

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**AN INVESTIGATION OF THE POTENTIAL OF GREEN
SYNTHESIZED ZINC OXIDE NANOPARTICLES FOR FOOD
PACKAGING APPLICATIONS**

MEHMET CAN DURMUŞ

**A THESIS OF MASTER OF SCIENCE
DEPARTMENT OF INTERDISCIPLINARY
NANOSCIENCE AND NANOENGINEERING
NANOSCIENCE AND NANOENGINEERING PROGRAM**

ADVISOR: ASSOC. PROF. DR. ŞÖLEN KINAYYİĞİT

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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**YEŞİL SENTEZ YÖNTEMİ İLE ÜRETİLEN ÇİNKO
OKSİT NANOPARTİKÜLLERİNİN GIDA AMBALAJ
UYGULAMALARINA YÖNELİK
POTANSİYELLERİNİN ARAŞTIRILMASI**

MEHMET CAN DURMUŞ

YÜKSEK LİSANS TEZİ
DİSİPLİNLER ARASI NANO BİLİM VE
NANOMÜHENDİSLİK ANABİLİM DALI
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*To my
Beloved Family*

ABSTRACT

In recent years, escalating environmental concerns about petroleum-based food packaging have prompted a surge in research on sustainable alternatives. Biopolymer-based films, such as those derived from chitosan and gelatin, have emerged as attractive greener options due to their biodegradability, biocompatibility, and renewability. Metal and metal oxide nanoparticles, including silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO₂), and aluminum oxide (Al₂O₃), are commonly used in food packaging applications to enhance tensile strength, gas and UV barrier properties, and ethylene scavenging while providing antimicrobial effects and extending shelf life. Among these, ZnO nanoparticles have garnered particular interest for their potent antibacterial activity, UV-blocking properties, and cost-effectiveness.

This thesis presents a comprehensive study on the green synthesis of ZnO nanoparticles using *Helichrysum arenarium* (dwarf everlasting) extract and their application in food packaging. *H.arenarium*, renowned for its analgesic, anti-inflammatory, antidiabetic, and antioxidant properties, contains rich bioactive compounds including flavonoids, chalcones, phenolic acids, coumarins, and pyrons, making it an ideal natural reducing and stabilizing agent. The study optimizes synthesis conditions including pH, temperature, and precursor concentration to achieve controlled nanoparticle formation.

The synthesized ZnO-HA nanoparticles were thoroughly characterized using SEM, EDX, FTIR, XRD, and UV-Visible Spectroscopy to elucidate their structural, morphological, optical, and chemical properties. Antimicrobial efficacy was evaluated using Kirby-Bauer disk diffusion test method against both Gram-positive and Gram-negative bacteria. Subsequently, the green-synthesized ZnO-HA nanoparticles were incorporated into chitosan/arabic gum (CH-AG) composite coatings at varying concentrations (0.2%, 0.5%, 0.8%, and 1.0%) for banana preservation applications. The effectiveness of these nanocomposite coatings was systematically evaluated through a 20-day storage study, monitoring key quality parameters including color change, mass loss, and firmness.

This integrated approach from green synthesis to practical application demonstrates the potential of sustainable nanotechnology in addressing food waste challenges. The findings contribute to both fundamental understanding of green nanoparticle synthesis and the development of eco-friendly food packaging solutions, potentially offering viable alternatives to conventional packaging while maintaining food quality and safety.

Keywords: Green-approach, Zinc oxide nanoparticles, Nanotechnology, Biodegradable materials, Sustainable packaging, Plant-Based innovation.

ÖZET

Son yıllarda, petrol bazlı gıda ambalajlarının çevresel etkileri konusundaki artan endişeler, sürdürülebilir alternatiflere yönelik araştırmalarda artışa neden olmuştur. Kitosan ve jelatin gibi biyopolimerlerden elde edilen filmler, biyobozunurluk, biyoyumumluluk ve yenilenebilirlik özellikleri sayesinde cazip yeşil alternatifler olarak öne çıkmıştır. Gümüş (Ag), Çinko oksit (ZnO), Titanyum dioksit (TiO₂) ve Alüminyum oksit (Al₂O₃) gibi metal ve metal oksit nanopartiküller, gıda ambalaj uygulamalarında çekme dayanımını, gaz ve UV bariyer özelliklerini ve etilen giderimini artırırken antimikrobiyal etki sağlayarak raf ömrünü uzatmak için yaygın olarak kullanılmaktadır. Bunlar arasında ZnO nanopartiküller, güçlü antibakteriyel aktiviteleri, UV bariyer özellikleri ve maliyet avantajları nedeniyle özel bir ilgi görmektedir.

Bu tez kapsamında, *Helichrysum arenarium* (ölmez çiçek) ekstraktı kullanılarak ZnO nanopartiküllerinin yeşil sentezi ve bunların gıda ambalajındaki uygulamaları üzerine kapsamlı bir çalışma yapılacaktır. Analjezik, anti-inflamatuar, antidiyabetik ve antioksidan özellikleriyle tanınan *H. arenarium*, flavonoidler, kalkanlar, fenolik asitler, kumarinler ve pironlar dahil olmak üzere zengin biyoaktif bileşikler içermekte olup, ideal bir doğal indirgeyici ve stabilize edici ajan oluşturmaktadır. Çalışmada, nanopartikül oluşumunu sağlamak için pH, sıcaklık ve öncül konsantrasyonu dahil olmak üzere sentez koşullarını optimize edilmiştir.

Sentezlenen ZnO-HA nanopartikülleri, yapısal, morfolojik, optik ve kimyasal özelliklerini aydınlatmak için SEM, EDX, FTIR, XRD ve UV-Görünür Spektroskopi kullanılarak kapsamlı bir şekilde karakterize edilmiştir. Antimikrobiyal etkinlik, hem Gram-pozitif hem de Gram-negatif bakterilere karşı Kirby-Bauer disk difüzyon yöntemi kullanılarak değerlendirilmiştir. Daha sonra, yeşil sentezlenmiş ZnO-HA nanopartikülleri, muz koruma uygulamaları için değişen konsantrasyonlarda (%0.2, %0.5, %0.8 ve %1.0) kitosan/arap zıncı (CH-AG) kompozit kaplamalarına dahil edilmiştir. Bu nanokompozit kaplamaların etkinliği, renk değişimi, kütle kaybı ve sertlik gibi temel kalite parametreleri izlenerek 20 günlük bir depolama çalışması ile sistematik olarak değerlendirilmiştir.

Yeşil sentezden pratik uygulamaya kadar bu entegre yaklaşım, sürdürülebilir nanoteknolojinin gıda atığı zorluklarını ele almadaki potansiyelini göstermektedir. Bulgular, hem yeşil nanopartikül sentezinin temel anlaşılmasına hem de çevre dostu gıda ambalajı çözümlerinin geliştirilmesine katkıda bulunmakta, gıda kalitesi ve güvenliğini korurken geleneksel ambalajlara potansiyel olarak uygulanabilir alternatifler sunmaktadır.

Anahtar Kelimeler: Yeşil yaklaşım, Çinko oksit nanopartikülleri, Nanoteknoloji, Biyobozunur malzemeler, Sürdürülebilir ambalaj, Bitki-bazlı inovasyon.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	: Absorption coefficient
β	: Peak broadening at half maximum intensity (FWHM)
ΔE	: Total color difference
ϵ	: Dielectric constant
γ	: Propagation constant
λ	: X-ray wavelength
ν	: Photon frequency
θ	: Bragg angle
∇	: Operator Nabla
D	: Crystallite size
E	: Electric field vector
E_g	: Bandgap energy
F	: Force
h	: Planck's constant
H	: Magnetic field vector
K	: Shape factor (in Scherrer equation)
V_o	: Oxygen vacancies
Zn_i	: Zinc interstitials
AG	: Arabic Gum
Ag	: Silver
Al_2O_3	: Aluminum Oxide
ATCC	: American Type Culture Collection
ATR	: Attenuated Total Reflectance
BPA	: Bisphenol A
CFU/mL	: Colony-Forming Units per milliliter
CH	: Chitosan
CH-AG	: Chitosan/Arabic Gum
CIE	: Commission Internationale de l'Éclairage
CLSI	: Clinical and Laboratory Standards Institute
CVD	: Chemical Vapor Deposition
DD	: Degree of Deacetylation
DLS	: Dynamic Light Scattering
EDS	: Energy Dispersive X-ray Spectroscopy
EUCAST	: European committee on antimicrobial susceptibility testing
FDA	: U.S. Food and Drug Administration
FTIR	: Fourier Transform Infrared Spectroscopy
FWHM	: Full Width at Half Maximum
GRAS	: Generally Recognized as Safe
GTU	: Gebze Technical University
HA	: <i>Helichrysum arenarium</i>
JCPDS	: Joint Committee on Powder Diffraction Standards
MCT	: Mercury Cadmium Telluride
MNPs	: Metal Nanoparticles
MONPs	: Metal Oxide Nanoparticles
MW	: Molecular Weight

PA	: Polyamide
PCA	: Principal Component Analysis
PE	: Polyethylene
PEG 400	: Polyethylene Glycol 400
PET	: Polyethylene Terephthalate
PG	: Polygalacturonase
PME	: Pectin Methylesterase
PP	: Polypropylene
PS	: Polystyrene
PTFE	: Polytetrafluoroethylene
PVC	: Polyvinyl Chloride
RH	: Relative Humidity
r.t	: Room Temperature
ROS	: Reactive Oxygen Species
SD	: Standard Deviation
SEM	: Scanning Electron Microscopy
TEM	: Transmission Electron Microscopy
TGS	: Triglycine Sulfate
TiO ₂	: Titanium Dioxide
UV-Vis	: Ultraviolet-Visible Spectroscopy
XRD	: X-Ray Diffraction
ZnO	: Zinc Oxide
ZnO-HA	: Zinc Oxide Nanoparticles synthesized with <i>Helichrysum</i> arenarium

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1. INTRODUCTION

Food packaging plays a crucial role in modern food industry by protecting food products from external factors, extending shelf life, and maintaining food quality throughout the supply chain [1]. The primary functions of food packaging include protection against physical damage, chemical alterations, and microbiological contamination while facilitating storage, transportation, and distribution processes [2]. In recent years, consumer demands have shifted towards high-quality, natural, fresh, and microbiologically safe food products with extended shelf life, making food packaging innovations increasingly important [3]. This shift in consumer preferences has led to significant developments in packaging technologies, particularly in areas of active and intelligent packaging systems that can monitor and maintain food quality [4].

The evolution of packaging technology has transformed its role beyond mere protection; modern food packaging systems are now designed to inform consumers, enhance product appeal, and incorporate innovative adaptive approaches to food preservation. These advancements in packaging technology are driven by both consumer expectations and industrial developments, leading to continuous innovations in the field. Furthermore, the global food packaging industry has become increasingly focused on developing solutions that not only meet functional requirements but also address growing environmental concerns and sustainability challenges [5]. This has resulted in a conscious shift towards more sustainable and environmentally conscious packaging alternatives that maintain the same level of functionality and food protection [6].

The growing environmental concerns regarding petrochemical-based packaging materials have led to an increased focus on biodegradable alternatives in the food industry. Traditional petroleum-derived packaging materials, such as polyethylene terephthalate (PET) [7], polyvinylchloride (PVC) [8], polyethylene (PE) [9], polypropylene (PP) , polystyrene (PS) [10], and polyamide (PA) [11], have been widely used due to their desirable properties including flexibility, lightweight nature, transparency, tensile strength, thermal stability, gas barrier properties, cost-effectiveness, and protective capabilities [12]. However, these materials pose

significant environmental challenges due to their non-biodegradable nature, persistence in ecosystems for hundreds of years, and potential release of harmful chemicals such as bisphenol A (BPA) during degradation [13]. In response to these environmental concerns and stricter regulations, the food packaging industry has turned its attention to biodegradable alternatives, particularly biopolymer-based materials. Natural biopolymers such as chitosan, gelatin, cellulose, and their derivatives have emerged as promising candidates for sustainable packaging solutions due to their inherent biodegradability, biocompatibility, non-toxicity, and renewability [14]. These biopolymer-based materials can be processed into edible films and coatings, offering unique advantages such as biodegradability and the ability to act as barriers against moisture, gas, and flavor transfer [15]. Moreover, the growing interest in composite structures has led to the development of enhanced biopolymer-based materials with improved mechanical and barrier properties, addressing some of the limitations of conventional biopolymers while maintaining their environmental benefits [16]. This shift towards biodegradable alternatives represents a crucial step in reducing the environmental impact of food packaging while meeting the increasing consumer demand for sustainable products and supporting the global initiative for a more environmentally conscious food industry [17].

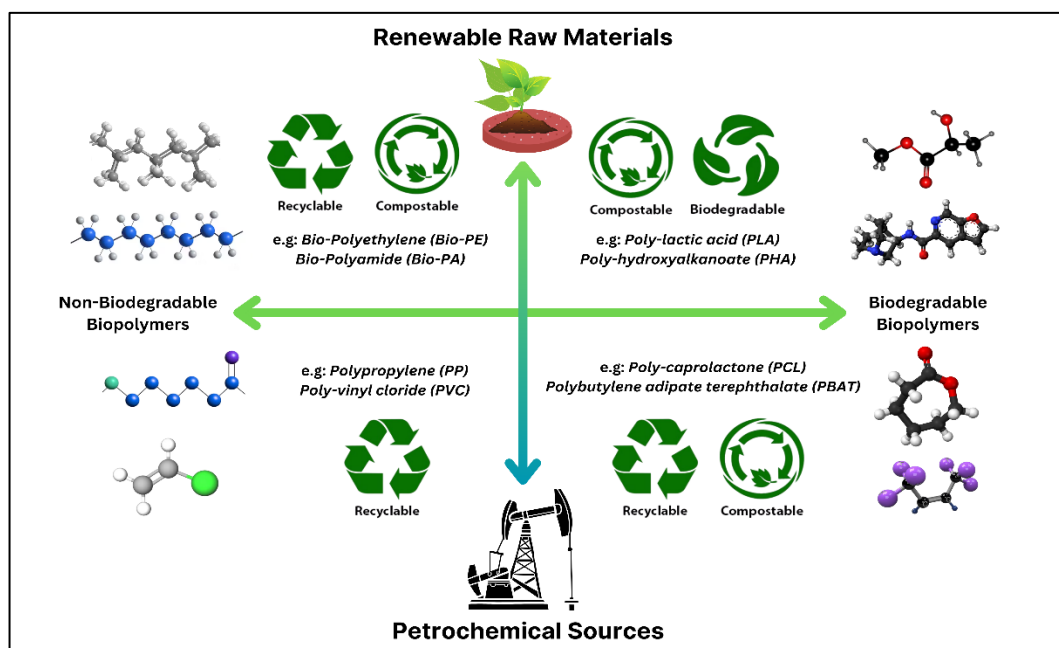


Figure 1.1: Detailed Classification of biopolymers by source and biodegradability.

Nanotechnology has emerged as a revolutionary approach in various industries, particularly transforming the food packaging sector through innovative solutions that address both functional requirements and environmental concerns [18]. The integration of nanotechnology in food packaging has opened new avenues for developing materials with enhanced performance characteristics, improved preservation capabilities, and reduced environmental impact [19].

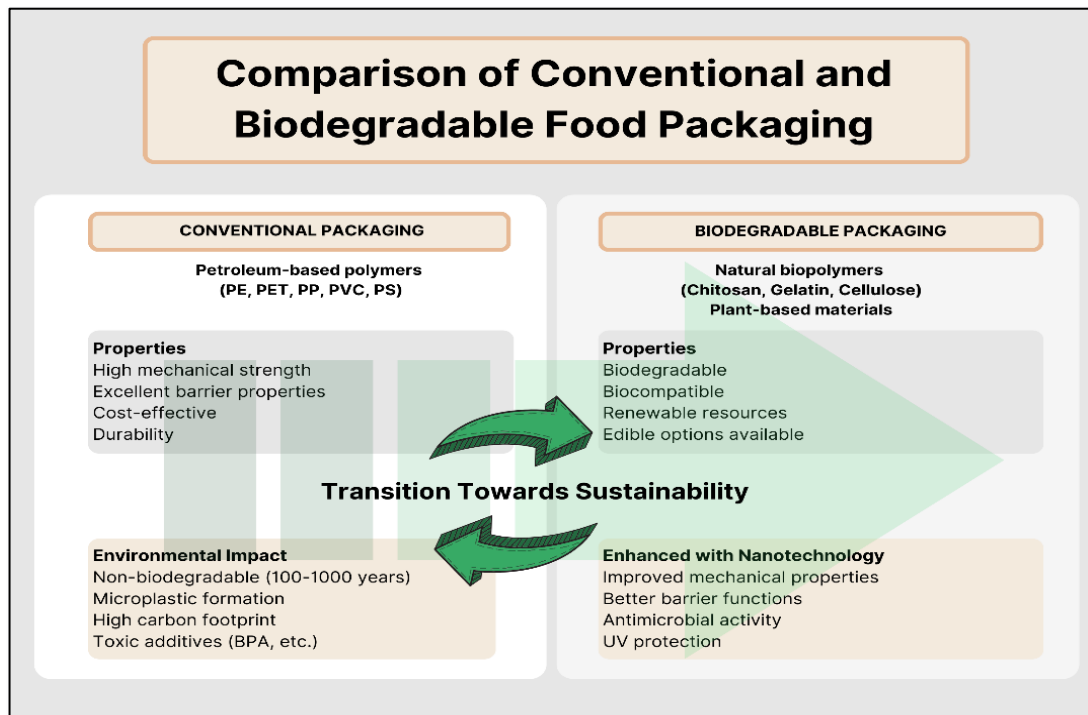


Figure 1.2: Comparison of conventional petroleum-based packaging and biodegradable packaging materials.

While conventional packaging materials offer excellent mechanical and barrier properties, their environmental persistence and toxic effects pose significant challenges [20]. Biodegradable alternatives, enhanced with nanotechnology, present a sustainable solution by combining natural biopolymers with advanced functional properties. This technological advancement has enabled the development of packaging materials with superior mechanical properties, enhanced barrier functions, and active antimicrobial features, contributing to extended shelf life and improved food safety [21]. Furthermore, the application of nanomaterials in food packaging has demonstrated remarkable potential in creating intelligent packaging systems that can monitor food quality, detect spoilage, and provide real-time information about product

conditions, thus addressing both environmental concerns and modern packaging requirements [22].

The incorporation of metal and metal oxide nanoparticles into packaging materials has been particularly significant in advancing packaging functionality. These nanoparticles, including silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO₂), and aluminum oxide (Al₂O₃), can significantly enhance the physical and chemical properties of packaging materials [23–25]. For instance, nanocomposites created by incorporating nanoclays into polymer films exhibit improved gas barrier properties, making them particularly suitable for oxygen or moisture-sensitive products. Similarly, metal oxide nanoparticles can provide antimicrobial protection, UV-blocking capabilities, and enhanced mechanical strength to the packaging materials [26–28]. The multifunctional nature of these nanoparticles allows for the development of active packaging systems that not only protect food products but also actively contribute to maintaining their quality and freshness [29]. Furthermore, the integration of nanotechnology with biodegradable polymers presents an opportunity to develop sustainable packaging solutions that combine the advanced properties of nanomaterials with environmental benefits [30, 31]. However, this technological advancement also necessitates careful consideration of safety aspects, particularly regarding the potential migration of nanoparticles into food products and their long-term environmental impact, emphasizing the importance of developing green synthesis approaches and conducting thorough safety assessments [32–34].

Among various metal oxide nanoparticles, zinc oxide (ZnO) nanoparticles have attracted considerable attention in food packaging applications due to their unique combination of properties and cost-effectiveness [35, 36]. ZnO nanoparticles exhibit remarkable antimicrobial activity against a broad spectrum of microorganisms, including both Gram-positive and Gram-negative bacteria, which is particularly valuable for food preservation [37]. Their antimicrobial mechanism involves the generation of reactive oxygen species (ROS) and the release of Zn²⁺ ions, providing effective protection against food spoilage organisms [38]. Additionally, ZnO nanoparticles possess excellent UV-blocking capabilities, which help protect packaged food from UV-induced degradation while maintaining optical transparency in the visible region [39]. These nanoparticles also demonstrate high thermal stability, mechanical strength, and chemical stability, making them ideal candidates for food

packaging applications [40]. Furthermore, ZnO nanoparticles are recognized as "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration (FDA), highlighting their potential for safe use in food contact materials [41]. The incorporation of ZnO nanoparticles into biopolymer matrices can significantly enhance the mechanical properties, barrier characteristics, and thermal stability of the resulting packaging materials while maintaining their biodegradability.

Traditional synthesis methods for ZnO nanoparticles often involve the use of hazardous chemicals, high energy consumption, and potentially toxic by-products, raising environmental and health concerns [42, 43]. In response to these challenges, green synthesis approaches using plant extracts have emerged as an environmentally friendly and sustainable alternative. The green synthesis of metal and metal oxide nanoparticles using plant extracts has gained significant attention due to its simplicity, cost-effectiveness, and environmental compatibility [44]. Various medicinal plants and their extracts have been successfully employed in the synthesis of nanoparticles, demonstrating the versatility and potential of this approach. These plant-mediated syntheses typically occur under mild conditions and utilize the natural reducing and stabilizing agents present in plant extracts, eliminating the need for harmful chemicals and complex procedures [45, 46].

Helichrysum arenarium (dwarf everlasting) presents a particularly promising option for the green synthesis of ZnO nanoparticles due to its rich phytochemical composition [47]. This medicinal herb contains a diverse array of bioactive compounds, including flavonoids, chalcones, phenolic acids, coumarins, and pyrans, which can serve as natural reducing and stabilizing agents in nanoparticle synthesis [48, 49]. The phenolic compounds present in the plant extract not only facilitate the reduction of metal ions but also provide stability to the formed nanoparticles through surface functionalization. These bioactive compounds enable the formation of stable nanoparticles through mechanisms involving electron transfer, metal ion reduction, and subsequent capping of the nanoparticle surface [50].

Moreover, *Helichrysum arenarium* possesses significant biological activities, including analgesic, anti-inflammatory, antidiabetic, and antioxidant properties, which could potentially enhance the functionality of the synthesized nanoparticles [51, 52]. The inherent therapeutic properties of the plant have been well-documented in traditional medicine, and its incorporation in nanoparticle synthesis could lead to the

development of multifunctional materials with enhanced biological activities. This green synthesis approach represents a sustainable method that aligns with green chemistry principles while potentially conferring additional beneficial properties to the resulting nanomaterials. Furthermore, the abundance and accessibility of the plant make it an economically viable option for large-scale green synthesis of nanoparticles [53]. Combination of antimicrobial properties of ZnO nanoparticles with the bioactive compounds from *H. arenarium* extract creates a synergistic effect that is particularly advantageous for food packaging applications.

