



WALLAGA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

**GREEN SYNTHESIS AND CHARACTERIZATION OF
ZnO/Ag₂O/Co₃O₄ NANOCOMPOSITE USING *Rhamnus
prinoides* LEAF EXTRACT FOR PHOTOCATALYTIC AND
ANTIBACTERIAL APPLICATIONS**

MSc Thesis

By:

Mosisa Dugasa Fite

June 2025

Nekemte, Ethiopia



WALLAGA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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LEAF EXTRACT FOR EVALUATION OF ITS PHOTOCATALYTIC
AND ANTIBACTERIAL ACTIVITY**

**A Thesis Submitted to the College of Natural and Computational Sciences
in Partial Fulfillment of the Requirements for the Degree of Master of
Inorganic Chemistry.**

Mosisa Dugasa Fite

Advisor: Asnake Lealem (PhD)

June 2025

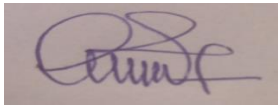
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As members of the Board of Examining of the Final MSc. thesis open defense, we certify that we have read and evaluated the thesis prepared by **Mosisa Dugasa Fite** under the title “**Green Synthesis and Characterization of ZnO/Ag₂O/Co₃O₄ Nanocomposite Using *Rhamnus Prinoides* Leaf Extract for Photocatalytic and Antibacterial Applications**” and recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in **Inorganic Chemistry**.

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STATEMENT OF THE AUTHOR

I **Mr. Mosisa Dugasa Fite** hereby declare and affirm that the thesis entitled “**Green Synthesis and Characterization of ZnO/Ag₂O/Co₃O₄ Nanocomposite Using *Rhamnus Prinoidea* Leaf Extract for Photocatalytic and Antibacterial Applications**” is my own work conducted under the supervision of Asnake Lealem (PhD). I have followed all the ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misrepresented, fabricated, or falsified any idea / data / fact / source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Post Graduate Studies at Wallaga University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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This is to certify that this thesis is entitled “**Green Synthesis and Characterization of ZnO/Ag₂O/Co₃O₄ Nanocomposite Using *Rhamnus Prinoides* Leaf Extract for Photocatalytic and Antibacterial Applications**” is accepted in partial fulfillment of the requirements for the award of M.Sc. degree in Inorganic Chemistry by the School of Graduate Studies, Wallaga University through the College of natural and Computational Sciences, done by **Mosisa Dugasa Fite** is genuine work carried out by him under my guidance. The matter embodied in this thesis work has not been submitted earlier for award of any degree. The assistance and help received during the course of this investigation have been dually acknowledged. Therefore, I recommend that it can be accepted as fulfilling the research thesis requirements.

Asnake Lealem (PhD)

Advisor

Signature

Date

ABBREVIATIONS AND ACRONOMYS

ATCC	:	American Type Culture Collection
BET	:	Brunauer Emmett Teller
CB	:	Conduction Band
DLS	:	Dynamic Light Scattering
DMSO	:	Dimethyl Sulfoxide
DNA		Deoxyribonucleic Acid
EDX	:	Energy Dispersive X-ray
E_g	:	Energy Band Gap
FTIR	:	Fourier Transformed Infrared
JCPDS	:	Joint Comitte on Powder Diffraction Standards
MB	:	Methylene Blue
NCs	:	Nanocomposites
NMs	:	Nanomaterials
NPs	:	Nanoparticles
pH	:	Power of Hydrogen
pHPZC	:	pH of Point Zero Charge
PL	:	Photoluminscence
ROS	:	Reactive Oxygen Species
SEM	:	Scanning Electron Microscope
SPR	:	Surface Plasmon Resonance
TEM	:	Transmission Electron Microscope
TGA	:	Thermal Gravimetric Analysis
UV-Vis	:	Ultra Violet –Visible
VB	:	Valence Band
XPS	:	X-ray Photoelectron Spectroscopy
XRD	:	Xray-Diffraction
ZOI	:	Zone of Inhibition

BIOGRAPHICAL SKETCH

The author was born on August 20, 1995, in Dini Andode Kebele, Leka Dullecha District, East Wallaga, Oromia Regional State, Ethiopia. He attended his primary school at Dini Andode from 2001 to 2008 G.C. and his secondary school at Getema Secondary School (grades 9–12) from 2009 to 2013 G.C. He then joined Wachemo University, where he earned a BSc in Applied Chemistry in 2017 G.C. In 2020, he was employed by Wallaga University. After gaining two years of work experience, he commenced his MSc studies in Inorganic Chemistry at Wallaga University in 2022.

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ABSTRACT

Recently, attention focuses on synthesis of nanomaterial by biosynthesis methods, which use plant extracts or microorganism's as alternative approach. The main objective of this study was to synthesize and characterize ZnO/Ag₂O/Co₃O₄ nanocomposite using *Rhamnus prinoides* leaf extract to evaluate its photocatalytic and antibacterial activities. Metal oxide nanoparticles, binary nanocomposites and ternary ZnO/Ag₂O/Co₃O₄ nanocomposites are synthesized from zinc acetate dihydrate, silver nitrate, and cobalt nitrate hexahydrate using green synthesis method. The synthesized nanoparticles and nanocomposite were characterized using ultraviolet spectroscopy, fourier transformed infrared spectroscopy, and x-ray-diffraction techniques, and its photocatalytic and antibacterial activities were evaluated. The ultraviolet-visible absorption spectra of metal oxide nanoparticles displayed characteristic peaks at 358 nm for ZnO nanoparticle, 420 nm for Ag₂O nanoparticle, 586 nm and 638 nm for Co₃O₄ nanoparticle. The ultraviolet-visible spectra of binary and ternary nanocomposite displayed characteristic absorption peak at 358 nm and 472 nm for Ag₂O/ZnO nanocomposite, 364 nm for Co₃O₄/ZnO nanocomposite, 338 nm and 486 nm for Ag₂O/Co₃O₄ nanocomposite, 364 nm for 1:1:1, 372 nm for 1:2:1, 368 nm for 1:1:2, and 360 nm for 2:1:1 ZnO/Ag₂O/Co₃O₄ nanocomposite. Fourier transformed infrared analysis displayed the functional group presents in the *Rhamnus prinoides* extract, and stretching vibration bands corresponding to Zn-O, Ag-O, O-Co²⁺, and O-Co³⁺. The x-ray diffraction analysis revealed that synthesized samples exhibit crystalline nature with average crystalline sizes of 28.78 nm for ZnO nanoparticle, 26.25 nm for Ag₂O nanoparticle, and 26.65 nm for Co₃O₄ nanoparticle. For binary and ternary nanocomposite, the average crystalline size was calculated as 25.82 nm for Ag₂O/ZnO nanocomposite, 27.52 nm for Co₃O₄/ZnO nanocomposite, 25.92 nm for Ag₂O/Co₃O₄ nanocomposite, 21.31 nm for 1:1:1, 14.37 nm for 1:2:1, 19.61 nm for 1:1:2, and 24.70 nm for 2:1:1 ZnO/Ag₂O/Co₃O₄ nanocomposite. The photocatalytic degradation efficiencies of methylene blue under sunlight after 40 minutes of irradiation were 72.79 % for ZnO nanoparticle, 82.76 % for Ag₂O nanoparticle, and 82.40 %, for Co₃O₄ nanoparticle. In addition photocatalytic activities of binary and ternary nanocomposites are 86.77 % for Ag₂O/ZnO nanocomposite, 91.62 % for Co₃O₄/ZnO nanocomposite, 83.50 % for Ag₂O/Co₃O₄ nanocomposite, 94.02 % for 1:1:1, 98.31 % for 1:2:1, 95.28 % for 1:1:2, and 93.01 % for 2:1:1 nanocomposite. The antibacterial activities of synthesized samples against *Escherichia coli*, *Enterobacter cloacae* (Gram-negative) and Gram-positive *Staphylococcus aureus* exhibited significant potential when compared to ciprofloxacin. The highest inhibition zones were observed for ZnO/Ag₂O/Co₃O₄ nanocomposite with ratios of, 1:1:2 2:1:1 and 1:2:1 against *Escherichia coli*, *Enterobacter cloacae*, and *Staphylococcus aureus*, with inhibition zone diameters of 17.6±0.58^d mm, 16.6±0.1^{efgh}, and 19.5±0.6^c, respectively. This finding demonstrates the promising photocatalytic and antibacterial activities of green synthesized nanoparticles and nanocomposite.

Keywords: antibacterial; crystalline size; methylene blue; nanocomposite; photocatalytic activity; *Rhamnus prinoides*.

1. INTRODUCTION

1.1. Background of Study

Unprincipled agricultural and industrial activities release substantial amounts of organic and inorganic pollutants into the soil and groundwater every year, either directly or indirectly. Among these pollutants, textile dyes have become a significant concern. For instance, methylene blue (MB) dye commonly used in industries like paper, rubber, pesticides, and varnishes, is associated with toxicity and gene mutations. Figure 1 shows the chemical structure of MB dye. Furthermore, in many developing countries, wastewater containing these carcinogenic dyes continues to be discharged into the environment without adequate treatment (Mahanthappa *et al.*, 2019).

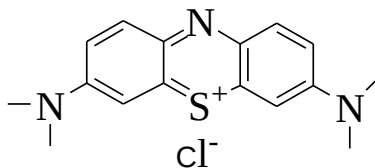


Figure 1. Chemical Structure of Methylene Blue

Various chemical methods have been used globally to treat textile effluents, including ozonation, sedimentation, water oxidation, and photocatalysis (Abdullah *et al.*, 2023). However, some of these techniques have certain limitations. For example, ozonation is expensive, sedimentation requires significant space for installation, water oxidation primarily addresses suspended particles without effectively eliminating pathogens. In contrast, photocatalysis is an eco-friendly and efficient approach known for its low cost, feasibility, non-toxicity, and ability to achieve better dye degradation and pollutant removal from industrial wastewater. Currently, researchers worldwide are focusing on advancing smarter and more efficient semiconducting nanostructures to enhance the removal of pollutants from contaminated water sources through photocatalysis.

Pathogenic Bacteria are among the most prevalent microorganisms responsible for various illnesses in humans, including chronic diseases, fatalities, and water contamination. For

instance, *Escherichia coli* (*E. coli*), a bacterium commonly found in the human intestine, can lead to conditions such as septicemia, cholecystitis, and urinary tract infections. While *E. coli* can be beneficial in certain contexts, it is also a cause of both community-acquired and hospital-acquired infections, and it is the leading contributor to bloodstream infections in humans (MacKinnon *et al.*, 2020). Another example is *Enterobacter cloacae* (*E. cloacae*), a gram-negative, short bacillus that naturally inhabits the intestines, and it can act as a pathogen capable of infecting multiple organs and affects soft tissues, the lungs, and the urinary tract (Elbehiry *et al.*, 2024). *Staphylococcus aureus* (*S. aureus*) is a gram-positive bacterium responsible for various severe conditions in animals, including suppurative diseases, arthritis, omphalitis, urinary tract infections, and both clinical and subclinical mastitis in ruminants. In humans, *S. aureus* is a leading cause of toxic shock syndrome, endocarditis, post-operative wound infections, and food poisoning (Pal *et al.*, 2020).

Conventional water disinfection methods, such as chlorination, ozonation, and UV (ultra violet) treatment, have notable limitations, including impracticality and the potential formation of carcinogenic byproducts (Sasi *et al.*, 2022). The widespread application of antibiotics in food production, animal husbandry, and household cleaners has accelerated the development of antibiotic-resistant microbial strains. One promising solution is the use of antibacterial nanoparticles or ions and its composites, which interact with bacterial cell walls and disrupt their internal components, particularly deoxyribonucleic acid (DNA). In recent years, semiconducting metal oxide nanostructured materials have garnered significant attention due to their unique properties and broad potential applications across various fields. Among these, ZnO nanoparticles (NPs), n-type wide-bandgap semiconductor, is widely used in photovoltaics, photocatalysis, ultraviolet protection, sensors, and pharmaceuticals, owing to its excellent optical and electrical properties. However, ZnO NPs faces several intrinsic limitations, such as its large bandgap (~3.37 eV), which restricts its optical absorption to the ultraviolet range of the solar spectrum and limits its effectiveness in water purification applications (Singh *et al.*, 2019). Additionally, ZnO NPs photocatalytic efficiency in aqueous solutions is hindered by limited photostability and rapid electron-hole recombination. To address these challenges and enhance the photocatalytic performance of ZnO NPs, researchers have used various strategies, including surface modification, doping, and

coupling with other semiconductors (Perumal *et al.*, 2023). Among these, Ag₂O nanoparticles, a p-type semiconductor with a narrow band gap (1.1-1.4 eV), which have been extensively studied for their synthesis, characterization, photocatalytic, and antibacterial activities. Their favorable optical properties and ability to absorb the entire solar spectrum make them particularly effective for visible light-driven photocatalytic removal of organic pollutants (Danish *et al.*, 2022). Similarly, Co₃O₄, another p-type semiconductor with a bandgap of 2.1 eV and 1.48 eV has also demonstrated significant potential in photocatalytic and other applications (Wang *et al.*, 2020).

Different researchers have synthesized metal oxides such as Ag₂O NPs (Muthukumar *et al.*, 2022), ZnO NPs (Sasi *et al.*, 2022), and Co₃O₄ NPs (Kombaiah *et al.*, 2019), as well as their binary nanocomposite (NCs), including ZnO/Ag₂O NCs (Ahmad *et al.*, 2020), Co₃O₄/ZnO NCs (Gendo *et al.*, 2024), and Ag₂O/Co₃O₄ NCs (Mohamed *et al.*, 2022), to enhance photocatalytic and antibacterial activities and investigated their characterization. A few reports on the synthesis of Ag₂O NPs coupled with two metal oxides were reported including Ag₂O/g-C₃N₄/Fe₃O₄ NCs (Zhang *et al.*, 2017), Ag₂O/CoFe₂O₄/GO NCs (Aftab *et al.*, 2023), Ag₂O/ZnO/NiO NCs (Noelson *et al.*, 2022), Ag₂O/SnO₂/TiO₂ NCs (Subhan *et al.*, 2020), Ag₂O/ZnO/CuO NCs (Masho *et al.*, 2024) and so on for enhancing photocatalytic and antibacterial activities. A literature study shows that different studies have used physical and chemical synthesis methods such as coprecipitation method (Ghanbari *et al.*, 2016), sol–gel method (Silva *et al.*, 2018), hydrothermal method (Bouremama *et al.*, 2015), wet chemical approach (Gupta *et al.*, 2021), mechanical ball milling (Akgul & Akgul, 2022) and *etc.* to synthesis nanocomposite. The drawbacks of physicochemical methods include complex synthesis processes, the production of hazardous byproducts, high energy demands, and the occasional formation of complex mixtures. Recently, there has been a growing focus on environmentally friendly approaches biosynthesis, for synthesis of nanoparticles and nanocomposites. This method utilizes bio-extracts or microorganisms as an eco-friendly ways for synthesizing nanomaterials (Nnadozie & Ajibade, 2020). Ag₂O-based nanocomposite have gained significant attention in plant-based synthesis, with extracts from various plant species being utilized to synthesize Ag₂O-based ternary nanocomposite (Tijani *et al.*, 2019).

Rhamnus prinoides (mostly known as Gesho in both Amharic and Afan Oromo) is an evergreen medicinal plant traditionally used to treat various infectious diseases. It also serves as a bittering agent in the preparation of traditional East African fermented beverages like tella and tej (Campbell *et al.*, 2019). Research has demonstrated that *Rhamnus prinoides* (*R. prinoides*) acts as an effective reducing, capping, and stabilizing agent in the green synthesis of nanoparticles (Habtemariam & Alemu, 2021). Although many researchers have investigated the synthesis of metal oxide Ag₂O NPs, ZnO NPs, Co₃O₄ NPs, binary Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄ NCs, and other Ag₂O-based ternary nanocomposite to study photocatalytic and antibacterial activities, no reports exist on the synthesis of ternary ZnO/Ag₂O/Co₃O₄ nanocomposite for such applications, except for the wet chemical synthesis of ZnO/Ag₂O/Co₃O₄ NCs reported by (Alam *et al.*, 2019) for sensor applications. So far, to the best of our knowledge, there have been no reports for the synthesis of ZnO/Ag₂O/Co₃O₄ nanocomposite via the green method using *R. prinoides* leaf extract as stabilizing agent for photocatalytic and antibacterial activities. Therefore, in the current study, ZnO/Ag₂O/Co₃O₄ NCs was synthesized by using *R. prinoides* for evaluation of its photocatalytic activity against MB dye under sunlight light irradiation and antibacterial activities towards two gram negative *E. coli* and *E. cloacae* and gram positive *S. aureus* was introduced for the first time.

1.2. Statement of the Problem

Synthesis methods of nanomaterials, including chemical and physical approaches, are typically costly, hazardous, and pose environmental risks (Cruz *et al.*, 2020). To address these issues, researchers have identified precise green synthesis pathways, utilizing naturally occurring sources for the synthesis of nanomaterials. The discharge of methylene blue (MB) dyes into water during production and application has become a major source of environmental contamination due to their toxicity and non-biodegradable nature which makes them difficult to remove through conventional water treatment methods (Oladoye *et al.*, 2022). These dyes pose significant hazards to living organisms, as overexposure can cause severe damage to internal organs, including the liver, kidneys, lungs, and vascular system. Photocatalysis is reported as a cost-effective way of treating wastewater containing organic pollutants using nanomaterials. Among these, ZnO NPs stands out as a promising semiconductor for photocatalytic applications. However, its use is restricted to the UV region because of its wide band gap. To extend ZnO NPs absorption band into the visible region, narrow band gap transition metal oxides like Ag₂O NPs and Co₃O₄ NPs can be incorporated. The synthesis of ZnO/Ag₂O/Co₃O₄ nanocomposite is highly beneficial for eliminating toxic dyes like methylene blue, contributing to environmental remediation and addressing the frequent need for enhanced photocatalytic activity. In parallel to this, pathogenic bacterial infections, including typhoid, cholera, and strep throat, are common in Ethiopia and contribute to a significant number of health-related cases (Qin & Ronchieri, 2022). The rise of antibacterials for bacterial pathogens has become a significant challenge in the healthcare sector, and needs continued research to develop new treatments. The green synthesis of nanoparticles and nanocomposites using plant extracts has gained widespread recognition as a promising approach due to its numerous advantages, including the absence of harmful chemicals, simplicity, and environmental friendliness. Thus, the current study focuses on the green synthesis and characterization of a ZnO/Ag₂O/Co₃O₄ nanocomposite using *R. prinoides* leaf extract, with enhanced photocatalytic and antibacterial efficiency. Additionally, no reports have been found on the synthesis of a ZnO/Ag₂O/Co₃O₄ ternary nanocomposite using *R. prinoides* leaf extract.

1.3. Objective of the Study

1.3.1. General Objective

The main objective of the study was to synthesize and characterize ZnO/Ag₂O/Co₃O₄ nanocomposite using *Rhamnus prinoides* leaf extract for evaluation of its photocatalytic and antibacterial activity.

1.3.2. Specific Objectives

- ✓ To synthesize metal oxide nanoparticles (Ag₂O, ZnO, and Co₃O₄), binary nanocomposites (Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄), and ternary nanocomposites (ZnO/Ag₂O/Co₃O₄) using *Rhamnus prinoides* leaf extract via the green synthesis method.
- ✓ To characterize green synthesized metal oxide nanoparticles (Ag₂O, ZnO, and Co₃O₄), binary nanocomposites (Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄), and ternary nanocomposites (ZnO/Ag₂O/Co₃O₄) using Ultraviolet-Visible spectra, Fourier Transformed Infrared spectra, and X-ray diffraction techniques.
- ✓ To examine the photocatalytic degradation efficiency of all synthesized nanoparticles and nanocomposites toward methylene blue under sunlight irradiation.
- ✓ To evaluate antibacterial activities of the all synthesized nanoparticles and nanocomposites using disk diffusion method.

1.4. Significance of the Study

This study offers significant information on the green synthesis of Ag₂O, ZnO, Co₃O₄ NPs, as well as Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and ZnO/Ag₂O/Co₃O₄ NCs using *R. prinoides* leaf extract, along with their characterization. To address the harmful effects of water pollutants from untreated industrial effluents, photocatalytic and antibacterial activities offer an efficient solution. Moreover, the study offers valuable information on the photocatalytic and antibacterial activities of Ag₂O, ZnO, Co₃O₄ NPs, as well as Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and ZnO/Ag₂O/Co₃O₄ NCs synthesized using *R. prinoides* leaf extract via the green synthesis method. Furthermore, the newly synthesized samples may serve as a baseline for future research by other researchers using different plants for the synthesis of these nanomaterials.

2. LITERATURE REVIEW

2.1. Environmental Pollution

Environmental pollution is one of the major global problems, which include land, air, and water pollution. This issue has become more complex in recent decades due to population growth, urbanization, and industrial activities. Therefore, it is crucial to mitigate the worsening of environmental pollution through the development of effective technologies for the disposal and treatment of various pollutants. These technologies will play a key role in reducing the impact of pollution on ecosystems, human health, and the planet as a whole (Gbair & Alshamsi, 2022). One of the most hazardous forms of pollution is water pollution, which poses significant risks to human health and the survival of organisms. The primary sources of aquatic pollutions are industrial wastewater, which contains a variety of organic and inorganic pollutants that harm both the environment and living beings. The widespread presence of organic dyes in industrial wastewater significantly causes to environmental pollution. Numerous industries, including paper, textile, pharmaceuticals, leather, printing, paint, food, and ink production, release dyes to wastewater. These dye degrade water quality and act as toxic, mutagenic, and carcinogenic agents (Dadi *et al.*, 2017). The risk of water contamination by dyes is compounded by the fact that many of these substances are resistant to microbial degradation, making them particularly challenging to remove and treat (Kishor *et al.*, 2021). Dyes can be categorized into two main types: natural dyes, which are derived from plants, and synthetic dyes. These dyes are further classified into anionic and cationic chemical structures. Cationic dyes contain functional groups that can dissociate in aqueous solutions, releasing positively charged ions. Examples include methylene blue, rhodamine, malachite green, and crystal violet. On the other hand, anionic dyes include acid dyes and reactive dyes, such as phenol red and methyl orange. These dyes typically contain sulfonic acid, anionic, or carboxylic acid groups (Lanjwani *et al.*, 2024). Methylene blue (MB), also known as methylthionine chloride, was initially used as a synthetic drug in clinical therapy. It is widely used in the textile industry for dyeing materials such as leather, wood, and cotton, and is also used in pharmaceutical production. However, MB poses significant health risks to humans, including increased heart rate, nausea, eye and skin irritation, and vomiting (Pinheiro *et al.*, 2020).

2.2. Nanotechnology and Nanomaterials

Nanotechnology, a key area of modern science, focuses on designing, synthesizing, and manipulating particle structures within the range of 1 to 100 nm (Ijaz *et al.*, 2021). Materials are classified as nanomaterials if their size, or at least one of their dimensions, falls within this range. In bulk materials, physical properties are independent of size, whereas in the case of nanomaterials (NMs), different physical properties can depend on the size and shape of the NMs. The importance of NMs became evident when researchers discovered that size influences the physicochemical properties of a substance, including its chemical, electrical, mechanical, and optical properties. Nanoparticles are defined as particulate dispersions or solid particles with sizes ranging from 10 to 100 nm in diameter. Metal NPs, such as gold, iron, silver, and other metallic NPs, have been studied, and it has been shown that these materials have unusual chemical, optical, and electrical properties as compared to bulk materials. Thus, NPs have a wide range of uses, including in the environmental health, mechanics and optics, biomedical sciences, chemical and electronic industries, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications (Remya *et al.*, 2017).

Metallic oxides synthesis are an interesting research area due to their intriguing physicochemical, electrical, magnetic and optical properties. Hence, the metal oxide nanoparticles such as nickel oxide, silver oxide, cobalt oxide, iron oxide, zinc oxide, tin oxide, titanium oxide, and copper oxide have been currently synthesized because of their fascinating properties for a large variety of applications in solar cells (Kumar *et al.*, 2020), gas sensors (Dey, 2018), photocatalysis (Mehtab *et al.*, 2022) and *etc.* Nanocomposites offer enhanced properties, such as increased mechanical strength, better thermal stability, optically efficient materials, and other beneficial features, when compared to pure polymers or traditional micro- and macro-sized composites. In recent years, the field of nanocomposites has attracted significant interest from scientists. This growing focus stems from the simple and powerful idea that incorporating nanoscale components into material design enables the creation of new materials with exceptional flexibility and improvements in their physical properties (Guerra *et al.*, 2018).

2.3. Synthesis Methods of Nanomaterials

Some synthesis techniques for nanocomposite was presented, and Figure 2 (Pal *et al.*, 2019) includes a schematic diagram for the synthesis of nanoparticles. Generally there are two approaches for nanomaterials synthesis. The top-down approach involves starting with a large material piece and using progressively finer tools to create smaller structures. The primary issue with this technique is the imperfections in the surface structure and the significant crystallographic damage in the processed patterns. The bottom-up approach involves smaller components at atomic or molecular scales self-assembling, either through natural physical principles or an externally applied driving force, to form larger and more organized systems. In nanotechnology, the bottom-up method is often preferred, though it is not a new concept.

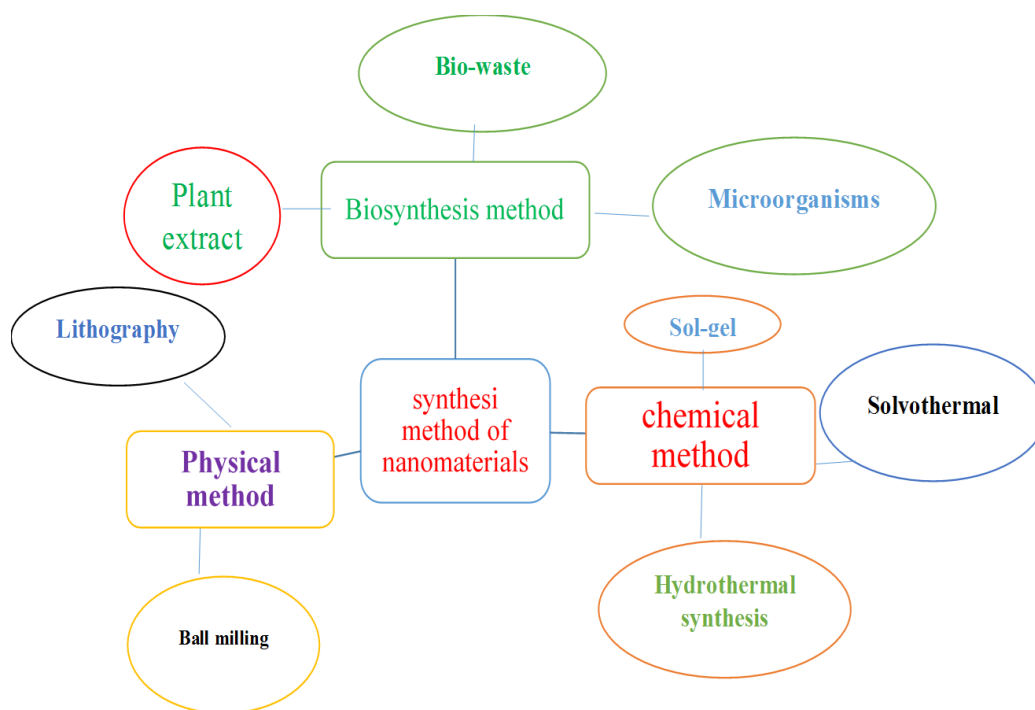


Figure 2. Different synthesis methods of nanomaterials

2.3.1. Biosynthesis Method

Biosynthesis refers to the process of synthesizing materials from environmentally friendly or sustainable sources by using an effective reducing agent, and a safe stabilizing agent (Danish

et al., 2022). As a result, scientists are increasingly interested in using biosynthesis to produce a variety of nanomaterials, including metal oxide nanoparticles, nanocomposite, and materials inspired by biological systems. Green synthesis is increasingly seen as a crucial method for reducing the harmful effects of traditional nanoparticle synthesis techniques used in laboratories and industry. In this context, conventional nanoparticle/nanocomposite synthesis methods, such as chemical and physical synthesis, are costly, hazardous, and environmentally unfriendly. To mitigate these harmful effects, researchers have identified specific pathways, naturally occurring sources, and their products that can be utilized to synthesize nanoparticles (Ahmed *et al.*, 2022). Therefore, there is a need for an eco-friendly, greener, and cost-effective approach to synthesize nanoparticles with high purity, crystallinity, and phase selectivity, homogeneity in particle shape, size, and texture (Singh *et al.*, 2017). In the literature, phytochemical analyses reveal that *R. prinoides* is rich in compounds such as tannins, flavonoids, saponins, glycosides, alkaloids, and phenols and applied for biosynthesis of nanomaterials (Habtemariam & Bekele, 2022).

