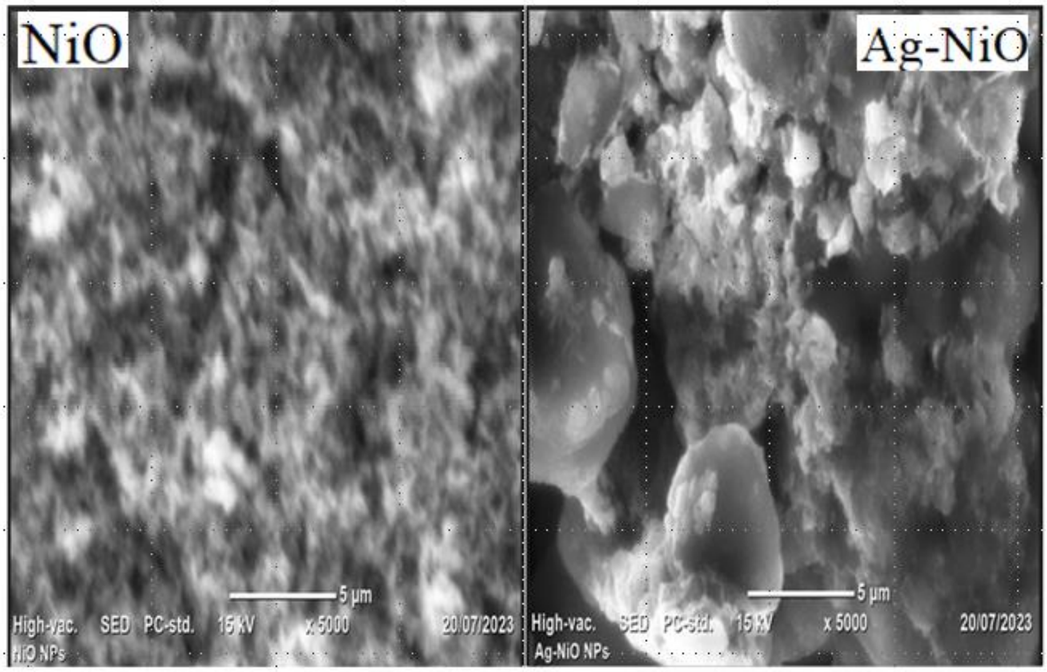

Figure 12. SEM microgrm of NiO and Ag-NiO NMs

4.5. Antibacterial activity

The effect of NiO and Ag-NiO NMs on selected bacteria (*Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*) at 100 µg/mL concentrations. The result of the antibacterial test was shown in table 2.

Table 2. Antibacterial test results

Strain	Zone of inhibition in mm				
	Gentamicin(+ve control)	DMSO(-ve control)	NiO NMs	Ag-NiO NMs	MAPE
S. typhi	26 (±0.3)	-	8 (±0.1)	14 (±0.3)	-
E. coli	25 (± 0.2)	-	9 (±0.2)	10 (±0.2)	-
S. aureus	27 (± 0.5)	-	14 (± 0.4)	17 (±0.6)	9 (±0.2)
B. cereus	28 (± 0.6)	-	11 (±0.3)	16 (±0.5)	7 (±0.1)

(-) indicate = No inhibition zone, MAPE = *Musa acuminate* peel extract

In this study, the antibacterial activity of the synthesized nickel oxide and silver-doped nickel oxide nanomaterials using *Musa acuminate* peel extract was examined against both Gram-negative (*S. typhi* and *E. coli*) and (*S. aureus* and *B. cereus*) bacteria by disc diffusion test. The zone of inhibition (ZOI) value for the above samples was recorded in (Table 2). The negative control (DMSO) was not shown any activity against the bacterial strains. On the other hand, antibacterial activity was observed against pathogens when *Musa acuminate* peel extract, NiO NMs, and Ag-NiO NMs used. The antibacterial effect of NiO NMs and Ag-NiO NMs against all four bacteria; and the bacterial effect of MAPE against two bacteria compared with control. It is seen that the growth of Gram-positive bacterial strains, *Staphylococcus aureus* was more effectively affected by NiO NMs than of the Gram-negative strains, *Salmonella typhi*, and *Escherichia coli*. The ZOI of the NiO and Ag-NiO NMs are higher when it is tested against *Staphylococcus aureus* than when tested against *Salmonella typhi*, *Escherichia coli*, and *Bacillus cereus*. These results refer to the differences in the cell wall of each strain; the cell walls of Gram-negative bacteria (*S. typhi* and *E.coli*) are composed of lipopolysaccharides which form a penetration barrier that allows the entrance of only macromolecules. In contrast, the cell wall of Gram-positive bacteria (*S. aureus* and *B.cereus*) have a thin layer of peptidoglycan and abundant pores that allows foreign molecules to penetrate, resulting in cell membrane damage and cell death. The antibacterial effect of Ag-NiO nanomaterial is higher than NiO due to the generation of ROS, which causes bacterial cell damage [98].