Application of green-synthesized ZnO nanoparticles in food packaging has gained considerable attention due to their enhanced antimicrobial properties and potential to extend the shelf life of perishable foods [54]. Edible coatings incorporating ZnO nanoparticles represent an innovative approach to reduce post-harvest losses and maintain food quality during storage. Among various biopolymers used for edible coatings, chitosan (CH) and arabic gum (AG) composites have emerged as promising materials due to their biodegradability, non-toxicity, and excellent film-forming properties [55, 56].

Chitosan, a deacetylated derivative of chitin, possesses inherent antimicrobial properties and forms transparent films with good oxygen barrier properties [57]. When combined with arabic gum, a natural polysaccharide with excellent emulsifying and stabilizing properties, the resulting composite exhibits enhanced mechanical strength and water vapor barrier characteristics [58]. Incorporation of ZnO nanoparticles into CH-AG composite coatings can synergistically improve their antimicrobial efficacy and physical properties, creating a multifunctional packaging material [59, 60].

Banana (*Musa acuminata*), as one of the most consumed fruits worldwide, faces significant post-harvest losses due to rapid ripening and susceptibility to microbial decay [61]. The application of nanocomposite edible coatings on bananas could effectively delay ripening processes by reducing respiration rate, minimizing moisture loss, and preventing microbial growth [62]. ZnO-incorporated CH-AG coatings can create a semi-permeable barrier that regulates gas exchange while maintaining the fruit quality attributes including color, firmness, and nutritional value during storage [63, 64].

Therefore, this study presents a dual approach: (1) developing an environmentally friendly method for the synthesis of ZnO nanoparticles using *Helichrysum arenarium* extract as a natural reducing and stabilizing agent, and (2) incorporating these green-synthesized ZnO-HA nanoparticles into chitosan/arabic gum (CH-AG) composite coatings for banana preservation. The research encompasses a systematic investigation of the synthesis parameters and their effects on the properties of the resulting nanoparticles. The synthesized ZnO nanoparticles have been characterized in detail using various analytical techniques including Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and UV-Visible Spectroscopy to determine their structural, morphological, and optical properties.

Furthermore, the study evaluates the antimicrobial efficacy of the synthesized nanoparticles against both Gram-positive and Gram-negative bacteria through standardized microbiological assays. The application phase investigates the optimal concentration of ZnO-HA nanoparticles (0.2%, 0.5%, 0.8%, and 1.0%) in CH-AG composite coatings and assesses their effectiveness in maintaining banana quality through monitoring color change, mass loss, and firmness during a 20-day storage period. This integrated approach not only contributes to the development of sustainable synthesis methods for metal oxide nanoparticles but also demonstrates their practical implementation in eco-friendly food packaging solutions. The findings are expected to provide valuable insights into both green nanotechnology and sustainable post-harvest management strategies, potentially offering a viable solution to reduce food waste while ensuring food safety and quality.

1.1. Synthesis Methods for Metal (MNPs) and Metal Oxide Nanoparticles (MONPs)

The field of nanoscience and nanotechnology has experienced unprecedented growth over the past decades, attracting significant attention from diverse scientific and engineering communities worldwide [65]. The exponential increase in research activities and publications in this field reflects its fundamental importance and transformative potential across multiple disciplines. A comprehensive analysis of scientific literature reveals a dramatic surge in nanoparticle-related research, with publication numbers showing remarkable growth from the early 2000s to the present

day [66, 67]. This intense scientific interest is driven by the extraordinary properties and versatile applications of nanoparticles, particularly metal and metal oxide nanoparticles, which have demonstrated remarkable potential in various fields including materials science , chemistry , physics , biology, medicine, and food packaging applications [68, 69].

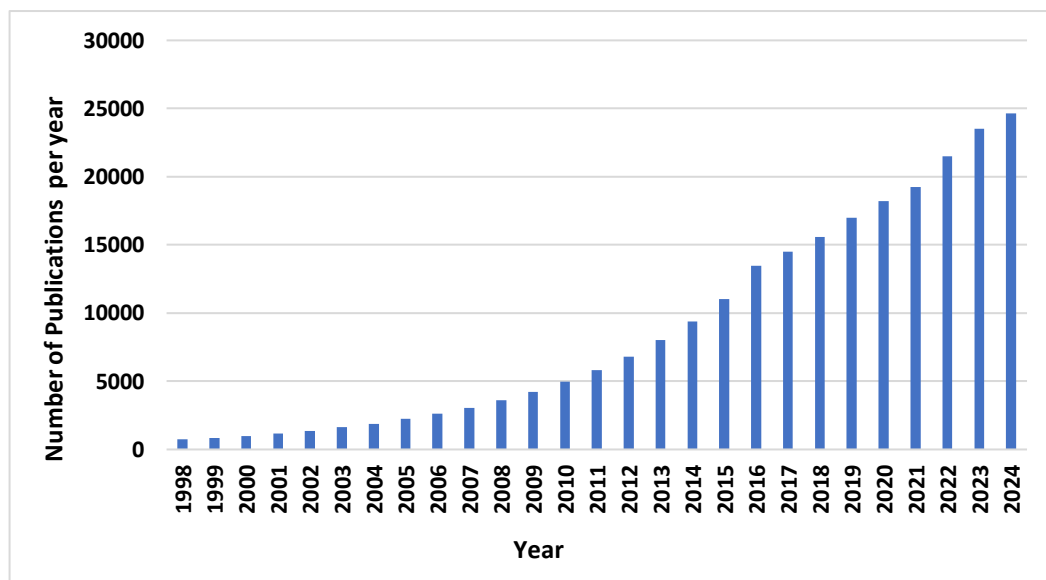


Figure 1.3: Graph showing the exponential increase in publications related to metal nanoparticle synthesis and applications from 1998 to 2024. Web of Science, Keyword: Metal nanoparticles.

Metal nanoparticles (MNPs) occupy a unique position between molecular and bulk materials, earning them the designation "nanoclusters.". This intermediate state presents fascinating properties as these particles are larger than individual atoms and molecules yet remain too small to exhibit bulk solid characteristics. This unique positioning results in behavior that defies both conventional quantum chemistry and classical physics principles, leading to remarkable and often unexpected properties [70, 71]. The electronic properties of MNPs demonstrate significant deviations from their bulk counterparts, particularly in aspects such as conductivity and magnetism [72]. As particle size decreases, a corresponding reduction occurs in the electronic states within both the valence and conductivity bands, resulting in dramatic alterations of electronic properties [73]. This phenomenon, known as the quantum size effect, illustrates how electronic properties progressively transform as dimensionality reduces from bulk three-dimensional systems to zero-dimensional quantum dots. Each nanoparticle essentially functions as a confined system containing thousands of atoms,

creating unique electron containment properties that contribute to their distinctive characteristics [74].

The synthesis of metal and metal oxide nanoparticles encompasses various methodologies, with effectiveness determined by several critical parameters. These include precise control over size, shape, and crystal structure, as well as the ability to maintain stable chemical and physical properties. The careful control of these parameters is essential as they directly influence the final properties and potential applications of the nanoparticles. For instance, particle size and morphology can significantly affect optical, electronic, and catalytic properties, while crystal structure influences mechanical and thermal stability [71, 75]. Furthermore, the surface chemistry of nanoparticles plays a crucial role in determining their interaction with surrounding media and their performance in specific applications.

The evolution of synthesis techniques has paralleled the growing understanding of nanoparticle formation mechanisms and the increasing demands for sustainable practices in chemical processes. Traditional synthesis methods, while effective in producing nanoparticles with controlled characteristics, often involve the use of harsh chemicals and energy-intensive processes [76]. This has led to a significant shift in research focus towards developing more environmentally friendly approaches. These green synthesis methods aim to maintain precise control over nanoparticle characteristics while minimizing environmental impact through the use of natural reducing agents, mild reaction conditions, and biocompatible stabilizing agents.

Equally important considerations in nanoparticle synthesis include managing aggregation rates, minimizing impurities, ensuring reproducibility, and developing cost-effective scaling strategies [77]. The prevention of particle aggregation is particularly crucial as it affects the stability and long-term usability of nanoparticle systems. The presence of impurities can significantly impact the performance and reliability of nanoparticles in their intended applications. Additionally, the ability to reproduce synthesis results consistently and scale up production while maintaining particle quality represents a significant challenge in the field.

Recent advances in characterization techniques have greatly enhanced our understanding of nanoparticle formation mechanisms and properties. Modern analytical methods allow for detailed investigation of particle size, shape, composition,

and surface characteristics, enabling better control over synthesis processes and more precise tailoring of nanoparticle properties for specific applications [78]. This improved understanding has led to the development of more sophisticated synthesis strategies that offer enhanced control over particle characteristics while addressing environmental and scaling concerns

Recent developments in nanoparticle synthesis have focused on improving control energy consumption and waste generation. Understanding the fundamental aspects of chemical synthesis techniques continues to be crucial for developing improved methods that balance control over particle properties with environmental sustainability.

1.1.1. Traditional Synthesis Routes for Metal Nanoparticles (MNPs)

The synthesis of metal and metal oxide nanoparticles can be broadly classified into two fundamental approaches: top-down and bottom-up methods. Each approach offers distinct advantages and limitations in terms of particle control, scalability, and application potential [79]. Understanding these basic approaches is crucial for developing effective synthesis strategies that meet specific requirements for various applications, including food packaging materials.

Top-down approaches involve the mechanical breakdown of bulk materials into nanoscale particles through various physical processes. These methods include mechanical grinding, high-energy ball milling, laser ablation, sputtering, and lithographic techniques [80]. During mechanical processes, bulk materials are subjected to intense mechanical forces that break them down into smaller particles. In mechanical grinding and ball milling, the size reduction is achieved through repeated deformation, fracture, and cold welding of particles under high-energy collisions [81]. Laser ablation involves the use of high-energy laser pulses to remove material from a bulk target, resulting in the formation of nanoparticles in either gas or liquid media. While these methods can produce nanoparticles in large quantities, they often face significant challenges. The primary limitations include broad size distributions, irregular morphologies, surface imperfections, and structural defects that can affect the final properties of the nanoparticles. Additionally, the high energy consumption

associated with these processes raises concerns about cost-effectiveness and environmental impact [82].

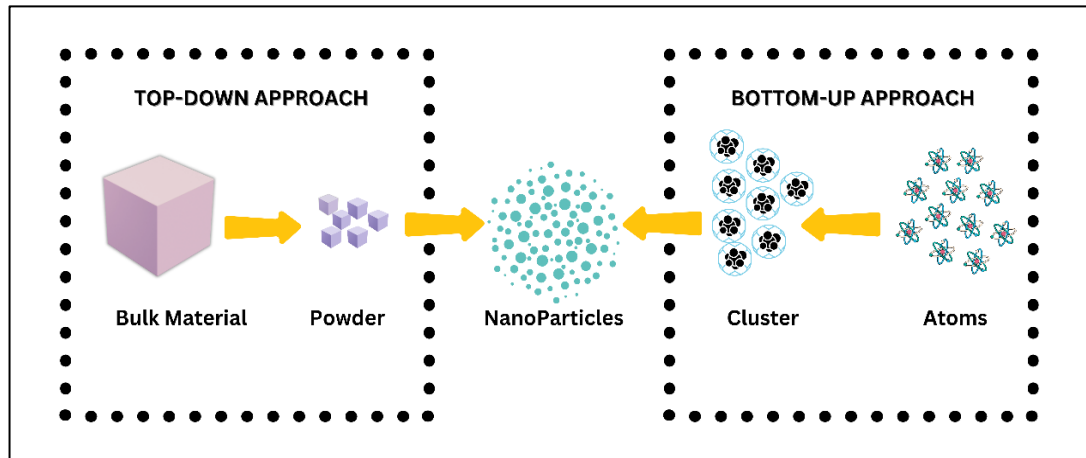


Figure 1.4: Schematic illustration comparing top-down and bottom-up approaches in nanoparticle synthesis.

Bottom-up approaches, in contrast, involve the assembly of nanoparticles from atomic or molecular precursors through chemical reactions. These methods are based on the principle of atomic or molecular self-assembly to form larger structures in the nanoscale range. Chemical reduction, sol-gel processing, chemical vapor deposition (CVD), and electrochemical methods are common examples of bottom-up synthesis techniques [83]. In chemical reduction methods, metal ions are converted to their zero-valent state through controlled reduction reactions. Sol-gel processing involves the formation of an inorganic colloidal suspension (sol) followed by its conversion to a gel-like network containing both a liquid and solid phase. CVD methods utilize vapor-phase precursors that react or decompose on a substrate surface to form nanoparticles. The primary advantage of bottom-up approaches lies in their ability to provide better control over particle size, shape, composition, and crystal structure [83, 84].

The chemical synthesis of nanoparticles through bottom-up approaches generally involves three essential components: metal precursors, reducing agents, and stabilizing agents. Metal precursors, typically metal salts such as chlorides, nitrates, or acetates, provide the source of metal ions for nanoparticle formation. The choice of metal precursor can significantly influence the reaction kinetics and final particle properties [85]. Common reducing agents such as sodium borohydride, citrate, and ascorbic acid facilitate the conversion of metal ions to metal atoms, initiating the nucleation process.

The strength of the reducing agent affects the reduction rate and, consequently, the size and size distribution of the resulting nanoparticles.

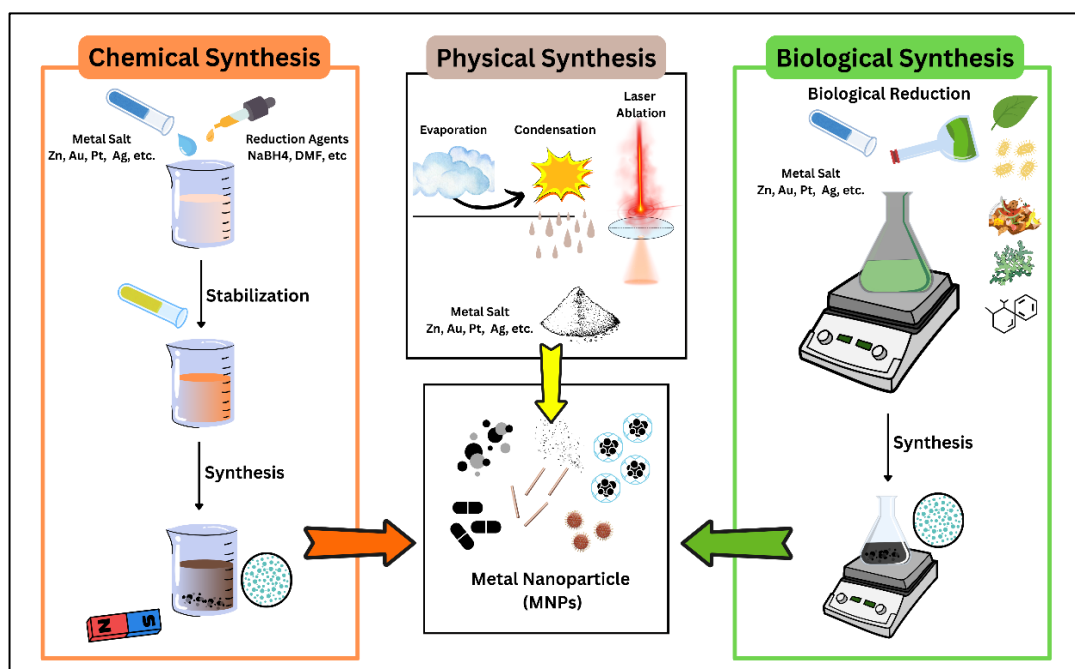


Figure 1.5: The diagram illustrates three methods for synthesizing metal nanoparticles: Chemical. Physical, and Biological method.

Stabilizing agents play a crucial role in preventing particle aggregation and controlling growth through either steric or electrostatic stabilization mechanisms. These molecules adsorb onto the surface of newly formed nanoparticles, creating a protective layer that maintains colloidal stability [86]. Common stabilizing agents include polymers, surfactants, and organic ligands. The interaction between stabilizing agents and particle surfaces not only prevents aggregation but also influences the final size, shape, and surface chemistry of the nanoparticles. This surface modification can be particularly important for applications in food packaging, where compatibility with the packaging matrix and long-term stability are essential considerations.

The reaction conditions in bottom-up synthesis methods require careful control to achieve desired particle characteristics. Temperature plays a significant role in determining reaction kinetics and particle formation mechanisms. Higher temperatures generally increase reaction rates and can affect particle size distribution [87]. The pH of the reaction medium influences the reduction potential of both metal ions and reducing agents, thereby affecting the overall synthesis process. Additionally, the

concentration ratios between metal precursors, reducing agents, and stabilizers must be optimized to achieve controlled particle growth.

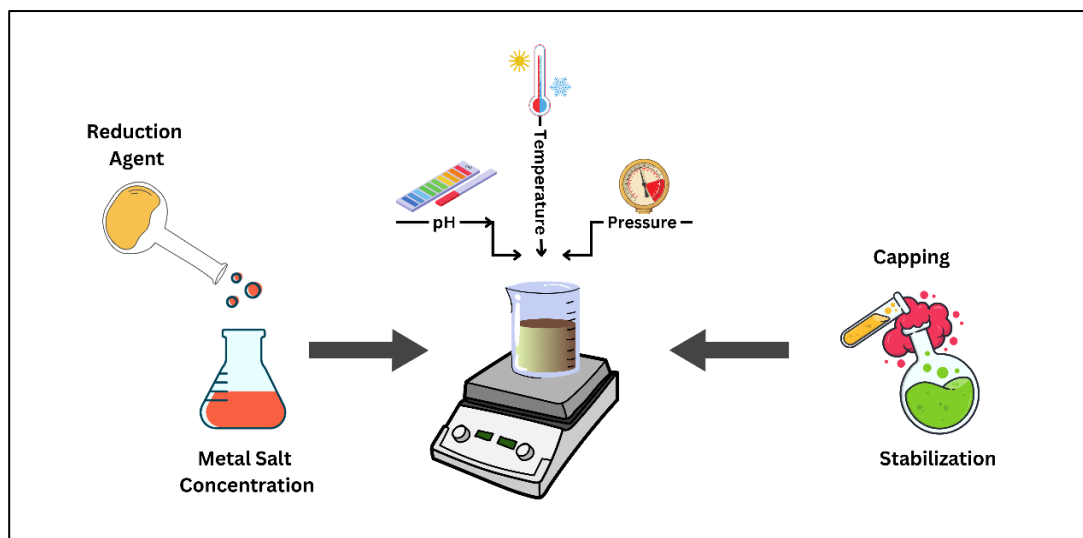


Figure 1.6: Effect of various reaction parameters on nanoparticle formation and characteristics.

The selection between top-down and bottom-up approaches depends on various factors including the desired particle characteristics, application requirements, scale of production, and economic considerations. For applications in food packaging materials, where precise control over particle size and uniformity is crucial for achieving specific functional properties, bottom-up approaches, particularly chemical synthesis methods, are generally preferred. These methods offer better control over particle characteristics and can be modified to incorporate green synthesis principles, aligning with the growing demand for sustainable manufacturing processes.

1.1.2. Green Synthesis Approaches for Metal Nanoparticles (MNPs)

The growing environmental concerns and the principles of sustainable chemistry have led to the development of green synthesis methods for metal (e.g., Ag, Au, Pt, Mg, Zn, Cu, etc.) and metal oxide (e.g., Ag_2O , CuO , TiO_2 , MnO_2 , ZnO , Fe_2O_3 etc.) nanoparticles. These environmentally friendly approaches aim to minimize the use of hazardous chemicals, reduce energy consumption, and generate fewer toxic by-products compared to conventional synthesis methods [88]. Green synthesis represents a paradigm shift in nanoparticle production, emphasizing the use of natural reducing and stabilizing agents, mild reaction conditions, and environmentally benign solvents.

This transition towards sustainable practices has become increasingly important as the demand for nanoparticles continues to grow across various industries.

The fundamental principles of green synthesis align with the twelve principles of green chemistry, focusing on waste prevention, atom economy, and the use of safer chemicals and solvents [89]. These methods typically operate under mild conditions, often at room temperature and atmospheric pressure, significantly reducing energy requirements. Furthermore, green synthesis approaches frequently employ water as the primary solvent, eliminating the need for toxic organic solvents commonly used in traditional methods. The elimination of harmful organic solvents not only reduces environmental impact but also simplifies the purification and isolation processes.

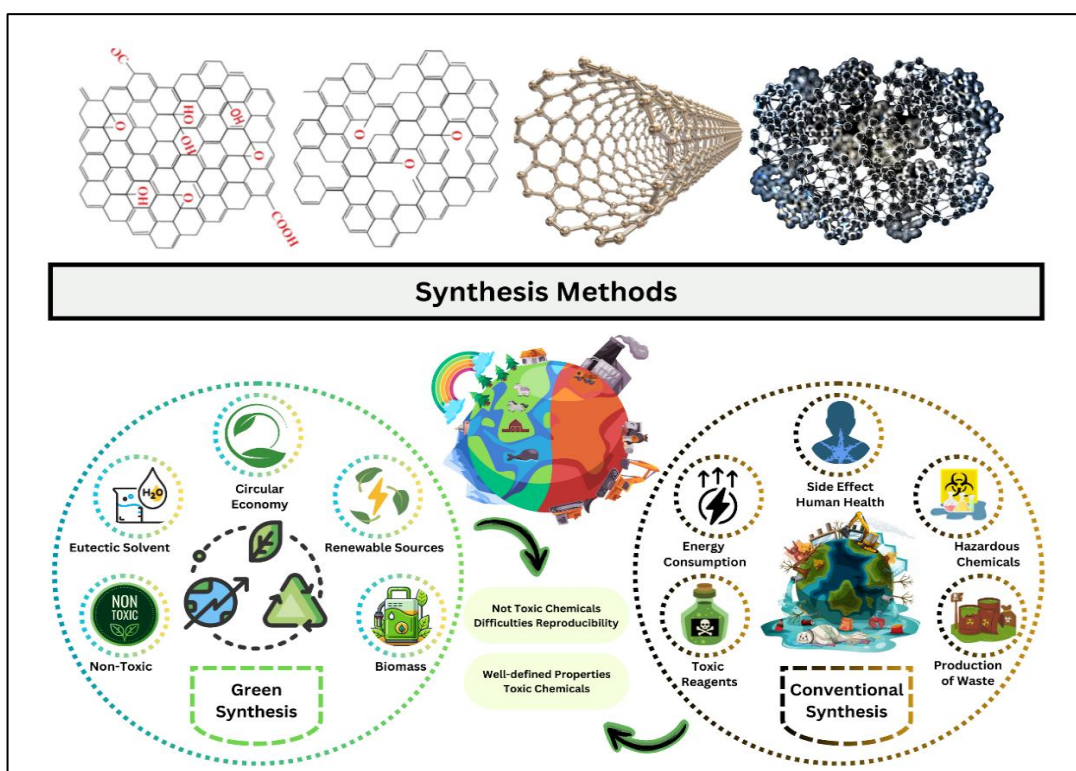


Figure 1.7: Comparison of conventional and green synthesis approaches highlighting environmental impacts.