2.4. Factors Affecting Green Synthesis of Nanomaterials

The synthesis of nanomaterials is influenced by several factors. Numerous studies have documented the dynamic properties of synthesized nanoparticles, which exhibit various properties due to changes in time, environmental conditions, and other variables. Key physicochemical parameters such as the pH of the solution, the molar ratio of metal salt concentration, temperature, volume of plant extract, and synthesis time significantly impact the rate of synthesis, as well as the stability, size, morphology, and quality of NPs and NCs (Shreyash *et al.*, 2021). Below, some of the primary factors influencing nanoparticle biosynthesis are discussed.

a) pH of The Reaction Medium: The pH of the reaction medium has a major role in the formation of nanoparticles. As the quantity of hydrogen ions fluctuates, so do the nanoparticles' size and its formation. Furthermore, researchers have demonstrated that larger particles develop at lower acidic pH levels than alkaline pH levels (Rahnama *et al.*, 2021). Mohammadi and Ghasemi (2018) utilized Cherry fruit extract for the synthesis of ZnO NPs at various pH levels (4, 6, 7, 8, and 10). The pH had an impact on the reaction, and at strongly alkaline conditions, the formation of ZnO NPs was hindered due to the presence of

abundant OH ions reacting with Zn^{2+} cations to form $\text{Zn}(\text{OH})_2$ complexes. The study found optimal pH for green synthesis of small-sized hexagonal ZnO NPs using cherry fruit extract was found to be pH 8.

b) Effect of Metal Precursor Concentration: The concentration of the reaction mixture is an important factor that controls the size of nanoparticles. Rahuman *et al.* (2021) conducted a study on the synthesis of silver NPs using *Carissa carandas* leaf extracts. They found that the optimal concentration of silver nitrate was 1.25 mmol/L. By varying the metal ion concentrations in the solution, which ranged from 0.25 to 2.5 mmol/L, they observed a pronounced surface plasmon resonance (SPR) peak at 410 nm indicating the successful synthesis of silver NPs.

c) Temperature: Temperature is another important factor that affects the three distinct methods of nanoparticle synthesis. The physical method requires the highest temperatures ($>350\text{ }^{\circ}\text{C}$), while the chemical method operates at lower temperatures. In contrast, green technologies typically require ambient temperature or temperatures below $100\text{ }^{\circ}\text{C}$ for nanoparticle synthesis. The type of nanoparticle formed depends on the temperature of the reaction medium (Oje *et al.*, 2021). Takele *et al.* (2023) investigated the effect of synthesis temperature on ZnO NPs within the temperature range of $25\text{--}100\text{ }^{\circ}\text{C}$, identifying $90\text{ }^{\circ}\text{C}$ as optimum temperature, as higher temperature accelerate the reduction of metal ions to nanoparticles.

d) Plant Extract Concentration: The concentration of plant extract plays a crucial role in the synthesis of NPs because it provides the electrons needed for the reduction and stabilization of metal ions. It significantly influences the particle size distribution and shape of green-synthesized NPs. Kora and Rastogi (2018) studied the synthesis of palladium NPs using *Anogeissus latifolia* gum and found that the gum concentration affected the reduction rate of palladium ions. Increasing the gum concentration from 0.1% to 0.5% promoted the formation of small-sized palladium NPs. The optimal concentration for synthesizing small-sized palladium NPs was found to be 0.5 % extract.

e) Time: The quality and shape of nanoparticles are significantly influenced by the length of incubation in the nanoparticle reaction medium (Pal *et al.*, 2019). Turki *et al.* (2022) examined the effect of reaction time on the synthesis of CdS nanoparticles. To determine the

optimal time for producing stable CdS particles, the reaction mixture containing CdCl₂, avocado extract, and Na₂S was stirred on a magnetic stirrer for various time intervals (0h, 2h, 4h, and 6h). The resulting solutions were then analyzed using a UV-Vis spectrophotometer, and the optimal time for nanoparticle formation was found to be 6 hours.

2.5. Synthesis of Metal Oxide Nanoparticles

2.5.1. Synthesis of Zinc Oxide Nanoparticles

Zinc oxide is a desirable n-type semiconductor and promising inorganic material that has gained significant attention in recent years due to its high stability, environmental benefits, exceptional electrical and optical characteristics (Song *et al.*, 2017). Numerous sources describe ZnO NPs as a unique material with a wide band gap of 3.37 eV, along with a range of attractive properties. ZnO NPs materials can therefore be used in a variety of industries, including optoelectronics, solar energy, catalysts, nanomedicine, pharmaceuticals, and cosmetics. ZnO NPs can only absorb UV light with a wavelength equal to or less than 385 nm. Therefore, to absorb visible light, the band gap of ZnO NPs must be narrowed, which can be achieved by doping with transition metal ions. Combining ZnO NPs with other materials that have different energy levels to form a heterojunction is an effective technique for enhancing its photocatalytic activity, maximizing the use of the solar spectrum, and improving its antibacterial properties. ZnO NPs are non-toxic, semiconducting materials known for their high transparency, excellent photocatalytic properties, and strong antibacterial activities. Recently, there has been growing interest in the biosynthesis of ZnO NPs, which uses biological agents as reducing, stabilizing, and capping agents.

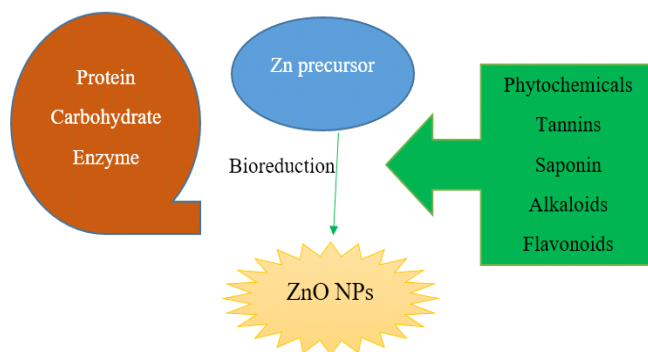


Figure 3.Biosyntheis of ZnO nanoparticles

The biosynthesis of ZnO NPs can be achieved using either plant extracts or microbes, providing a sustainable and safer alternative to conventional chemical synthesis methods (Figure 3) (Ahmed *et al.*, 2017). ZnO NPs synthesized by *Euphorbia prolifera* leaf extract, which acted as both a reducing and stabilizing agent, showed effective catalytic activity in the degradation of methylene blue (MB) dye, as reported by (Momeni *et al.*, 2016). Karnan and Selvakumar (2016) synthesized ZnO NPs via a green route using *Nephelium lappaceum* L. fruit peel extract. They reported remarkable photocatalytic degradation (83.99 %) of methyl orange azo dye by the ZnO NPs within 120 min under UV light. Rambabu *et al.* (2021) used *Phoenix dactylifera* as a reducing and capping agent for the synthesis of ZnO NPs to evaluate their photocatalytic and antibacterial activities. The study reported a final methylene blue (MB) degradation efficiency of 90.5 % after 180 minutes. Antibacterial tests demonstrated that ZnO NPs acted as an effective bactericide, with performance comparable to the commercial antibiotic ciprofloxacin. The ZnO NPs exhibited strong antibacterial effects against both Gram-positive and Gram-negative bacterial strains. Anand *et al.* (2019) investigated the antibacterial properties of ZnO NPs synthesized using *Prunus dulcis* extract through a disk diffusion method. Their study found that the synthesized ZnO NPs effectively inhibited the growth of *S.aureus*, *E.coli*, and *Salmonella paratyphi*.

2.5.2. Synthesis of Silver Oxide Nanoparticles

Green synthesis is a promising alternative to many physicochemical approaches due to its safety, low cost, low toxicity, reproducibility, and potential for large-scale synthesis of Ag₂O NPs. Recently, it has been discovered that Ag₂O NPs possess favorable characteristics for use in photovoltaic cells, sensors, data storage devices, medicinal applications, and photocatalysis. Ag₂O NPs have been explored in many studies focusing on their synthesis, characterization, and photocatalytic, and antibacterial performance. Widyaningtyas *et al.* (2019) investigated the photocatalytic activity of *Vernonia amygdalina* Del.-mediated Ag₂O NPs, which exhibited good performance in the visible region, degrading 71.59 % of methylene blue (MB) in 120 min with a maximum catalyst weight of 3 mg. Additionally, (El-Ghmari *et al.*, 2021) used *Herniaria hirsuta*-mediated Ag₂O nanoparticles to achieve better catalytic activity for the degradation of MB dye under standard conditions, reaching a degradation rate of 89 % due to their morphology and small size. Muthukumar *et al.* (2022)

utilized green amaranth aqueous leaf extract-mediated Ag₂O NPs for antibacterial and photocatalytic activities. Antibacterial tests using the disk diffusion method showed that the synthesized NPs were more effective against Gram-negative *P. aeruginosa* than against Gram-positive *S.aureus*. Furthermore, photocatalytic degradation of caffeine using the NPs resulted in 99 % degradation at an initial concentration of 50 ppm. Flores-Lopez *et al.* (2021) synthesized Ag₂O nanoparticles using *Aloe vera* plant extract via a green synthesis method, demonstrating excellent antibacterial activity against both Gram-negative *E. coli* and Gram-positive *S.aureus*.

2.5.3. Synthesis of Cobalt Oxide Nanoparticles

Due to its versatile properties, Co₃O₄ NPs has been widely applied in various fields, including catalysis, sensors, batteries, electrochromic films, heterogeneous catalytic materials, magnetic materials, electrochemical devices, and solar selective absorbers (Waris *et al.*, 2021). The synthesis of Co₃O₄ NPs using plant extracts has gained significant attention due to its environmentally friendly, simple, rapid, non-toxic, and cost-effective nature. Mulya Dewi and co-workers synthesized Co₃O₄ NPs using *Euphorbia heterophylla* leaf extract and investigated photocatalytic activity. The synthesized NPs were spherical in shape, with sizes of 69.75 nm. The photocatalytic activity of the green-synthesized Co₃O₄ NPs was evaluated using MB dye, and the results showed that the Co₃O₄ NPs exhibited good photocatalytic performance, achieving up to 63.10 % degradation under visible light irradiation (Mulya Dewi *et al.*, 2019). A graphical description of the synthesis of Co₃O₄ NPs from plant materials for effective application is provided in Figure 4(Pagar *et al.*, 2019).

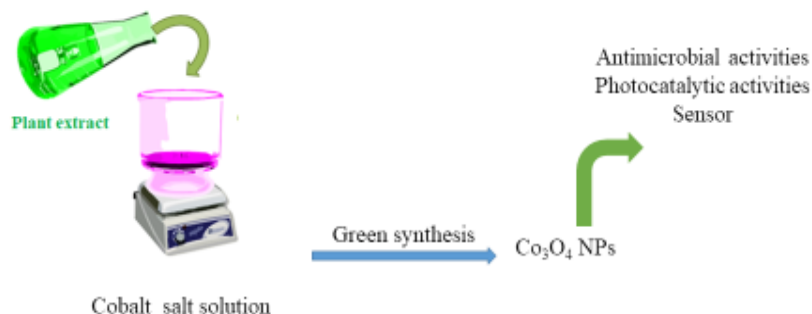


Figure 4. Green synthesis of cobalt oxide nanoparticle

Moreover, Bibi and co-workers synthesized Co_3O_4 NPs using *Punica granatum* peel extract and studied the photocatalytic activity of the NPs. The synthesized NPs were spherical with the particle size of 43.78-73.10 nm. The photocatalytic activity of the green synthesized Co_3O_4 NPs was evaluated to degrade remazol brilliant orange 3R (RBO 3R) dye and results indicate Co_3O_4 NPs shows excellent photocatalytic activity (degraded up to 78.45% (Bibi *et al.*,2017). Chelliah *et al.* (2023) used a green, cost-effective, and environmentally friendly biosynthesis method to produce cobalt oxide (Co_3O_4) nanoparticles using *Curcuma longa* (turmeric) plant extract for photocatalytic and antibacterial evaluations. The biogenic Co_3O_4 nanoparticles were tested against both Gram-positive (*S. aureus*) and Gram-negative (*E.coli*) bacteria. The results showed that the Gram-positive bacterial strain exhibited a greater resistance to the Co_3O_4 nanoparticles. Additionally, the photocatalytic activity of the Co_3O_4 nanoparticles was assessed under visible light for the degradation of Methylene Blue (MB) dye, achieving a significant degradation rate of 90%.

Bekele *et al.* (2022) synthesized *Solanum tuberosum* extract-mediated Co_3O_4 NPs for antibacterial applications. The synthesized Co_3O_4 NPs were thoroughly characterized using various techniques, including X-ray diffraction (XRD), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX), transmission electron microscopy (TEM), surface area electron diffraction,UV-Vis diffuse reflectance spectroscopy, and Fourier transform infrared (FTIR) spectroscopy. The Co_3O_4 nanoparticles exhibited enhanced antibacterial activity, showing a zone of inhibition of 16 mm against *S. aureus*. This activity was attributed to the formation of enhanced reactive oxygen species (ROS) by the nanoparticles. Sivachidambaram *et al.* (2017) synthesized 15 nm Co_3O_4 NPs using *Azadirachta indica* (neem) leaf extract and reported antimicrobial activity against both Gram-positive and Gram-negative bacteria. In addition to their antibacterial properties, the Co_3O_4 NPs were used as catalysts in the hydrogenation of 4-nitrophenol and 4-nitroaniline, demonstrating their potential in catalytic applications.

2.6. Synthesis Binary Nanocomposite

Multi-nanostructure materials, such as nanocomposite, demonstrate enhanced properties and activity due to the synergistic improvement of the physicochemical characteristics of their

individual nanocomponent (Fatimah *et al.*, 2020). The synthesis of heterogeneous nanomaterials within a single nanocomposite has attracted significant attention from scientists in recent years. This is because the combination of multifunctional intrinsic properties generates new characteristics that are not found in single nanocomponents, enabling the synthesis of efficient hybrid nanocomposite with high activity.

2.6.1. Synthesis of Ag₂O/ZnO Nanocomposite

Ag₂O NPs is an ideal co-catalyst for ZnO to enhance photocatalytic performance (Lamba *et al.*, 2017). Fayyadh and Alzubaidy (2021) reported an eco-friendly biological synthesis method using silver nitrate, zinc nitrate hexahydrate, and *Lawsonia inermis* (Henna) plant extract to synthesize ZnO/Ag₂O NCs. The synthesized nanocomposite were characterized using various techniques, including XRD(X-ray diffraction),scanning electron microscopy (SEM), energy dispersive X-ray (EDX),FTIR(Fourier Infrared Transformed), zeta potential, and dynamic light scattering (DLS).Finally, the antifungal activity of the ZnO/Ag₂O NCs was investigated.A comparison of various synthetic methods and the applications of Ag₂O/ZnO NCs is presented in Table 1. Li *et al.* (2024) proposed the potential antibacterial and photocatalytic activities of ZnO/Ag₂O NCs, which were characterized by UV-Visible spectrophotometry(UV-Vis), X-ray photoelectron spectroscopy (XPS), XRD, TEM(Tranission electron microscope), and SEM. The results showed that the nanocomposite were capable of degrading 95.6 % of Congo red dye within 180 min. Additionally, the nanocomposite exhibited inhibition zones of 13.5 mm for the Gram-negative *E. coli* and 12.9 mm for the Gram-positive *Bacillus subtilis*, indicating strong antibacterial properties.

Table 1. Synthetic method, characterization techniques, and properties/applications of some Ag₂O/ZnO nanocomposite

Composition and morphology	Synthetic method	Characterization Techniques	Application	Reference
ZnO/Ag ₂ O NCs	Biosynthesis method	SEM,TEM,XPS	Photocatalytic activities and antibacterial activities	Ahmad <i>et al.</i> ,2020
ZnO/Ag ₂ O irregular nanoflakes	Solid state method	XRD,TEM,SE,F TIR	Photocatalytic activities	Yang <i>et al.</i> ,2018
ZnO/Ag ₂ O nanoflowers	Ionic liquid assisted method	XRD,TEM,EDX ,BET	Photocatalytic activities	Zhao <i>et al.</i> ,2017
ZnO/Ag ₂ O NPs	Thermolysis and deposition	XRD,FTIR, EDX,SEM, TEM, UV–Vis	Photocatalytic activities	Kadam <i>et al.</i> ,2016
ZnO/Ag ₂ O NPs	Biosynthesis method	XRD,DLS,EDX, SEM,FTIR	Antifungal efficacy	Fayyadh & Alzubaidy,2021
ZnO/Ag ₂ O NPs	Photochemical synthesis	TEM,EDX,UV-Visible	Photocatalytic activities	Ma <i>et al.</i> ,2014
ZnO/Ag ₂ O NPs	chemical method	SEM,PL	Antibacterial activity	Gultom <i>et al.</i> ,2024
ZnO/Ag ₂ O NCs	Biosynthesis method	XRD,FTIR,TEM	Antibacterial activities	Da Silva <i>et al.</i> 2024

2.6.2. Synthesis of Co₃O₄/ZnO Nanocomposite

The combination of p-type Co₃O₄ NPs and n-type ZnO NPs represents the right approach for successful development of p-n heterojunctions as these heterojunctions can provide built-up the p-n interface that can subsequently enhance the overall photocatalytic and antibacterial

activity of fabricated composite material. $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite have been synthesized by using the chemical, biosynthesis, and physical methods. Table 2 presents the synthesis method, characterization, and some applications of $\text{Co}_3\text{O}_4/\text{ZnO}$ NCs. Shirzadi-Ahodashi *et al.* (2020) reported the chemically synthesized $\text{Co}_3\text{O}_4/\text{ZnO}$ NCs appeared to possess a particle size of 12 nm and had a band gap value of 4.97 eV which was larger than their parent materials. The hydrothermal/sol-gel method synthesized $\text{Co}_3\text{O}_4/\text{ZnO}$ core-shell NPs with an average inner core material size of 22 nm and outer shell with 56 nm particle sizes from the TEM image was also reported by (Tadesse *et al.*, 2023).

Table 2. Synthesis method, characterization techniques, and properties/applications of some $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite

Synthetic method	Characterization Techniques	Application/properties	reference
Green synthesis method	XRD, SEM, FTIR	photocatalytic activities	Aragaw <i>et al.</i> , 2020
Hydrothermal synthesis	UVVis, XRD, XPS, SEM	sensor application	Yang <i>et al.</i> , 2019
Solid state reaction	SEM, TEM, XRD, BET, PL	Photocatalytic activities	Reda <i>et al.</i> , 2017
thermal decomposition	XRD, FTIR, TEM	magnetic properties	Shafiu <i>et al.</i> , 2014
Biosynthesis method	XRD, TEM, SEM, FTIR	antibacterial activities	Dwivedi <i>et al.</i> , 2023
Biosynthesis method	XRD, SEM, EDX	antibacterial activities	Tadesse <i>et al.</i> , 2023

2.6.3. Synthesis of $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ Nanocomposite

$\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ nanocomposite are a combination of Ag_2O NPs and Co_3O_4 NPs that have been used in various applications. Table 3 provides the synthesis method, characterization, and some applications of $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs. Saeed *et al.* (2022) reported the synthesis and characterization of an $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ Z-scheme photocatalyst and evaluated its photocatalytic performance using an aqueous solution of reactive yellow dye as a model organic pollutant. The $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ photocatalyst was synthesized using precipitation and impregnation methods, respectively. Advanced techniques including XRD, UV-Vis, SEM, TEM, and XPS were used for the characterization of the synthesized $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs. It was found that coupling Ag_2O NPs with Co_3O_4 NPs enhanced the photocatalytic performance from 55% to

100% for a 50 mL dye solution containing 100 mg/L of dye. In another study, (Li *et al.*,2017) reported the solvothermal synthesis of Ag₂O/Co₃O₄ NCs to investigate its electrochemical properties, demonstrating excellent cycle performance, with 65.3% of specific capacitance retained after 200 cycles. Additionally, the structures and morphologies of the synthesized samples were characterized using XRD, SEM, and TEM.

Table 3.Synthetic method, characterization techniques, and properties/applications of some Ag₂O/Co₃O₄ nanocomposite

Synthetic method	Characterization Techniques	Application/properties	reference
Chemical process	XRD,SEM-EDX	Sensor	Khan <i>et al.</i> ,2024
Coprecipitation and sol gel method	XRD,SEM,BET	catalytic activities	Balikci <i>et al.</i> ,2008
chemical method	XRD,PL	photocatalytic activities	Mohamed <i>et al.</i> ,2022

2.7. Synthesis of Ternary Nanocomposite

Recently, researchers have expanded the development of binary nanomaterial systems to ternary nanostructures, further improving the performance of these materials. These newly developed ternary nanostructures exhibited higher efficiency and novel properties compared to binary systems, offering enhanced potential for various applications (Maguire *et al.*, 2021).Numerous studies have detailed the synthesis of ternary nanocomposite through various nanomaterial synthesis methods. For example, Meena *et al.* (2021) synthesized ZnO/CuO/Ag₂O nanocomposite via stepwise grafting, achieving a 99.05 % degradation efficiency of crystal violet under solar irradiation within 105 min. Similarly, Kale *et al.* (2020) developed rGO-ZnS-TiO₂ nanocomposite using a sonochemical approach, which resulted in 97 % degradation of crystal violet after 50 minutes of UV light irradiation. A summary of selected Ag₂O-based ternary nanocomposite is provided in Table 4.

Table 4. Different techniques for the synthesis and characterization of various Ag₂O based ternary nanocomposite and their prospective applications in various fields.

Name of composite	Synthesis route	Techniques used for characterization	application	Reference
Ag ₂ O/SrO/CaO	Co-precipitation method	SEM, XRD	antibacterial activity	Aghaee & Manteghi, 2022
Ag ₂ O/B ₂ O ₃ /TiO ₂	combination of green and precipitation methods	SEM, UV-Vis, XRD, XPS	Photocatalysis	Tijan <i>et al.</i> , 2019
Ag ₂ O/ZnO/CuO	Temperature-programmed oxidation method	SEM, UV-Vis, XRD, EDX, XPS	Photocatalysis	Salari & Sadeghinia, 2019
Ag/Ag ₂ O/ZnO	green synthesis method	SEM, TEM, UV-Vis, XRD, FTIR, EDX	Photocatalysis	Ding <i>et al.</i> , 2019
ZnO/Ag ₂ O/Co ₃ O ₄	wet chemical method	FTIR, XPS, XRD, SEM, TEM, EDX, and UV-vis	Sensor	Alam <i>et al.</i> , 2019
ZnO/CuO/Co ₃ O ₄	wet chemical method	SEM, XRD, FTIR, UV-Vis, XPS	Sensor	Alam <i>et al.</i> , 2019

2.8. Botanical Description of *Rhamnus prinoides* and Its Medicinal Values

Rhamnus prinoides L'Herit (*R. prinoides*), Gesho in (Tigrigna, Afan Oromo, Amharic), Gishe (Guragegna) and Geshu (Agewgna), is an ever-green medicinal plant that has been used traditionally for the treatment of different infectious diseases (Molla *et al.*, 2016). In Ethiopia, the leaves, fruits, or roots of *R. prinoides* (Figure 5) are used to treat scabies, hepatitis, tinea capitis, “chifea” (eczema), ringworm, and dandruff, and for the management of waterborne and related diseases (Chen *et al.*, 2020).



Figure 5. *Rhamnus prinoides* (Picture by Mosisa Dugasa, April 2024, Nekemte, Ethiopia)

Agidew (2022) reported the taxonomical classification of *R.prinoides*, as shown in Table 5.

Table 5. Taxonomical classification of *R.prinoides*

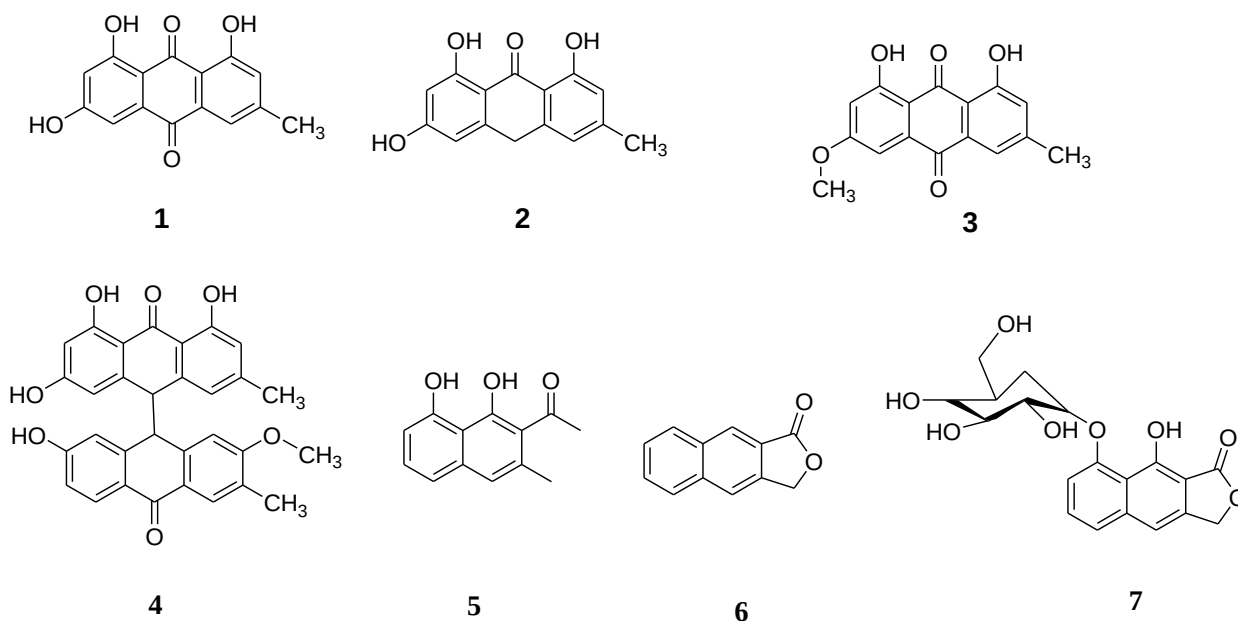
Kingdom	Plantae
Order	Rhamnales
Family	Rhamnaceae.
Genus	Rhamnus
Species	Prinoides
Botanical Name	<i>Rhamnus prinoides</i> L'Herit

Nigussie *et al.* (2021) provided a comprehensive review of the pharmacological and traditional uses of *R. prinoides* (Table 6). Their study revealed that in Angola, the bark is used to induce vomiting, while in South Africa, a decoction of the root is consumed to cleanse the blood by the Zulu people and to treat pneumonia by the Sotho peoples. In Kenya, the boiled soup of the root is used to treat the common cold, the leaves are used for chest pain, and both leaves and stems are used for treating tonsillitis in central Kenya. Additionally, in Kenya, the leaves are used to prepare "muteta," a soup that acts as an appetizer. In Ethiopia, a mixture of the leaves and roots of *Rubus apetalus* Poir is boiled, decocted, and consumed before meals as a remedy for sexually transmitted diseases (Tuasha *et al.*, 2018).

Various classes of secondary metabolites such as alkaloids, triterpenes, saponins, tannins, phenols, cardiac glycosides, anthraquinones, polyphenols, and flavonoids have been

identified in different parts of *R. prinoides*, with tremendous antioxidant and anti-inflammatory activities provided further scientific evidences for its use as folk medicine and with good potential for future healthcare practice (Chen *et al.*, 2020). A range of specific compounds have also been reported, including Emodin (**1**), Emodinanthrone (**2**), Physcion (**3**), Emodinbianthrone (**4**), Musizin (**5**), β -sorigenin (**6**), Geshoidin (**7**), Rhamnocitrin (**8**), Rhamnazin (**9**), Prinoidin (**10**), Rhamnetin (**11**), Quercetin (**12**), Chrysophanol (**13**), and 3-O-Methylquercetin (**14**), Bufotenine (**15**). Additionally, essential oils such as 4-hydroxy-4-methyl-2-pentanone (**16**) and ethyl 4-ethoxybenzoate (**17**) have been isolated from the leaves (Figure 6). It has been shown that *R. prinoides* is an effective reducing and stabilizing agent for the successful synthesis of Ag, and WO₃ nanoparticles (Habtemariam & Alemu, 2021).

The plant also serve as an important hopping agent, making traditional alcoholic beverages like tella and tej (in Ethiopia), animal feed, medicine, nectar for bees, and soil conservation.



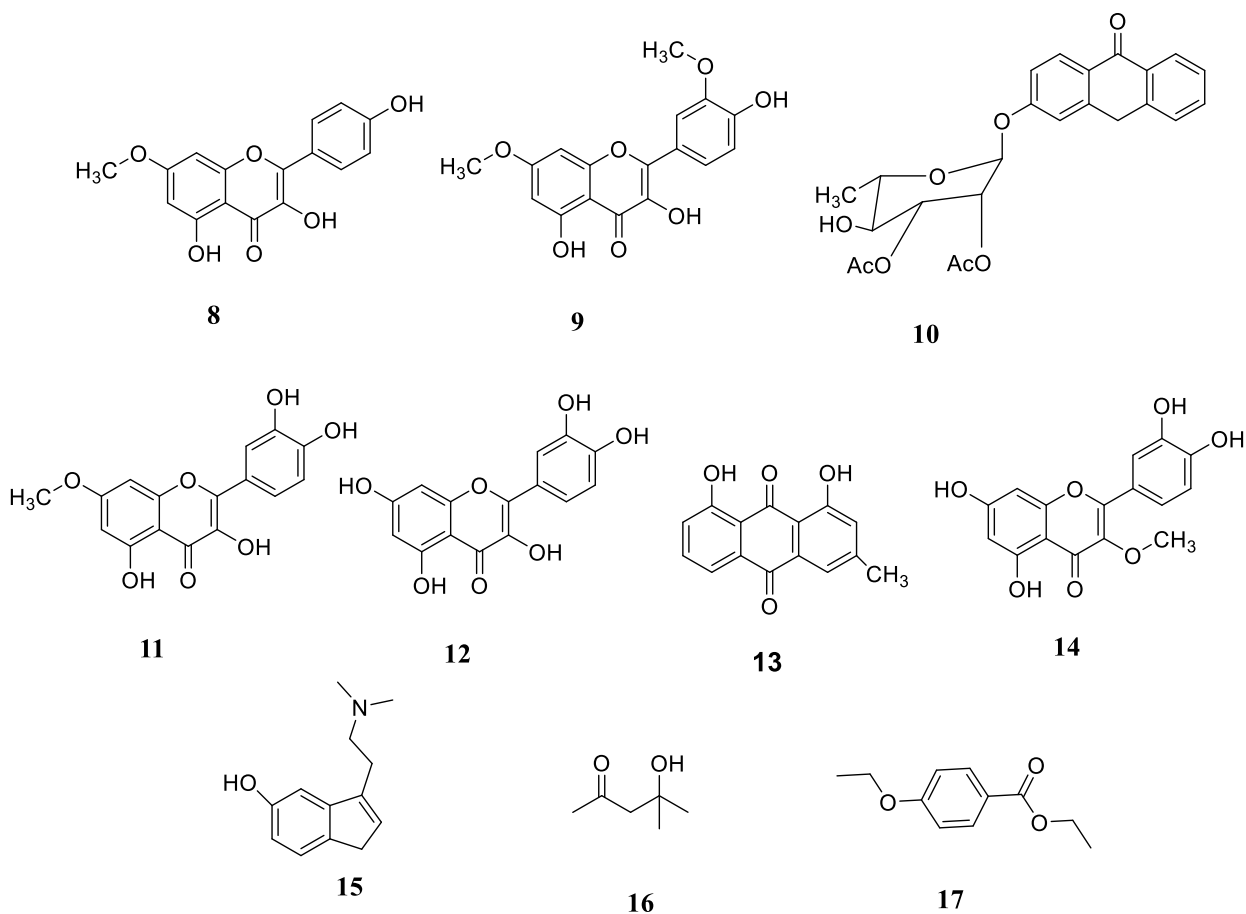


Figure 6.Structure of compounds isolated from *Rhamnus prinoides*

Table 6.Ethnomedicinal uses of *R. prinoides* in Africa

Disease treated	Plant part used	Mode of preparation	Country practiced	Reference
Tonsillitis	Leaves	Leaves infusion Gargle to rinse the throat	Ethiopia	Teka <i>et al.</i> , 2020
Skin infection	Leaves	Fresh leaves rubbed it the affected part	Ethiopia	Tamene, 2020
Malaria	Bark roots		South Africa	Cock <i>et al.</i> , 2019
Sexually transmitted diseases	Leaves	The leaf is boiled, and drunk before meal	Ethiopia	Tuasha <i>et al.</i> , 2018

2.9. Characterization Techniques of Nanomaterials

Characterization is an important step in identifying the nanoparticles morphology, shape, pore size; particles size distribution *etc.* The general techniques for nanomaterial characterization is given on Figure 7. The optical properties, functional group presents, crystal structure, morphology, and chemical composition of the various prepared nanomaterials are determined by Ultraviolet Visible Spectroscopy (UV-Vis), Fourier Transformed Infrared (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Electron Dispersive X-ray (EDX), respectively (Mourdikoudis *et al.*, 2018).