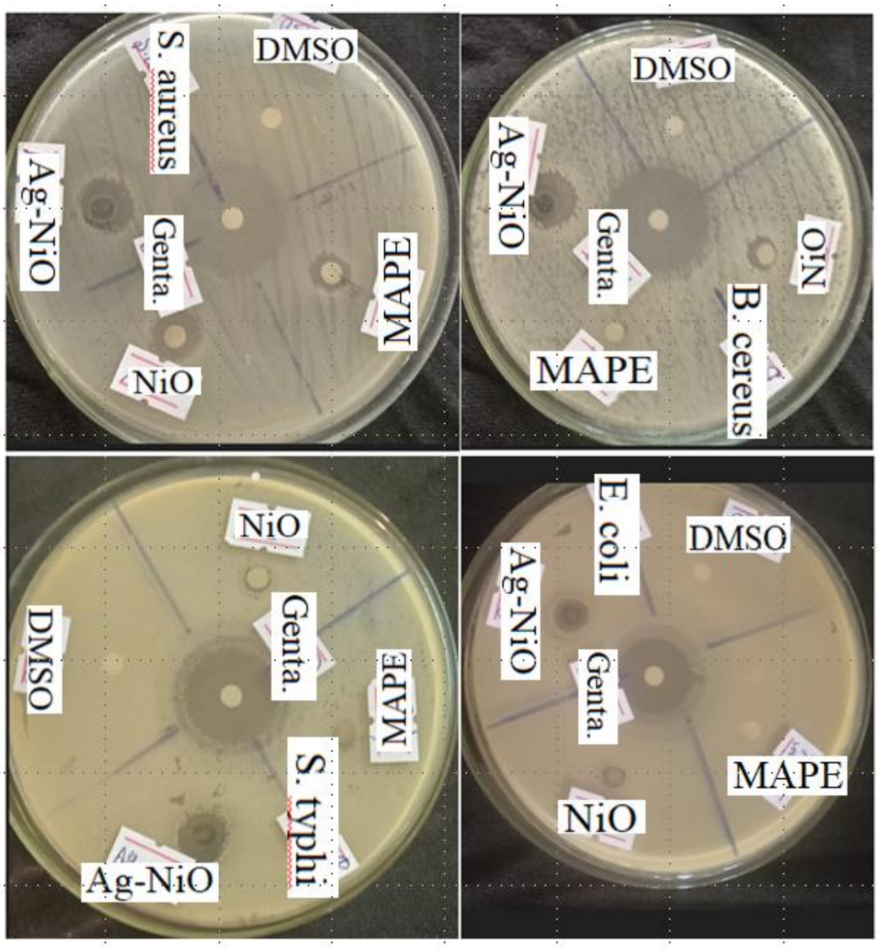

Figure 13. Zone of antibacterial activity

4.6. Antioxidant activity

Antioxidants were characterized by the capacity to scavenge free radicals and major aspect of antioxidants is the ability to donate hydrogen or electron to the oxidants [14]. The results showed that the colour of the DPPH solution changed from purple to light yellow due to accepting hydrogen from nanomaterials to form a stable molecule DPPH-H. This means that it possesses antioxidant activity because it contains the proton that can give it to scavenge free radicals [73]. The scavenging activities of the particles were estimated using the following concentrations: 2, 4, 8, 16, 32, and 64 μg/mL. The results (Figure 14) showed the effective free radical scavenging activityof the synthesized samples with calculated IC_{50} values of 35.62, 21.50, and 19.74 μg/mL for MAPE, NiO NMs, and Ag-NiO NMs respectively, which is in line with the literature [77] that was reported as IC_{50} of

22.19 µg/mL by plant extract mediated synthesis-reduced nickel oxide nanomaterials for antioxidant and bactericidal activity. The IC_{50} values demonstrate the ability to inhibit radicals. The result may be due to the presence of Polyphenols. Phenols are the most common secondary metabolites in the plant kingdom. They have numerous biological properties including antioxidant capacities for which they are indicated in the management of several diseases [99] .