Various natural sources can serve as reducing and stabilizing agents in green synthesis, offering renewable and biodegradable alternatives to synthetic chemicals. These bio-based reducing agents often contain multiple functional groups that can facilitate both the reduction of metal ions and the stabilization of formed nanoparticles [90]. The mild nature of these reducing agents typically results in slower reaction kinetics, which can actually provide better control over particle formation and growth processes. This

controlled growth often leads to more uniform particle size distributions and well-defined morphologies [91].

The reaction mechanisms in green synthesis typically involve complex interactions between the metal precursors and natural reducing agents. The formation of metal oxide nanoparticles through green synthesis often follows a more gradual and controlled pathway compared to conventional methods. This controlled process can lead to the formation of stable nanoparticles with unique surface properties. The slower kinetics also allows for better monitoring and control of the reaction progress, enabling the optimization of synthesis conditions for specific applications [92].

The advantages of green synthesis extend beyond environmental benefits. Nanoparticles produced through green methods often exhibit enhanced biocompatibility and reduced toxicity, making them particularly suitable for applications in food packaging, biomedical devices, and other sensitive applications [93]. The surface functionalization achieved through green synthesis can provide additional stability and functionality to the nanoparticles. Additionally, these methods can be cost-effective and readily scalable, offering practical advantages for industrial production. The economic viability of green synthesis methods, combined with their environmental benefits, makes them increasingly attractive for commercial applications.

Recent advances in characterization techniques have enabled better understanding of green synthesis mechanisms, leading to improved control over particle properties. The ability to monitor and optimize reaction conditions has resulted in the development of more efficient and reliable green synthesis protocols. Ongoing research continues to explore new sustainable approaches and improve existing methods, expanding the possibilities for environmentally friendly nanoparticle synthesis.

1.1.3. Phytochemical-Mediated Synthesis: Bioactive Plant Compounds in NPs Formation

Among various green synthesis approaches, the utilization of plant extracts for nanoparticle synthesis has emerged as a particularly promising strategy, offering a unique combination of efficiency, sustainability, and biological functionality. This approach harnesses the rich biochemical complexity of plants, where naturally

occurring compounds serve as both reducing and stabilizing agents in the synthesis process. The remarkable diversity of phytochemicals present in plant extracts provides a sophisticated natural laboratory for nanoparticle synthesis, often resulting in products with enhanced properties and reduced environmental impact [90, 94].

Plant extracts contain an intricate mixture of bioactive compounds including polyphenols, flavonoids, terpenoids, alkaloids, proteins, and carbohydrates. These biomolecules possess specific chemical structures featuring hydroxyl, carboxyl, and amine functional groups, which play crucial roles in the reduction and stabilization processes. The reducing capabilities of these compounds stem from their electron-rich nature and ability to undergo oxidation reactions, facilitating the conversion of metal ions to nanoparticles [95]. Simultaneously, these molecules can act as capping agents, providing stability to the newly formed nanoparticles through surface functionalization [96].

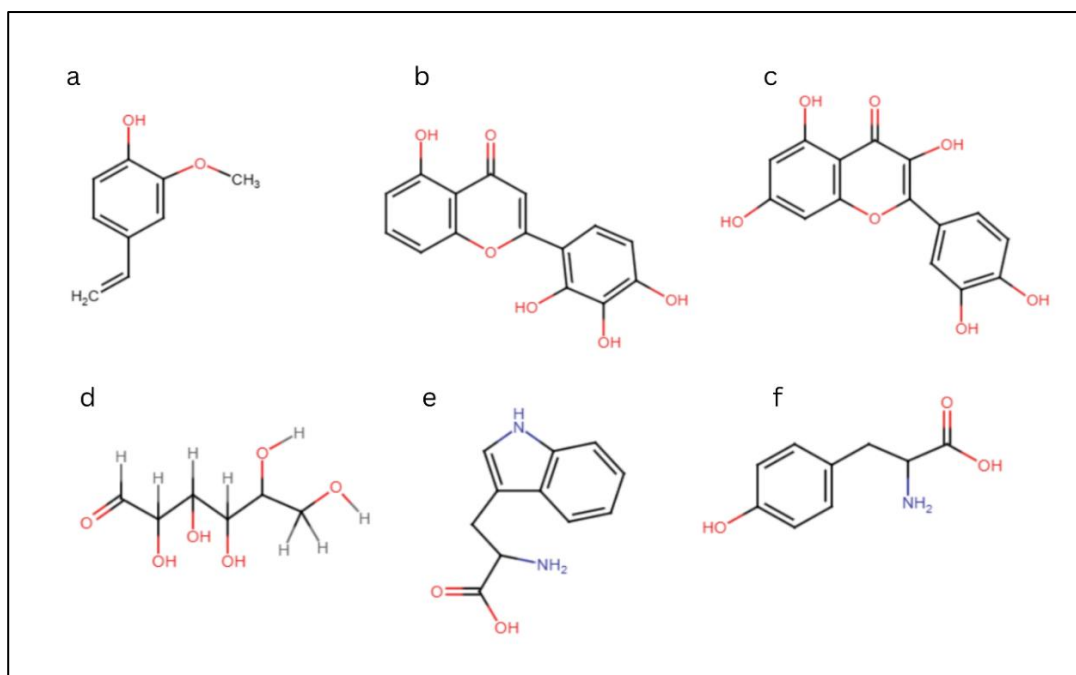


Figure 1.8: Major plant metabolites involved in metal nanoparticle synthesis: (a) Terpenoids (e.g., eugenol), (b) Flavonoids (e.g., luteolin), (c) Flavonoids (e.g., quercetin), (d) Reducing sugars, (e) Amino acids (e.g. tryptophan), (f) Amino acids (e.g., tyrosine). Chemical structures showing key functional groups responsible for reduction and stabilization.

The mechanism of phytogenic synthesis involves several distinct stages and complex molecular interactions. Initially, metal ions undergo complexation with phytochemicals through various functional groups. Phenolic compounds, particularly flavonoids and polyphenols, play a primary role in the reduction process through their hydroxyl groups. These groups can donate electrons to metal ions, facilitating their reduction to zero-valent state. Terpenoids contribute to the process through their diverse functional groups, while proteins and amino acids can both reduce metal ions and provide stability through surface binding [95].

The reduction process is typically followed by nucleation, where reduced metal atoms aggregate to form initial nuclei. The presence of various plant metabolites significantly influences this stage, affecting both the rate of nucleation and the stability of formed nuclei. Subsequently, these nuclei undergo growth processes, leading to the formation of stable nanoparticles. The diversity of plant metabolites present during these stages can result in unique particle morphologies and size distributions.

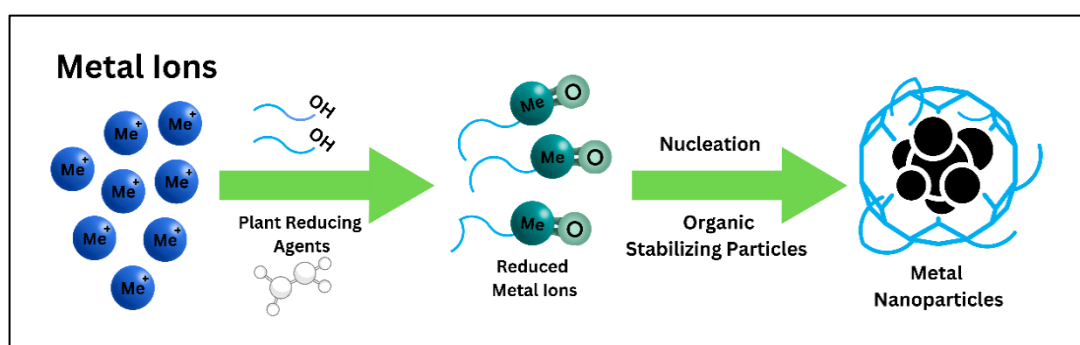


Figure 1.9: Schematic representation of the phytogenic synthesis mechanism showing different stages of nanoparticle formation and the role of plant metabolites.

The efficiency of plant-mediated synthesis is influenced by various factors including the type of plant extract, concentration of phytochemicals, pH, temperature, and metal ion concentration. Different plants contain varying compositions of metabolites, leading to different reduction capabilities and stabilization mechanisms. The pH of the reaction medium affects both the ionization state of biomolecules and their reducing capability, while temperature influences reaction kinetics and the stability of formed particles [97].

One significant advantage of phytogenic synthesis is the potential for enhanced functionality of the resulting nanoparticles. The natural compounds involved in the

synthesis can impart additional properties to the nanoparticles, such as antimicrobial activity, antioxidant properties, or improved biocompatibility. This is particularly relevant for applications in food packaging and biomedical fields, where such additional functionalities can provide enhanced performance.

Recent advances in understanding the role of specific plant metabolites have led to more targeted approaches in phytogenic synthesis. The ability to identify and optimize the contributions of different compounds has enabled better control over particle characteristics and properties. This growing understanding continues to expand the possibilities for developing more efficient and controlled synthesis protocols using plant-based approaches.

1.1.4. *Helichrysum Arenarium* Extract in Nanoparticles Synthesis

Among various medicinal plants employed in green synthesis, *Helichrysum arenarium* (dwarf everlasting) represents a particularly promising candidate for the synthesis of metal oxide nanoparticles. This medicinal plant, belonging to the Asteraceae family, has garnered significant attention due to its rich phytochemical composition and traditional therapeutic applications [49]. The exceptional potential of this species in nanoparticle synthesis stems from its diverse array of bioactive compounds, which can effectively facilitate both the reduction of metal ions and the stabilization of formed nanoparticles.

The phytochemical profile of *Helichrysum arenarium* is characterized by an abundance of biologically active compounds, including flavonoids (such as kaempferol, quercetin, and their glycosides), chalcones, phenolic acids (chlorogenic acid, caffeic acid), coumarins, and pyrones [47]. These compounds feature multiple hydroxyl and other functional groups that play crucial roles in the reduction and stabilization processes during nanoparticle synthesis. The synergistic effects of these various compounds contribute to the plant's effectiveness in green synthesis applications.

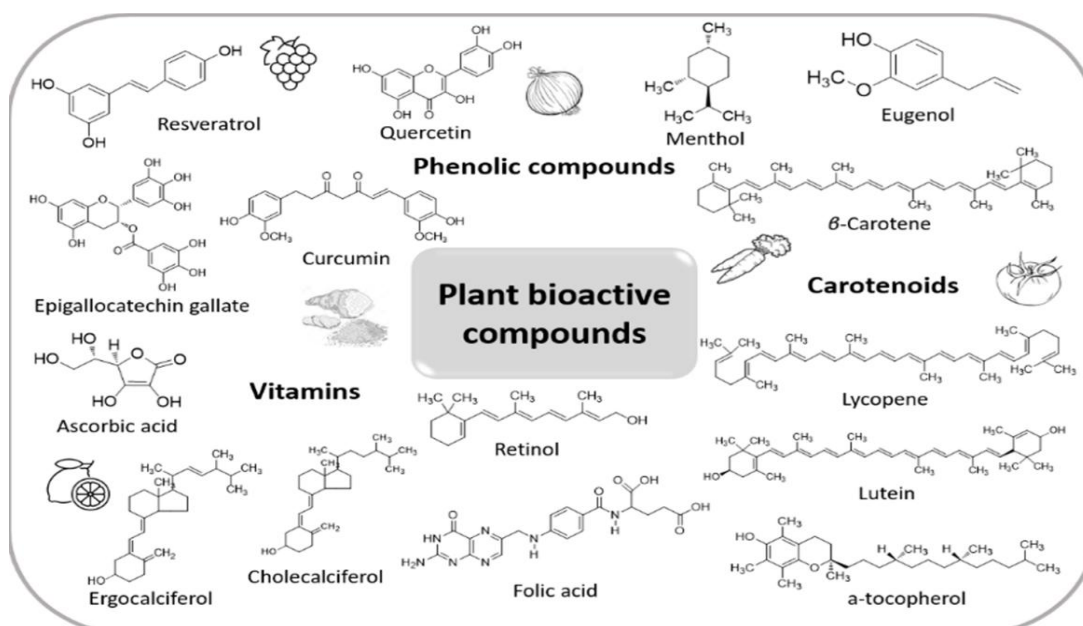


Figure 1.10: Chemical structures of major bioactive compounds present in a plant extract [98].

The reducing capability of the *Helichrysum arenarium* can be attributed primarily to its polyphenolic compounds, particularly flavonoids and phenolic acids. These molecules can donate electrons to metal ions while simultaneously undergoing oxidation, facilitating the formation of metal nanoparticles. The presence of multiple hydroxyl groups in these compounds enables efficient electron transfer processes, leading to controlled reduction of metal ions. Additionally, the structural diversity of these compounds provides various binding sites for stabilizing the formed nanoparticles.

Beyond its role in nanoparticle synthesis, the *Helichrysum arenarium* possesses significant biological activities including antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties [51]. These inherent therapeutic properties can potentially be imparted to the synthesized nanoparticles, enhancing their functionality for specific applications. The combination of the plant's reducing capabilities and its biological activities makes it particularly suitable for synthesizing nanoparticles intended for food packaging and biomedical applications.

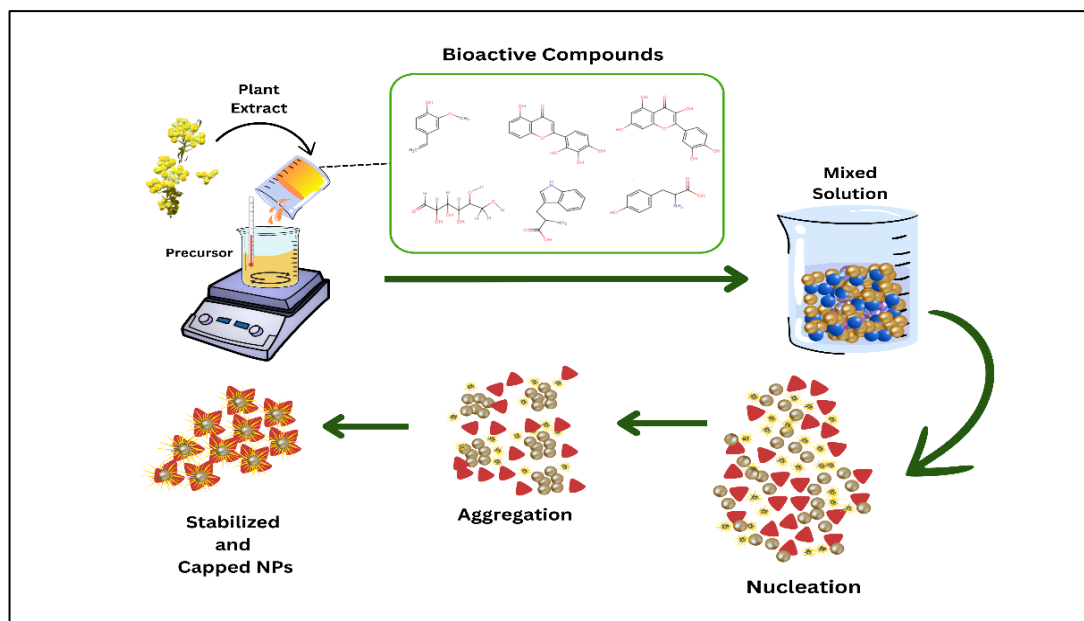


Figure 1.11: Schematic representation of nanoparticle synthesis using the plant extract, highlighting the roles of different bioactive compounds.

The extraction conditions and processing parameters significantly influence the efficiency of nanoparticle synthesis using this plant species. Factors such as extraction temperature, solvent type, pH, and concentration affect the availability and activity of the bioactive compounds [99]. Understanding and optimizing these parameters is crucial for developing reproducible and efficient synthesis protocols. The selection of appropriate extraction conditions can maximize the yield of active compounds and enhance their reducing and stabilizing capabilities.

Recent studies have demonstrated that the effectiveness of this plant extract in nanoparticle synthesis can be attributed to its unique combination of secondary metabolites [100]. The presence of multiple reducing agents in the extract allows for controlled reduction rates, potentially leading to more uniform particle size distributions. Furthermore, the natural stabilizing agents present in the extract can provide long-term colloidal stability without the need for additional synthetic stabilizers [101]. This comprehensive approach to green synthesis, utilizing the plant's natural biochemical complexity, offers a sustainable and efficient pathway to produce metal oxide nanoparticles with enhanced properties and reduced environmental impact.

1.2. ZnO Nanoparticles: Properties and Characteristics

Zinc oxide (ZnO) nanoparticles have emerged as significant materials in nanotechnology research due to their unique properties and diverse applications. The transition from bulk ZnO to nanoscale dimensions results in distinctive characteristics that have attracted considerable scientific interest. These nanostructures exhibit remarkable physical and chemical properties, making them suitable for various technological applications while maintaining cost-effectiveness and environmental compatibility [102].

The crystal structure of ZnO nanoparticles plays a fundamental role in determining their properties. Under ambient conditions, ZnO typically crystallizes in the hexagonal wurtzite structure, although zinc blende and rocksalt forms can also exist. In the wurtzite structure, each zinc ion is surrounded by four oxygen ions in a tetrahedral configuration, creating a characteristic polar surface [103]. This structural arrangement significantly influences the material's surface chemistry and reactivity patterns.

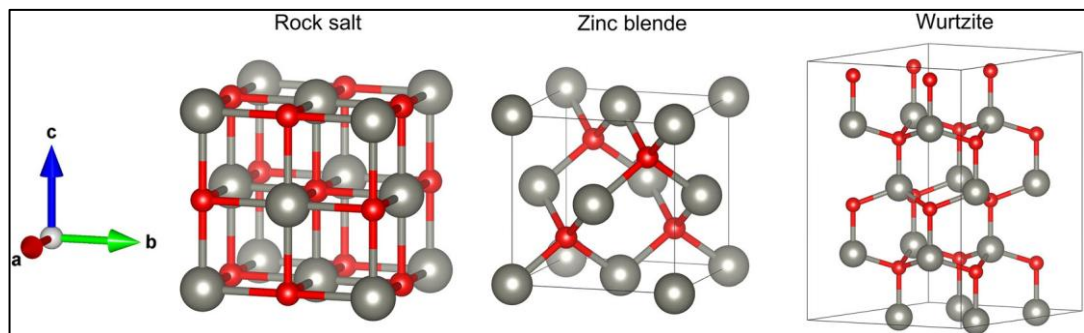


Figure 1.12: The crystal structure representation of ZnO, showing the Rock Salt, Zinc Blende, and Wurtzite crystal arrangements (from left to right) [104].

At the nanoscale, ZnO demonstrates unique electronic and optical properties that distinguish it from its bulk form. The material exhibits a wide bandgap of 3.37 eV at room temperature, contributing to its semiconductor characteristics. This electronic structure, combined with a high exciton binding energy of 60 meV, enables efficient electronic transitions at room temperature. As particle size decreases to the nanoscale, quantum confinement effects become more pronounced, leading to size-dependent variations in optical and electronic properties [105].

The surface properties of ZnO nanoparticles significantly influence their behavior and potential applications. Their high surface-to-volume ratio enhances surface reactivity

and catalytic properties. Surface modifications can be achieved through various methods, allowing for the customization of particle properties. The presence of surface defects, such as oxygen vacancies and zinc interstitials, affects the material's photocatalytic activity and electronic behavior [106].

When compared to other metal oxide nanoparticles, ZnO offers several advantages. Its biocompatibility and safety profile make it suitable for various applications. The material shows strong UV absorption while remaining transparent to visible light, a characteristic that sets it apart from many other metal oxides [107]. Additionally, the versatility in synthesis methods allows for controlled production of particles with specific characteristics.

1.2.1. Structural and Physicochemical Properties

The structural and physicochemical properties of ZnO nanoparticles are fundamentally linked to their atomic arrangement and electronic configuration. Understanding these properties is essential for controlling their synthesis and optimizing their performance in various applications. The characteristic features of ZnO nanoparticles arise from their unique crystal structure, electronic properties, and surface chemistry, which collectively determine their behavior at the nanoscale.

The wurtzite structure of ZnO consists of alternating planes of tetrahedrally coordinated Zn^{2+} and O^{2-} ions, stacked along the c-axis [108]. This arrangement results in a hexagonal unit cell with lattice parameters $a = 3.25 \text{ \AA}$ and $c = 5.2 \text{ \AA}$, yielding a c/a ratio of approximately 1.60, which is close to the ideal value for hexagonal cell (1.633) [109]. Each Zn^{2+} ion is surrounded by four O^{2-} ions at the corners of a tetrahedron, and vice versa, forming a sp^3 covalent bonding configuration. The polar nature of the Zn-O bond, arising from the significant electronegativity difference between zinc and oxygen atoms, combined with the crystallographic arrangement, creates polar surfaces perpendicular to the c-axis. The (0001)-Zn and (000)-O polar surfaces exhibit different chemical and physical properties, influencing the growth behavior and surface reactivity of ZnO nanoparticles [110].

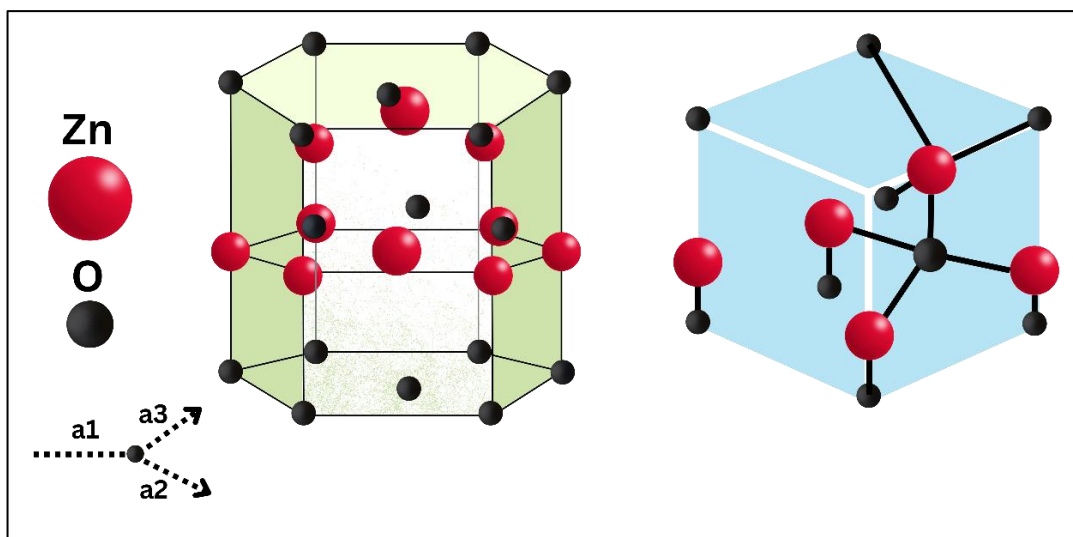


Figure 1.13: Detailed Crystal structure of hexagonal wurtzite ZnO, showing tetrahedral coordination between Zn and O atoms (left), and the tetrahedral coordination of ZnO (right).