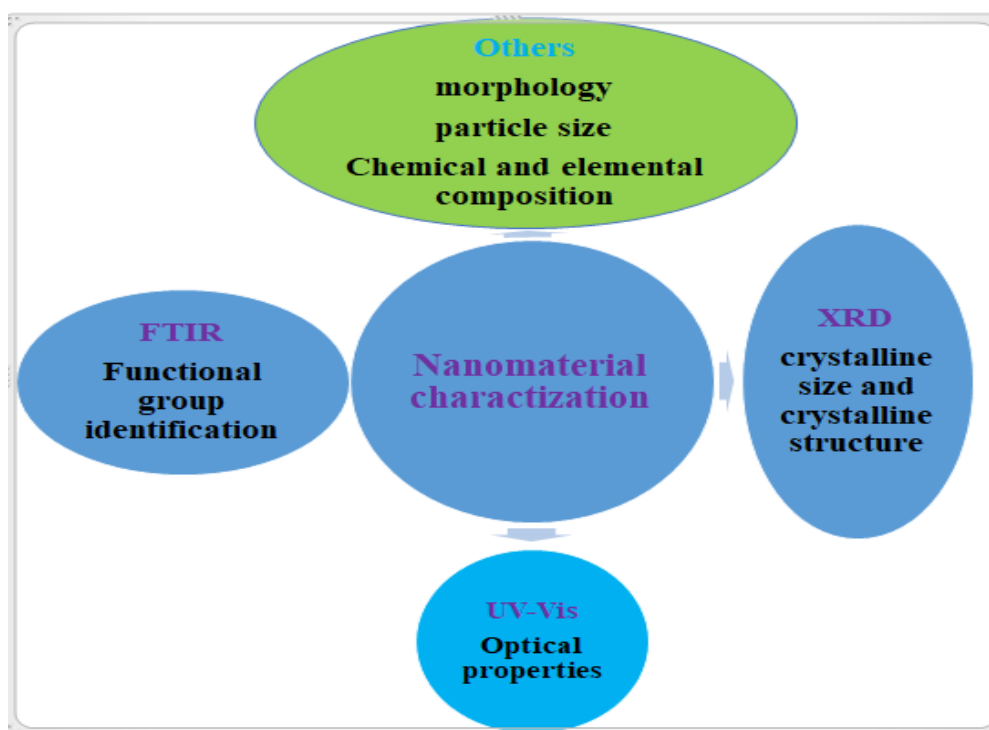


Figure 7. General techniques available for the characterization of nanomaterials

2.9.1. UV-Visible Spectroscopy

UV-visible absorption spectroscopy is commonly used to analyze the optical properties of nanosized particles (Rathinavel *et al.*, 2020). This technique is particularly useful for determining the energy band gap (E_g) of nanoparticles. UV-visible spectroscopy focuses on

the interactions between matter and electromagnetic radiation in the ultraviolet-visible range. It is often referred to as "electronic spectroscopy" because it involves the excitation of the outermost electrons of atoms, which are involved in molecule formation. Alam *et al.* (2019) studied the optical absorbance of ZnO/Ag₂O/Co₃O₄ nanocomposite and found the optical band gap energy of 4.31 eV based on the average absorption band observed.

2.9.2. Fourier-Transformed Infrared (FTIR) Spectroscopy

Fourier transformed infrared (FTIR) spectrophotometer is used to detect functional groups involved in synthesized nanoparticle and nanocomposite (Manikandan *et al.*, 2022). This technique helps us to determine which bonds are responsible for the synthesis of NCs. FTIR spectrum is usually given as a graph of transmittance or absorbance percentage as a wavelength function in the mid-infrared, with a wavelength range between 400 cm⁻¹ to 4000 cm⁻¹. For example, the FTIR spectrum of ZnO/Ag₂O/Co₃O₄ NCs shows three absorption peaks at 560, 660 and 1100 cm⁻¹. The peaks at 560 and 660 cm⁻¹ are associated with the stretching vibrations of the Ag–O and Zn–O bonds, respectively, as reported previously by (Alam *et al.*, 2019) and the peak at 1100 cm⁻¹ was generated due to the stretching vibration of the C–O bond.

2.9.3. X-ray Diffraction (XRD)

XRD is a quick analytical technique primarily utilized for crystalline material structural characteristics and phase identification. The sample being studied, an x-ray source (monochromatic or with varying wavelengths), and a detector that captures the diffracted x-rays are all necessary for this technique. Powder x-ray diffraction is used for phase determination and unit cell information of the nanocomposite under investigation (Farrokhi *et al.*, 2014). Alam *et al.* (2019) also investigated XRD analysis of ZnO/Ag₂O/Co₃O₄ ternary nanocomposite to confirm the formation of ternary nanocomposite.

2.10. Application of Nanomaterials

2.10.1. Photocatalytic Activity

A special technique called photocatalysis can be used for a number of things, including hydrogen production, and the breakdown of several organic contaminants in wastewater,

antibiotic activity, and air cleansing. In recent years, there has been a rise in interest in one of these applications on the degradation of organic contaminants in wastewater. The photocatalytic process is drawing more interest in wastewater treatment than other techniques since it removes dyes under conditions of moderate temperature and pressure. Additionally, since no sludge is created and visible or near-UV light is used as the irradiation source, the photocatalytic process is more advantageous advanced oxygen process (Fagier, 2021).

The use of photocatalytic degradation of methylene blue (MB) as a model reaction to assess the photocatalytic capabilities of nanomaterials has become a widely adopted practice in recent years (El-Sheshtawy *et al.*, 2019). Several factors contribute to the popularity of this reaction: a) MB is a known carcinogenic pollutant, making its removal from aqueous media is highly desirable; b) MB is a UV-visible (UV-Vis) active species with distinct peaks in the UV-Vis spectrum, enabling easy qualitative analysis using simple UV-Vis spectroscopy; c) MB is a colored dye that exhibits a blue color in its oxidized state, making its presence visually detectable in any medium; d) Tracking the progress of the degradation reaction is simple, as the blue color of MB fades when it degrades into smaller molecules under the influence of radiation-activated photocatalytic nanomaterials in the reaction medium. Experimental evidence for the degradation can also be easily obtained by monitoring the UV-Vis spectrum, where the characteristic absorption peaks of MB disappear; e) Following photocatalysis, MB undergoes transformation into water (H_2O), carbon dioxide (CO_2), and other by-products, such as ammonium, nitrate, or sulfate ions. This process converts the harmful MB dye into much less toxic by-products. The first step of the photocatalysis is the activation of the NMs by the reconstruction of the NMs surface via electromagnetic radiation illumination as shown by equation 1 and (Figure 8) as reported by (Karnan & Selvakumar, 2016). The electromagnetic radiations interact with the surface Plasmon's of the NMs (*e.g.*, ZnO NPs) and excite the electrons into the higher states. Upon the excitation of the negatively charged electron (e^-), the positively charged hole (h^+) is created in its place. These e^-/h^+ pairs are responsible for the photocatalytic capability of the NMs. The energy required for this activation is equal to the band gap (the difference of the energy values between the valence and conduction band) of the NMs. This required energy is provided by the incident electromagnetic radiations. These generated e^-/h^+ can undergo recombination

reactions via relaxation processes as indicated in equation (1) which would lead to the deactivation of the photocatalyst.

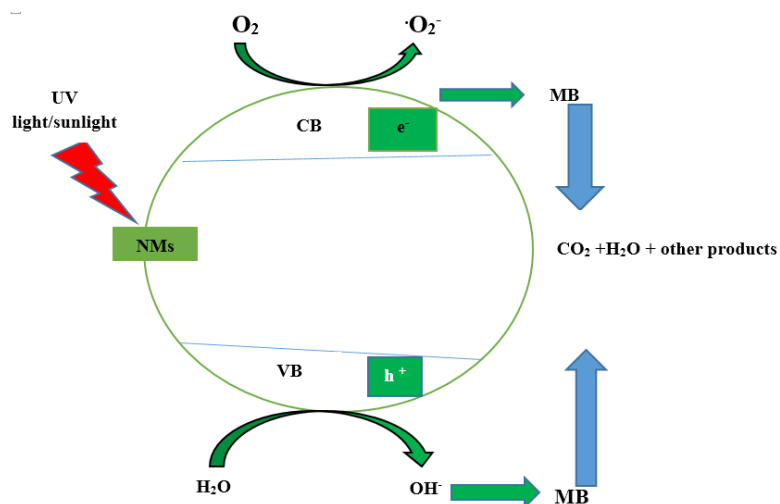
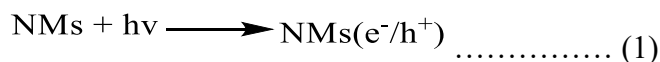
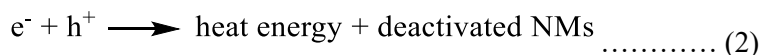
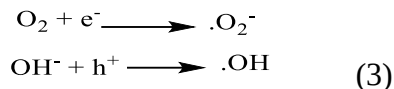


Figure 8.General Mechanism for Photodegradation of Methylene blue

Therefore, avoiding recombination reactions is essential for achieving the maximum photocatalytic efficacy of the NMs. The generated e^- reacts with the atmospheric oxygen and expedites the formation of superoxide radicals ($\cdot\text{O}_2^-$) as indicated by equation (2).



The presence of the H_2O further facilitate the photocatalytic reaction by the interaction of the hydroxyl ions (OH^-) with the h^+ and results in the formation of the hydroxyl free radicals ($\cdot\text{OH}$) in the reaction medium as shown by equation (3). These reactive and non-selective radicals interact with the dye (MB) and the process of demethylation occur leading to the breakage of the N- CH_3 bond. This step is followed by the attack of the radical species on the aromatic ring. Most of the reaction intermediates come from the breakage of the aromatic ring of the MB. Few case studies also document the generation of hydroperoxyl species ($\cdot\text{OOH}$) achieved by the protonation of $\cdot\text{O}_2^-$ which results in the formation of hydrogen peroxide (H_2O_2) in the reaction medium (King *et al.*, 2019).



Various parameters influence the rate of dye adsorption onto nano-adsorbents, including pH, catalyst load, dye concentration, and contact time. Analyzing these parameters will help elucidate the mechanism of adsorption and degradation. Several physicochemical adsorption mechanisms are used for dye treatment, including electrostatic interactions, hydrogen bond formation, surface complexity, and π - π^* interactions, among others. Apart from adsorption, the photodegradation mechanism is also used by researcher for the removal of colored dyes which are not easily removed by using simple techniques (Saharan *et al.*, 2023). Various factors that effect of photocatalysts are discussed in brief as follows:

a) Effect of pH: pH is a parameter that indicates the acidity or basicity of a given sample. Not only that, pH is an extremely important and simple parameter which determines most chemical reaction in aquatic medium. Too high or too low pH value can be harmful to aquatic life, which suggests that aquatic species are sensitive to change in pH. The optimum solution pH that maximizes degradation efficiency may vary depending on the synthesis method used, whether pure or modified nanoparticles are used, and the nature of the azo dyes. For example, (Rahnama *et al.*, 2021) investigated the photodegradation of Congo red in a universal buffer across a pH range of 6.0–12.0 using Ag_2O - Ag_2CO_3 /rGO ternary nanocomposite. They observed that the color of Congo red was blue in the pH range of 2.0–6.0. Higher degradation of Congo red was observed at pH 7.0 within 10 minutes of irradiation. In another study, (Subhan *et al.*, 2020) reported an optimal pH of 9 for methyl violet degradation, observed during 60 minutes of irradiation. Similarly, (Tijani *et al.*, 2019) found the highest degradation efficiency at an optimal pH of 7.60 for local dyes (total organic carbon and chemical oxygen demand) over a period of 150 minutes. Zhang *et al.* (2017) studied the effect of initial pH in the range from 3 to 11 and found an optimum pH of 7 after 60 minutes of irradiation for the degradation of RhB.

b) Effect of Catalyst Dose: Generally, the amount of photo mineralization of water pollutant increases with an increase in the amount of catalyst dosage. This is due to the exposure of more active sites on the photocatalyst, which adsorb more photons and produces more OH radicals and positive holes under irradiation (Murillo-Sierra *et al.*, 2021). However, adding

more catalyst than optimum value can hinder rate of catalysis owing to formation of particle agglomeration, and screening of light due to solution turbidity. Thus, the drive to using an optimum amount of catalyst is not only for achieving maximum degradation efficiency but also avoiding excessive usage of catalyst. Salari and Sadeghinia (2019) investigated the effect of photocatalyst dosage (ranging from 100 to 300 mg) on photocatalytic activity. They found that as the catalyst concentration increased, the photocatalytic activity also increased. The optimal dosage was determined to be 250 mg for the best photocatalytic performance. Zhang *et al.* (2017) also studied the effect of the catalyst dose of $\text{Ag}_2\text{O/g-C}_3\text{N}_4/\text{Fe}_3\text{O}_4$ nanocomposite with different dosages (2, 5, 10, and 15 mg) for the degradation of Rhodamine B (RhB) solution, respectively. The degradation effect was 98.3% with the use of 10 mg $\text{Ag}_2\text{O/g-C}_3\text{N}_4/\text{Fe}_3\text{O}_4$ ternary nanocomposite, which shows the optimal degradation effect. 15 mg $\text{Ag}_2\text{O/g-C}_3\text{N}_4/\text{Fe}_3\text{O}_4$ NCs gives a degradation effect of 83.1%. It may be attributed to the relatively weaker light transmittance with more photocatalysts.

c) Effect of Initial Dye Concentration: The photocatalytic removal efficiency of an azo dye is also influenced by the initial concentration of the dye (Murillo-Sierra *et al.*, 2021). Increasing the initial concentration beyond the optimal level reduces the removal rate for several reasons. First, as the concentration increases, the ratio of photogenerated reactive radicals to dye molecules decreases, this leads to lower degradation efficiency. Second, according to Beer-Lambert's law, higher dye concentrations result in greater light absorption, preventing more photons from reaching the catalyst surface, especially in the deeper parts of the solution. This reduces charge carrier generation and the overall efficiency of degradation. Finally, OH^- ions may repel with anionic dye molecules for adsorption sites on the catalyst surface, leading to a reduced generation of OH radicals and consequently lower removal efficiency (Zubair *et al.*, 2021). Salari & Sadeghinia (2019) examined the effect of initial dye concentration (5, 10, 15, 20, and 25 ppm) using $\text{Ag}_2\text{O/ZnO/CuO}$ ternary nanocomposite and found that the optimal concentration was around 20 ppm. The measured reduced activity at higher concentrations can be likely attributed to the decrease in the number of the adsorbed photon due to completion from the dye molecule for light absorption. In another study, (Zhang *et al.*, 2017) studied the effect of RhB concentration on the degradation of RhB in 10, 20, 30, 40, and 50 mg/L RhB solutions by using $\text{Ag}_2\text{O/g-C}_3\text{N}_4/\text{Fe}_3\text{O}_4$ ternary nanocomposite.

The degradation effect decreases from 98.3% to 34.8% when the RhB concentration increases from 10 mg/L to 50 mg/L. Kalpana & Selvaraj (2015) studied the effect of initial dye concentration at various concentration of 10, 12, 14, 16 and 20 ppm solutions to obtain optimum concentration of 12 ppm with ZnO/TiO₂/Ag₂O ternary nanocomposite within 30 minutes of irradiation time. On increasing dye concentration, number of dye molecules adsorbed on the catalyst surface is also increased. However, at the initial stage, as the dye concentration increases, the penetration of photons toward the catalyst surface is reduced. This limits the generation of hydroxyl radicals required for the dye degradation process, causing the rate of photodegradation to decrease as the dye concentration increases.

d) Effect of Irradiation Time: Another important parameter for optimizing the photocatalytic process is the exposure time between pollutants and the photocatalyst. Subhan *et al.* (2020) investigated the effect of irradiation time using Ag₂O/SnO₂/TiO₂ NCs for the degradation of methylene violet. They found that 98% degradation was achieved after 60 minutes of exposure.

2.10.2. Antibacterial Activity

Bacterial growth has become a growing concern in daily life, causing significant damage across various industries, including textiles, water treatment, biomedicine, and food packaging. The rise of microorganisms resistant to antibiotics has leads to an increased search for new treatments for infectious diseases (Díez-Pascual, 2020). For example, certain Gram-positive bacteria, such as *S.aureus*, and Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*), have developed into multi-drug resistant pathogens, which are major contributors to hospital-acquired infections. Therefore, new, cheap and effective antimicrobial agents for controlling bacterial activity are urgently needed, and nanomaterials constitute a very promising approach. The extensive use of traditional antibacterial drugs leads to the bacterial resistance toward these antibacterial drugs, further intensifying the problem. Recent advancement in the field of nanotechnology has opened new channels for its application in a variety of fields. Among the various nanomaterials, metal oxides are extensively studied due to their non-toxicity, stability, efficient biological properties, etc. Table 7 provides a summary of some antibacterial

activities exhibited by different nanocomposites. A range of nanomaterials have been utilized as effective bactericidal agents against bacteria, including ZnO NPs (Ramesh *et al.*, 2015), Ag₂O NPs (Asif *et al.*, 2024), Co₃O₄ NPs (Sarvalkar *et al.*, 2024), Ag₂O/ZnO NCs (El-Mohsnawy *et al.*, 2023), Co₃O₄/ZnO NCs (Gendo *et al.*, 2024), Ag/Ag₂O/ZnO NCs (Peng *et al.*, 2022), and Ag₂O/SrO/CaO NCs (Aghaee & Manteghi, 2022). These materials have shown promising antimicrobial properties and have been studied for their potential in addressing bacterial infections. Subhan *et al.* (2015) synthesized mixed metal-oxide nanocomposite NiO–CeO₂–ZnO NCs for antibacterial activities. Munawar *et al.* (2020) synthesized triphase ZnO–Yb₂O₃–Pr₂O₃ nanocomposite by the coprecipitation method. The grown nanocomposite exhibited superior activity with 26, 29, and 31 mm zone of inhibition (ZOI) against *S. aureus* and 29, 29, and 30 mm ZOI against *E. coli* bacterial strains at concentrations of 10, 20, and 40 g/mL. They also compared the antibacterial activity of ZnO–Yb₂O₃–Pr₂O₃ nanocomposite with other researchers' work. Their work shows high antibacterial activity than others. They suggested that a small crystallite size and larger surface area of the nanocomposite could cause a high production rate of reactive oxygen species that can cause the destruction of cellular proteins and DNA or may cause the death of the cell, facilitating the transportation and diffusion of reactant molecules. The nanoscale surface effect and small size effect give these materials a unique antibacterial mechanism, mainly including three viewpoints (Figure 9) as proposed by (Qi *et al.*, 2020): generate reactive oxygen species (ROS) or release metal ions to destroy bacterial DNA and protein; nanoparticles gather on the surface of the bacterial cell membrane and destroy the cell membrane and interrupt transmembrane electron transfer.

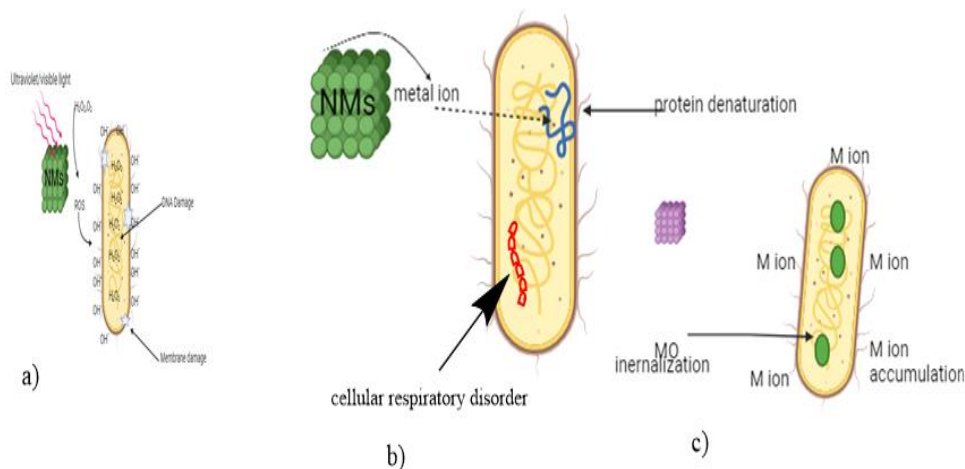


Figure 9. Mechanism of antibacterial activities of nanomaterials

Table 7. Antibacterial activities of some nanocomposites

Nanocomposites	Target Bacteria	Zone of inhibition	Summary	Reference
ZnO/Ag ₂ O NCs	<i>E.coli</i> , <i>B.subtilis</i>	<i>E.coli</i> : 17-34 mm and <i>B.subtilis</i> : 199-30 mm	ZnO/Ag ₂ O NCs showed high antibacterial activities than ZnO NPs	Ahmad <i>et al.</i> , 2020
CdO/CuO/ZnO NCs (100 mg/L)	<i>S.aureus</i> and <i>S.typhi</i>	<i>S.aureus</i> : 28 mm and <i>S.typhi</i> : 22 mm	Effective antimicrobial activities at high concentration	Kannan <i>et al.</i> , 2020
CuO/Fe ₂ O ₃ /Ag ₂ O NCs	<i>Klebsiella pneumoniae</i> , <i>E.coli</i> , <i>Staphylococcus epidermidis</i> , <i>S.aureus</i>	<i>Staphylococcus epidermidis</i> : 15 mm, <i>S.aureus</i> : 12 mm, <i>Klebsiella pneumoniae</i> : 11 mm, <i>E.coli</i> : 13 mm	High antibacterial activities	Rahmah, 2023

3. MATERIALS AND METHODS

3.1. Apparatus and Instruments

The different apparatus and instruments used for this research work were: an oven, refrigerator, droppers, test tubes, cuvette, mortar and pestle, an electronic balance, miller, crucibles, a different size beakers, a different size conical flasks, different size measuring cylinders, different size volumetric flasks, centrifuge, muffle furnace, magnetic stirrer with hotplate, funnel, filter paper (whatman number 1), pH meter (pH-013), UV-Vis spectrophotometer (DU800R DRAWELL), X-Ray Diffractometer (SHIMADZU Corporation (Japan), XRD-7000), and Fourier transfer infrared spectrophotometer (IS50 ABX, USA).

3.2. Chemicals and Reagents

All chemicals and reagents used were of analytical grade and were used without any further treatment. Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 98 %), silver nitrate (AgNO_3 , 99.5 %), cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 99 %), sodium hydroxide (NaOH , 99.8 %), ethanol ($\text{C}_2\text{H}_5\text{OH}$, 99.9 %), ferric chloride (FeCl_3 , 99 %), hydrochloric acid (HCl , 37 %), sulfuric acid (H_2SO_4 , 98 %), acetic anhydride ($(\text{CH}_3\text{CO})_2\text{O}$, 97 %), chloroform (CHCl_3 , 98 %), dimethyl sulfoxide ($(\text{CH}_3)_2\text{SO}$, 99 %), potassium iodide (KI , 99.7 %), iodine (I_2 , 99 %), acetic acid (CH_3COOH , 99.5 %), lead acetate ($\text{Pb}(\text{CH}_3\text{COOH})_2$, 99 %), and methylene blue ($\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$).

3.3. Plant Material Collection and Its Extract Preparation

The leaf of *R. prinoides* was collected in April 2024 from the Botanical garden of Wallaga University in Nekemte, Ethiopia, and were later authenticated and identified at the university's Biology Department by Dr. Fekadu Gurmessa (Botanist). To remove dirt and other contaminants, the leaves were thoroughly washed with running tap water, followed by deionized water, and then dried in the absence of sunlight at ambient temperature for two weeks to remove moisture. The leaf extract was prepared following the method described by (Habtemariam & Bekele, 2022). 10 g of finely crushed *R. prinoides* leaf was added to 100 mL of deionized water and heated at 60 °C for 30 minutes in beaker (Figure 10), and then

cooled. After cooling, the extract was filtered through filter paper to remove any remaining particles. The resulting solution was used immediately.



Figure 10. Schematic Representations of the *R. prinoides* leaf extract preparation

3.4. Phytochemical Screening of *Rhamnus prinoides* Leaf Extract

Plant extracts contain secondary metabolites such as phenols, alkaloids, flavonoids, and terpenoids, which play a role as capping and stabilizing agent. Phytochemical screening of the *R. prinoides* leaf extract, was conducted at the Chemistry Laboratory of Wallaga University. The preliminary phytochemical tests followed standard procedures and methods previously reported by (Agidew, 2022) to identify various compound classes.

a) Test for Alkaloids (Wagner test). 4 mL of *R. prinoides* leaf extract was acidified by adding 1.5% (v/v) of HCl followed by a 5 drops of Wagner's reagent (iodine solution in potassium iodide). Brown-reddish formation confirms the presence of alkaloids (Nortjie *et al.*, 2022).

b) Test for Flavonoids (Lead acetate test). 1mL of lead acetate solution was added to the 2 mL of extract. A yellow-colour precipitate indicates the presence of flavonoids (Gizaw, 2022)

c) Test for steroids (Salkowski's test): 1mL of extract mixed with equal amount of chloroform was treated with 2 mL of concentrated sulfuric acid. Appearance of bilayer dark red/brown precipitate indicated the presence of steroids (Low *et al.*, 2021)

d) Test for Saponins (Froth test). About 1 mL of the extract was diluted separately with 20 mL of deionized water and shaken in test tube for 15 seconds. A 1 cm layer of foam indicates the presence of saponins (Amabye, 2015)

e) Test for Glycosides (Keller-Kiliani test). To 1 mL of *R. prinoides* fruit extract, 1 mL of acetic acid, a drop of ferric chloride solution and then 1 mL of sulfuric acid (concentrated) were added. The formation of reddish-brown color confirmed the presence of glycosides (Gul *et al.*, 2017)

f) Test for Phenols (Ferric chloride test). About 3 drops of ferric chloride solution was added to 4 mL of *R. prinoides* leaf extract. A bluish-black color formed indicates the presence of phenol (Nortjie *et al.*, 2022).

g) Test for Tannins (Ferric chloride test). 3 mL of the extract was dissolved in 2 mL of deionized water and filtered. 2 drops of ferric chloride was added to the extract. Development of a blue-black precipitate indicates the presence of tannins (Kurniawan *et al.*, 2023).

3.5. Optimization Methods for Green Synthesis of ZnO/Ag₂O/Co₃O₄ Nanocomposite

Optimization is required to obtain a high-quality product, stability, and proper particle size. To optimize the synthesis of ZnO/Ag₂O/Co₃O₄ NCs, the effects of solution pH (3, 5, 7, 9, 11, 12, 13), molar ratios of precursor metal salt Zn:Ag:Co (1:1:1, 1:2:1, 1:1:2, 2:1:1), temperature (25, 50, 60, 70, 80, 90, 100 °C), plant extract volume (10, 20, 30, 40, 50 mL), and reaction time (60, 90, 120, and 150 minutes) were examined based on the procedure outlined by (Mukhtar *et al.*, 2020). To determine optimal pH, a solution containing 100 mL of 1:2:1 molar ratio of Zn: Ag: Co was mixed with 30 mL of volume plant extract and stirred for 120 minutes at 60°C. Under these conditions optimum pH was found to be 11. To optimize molar ratio of Zn: Ag: Co, 100 mL of different molar ratio of Zn: Ag: Co (1:1:1, 1:2:1, 1:1:2, 2:1:1) was mixed with 30 mL of plant extract. Then, pH of each mixture was adjusted to 11, followed by stirring for 120 minutes at 60°C. Under these conditions, the 1:2:1 ratio was identified as optimum. To get optimum point for effect of temperature, 100 mL of 1:2:1 of Zn: Ag: Co was mixed together with 30 mL of plant extract, and the pH was adjusted to 11 using 2M NaOH. Then the mixture was stirred for 120 minutes. Under these conditions, 60

°C was found as optimum temprature. To determine optimum volume of plant extract, various volume of the *R. prinoides* leaf extract was mixed with 100 mL of 1:2:1 molar ratio of Zn: Ag: Co. The pH was adjusted to 11, and the mixture were stirred for 120 minute at 60 °C. Among the studied volume of extract ,30 mL was found to be optimum. To get optimum time of reaction, 100 mL of 1:2:1 molar ratio of Zn: Ag: Co was mixed with 30 mL of volume of extracts, then pH was adjusted to 11 ,and stirred for 120 minutes at 60 °C. The colours of solutions were expected to change after some minutes to indicating the formation of corresponding nanoparticles and nanocomposite. At the last obtained precipitate was washed with ethanol several times to remove impurities, followed by centrifuging for 15 minute at 10000 rpm, and kept for further use for characterizations and applications. The synthesized NPs and NCs were monitored by using UV -Visible spectrophotometer.

3.6. Green Synthesis of ZnO, Ag₂O, and Co₃O₄ Nanoparticles

ZnO NPs was synthesized following a modified procedure used by (Kebede *et al.*, 2023) with some modifications. In this process, 30 mL of 0.1 M zinc acetate dihydrate was mixed with 10 mL of the *R. prinoides* leaf extract. To adjust the pH to 11, 2 M NaOH was added, and the solution was stirred at 60°C for 2 hours. After 2 hours of stirring, a yellow paste precipitate formed, indicating the conversion of the zinc salt to zinc hydroxide (Zn(OH)₂), which subsequently transformed into ZnO NPs upon calcination, confirming the formation of zinc oxide nanoparticles (Figure 11). A similar color change was reported by (Rajakumar *et al.*, 2018) when *Andrographis paniculata* leaf extract was added to a zinc acetate dihydrate precursor. The precipitate was then centrifuged at 5,000 rpm for 10 minutes, followed by drying in an oven at 60°C for 7 hours. The dried sample was subsequently calcined in a muffle furnace at 450 °C for 2 hours, and the resulting white powder was collected. Finally, the material was ground into a fine powder using a mortar and pestle and stored for further analysis.