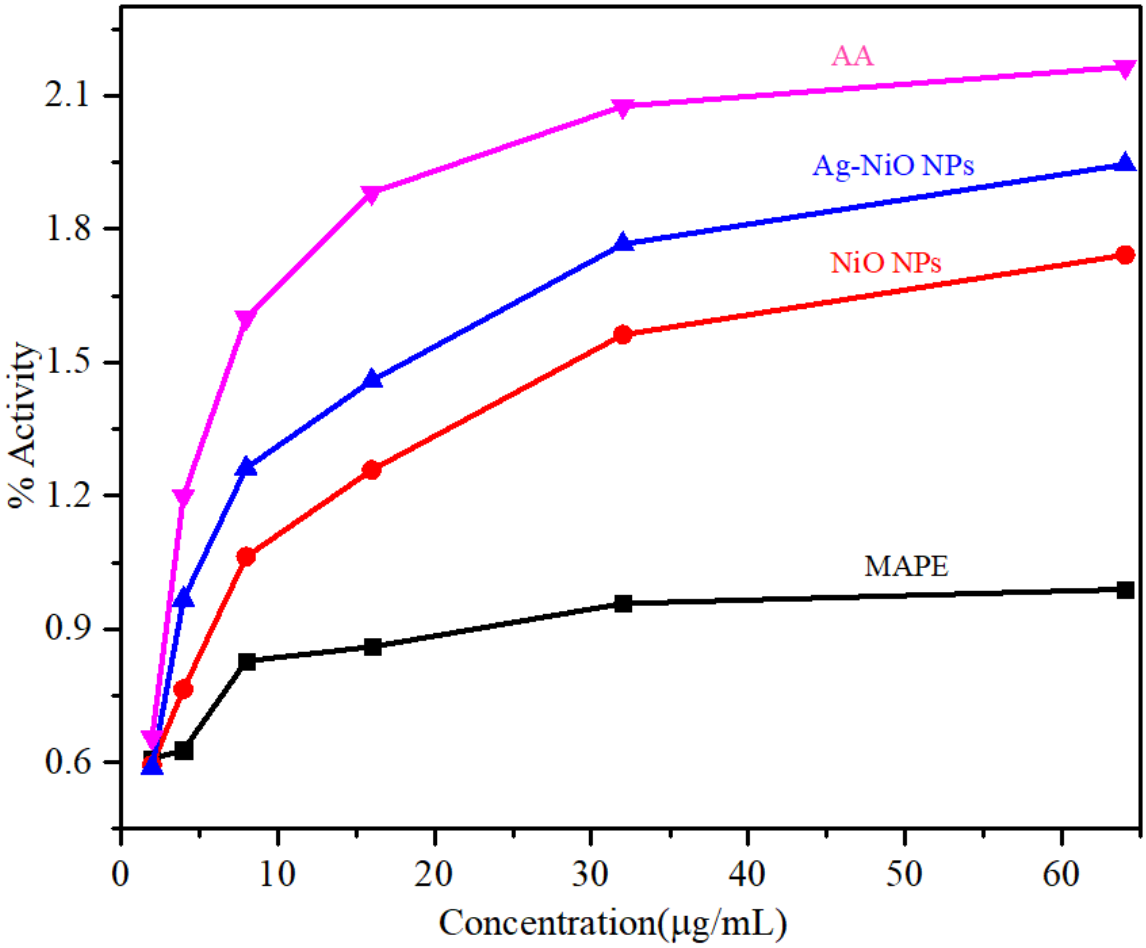

Figure 14. Antioxidant activities of synthesized NMs, *Musa acuminate* peel extract, and ascorbic acid

Enhancement in the antioxidant activity of the plant extract mediated NiO NMs might be an effect of metal ions present in the particles which is agreement with literature report [99] which confirms that the presence of Ni and Ag ions in particles might be responsible for higher DPPH free radical scavenging activity. The DPPH radical scavenging potential of Ag-NiO NMs was found to be more significant than NiO NMs.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In general, green method for the synthesis of NiO and Ag-NiO nanomaterials using peel extracts of *Musa acuminate* has been demonstrated. The metal oxide nanoparticles were characterized by UV-Vis, FTIR, XRD, and SEM. The Synthesized of NiO and Ag-NiO nanomaterials was optimized based on parameters like metal ion concentration, pH, temperature, plant extract, and reaction time to demonstrate the sharpness of the peaks. The UV-Vis absorption peaks at 302 and 309 nm indicate the synthesis of NiO and Ag-doped NiO NMs respectively. The functional groups were identified by FTIR spectra. XRD pattern showed distinctive peaks corresponding to (111), (200), (202), (311), and (222) planes that can be indexed. Crystalline sizes of the formed particle were obtained to be 11.89 and 13.27 nm from the XRD data by using Scherer's formula for NiO and Ag-doped NiO NMs respectively. SEM results also demonstrate that particles are agglomerated, spherical, and crystalline.