The electronic structure of ZnO nanoparticles is characterized by a direct bandgap of 3.37 eV at room temperature. The valence band is primarily formed by the oxygen 2p orbitals, while the conduction band originates from the zinc 4s levels. The electronic configuration of Zn^{2+} ($3d^{10}$) and O^{2-} ($2p^6$) ions results in strong sp^3 hybridization, contributing to the material's semiconductor properties. The large exciton binding energy (60 meV) exceeds the thermal energy at room temperature (26 meV), enabling efficient excitonic emission under ambient conditions. Surface states and defects, including oxygen vacancies (V_O) and zinc interstitials (Zn_i), create additional energy levels within the bandgap, significantly affecting the optical and electronic behavior of the nanoparticles [111].

Surface chemistry plays a crucial role in determining the reactivity and stability of ZnO nanoparticles. The high surface-to-volume ratio characteristic of nanostructured materials results in enhanced surface energy and reactivity. The surface of ZnO nanoparticles typically contains various functional groups, primarily hydroxyl (OH) groups, which can undergo protonation or deprotonation depending on the pH of the surrounding medium. Oxygen vacancies and other defect sites act as active centers for surface reactions and can significantly influence the particle's photocatalytic activity and chemical reactivity. These surface features can be modified through various chemical treatments to achieve desired properties for specific applications.

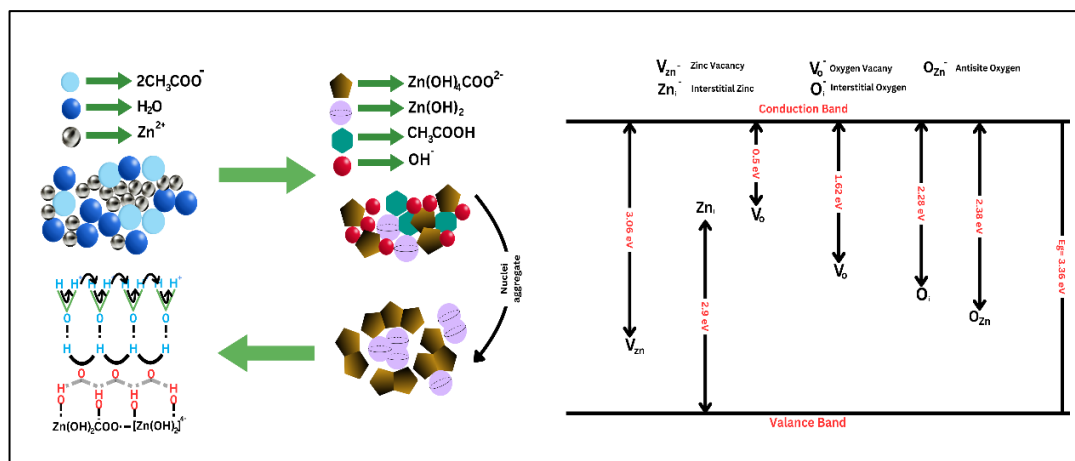


Figure 1.14: Schematic representation of surface defects, energy levels, and chemical interactions in ZnO nanoparticles.

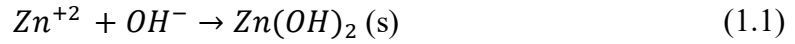
The morphology of ZnO nanoparticles exhibits remarkable diversity depending on synthesis conditions. The anisotropic nature of the wurtzite structure, combined with the presence of polar surfaces, leads to preferential growth along specific crystallographic directions. Common morphologies include spherical particles, nanorods, nanowires, nanobelts, and more complex hierarchical structures. The particle size and shape distribution directly influence their properties, including surface area, reactivity, and quantum confinement effects. Control over morphology can be achieved through careful adjustment of synthesis parameters such as precursor concentration, pH, temperature, and the presence of surface-modifying agents.

1.2.2. Synthesis and Characterization

The synthesis and characterization of ZnO nanoparticles represent critical aspects in controlling their properties and optimizing their performance. Various synthetic approaches have been developed, each offering distinct advantages in terms of particle size control, morphology, and physicochemical properties [112]. The selection of appropriate synthesis methods and characterization techniques is crucial for achieving desired nanoparticle characteristics.

Synthesis methods for ZnO nanoparticles can be broadly categorized into physical, chemical, and biological approaches. Chemical methods, including sol-gel processing, precipitation, hydrothermal/solvothermal synthesis, and chemical vapor deposition, offer precise control over particle characteristics. The sol-gel method involves the formation of a colloidal solution (sol) followed by gel formation through hydrolysis

and condensation reactions of zinc precursors. Hydrothermal synthesis, conducted under elevated temperature and pressure conditions, enables the growth of crystalline particles with controlled morphology. The reaction mechanisms in these processes typically involve (1.1) and (1.2) [113].



The synthesis parameters significantly influence the final properties of ZnO nanoparticles. Temperature affects both reaction kinetics and particle crystallinity, with higher temperatures generally promoting better crystallization and larger particle sizes. The pH of the reaction medium plays a crucial role in controlling particle nucleation and growth processes. Under basic conditions ($\text{pH} > 7$), the formation of $\text{Zn}(\text{OH})_2$ intermediates is favored, while acidic conditions can lead to dissolution effects. Precursor concentration affects supersaturation levels and subsequent nucleation rates, thereby influencing particle size distribution. The presence of surfactants or capping agents can modify surface energy and direct particle growth along specific crystallographic directions [114].

Comprehensive characterization of ZnO nanoparticles involves multiple analytical techniques to evaluate their structural, morphological, and physicochemical properties. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) provide detailed information about particle size, shape, and surface morphology. High-resolution TEM enables visualization of crystal lattice planes and defect structures. X-ray Diffraction (XRD) analysis reveals crystalline phase, lattice parameters, and crystallite size through the Scherrer equation (1.3) [115].

$$D = K\lambda/\beta\cos\theta \quad (1.3)$$

where D represents crystallite size, K is the shape factor, λ is the X-ray wavelength, β is the peak broadening at half maximum intensity (FWHM), and θ is the Bragg angle. Fourier Transform Infrared Spectroscopy (FTIR) provides information about surface functional groups and chemical bonding. The characteristic Zn-O stretching vibration

typically appears in the range of 400-600 cm⁻¹. UV-Visible spectroscopy enables the determination of optical properties and bandgap energy through the Tauc relation (1.4) [116].

$$(\alpha h\nu)^2 = A(h\nu - E_g) \quad (1.4)$$

where α is the absorption coefficient, h is Planck's constant, ν is the photon frequency, A is a constant, and E_g is the bandgap energy.

Dynamic Light Scattering (DLS) measurements provide information about particle size distribution and colloidal stability in solution. Zeta potential measurements indicate surface charge characteristics and stability of nanoparticle suspensions, with values exceeding ± 30 mV generally indicating good colloidal stability [117].

1.2.3. Functional Properties and Applications

The functional properties of ZnO nanoparticles, particularly their antimicrobial and antibacterial activities, have attracted significant attention in various applications. The mechanisms underlying these biological activities are complex and multifaceted, involving several simultaneous processes that contribute to their effectiveness against microorganisms [118]. Understanding these mechanisms is crucial for optimizing their performance in practical applications.

The antimicrobial activity of ZnO nanoparticles primarily operates through the generation of Reactive Oxygen Species (ROS), which occurs when the nanoparticles are exposed to light, especially UV radiation. This photocatalytic process creates electron-hole pairs on the nanoparticle surface, leading to the formation of various reactive species including superoxide anions, hydroxyl radicals, and hydrogen peroxide [119, 120]. These reactive species cause severe oxidative stress in microbial cells, resulting in membrane lipid peroxidation, protein oxidation, and DNA damage. The extent of ROS generation and subsequent antimicrobial activity depend significantly on particle size, surface area, and crystallinity of the nanoparticles [121].

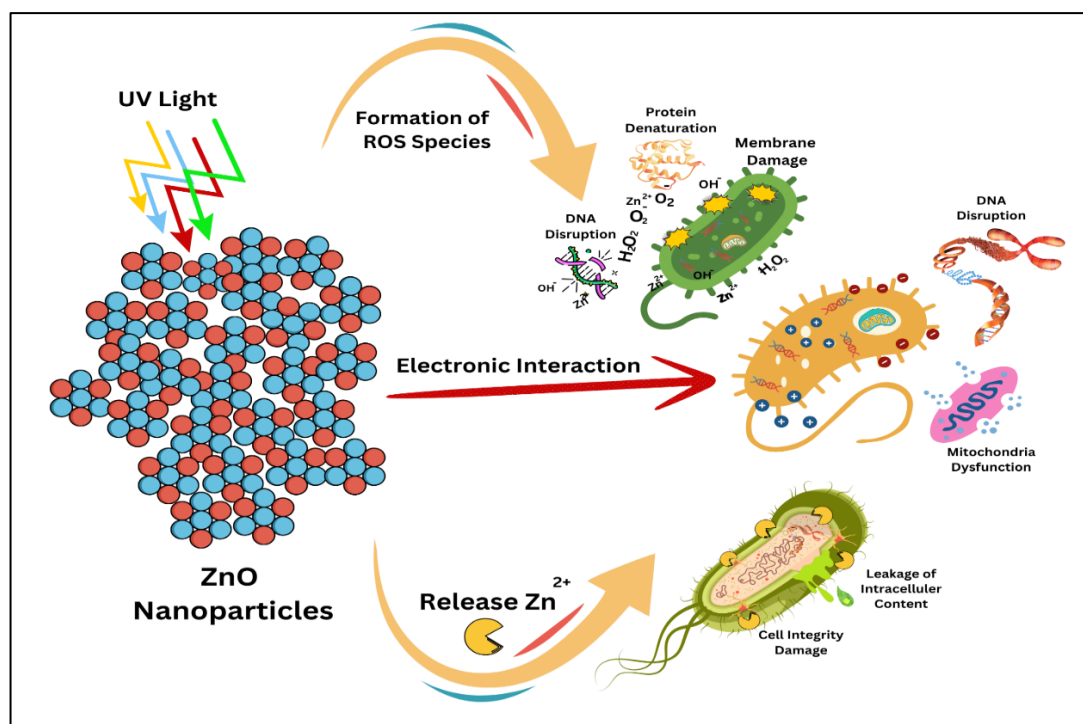


Figure 1.15: Detailed illustration of antimicrobial mechanisms of ZnO nanoparticles.

The release of zinc ions from the nanoparticle surface provides another significant antimicrobial mechanism. These ions can penetrate bacterial cell membranes and interfere with cellular metabolism through multiple pathways. Zinc ions disrupt protein function and enzyme systems, affecting crucial cellular processes and inhibiting bacterial growth and reproduction [120, 122]. The rate and extent of ion release are influenced by environmental conditions such as pH and temperature, as well as the specific surface characteristics of the nanoparticles [123].

Direct physical interaction between ZnO nanoparticles and bacterial cells represents a third mechanism of antimicrobial action. The nanoparticles can accumulate on the bacterial cell surface, disrupting membrane integrity and affecting cellular functions. This mechanical damage is particularly effective due to the high surface-to-volume ratio of nanoparticles and their ability to interact closely with bacterial cell membranes. The effectiveness of this mechanism varies depending on particle morphology and the specific characteristics of the target microorganisms [124].

The antibacterial efficacy of ZnO nanoparticles shows varying effectiveness against different types of bacteria due to fundamental differences in cellular structure. Gram-positive bacteria, with their thick peptidoglycan layer, respond differently to nanoparticle exposure compared to Gram-negative bacteria, which possess an

additional outer membrane [125]. These structural variations influence the interaction mechanisms and overall antimicrobial efficiency of the nanoparticles.

Environmental conditions play a crucial role in determining the antimicrobial activity of ZnO nanoparticles. The pH of the environment affects both nanoparticle stability and bacterial susceptibility, while temperature influence reaction kinetics and bacterial metabolism [126]. Light exposure, particularly UV radiation, significantly enhances the generation of ROS and subsequent antimicrobial activity [127]. Additionally, the presence of organic matter in the environment can modify the interactions between nanoparticles and bacterial cells, affecting overall efficacy.

The combination of these various antimicrobial mechanisms makes ZnO nanoparticles particularly effective against a broad spectrum of microorganisms. Their activity can be enhanced through careful control of particle characteristics and optimization of environmental conditions. Understanding and manipulating these factors enables the development of more effective antimicrobial applications while maintaining the inherent advantages of ZnO nanoparticles.

1.3. Biopolymer-based Materials

The development of biopolymer-based materials represents a significant advancement in sustainable material science. These materials, derived from natural sources, offer unique advantages including biodegradability, biocompatibility, and renewability [128]. The growing interest in biopolymers stems from both environmental concerns and their versatile functional properties, which can be further enhanced through the incorporation of nanoparticles.

Natural biopolymers encompass a diverse range of materials including polysaccharides (such as chitosan, cellulose, and starch), proteins (gelatin, collagen), and various plant-derived polymers. Each type of biopolymer exhibits distinct characteristics based on its molecular structure and composition. Chitosan, for example, derived from the deacetylation of chitin, possesses unique properties due to its cationic nature and reactive amino groups. The molecular structure of these biopolymers significantly influences their physical properties, chemical reactivity, and potential applications. Similarly, gelatin, derived from collagen, offers excellent film-

forming properties and biocompatibility, making it particularly suitable for various applications [129].

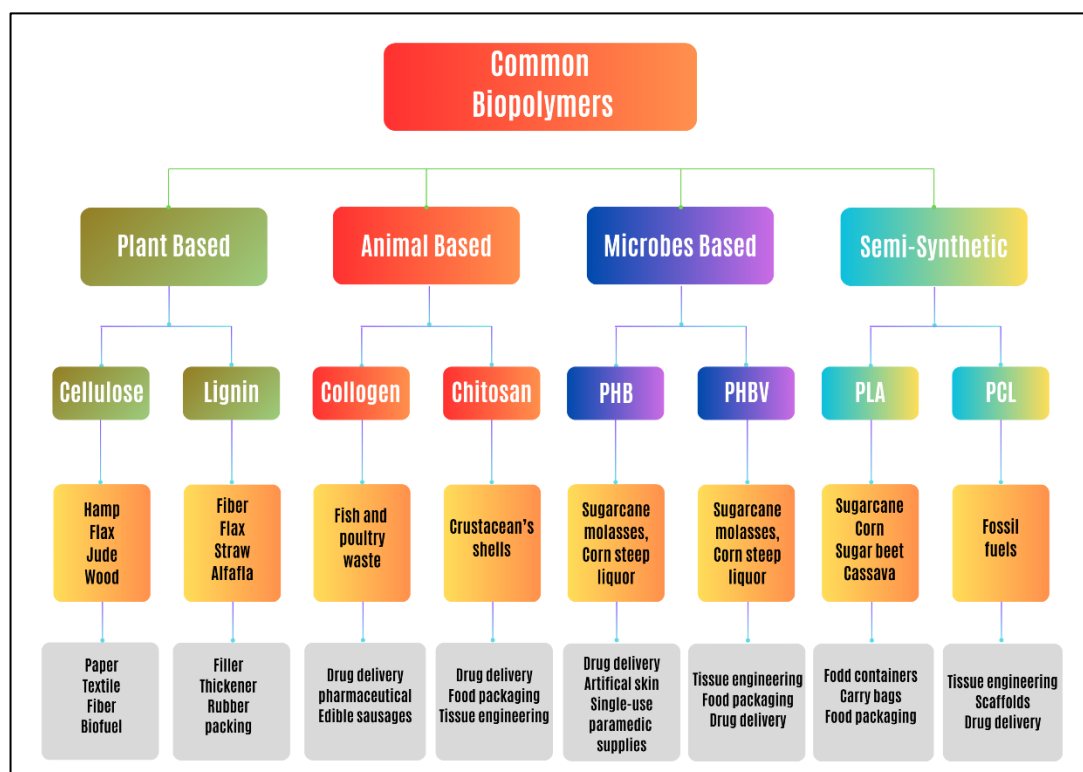


Figure 1.16: Common biopolymers and their functional properties.

The physical and chemical properties of biopolymer materials depend on several factors including molecular weight, degree of polymerization, and intermolecular interactions. The presence of various functional groups along the polymer chains enables modification and crosslinking reactions, allowing for property enhancement. Hydrogen bonding, electrostatic interactions, and hydrophobic effects play crucial roles in determining the material's behavior in different environments. These interactions affect properties such as mechanical strength, water resistance, and thermal stability. The molecular weight distribution particularly influences the mechanical properties and processability of the materials, while the degree of crystallinity affects their barrier properties and stability [130].

Biopolymer processing techniques significantly influence the final material properties. Common processing methods include solution casting, electrospinning, and various molding techniques. The selection of appropriate processing conditions affects the material's morphology, crystallinity, and performance characteristics. Factors such as

solution concentration, temperature, pH, and drying conditions must be carefully controlled to achieve desired material properties [131]. For instance, the rate of solvent evaporation during solution casting significantly impacts the final film structure and properties, while processing temperature can affect the degree of crystallinity and thermal stability of the materials.

The biodegradability of these materials occurs through various mechanisms including enzymatic degradation, hydrolysis, and microbial decomposition. The rate and extent of biodegradation depend on environmental conditions and the material's chemical structure [132]. Understanding these degradation mechanisms is crucial for developing materials with appropriate lifespans for specific applications. The presence of specific functional groups, degree of crosslinking, and crystallinity significantly influence the biodegradation behavior. Environmental factors such as temperature, humidity, and microbial activity also play crucial roles in determining the degradation rate and patterns.

1.3.1. Properties of Biopolymers

The fundamental properties of biopolymers are directly related to their molecular structure, chemical composition, and hierarchical organization. Understanding these properties is essential for optimizing their performance in various applications and for developing enhanced materials through nanoparticle incorporation.

Chitosan, one of the most extensively studied biopolymers, exhibits unique characteristics due to its linear polysaccharide structure composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine units [133]. The presence of amino groups along its backbone provides chitosan with distinctive properties including positive charge in acidic conditions, mucoadhesiveness, and the ability to form complexes with various molecules. The degree of deacetylation significantly influences its properties, with higher values typically resulting in enhanced mechanical strength and chemical reactivity [134].

Gelatin, derived from the partial hydrolysis of collagen, possesses a unique amino acid composition that determines its functional properties. The presence of specific amino acids in its structure enables the formation of triple-helix conformations, contributing to its gel-forming ability and mechanical properties. The molecular weight distribution

and isoelectric point of gelatin influence its behavior in solution and its interaction with other materials.

The mechanical and barrier properties of biopolymers are influenced by several factors including molecular weight, crystallinity, and intermolecular forces. Hydrogen bonding between polymer chains contributes significantly to material strength, while the presence of crystalline regions affects both mechanical and barrier properties. The inherent hydrophilic nature of many biopolymers can lead to moisture sensitivity, which may affect their dimensional stability and mechanical properties [135]. Various modification strategies, including chemical modification and nanoparticle incorporation, can be employed to enhance these properties.

1.3.2. Role of Nanoparticles in Biopolymer Systems

The incorporation of nanoparticles into biopolymer matrices represents a significant strategy for enhancing material properties and introducing new functionalities. Metal and metal oxide nanoparticles, when properly dispersed within biopolymer systems, can significantly modify the physical, mechanical, and functional properties of the resulting materials. The synergistic effects between nanoparticles and biopolymers often lead to materials with superior properties compared to their individual components [136].

Nanoparticle incorporation affects biopolymer properties through various mechanisms. The presence of nanoparticles can alter the polymer chain organization and crystallinity, influencing the material's mechanical behavior. The interaction between nanoparticles and polymer chains occurs through various forces including electrostatic interactions, hydrogen bonding, and van der Waals forces [137]. These interactions create an interfacial region around the nanoparticles that exhibits different properties from the bulk polymer. The extent and nature of these interfacial interactions significantly influence the overall performance of the nanocomposite materials [138].

The dispersion state of nanoparticles within the biopolymer matrix plays a crucial role in determining composite properties. Proper dispersion leads to enhanced mechanical properties through effective stress transfer between the matrix and nanoparticles. The improved mechanical properties often include increased tensile strength, enhanced

elastic modulus, and better impact resistance. However, achieving uniform dispersion presents significant challenges due to the high surface energy of nanoparticles and their tendency to aggregate. Surface modification of nanoparticles through various chemical treatments or the optimization of processing conditions can help achieve better dispersion and stronger interfacial interactions with the polymer matrix. The functional properties introduced by nanoparticles depend on their specific characteristics and concentration. Metal oxide nanoparticles, particularly ZnO, can provide multiple functional improvements including antimicrobial activity, UV protection, and enhanced barrier properties [139]. The size and shape of nanoparticles significantly influence their effectiveness in these applications. For example, smaller particles typically provide better antimicrobial activity due to their increased surface area and reactivity. The surface chemistry of nanoparticles can be modified to enhance their compatibility with the biopolymer matrix and improve their functional performance [140].

The concentration of nanoparticles must be carefully optimized to achieve desired properties without compromising the material's integrity. While higher concentrations may enhance certain properties, they can also lead to aggregation and potentially deteriorate other characteristics. The optimal concentration depends on various factors including the type of nanoparticles, the nature of the biopolymer matrix, and the desired final properties [141]. Furthermore, the processing conditions during nanocomposite manufacturing significantly influence the final distribution, shape and effectiveness of the synthesized nanoparticles.

1.3.3. Current Trends and Challenges

The development of biopolymer-based materials incorporating nanoparticles continues to evolve, presenting both promising opportunities and significant challenges. Understanding these current trends and challenges is crucial for advancing the field and developing practical applications. One of the primary challenges in developing biopolymer-nanoparticle systems lies in achieving uniform and stable nanoparticle dispersion. Despite various modification strategies and processing techniques, maintaining long-term stability and preventing nanoparticle aggregation remains difficult. The tendency of nanoparticles to form clusters can significantly reduce their effectiveness and potentially compromise the material's properties [142].

This challenge becomes particularly significant during scale-up processes, where maintaining uniform dispersion in larger volumes presents additional complications.

The optimization of synthesis parameters represents another significant challenge. Factors such as pH, temperature, concentration, and processing conditions must be carefully controlled to achieve desired properties [143]. The interaction between these parameters is often complex, making it difficult to predict and control the final material properties. Additionally, the reproducibility of synthesis methods and the consistency of material properties across different batches remain important concerns.

Environmental factors significantly influence the performance and stability of biopolymer-nanoparticle composites. The inherent sensitivity of biopolymers to moisture and temperature variations can affect their structural integrity and functional properties. Understanding and controlling these environmental effects is crucial for developing materials with consistent and reliable performance [144]. The development of strategies to enhance material stability under various environmental conditions continues to be an active area of research.

Current research trends focus on developing more efficient and sustainable synthesis methods, improving material performance, and expanding potential applications. The exploration of new surface modification techniques, the development of novel processing methods, and the investigation of synergistic effects between different types of nanoparticles represent some of the key areas of ongoing research projects. Additionally, increasing attention is being paid to understanding the long-term stability and environmental impact of these materials.