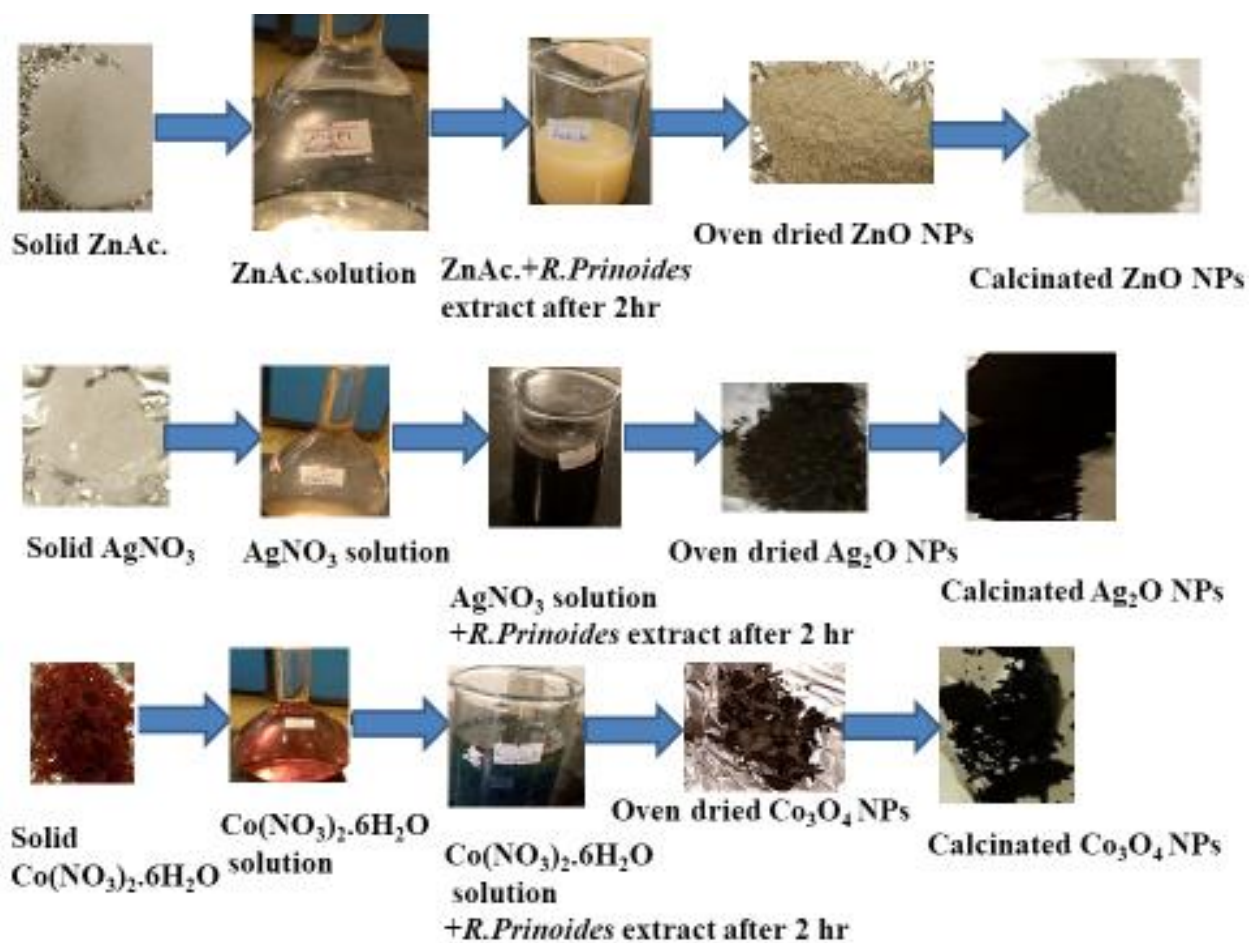


Figure 11. Schematic diagram of Green synthesis of ZnO NPs, Ag₂O NPs, and Co₃O₄ NPs

In addition, Ag₂O NPs were synthesized using a procedure described by (Pradheesh *et al.*, 2020), with some modifications. To prepare the NPs, 10 mL of *R. prinoides* leaf extract was added to 30 mL of a solution containing 0.1 M AgNO₃. A 2 M NaOH solution was then added until the pH reached 10, and the mixture was stirred continuously for 2 hours at 65°C. During this process, a noticeable black color change occurred, indicating the formation of Ag₂O NPs through the capping agent capabilities of *R. prinoides* leaf (Figure 11). A similar color change was observed in the synthesis of Ag₂O NPs using *Vernonia amygdalina*, as reported by (Widyaningtyas *et al.*, 2019). After the reaction, the mixture was centrifuged at 8,000 rpm for 5 minutes. The precipitate was then washed multiple times with deionized water, dried in an oven at 70°C for 12 hours, and calcined at 450°C for 2 hours. The resulting black powder was then subjected to extensive characterization and applications.

A Co_3O_4 NPs was synthesized following the procedure outlined by (Safdar *et al.*,2023) with some modifications. First, 100 mL of a 0.1 M solution of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was mixed with 30 mL of *R. prinoides* leaf extract in a beaker. To adjust the pH to 10, 2 M NaOH was added, and the beaker was placed on a hot plate with a magnetic stirrer, then it was heated and stirred at 60°C for 2 hours (Figure 11). During the synthesis of Co_3O_4 NPs, the color of the solution changed from greenish-blue to brown, and then to a black precipitate upon calcination, indicating successful formation of Co_3O_4 NPs. A similar color change was reported by (Hsu *et al.*,2018) during the synthesis of Co_3O_4 NPs. After the reaction, the solution was allowed to cool to room temperature and then centrifuged at 5,000 rpm for 10 minutes to collect the pellet. The pellet was washed several times with deionized water and ethanol to remove impurities. Next, the solution was transferred to an oven at 130°C for 5 hours to obtain the dried precipitate. The residue was then annealed at 500°C for 2 hours. Finally, the resulting black powder was stored in an air-tight container for further analysis.

3.7. Green Synthesis of $\text{Ag}_2\text{O}/\text{ZnO}$, $\text{Co}_3\text{O}_4/\text{ZnO}$, and $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ Nanocomposite

For $\text{Ag}_2\text{O}/\text{ZnO}$ NCs synthesis, the procedure described by (Basit *et al.*,2023) was followed. In this method, 50 mL of 0.1 M zinc acetate dihydrate solution (dissolved in 50 mL of deionized water) was added dropwise with continuous stirring to 50 mL of 0.1 M silver nitrate solution. Afterward, 30 mL of *R. prinoides* leaf extract was introduced into the mixture. Sodium hydroxide (2 M) was then added dropwise to adjust the pH to 11. The resulting mixture was stirred continuously for 2 hour at 70°C . Following this, a dark brown precipitate formed which was collected by centrifugation, washed multiple times with deionized water and ethanol to remove impurities, and then dried in a hot air oven at 120°C for 2 hours (Figure 12). A similar dark brown color change was reported by (Pandi *et al.*,2023) during the synthesis of $\text{Ag}_2\text{O}/\text{ZnO}$ nanocomposite using *Millingtonia hortensis* leaf extract and $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ and AgNO_3 as precursor salts,confirming the formation of $\text{Ag}_2\text{O}/\text{ZnO}$ nanocomposite.The obtained product was then annealed at 450°C for 2 hours, ground into powder, and stored for further analysis.

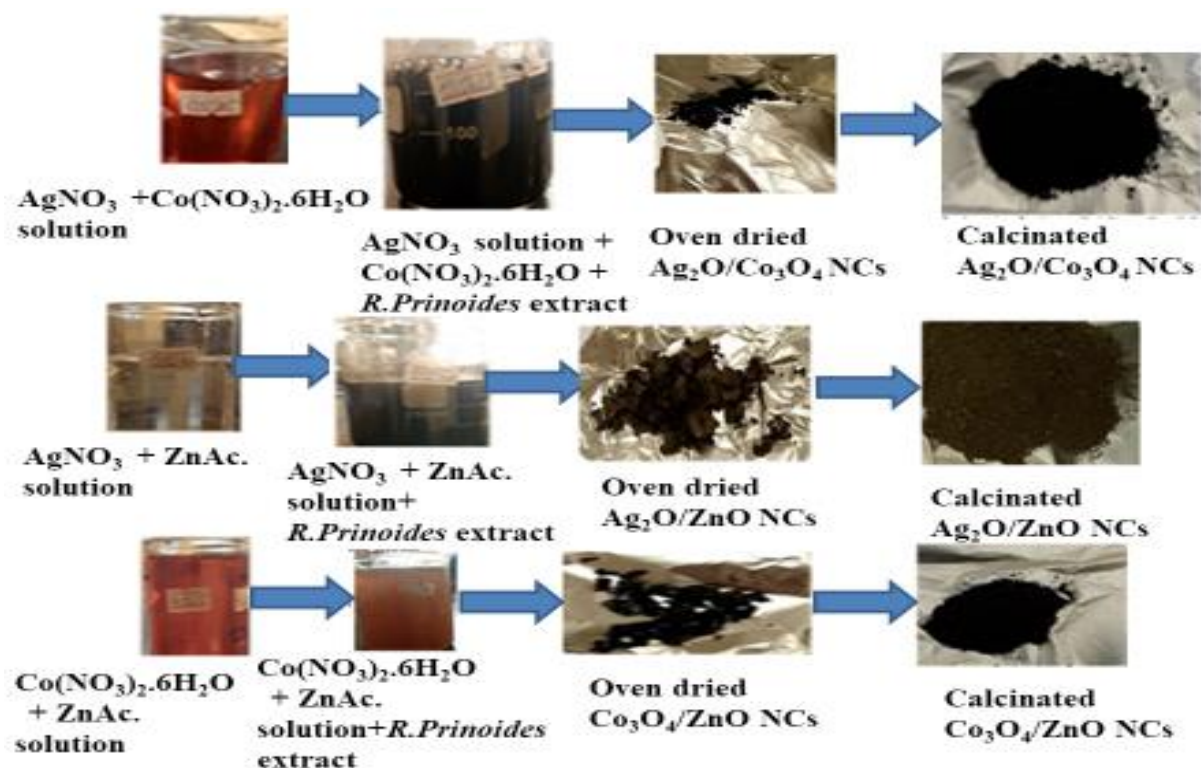


Figure 12. Schematic diagram of Green synthesis of $\text{Ag}_2\text{O}/\text{ZnO}$ NCs, $\text{Co}_3\text{O}_4/\text{ZnO}$ NCs, $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs and $\text{ZnO}/\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs

In addition, $\text{Co}_3\text{O}_4/\text{ZnO}$ NCs was synthesized using the procedure outlined by (Aragaw *et al.*, 2020) with some modifications. 40 mL of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ (0.1 M) and 40 mL of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.1 M) solutions were added to 30 mL of the *R. prinoides* leaf extract (Figure 12). Subsequently, 2 M NaOH was added to adjust the pH to 11, forming a hydroxide colloidal solution, and the mixture was stirred for 2 hours at 60°C. During the synthesis of $\text{Co}_3\text{O}_4/\text{ZnO}$ NCs, the color of the reaction mixture changed from light brown to dark brown and then to a black precipitate, indicating the formation of the nanocomposite. A similar color change was previously reported by (Dwivedi *et al.*, 2023). The solution was centrifuged and washed with deionized water followed by ethanol. The precipitate was oven-dried at 60°C for 6 hours and calcined at 500°C for 2 hours. Finally, the black powder obtained was stored for further analysis.

Furthermore, $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs was synthesized using the procedure applied by (Mohamed *et al.*, 2022) with some modifications. Accordingly, 30 mL of 0.1 M $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 30 mL of 0.1 M AgNO_3 solutions were added in a 250 mL conical flask. Following that, 20 mL of

R. prinoides leaf extract was added to the mixture (Figure 12). Then, 2 M NaOH was added to adjust the pH to 8, and the mixture was stirred for 2 hours at 70°C. During the synthesis of Ag₂O/Co₃O₄ NCs, a black precipitate formed, indicating the formation of the NCs. The solution was centrifuged and washed with deionized water followed by ethanol. The final solution was oven-dried at 75°C for 4 hours, and then calcined for 2 hours at 450°C to obtain a black powder. A similar color change was reported for the synthesis of Ag₂O/Co₃O₄ NCs by (Khan *et al.*,2024).

3.8. Green Synthesis of ZnO/Ag₂O/Co₃O₄ Nanocomposite

ZnO/Ag₂O/Co₃O₄ NCs were synthesized using the procedure reported by (Ishaque *et al.*,2023), with modifications. For the synthesis of equimolar ZnO/Ag₂O/Co₃O₄ NCs (1:1:1), 2.19 g of Zn(CH₃COOH)₂·2H₂O, 1.69 g of AgNO₃, and 2.91 g of Co(NO₃)₂·6H₂O were dissolved in 100 mL of deionized water. Following this, 30 mL of *R. prinoides* leaf extract was added. To adjust the pH to 11, 2 M NaOH was added, and the mixture was stirred for 2 hours at 60°C. After stirring, the mixture was allowed to cool to room temperature, resulting in the formation of a black precipitate. This precipitate was filtered and washed multiple times with deionized water and ethanol. The sample was then dried in an oven at 60°C for 4 hours and ground into a fine black powder using a mortar and pestle. The powder was annealed at 500°C for 2 hours and stored for further use. For the synthesis of ZnO/Ag₂O/Co₃O₄ NCs with different molar ratios (1:2:1, 1:1:2, and 2:1:1), the same procedure was followed, with the corresponding precursor salt ratios (Figure 13).

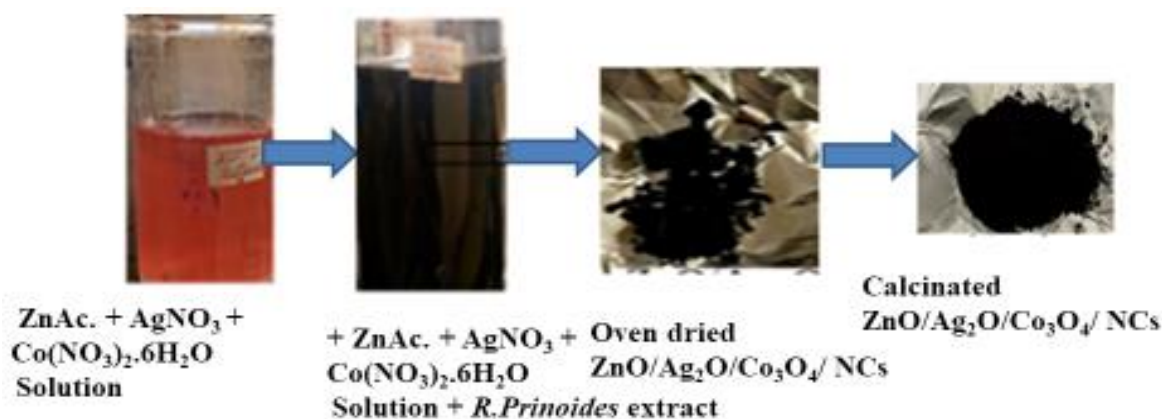


Figure 13. Schematic diagram of green synthesis of ZnO/Ag₂O/Co₃O₄ NCs

3.9. Characterization Techniques for Green Synthesized Nanoparticles and Nanocomposites

3.9.1. UV –Vis Spectroscopy

The UV-Visible analysis of synthesized nanoparticles and nanocomposites was analysed at Chemistry department of Wallaga University, Ethiopia. The 2 µg of the synthesized nanoparticles and nanocomposites were taken and dissolved in deionized water. The resulting solution was then poured into a quartz cuvette, and the spectra in the wavelength range of 200–800 nm were recorded. The analysis was conducted using a UV-visible spectrophotometer (DU800R DRAWELL) as reported by (Ifeanyichukwu *et al.*, 2020). Generally, surface plasmon resonance of the synthesized nanoparticles and nanocomposite was analyzed using UV-Vis spectroscopy. The energy band gap of the synthesized samples was also calculated from the UV-Vis spectra by extrapolating the linear part of the plot to the x axis, based on Tauc's equation, as shown in Equation 4.

$$\alpha h\nu = A(h\nu - E_g)^n \dots (4)$$

Where, A is a constant, hV is photon energy, E_g is the allowed energy band gap, α is absorption coefficient = ½ for allowed direct transition and n=2 for allowed indirect transition (Okeke *et al.*, 2020).

3.9.2. Fourier Transformed Infrared (FTIR) Spectroscopy

FTIR analysis was conducted at Addis Ababa Science and Technology, Ethiopia, using FTIR spectrophotometer (IS50 ABX, USA). The measurements were taken at room temperature, covering a spectral range of 400–4000 cm⁻¹, following the KBr method. For this method, the sample was finely ground and mixed with dry KBr. The mixture was then pressed in a hydraulic press to form a clear and transparent pellet for analysis as previously reported (Gottimukkala *et al.*, 2017). FTIR was performed to detect the surface functional groups, stretching, and bending vibrations in *R. prinoides* and the synthesized samples.

3.9.3. X-ray Diffraction (XRD)

The crystal structure and crystallite size of the synthesized samples were analyzed using X-ray diffraction (XRD) profiling with a SHIMADZU XRD-7000 instrument (Japan) at Adama

Science and Technology University, Ethiopia. The analysis was carried employing Cu-K α radiation ($\lambda = 1.54 \text{ \AA}$) over 2θ range of 10° to 85° , with the scan speed was set to $3^\circ/\text{min}$, an accelerating voltage of 40 kV and a current of 40 mA. Approximately 0.5 g of each synthesized samples was ground, homogenized, and mounted for analysis, following the procedure described by (Bunaciu *et al.*, 2015). The Miller index, diffraction peak positions, and relative intensities were identified by comparing the diffraction data with the standard diffraction patterns from the Joint Committee on Powder Diffraction Standards (JCPDS). The crystalline sizes of the synthesized samples were determined using Scherrer's formula, as shown in Equation 5:

$$D = \frac{0.94\lambda}{\beta \cos\theta} \dots\dots\dots (5)$$

Where θ is the Bragg angle, λ is the x-ray wavelength (1.54 $\text{\AA}$), and β illustrates full width at half maximum of a particular peak (Kumar *et al.*, 2020)

3.10. Photocatalytic Activity of Green Synthesized Nanoparticles and Nanocomposites

3.10.1. pH of Point Zero Charge Determination

To measure the pH of point zero charge (pHPZC) values, the pH drift method was applied in sequential order. The pH drift method is preferred over other conventional methods, such as titrations, because it is less time-consuming, provides results in fewer steps using common laboratory apparatus, and lowers the overall cost of the experiment (Gatabi *et al.*, 2016). A plot of initial pH values and ΔpH (final pH - initial pH) was then used to determine the pH of point zero charge. According to this method, solutions of 0.01 mol/L NaCl in 100 mL beakers were adjusted to pH values ranging from 2 to 12 as the initial pH using 0.1 mol/L NaOH and 0.1 mol/L HCl. Then, 0.04 g of the 1:2:1 molar ratio sample was added to the beakers and shaken at 300 rpm at room temperature. After 24 hours, the final pH (pHPZC) of the samples was measured using a pH meter (pH 013).

3.10.2. Optimum Experimental Condition for Methylene Blue Degradation

Optimization was carried out to maximize the degradation efficiency of Methylene Blue (MB) dye by adjusting selected parameters. The optimization process followed the procedure

used by (Safo *et al.*,2022), with some modifications, to determine the optimal conditions for the highest degradation efficiency of MB dye. These parameters included pH (ranging from 3 to 13), initial dye concentration (10, 15, 20, 25 ppm), catalyst dose (0.01, 0.02, 0.03, 0.04, 0.05 g), and irradiation time (0-40 min). The degradation performance of methylene blue (MB) by the synthesized nanocomposite was evaluated under sunlight irradiation. A specific amount of sample was taken and dispersed into the 100 mL of desired concentration of aqueous MB dye solution, then mixed in the dark for 30 minutes to reach the adsorption-desorption equilibrium. Optimization of the experiments was repeated until the optimum condition was reached. At the end of each experiment, the nanocomposite were separated from the solution by centrifugation. A UV-visible spectrophotometer was used to measure the absorption at λ max of 664 nm and the degradation efficiency (DE) of MB was evaluated by:

$$DE = \frac{C_0 - C_t}{C_0} \times 100 \dots \dots (6)$$

Where DE is degradation efficiency, C_0 is initial Concentration of dye and C_t is degradation at concentration after time t .

To compare MB degradation with and without synthesized NPs and NCs, the same optimum conditions used for photocatalytic degradation of synthesized NCs is applied, following procedure used by (Appia *et al.*, 2025). Accordingly, the photocatalytic degradation of methylene blue was studied using 100 mL of 10 ppm methylene blue dye at pH 11 for 40 minute of sunlight irradiation and monitored for 5 minute intervals without photocatalyst. The degradation of MB was evaluated by measuring its absorption at the maximum wavelength (λ_{max}) of 664 nm using a UV-Visible spectrophotometer (DU800R DRAWELL).

3.10.3. Reusability of ZnO/Ag₂O/Co₃O₄ Nanocomposite

ZnO/Ag₂O/Co₃O₄ NCs were separated from the sample solution, washed with deionized water, and then dried in an oven at 60°C for 3 hours. To evaluate the durability of the sample, the sample was kept in air without illumination for three cycles. After each cycle, the photocatalysts were reused to degrade the dye under the same experimental conditions. The degradation efficiency was monitored across these cycles to assess the synthesized

nanocomposite stability and performance over time, following the procedure described by (Song *et al.*,2019).

3.11. Antibacterial Activity of Green synthesized Nanoparticles and Nanocomposites

The antibacterial activities of the synthesized nanoparticles and nanocomposite were studied using the same procedure as reported by (Asamoah *et al.*,2020). The tested microorganisms, *E. coli* ATCC 25922 (gram-negative), *E. cloacae* ATCC 13047 (gram-negative), and *S. aureus* ATCC 25926 (gram-positive), were obtained from the Ethiopia Public Health Institute and freeze-preserved in the Microbiology Laboratory, Biology Department at Wallaga University. Bacterial cultures were maintained on nutrient Muller-Hinton agar (biomark laboratories, India) at 37 °C and the cultures were kept in appropriate media slants and stored at 4 °C until used.

The antibacterial activity in this study was assessed using the disk diffusion method on Mueller-Hinton agar. Approximately 20 mL of molten Mueller-Hinton agar was poured into Petri dishes and allowed to solidify. Bacterial suspensions were spread evenly over the agar surface using a cotton swab. Disks loaded with varying concentrations (100–12.5 mg/mL) of ZnO, Ag₂O, Co₃O₄ NPs, Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and different molar ratios of synthesized ZnO/Ag₂O/Co₃O₄ NCs were then placed on the inoculated Mueller-Hinton agar. Dimethyl sulfoxide (DMSO, 10%) was used as the negative control, while ciprofloxacin (10 µg/mL) served as the positive control against both Gram-positive and Gram-negative bacteria. After incubation for 24 hours at 37°C, the plates were photographed, and the diameter of the inhibition zones was measured in millimeters. The results were recorded as the mean ± standard deviation (SD) of the triplicate experiments.

3.12. Data Analysis

All the data obtained from UV-Vis, FTIR, XRD, photocatalytic degradation, and antibacterial results were analyzed using Microsoft Excel 2010, IBM SPSS Version 16, and Origin 8 software.

4. RESULTS AND DISCUSSIONS

4.1. Phytochemical Analysis of *Rhamnus Prinoides* Aqueous Leaf Extract

In this study, *R. prinoides* leaf was extracted using deionized water as the solvent. The presence of various phytochemicals, such as alkaloids, flavonoids, steroids, saponins, glycosides, phenols, and tannins, was tested and identified (as shown in Table 8 and Appendix Figure 1). During the synthesis process, these bioactive compounds present in the *R. prinoides* leaf extract acted as stabilizing agent facilitating the formation of NPs and NCs. Additionally, these compounds served as capping agents, helping to prevent agglomeration of the NCs.

Table 8. The result of phytochemical screening of *R. prinoides* leaf extract

Phytochemicals	Tests	Observations	Result
Alkaloids	Wagner test	Brown-reddish colour	++
Flavonoids	Lead acetate test	Formation of yellow precipitate	++
Steroids	Salkowski's test	Appearance of bilayer dark brown precipitate	+
Saponin	Froth tests	Formation of foam that was stable for nearly 3 min	+
Glycosides	Keller-Kiliani test	Reddish-brown color	+
Phenol	Ferric chloride test	Bluish black colour	++
Tannins	Ferric chloride test	Bluish black colour	+ +

‘+’ present, and ‘++’ indicates mostly presence of phytochemicals

The tests confirmed the presence of alkaloids, flavonoids, steroids, saponins, glycosides, phenols, and tannins in the plant extract, and a comparable color change was reported (Habtemariam & Alemu, 2021).

4.2. The Effects of Reaction Parameters on Synthesis of ZnO/Ag₂O/Co₃O₄ Nanocomposite

The green synthesis of ZnO/Ag₂O/Co₃O₄ NCs is highly sensitive to several parameters, including pH, molar ratios of metal salts, volume of plant extract, reaction temperature, and reaction time. These factors significantly influence the size, shape, yield, and agglomeration state of the NCs, which in turn affect their unique properties. UV–Vis spectroscopy, was used as a preliminary tool to confirm the formation of NCs and to determine the optimum reaction conditions for the synthesis process. In this study, the reaction parameters were carefully optimized for the effective green synthesis of ZnO/Ag₂O/Co₃O₄ NCs mediated by *R. prinoides* leaf extract.

a) Effects of pH: In the green synthesis of ZnO/Ag₂O/Co₃O₄ NCs, pH plays a crucial role. Previous studies have reported that pH significantly influence on the size of nanocomposite synthesized using plant extracts (Nhi *et al.*, 2022). Furthermore, variations in pH have been used to control the shape, composition, and size of the synthesized nanocomposite. The pH also influences the electrical charges of biomolecules, potentially affecting their capping and stabilizing properties, which in turn impacts nanoparticle growth. The effect of pH on the formation of ZnO/Ag₂O/Co₃O₄ NCs was confirmed through UV-Visible spectroscopy. To determine the optimal pH value, a range from 3 to 13 was used. At a lower pH (pH = 3), almost no absorption peak was observed, likely because acidic conditions are unsuitable for efficient synthesis due to the slow reaction rate and electrostatic repulsion of anions in the solution. A characteristic absorption peak was observed at pH 11, which was identified as the optimal pH for the reaction system due to the high-intensity peak observed under these conditions (Figure 14a). Additionally, the observed absorption intensity shift with increase with rising pH suggest that an alkaline environment enhances the capping and stabilizing capabilities of the *R. prinoides* leaf extract as supported by (Beldjilali *et al.*, 2020). The addition of NaOH is believed to act as a catalyst in the formation of the nanocomposite, facilitating particle collisions and connections to produce well-dispersed nanocomposite. However, increasing the pH beyond 11 may hinder the formation of NCs due to nanoparticle aggregation (Gebremedhn *et al.*, 2019).