The designed NiO and Ag-doped NiO NMs are highly stable and shown significant antibacterial activity against these bacterial strains with a maximum inhibition zone of 14, and 11 mm in *S. aureus*, and *B. cereus* respectively, and a minimum inhibition zone of 8 mm in *S. typhi* and also Ag-doping NiO NMs increasing zone of inhibition. In the antioxidant test, NiO and Ag-doped NiO NMs prevented the oxidation of 50% of the DPPH at a concentration of 21.50 µg/mL and 19.74 µg/mL respectively. Considering the obtained results, NiO and Ag-doped NiO NMs containing *Musa acuminate* peel extract may be utilized as an efficient drug/supplement in treating diseases in humans after sufficient clinical studies.

5.2. Recommendation

For further work, it was recommended that additional characterization for further size determination and morphology of the sample using instruments; TEM and EDX. Therefore the government or other science inclined companies can take this up as a project to develop nanomaterials that serve the purpose of most indispensable toxic material emitting machines today, thus bringing about a safer environment but at the same time not hindering technological advancement.

The advanced study should be carried out using other characterization of compounds acting as the reducing agents in each plant extract and nanomaterials which were not addressed by this study. Also, the antibacterial and anti-oxidant activities of the synthesized samples should be tested on other microbial species and medicinal application which were not addressed by this study.