2. PURPOSE OF THE STUDY

The primary objective of this study is to develop an environmentally friendly synthesis method for ZnO nanoparticles using *Helichrysum arenarium* (HA) plant extract as a natural reducing and stabilizing agent. The widespread use of conventional synthesis methods for metal oxide nanoparticles has raised significant environmental concerns due to their reliance on hazardous chemicals, high energy consumption, and generation of toxic by-products. By utilizing plant extracts as natural reducing agents, this research seeks to establish a sustainable approach to nanoparticle synthesis that minimizes environmental impact while maintaining control over particle characteristics. The selection of *Helichrysum arenarium* (HA) is particularly significant due to its rich composition of bioactive compounds, which can facilitate both the reduction of metal ions and the stabilization of formed nanoparticles under mild reaction conditions.

This research aims to contribute to the field of sustainable nanotechnology through systematic investigation of synthesis parameters and comprehensive characterization of the resulting nanoparticles. The study will examine the influence of various reaction conditions on particle formation and properties, providing crucial insights into the green synthesis mechanism. Through detailed analysis of structural, morphological, and antimicrobial properties, this work seeks to demonstrate the viability of plant-mediated synthesis as an alternative to traditional methods. The findings will advance our understanding of environmentally friendly nanoparticle production while establishing a foundation for future developments in sustainable nanomaterial synthesis.

Furthermore, this study aims to demonstrate the practical application of these green-synthesized ZnO-HA nanoparticles in sustainable food packaging solutions. The incorporation of these nanoparticles into chitosan/arabic gum composite coatings represents an innovative approach to address post-harvest losses and food waste, which are critical global challenges. By developing and evaluating nanocomposite edible coatings for banana preservation, this research seeks to establish a direct link between green nanotechnology and practical food preservation applications. The systematic investigation of different nanoparticle concentrations in the coating formulations will

provide valuable insights into optimizing preservation effectiveness while maintaining food safety standards. This integrated approach from green synthesis to practical application that exemplifies how sustainable nanotechnology can contribute to solving real-world problems in food security and waste reduction. The successful development of such eco-friendly packaging solutions could have significant implications for extending the shelf life of perishable fruits, particularly in regions where post-harvest losses pose major economic and nutritional challenges.

2.1. Main Objectives and Research Strategy

The fundamental aim of this research focuses on developing and optimizing a green synthesis protocol for ZnO nanoparticles using *Helichrysum arenarium* (HA) extract. The optimization process will investigate the effects of various synthesis parameters on nanoparticle formation and characteristics. These parameters include reaction conditions such as pH, temperature, and precursor concentration, which play crucial roles in the synthesis process. Understanding the influence of these parameters is essential for developing a reproducible synthesis method that can yield nanoparticles with controlled properties. The research strategy involves systematic examination of reaction conditions to establish optimal synthesis parameters.

Comprehensive characterization of the synthesized nanoparticles will be conducted using various analytical techniques. Electron microscopy (TEM and SEM) will examine particle morphology and size distribution. XRD analysis will provide information about crystal structure, while FTIR spectroscopy will help understand the role of plant extract components in the synthesis process. UV-visible spectroscopy will be used to confirm nanoparticle formation and study optical properties. This combination of characterization techniques will provide thorough understanding of the synthesized materials' properties and characteristics.

A significant aspect of this research includes the evaluation of antimicrobial properties of the synthesized nanoparticles, addressing the growing concern of antimicrobial resistance in healthcare. The study will employ standardized Kirby-Bauer disk diffusion methods to assess the antimicrobial activity against various microorganisms. The investigation will examine the inhibitory effects of the synthesized nanoparticles at different concentrations against both Gram-positive and Gram-negative bacteria. The effectiveness will be evaluated through careful measurement and analysis of

inhibition zones, providing quantitative data on the antimicrobial properties of the synthesized materials.

The final objective of this research involves the development and evaluation of nanocomposite edible coatings for food preservation applications. This phase will focus on incorporating the green-synthesized ZnO-HA nanoparticles into chitosan/arabic gum (CH-AG) composite matrices at varying concentrations (0.2%, 0.5%, 0.8%, and 1.0%). The research strategy includes systematic preparation of these nanocomposite coatings following established protocols, with careful attention to achieving homogeneous distribution of nanoparticles within the polymer matrix. The effectiveness of these coatings will be evaluated through a comprehensive banana preservation study over a 20-day storage period. Key quality parameters including color change (through every other day photographic documentation and color indexing), mass loss (through regular gravimetric measurements), and firmness will be monitored to assess the coatings' protective efficacy. This application-oriented approach will establish the practical viability of using green-synthesized ZnO nanoparticles in sustainable food packaging solutions, bridging the gap between laboratory synthesis and real-world applications. The systematic evaluation of different nanoparticle concentrations will help identify optimal formulations that maximize preservation effectiveness while ensuring food safety and maintaining the biodegradable nature of the coating materials.

2.1.1. Expected Outcomes and Significance

The research outcomes are expected to make significant contributions to the field of sustainable nanotechnology, particularly in the development of green synthesis methods for metal oxide nanoparticles. Through systematic investigation of synthesis parameters and comprehensive characterization studies, this work aims to establish a reliable protocol for producing ZnO nanoparticles using plant extracts. The findings will provide valuable insights into the relationship between synthesis conditions and particle properties, contributing to the fundamental understanding of plant-mediated synthesis mechanisms.

The antimicrobial studies represent a crucial component of this research, addressing current challenges in developing new antimicrobial agents. The investigation of ZnO

nanoparticles' effectiveness against various microorganisms will provide important data regarding their potential applications. The systematic evaluation of antimicrobial activity through standardized methods, including the analysis of inhibition zones at different concentrations, will establish a clear understanding of their biological effectiveness. These findings are particularly relevant given the growing concern over antimicrobial resistance and the need for alternative antimicrobial agents.

Furthermore, this study aims to demonstrate the viability of using *Helichrysum arenarium* (HA) extract in nanoparticle synthesis, potentially opening new avenues for the utilization of plant-based materials in nanoscience. The successful development of this green synthesis method could provide an environmentally friendly alternative to conventional synthesis approaches, contributing to the broader goals of sustainable chemistry and materials science. The knowledge gained from this research will serve as a foundation for future studies in the field of green nanotechnology and may lead to practical applications in various sectors where antimicrobial properties are desired.

The application of these green-synthesized ZnO-HA nanoparticles in food packaging represents another significant contribution of this research. The development of chitosan/arabic gum composite coatings incorporated with optimal concentrations of ZnO-HA nanoparticles is expected to provide an effective solution for extending the shelf life of perishable fruits, particularly bananas. The comprehensive evaluation of different nanoparticle concentrations (0.2%, 0.5%, 0.8%, and 1.0%) will establish optimal formulations that balance antimicrobial efficacy with food safety considerations.

The banana preservation studies will generate valuable data on the effectiveness of these nanocomposite coatings in maintaining fruit quality during storage. Through systematic monitoring of color change, mass loss, and firmness over a 30-day period, this research will provide quantitative evidence of the coatings' protective effects. These findings have significant implications for reducing post-harvest losses, which is a critical issue in global food security. The successful implementation of such coatings could contribute to reducing food waste, extending supply chain efficiency, and improving economic outcomes for farmers and retailers.

Moreover, the integration of green-synthesized nanoparticles with biodegradable polymers represents a sustainable approach to food packaging that addresses both

environmental and food safety concerns. This research could pave the way for the commercial development of eco-friendly packaging solutions that combine the antimicrobial properties of ZnO nanoparticles with the biodegradability of natural polymers. The economic viability of this approach, considering the use of readily available plant materials and simple synthesis procedures, makes it particularly attractive for practical implementation in developing countries where post-harvest losses are most severe.

3. EXPERIMENT

3.1. Materials and Reagents

The experimental methodology adhered strictly to green chemistry principles, prioritizing environmental sustainability and minimal chemical intervention. The synthesis process was designed to reduce the use of harmful chemicals and minimize environmental impact. Moderate temperature conditions (at $\sim 80^{\circ}\text{C}$) were maintained during extract preparation, optimizing the extraction of bioactive compounds while avoiding excessive energy consumption. The *Helichrysum arenarium* extract served as a natural reducing and stabilizing agent, replacing traditional toxic chemical reducers with a biodegradable, plant-derived alternative. Water was employed as the primary solvent, representing an environmentally friendly and non-toxic medium for nanoparticle synthesis.

The approach emphasized controlled and precise experimental conditions while maintaining a commitment to sustainable scientific practices. All materials were used in their original form, without additional purification, to reduce chemical waste and processing energy. The pH adjustment was carefully managed using minimal quantities of potassium hydroxide, ensuring precise control over reaction conditions without introducing excessive chemical interventions. This methodology represents a significant step towards developing more environmentally conscious nanomaterial synthesis techniques, demonstrating that advanced scientific research can be conducted with minimal ecological footprint.

3.1.1. Chemical Reagents

The chemical reagents used in this study were selected based on their purity, compatibility with green synthesis principles, and minimal environmental impact. All chemicals were used as received without further purification. **Table 3.1** presents the detailed specifications of chemical reagents employed in the ZnO nanoparticle synthesis.

Table 3.1: Chemical reagents used in ZnO-HA nanoparticle synthesis.

Chemical	Manufacturer	CAS No.	Purity	Molecular Weight	Physical Appearance
Zinc Nitrate Hexahydrate	Tekkim	10196-18-6	Extra pure	297.49 g/mol	White crystalline powder
Polyethylene Glycol 400	Tekkim	25322-68-3	Extra pure	~400 g/mol	Clear, colorless liquid (Viscosity: 45-55 cSt at 25°C)
Potassium Hydroxide	Merck	1310-58-3	-	56.11 g/mol	0.1 M solution (freshly prepared)
Pentane	Sigma-Aldrich	109-66-0	98%	72.15 g/mol	-
Deionized Water	Lab system	-	Resistivity: >18.2 MΩ·cm at 25°C, TOC: <5 ppb	18.015 g/mol	-

3.1.2. Plant Material

Helichrysum arenarium was selected as the source of natural reducing and stabilizing agents due to its rich phytochemical composition. The dried flowers were commercially obtained to ensure consistency and reproducibility of the synthesis process. The specifications of the plant material are detailed in **Table 3.2**.

Table 3.2: Plant material specifications.

Parameter	Description
Scientific Name	<i>Helichrysum arenarium</i>
Commercial name	Altın Otu / Draf Forever
Species	<i>Helichrysum</i> Sp.
Supplier	Arifoğlu Baharat
Part used	Dried flowers
Collection location	Not known
Drying method	Air-dried, away from direct sunlight
Purpose	Natural reducing and stabilizing agent

3.1.3. Microorganisms for Antimicrobial Testing

The antimicrobial efficacy of the synthesized ZnO nanoparticles was evaluated against a panel of pathogenic microorganisms representing both Gram-positive and Gram-negative bacteria, as well as a fungal strain. All microorganisms were obtained from the American Type Culture Collection (ATCC) to ensure standardization and reproducibility of results. **Table 3.3** lists the microorganisms and culture conditions used in this study.

Table 3.3: Microorganisms and culture media for antimicrobial testing.

Microorganism	ATCC Number	Type	Culture Medium	Inoculum Density
<i>Staphylococcus aureus</i>	25923	Gram-positive	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Bacillus cereus</i>	14579	Gram-positive	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Escherichia coli</i>	25922	Gram-negative	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Salmonella enterica</i>	13314	Gram-negative	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Shigella flexneri</i>	24570	Gram-negative	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Pseudomonas aeruginosa</i>	27853	Gram-negative	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)
<i>Candida albicans</i>	14053	Yeast	Mueller-Hinton Agar	0.5 McFarland (~1.5 × 10 ⁸ CFU/mL)

3.1.4. Materials for Food Packaging Application

The development of edible coatings required food-grade biopolymers and supporting chemicals that meet safety standards for food contact applications. Chitosan and arabic gum were selected as the primary biopolymer matrix due to their excellent film-forming properties, biodegradability, and proven safety in food applications. The materials used for coating formulation and banana preservation studies are presented in **Table 3.4**.

Table 3.4: Materials for edible coating formulation and application

Material	Manufacturer	CAS No.	Specifications	Physical Appearance	Purpose
Chitosan	Sigma-Aldrich	9012-76-4	Medium MW (190-310 kDa), DD>85%	Off-white powder	Primary biopolymer
Arabic Gum	Sigma-Aldrich	9000-01-5	Food grade	White to light yellow powder	Co-polymer
Acetic Acid (Glacial)	Merck	64-19-7	≥99.7%, MW: 60.05 g/mol	Clear liquid	Chitosan solvent
Sodium Hydroxide	Merck	1310-73-2	≥98%	White pellets	pH adjustment
Sulfuric Acid	Merck	7664-93-9	0.1 M solution	Clear liquid	pH adjustment
Sodium Hypochlorite	Sigma-Aldrich	7681-52-9	0.01% solution (diluted)	Clear liquid	Surface disinfection

3.2. Preperation of *Helichrysum arenarium* Extract

Dried *Helichrysum arenarium* flowers were cleaned using a soft brush to remove surface dust and contaminants. The plant material was dried at room temperature and ground into fine powder using a porcelain mortar and pestle. The grinding was performed in short intervals to avoid overheating. The powdered material was sieved through a 60-mesh sieve and stored in amber glass containers with tight-sealing lids in a desiccator at room temperature.

Exactly 10.000 ± 0.001 g of powdered *H. arenarium* were weighed using an analytical balance and mixed with 200 ± 1 mL of deionized water in a borosilicate glass beaker (250 mL). The mixture was heated to $80 \pm 1^\circ\text{C}$ on a magnetic stirrer with continuous stirring at 800 ± 10 rpm for 1.5 hours. Temperature was monitored using a digital thermocouple ($\pm 1^\circ\text{C}$ precision). After extraction, the beaker was removed from heat and cooled to room temperature, then left for 15 minutes to allow settling.

Filtration was performed using Whatman Q 125 Grade 4 filter paper (ISOLAB) on a Büchner funnel connected to vacuum. The filtration setup was rinsed with deionized water before use. The filtered extract was collected in a clean glass flask, then further filtered through $0.45\ \mu\text{m}$ PTFE syringe filters using disposable syringes. Filtration was done in small portions to prevent filter clogging.

The pH of the filtered extract was measured using a calibrated HANNA HI2002 pH meter. Across multiple extractions ($n=20$), the pH consistently ranged between 4.90-

4.95. The final extract was stored at $4 \pm 1^\circ\text{C}$ in amber glass containers with ground glass stoppers. A portion was kept separately for repeated analysis if needed.

Short grinding intervals prevented heat buildup that could damage bioactive compounds. The 60-mesh size gave uniform particles for better extraction consistency. The 800 rpm stirring provided good mixing without creating excessive turbulence. The 15-minute settling period reduced particulate matter before filtration. The $0.45\ \mu\text{m}$ PTFE filters removed fine particles for clearer extract. The narrow pH range (4.90-4.95) showed good extraction reproducibility and indicated the acidic nature of the extract, which affects the zinc precursor reaction in the next step.

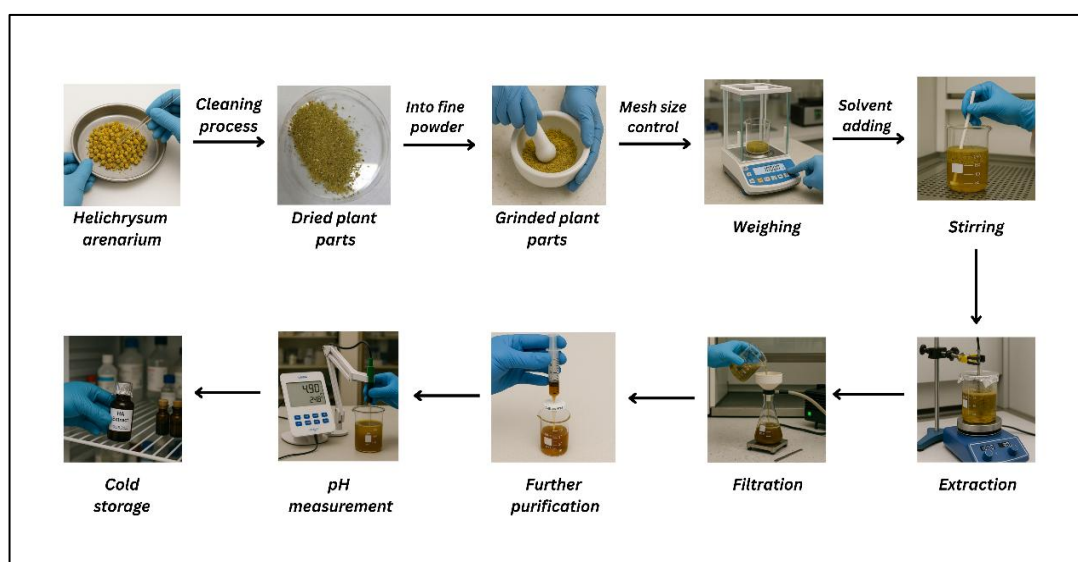


Figure 3.1: Detailed schematic diagram of extract preparation.

3.3. ZnO Nanoparticle Fabrication Methodology

A zinc precursor solution was prepared by dissolving 15.000 ± 0.001 g of zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\geq 99\%$ purity, Sigma-Aldrich) in 100 mL of deionized water (resistivity $>18.2\ \text{M}\Omega \cdot \text{cm}$ at 25°C) to obtain a final concentration of 0.504 ± 0.002 M. The solution was stirred magnetically at 400 ± 5 rpm at room temperature ($23 \pm 1^\circ\text{C}$) until complete dissolution was achieved. Separately, 100 mL of the previously prepared *H. arenarium* extract was equilibrated from storage temperature ($4 \pm 0.5^\circ\text{C}$) to room temperature over 30 minutes.

The synthesis was initiated by mixing the zinc precursor solution with the *H. arenarium* extract in a 250 mL glass beaker. Potassium hydroxide (KOH, 1 M) solution was added dropwise under continuous stirring until the pH reached 9.5 ± 0.1 , as measured by a calibrated digital pH meter (accuracy ± 0.01). Following the pH adjustment, two distinct protocols were employed to investigate the effect of surfactant addition time on morphology. For the *one-pot synthesis*, 1 mL of Polyethylene Glycol 400 (PEG 400) was added to the reaction medium immediately after the pH stabilization and prior to the heating step. Conversely, for the *sequential synthesis*, the mixture was first heated to $80 \pm 0.5^\circ\text{C}$, and the same amount of PEG 400 (1 mL) was introduced 15–20 minutes into the reaction, specifically after the initial color transition. The reaction mixture was then heated to $80 \pm 0.5^\circ\text{C}$ and maintained at this temperature with magnetic stirring at 1000 ± 10 rpm for 2 hours. After the reaction period, the mixture was allowed to cool to r.t naturally, followed by a 15-minute stabilization period.

The synthesized nanoparticles were separated by centrifugation at 6000 ± 50 rpm for 10 minutes. The precipitate was washed twice with deionized water to remove ionic impurities and twice with n-pentane to eliminate organic residues. After each washing step, centrifugation was repeated, and the supernatant was carefully decanted. The final product was dried in a vacuum oven at $80 \pm 2^\circ\text{C}$ under 0.8 ± 0.05 bar pressure for 24 hours. The dried ZnO nanoparticles were collected and stored in sealed amber glass vials at $4 \pm 1^\circ\text{C}$ until further characterization. All synthesis procedures were performed in triplicate to ensure reproducibility, and the results presented represent the average of three independent experiments.

Upon initial mixing of the zinc precursor with the plant extract, the pH dropped immediately to approximately 3.5 ± 0.2 , attributed to the presence of organic acids in the extract. Preliminary experiments indicated that maintaining this acidic pH prevented effective reduction of zinc ions; therefore, pH adjustment to 9.5 was essential for successful nanoparticle formation. A significant visual indicator of the reaction progress was the color transition from dark yellow to pale yellow, which occurred approximately 15 minutes after the mixture reached $80 \pm 2^\circ\text{C}$; this shift confirmed the nucleation of ZnO nanostructures. The high stirring speed of 1000 rpm was employed to ensure uniform heat distribution and prevent particle aggregation during synthesis. Following the cooling phase, the final pH stabilized at 6.5 ± 0.3 ,

suggesting neutralization of reactive species. The careful consideration of solution preparation parameters is crucial for maintaining consistent nucleation and growth conditions during the subsequent nanoparticle synthesis process [145].

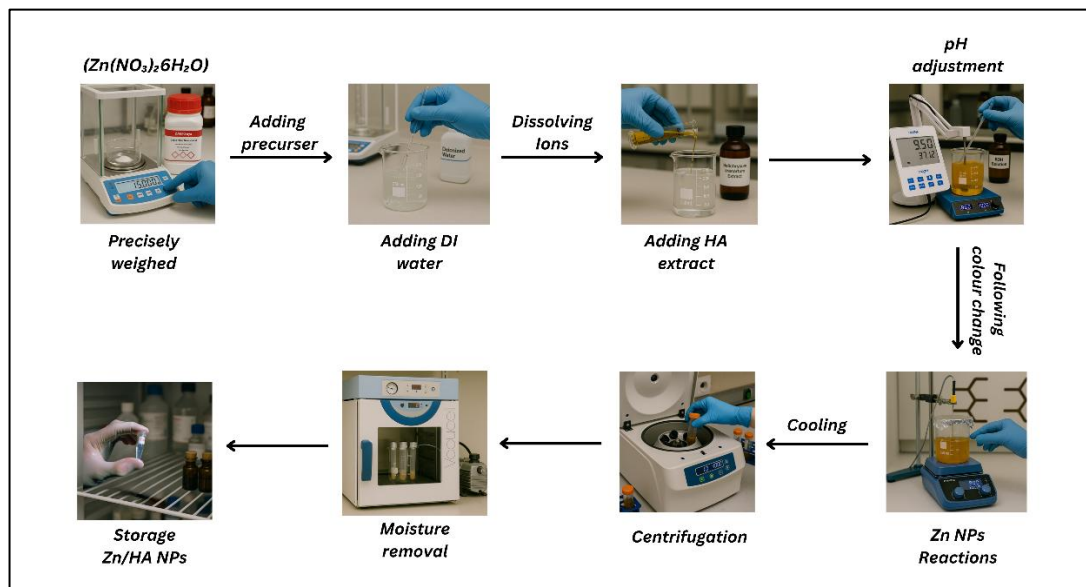


Figure 3.2: Comprehensive overview of the green-mediated ZnO nanoparticle synthesis procedure.