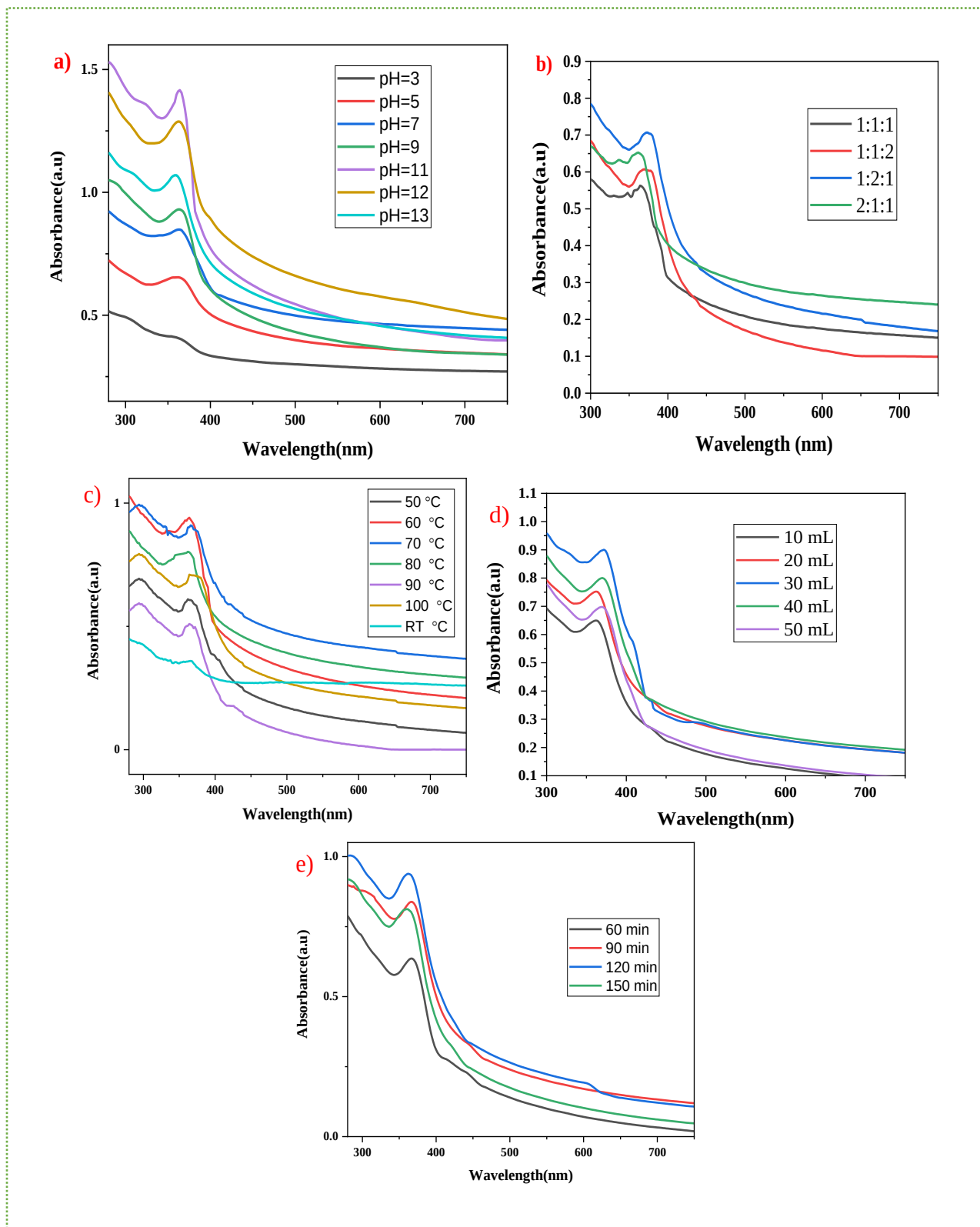


Figure 14. UV-Visible spectrum of ZnO/Ag₂O/Co₃O₄ NCs at different pH (a); different molar ratios (b); Temperature (c); Volume of extract (d); time (e)

b) Effects of Molar Ratios of Metal Salt Precursors: The ratio of precursor concentrations can also influence the green synthesis of NCs. In the present study, the effect of the molar ratio of Zn:Ag:Co was investigated by preparing solutions with the following proportions: 2.19 g, 1.69 g, 2.9 g (1:1:1); 2.19 g, 3.39 g, 2.9 g (1:2:1); 2.19 g, 1.69 g, 5.82 g (1:1:2); and 4.39 g, 1.69 g, 2.9 g (2:1:1) of zinc acetate dihydrate $[(\text{CH}_3\text{COO})_2\text{Zn}\cdot 2\text{H}_2\text{O}]$, silver nitrate (AgNO_3), and cobalt nitrate hexahydrate $[\text{Co}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}]$ dissolved in 100 mL of deionized water. Each solution was then mixed with 30 mL of *R.prinoides* leaf extract. Sharp and high-intensity peaks, along with a maximum red shift, were observed for the 1:2:1 and 1:1:2 molar ratios compared to 1:1:1 and 2:1:1 (Figure 14b). This result indicates that the intensity of the surface plasmon resonance peak is directly proportional to the concentration of the synthesized nanocomposite in the solution (Ahmad *et al.*, 2015).

c) Effects of Temperature: Temperature is another crucial factor influencing the synthesis of NCs, as it affects the size, shape, and yield of metallic nanoparticles. Higher temperatures promote the formation of nucleation centers, leading to increased nanoparticle formation. For the synthesis of metal oxide nanoparticles using plant extracts, a temperature range of 25°C to 100°C is recommended (Azad *et al.*, 2023). To investigate the effect of temperature on the formation of ZnO/Ag₂O/Co₃O₄ NCs, experiments were conducted at seven different temperatures (room temperature, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C). The volume of extract (30 mL), the molar ratio of metal precursors (1:2:1), and the pH (optimized to 11) were kept constant, and the mixtures were stirred for 60 minutes. The optimal temperature for synthesizing ZnO/Ag₂O/Co₃O₄ NCs was found to be 60°C (Figure 14 c). However, further increases in temperature beyond this optimal value resulted in a decline in absorption peak intensity. This decline may be attributed to the prolonged high-temperature heating, which can decompose the phytoconstituents in *R. prinoides* leaf extract, leading to the agglomeration of nanocomposite as supported by (Youssef & Mokhtar, 2014).

d) Effects of Volume of Plant Extract: The synthesis of nanomaterials using plant extract is primarily influenced by the volume of the extracts (Akintelu *et al.*, 2020). To determine the optimal volume, different volumes (10, 20, 30, 40, and 50 mL) were treated at the optimized molar ratio of precursors (1:2:1), with pH adjusted to 11 and the temperature set to 60°C, stirring for 60 minutes. The volume of 30 mL was identified as optimal; as it resulted in a

sharp, high-intensity absorption peak, indicating the formation of nanocomposite (Figure 14d). This outcome can be attributed to the phytochemicals in the *R. prinoides* leaf extract, which play a crucial role in facilitating the formation and stabilization of the nanocomposite through interactions with the precursors. Increasing the concentration of *R. prinoides* leaf extract leads to a higher quantity of secondary metabolites, which promotes the synthesis of smaller crystalline size of nanoparticles, thus preventing aggregation. Any increase or decrease in extract volume beyond 30 mL resulted in a decrease in the absorption peak intensity (Vijayaraghavan & Ashokkumar, 2017).

e) Effects of Reaction Time: Reaction time is a crucial parameter in the synthesis of NPs. In this study, the effect of reaction time ranging from 60 to 150 minutes was investigated. The results showed that the absorbance of ZnO/Ag₂O/Co₃O₄ NCs increased with reaction time, indicating that the reaction medium produced effective nanocomposite as the reaction time extended up to 120 minutes. However, after 120 minutes, the absorbance significantly not shifted or increased, suggesting that the stability of the NCs had reached its maximum. Therefore, 120 minutes was selected as the optimal reaction time for synthesizing high-yield ZnO/Ag₂O/Co₃O₄ NCs (Figure 14e). The intensity of the surface plasmon resonance (SPR) peak increased with longer reaction times, indicating higher concentrations of ZnO/Ag₂O/Co₃O₄ NCs. This suggests that the nanocomposite synthesized by this green synthesis method at this reaction time remain stable and do not aggregate. After 120 minutes, the intensity of the peak slightly decreased, implying that prolonged reaction time beyond this point (after 120 minutes) may lead to the aggregation of NCs rather than the formation of capped nanocomposite, and the result was supported by (Khalil *et al.*, 2014).

4.3. Characterization of Green Synthesized Nanoparticles and Nanocomposites

4.3.1. UV-Vis Absorption Spectral Analysis

The synthesized nanoparticles and nanocomposite were characterized using UV-Visible spectroscopy within the range of 200–800 nm. The *R. prinoides* leaf extract displayed one major peak at 362 nm and two additional peaks in the ultraviolet region, which are attributed to the presence of various phytochemicals (Figure 15a). After the reaction, these peaks disappeared, indicating that the phytochemicals played a significant role in converting metal salts to metal ions, resulting in the formation of the corresponding nanoparticles and

nanocomposite. This observation aligns with findings from previous studies (Habtemariam & Alemu, 2021). The absorption peak of the green-synthesized ZnO NPs at 358 nm (Figure 15a) aligns with previous studies on ZnO NPs synthesized using neem leaf extract as a capping agent (Almarhoon *et al.*, 2022). The slight blue shift observed in the ZnO NPs peak compared to the bulk ZnO NPs UV-Visible spectra at 385 nm is attributed to the quantum confinement effect. This effect arises from the reduced particle size of ZnO NPs during synthesis, facilitated by the *R. prinoides* leaf extract, and align with previous studies (Siripireddy & Mandal, 2017).

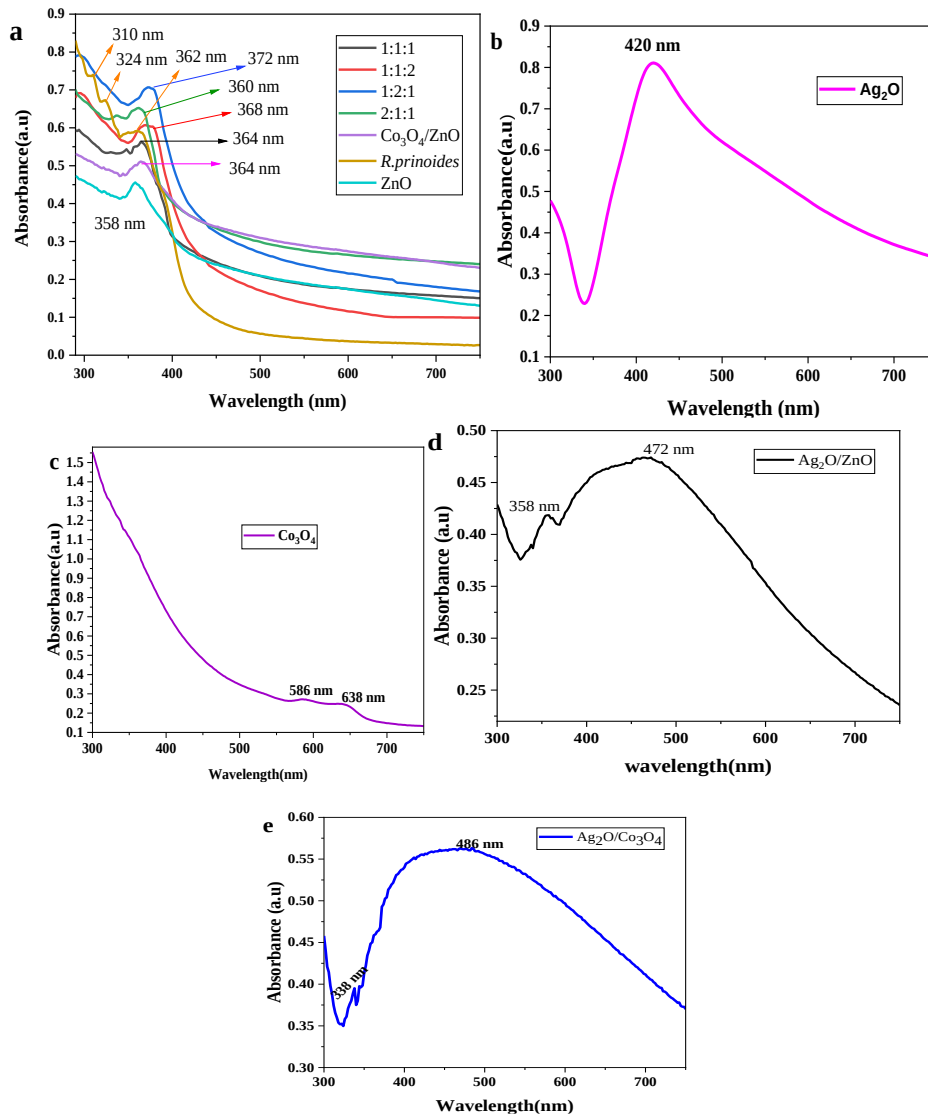


Figure 15. UV-Vis spectrum of *R. prinoides* (a), ZnO NPs (a), Co₃O₄/ZnO NCs (a), ZnO/Ag₂O/Co₃O₄ NCs (a), Ag₂O NPs (b), Co₃O₄ (c), Ag₂O/ZnO NCs (d), and Ag₂O/Co₃O₄ NCs (e)

Ag₂O NPs exhibited a high-intensity absorption peak at 420 nm (Figure 15b). The narrow absorption peak confirms the nanocrystalline nature of the Ag₂O NPs sample. The localized surface plasmon resonance (SPR) band appeared in the visible region at 420 nm which can be attributed to the small particle size of the Ag₂O nanoparticles and their surface plasmonic resonance and align with the report of (Rokade *et al.*,2016),confirming the successful formation of Ag₂O NPs using plant extracts. For instance, Ag₂O nanoparticles synthesized with *Lawsonia inermis* also exhibited a peak at 419 nm (Fayyadh & Jaduaa Alzubaidy, 2021), with *Moringa oleifera* at 419 nm (Muhammad *et al.*, 2023), and with *Ficus benghalensis* at 420 nm (Manikandan *et al.*,2017).UV-Visible spectroscopy revealed characteristic absorption peaks for Co₃O₄ NPs at 586 nm and 638 nm (Figure 15c). These two distinct wavelengths are likely associated with charge transfer processes from ligand to metal as: the absorption peak at 586 nm corresponds to charge transfer from O²⁻ to Co²⁺, while the peak at 638 nm is attributed to charge transfer from O²⁻ to Co³⁺. Furthermore, these peaks can also be linked to the surface plasmon resonance behavior of the Co₃O₄ NPs as confirmed by (Al Jahdaly *et al.*,2022).The findings are consistent with previous studies of (Farhadi *et al.*, 2014), confirming similar absorption characteristics for Co₃O₄ NPs.Ag₂O/ZnO nanocomposite exhibited characteristic absorption peaks at 358 nm and 472 nm (Figure 15d). The broad peak at 472 nm is attributed to the surface plasmonic resonance (SPR) of Ag₂O NPs, which is a typical feature of Ag₂O nanoparticles in the composites as supported by (Sampath *et al.*, 2022). Additionally, this absorption peak lying in the visible region is likely a result of electron trapping, a property enhancing the performance of the composite.The other peak at 358 nm corresponds to the exciton peak of ZnO nanoparticles, reflecting their presence in the Ag₂O/ZnO nanocomposite structure. This dual-peak behavior underscores the synergistic interaction between Ag₂O NPs and ZnO NPs within the nanocomposite. The UV-Visible spectra of Co₃O₄/ZnO NCs showed a peak at 364 nm (Figure 15a). This result is align with previous studies of (Gendo *et al.*,2023). The spectra display typical absorption with a sharp transition in the UV region, which is attributed to the intrinsic band gap absorption of ZnO NPs, caused by electron transitions from the valence band to the conduction band (Nadeem *et al.*, 2022). The UV-Visible spectra of Ag₂O/Co₃O₄ nanocomposite showed two characteristic peaks at 338 nm and 486 nm (Figure 15e). The moderate intensity peak at 486 nm is attributed to the surface plasmonic resonance (SPR) of

Ag₂O nanoparticles. The absorption band around 338 nm can be linked to the presence of Co (II) species in the nanocomposite as supported by (Orfi *et al.*, 2022). Additionally; it was observed that the amount of silver in the composite increased the absorbance values in the visible region. This suggests that silver enhances the optical density of the Co₃O₄ NPs, as higher absorption levels correlate with greater optical density (Alem *et al.*, 2023). Furthermore, the UV-Visible spectra of the ternary ZnO/Ag₂O/Co₃O₄ nanocomposite with different molar ratios (1:1:1, 1:2:1, 1:1:2, and 2:1:1) exhibited absorption peaks at 364 nm, 372 nm, 368 nm, and 360 nm respectively (Figure 15a). The decoration of ZnO NPs with the narrow bandgap semiconductor Ag₂O NPs caused a slight red shift in the absorption peaks, with optical absorbance observed at 360 nm, 364 nm, and 368 nm. The absorption peak for the 1:2:1 ratio shifted further to a higher wavelength at 372 nm. This shift is attributed to the deposition of Ag₂O NPs and Co₃O₄ NPs onto ZnO nanoparticles, enhancing the optical properties of the nanocomposite as supported by (Meena *et al.*, 2021). The optical band gap energies were determined by extrapolating the linear portion of the $(\alpha h\nu)^2$ versus $h\nu$ plot using Tauc plots for the green-synthesized ZnO, Ag₂O, Co₃O₄ NPs, their respective nanocomposite (Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/ Co₃O₄), and different molar ratios of ZnO/Ag₂O/Co₃O₄ nanocomposite (1:1:1, 1:2:1, 1:1:2, and 2:1:1). The resulting optical band gap energies were 2.85 eV, 2.51 eV, 2.30 and 1.95 eV, 2.85 and 2.44 eV, 2.72 eV, 1.9 eV, 2.72 eV, 2.53 eV, 2.68 eV, and 2.75 eV, respectively (Figure 16 and Figure 17). The optical band gap of ZnO NPs in the present study was found to be 2.85 eV, which aligns with the report by (Kumar Pal *et al.*, 2024), who used *Dillenia indica* extract for the synthesis. This band gap is smaller than that of bulk ZnO NPs (3.37 eV) and also smaller compared to other studies using different plant extracts. For example, (Gawade *et al.*, 2017) reported a band gap of 3.2 eV for ZnO NPs synthesized using *Calotropis procera*, while (Dhanmozhi *et al.*, 2017) reported a band gap of 3.4 eV using *Camellia sinensis*. These variations in band gap values are attributed to the differences in synthesis methods, plant extracts, and possibly the particle sizes of the synthesized ZnO nanoparticles.

The optical band gap of green-synthesized Ag₂O NPs was found to be 2.51 eV. Based on these findings, the band gap of green-synthesized Ag₂O NPs is higher than that of the bulk material (1.4 eV) and related to reported values using *Phragmanthera macrosolen* extract by

(Gungure *et al.*,2024). This increase is likely due to the particle size and the quantum confinement effect (Fayyadh & Jaduaa Alzubaidy, 2021).The optical band gap of the green-synthesized Co_3O_4 NPs was calculated to be 1.95 eV and 2.30 eV. The lower band gap value is attributed to the d–d spin-allowed transition between Co^{3+} and Co^{2+} ions, while the higher band gap value is associated with p–d charge transfer between Co^{3+} and O^{2-} in Co_3O_4 NPs as supported by (Dhas *et al.*, 2016).Based on these findings, the obtained optical band gap energy is in agreement with report of (Hassanpour *et al.*, 2017).The optical band gap of the green-synthesized $\text{Ag}_2\text{O}/\text{ZnO}$ NCs was calculated to be 2.85 and 2.44 eV, which aligns closely with the previous report by (Mohamed *et al.*,2020). The presence of Ag_2O in the $\text{Ag}_2\text{O}/\text{ZnO}$ NCs introduces a lower band gap (2.44 eV), enhancing visible light absorption.This hetrojunction structure promotes separation of photogenerated electron–hole pairs, improving photocatalytic activities compared to ZnO and Ag_2O NCs, as suggested by (Rini *et al.*,2023).

For $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite, the band gap was calculated to be 2.72 eV, which is an intermediate value between the band gaps of ZnO NPs and Co_3O_4 NPs. This indicates that the combination of ZnO NPs and Co_3O_4 NPs creates a structure that is more favorable for visible light absorption, making $\text{Co}_3\text{O}_4/\text{ZnO}$ photocatalyst more efficient than ZnO NPs alone in utilizing visible light.A related study using *Datura stramonium* leaf for synthesizing $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite reported a band gap value of 4.97 eV which was higher than present study (Tadesse *et al.*, 2023). The calculated optical band gap of $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs was found to be 1.90 eV. Incorporating Co_3O_4 nanoparticles into narrow band gap Ag_2O NPs results in a decrease in the overall band gap energy. The reduced band gap facilitates the absorption of visible light, leading to improved photocatalytic activity, as well as enhanced antibacterial properties due to the increased efficiency in generating ROS under light irradiation. This is consistent with findings by (Pradhan *et al.*,2024), who observed similar trends in the behavior of nanocomposites with altered band gap energies.

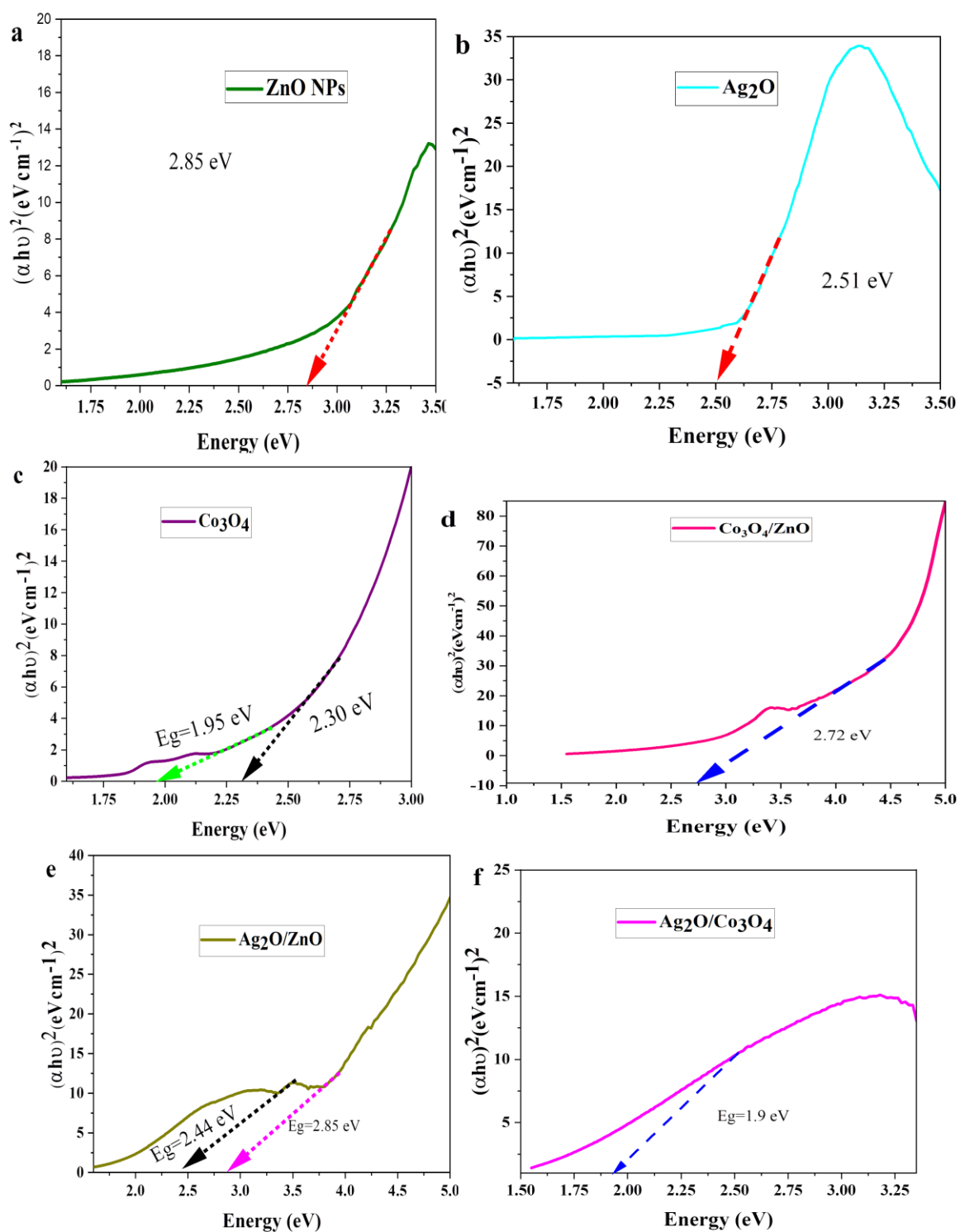


Figure 16. Band gap energy of green synthesized ZnO NPs (a), Ag₂O NPs (b), Co₃O₄ NPs(c), Co₃O₄/ZnO NCs (d), Ag₂O/ZnO NCs (e), and Ag₂O/Co₃O₄ NCs (f)

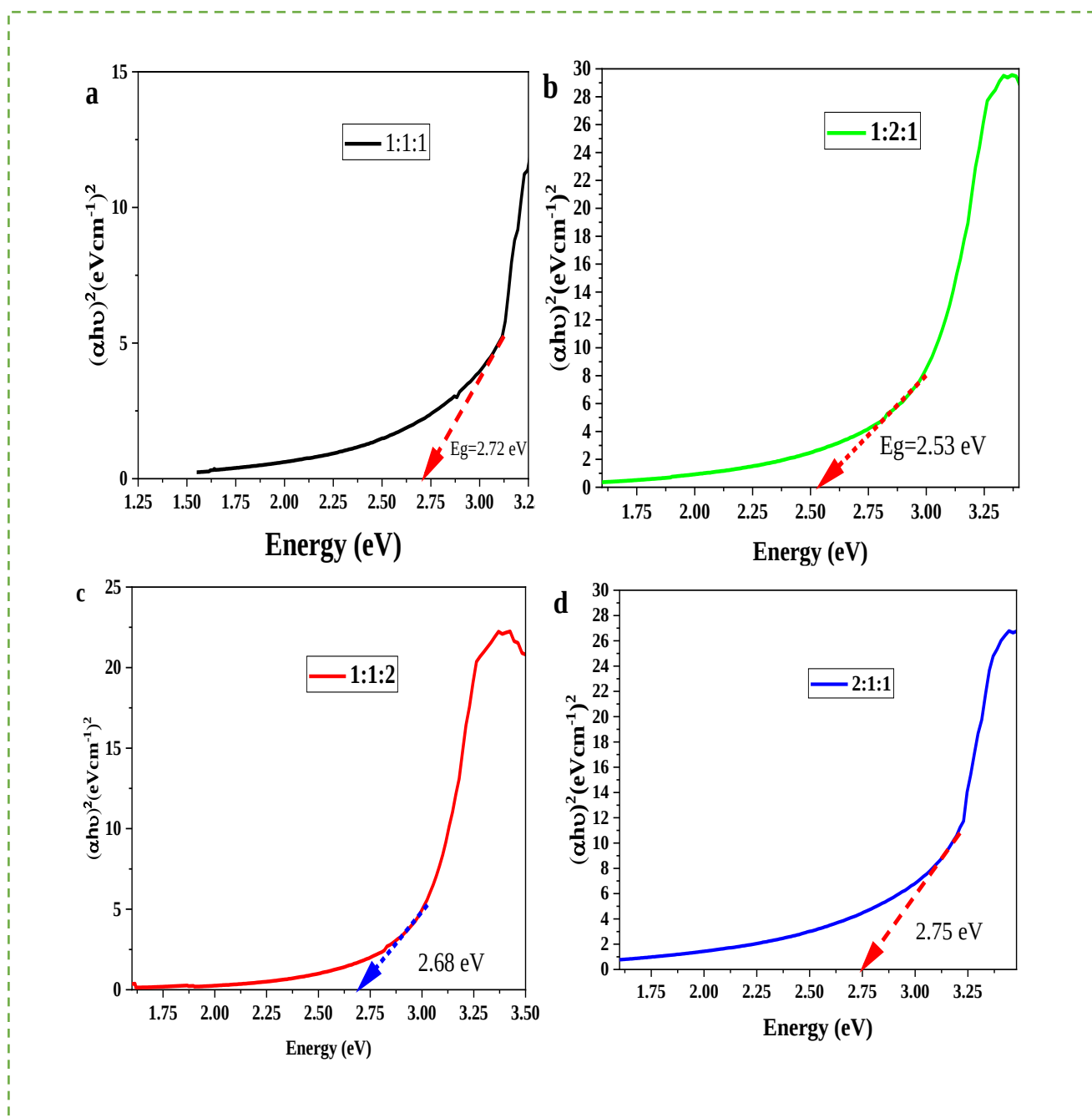


Figure 17. Band gap energy of green synthesized 1:1:1 (a), 1:2:1(b), 1:1:2(c), and 2:1:1(d) ZnO/Ag₂O/Co₃O₄ nanocomposite

The optical band gap energies of different molar ratios of ZnO/Ag₂O/Co₃O₄ nanocomposite (1:1:1, 1:2:1, 1:1:2, and 2:1:1) were 2.72 eV, 2.53 eV, 2.68 eV, and 2.75 eV, respectively. This indicates that the interaction between Co₃O₄ NPs and Ag₂O NPs influences the band gap

of ZnO NPs, narrowing it towards the smaller band gaps compared to ZnO NPs. The interaction at the interface between the different metal oxides in the composites facilitates the charge transfer and band alignment, resulting in an effective narrowing of the band gap compared to ZnO NPs. As the molar ratio of ZnO nanoparticles increases in the ZnO/Ag₂O/Co₃O₄ NCs (2:1:1), the larger band gap of ZnO NPs dominates, leading to a higher overall band gap for the composites. Conversely, when the molar ratios of Ag₂O and Co₃O₄ nanoparticles increase in ZnO/Ag₂O/Co₃O₄ nanocomposite (1:2:1 and 1:1:2), the smaller band gaps of Ag₂O and Co₃O₄ NPs dominate, resulting in a reduced overall band gap. The specific properties and electronic structures of each metal oxide play a significant role in determining the overall band gap of the nanomaterial, which can be modified by adjusting the molar ratios of the individual components (Ishaque *et al.*, 2023).

4.3.2. Fourier Transformed Infrared (FTIR) Spectroscopy Analysis

FTIR spectroscopy in the range of 4000 cm⁻¹ to 400 cm⁻¹ was utilized to confirm the presence of functional groups and plant metabolites involved in the capping, and stabilization of the green-synthesized nanoparticles and nanocomposite, as shown in Figures 18(a-c). The FTIR spectrum of *R. prinoides* (Figure 18 a) displays a strong broad band at 3390 cm⁻¹, which corresponds to the O-H stretching group common in alcohols or phenolic groups (polyphenols and flavonoids). Additionally, the FTIR spectra reveal several peaks at 890 cm⁻¹, 1029 cm⁻¹, 1403 cm⁻¹, 1574 cm⁻¹, and 2940 cm⁻¹. These peaks are attributed to the aromatic C-H out of plane bending, C-O stretching, C-N stretching or COO⁻ symmetric stretching (often found in alkaloids), C=C bond of aromatic rings (indicating the presence of phenol in the *R. prinoides* extract), and C-H stretching, respectively. Thus, the FTIR results demonstrate the presence of secondary metabolic groups in the leaf extract of *R. prinoides*, consistent with findings from other researchers (Debalkew & Teklu, 2024).

The FTIR spectra of the biosynthesized Co₃O₄ NPs display absorption bands at 3445 cm⁻¹ which are attributed to the O-H stretching vibration, as shown in Figures 18 c. The presence of the O-H functional group indicates the adsorption of water on the surface of the synthesized nanoparticles and nanocomposite (Yang *et al.*, 2016). Additional peaks are observed at 2940 cm⁻¹ and 2952 cm⁻¹ in the FTIR spectra of Co₃O₄ NPs and 1:1:2

(ZnO/Ag₂O/Co₃O₄ NCs), respectively. These peaks are attributed to C-H stretching vibrations, as reported by (Suriyakala *et al.*, 2022). Absorption bands at 2348 cm⁻¹ in the FTIR spectra of green-synthesized ZnO NPs are attributed to the C=O stretching vibration, indicating the adsorption of atmospheric CO₂ on the surface of the nanoparticles, as confirmed in the previous literature (Prasad *et al.*, 2019). The absorption peaks at 1741 cm⁻¹, 1740 cm⁻¹, 1650 cm⁻¹, 1745 cm⁻¹, 1776 cm⁻¹, 1774 cm⁻¹, 1695 cm⁻¹, 1650 cm⁻¹, and 1770 cm⁻¹ in the FTIR spectra of green-synthesized Ag₂O NPs, Co₃O₄ NPs, Ag₂O/ZnO NCs, Co₃O₄/ZnO NCs, Ag₂O/Co₃O₄ NCs, 1:1:1, 1:2:1, 1:1:2, and 2:1:1 (ZnO/Ag₂O/Co₃O₄ NCs) are due to C=O stretching, which is in agreement with the previous finding of (Rajeshkumar *et al.*, 2023).