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7. APPENDIX

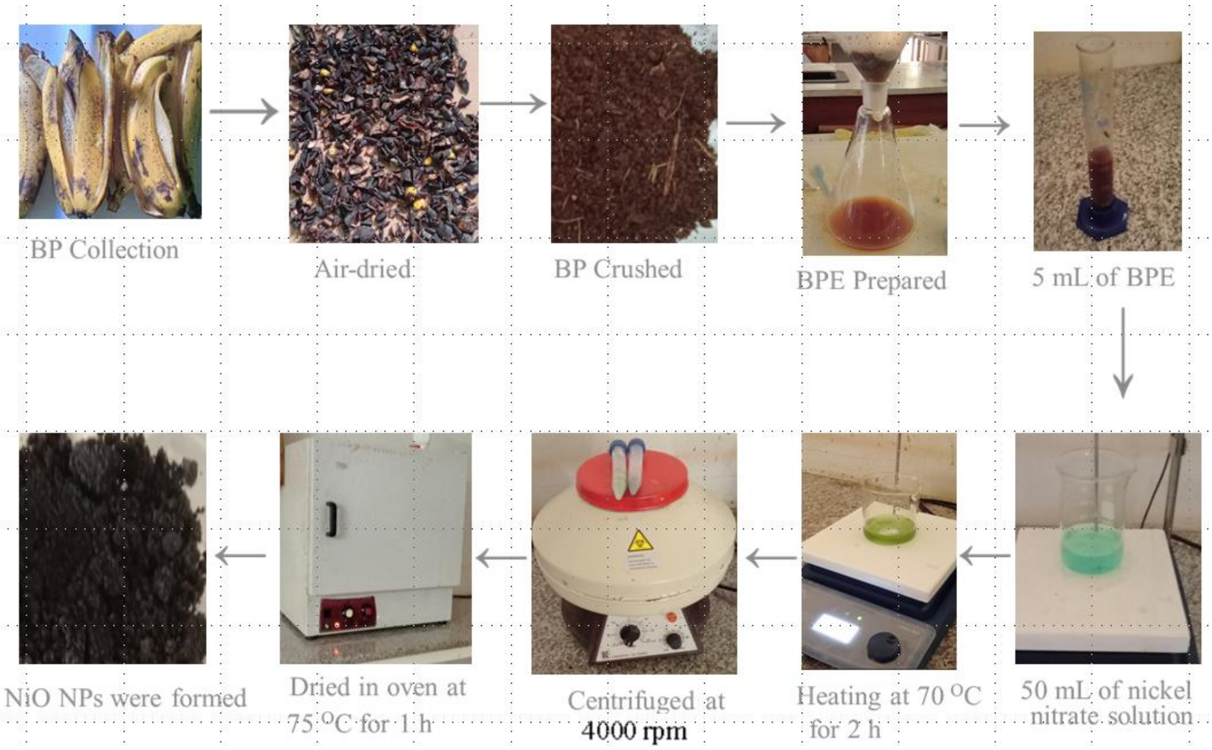
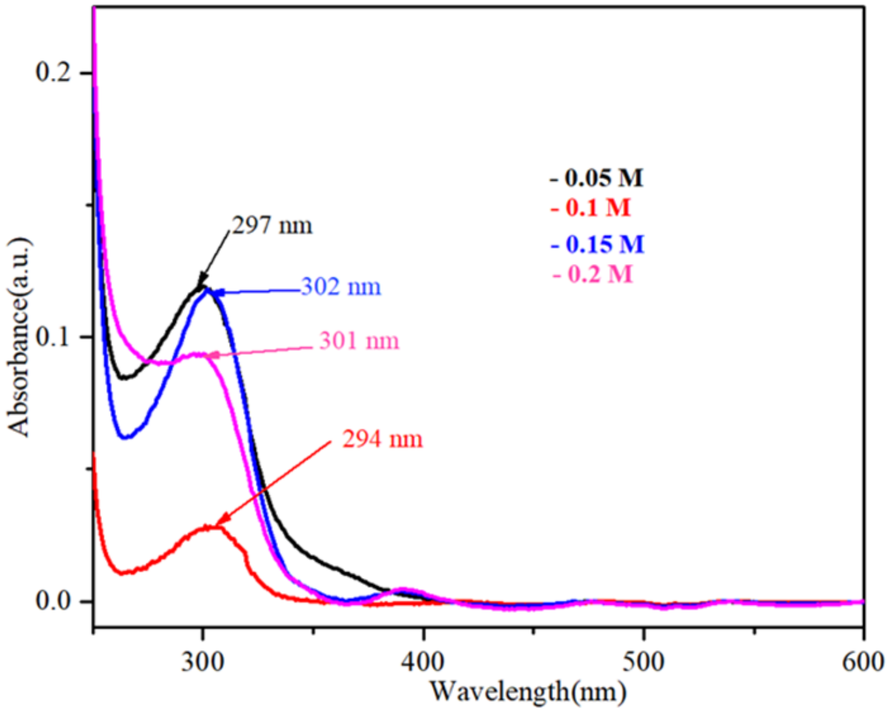
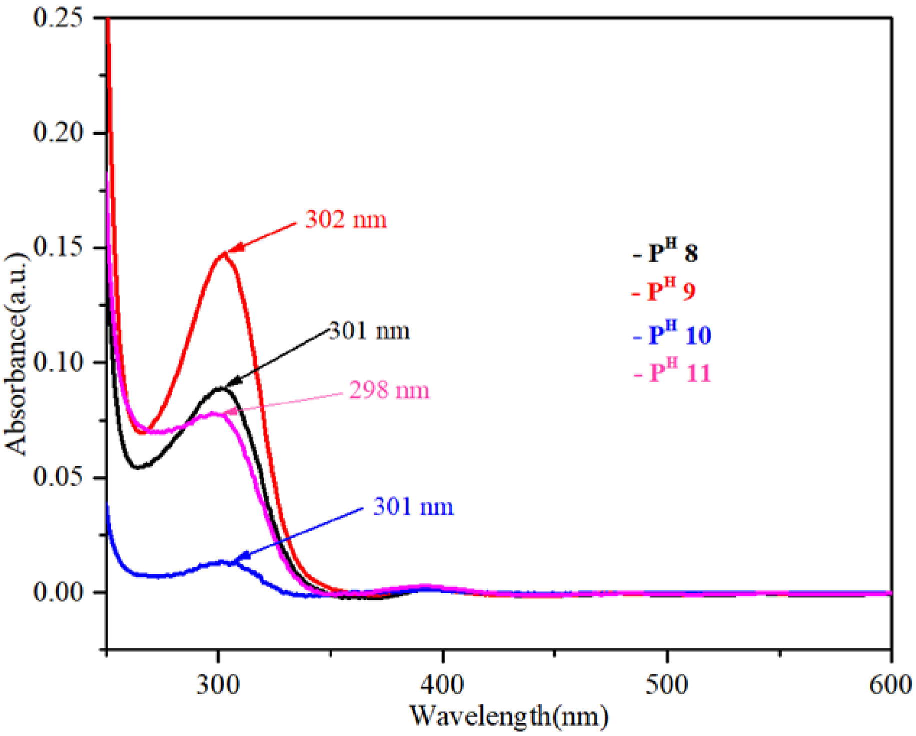