3.4. Preparation of Chitosan/Arabic Gum Composite Solutions

3.4.1. Chitosan Solution Preparation

The chitosan solution was prepared following modified protocols from previous studies [146, 147]. Precisely 2.000 ± 0.001 g of medium molecular weight chitosan (degree of deacetylation >85%) was dispersed in 100 mL of distilled water at room temperature ($23 \pm 1^\circ\text{C}$). To facilitate chitosan dissolution, 0.5 mL of glacial acetic acid was added to the suspension, creating a slightly acidic environment essential for chitosan solubilization. The mixture was magnetically stirred at room temperature for 2 hours at 600 rpm, with the reaction vessel covered to prevent evaporation. The stirring speed and duration were optimized to ensure complete dissolution while avoiding excessive foam formation, which could compromise coating uniformity [146, 147].

3.4.2. Arabic Gum Solution Preparation

Concurrently, the arabic gum solution was prepared by dissolving 20.000 ± 0.001 g of food-grade arabic gum in 100 mL of distilled water. The dissolution was performed at 40°C with continuous magnetic stirring for 2 hours, as elevated temperature enhances the solubility and reduces the viscosity of arabic gum solutions [147]. The temperature was maintained using a digital temperature-controlled magnetic stirrer to ensure consistent dissolution conditions. When necessary, the solution was subjected to ultrasonic treatment for 15 minutes to eliminate any remaining aggregates and achieve molecular level dispersion.

3.4.3. CH-AG Composite Formation

Both chitosan and arabic gum solutions were adjusted to $\text{pH } 5.6 \pm 0.1$ using 0.1 M NaOH or 0.1 M H_2SO_4 solutions, as this pH range optimizes the electrostatic interactions between the cationic chitosan and anionic arabic gum [147]. The pH adjustment was performed gradually with continuous monitoring using a calibrated pH meter.

The composite solution was formed by mixing the pH-adjusted solutions at a chitosan: arabic gum weight ratio of 1:10, which has been demonstrated to provide optimal film-forming properties and mechanical strength [146]. The arabic gum solution was added to the chitosan solution under vigorous stirring (800 rpm) to ensure homogeneous mixing. The combined solution was further stirred for 30 minutes and subjected to ultrasonic treatment when necessary to achieve complete homogenization. This CH-AG (1:10) composite served as the control coating solution.

3.5. Preparation of CH-AG/ZnO-HA Nanocomposite Coatings

3.5.1. Nanoparticle Dispersion Protocol

The incorporation of ZnO-HA nanoparticles into the CH-AG matrix followed established protocols for nanocomposite preparation [Santhosh & Sarkar, 2022; Aruwajoye et al., 2025]. Four different concentrations of ZnO-HA nanoparticles were prepared: 0.2%, 0.5%, 0.8%, and 1.0% (w/v) based on the total solution volume. For

each concentration, the appropriate weight of ZnO-HA nanoparticles was first dispersed in 20 mL of distilled water using ultrasonic treatment for 10 minutes to prevent agglomeration.

3.5.2. Nanocomposite Formulation

The nanoparticle dispersions were added dropwise to the CH-AG composite solution (180 mL) under continuous stirring at room temperature. The dropwise addition over 15 minutes ensured uniform distribution and prevented localized aggregation [146]. Following complete addition, the mixtures were stirred vigorously for 2 hours and subjected to ultrasonic treatment for 20 minutes to achieve homogeneous nanoparticle distribution throughout the polymer matrix. The final nanocomposite solutions were:

- CH-AG/ZnO-HA (0.2%)
- CH-AG/ZnO-HA (0.5%)
- CH-AG/ZnO-HA (0.8%)
- CH-AG/ZnO-HA (1.0%)

3.6. Banana Coating Application and Storage Study

3.6.1. Fruit Preparation

Fresh bananas (*Musa acuminata*, Cavendish variety) at ripeness stage 2-3 (green to yellow-green) were carefully selected based on uniform size, absence of physical damage, and similar maturity indices. The fruits were washed with 0.01% sodium hypochlorite solution for 5 minutes to ensure surface disinfection, followed by thorough rinsing with distilled water. The sanitized bananas were allowed to dry completely at room temperature ($23 \pm 1^\circ\text{C}$) on sterile filter paper for 30 minutes [147].

3.6.2. Coating Application Procedure

The coating application followed the dipping method as described by La et al. 2021 with modifications. Each banana was individually weighed and numbered for tracking purposes. The fruits were completely immersed in the respective coating solutions for 2 minutes, ensuring uniform surface coverage. After removal, excess coating was allowed to drain for 30 seconds, and the coated bananas were placed on

wire racks for drying at room temperature for 2 hours [146]. A total of 12 bananas were prepared for each treatment group:

- 2 uncoated controls
- 2 coated with CH-AG (0% ZnO/HA)
- 2 coated with CH-AG/ZnO-HA (0.2%)
- 2 coated with CH-AG/ZnO-HA (0.5%)
- 2 coated with CH-AG/ZnO-HA (0.8%)
- 2 coated with CH-AG/ZnO-HA (1.0%)

3.6.3. Storage Conditions and Quality Monitoring

The coated and control bananas were stored at ambient conditions (temperature: $26 \pm 2^\circ\text{C}$, relative humidity: $65 \pm 5\%$) for 20 days. The storage conditions were monitored daily using a digital thermo-hygrometer.

Color Change Assessment: Digital photographs were captured every two days at the same time around 14:00 under consistent lighting conditions using an iPhone 14 Pro camera with fixed settings and positioning. A standardized color index scale from 1-7 was developed to quantify peel color progression:

- Stage 1: Green
- Stage 2: Green-Yellow
- Stage 3: Light Yellow
- Stage 4: Yellow (Peak ripeness)
- Stage 5: Yellow with speckles
- Stage 6: Brown
- Stage 7: Very Dark/Overripe

The color assessment was performed through visual comparison with the developed color reference chart. This 7-point scale provides sufficient resolution to track the ripening progression while maintaining practical applicability and it was modified from Maqbool et al., 2011 [147].

Mass Loss Analysis: Each banana was weighed daily using an analytical balance (± 0.001 g). Mass loss percentage was calculated using the formula:

$$Mass\ loss(\%) = \frac{(Initial\ weight - Final\ weight)}{Initial\ weight} \times 100 \quad (3.1)$$

Firmness Evaluation: Fruit firmness was measured every 2 days using a digital fruit firmness tester (Next Gen Testing Equipment, Model: Fruit Firmness Tester) with an 8 mm cylindrical probe, which is the standard size recommended for banana firmness measurements. The device, specifically designed for measuring fruit texture parameters, provides direct readings in Newtons (N) with high precision. Three measurements were taken at the equatorial region of each fruit, avoiding areas with visible defects or bruising. The average of three measurements was calculated to represent the firmness value for each banana.

All measurements were performed with minimal handling, using gloves to prevent contamination and additional stress to the fruit surface. The firmness tester was calibrated before each measurement session according to the manufacturer's instructions. The data collection forms were maintained systematically to ensure complete documentation of the 20-day storage study

3.7. Chactarization Techniques

A comprehensive suite of advanced analytical techniques was employed to thoroughly characterize the synthesized ZnO nanoparticles. These techniques were carefully selected to provide detailed insights into the structural, morphological, chemical, and optical properties of the nanoparticles [148]. The characterization was performed using state-of-the-art instrumentation, with measurements conducted both in-house and through collaborative research facilities.

3.7.1. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique that provides comprehensive information about the molecular structure, functional groups, and chemical bonding in synthesized nanoparticles. This technique offers unique insights into the surface chemistry and molecular interactions of ZnO nanoparticles, enabling detailed characterization of their chemical composition.

FTIR characterizations were performed using a Perkin Elmer Spectrum 100 FTIR Spectrometer located in the Chemistry Department of Gebze Technical University. The instrument was configured with specific technical parameters, including a measurement range of 4000-450 cm^{-1} and a Mercury Cadmium Telluride (MCT) detector with exceptional sensitivity exceeding 1500:1 using CSI optics and TGS technology. The sample preparation utilized the Attenuated Total Reflectance (ATR) method, an advanced sampling technique that allows direct analysis of solid samples without extensive sample preparation. This approach involves placing the nanoparticle sample directly onto a high-refractive-index crystal (typically diamond or germanium), which enables direct infrared spectral analysis with minimal sample manipulation. The ATR technique provides enhanced sensitivity and reduces potential sample alteration during measurement.

FTIR analysis provides critical insights into the chemical composition and surface characteristics of the synthesized ZnO nanoparticles. By measuring the absorption of infrared radiation at different frequencies, the technique reveals detailed information about molecular vibrations, functional groups, and chemical bonding. The resulting spectrum serves as a molecular fingerprint, enabling researchers to identify surface functional groups, confirm chemical interactions, detect potential organic capping agents, and understand structural changes that occurred during nanoparticle synthesis.

3.7.2. Ultraviolet-Visible (UV-VIS) Spectroscopy

Ultraviolet-Visible (UV-Vis) spectroscopy is a powerful analytical technique that provides critical insights into the optical properties and electronic structure of nanomaterials. In this study, characterization was performed using a Shimadzu UV-1800 spectrophotometer located in the Chemistry Department of Gebze Technical University, specifically chosen for its precision in nanomaterial optical analysis.

The UV-Vis spectroscopy technique offers a unique window into the electronic transitions and quantum confinement effects of ZnO nanoparticles. By measuring the absorption of electromagnetic radiation across the ultraviolet and visible spectrum, researchers can extract fundamental information about particle size, electronic band structure, and optical properties. The instrument's dual-beam photometric system enables simultaneous reference and sample measurements, significantly reducing

potential experimental errors and enhancing the reliability of optical property determinations.

For ZnO nanoparticles, UV-Vis spectroscopy is particularly valuable in understanding size-dependent optical characteristics. As nanoparticle dimensions approach quantum confinement regimes, distinct shifts in absorption spectra can reveal critical information about particle size, morphology, and electronic structure. The absorption spectrum serves as a molecular fingerprint, providing insights into the nanoparticles' formation mechanism, crystallinity, and potential quantum effects that emerge at the nanoscale.

3.7.3. Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

Morphological and compositional characterization of the synthesized ZnO nanoparticles was conducted using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) at the Materials Science and Engineering Laboratory of Gebze Technical University. This powerful analytical approach provides unprecedented insights into the structural and chemical characteristics of nanomaterials at high magnifications.

Scanning Electron Microscopy offers a comprehensive view of nanoparticle surface morphology, enabling researchers to visualize particle shape, size distribution, and surface topographical features with exceptional clarity. Unlike transmission electron microscopy, SEM provides a remarkable depth of field that allows three-dimensional-like imaging of sample surfaces. The technique operates by scanning a focused electron beam across the sample, generating various signals that reveal intricate surface details and structural information.

To ensure accurate quantitative analysis and optimal spectral resolution, the EDS data acquisition was performed under strictly controlled operating conditions. The analysis was conducted using an accelerating voltage of 15 kV, which was selected to balance adequate excitation of zinc L-lines and K-lines while minimizing beam penetration depth. The detector energy resolution was set to 126 eV. Signal collection was standardized with a live acquisition time of 30 seconds and an amplification time of 12.8 seconds to ensure sufficient count statistics. Additionally, a takeoff angle of 31.2°

was maintained throughout the measurements to optimize the X-ray collection efficiency and minimize topographic shadowing effects. Following the spectral acquisition, quantitative elemental analysis was performed to determine the chemical purity of the samples. The analysis revealed a primary composition of 82.99 wt% zinc (Zn) and 15.2 wt% oxygen (O), values that are consistent with the stoichiometric ratios expected for the ZnO wurtzite structure. Apart from the major constituents, the spectra indicated the presence of trace elements, specifically potassium (K) and chlorine (Cl). These minor components are attributed to the residual minerals from the *Helichrysum arenarium* extract acting as stabilizing agents on the nanoparticle surface during the green synthesis process, a phenomenon consistently observed in plant-mediated synthesis studies.

3.7.4. X-Ray Diffraction (XRD)

X-Ray Diffraction (XRD) analysis was performed using a Bruker D-8 Advance X-Ray Device, located in the Materials Science and Engineering Laboratory at Gebze Technical University. At its fundamental core, XRD exploits the wave-like properties of X-rays and their interaction with crystalline atomic structures, providing a sophisticated method for investigating material composition and arrangement.

The technique's theoretical foundation rests on Bragg's Law, a pivotal equation proposed by William Lawrence Bragg in 1921. This law mathematically describes the conditions under which X-rays are coherently scattered by a crystal lattice. The fundamental equation, $n\lambda = 2d \sin\theta$, relates the wavelength of incident X-rays (λ), the spacing between atomic planes (d), and the angle of incidence (θ). When X-rays interact with crystalline materials, they undergo constructive interference at specific angles, creating distinctive diffraction patterns that serve as a unique "fingerprint" of the material's atomic structure.

The sample preparation involved carefully mounting nanoparticle powder onto glass stages for precise analysis. Measurements were conducted using a comprehensive scanning protocol: samples were scanned over a 2θ range of 5-80 degrees, with a highly precise step-size of 0.0167 degrees. Each scanning step was maintained for 212 seconds, ensuring high-resolution data collection. The system utilized Cu-K α

radiation, powered at 40 kV and 40 mA, which provides optimal penetration and detailed crystallographic information.

When X-rays interact with a crystalline sample, they encounter a complex three-dimensional array of atomic planes. Some X-ray photons are transmitted, some absorbed, and critically, some are diffracted at specific angles determined by the atomic spacing and orientation. This diffraction process creates a series of constructive and destructive interference patterns that can be mathematically analyzed to reveal intricate details about the material's internal structure.

For ZnO nanoparticles, XRD provides critical insights into crystal structure, phase purity, and potential structural variations introduced during green synthesis. The technique allows researchers to determine key parameters such as average crystallite size, lattice strain, and the presence of any structural defects. By comparing the obtained diffraction patterns with comprehensive crystallographic databases using X'pert HighScore software, precise identification and characterization of the material become possible.

The unique ability of XRD to provide a non-destructive, atomic-level investigation makes it an invaluable tool in materials science. For green-synthesized ZnO nanoparticles, this technique offers a comprehensive understanding of how synthesis conditions influence the final material's structural characteristics, bridging the gap between synthesis parameters and resulting material properties.

3.7.5. Antimicrobial Activity Characterization

Antimicrobial activity characterization represents a critical functional assessment of synthesized ZnO nanoparticles, providing insights into their potential biological applications. This evaluation employs the standardized Kirby-Bauer disk diffusion method, a widely recognized technique for screening antimicrobial properties of novel materials. These analyses were conducted at the Medical Microbiology Laboratory of Altınbaş University, ensuring professional and standardized evaluation of the nanoparticles' antimicrobial properties.

The characterization methodology involves a comprehensive screening approach using carefully selected reference bacterial strains representing diverse microbial groups. The chosen microorganisms include both Gram-positive and Gram-negative

bacteria, providing a robust platform for evaluating the nanoparticles' broad-spectrum antimicrobial potential. The selection of standard ATCC reference strains ensures reproducibility and comparability with established scientific protocols.

The Kirby-Bauer disk diffusion method offers a quantitative approach to assessing antimicrobial activity by measuring inhibition zone diameters. This technique provides direct visual and measurable evidence of the nanoparticles' ability to inhibit microbial growth. By utilizing multiple concentrations and diverse bacterial strains, researchers can comprehensively map the antimicrobial characteristics of the synthesized ZnO nanoparticles.

The experimental design incorporates critical control mechanisms, including positive and negative controls, to ensure the reliability and accuracy of the antimicrobial assessment. Standardized protocols, such as the McFarland standard for microbial suspension preparation and EUCAST guidelines for result interpretation, further enhance the scientific rigor of the characterization process [149].

This functional characterization goes beyond traditional structural analysis, offering insights into the potential practical applications of ZnO nanoparticles in antimicrobial technologies. The approach bridges the gap between material synthesis and biological functionality, providing a comprehensive understanding of the nanoparticles' interaction with biological systems.

3.7.6. Color Analysis Using X-Rite Colorimeter

Objective color measurements were performed using an X-Rite colorimeter to complement the visual color assessment. The instrument was calibrated using a standard white calibration plate before each measurement session. Color parameters including L* (lightness), a* (red-green coordinate), and b* (yellow-blue coordinate) values were measured on the CIE Lab* color space system.

Three measurements were taken at different positions on each banana surface (stem, middle, and blossom end), and the average values were calculated. The total color difference (ΔE) was calculated using the formula:

$$\Delta E = \sqrt{[(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)^2]} \quad (3.2)$$

where subscript 0 represents initial values and t represents values at time t. The color measurements were performed every two days, coinciding with the photographic documentation schedule.

3.7.7. Data Analysis and Visualization

All experimental data were processed and analyzed using Microsoft Excel (Version 2021) for basic calculations and preliminary data organization. Advanced data visualization and spectroscopic data analysis were performed using OriginPro 2026 (OriginLab Corporation, USA).

For UV-Vis spectroscopy data, Origin was used to:

- Process absorption spectra and identify peak positions
- Calculate Tauc plots for optical bandgap determination
- Generate overlay plots for comparing different synthesis conditions
- Perform baseline corrections and smoothing operations

Statistical parameters including mean values and standard deviations were calculated for all quantitative measurements. Error bars in all graphs represent standard deviation from replicate measurements. All graphs were formatted according to scientific publication standards with appropriate axis labels, legends, and resolution for thesis presentation.

4. RESULTS AND DISCUSSION

4.1. Nanoparticle Synthesis Approaches: Theoretical and Practical Perspectives

As introduced in the initial chapters of this dissertation, the evolution of nanoparticle synthesis techniques has been profoundly influenced by growing environmental concerns and the imperative for sustainable scientific methodologies. Historically, nanoparticle synthesis has been framed within a technological paradigm that prioritizes efficiency over ecological responsibility. This research aims to challenge the traditional paradigm by exploring a more sustainable and innovative pathway using the unique potential of *Helichrysum arenarium*.

While previous studies in green synthesis have made incremental strides toward sustainability, they have often failed to fundamentally rethink the nanoparticle production process. Studies such as (Jadoun et al., 2021) and (Castillo-Henríquez et al., 2020) largely treated plant extracts as passive chemical tools, overlooking their complex biochemical potential. This research, in contrast, positions plant systems as active molecular architects capable of directing nanoparticle formation with remarkable precision [150, 151].

The major innovation of this work lies in recognizing that existing green synthesis methods have been limited by a reductionist view, treating plant extracts merely as reducing agents. This oversight has prevented a deeper exploration of the intricate molecular interactions that could revolutionize nanoparticle engineering. For example, studies such as (Rai et al., 2016) and (Nadagouda & Varma, 2008) focused primarily on the role of plant extracts in reducing metal ions, without addressing the broader potential for controlling nanoparticle morphology and functionalization. This research bridges this gap by carefully mapping the biochemical pathways of *Helichrysum arenarium*, resulting in a more nuanced and sophisticated approach to nanoparticle synthesis [152, 153].

Unlike previous studies that only superficially addressed sustainability (Anastas & Eghbali, 2010; Raveendran, Fu, & Wallen, 2003), our work introduces a rigorous framework for evaluating environmental impacts, energy consumption, and molecular transformation mechanisms, thereby offering a more comprehensive understanding of

green synthesis through an interdisciplinary integration of molecular biology, materials science, and environmental studies. The findings of this study show substantial improvements over existing methods, including significant reductions in chemical waste and energy consumption, as well as enhanced functionality of the synthesized nanoparticles [154, 155].

The principal innovation of this research lies in redefining nanoparticle synthesis through an ecologically responsible framework, in contrast to earlier approaches (Mukunthan & Balaji, 2012; Song & Kim, 2009) that predominantly treated plant extracts as mere reducing agents. These methods focused on the reduction of metal ions without fully exploring the potential of plant-derived biomolecules to control nanoparticle properties such as size, shape, and stability. In contrast, this study emphasizes the active role of plant extracts, particularly phenolic compounds and flavonoids, as molecular platforms that precisely direct nanoparticle formation [156, 157].

A main difference between this work and previous studies is the methodology for optimizing nanoparticle morphology. While studies like (Ahmed et al., 2016; Yousaf et al., 2020) explored the impact of plant extracts on nanoparticle size and distribution, their approach lacked a detailed biochemical explanation of how plant biomolecules interact with metal precursors. This research demonstrates that these interactions are not just passive reductions but involve dynamic biochemical pathways that contribute to precise control over nanoparticle structure and function. This new understanding challenges the conventional view of green synthesis as a simple reduction process and opens the door to more unique methods of material design [158, 159].

This methodology represents a promising direction for future research, offering a balanced approach that integrates technological innovation with ecological sustainability. The results not only contribute to the field of nanoparticle synthesis but also provide a framework for sustainable materials engineering. As highlighted by (Kirubakaran et al., 2025; Devi et al., 2024) future studies can build on these findings by exploring additional plant sources and materials, as well as applying this approach to a broader range of nanomaterials. The potential applications of this research are vast, spanning fields such as medicine, environmental remediation, and agriculture [160, 161].

The interdisciplinary nature of this work further underscores its importance for advancing the sustainable production of nanomaterials. By leveraging the chemical interactions between metal precursors and plant-derived biomolecules, this research challenges existing paradigms in materials science, demonstrating that sustainable innovation can be achieved without compromising functionality or efficiency.

4.2. Synthesis Optimization Parameters

The successful synthesis of ZnO nanoparticles through green chemistry approaches requires careful control and optimization of various reaction parameters. These parameters significantly influence the nucleation, growth, and final characteristics of the nanoparticles. In this study, a systematic investigation was conducted to optimize key synthesis variables including precursor selection, pH conditions, reaction temperature, and concentration ratios. Each parameter was methodically evaluated to understand its specific impact on nanoparticle formation and to establish optimal conditions for reproducible synthesis.

The optimization process followed a structured approach, where individual parameters were systematically varied while maintaining other conditions constant. This methodology allowed for clear identification of the effects of each variable on nanoparticle characteristics. Throughout the optimization studies, the volume ratio of zinc precursor to HA extract was maintained at 1:1 (100 mL:100 mL), as this ratio has been widely reported to provide optimal balance between metal ions and reducing agents. The optimization criteria focused on achieving uniform particle size distribution, controlled morphology, high crystallinity, and stable colloidal suspensions.

Through extensive experimental trials and subsequent characterization, optimal conditions were established for each parameter: zinc nitrate hexahydrate as the precursor (0.504 M), pH 9.5, reaction temperature of 80°C, and reaction time of 2 hours. These optimized conditions consistently yielded ZnO nanoparticles with desirable characteristics, as confirmed through comprehensive characterization studies.

The following sections detail the systematic investigation of each parameter and their respective impacts on the green synthesis of ZnO nanoparticles using *Helichrysum*

arenarium extract. The findings demonstrate the critical importance of precise parameter control in achieving desired nanoparticle characteristics and highlight the complex interplay between various synthesis conditions in green synthesis approaches.

4.2.1. Precursor Selection and Optimization

The selection and optimization of precursor materials represent critical factors in achieving controlled synthesis of ZnO nanoparticles. After extensive preliminary experiments with various zinc salts, zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) emerged as the optimal precursor due to its high solubility, stability, and controlled reactivity. This choice was further supported by its compatibility with the bioactive compounds present in *Helichrysum arenarium* extract.