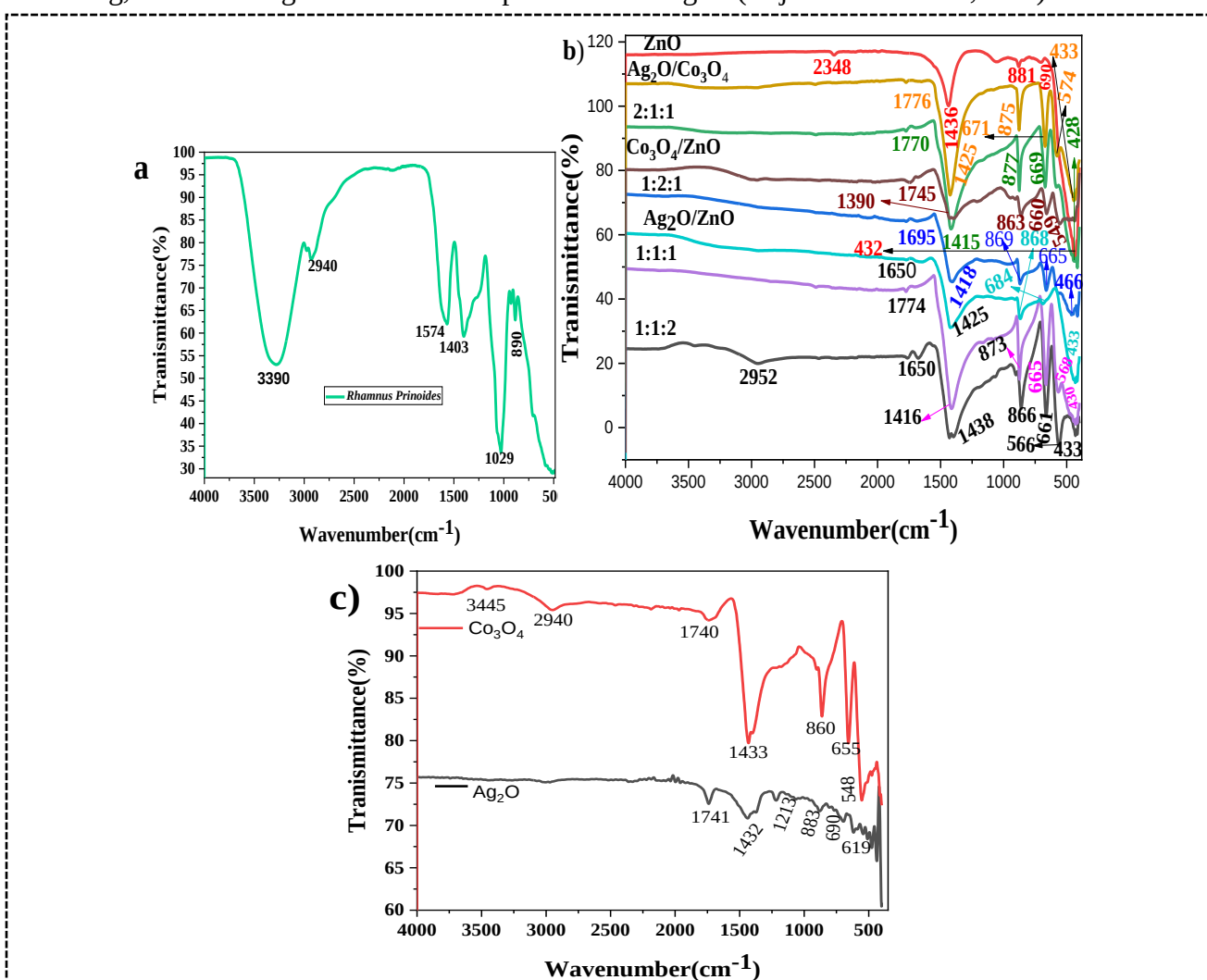


Figure 18. The FTIR spectrum of *R. prinoides* (a), Green synthesized ZnO, Ag₂O, Co₃O₄ NPs (b) and Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄ (c), and ZnO/Ag₂O/Co₃O₄ (1:1:1, 1:2:1, 1:1:2, and 2:1:1) nanocomposite (d)

Additionally, the characteristic peaks observed at 1436 cm^{-1} , 1432 cm^{-1} , 1433 cm^{-1} , 1425 cm^{-1} , 1390 cm^{-1} , 1425 cm^{-1} , 1416 cm^{-1} , 1418 cm^{-1} , 1438 cm^{-1} , and 1415 cm^{-1} in the FTIR spectra of synthesized ZnO NPs, Ag₂O NPs, Co₃O₄ NPs, Ag₂O/ZnO NCs, Co₃O₄/ZnO NCs, Ag₂O/Co₃O₄ NCs, 1:1:1, 1:2:1, 1:1:2, and 2:1:1 (ZnO/Ag₂O/Co₃O₄ NCs) were attributed to the C–C stretching vibration in the aromatic ring, originating from the biomolecules of *R. prinoides*, and the result is supported by (Ahmad *et al.*, 2022). The absorption bands at 1213 cm^{-1} in the FTIR spectra of green-synthesized Ag₂O NPs (Figure 18c) were attributed to the stretching vibrations of C–O in polyols or hydroxyl groups of flavonoids, align with the result of (Kiraz *et al.*, 2023). Additional characteristic peaks observed at 881 cm^{-1} and 860 cm^{-1} for green-synthesized ZnO NPs and 1:2:1 (ZnO/Ag₂O/Co₃O₄ NCs) indicate C–H out-of-plane bending, which is associated with the weak band, a result consistent with the finding of (Hunde Gonfa & Lealem Birhanu, 2024).

Moreover, a small intense peak observed at 432 cm^{-1} and 690 cm^{-1} belongs to the stretching vibrations within ZnO NPs. Similar results were reported by (Khalil *et al.*, 2023) for ZnO NPs at 432 cm^{-1} and by (Durai *et al.*, 2024) at 690 cm^{-1} . In the FTIR spectrum of Ag₂O NPs, characteristic peaks observed at 619 cm^{-1} and 883 cm^{-1} are likely due to the formation of the Ag–O bond. Previous work by (Kumar *et al.*, 2021) has shown similar findings. The absorption peak at 690 cm^{-1} corresponds to overtones produced by the overlap of stretching and bending vibrations of the Ag–O bonds. This finding was also reported by (Bibi *et al.*, 2021), who used another extract as a capping agent. The FTIR spectrum of Co₃O₄ NPs displays two strong peaks at 655 cm^{-1} and 548 cm^{-1} . The band at 548 cm^{-1} may correspond to O–Co²⁺, while the characteristic band at 655 cm^{-1} is attributed to O–Co³⁺. Similar results were found by (Safdar *et al.*, 2023) using *Hyphaene thebaica* fruits. In the FTIR spectrum of Ag₂O/ZnO nanocomposite, the characteristic peaks observed at 433 cm^{-1} , 684 cm^{-1} , and 868 cm^{-1} were attributed to M–O–M stretching vibrations, with Ag–O at 684 cm^{-1} and ZnO NPs at 433 cm^{-1} . These findings align with previous reports (Mohanani *et al.*, 2024). Additionally, in the FTIR spectrum of Co₃O₄/ZnO nanocomposite, the characteristic peaks at 549 cm^{-1} , 660 cm^{-1} , and 863 cm^{-1} correspond to the stretching vibrations of O–Co²⁺, O–Co³⁺, and Zn–O, respectively, which are consistent with the literature (Hassanpour *et al.*, 2017). These results confirm the successful synthesis of Co₃O₄/ZnO nanocomposite. In the FTIR spectrum

of Ag₂O/Co₃O₄ nanocomposite, the characteristic peaks at and 574 cm⁻¹ and 671 cm⁻¹ are attributed to the stretching vibrations of O–Co²⁺ and O–Co³⁺, while the peaks at 443 cm⁻¹ correspond to Ag–O, as confirmed by (Kumar *et al.*,2021). The peak around to 875 cm⁻¹ corresponds to weak M–M–O vibrations. Similar results were reported by Hassanin and colleagues using neem leaf extracts (Hassanin *et al.*, 2021), confirming the formation of nanocomposite. In the ternary metal oxide ZnO/Ag₂O/Co₃O₄ NCs, the characteristic absorption bands observed at 430 cm⁻¹ (1:1:1), 466 cm⁻¹ (1:2:1), 433 cm⁻¹ (1:1:2), and 428 cm⁻¹ (2:1:1) are attributed to the stretching vibrations of Zn–O in the nanocomposite, which is aligned with the report of (Khalil *et al.*,2023). Additionally, the characteristic peaks observed at 565 cm⁻¹ to 568 cm⁻¹ and 661 cm⁻¹ to 669 cm⁻¹ in the FTIR spectrum of ZnO/Ag₂O/Co₃O₄ NCs correspond to O–Co²⁺ and O–Co³⁺, respectively, which is in agreement with the report of (Meena *et al.*,2021).

Furthermore, the peaks observed at 866 cm⁻¹ to 877 cm⁻¹ are likely due to the presence of the silver-oxide (Ag–O) bond in the sample, which align with the finding of (Janthima *et al.*,2018). The FTIR spectra of the biosynthesized NPs and NCs showed slight shifts and changes in the intensities of some peaks, indicating that the major biomolecules from the extracts were capped or bonded to the surface of the synthesized NPs and NCs. These changes suggest that polyols and phenols, acted as both capping and stabilizing agents. During the synthesis process, these compounds may be oxidized aldehyde or ketone as confirmed by the presence of broad peak observed at ~1650 cm⁻¹ to 1776 cm⁻¹. These carbonyl groups confirm that the green synthesized nanoparticles and nanocomposite are functionalized or capped by secondary metabolites such as terpenoids, which contain amine, alcohol, ketone, aldehyde, and carboxylic acid functional groups, as confirmed by (Ullah *et al.*,2023).

4.3.3. X-ray Diffraction (XRD) Analysis

The XRD patterns for the synthesized nanoparticles Ag₂O and Co₃O₄ NPs are shown in Figure 19 a. The XRD pattern of Ag₂O nanoparticles displayed clear peaks at 2θ ≈ 32.5°, 38.1°, 44.3°, 64.5°, 77.4°, and 81.6°, corresponding to the (110), (111), (200), (220), (311), and (222) crystallographic planes, respectively. These peaks are consistent face-

centered cubic structure as the data provided by JCPDS card No. 00-041-1106. Similar findings were reported by (El Messaoudi *et al.*, 2023) who synthesized Ag₂O NPs using *Punica granatum* leaf extract, further confirming the presence of Ag₂O nanoparticles with no additional peaks, indicating the purity of the synthesized nanoparticles.

For Co₃O₄ nanoparticles, the XRD pattern displayed peaks at $2\theta \approx 31.9^\circ, 36.9^\circ, 44.8^\circ, 59.4^\circ, 65.3^\circ$, and 77.7° , corresponding to the (331), (210), (216), (416), (503), and (424) crystal planes, respectively. These peaks are consistent with JCPDS No. 01-1278 and JCPDS No. 15-0806, which is in agreement with (Mohammadi *et al.*, 2021) report. Additional smaller peaks at 2θ values of $18.96^\circ, 42.5^\circ$, and 55.7° match with JCPDS cards No. 073-1701, No. 01-073-1701, and No. 42-1467, respectively, which correspond to the (111), (200), and (422) planes of Co₃O₄ nanoparticles. These results indicate that the Co₃O₄ nanoparticles crystallized into a face-centered cubic phase, as supported by previous studies (Saeed *et al.*, 2021). A close analysis of the diffraction peaks shows good agreement with literature values, confirming that the Co₃O₄ nanoparticles were synthesized from the reaction between cobalt nitrate hexahydrate and *R. prinoides* leaf extract. For ZnO nanoparticles (Figure 19 c), the XRD pattern revealed seven distinct peaks at $2\theta \approx 31.8^\circ, 34.5^\circ, 36.3^\circ, 47.6^\circ, 56.7^\circ, 63.01^\circ$, and 68.3° , corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes, respectively. These diffraction peaks align well with those reported in the Joint Committee on Powder Diffraction Standards (JCPDS, card No. 89-7102), confirming the hexagonal Wurtzite crystal structure, which is align with previous studies (El-Belely *et al.*, 2021), confirming successful synthesis of ZnO NPs. The XRD pattern of green-synthesized Ag₂O/ZnO, Co₃O₄/ZnO, and Ag₂O/Co₃O₄ NCs is presented in Figure 19 b. In the XRD spectrum of Ag₂O/ZnO NCs, diffraction peaks observed at 2θ values of $31.8^\circ, 34.46^\circ, 36.3^\circ, 47.6^\circ, 56.7^\circ, 62.9^\circ$, and 68.06° correspond to the (100), (002), (101), (102), (110), (103), and (112) miller indices, respectively, matching the standard data (JCPDS, card No. 89-7102) for the hexagonal Wurtzite structure of ZnO nanoparticles. Additional peaks at $38.1^\circ, 44.3^\circ, 64.5^\circ, 77.4^\circ$, and 81.6° are consistent with the (111), (200), (220), (311), and (222) planes, which correspond to the face-centered cubic crystal structure of Ag₂O nanoparticles, as referenced in (JCPDS No. 00-041-1106). This is in agreement with previous studies (Abad *et al.*, 2023). The presence of prominent peaks from both components indicates the formation of

the Ag₂O/ZnO nanocomposite. The slight peak shifts compared to ZnO nanoparticles are due to differences in ionic radii between Zn and Ag, resulting in lattice compression caused by the substitution of Zn²⁺ and Ag⁺ ions, as in earlier reports (Suganya *et al.*, 2022).

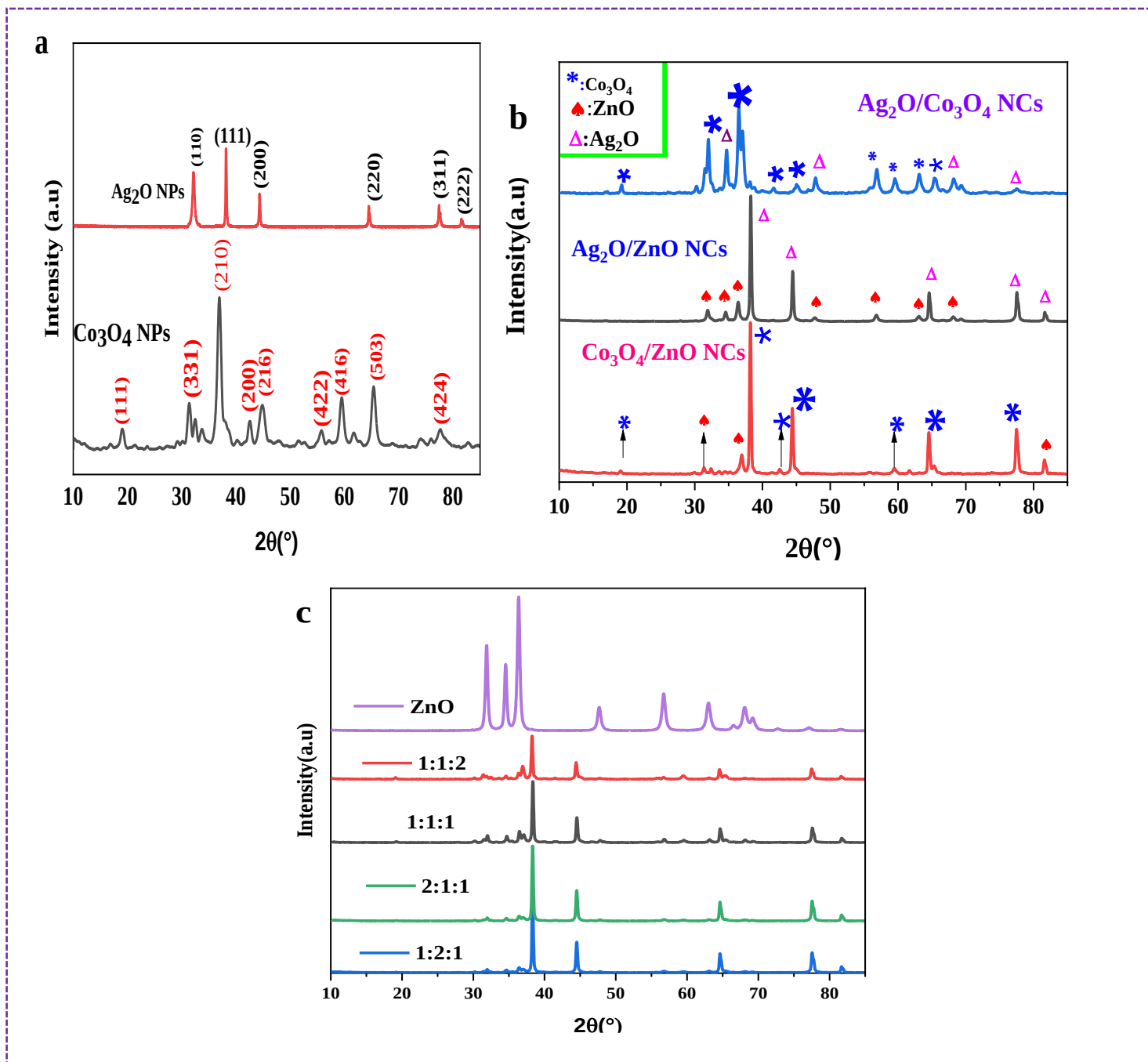


Figure 19. The XRD spectrum of green synthesized ZnO, Ag₂O, and Co₃O₄ nanoparticles (a), Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄ (b), 1:1:1, 1:2:1, 1:1:2, and 2:1:1 nanocomposite (c)

In the XRD pattern of the $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite, diffraction peaks corresponding to the face-centered cubic phase of Co_3O_4 NPs are observed at $2\theta \approx 18.89^\circ$, 38.32° , 42.75° , 44.52° , 59.6° , 64.6° , and 77.58° , corresponding to the (111), (220), (222), (216), (416), (503), and (424) crystallographic planes. The hexagonal Wurtzite structure of ZnO NPs is also evident at $2\theta \approx 31.38^\circ$, 36.8° , and 81.7° , attributed to the (100), (101), and (104) planes, in accordance with JCPDS card No. 36-1451. Overall, the diffraction patterns of the $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite show no peaks other than those of Co_3O_4 and ZnO nanoparticles, confirming the absence of impurities and the successful synthesis of the $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite. Interestingly, compared to the peak intensity of pure ZnO nanoparticles synthesized in this study, some peaks of the ZnO phase are absent in the XRD of the $\text{Co}_3\text{O}_4/\text{ZnO}$ nanocomposite. This phenomenon is attributed to the fact that the ZnO NPs is partially shielded by the outer Co_3O_4 NPs shell in the composite, as supported by findings from other studies (Jana *et al.*, 2015). Additionally in the XRD spectrum of $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs, peak observed at $2\theta \approx 34.6^\circ$ (100), 47.7° (200), 68.1° (222), and 77.4° (311) match with the reflections of the face-centered cubic structure of Ag_2O NPs (JCPDS No. 00-041-1106) and JCPDS No. 72-2108.

In contrast, the major diffraction peaks of Co_3O_4 nanoparticles appear at $2\theta \approx 19.02^\circ$, 31.8° , 36.6° , 41.52° , 44.97° , 56.75° , 59.47° , 63° and 65.35° , closely matching the crystalline planes of (111), (331), (210), (311), (216), (396), (220), (416), and (503) in accordance with JCPDS No. 01-1278, 15-0806, 42-1467 indicating the face-centered cubic phase of Co_3O_4 nanoparticles, also supported by (Al-Qasmi, 2022). Interestingly, the diffraction peaks corresponding to crystalline Co_3O_4 NPs (31.8° , 36.6° , and 44.97°) in the $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ nanocomposite slightly shifted in diffraction angles compared to those of pure Co_3O_4 nanoparticles. This shift is likely due to the expansion of the lattice parameters of the unit cells, caused by the incorporation of Ag_2O NPs into the Co_3O_4 NPs lattice, as confirmed by (Gao *et al.*, 2018).

The XRD patterns of $\text{ZnO}/\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ nanocomposite with different molar ratios of metals (Zn: Ag: Co) of 1:1:1, 1:2:1, 1:1:2, and 2:1:1 ($\text{ZnO}/\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs) are shown in Figure

19 c. The XRD spectra of all synthesized ZnO/Ag₂O/Co₃O₄ nanocomposite with varying molar ratios match the XRD libraries, confirming the presence of the hexagonal Wurtzite structure of ZnO NPs (JCPDS card No. 89-7102), the face-centered cubic crystal structure of Ag₂O NPs (JCPDS No. 00-041-1106), and the face-centered cubic phase of Co₃O₄ NPs (JCPDS No. 01-1278 and JCPDS No. 15-0806). The XRD peaks indicate the coexistence of three phases within a single matrix. For the 1:1:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite, ZnO NPs peaks were observed at $2\theta \approx 31.8^\circ$ (100), 34.7° (002), 36.7° (101), 47.8° (102), 56.8° (110), 63.1° (103), and 68.2° (112). In the 1:2:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite, ZnO NPs peaks appeared at $2\theta \approx 31.9^\circ$ (100), 34.6° (002), 36.4° (101), and 56.7° (110) respectively. For the 1:1:2 (ZnO/Ag₂O/Co₃O₄) nanocomposite, ZnO NPs peaks were found at $2\theta \approx 31.6^\circ$ (100), 34.5° (002), 36.8° (101), 56.8° (110), and 63.1° (103) respectively. In the 2:1:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite, ZnO NPs peaks were observed at $2\theta \approx 31.8^\circ$ (100), 34.5° (002), 36.4° (101), 47.6° (102), 56.6° (110), 62.99° (103), and 68.01° (112). The XRD diffraction peaks for the 1:1:1 (ZnO/Ag₂O/Co₃O₄) and 1:2:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite appeared at diffraction angles of $2\theta \approx 38.3^\circ$, 64.7° , 77.6° , and 81.7° , corresponding to the Ag₂O NPs phase. Similarly, the XRD patterns for the 1:1:2 and 2:1:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite also displayed Ag₂O NPs peaks at $2\theta \approx 38.2^\circ$, 64.4° , 77.5° , and 81.6° . These diffraction peaks correspond to the Miller indices (111), (220), (311), and (222) for Ag₂O NPs. Moreover, in the XRD patterns of 1:1:1, 1:1:2, and 2:1:1 (ZnO/Ag₂O/Co₃O₄ NCs), peaks at $2\theta \approx 19.09^\circ$, 44.5° , and 59.4° were observed, which match the Miller indices of (111), (216), and (416) of Co₃O₄ NPs. Similarly for 1:2:1, the peaks at 44.5° and 59.4° also observed, consistent with JCPDS No. 01-1278 and JCPDS No. 15-0806. These results align with previous studies (Masrat *et al.*, 2022). When comparing the intensities of the ZnO NPs and Ag₂O NPs peaks to the Co₃O₄ NPs peaks, it is evident that the Co₃O₄ peak is smaller and weaker in the nanocomposite. This likely reflects the lower content of Co₃O₄ NPs in the composite and peak overlapping may occur (Hamad *et al.*, 2015). From the XRD patterns of all molar ratios of ZnO/Ag₂O/Co₃O₄ nanocomposite, it is evident that a small variation in the intensity of the peaks is observed, which may be due to changes in the ratios of the synthesized nanocomposite. Moreover, the XRD patterns exhibited no ambiguous peaks, confirming the purity and high crystallinity of the NCs. The appearance of three phases corresponding to ZnO, Ag₂O, and Co₃O₄ NPs in the samples

confirmed the successful synthesis of the ZnO/Ag₂O/Co₃O₄ NCs. The average crystalline size of the synthesized nanoparticles and nanocomposite was computed from the selected major peaks using Scherrer's formula (Equation 5 in Chapter 3). The average crystalline sizes of the green-synthesized ZnO, Ag₂O, Co₃O₄ nanoparticles, and the Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, 1:1:1, 1:2:1, 1:1:2, and 2:1:1 ZnO/Ag₂O/Co₃O₄ nanocomposite, were calculated as 28.78 nm, 26.25 nm, 26.65 nm, 25.82 nm, 27.52 nm, 25.92 nm, 21.31 nm, 14.37 nm, 19.61 nm, and 24.70 nm respectively, as shown in Table 9.

The average crystalline sizes of the synthesized ZnO, Ag₂O, and Co₃O₄ nanoparticles were comparable with previous reports: 28.78 nm for ZnO NPs (Islam *et al.*, 2025), 26 nm for Ag₂O NPs (Azzi *et al.*, 2024), 26 nm for Co₃O₄ NPs (Chelliah *et al.*, 2023), whereas for binary nanocomposite, 24.99 nm for Ag₂O/ZnO NCs (Gungure *et al.*, 2025), and 25 nm for Co₃O₄/ZnO NCs (Tadesse *et al.*, 2023). Based on the average crystalline size, it is evident that the ternary nanocomposite exhibits smaller crystalline size than synthesized binary nanocomposites and metal oxide nanoparticles. This reduction in size may be attributed to lattice strain which arises from the difference in ionic radii of zinc, silver, and cobalt ions, as suggested by (Satdeve *et al.*, 2019).

Table 9. XRD peak position and average crystalline size of Green synthesized ZnO, Ag₂O, Co₃O₄ NPs, Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and ZnO/Ag₂O/Co₃O₄ nanocomposite using major intense peak

Samples	Miller indices(hkl)	2 θ (°)	FWHM(°)	Crystalline size(nm)	Average crystalline size(nm)
ZnO NPs	100	31.8	0.280	29.52	28.78
	002	34.5	0.243	34.52	
	101	36.3	0.374	22.30	
Ag ₂ O NPs	111	38.1	0.274	30.68	26.25
	200	44.3	0.321	26.75	
	311	77.4	0.477	21.32	
Co ₃ O ₄ NPs	210	36.9	0.235	35.68	26.65
	416	59.4	0.361	25.32	
	503	65.3	0.498	18.95	
Ag ₂ O/ZnO NCs	111	38.1	0.264	31.84	25.82
	200	44.3	0.344	24.91	
	311	77.4	0.473	21.52	
Co ₃ O ₄ /ZnO NCs	222	38.3	0.265	31.76	27.52
	111	44.5	0.304	28.3	
	503	64.6	0.421	22.5	
Ag ₂ O/Co ₃ O ₄ NCs	331	31.8	0.323	26.18	25.92
	210	36.6	0.341	24.93	
	396	56.7	0.340	26.65	
1:1:1 ZnO/Ag ₂ O/Co ₃ O ₄ NCs	111	38.3	0.310	27.54	21.31
	112	44.5	0.380	22.53	
	220	64.7	0.680	13.86	
1:2:1 ZnO/Ag ₂ O/Co ₃ O ₄ NCs	111	38.3	0.390	21.21	14.37
	112	44.5	0.911	9.41	
	311	77.6	0.822	12.51	
1:1:2 ZnO/Ag ₂ O/Co ₃ O ₄ NCs	111	38.2	0.672	19.97	19.61
	112	44.5	0.423	20.51	
	311	77.5	0.565	18.35	
2:1:1 ZnO/Ag ₂ O/Co ₃ O ₄ NCs	111	38.2	0.292	28.15	24.70
	101	36.7	0.351	23.95	
	311	77.5	0.464	22.00	

4.4. Photocatalytic Activity

4.4.1. pH of Point Zero Charge Determination

pH of point zero charge is an important parameter to study the pH effect. For the values more and less than pHPZC, the surface charge of the photocatalyst is negative and positive, respectively. In this study pHPZC for ZnO/Ag₂O/Co₃O₄ NCs was calculated by using drift method and the result is shown in Figure 20.

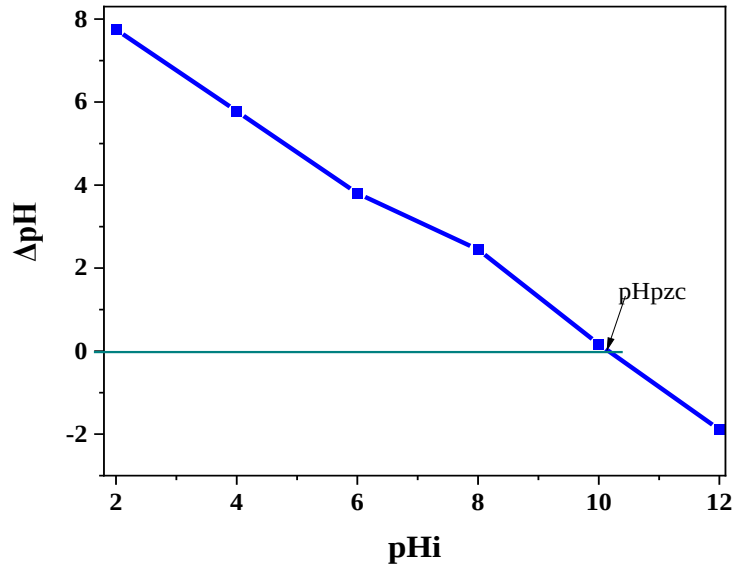


Figure 20. pHPZC of 1:2:1 ZnO/Ag₂O/Co₃O₄ nanocomposite

Additionally, the results obtained are in good agreement with previous studies (Gezici & Bişgin, 2021). In this result pH final was the pH initial changed after 24 hr and pH=10.1 was shown for this sample as pHPZC. At pH of solution higher than 10.1 the surface of ZnO/Ag₂O/Co₃O₄ NCs is negatively charged and for less than 10.1 the surface of photocatalyst is expected to be positively charged. Because methylene blue, as the case study is positive charge molecule, its degradation efficiency is generally low below the pHPZC and increase when the pH of solution exceeds the pHPZC (Zarrabi *et al.*, 2018).

4.4.2. Factors Affecting Photocatalytic Activity

a) Effect of pH

It is well known that pH is one of the most important parameters in photocatalytic wastewater treatment, as it affects the surface charge of the photocatalyst and its photocatalytic efficiency (Zarrabi *et al.*, 2018). The pH of the solution was adjusted using 0.1

M NaOH and 0.1 M HCl. To study the effect of solution pH, experiments were conducted at pH values of 3, 5, 7, 9, 11, and 13, with an initial dye concentration of 100 mL of 10 ppm and a catalyst load of 0.04 g, for 30 minutes of irradiation (Figure 21a).