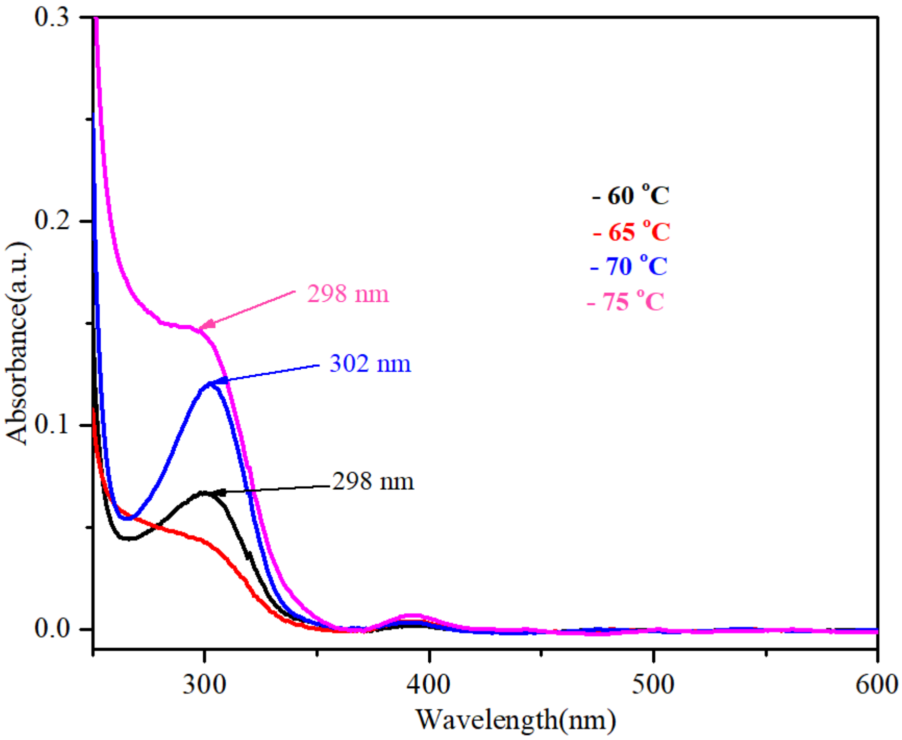
Figure A$_1$. Nickel oxide nanoparticles synthesis



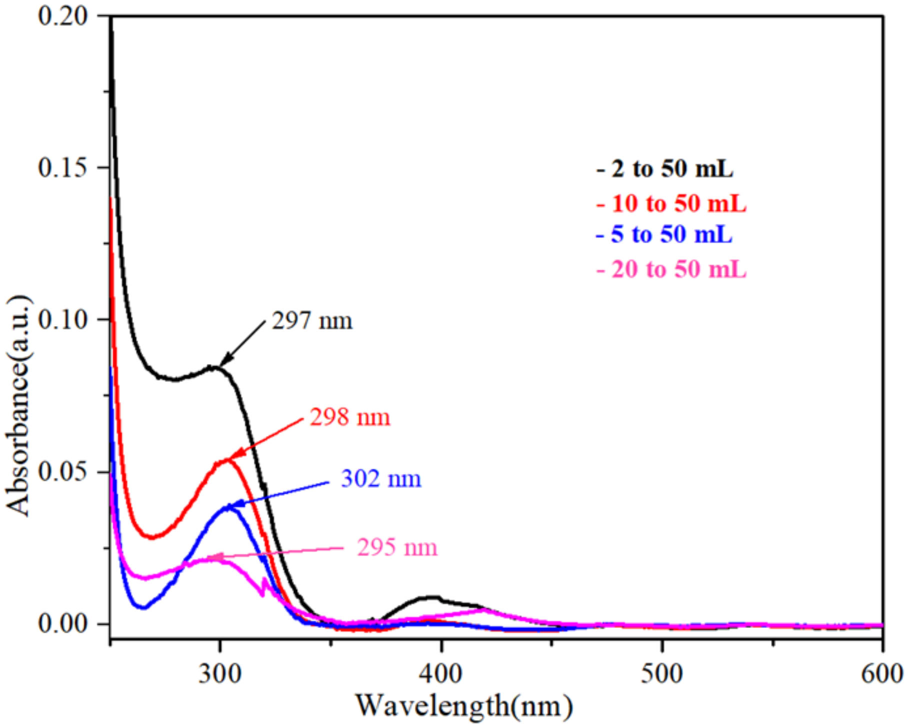
FigureA$_2$. Optimization of precursor concentration; $Ni(NO_3)_2$. $6H_2O$