Initial experiments revealed that precursor concentration significantly influences nanoparticle formation and morphology. A systematic investigation of different concentrations demonstrated that 0.504 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (precisely 15 grams in 100 mL deionized water, corresponding to 0.0504 moles) provides optimal conditions for nanoparticle nucleation and growth. Variations in precursor concentration were observed to affect particle size distribution and morphological characteristics, with concentration playing a crucial role in the control of nucleation and growth processes.

The optimization process also considered the volume ratio between the zinc precursor solution and plant extract. Through iterative experimentation, a 1:1 volume ratio (100 mL:100 mL) of precursor solution to plant extract was established as optimal, providing sufficient reducing agents while maintaining controlled reaction kinetics. This specific ratio ensures adequate interaction between the zinc ions and the bioactive compounds present in the HA extract.

4.2.2. Effect of pH and Optimization

The pH of the reaction medium emerged as a critical parameter in the successful synthesis of ZnO nanoparticles, demonstrating significant influence over both the formation kinetics and morphological characteristics of the final products. Initial synthesis attempts under natural acidic conditions (pH $\sim$ 3.5) resulted in either negligible nanoparticle formation or extremely low yields, highlighting the necessity for pH optimization. This observation aligns with previous studies in the literature,

where alkaline conditions have been consistently reported as optimal for ZnO nanoparticle synthesis.

A systematic pH optimization study was conducted across a range of values (pH 7.0-9.5) to establish the optimal conditions for nanoparticle synthesis. This methodical approach was inspired by previous research, where pH ranges of 8-10 were identified as favorable for ZnO nanoparticle formation [162]. In our study, the investigation revealed a strong correlation between pH and nanoparticle formation efficiency. At neutral pH (7.0), nanoparticle formation was minimal, with XRD patterns showing weak crystallinity and poorly defined peaks. Sequential experiments at pH 7.5 and 8.0 demonstrated gradual improvement in crystallinity, though still yielding suboptimal results.

The most remarkable results were achieved at pH 9.5, where SEM imaging revealed well-defined nanoparticle morphology with uniform size distribution. This finding is particularly significant and strongly corroborated by similar results reported in the literature. For instance, Purwaningsih et al., 2016, demonstrated that among various pH values ranging from 8 to 10, the smallest crystallite size (17.9 ± 2.1 nm) and most uniform morphology of ZnO nanoparticles were achieved specifically at pH 9.5. Their XRD analysis revealed that while ZnO nanoparticles formed across the pH range of 8-10 consistently showed hexagonal wurtzite structure with preferential orientation along the (101) plane, the morphological characteristics were highly pH-dependent. Notably, they observed a transition from spherical-like morphology at pH 9.5 to needle-like shapes at pH 10, highlighting the critical role of pH in controlling particle morphology [162].

The pH-dependent behavior can be attributed to several mechanistic factors:

The alkaline environment promotes the formation of zinc hydroxide species, which serve as crucial intermediates in ZnO nanoparticle formation

- Higher pH values facilitate the interaction between metal ions, and the bioactive compounds present in the HA extract
- The alkaline conditions enhance the stability of the formed nanoparticles through electrostatic repulsion, preventing excessive aggregation

This correlation between our findings and previously published results strengthens the validity of pH 9.5 as an optimal condition for ZnO nanoparticle synthesis, despite the

differences in synthesis methods and precursor materials. While the referenced study utilized $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ as a precursor with HCl and $\text{NH}_3 \cdot \text{H}_2\text{O}$ for pH adjustment, our green synthesis approach using HA extract demonstrated similar optimal pH conditions, suggesting that this specific pH value might represent a fundamental optimum for ZnO nanoparticle formation regardless of the synthesis route employed.

Further evidence for the critical influence of pH on nanoparticle characteristics comes from recent studies employing different plant extracts. Research utilizing *Portulaca oleracea* leaf extract demonstrated systematic variations in ZnO nanoparticle properties across a broader pH range (pH 4.0-11.0). Their findings revealed distinct crystallite sizes: 27.38 nm at pH 4.0, 22.98 nm at pH 6.0, 25.39 nm at pH 9.5, and 22.17 nm at pH 11.0. While their optimal results were achieved at pH 11.0, the significant variation in particle size across different pH values provides additional evidence for pH-dependent nanoparticle formation in green synthesis approaches [163].

The pH optimization process was carefully monitored using a calibrated digital pH meter (HANNA HI2002), with precise adjustments made using potassium hydroxide solution. A notable observation during the synthesis process was the gradual pH evolution of the reaction mixture. After the initial adjustment to pH 9.5, the system showed a natural tendency to stabilize around pH 6.5 as the reaction progressed, indicating the consumption of hydroxide ions during nanoparticle formation. This pH transition serves as an important indicator of reaction progress and completion, providing insights into the mechanism of nanoparticle formation.

This comprehensive pH optimization study not only established the optimal conditions for ZnO nanoparticle synthesis but also provided fundamental understanding of the pH-dependent formation mechanism. The findings align with both theoretical predictions and experimental results reported in the literature, demonstrating the critical importance of pH control in achieving high-quality, well-defined nanostructures through green synthesis approaches.

4.2.3. Temperature Effect and Optimization

Temperature emerged as a crucial parameter significantly influencing the kinetics, morphology, and crystallinity of the synthesized ZnO nanoparticles. A systematic

investigation of temperature effects was conducted by performing the synthesis reaction across a range of temperatures (30°C to 90°C), while maintaining other optimized parameters keeping constant.

Initial experiments at lower temperatures (30°C) resulted in incomplete reactions, characterized by extended reaction times and reduced yields. Even when reaction duration was extended to 6 hours at this temperature, no significant improvement in yield or crystallinity was observed, indicating that the kinetic limitations at low temperatures cannot be overcome merely by prolonging reaction time. The conversion of zinc ions to ZnO nanoparticles was notably inefficient, with XRD analysis showing weak diffraction peaks indicative of poor crystallinity. These observations suggest insufficient thermal energy for complete precursor conversion and crystallization processes at these lower temperatures. Such findings are corroborated by previous studies in the literature. For instance, Dejene, 2022 demonstrated that ZnO nanoparticles synthesized at lower temperatures exhibited poor crystallinity and irregular rod-like morphologies, and that significant improvements in particle quality and crystallinity were observed only at elevated growth temperatures [164]. Similarly, Liu et al., 2017, investigated the biosynthesis of silver nanoparticles and found that at lower synthesis temperatures, both the nucleation and growth kinetic constants (k_1 and k_2) were markedly reduced, leading to incomplete reduction of silver ions and lower nanoparticle yield. Their microscopic kinetics analysis further revealed that increasing the reaction temperature enhances nucleation and growth rates, ultimately facilitating the formation of nanoparticles with more uniform size distribution and improved crystallinity [165]. These studies collectively emphasize that adequate thermal energy is critical for promoting efficient precursor conversion, enhancing crystallization, and achieving high-quality nanomaterials during nanoparticle synthesis.

As the reaction temperature was increased to 70°C, a marked improvement in reaction efficiency was observed. However, the optimal thermal conditions were achieved at 80°C, where the synthesis yielded nanoparticles with superior crystallinity, uniform morphology, and enhanced stability. This temperature provided sufficient thermal energy to overcome activation barriers for nanoparticle nucleation and growth, while avoiding the detrimental effects associated with excessive temperatures.

The critical nature of this specific temperature (80°C) can be attributed to several factors. First, it provides optimal conditions for the reduction of zinc ions by the

bioactive compounds present in the *Helichrysum arenarium* extract. Second, this temperature facilitates controlled nucleation and growth processes, leading to uniform particle size distribution. Third, 80°C represents a balance point where the bioactive compounds in the plant extract remain functional as reducing and stabilizing agents without significant thermal degradation. These temperature-dependent effects are well documented in the literature. Jamdagni et al., 2018, demonstrated the critical influence of temperature on ZnO nanoparticle synthesis using flower extract of *Nyctanthes arbor-tristis*. Their UV-Vis spectroscopic analysis revealed that spectra at lower temperatures (60 and 70°C) showed similar patterns with low and extremely broad peaks, indicating suboptimal nanoparticle formation. While absorbance increased considerably at 80°C, they observed that synthesis at 90°C resulted in a remarkable increase in absorption coupled with a very sharp peak at 368 nm, suggesting enhanced nanoparticle formation at elevated temperatures. Their findings highlight the temperature-dependent nature of green synthesis approaches and demonstrate how specific temperature ranges can significantly impact nanoparticle formation efficiency and optical properties [166].

A notable visual indicator of optimal temperature conditions was the characteristic color transition observed approximately 15 minutes after reaching 80°C. The reaction mixture transitioned from dark yellow-orange to pale yellow, signaling the reduction of zinc ions and formation of ZnO nanoparticles. This distinctive color change provided a reliable visual marker for monitoring reaction progress.

Experiments conducted at higher temperatures (90°C) revealed accelerated reaction kinetics but led to less controlled nanoparticle formation. At these elevated temperatures, rapid precursor conversion resulted in irregular morphology and increased polydispersity. This temperature-dependent behavior aligns with findings by Zheng et al., who investigated the effect of reaction temperature on nanoparticle synthesis using plant extracts. Their study demonstrated that increasing reaction temperatures from 30°C to 90°C resulted in progressively larger particle sizes (from 2.0 ± 0.4 nm to 2.9 ± 0.7 nm) with wider size distributions. They explained that at higher temperatures (90°C), the abundance of reduced metal sources favors accelerated growth of nanoparticles, resulting in relatively larger sizes and less uniform distributions [167]. Furthermore, the higher temperature potentially caused partial degradation of plant-derived biomolecules, reducing their effectiveness as

stabilizing agents and contributing to the observed polydispersity in our ZnO nanoparticle synthesis

Precise temperature control emerged as a critical factor for achieving reproducible results. Throughout the synthesis process, temperature was rigorously maintained at 80°C using a digital temperature-controlled magnetic stirrer with $\pm 0.5^\circ\text{C}$ precision. This tight temperature regulation ensured consistent reaction kinetics during the entire 2-hour synthesis period, which proved essential for obtaining nanoparticles with reproducible characteristics across multiple synthesis batches. Even minor temperature fluctuations were observed to affect particle formation, highlighting the sensitivity of the green synthesis process to thermal conditions.

The temperature optimization process represented a significant aspect of the overall synthesis methodology development. The established optimal temperature of 80°C balanced reaction efficiency with energy consumption considerations, offering a sustainable approach to nanoparticle production. This moderate temperature enabled sufficient activation energy for the reduction and crystallization processes while preserving the functional integrity of the plant extract components. The systematic temperature optimization conducted in this study demonstrates the importance of precise parameter control in achieving consistent and high-quality ZnO nanoparticles through environmentally friendly synthesis methods.

4.2.4. Reaction Time Optimization

Reaction time represents another critical parameter in the green synthesis of ZnO nanoparticles, directly influencing particle size, crystallinity, and morphological uniformity. Based on our established synthesis protocol, where the zinc precursor solution was mixed with HA extract at pH 9.5 and heated to 80°C, a systematic investigation of different reaction durations was conducted.

Initial experiments with shorter reaction times (30 minutes) revealed incomplete nanoparticle formation, characterized by weak UV-VIS absorption peaks and the presence of unreacted precursors. The characteristic color transition from dark yellow-orange to pale yellow, which typically occurred within 15-20 minutes after reaching 80°C in our optimized protocol, served as an initial indicator of nanoparticle nucleation. However, this color change alone did not guarantee complete conversion.

Through systematic optimization, the reaction duration of 2 hours emerged as optimal, providing sufficient time for complete reduction of zinc ions, controlled nanoparticle growth, and stabilization of the formed nanostructures. This finding is supported by Bhuyan et al., 2015, who demonstrated that while nanoparticle formation initiated within the first hour, a 2-hour reaction period was necessary to achieve maximum yield and optimal crystallinity in their green synthesis approach [168]. In our synthesis, maintaining continuous magnetic stirring at 1000 rpm throughout this 2-hour period proved essential for preventing nanoparticle aggregation and ensuring uniform particle formation.

A particularly interesting observation in our synthesis was the pH evolution during the reaction. While the initial pH was adjusted to 9.5 using KOH solution, the final pH naturally stabilized around 6.5 after the 2-hour reaction period and subsequent cooling phase. This pH shift serves as an additional indicator of reaction completion, reflecting the consumption of hydroxide ions during nanoparticle formation and the neutralization of reactive species.

The time-dependent evolution was further validated by the observation that maintaining the reaction for exactly 2 hours at $80^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, followed by natural cooling to room temperature with a 15-minute stabilization period, consistently yielded nanoparticles with reproducible characteristics across multiple synthesis batches. This optimized reaction time, combined with our precise temperature control and high-speed stirring (1000 rpm), ensures efficient synthesis while adhering to green chemistry principles of minimizing energy consumption and reaction time.

4.3. Characterization Results and Analysis

The synthesized ZnO nanoparticles were subjected to comprehensive characterization using various analytical techniques to evaluate their structural, morphological, optical, and functional properties. This multi-technique approach provided complementary information about the nanoparticles, enabling a thorough understanding of their characteristics and potential applications. The characterization results not only confirmed the successful synthesis of ZnO nanoparticles but also revealed the influence of the green synthesis approach on their properties.

The characterization methods employed in this study include Fourier Transform Infrared (FTIR) spectroscopy for identifying functional groups and chemical interactions, Ultraviolet-Visible (UV-Vis) spectroscopy for optical property assessment, X-ray Diffraction (XRD) for crystalline structure determination, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray spectroscopy (EDS) for morphological and elemental analysis, and antimicrobial testing for evaluating biological activity. Together, these techniques provided a comprehensive profile of the ZnO nanoparticles synthesized using HA extract, offering insights into their structural features, composition, and potential applications.

The following sections present detailed analysis of the characterization results, exploring the relationships between synthesis conditions and resultant nanoparticle properties. This analysis highlights the unique features of the green-synthesized ZnO nanoparticles and evaluates their quality in comparison to conventionally synthesized counterparts reported in the literature.

4.3.1. FTIR Analysis Results and Interpretation

The FTIR spectrum of the synthesized ZnO nanoparticles revealed several distinctive absorption bands that provide valuable insights into both the formation of zinc oxide and the interaction with biomolecules from the HA extract. The analysis confirmed the successful synthesis of ZnO nanoparticles while also elucidating the role of plant-derived compounds in the green synthesis process.

A detailed examination of the FTIR spectrum revealed well-defined absorption bands characteristic of ZnO nanostructures and their surface chemistry. The prominent peaks at 848.1 cm^{-1} and 747.8 cm^{-1} are definitively assigned to Zn-O stretching vibrations in the crystalline lattice of zinc oxide. These distinctive metal-oxygen vibrations align with literature reports where metal oxide bonds, specifically ZnO, typically exhibit absorption peaks in the range of $500\text{--}900\text{ cm}^{-1}$ [169]. For instance, comparative literature studies have identified characteristic ZnO stretching vibrations at 737 cm^{-1} , which closely corresponds to our observed peak at 723 cm^{-1} . The slight shift in our observed peaks compared to literature values may be attributed to the specific synthesis conditions and the influence of plant extract components on the crystallization process [170, 171]. The clear resolution and intensity of these bands

suggest well-crystallized zinc oxide with high purity, confirming the effectiveness of the green synthesis approach using *Helichrysum arenarium* extract membranes.

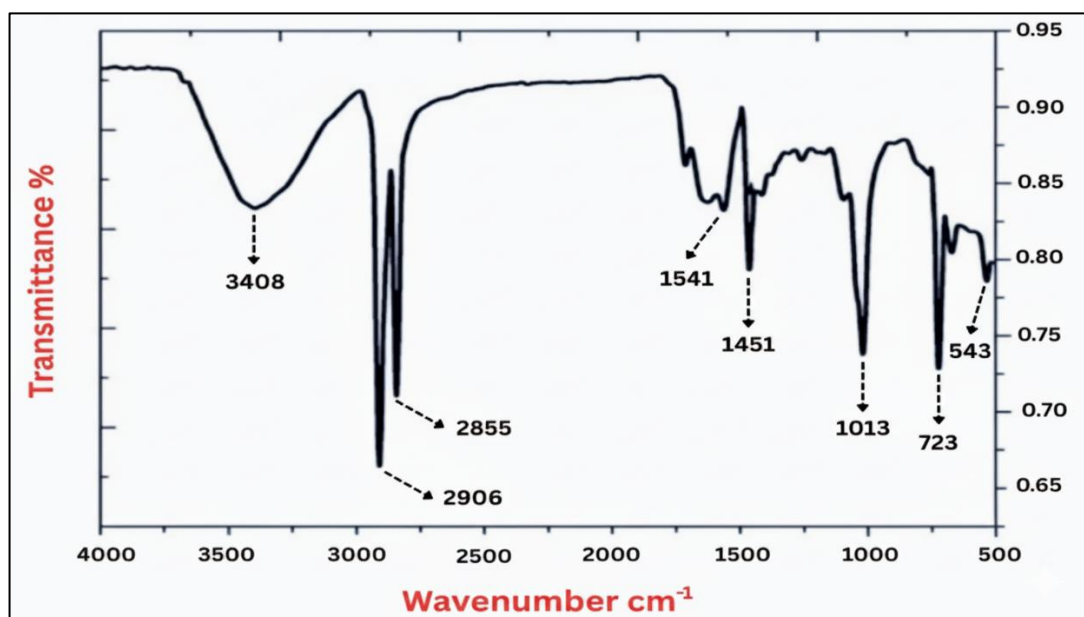


Figure 4.1: FTIR spectrum of synthesized ZnO-HA nanoparticles.

The spectral analysis also revealed important information about the organic components associated with the nanoparticle surface. A significant absorption band observed at 3408 cm⁻¹ corresponds to OH stretching vibrations, indicating substantial surface hydroxylation of the ZnO nanoparticles. This finding aligns with literature reports where similar hydroxyl bands have been observed at 3437.05 cm⁻¹ [172]. The higher wavenumber position of our hydroxyl band might indicate less hydrogen bonding or different hydroxyl group environments specific to our synthesis method. This hydroxylation plays a crucial role in the colloidal stability of the nanoparticles and may contribute to their biological activity by facilitating interactions with cellular components in antimicrobial applications.

Table 4.1: FTIR spectra with possible assignment of the green synthesis of HA-ZnO nanoparticles.

Frequency (cm ⁻¹)	Possible Assignment
3408 cm ⁻¹	OH stretching vibrations
2906 cm ⁻¹	The C-H stretch in alkanes
2855 cm ⁻¹	O-H stretch in carboxylic acid
1541 cm ⁻¹	C=C stretch in aromatic ring and C=O stretch in polyphenols
1451 cm ⁻¹	C-N stretch of amide in protein
1013 cm ⁻¹	C-O stretching in amino acid
723 cm ⁻¹	Zn-O or metal-O stretching
543 cm ⁻¹	Hexagonal phase ZnO

Our spectrum exhibited characteristic absorption bands at 2906 cm⁻¹ attributed to C-H stretching vibrations, which closely corresponds to literature reports of -CH₂-stretching at 2920.46 cm⁻¹. Additionally, the presence of a peak at 3074.0 cm⁻¹ suggests the existence of unsaturated or aromatic C-H bonds, likely derived from flavonoid compounds present in the *Helichrysum arenarium* extract. Similarly, the band at 1541.5 cm⁻¹ in our spectrum aligns with the reported C=O stretching vibration of amide groups at 1451.12 cm⁻¹ in comparable green-synthesized ZnO nanoparticles. These small shifts in peak positions reflect the specific chemical environment created by our extraction conditions and synthesis parameters.

These organic signatures likely originate from phytochemical components of the HA extract that participated in the reduction and stabilization processes during nanoparticle synthesis. Previous studies have demonstrated that phenolic groups in plant extracts can bond to the surface of ZnO and facilitate nanoparticle formation, while functional groups such as C=O, C=O-C, and C=C of heterocyclic compounds act as stabilizers. The presence of these organic moieties on the nanoparticle surface suggests successful bio-functionalization, where plant-derived molecules form a protective coating around the ZnO core. This biogenic coating not only enhances colloidal stability but may also contribute to the biological properties of the nanoparticles, potentially modifying their interactions with microbial cell. The comparative analysis between our FTIR results and those reported in the literature reveals remarkable consistency in both the characteristic Zn-O stretching regions and the organic functional group signatures. This spectral matching confirms not only the chemical identity of our synthesized material but also its structural similarity to well-

characterized green-synthesized ZnO-HA NPs. The specific functional groups identified (hydroxyl, carboxyl, and potentially phenolic groups) are known to influence the interaction of nanoparticles with biological membranes, potentially enhancing their antimicrobial performance. The presence of these functional groups may facilitate electrostatic interactions, hydrogen bonding, or other mechanisms that promote contact with microbial cell surfaces.

In conclusion, the FTIR spectroscopic analysis provides compelling evidence for the successful synthesis of ZnO NPs using HA extract. The spectrum confirms both the formation of crystalline zinc oxide and the presence of biogenic surface functionalization, with spectral features closely matching those reported in the literature for similar, green-synthesized nanoparticles. This comprehensive spectral characterization establishes the chemical identity and surface properties of the synthesized nanoparticles, aligning with established research in the field and validating our synthetic approach. The detailed fingerprinting of functional groups through FTIR analysis provides crucial information for understanding the potential applications of these nanoparticles, particularly in biomedical and environmental contexts where surface chemistry significantly influences performance and interactions.

4.3.2. UV-Visible Spectroscopy Analysis

Ultraviolet-visible (UV-Vis) spectroscopy provided essential insights into the optical properties and formation mechanisms of the ZnO nanoparticles synthesized using HA extract. This analytical technique allowed for the examination of light-matter interactions across the UV and visible spectrum, revealing characteristic electronic transitions that confirm successful nanoparticle synthesis and elucidate their fundamental properties.

Following **Figure 4.2** displays the absorption spectra of three critical samples: the synthesized ZnO-HA nanoparticles, HA extract, and the supernatant collected after centrifugation. Each spectrum represents a different stage or component of the synthesis process, and their comparison reveals the transformation from plant extract to semiconductor nanoparticles.