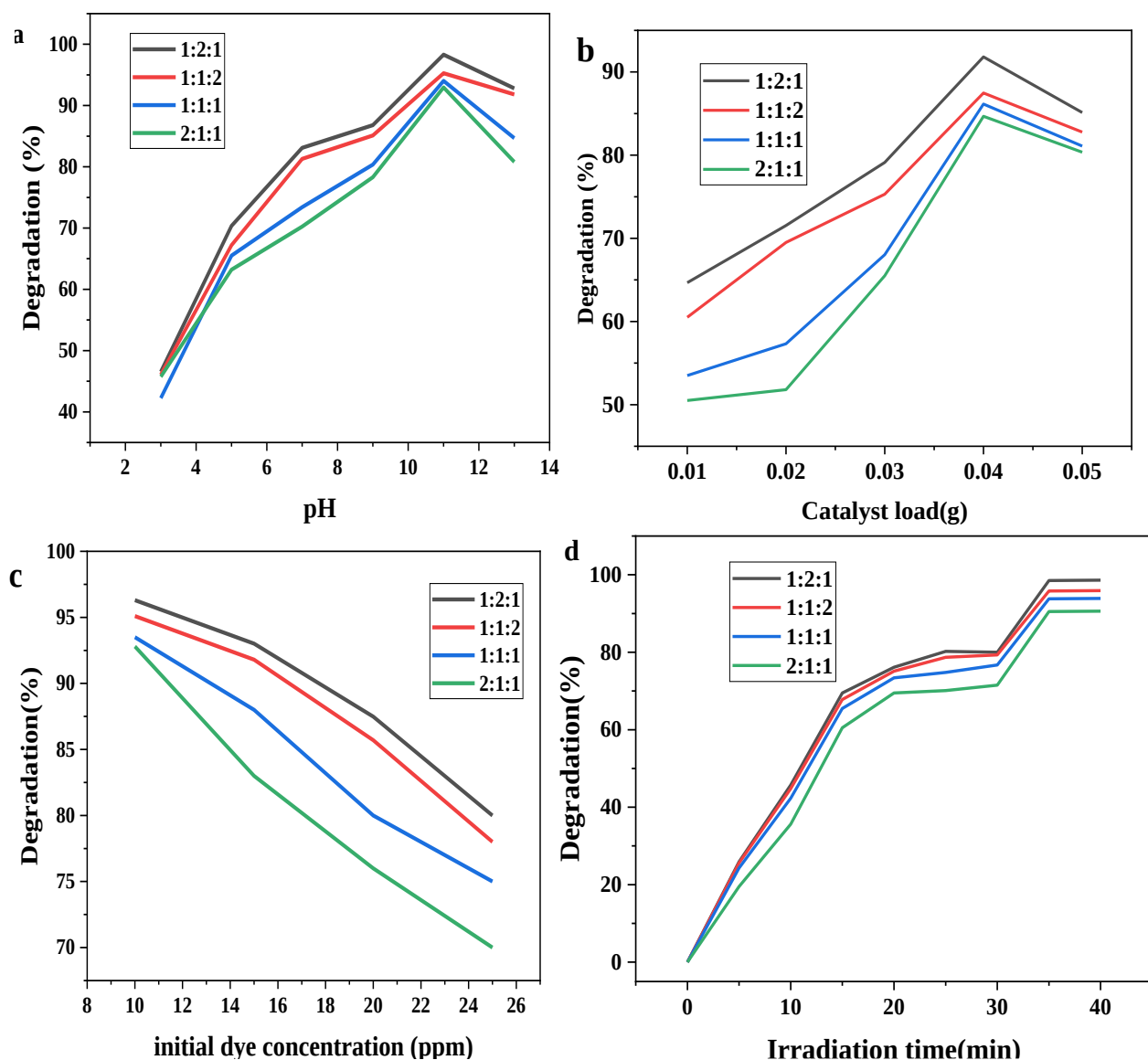


Figure 21. The effect of pH (a), catalyst dose (b), initial dye concentration (c), and irradiation time (d) on degradation of methylene blue using synthesized photocatalyst

An increase in the degradation of methylene blue (MB) dye was observed as the pH increased from 3 to 11. The ZnO/Ag₂O/Co₃O₄ nanocomposite with the different molar ratios of 1:1:1, 1:2:1, 1:1:2, and 2:1:1 exhibited maximum degradation rates of 94.02 %, 98.31 %, 95.28 %, and 92.95 %, respectively, at pH 11 after 30 minutes of sunlight irradiation (Figure 21a). The increase in photocatalytic degradation efficiency with rising pH is likely due to the higher availability of hydroxyl ions (OH⁻) in the alkaline medium, which combine with holes formed during the electronic excitation of the catalyst to generate more hydroxyl radicals, which are highly reactive species responsible for the degradation of methylene blue. In contrast, the lower removal efficiency observed under acidic condition (pH <7) may be attributed to the higher concentration of H⁺ ions, which repel the MB dye molecules and reduce their interaction with the catalyst's active sites. Moreover, since the optimum pH is greater than pHPZC, the surface of the ZnO/Ag₂O/Co₃O₄ NCs is negatively charged under these conditions, increasing the electrostatic attraction with the cationic MB molecule. This is likely because the ZnO/Ag₂O/Co₃O₄ nanocomposite surface is negative since the optimum pH is greater than pHPZC. However, in acidic conditions the catalyst surface is positively charged, resulting in electrostatic repulsion with MB dye and thus decrease degradation efficiency (Zyoud *et al.*, 2023).

b) Effect of Catalyst Dose

The effect of catalyst loading on MB degradation was further investigated by using 0.01, 0.02, 0.03, 0.04, and 0.05 g of catalyst in a 100 mL of 10 ppm MB solution at pH 11. A sample of the reaction mixture was taken for analysis after 30 minutes of sunlight irradiation (Figure 21). As shown in Figure 21b, the percentage removal of methylene blue was 86.15 %, 91.87 %, 87.48 %, and 84.75 % after 30 minutes of irradiation for the 1:1:1, 1:2:1, 1:1:2, and 2:1:1 nanocomposite, respectively. The photodegradation efficiency gradually increased with the catalyst load. However, further increasing the catalyst loading to 0.05 g leads to a decrease in efficiency under the same experimental conditions. Thus, the optimal catalyst loading for maximum degradation of MB was 0.04 g. The increase in catalyst loading is associated with an increase in the number of active sites on the photocatalyst surface, which in turn increases the formation rate of hydroxyl radicals. When the catalyst loading exceeded the optimal value, the MB degradation efficiency decreased due to light interception by the

suspension. Additionally, at higher catalyst loading, aggregation of catalyst particles is expected, reducing surface active sites, increasing light scattering, and ultimately decreasing the passage of irradiation through the sample (Al-Bedairy & Alshamsi, 2018).

c) Effect of Initial Dye Concentration

The effect of initial dye concentration on the photocatalytic degradation efficiency of methylene blue (MB) was studied in the concentration range of 10 mg/L to 25 mg/L, as shown in Figure 21c. Other parameters, such as catalyst dose (0.04 g), pH (11), and irradiation time (30 minutes), were kept constant. The photodegradation efficiency decreased with increasing dye concentration, indicating that the removal of MB was more efficient at lower concentrations, specifically at 10 mg/L (Figure 21c). At higher dye concentrations, a greater number of dye molecules are adsorbed onto the catalyst surface. This results in a reduction in the formation of hydroxyl radicals, as more active sites on the catalyst are occupied by dye molecules. Consequently, the photodegradation efficiency decreases (Kumar & Pandey, 2017).

d) Effect of Irradiation Time

Before conducting the degradation test, an adsorption test of the samples was performed for 30 minutes to determine the adsorption-desorption equilibrium. The reaction time influences the duration the photocatalyst has to generate reactive species and promote the degradation of pollutant molecules. In the current study, the effect of irradiation time was investigated by measuring the degradation efficiency at different irradiation durations upto 40 min under the optimum conditions of photocatalyst dosage (0.04 g), initial dye concentration (10 mg/L), and solution pH of 11. Maximum photodegradation of MB dye was attained as 93.90 %, 98.3 %, 95.9 %, and 90.6 % at an irradiation time of 40 minutes using green-synthesized 1:1:1, 1:2:1, 1:1:2, and 2:1:1 samples of ZnO/Ag₂O/Co₃O₄ nanocomposite, respectively (Figure 21d). The findings (Figure 21d) indicate that with increased reaction time, the degradation of MB improves, and the nanocomposite performance remains high. Initially, the rate of MB degradation is typically low, as the photocatalyst requires time to generate sufficient reactive species to initiate the degradation process. As the reaction time progresses, the availability of reactive species increases, leading to a higher rate of MB degradation. This trend continues

until a saturation point is reached, where efficiency plateaus due to factors such as catalyst deactivation or saturation of active sites on the catalyst surface (Merenda *et al.*, 2019). The initial rapid increase in dye adsorption can be attributed to the availability of vacant active sites for dye adsorption. Moreover, the percentage removal of the dye was observed to stabilize after the optimum time of 40 minutes. This stabilization may be due to slow pore diffusion, saturation of the adsorbent, or reduced availability of sorption sites (Chauhan *et al.*, 2020).

4.5. Evaluation of Photocatalytic Activities of Green Synthesized Nanoparticles and Nanocomposites for Methylene blue Dye Degradation.

The photodegradation of MB dye was carried out under sunlight exposure using all synthesized nanoparticles and nanocomposite. A 10 ppm MB dye solution and 0.04 g of synthesized photocatalyst were used, with the reaction conducted for a total of 40 minutes and monitored at 5-minute intervals. The degradation of MB was evaluated by measuring its absorption at the maximum wavelength (λ_{max}) of 664 nm using a UV-Visible spectrophotometer. UV-Visible analysis of MB is crucial, as most calculations rely on its spectral data. In the UV-Visible spectrum of MB dye, the most prominent absorption peak appears at 664 nm corresponding to the MB monomer, while a shoulder peak at 612 nm is associated with the MB dimer. A sharp decrease in the absorbance spectra at $\lambda_{\text{max}} = 664$ nm as well as a reduction in color intensity (Figure 22), was observed for all green-synthesized nanoparticles and nanocomposite.

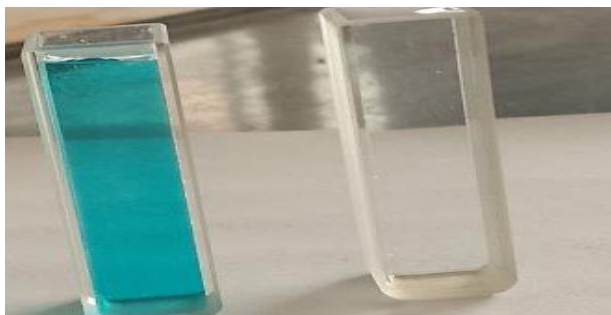


Figure 22. Colour of methylene blue before and after sunlight irradiation using photocatalyst

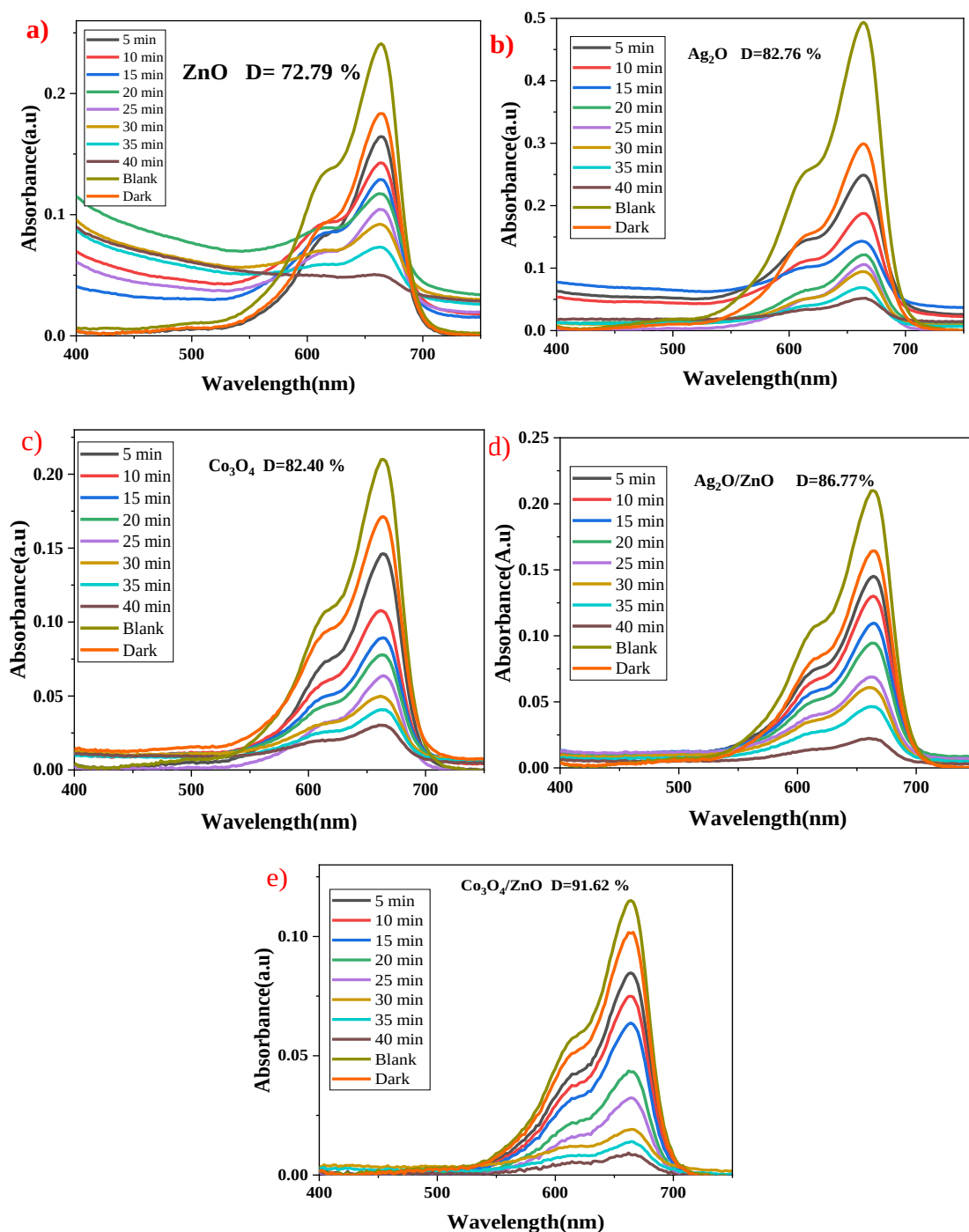


Figure 23. UV-Visible spectra of photocatalytic degradation of methylene dye using ZnO NPs (a), Ag_2O NPs (b), Co_3O_4 NPs (c) nanoparticles, Ag_2O/ZnO NCs (d), Co_3O_4/ZnO NCs (e)

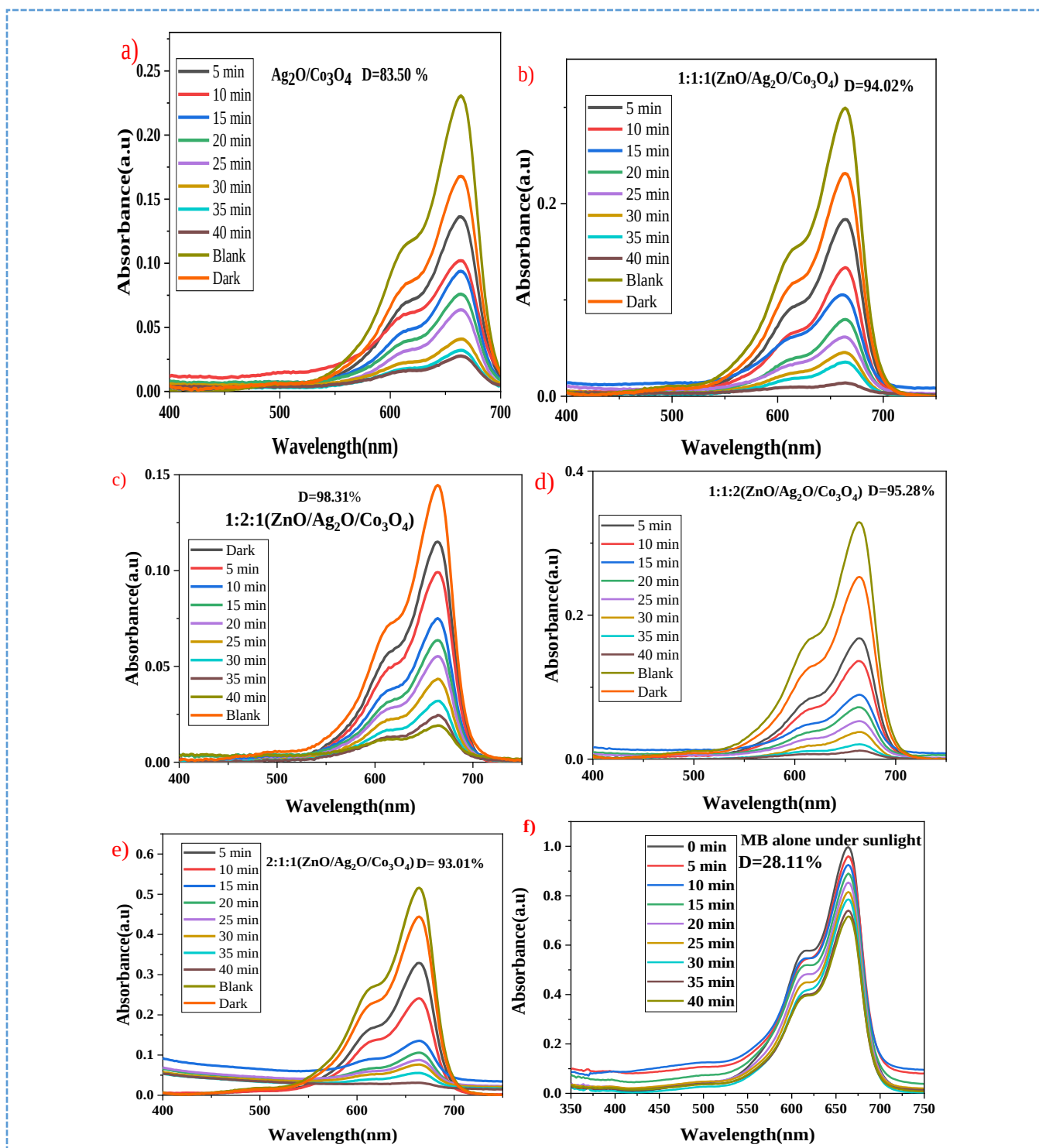


Figure 24. UV-Visible spectra of photocatalytic degradation of methylene dye using $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ (a), 1:1:1(b), 1:2:1(c), 1:1:2(d), 2:1:1 $\text{ZnO}/\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ nanocomposite (e), and without photocatalyst (f)

This significant decrease in peak intensity under sunlight irradiation confirms the photocatalytic degradation of MB dye. Notably, the color of MB in the aqueous solution changed from blue to colorless, indicating the presence of hydroxyl radicals as reported by (Kurniawan *et al.*, 2020). Degradation efficiency was calculated using Equation 6. In this study, the photocatalytic activities of ZnO, Ag₂O, Co₃O₄ nanoparticles, Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and ZnO/Ag₂O/Co₃O₄ (1:1:1, 1:2:1, 1:1:2, and 2:1:1) nanocomposite were evaluated under sunlight irradiation for the degradation of MB dye. The corresponding results are shown in Figures 23 and 24.

The green-synthesized ZnO NPs, Ag₂O NPs, Co₃O₄ NPs, Ag₂O/ZnO NCs, Co₃O₄/ZnO NCs, Ag₂O/Co₃O₄ NCs, and ZnO/Ag₂O/Co₃O₄(1:1:1, 1:2:1, 1:1:2, and 2:1:1) nanocomposite exhibited degradation efficiencies of 72.79 %, 82.76 %, 82.40 %, 86.77 %, 91.62 %, 83.50 %, 94.02 %, 98.31 %, 95.28 %, and 93.01 %, respectively, after 40 minutes of sunlight exposure. From the results, it is evident that green-synthesized ZnO nanoparticles demonstrated lower degradation efficiency compared to all samples. This lower efficiency may be attributed to the poor absorption of visible light due to the wide band gap and low charge-separation efficiency of pure ZnO nanoparticles. However, the deposition of Ag₂O nanoparticles onto the surface of ZnO NPs significantly improved the photocatalytic efficiency of the Ag₂O/ZnO binary nanocomposite compared to green-synthesized ZnO NPs. This improvement is primarily due to a shift in absorption to higher wavelengths in the visible range, enhancing the generation of charge carriers and improving charge-separation efficiency. Furthermore, this enhancement may also be attributed to the synergistic interaction between ZnO and Ag₂O nanoparticles (Mohamed *et al.*, 2020). In addition, when bare ZnO nanoparticles are compared with the synthesized Co₃O₄/ZnO nanocomposite, the enhanced photocatalytic activity of the Co₃O₄/ZnO nanocomposite over pure ZnO NPs is attributed to the synergistic interaction between Co₃O₄ and ZnO NPs, along with improved charge separation compared to pure ZnO nanoparticles. This evidence is supported by literature (Kumar *et al.*, 2021). Regarding Ag₂O/Co₃O₄ nanocomposite; the degradation efficiency is higher than that of the corresponding metal oxide nanoparticles. The formation of the Ag₂O/Co₃O₄ heterostructure decreases the recombination rate of electron-hole pairs

through surface plasmon resonance of Ag₂O NPs, which enhances the photocatalytic activity (Saeed *et al.*, 2021).

As shown in Figures 24 b-e, the ternary ZnO/Ag₂O/Co₃O₄ nanocomposite exhibit the highest photocatalytic performance under sunlight irradiation compared to bare ZnO NPs and binary nanocomposite. This is due to the decoration of the p-type semiconductor Ag₂O NPs on the metal oxide surface, which extends the optical absorption of the ternary ZnO/Ag₂O/Co₃O₄ nanocomposite towards higher wavelengths and reduces the band gap from 2.85 eV (ZnO) to 2.53 eV (ZnO/Ag₂O/Co₃O₄) NCs. The positively charged cationic dye MB can be adsorbed onto the surface of the highly negatively charged nanomaterials via strong electrostatic attraction. This electrostatic interaction enhances the adsorptive properties and, thus, the degradation efficiency. Ag₂O NPs shows small photodegradation activity under sunlight than that of Ag₂O/ZnO NCs, likely due to the rapid recombination of e⁻/h⁺ pairs (Yang *et al.*, 2018). Therefore, the photocatalytic activity of the ternary ZnO/Ag₂O/Co₃O₄ nanocomposite is higher than that of other photocatalysts. The high photocatalytic activity of the ZnO/Ag₂O/Co₃O₄ photocatalyst under sunlight irradiation can be attributed to the synergistic effects of high separation efficiency of photogenerated charges, facilitated by rapid photo-induced charge transfer, and the high adsorption capacity of the ZnO/Ag₂O/Co₃O₄ nanocomposite. Indeed, the Ag₂O NPs and Co₃O₄ NPs act as electron sinks, reducing the recombination rate of photogenerated electron-hole pairs, which improves the photocatalytic activity of ZnO nanoparticles.

The degradation efficiency of methylene blue without any photocatalyst after 40 minute of sunlight irradiation was found to be 28.11% (Figure 24 f). This result indicated that direct degradation of methylene blue alone is insufficient to significantly degrade under sunlight irradiation. In direct photolysis MB absorb light directly, leading to the breakdown of its molecular structure. However the major disadvantage of photolysis method is prolonged degradation time and frequent conversion of the original compound into intermediate products which can sometimes more toxic (Meng *et al.*, 2022). However, in this study, the degradation efficiency of MB was highly enhanced to 98.3% in the presence of ZnO/Ag₂O/Co₃O₄ NCs under sunlight irradiation. This confirmed that sunlight alone by itself is insufficient for degradation.

4.6. Possible Photodegradation Mechanism

The migration direction of photoinduced holes and electrons is primarily determined by the band edge positions of the semiconductors. Before the formation of heterojunctions, the conduction band (CB) edge position of ZnO NPs is more negative than those of Co₃O₄ NPs and Ag₂O NPs, as illustrated in Figure 25. When these materials are combined to form heterojunctions between p-type (Co₃O₄ NPs and Ag₂O NPs) and n-type (ZnO NPs) semiconductors, the Fermi level of ZnO NPs shifts downward, while the Fermi levels of Co₃O₄ NPs and Ag₂O NPs shift upward until equilibrium is reached among the three components. As a result of this Fermi level alignment, the CB and valence band (VB) potentials of Co₃O₄ NPs and Ag₂O NPs become more negative compared to those of ZnO NPs (Sang *et al.*, 2020). Upon sunlight irradiation, photons are absorbed by ZnO, Co₃O₄, and Ag₂O on the photocatalyst surface. This leads to the generation of electron-hole pairs in the corresponding VB, which then move to their respective CBs. Due to the wide band gap of ZnO NPs ($E_g = 3.1$ eV), the photoinduced electrons in ZnO NPs cannot be directly transferred from its valence band (VB) to its conduction band (CB) under sunlight illumination. However, these photoexcited electrons in ZnO NPs may be transferred from its VB to the VB of Co₃O₄ NPs and Ag₂O NPs. These electrons, in turn, can be further transferred to the CBs of Co₃O₄ NPs and Ag₂O NPs. Additionally, photoexcited electrons accumulated in the CBs of Co₃O₄ NPs and Ag₂O NPs will eventually move to the CB of ZnO NPs, while the holes will accumulate in the VBs of Co₃O₄ NPs and Ag₂O NPs. The accumulated electrons in the CB of ZnO NPs interact with O₂ molecules to produce superoxide ($\cdot\text{O}_2^-$) species, while the holes in the VBs of Co₃O₄ and Ag₂O NPs interact with H₂O, oxidizing it into hydroxyl ($\cdot\text{OH}$) radicals. These OH radicals can participate in the photodegradation of methylene blue (MB) under sunlight illumination. Based on the experimental findings, a mechanism for the photodegradation of the ternary ZnO/Co₃O₄/Ag₂O nanocomposite has been proposed, which is schematically illustrated in Figure 25. The possible reactions involved in the photodegradation process over the ZnO/Co₃O₄/Ag₂O nanocomposite are described by Equations 7-15.

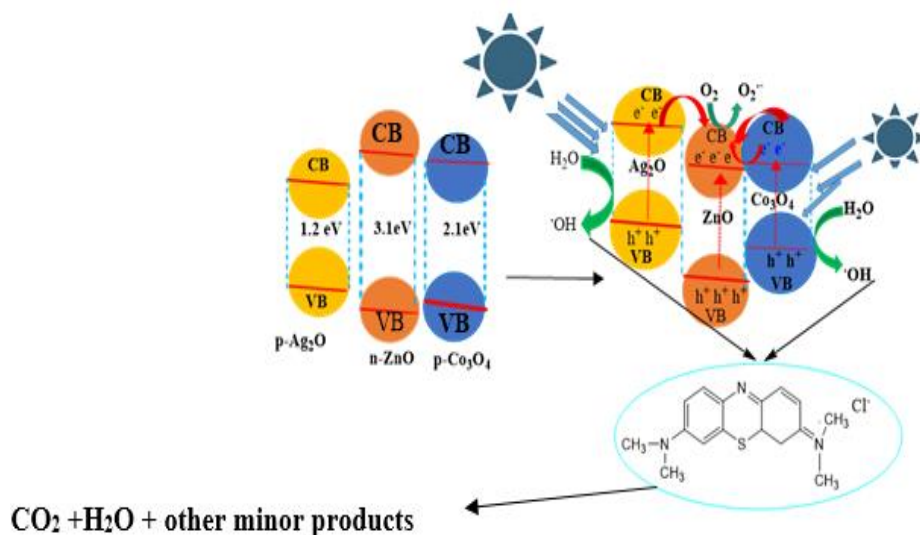
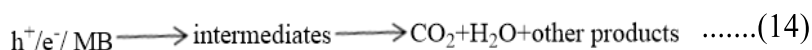
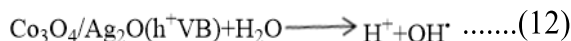
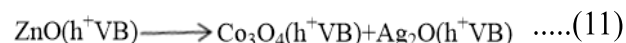
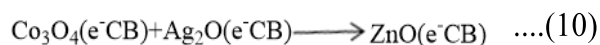
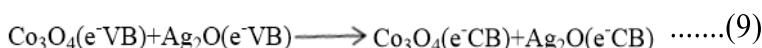
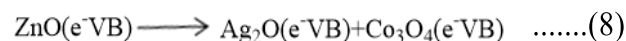
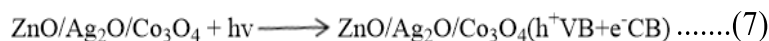


Figure 25. The proposed photocatalytic degradation mechanism of ZnO/Ag₂O/Co₃O₄ nanocomposite under sunlight irradiation



4.7. Comparative Studies

To assess the photocatalytic performance of the ZnO/Ag₂O/Co₃O₄ nanocomposite for methylene blue (MB) degradation, it is essential to compare the results from this study with those reported in previous literature. Table 10 provides a summary of the MB dye removal performance using Ag₂O, ZnO, and Co₃O₄-based nanocomposite. The findings indicate that

the green-synthesized ZnO/Ag₂O/Co₃O₄ nanocomposite is a promising candidate removal of MB dye from effluents under sunlight irradiation.

Table 10. Comparison of photodegradation efficiency of present work with other published materials.

Photocatalyst	light source	Efeciency (%)	Reference
ZnO/TiO ₂ /CdO	200 W tungsten lamp	96	Upadhyay <i>et al.</i> ,2020
Ag ₂ O/NiO/CuFe ₂ O ₄	Sunlight	78.64	Liu <i>et al.</i> ,2020
ZnO/CdO/Pr ₂ O ₃	Visible light	93	Munawar <i>et al.</i> ,2023
Ag doped ZnO/MgO	UV Visible	90	Sabouri <i>et al.</i> ,2024
NiO/ZnO/Ag	UV irradiation	94	Bozetine <i>et al.</i> ,2021
CuO/TiO ₂ /ZnO	Visible light	94	Rehman <i>et al.</i> ,2024
ZnO/ZnS/MnO ₂	200 W visible light	97	Abdullah <i>et al.</i> ,2021
WO ₃ /ZnO/rGO	200 W tungsten filament lamp	94.12	Chaudhary <i>et al.</i> ,2020
MgO/AgO/Ag ₂ O	Sunlight	76.8	Zidane <i>et al.</i> ,2022
TiO ₂ /ZnO/rGO	300 W Xenon lamp	92	Raghavan <i>et al.</i> ,2015
ZnO/Ag ₂ O/Co ₃ O ₄	Sunlight	98.31	This Study

4.8. Reusability of Photocatalyst

The ZnO/Ag₂O/Co₃O₄ (1:2:1) photocatalyst was evaluated for its recyclability, as shown in Figure 26. In this experiment, the same optimum operating conditions were used for the ZnO/Ag₂O/Co₃O₄ NCs, with an initial dye concentration of 100 mL 10 ppm, catalyst load of 0.04 g, pH of 11, and an irradiation time of 40 minutes for the recyclability study.

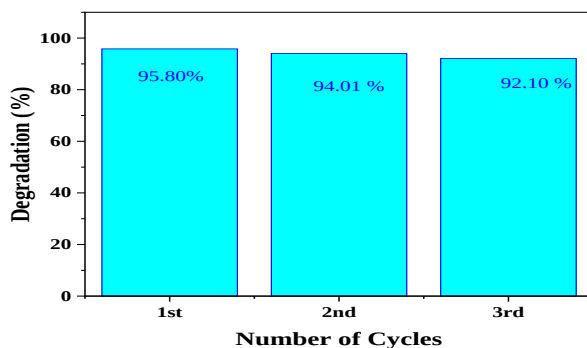


Figure 26. Recyclability of ZnO/Ag₂O/Co₃O₄ nanocomposite for photodegradation of MB for three successive cycles

A small and gradual decrease in the activity of the sample was observed during the first to third cycles: 95.80 %, 94.01 %, and 92.10 %, respectively. However, a gradual decrease in the photocatalytic degradation activity of the ZnO/Ag₂O/Co₃O₄ ternary nanocomposite occurred, likely due to the loss of the recycled catalyst during sampling (Khairnar & Shrivastava, 2019). This experiment confirms the potential recyclability of the synthesized ZnO/Ag₂O/Co₃O₄ photocatalyst over three subsequent cycles with a slight decrease in efficiency. The result indicates that the synthesized ZnO/Ag₂O/Co₃O₄ NCs is not suffering from photo-corrosion during degradation, as reported in the literature (Kazemi *et al.*, 2016).