FigureA3. Optimization PH effect of NiO NPs



FigureA4. Optimization of temperature effect



FigureA5. Optimization of plant extract

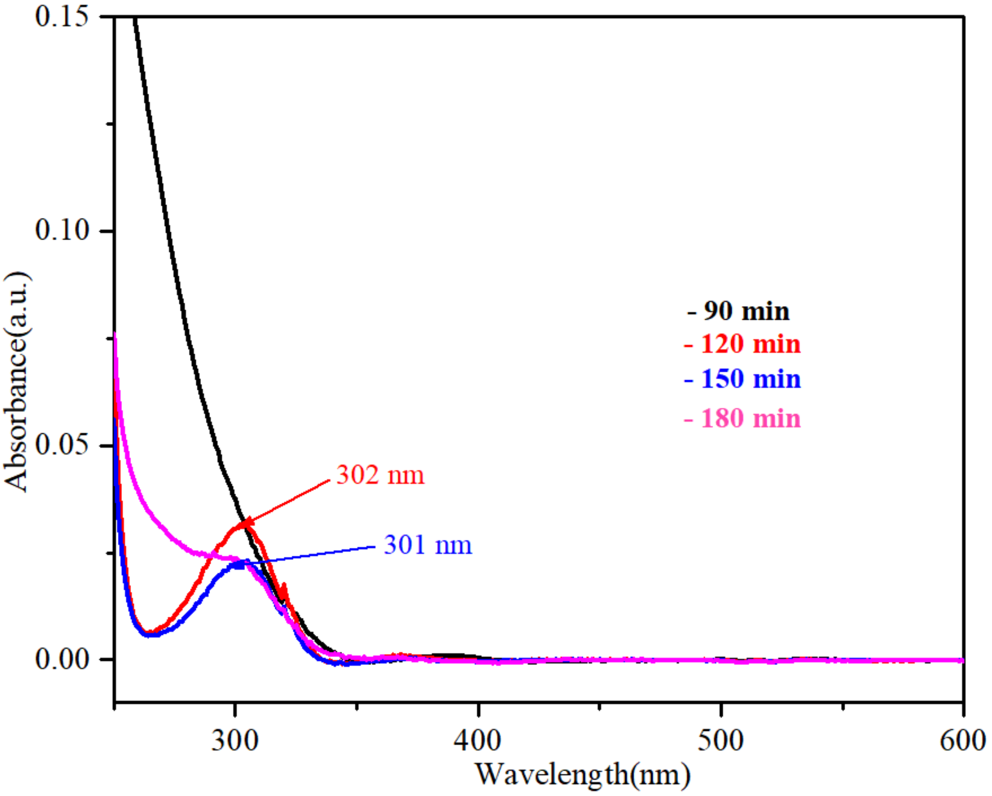
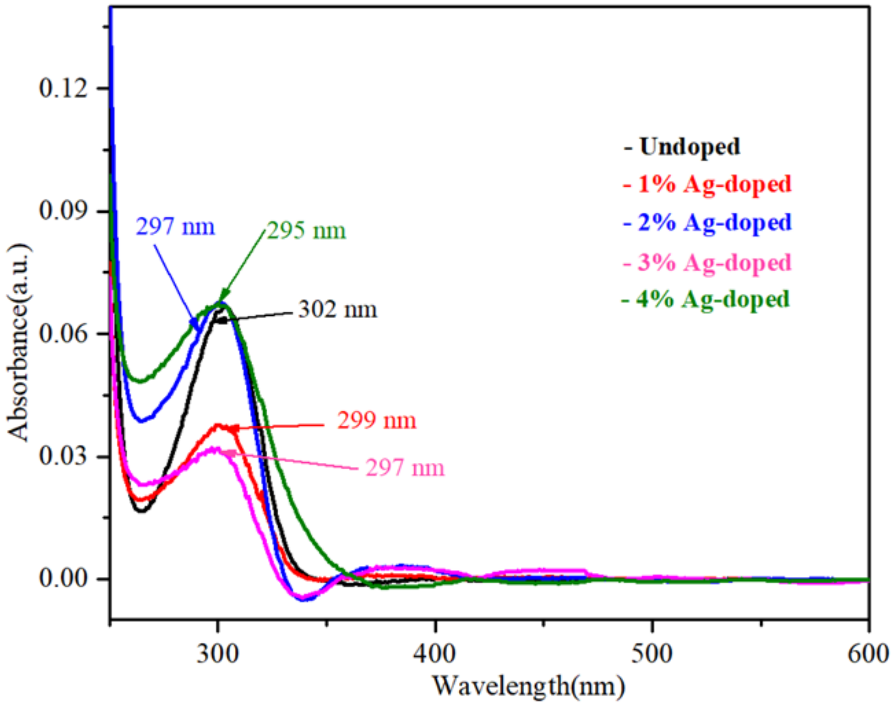