4.9. Evaluation of Antibacterial Activity of Green Synthesized Nanoparticles and Nanocomposites

The antibacterial activity of all green-synthesized nanoparticles and nanocomposite was examined using the disk diffusion method with different bacterial strains, including two gram-negative (*E. coli* and *E. cloacae*) bacteria and one gram-positive (*S. aureus*), at varying concentrations (12.5 to 100 mg/mL). Ciprofloxacin (10 µg/mL) and DMSO (10%) were used as the positive and negative controls, respectively. The antibacterial efficiency was identified by the appearance of an inhibition zone on the disk. The results for all synthesized samples at at concentration of 100 mg/mL are presented in Table 11. Data for concentrations ranging from 12.5 mg/mL to 75 mg/mL are compiled in Appendix Table 1 and Appendix Table 2. The antibacterial activities of the synthesized nanoparticles and nanoparticles were compared with ciprofloxacin. Inhibition against the bacterial strains was observed after incubation for 24 hours.

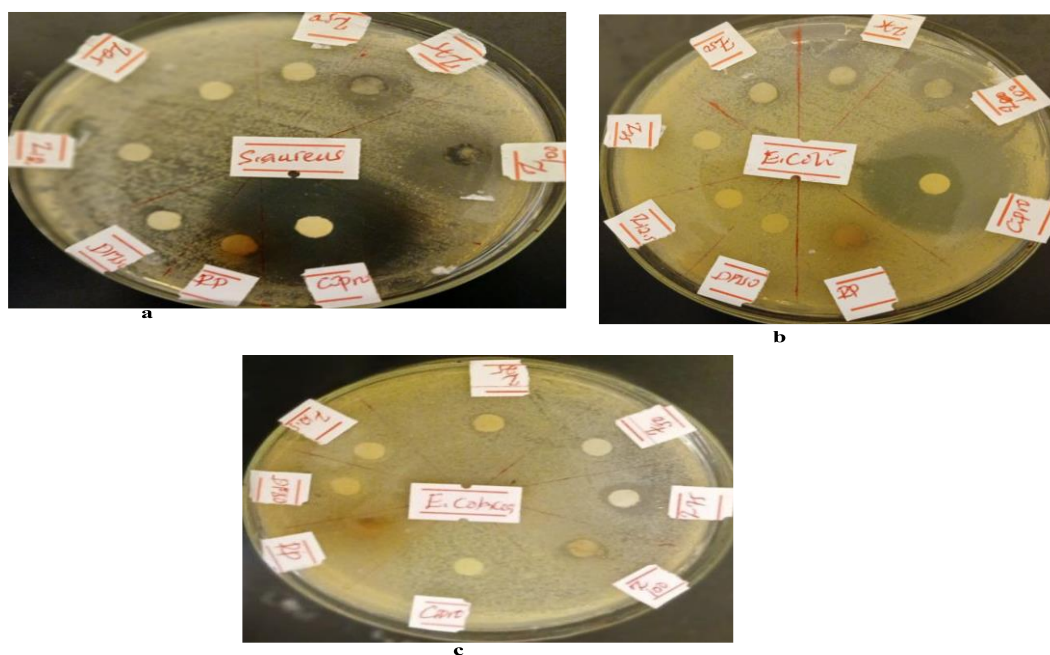
A very small but noticeable zone of inhibition was observed for *R. prinoides* leaf extract, and zone of inhibition of was recorded as 6.4 ± 0.2^a , 6.3 ± 0.02^a , and 6.26 ± 0.05^a mm against *S. aureus*, *E. coli*, and *E. cloacae* respectively. However these values improved significantly after treatment with synthesized NPs and NCs, indicating enhanced antibacterial activities. Similar enhancement of plant extract efficacy through NPs incorporation has also been reported by (Salem *et al.*, 2014).

Table 11.Antibacteria activities of green synthesized ZnO, Ag₂O, Co₃O₄ NPs, Ag₂O/ZnO, Co₃O₄/ZnO, Ag₂O/Co₃O₄, and ZnO/Ag₂O/Co₃O₄ NCs at concentration of 100 mg/mL

Sample	Inhibition zone (mm) for 100 mg/mL		
	<i>S.aureus</i>	<i>E.coli</i>	<i>E.Cloacae</i>
ZnO NPs	14.0±0.20 ^k	14.9±0.15 ^j	9.7±0.25 ^o
Ag ₂ O NPs	14.1±0.26 ^k	13.7±0.21 ^k	9.8±0.2 ^o
Co ₃ O ₄ NPs	15.8±0.2 ^{hi}	16.0±0.4 ^{ghi}	7.8±0.15 ^p
Ag ₂ O/ZnO NCs	16.25±0.07 ^{fghi}	16.2±0.3 ^{fghi}	15.9±0.2 ^{hi}
Co ₃ O ₄ /ZnO NCs	13.6±0.15 ^k	14.8±0.15 ^j	10.9±0.25 ⁿ
Ag ₂ O/Co ₃ O ₄ NCs	15.5±0.05 ^{ij}	16.2±0.05 ^{fghi}	16.1±0.15 ^{ghi}
1:1:1	16.7±0.05 ^{defg}	11.9±0.11 ^{lm}	11.8±0.2 ^m
1:2:1	19.5±0.6 ^c	17.5±0.6 ^d	14.8±0.38 ^j
1:1:2	17.1±0.36 ^{de}	17.6±0.58 ^d	12.6±0.41 ^l
2:1:1	16.4±0.05 ^{efgh}	16.9±0.1 ^{def}	16.6±0.1 ^{efgh}
Ciprofloxacin(10 µg/mL)	30.0±0.0 ^b	34.5±0.5 ^a	30.3±0.57 ^b
<i>R.Prinoides</i>	6.4 ±0.2 ^q	6.3±0.02 ^q	6.26±0.05 ^q
DMSO (10%)	null	null	null

Values of the inhibition zone superscripted by the same letter across the rows are not significantly different from each other at $\alpha=0.05$.

A decrease in the concentration of all synthesized nanoparticles and nanocomposite corresponded to a reduction in bacterial growth inhibition for all samples. The maximum zones of inhibition observed for ZnO NPs, Ag₂O NPs, and Co₃O₄ NPs against *S. aureus* in this study were comparable to those reported for ZnO NPs (Irshad *et al.*, 2018), Ag₂O NPs (Mani *et al.*, 2021), and Co₃O₄ NPs (Govindasamy *et al.*, 2022). Similarly, the maximum zones of inhibition observed for ZnO NPs, Ag₂O NPs, and Co₃O₄ NPs against *E. coli* were align with the inhibition zones reported in the literature for ZnO NPs (Al-Badaii *et al.*, 2024), Ag₂O NPs (Tabassum *et al.*, 2025), Co₃O₄ NPs (Moawad., 2025). For simplicity and space saving on disk, the samples were coded as Z,A,C,AZ,CZ,AC,ZAC,ZA₂C,ZAC₂, and Z₂AC for ZnO ((Figure 27), Ag₂O(Figure 28), Co₃O₄(Figure 29), Ag₂O/ZnO(Figure 30), Co₃O₄/ZnO(Figure 31), Ag₂O/Co₃O₄(Figure 32), 1:1:1(Figure 33), 1:2:1(Figure 34), 1:1:2(Figure 35), and 2:1:1(Figure 36), respectively.



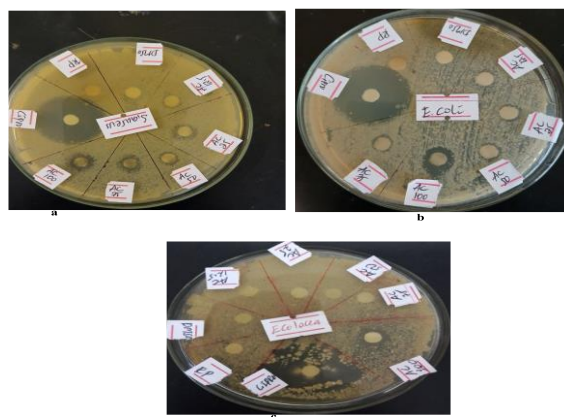


Figure 32.Inhibition Zone of $\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$ NCs against *S.aureus* (a), *E.coli* (b), *E.cloacae* (c)

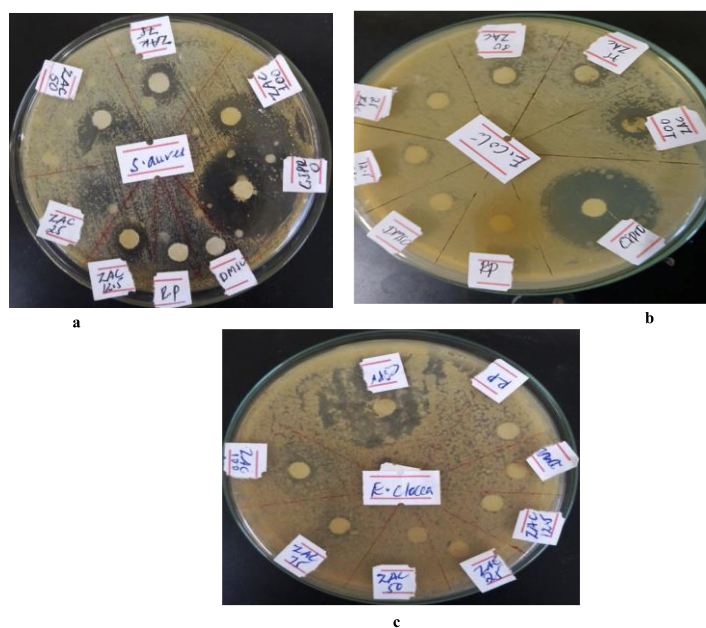


Figure 33.Inhibition Zone of 1:1:1($\text{ZnO}/\text{Ag}_2\text{O}/\text{Co}_3\text{O}_4$) Nanocomposite against *S.aureus* (a), *E.coli* (b), *E.cloacae*(c)

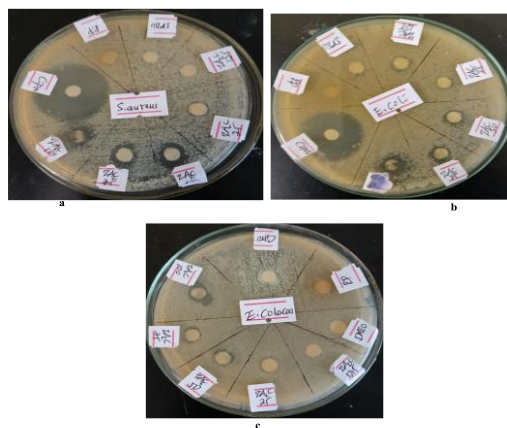


Figure 34.Inhibition Zone of 1:2:1(ZnO/Ag₂O/Co₃O₄) Nanocomposite against *S.aureus* (a), *E.coli* (b), *E.cloacae* (c)

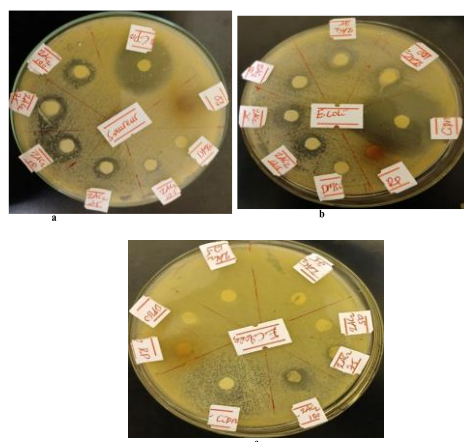


Figure 35.Inhibition Zone of 1:1:2(ZnO/Ag₂O/Co₃O₄) Nanocomposite against *S.aureus* (a), *E.coli* (b), *E.cloacae* (c)

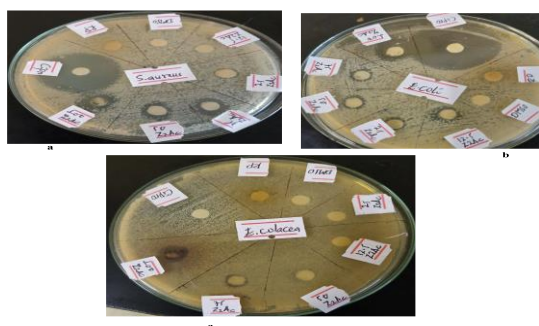


Figure 36.Inhibition Zone of 2:1:1(ZnO/Ag₂O/Co₃O₄) Nanocomposite against *S.aureus* (a), *E.coli* (b), *E.cloacae* (c)

The maximum inhibition zones were observed for the 1:2:1 (ZnO/Ag₂O/Co₃O₄) against *S. aureus* with inhibition zone of 19.5±0.6^c mm. For *E. coli*, 1:1:2 demonstrated the maximum inhibition of 17.6±0.58^d mm. In case of *E. cloacae*, the 2:1:1 is the most efficient than others with inhibition zone of 16.6±0.1^{efgh}. The highest antibacterial activities of 1:2:1 NCs may be attributed to its smaller crystalline size compared to other sample. Smaller crystalline size provides large surface area enhancing surface reactivity and interaction with bacterial cell.

4.10. Possible Antibacterial Activity Mechanism

Figure 37 illustrates the proposed antibacterial mechanism of ZnO/Ag₂O/Co₃O₄ nanocomposite, which primarily involves two main processes: metal ion release and the formation of reactive oxygen species (ROS).

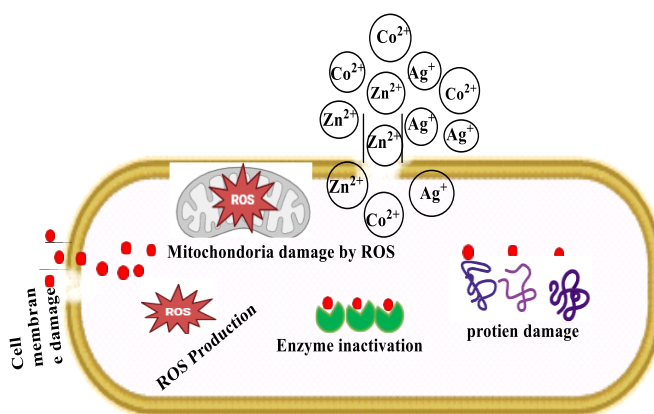


Figure 37. Proposed antibacterial activity mechanism of ZnO/Ag₂O/Co₃O₄ nanocomposite

The ZnO/Ag₂O/Co₃O₄ nanocomposite releases Zn²⁺, Ag⁺, and Co²⁺ ions, which interact with the protein-SH functional groups, leading to their denaturation. The inactivation of these crucial proteins disrupts DNA replication, ultimately resulting in bacterial death. Additionally, the heterogeneous structure formed between ZnO NPs, Ag₂O NPs, and Co₃O₄ NPs facilitates the separation of electron-hole pairs, which leads to the generation of a significant number of ROS, including superoxide radicals ($\cdot\text{O}_2^-$) and hydroxyl radicals ($\cdot\text{OH}$). These radicals can induce lipid peroxidation, DNA damage, mitochondrial dysfunction, and protein oxidation, all of which contribute to bacterial cell death. Furthermore, nanoparticles can penetrate the cell membrane and internalize into the interior

of eukaryotic cells, disrupting the membrane and causing cytoplasmic leakage (Li *et al.*, 2024).

In this study, antibacterial activity of ZnO NPs, Co₃O₄ NPs, Co₃O₄/ZnO NCs, Ag₂O/Co₃O₄ NCs, and the 1:1:2, and 2:1:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite were comparatively more effective against Gram-negative bacteria than against Gram-positive bacteria. This can be attributed to the structural differences between Gram-positive and Gram-negative bacteria. While both types share similar internal structures, their external structures differ significantly. Gram-positive bacteria have a thick peptidoglycan layer that contains teichoic and lipoteichoic acids, while Gram-negative bacteria possess a thin peptidoglycan layer and an outer membrane made up of lipopolysaccharides, phospholipids, and proteins. Therefore, the photoinactivation rate of bacteria may be influenced not only by cell wall thickness but also by the morphology of the cell envelope and the resistance of the outer membrane to the reactive oxygen species produced at the photocatalyst surface (Ishaque *et al.*, 2023). The antibacterial activity of the green-synthesized nanoparticles and nanocomposites was insufficient and partially inactive against *E. cloacae* (a Gram-negative bacterium). This effect may be attributed to the cell-specific presence of lipopolysaccharide-lipoprotein complexes in the cell wall of Gram-negative bacteria, as well as the presence of transport pumps (EmmDR pump) that enable bacteria to regulate their internal environment. These pumps help bacteria remove toxic substances, such as antimicrobial agents, and prevent antibiotics from reaching the intracellular sites needed to exert their antibacterial effects (Hernández-Díaz *et al.*, 2021).

Moreover, the inhibition zone of the green-synthesized ZnO/Ag₂O/Co₃O₄ nanocomposite demonstrates significantly stronger antibacterial activity than that of the binary nanocomposites and nanoparticles, due to the synergistic effect of Ag₂O, Co₃O₄, and ZnO NPs against both Gram-negative and Gram-positive bacteria. Several studies have shown that the combination of Ag₂O NPs with nano-ZnO can result in synergistic effects (Munnawar *et al.*, 2017). Additionally, ZnO/Ag₂O/Co₃O₄ nanocomposite possess a greater number of free surfaces to generate reactive oxygen species (ROS) compared to ZnO, Ag₂O, and Co₃O₄ NPs. The addition of Ag₂O and Co₃O₄ NPs to ZnO NPs introduces more surface defects in the ZnO/Ag₂O/Co₃O₄ nanocomposite.

4.11. Comparative Studies of Antibacterial Activities

To evaluate the antibacterial activities of the present study with previous work, the comparative studies were conducted. The current study demonstrates significantly enhanced and comparable antibacterial activities of the synthesized ternary nanocomposite using *R. prinoides*, as compared to previously reported photocatalysts, as shown in Table 12.

Table 12. Comparison of present antibacterial with previous reports

Sample	Synthesis method	Bacteria	ZOI(mm)	Reference
TiO ₂ /ZnO/MgO	Solgel method	<i>S.aurues</i> , <i>E.coli</i>	13.85±0.75,14.76±0.35 mm	Anaya-Esparza <i>et al.</i> ,2019
Ag ₂ O/CoFe ₂ O ₄ /rGO	Chemical methods	<i>S.aurues</i> , <i>E.coli</i>	14 mm,13 mm	Aftab <i>et al.</i> ,2023
Ag/ZnO/Fe ₃ O ₄	Copericipitation method	<i>S.aurues</i> , <i>E.coli</i>	11 mm,19 mm	Babu <i>et al.</i> ,2020
CuO/ZnO/Co ₃ O ₄	Copericipitation method	<i>S.aurues</i> , <i>E.coli</i>	14 mm,12 mm	Aziz <i>et al.</i> ,2024
MS ₂ /ZnO/rGO	Hydrothermal method	<i>S.aurues</i> , <i>E.coli</i>	18.3,20.6 mm	Priyadharsan <i>et al.</i> ,2018
Ag ₂ O/SrO/CaO	Copericipitation method	<i>S.aurues</i> , <i>E.coli</i>	13.78,16.45 mm	Aghaee & Manteghi,2022
ZnO/Ag ₂ O/Co ₃ O ₄	Green Synthesis method	<i>S.aureus</i> , <i>E.coli</i> , <i>Ecloacae</i>	19.5±0.6 ^c 17.6±0.58 ^d , and 16.6±0.1 ^{efgh} mm	This work

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In the current study, a cost-effective and green approach was successfully applied for the synthesis of metal oxide nanoparticles (ZnO, Ag₂O, and Co₃O₄), binary nanocomposite (Ag₂O/ZnO, Co₃O₄/ZnO, and Ag₂O/Co₃O₄), and ZnO/Ag₂O/Co₃O₄ nanocomposite using *R. prinoides* leaf extract. The optical, bonding, and crystal structure of the synthesized samples were characterized using UV-Vis, FTIR, and XRD respectively. The surface plasmonic resonance confirmed the formation of synthesized NPs and NCs. The functional groups present in *R. prinoides*, were used as capping and stabilizing agent as confirmed by FTIR analysis. XRD analysis confirmed crystalline structure of the synthesized NPs and NCs. The photocatalytic degradation efficiency of methylene blue (MB) under sunlight irradiation, were 72.79 %, 94.02 %, 98.31 %, 95.28 %, and 93.01 % for ZnO NPs, 1:1:1, 1:2:1, 1:1:2, and 2:1:1 ZnO/Ag₂O/Co₃O₄ NCs, respectively. The disk diffusion method was used to evaluate the antibacterial activities of the green-synthesized nanoparticles and nanocomposite against Gram-negative bacteria (*E. coli* and *E. cloacae*) and Gram-positive (*S. aureus*). Maximum inhibition zones of 17.6 ± 0.58^d mm, 16.6 ± 0.1^{efgh} , and 19.5 ± 0.6^c were observed for 1:1:2 (ZnO/Ag₂O/Co₃O₄), 2:1:1 (ZnO/Ag₂O/Co₃O₄) and 1:2:1 (ZnO/Ag₂O/Co₃O₄) nanocomposite against *E. coli*, *E. cloacae*, and *S. aureus*, respectively. Overall, the study demonstrated that biologically synthesized nanoparticles and nanocomposite are suitable for use in a variety of biomedical and water treatment applications.

5.2. Recommendations

In the present study, ZnO/Ag₂O/Co₃O₄ nanocomposite was synthesized through low cost, environmentally friendly, and inexpensive method using *R.prinoides* leaf extract for photocatalytic and antibacterial activities. As a result the following recommendations are made for further research based on the present study:-

- It is better to synthesize ZnO/Ag₂O/Co₃O₄ nanocomposite using other parts *R.prinoides* (eg. roots and stem) extract as capping and stabilizing agent.
- The advanced study should be carried out through other characterizing techniques that were not addressed in this study to study surface morphology, composition and oxidation state, stability, surface area of biosynthesized nanoparticles and nanocomposite and *etc.* by using SEM, TEM, EDX, XPS, TGA, and BET.
- Additional potential applications study which were not addressed in this study like antifungi, sensor, antioxidant, anticancer activities are among that needs attention by further research in the future for better efficiency.

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7. APPENDICES

7.1. Appendix Tables

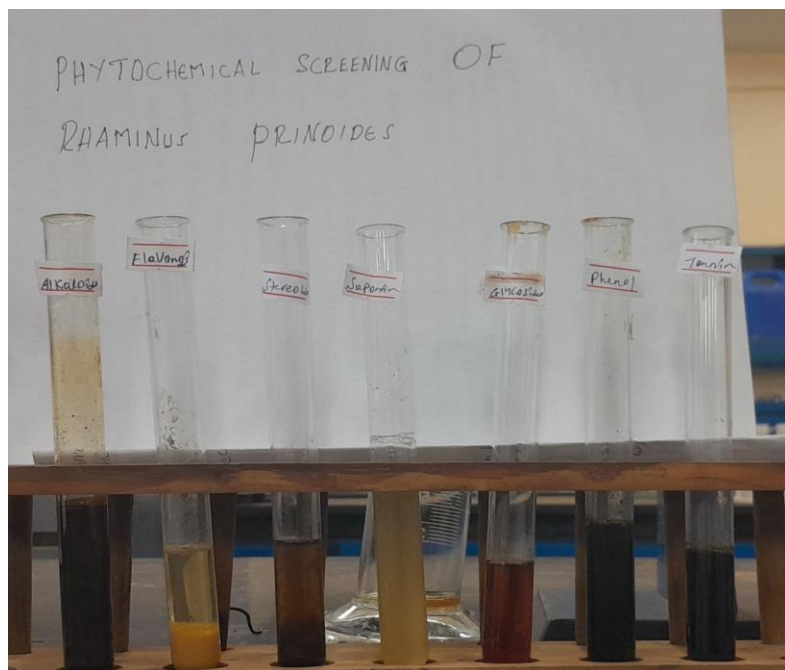
Appendix Table 1.Antibacterial studies of green synthesized nanoparticles and nanocomposites at concentration of 75 mg/mL and 50 mg/mL

Treatment	Concentration (mg/mL)	Inhibition zone (mm)		
		<i>S.aureus</i>	<i>E.coli</i>	<i>E.Cloacae</i>
ZnO NPs	75	12.7±0.17 ^e	10.03±0.15 ^{ij}	8.8±0.15 ^k
Ag ₂ ONPs		11.7±0.1 ^g	9.7±0.25 ^j	9.6±0.28 ^j
Co ₃ O ₄ NPs		14.2±0.1 ^d	9.96±0.15 ^{ij}	7.6±0.11 ^l
Ag ₂ O/ZnONCs		15.6±0.1 ^c	15.46±0.05 ^c	14.26±0.11 ^d
Co ₃ O ₄ /ZnO NCs		12.3±0.26 ^{ef}	9.8±0.15 ^{ij}	6.8±0.11 ^m
Ag ₂ O/Co ₃ O ₄ NCs		10.9±0.11 ^h	7.0±0.2 ^m	0.0±0.0
1:1:1		14.7±0.25 ^d	8.5±0.43 ^k	7.9±0.17 ^l
1:2:1		11.6±0.53 ^g	9.8±0.15 ^{ij}	11.9±0.17 ^{fg}
1:1:2		9.8±0.15 ^{ij}	9.9±0.11 ^{ij}	6.9±0.15 ^m
2:1:1		10.7±0.25 ^h	10.4±0.37 ^{hi}	9.8±0.20 ^{ij}
Ciprofloxacin(10 µg/mL)		30.0±0.0 ^b	39.56±0.4 ^a	30.3±0.57 ^b
<i>Rhamnus prinoides</i>		6.4±0.2 ⁿ	6.3±0.02 ⁿ	6.26±0.05 ⁿ
DMSO (10%)		null	null	null
ZnO NPs	50	12.3±0.2 ^e	6.7±0.25 ^{klm}	0±0.0
Ag ₂ ONPs		10.36±0.15 ^{fg}	9.7±0.26 ^h	0±0.0
Co ₃ O ₄ NPs		13.04±0.21 ^d	9.9±0.11 ^{gh}	0±0.0
Ag ₂ O/ZnONCs		11.8±0.26 ^e	14.2±0.1 ^c	0±0.0
Co ₃ O ₄ /ZnO NCs		9.8±0.2 ^h	6.8±0.23 ^{kl}	0±0.0
Ag ₂ O/Co ₃ O ₄ NCs		6.9±0.11 ^k	6.8±0.11 ^{kl}	0±0.0
1:1:1		11.9±0.17 ^e	6.7kl±0.11 ^{kl}	0±0.0
1:2:1		10.6±0.52 ^f	8.9±0.17 ⁱ	9.8±0.28 ^h
1:1:2		8.2±0.25 ^j	8.7±0.25 ⁱ	0±0.0
2:1:1		9.7±0.25 ^h	9.1±0.11 ⁱ	0±0.0
Ciprofloxacin(10 µg/mL)		30.0±0.0 ^b	34.5±0.5 ^a	30.3±0.57 ^b
<i>Rhamnus prinoides</i>		6.4±0.2 ^{klmn}	6.3±0.02 ^{lmn}	6.26±0.05 ^{mn}
DMSO (10%)		null	null	null

Appendix Table 2.Antibacterial studies of green synthesized nanoparticles and nanocomposites at concentration of 25 mg/mL and 12.5 mg/mL

Treatment	Concentration (mg/mL)	<i>S.aureus</i>	<i>E.coli</i>	<i>E.Cloacae</i>
ZnO NPs	25	0.0±0.0	0.0±0.0	0.0±0.0
Ag ₂ ONPs		7.1±0.15 ^f	7.8±0.2 ^e	0.0±0.0
Co ₃ O ₄ NPs		0.0±0.0	0.0±0.0	0.0±0.0
Ag ₂ O/ZnO NCs		0.0±0.0	0.0±0.0	0.0±0.0
Co ₃ O ₄ /ZnO NCs		8.8±0.15 ^d	6.7±0.1 ^h	0.0±0.0
Ag ₂ O/Co ₃ O ₄ NCs		6.6±0.05 ^g _h	6.7±0.2 ^h	0.0±0.0
1:1:1		9.7±0.2 ^c	0.0±0.0	0.0±0.0
1:2:1		7.1±0.15 ^f	7.1±0.2 ^f	7.7±0.2 ^e
1:1:2		7.2±0.2 ^f	7.9±0.17 ^e	0.0±0.0
2:1:1		6.86±0.2 ^g	6.96±0.15 ^g	0.0±0.0
<i>Rhamnus prinoides</i>		6.4±0.2 ^h	6.3±0.02 ^h	6.26±0.05 ^h
Ciprofloxacin(10 µg/mL)		30.0±0.0 ^b	34.5±0.5 ^a	30.3±0.57 ^b
DMSO (10%)		null	null	null
ZnO NPs	12.5	0.0±0.0	0.0±0.0	0.0±0.0
Ag ₂ ONPs		0.0±0.0	7.13±0.11 ^{ef}	0.0±0.0
Co ₃ O ₄ NPs		0.0±0.0	0.0±0.0	0.0±0.0
Ag ₂ O/ZnO NCs		0.0±0.0	0.0±0.0	0.0±0.0
Co ₃ O ₄ /ZnO NCs		7.9±0.11 ^c	6.8±0.2 ^{fgh}	0.0±0.0
Ag ₂ O/Co ₃ O ₄ NCs		6.76±0.05 ^{fgh}	6.5±0.6 ^{ghi}	0.0±0.0
1:1:1		7.7±0.2 ^c	0.0±0.0	0.0±0.0
1:2:1		0.0±0.0	6.9±0.15 ^{efg}	0.0±0.0
1:1:2		0.0±0.0	7.3±0.28 ^{de}	0.0±0.0
2:1:1		0.0±0.0	7.06±0.05 ^{11ef}	0.0±0.0
<i>Rhamnus prinoides</i>		6.4±0.2 ^{hi}	6.3±0.02 ^{hi}	6.26±0.05 ⁱ
Ciprofloxacin (10 µg/mL)		30.0±0.0 ^b	34.5±0.5 ^a	30.3±0.57 ^b
DMSO (10%)		null	null	null

7.2. Appendix Figure



Appendix Figure 1.Phytochemical screening of *R.prinoides* leaf extract