Figure A6. Optimization of reaction time



FigureA7. Optimization volume of dopant (1- 4% Ag-doped)

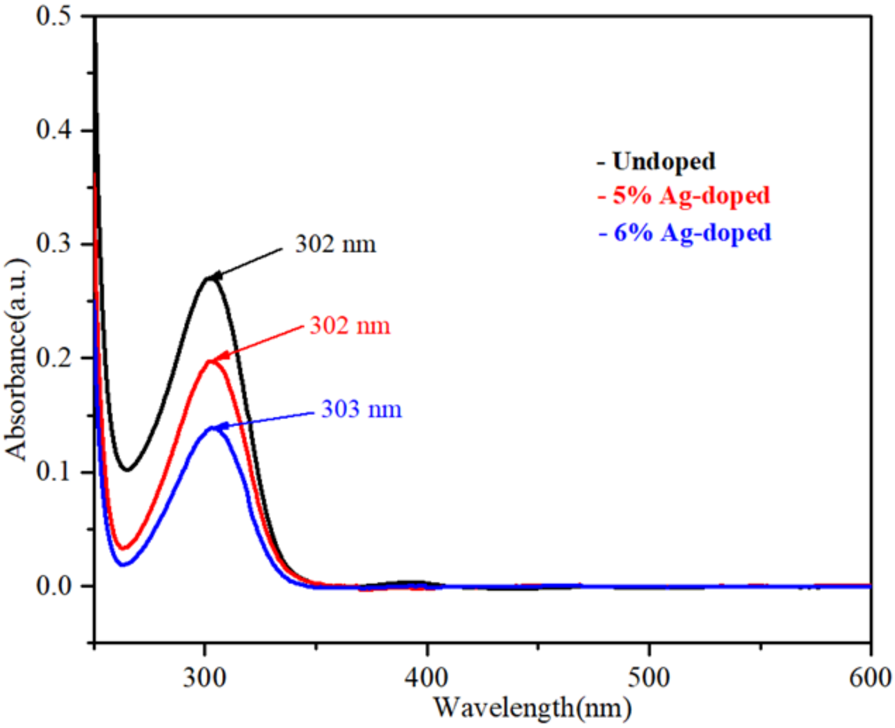


Figure A8. Optimization volume of dopant (5 and 6% Ag-doped)

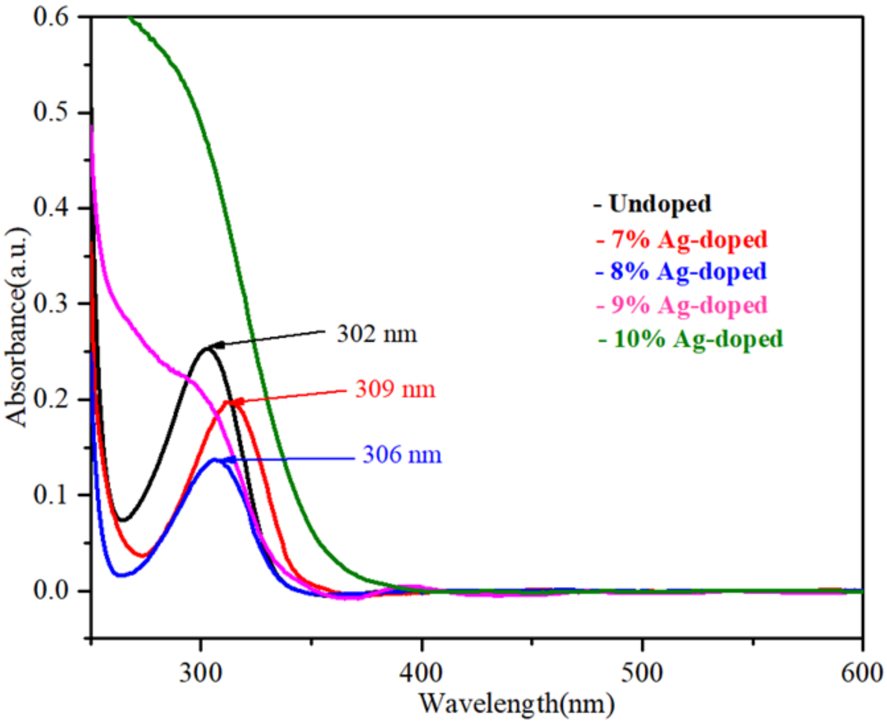


Figure A9. Optimization volume of dopant (7-10% Ag-doped)