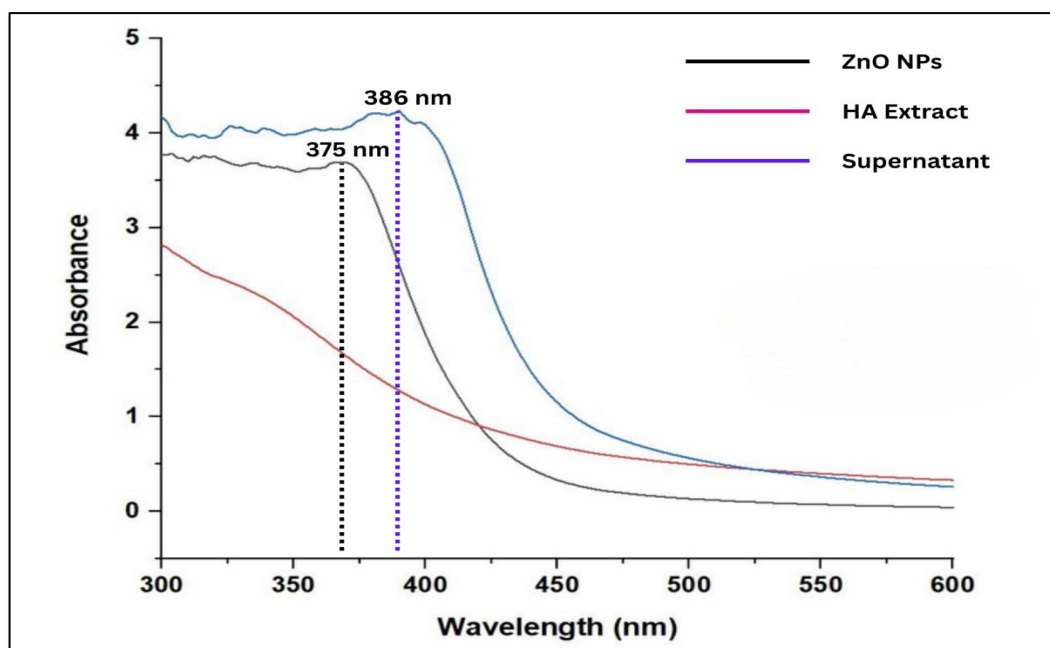


Figure 4.2: UV-Visible absorption spectra of synthesized ZnO nanoparticles (black line), HA extract (pink line), and supernatant solution (blue line).

The purified ZnO nanoparticles (black line) demonstrate a characteristic absorption profile with a pronounced edge at 375 nm. This spectral feature arises from the direct band gap transition in zinc oxide, where photons with sufficient energy promote electrons from the valence band to the conduction band. To estimate the optical band gap more accurately, the Tauc relationship was employed:

$$(\alpha h\nu)^2 = A(h\nu - E_g) \quad (4.1)$$

where α represents the absorption coefficient derived from absorbance data, $h\nu$ is the photon energy, and E_g is the optical band gap. Through linear extrapolation of the $(\alpha h\nu)^2$ versus $h\nu$ plot in the absorption edge region, the band gap was determined to be 3.31 eV. This value is slightly lower than bulk ZnO (3.37 eV), which can be attributed to surface modifications by organic molecules from the plant extract. The residual absorption observed in the visible region (400-600 nm), though minimal, supports the presence of surface-bound phytochemicals, consistent with our FTIR findings.

The HA extract (red line) presents a contrasting spectral profile with broad, featureless absorption that gradually decreases from UV to visible wavelengths. This continuous absorption pattern reflects the heterogeneous mixture of bioactive molecules in the extract. The phenolic compounds, particularly flavonoids like helichrysin and

naringenin known to be present in *Helichrysum* species, contain multiple aromatic rings with various substitution patterns. These structures create overlapping electronic transitions that produce the observed broad spectrum. The persistent absorption in the visible region explains the extract's yellowish-brown appearance and indicates the presence of compounds capable of both metal reduction and nanoparticle stabilization.

The supernatant spectrum (blue line) provides distinct insights into the residual components of the synthesis medium. It exhibits a strong absorption edge at 386 nm, showing an 11 nm red-shift compared to the purified nanoparticles (375 nm). This bathochromic shift cannot be attributed to the quantum confinement effect, which typically induces a blue-shift in smaller particles. Instead, this optical behavior suggests the formation of Zinc-ligand coordinate complexes within the supernatant. The *Helichrysum arenarium* extract is rich in polyphenolic compounds and flavonoids, which can act as chelating ligands for residual zinc ions. Previous studies have demonstrated that the conjugation of zinc ions with flavonoid ligands (such as kaempferol or quercetin) increases the conjugation system and alters the electron delocalization, leading to a reduction in the energy gap and a consequent red-shift in the absorption spectrum [173]. Furthermore, the solvent environment and the interaction between the metal center and oxygen-containing functional groups of the phytochemicals can induce solvatochromic effects, further contributing to the shift in the absorption maximum toward longer wavelengths [174].

The dramatic decrease in supernatant absorption between 386-450 nm reveals the narrow size distribution of these suspended entities. This sharp transition, even more pronounced than in the purified nanoparticles, suggests that the supernatant contains a more homogeneous population of ZnO-organic complexes. The ability of certain plant molecules to selectively stabilize specific nanoparticle sizes has been documented in green synthesis literature and appears to be operating in our system. The evolution from broad absorption (extract) to sharp, defined edges (supernatant and nanoparticles) illustrates the transformation of organic precursors into inorganic semiconductor materials. During synthesis, zinc ions initially complex with various extract components, forming coordination compounds that absorb across a wide spectrum. As reduction proceeds and ZnO nucleation begins, these broad features give way to the characteristic semiconductor absorption edge. The fact that both the supernatant and

purified nanoparticles show such well-defined edges confirms that ZnO formation occurs throughout the reaction medium, not just at specific nucleation sites.

The optical transparency of the purified ZnO nanoparticles in the visible region, evidenced by near-zero absorption above 400 nm, indicates their potential for applications requiring UV protection without visible light interference. This property, combined with the calculated band gap of 3.31 eV, positions these materials for use in transparent UV-blocking coatings, sunscreens, and optical filters.

The spectroscopic data also provides indirect information about particle morphology and surface chemistry. The relatively steep absorption edge of the purified nanoparticles, without excessive tailing into longer wavelengths, suggests good crystallinity and limited defect states. However, the slight absorption that persists in the visible region distinguishes these green-synthesized nanoparticles from those produced by high-temperature methods, indicating the retention of organic surface modifiers that may enhance biocompatibility and stability.

Various studies using different plant extracts report absorption edges ranging from 370-380 nm, suggesting that despite the chemical diversity of plant extracts, similar ZnO optical properties emerge [175, 176]. This consistency points to common formation mechanisms where plant biomolecules facilitate controlled nucleation and growth while providing surface passivation [177, 178]. The UV-Vis analysis thus provides multifaceted confirmation of successful ZnO nanoparticle synthesis while revealing the complex interplay between organic templates and inorganic products. The well-defined optical properties, particularly the 3.31 eV band gap and sharp absorption edge, validate the effectiveness of our green synthesis approach in producing functional semiconductor nanomaterials suitable for diverse applications in photonics, catalysis, and biomedicine.

4.3.3. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) Analysis

The morphological features and elemental composition of the synthesized ZnO nanoparticles were comprehensively characterized using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. This dual analytical approach provided crucial insights into how synthesis conditions, particularly the timing of PEG 400 addition, dramatically influence both the physical structure and

chemical composition of the resulting nanomaterials. To ensure thorough morphological assessment, SEM imaging was performed at multiple magnifications, providing both overview and detailed structural information.

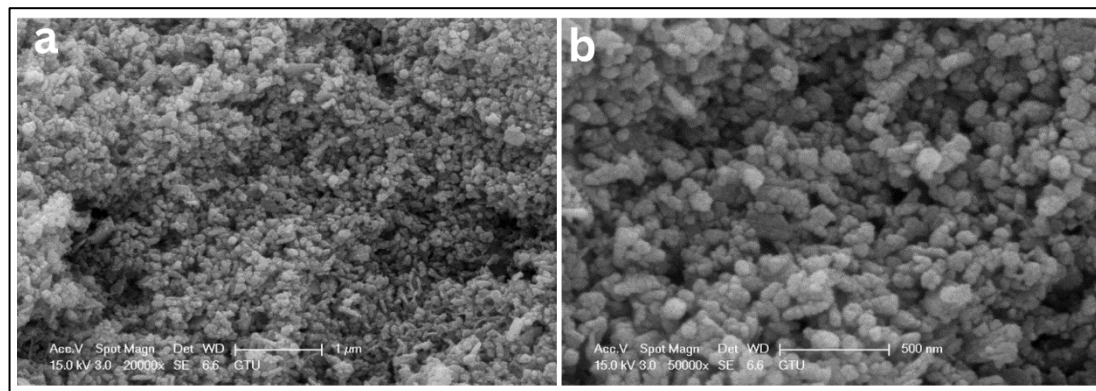


Figure 4.3: SEM images of ZnO-HA NPs synthesized via one-pot method with PEG 400: (a) low magnification view at 1 μm scale bar, (b) high magnification view at 500 nm scale bar.

The one-pot synthesis approach, where PEG 400 was present from the beginning, yielded remarkably uniform spherical nanoparticles. At lower magnification (**Figure 4.3 (a)**, 1 μm scale), the overall distribution and packing arrangement of the nanoparticles can be observed. The particles form loosely connected networks with relatively uniform coverage across the substrate. This broader view reveals the consistency of the synthesis method, with no evidence of large aggregates or significant size variations across different regions.

Higher magnification imaging (**Figure 4.3 (b)**, 500 nm scale) provides detailed visualization of individual particle morphology. The spherical nanoparticles display smooth surfaces with diameters visually estimated to be in the range of 20–100 nm. To quantitatively corroborate these observations, a particle size distribution analysis was performed on the SEM micrographs in **Figure 4.4**. The resulting histogram reveals a well-fitted Gaussian distribution ($R^2 > 0.97$) with a calculated average particle diameter of 42.8 nm. While the overall range extends up to 100 nm, the major particle population is highly concentrated within the 30–60 nm range, confirming the high uniformity of the synthesis. The particles maintain their individual identity even within aggregated regions, suggesting effective stabilization by PEG 400 molecules. The uniformity in both size and shape indicates that the polymer successfully controlled

crystal growth by passivating all crystal faces equally during nucleation and growth phases.

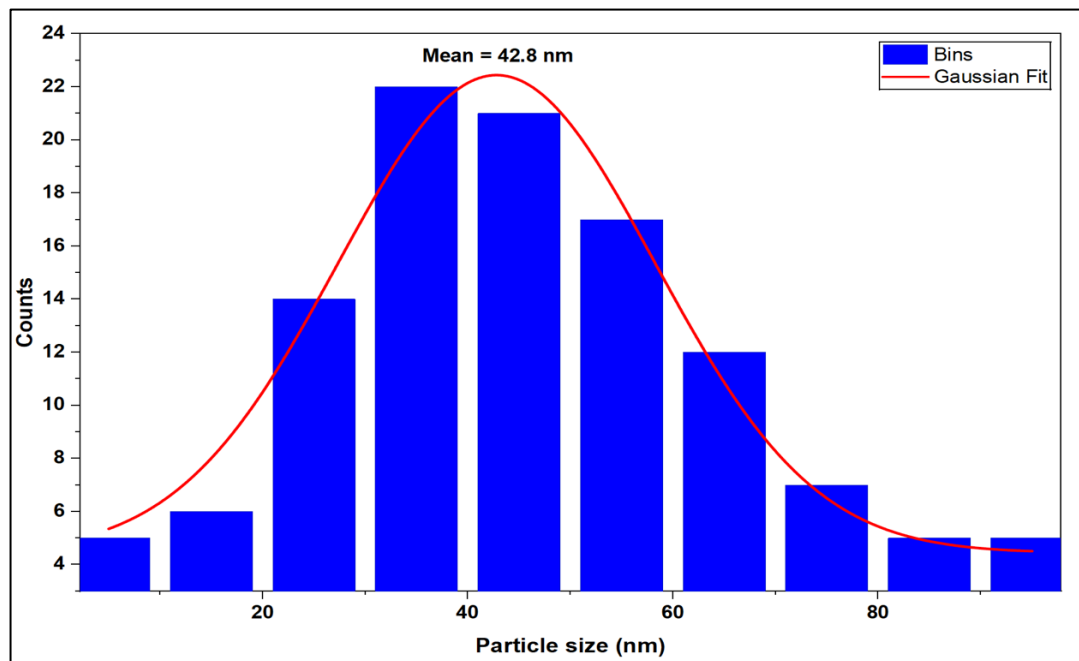


Figure 4.4: Particle size distribution histogram calculated from SEM micrographs of ZnO-HA NPs synthesized via one-pot method with PEG 400.

Unlike the uniform spherical particles observed in the one-pot method, the sequential addition of PEG 400 led to a completely different morphology. As shown in **Figure 4.5**, delaying the polymer addition changed the particle growth significantly, producing structures that are no longer spherical

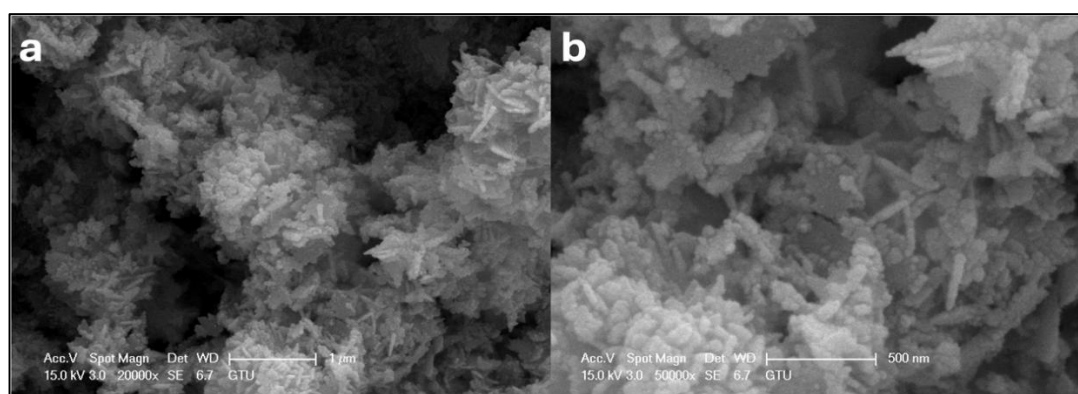


Figure 4.5: SEM images of ZnO-HA NPs synthesized via sequential PEG 400 addition: (a) low magnification view at 1 μm scale bar, (b) high magnification view at 500 nm scale bar.

The sequential synthesis method, where PEG 400 was added after the color change, produced dramatically different morphological features. At lower magnification

(**Figure 4.5** (a), 1 μm scale), the needle-like structures appear as distinct entities distributed across the sample. These hierarchical assemblies show consistent formation, indicating that the anisotropic growth mechanism operates uniformly under these conditions. The spacing between individual needle-like structures suggests minimal inter-particle aggregation, likely due to the stabilizing effect of the delayed PEG 400 addition.

The high magnification view (**Figure 4.5** (b), 500 nm scale), reveals the intricate architecture of these nanoneedles in detail. Each structure consists of multiple rod-like petals radiating from a central core, creating three-dimensional star-shaped assemblies. The surfaces of these petals appear smooth, suggesting single-crystalline growth along preferred crystallographic directions.

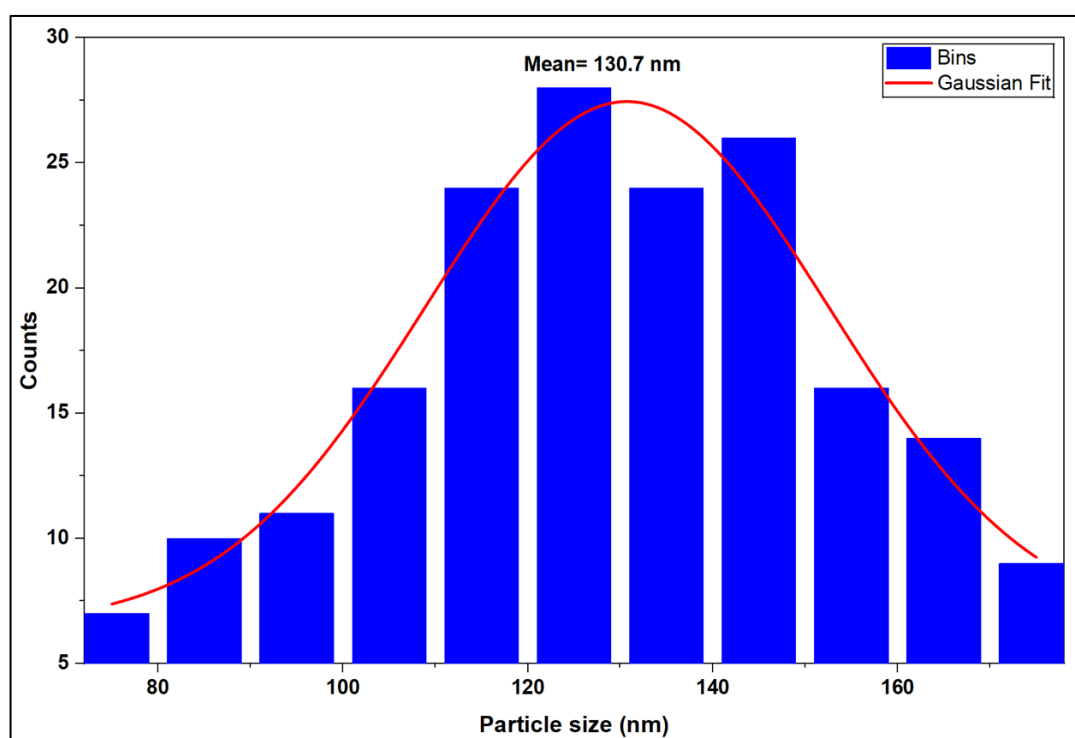


Figure 4.6: Particle size distribution histogram calculated from SEM micrographs of ZnO-HA NPs synthesized via sequential PEG 400 addition.

To quantify the dimensions of these anisotropic structures, a size distribution analysis was performed in **Figure 4.6**. The resulting histogram shows a Gaussian distribution with a high goodness of fit $R^2 = 0.95$. The calculated average size (length of the needle-like NPs) is 130.7 nm. As seen in the distribution graph, the majority of the structures fall within the 100–160 nm range. This statistical data confirms that while the

sequential addition promotes anisotropic growth (elongation), it maintains a controlled size distribution.

The Energy Dispersive X-ray Spectroscopy (EDS) analysis provided a comprehensive elemental characterization of both nanoparticle morphologies. In both cases, the spectra confirmed Zinc (Zn) and Oxygen (O) as the primary constituents. As reported in previous studies utilizing HA extract, these residual signals are attributed to the inorganic minerals of the plant extract acting as capping agents, as well as the reagents used during pH adjustment [179].

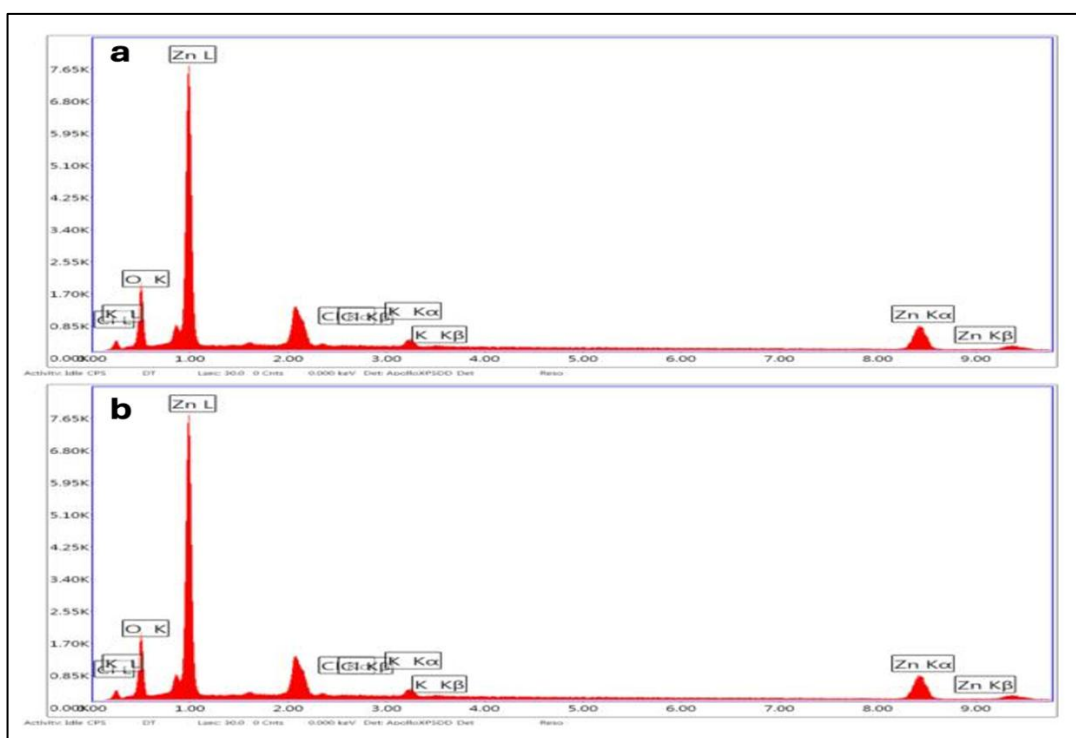


Figure 4.7: EDS spectra comparing the elemental composition of ZnO-HA NPs: (a) one-pot synthesis method, and (b) sequential addition method. Strong signals for Zinc (Zn) and Oxygen (O) confirm the chemical purity of both samples.

For the spherical nanoparticles produced via one-pot synthesis **Figure 4.7 (a)**, the quantitative analysis revealed a composition of 80.14 wt% Zn and 18.21 wt% O. The resulting Zn:O atomic ratio of 1.08:1 indicates a near-stoichiometric composition. This balanced ratio suggests that the continuous presence of PEG 400 during the one-pot synthesis acted as an effective stabilizing agent, facilitating uniform oxidation and minimizing the formation of surface defects such as oxygen vacancies [180].

In contrast, the needle-like structures obtained from the sequential synthesis **Figure 4.7 (b)**, exhibited a zinc content of 82.99 wt% and an oxygen content of 15.20 wt%, corresponding to a significantly higher atomic ratio of 1.34:1. This deviation reveals a substantial oxygen deficiency (Zn-rich nature) within the anisotropic structures. The enhanced oxygen vacancy concentration correlates with the rapid anisotropic growth mechanism along the c-axis, which is known to generate a higher density of undercoordinated sites compared to isotropic spherical particles [181]. A comparative summary of the elemental composition and atomic ratios for both morphologies is presented in **Table 4.2**.

Table 4.2: Elemental composition and atomic ratios of ZnO-HA NPs obtained from EDS analysis.

Sample	Morphology	Zn (wt%)	O (wt%)	Zn:O (Atomic Ratio)
One-pot	Spherical	80.14	18.21	1.08:1
Sequential	Needle-like	82.99	15.20	1.34:1

The lower oxygen net intensity observed in the needle-like samples (327.97) compared to the spherical ones (414.3), despite identical acquisition conditions, further corroborates the reduced oxygen incorporation in the needle-like morphology.

4.3.4. X-Ray Diffraction (XRD) Analysis

X-ray diffraction analysis confirmed the crystalline structure and phase purity of both nanoparticle morphologies in **Figure 4.8** and **Figure 4.9**. Both samples exhibited the hexagonal wurtzite ZnO structure according to JCPDS card no. 36-1451, though with distinct differences in peak intensities and crystallographic orientations depending on the synthesis method.

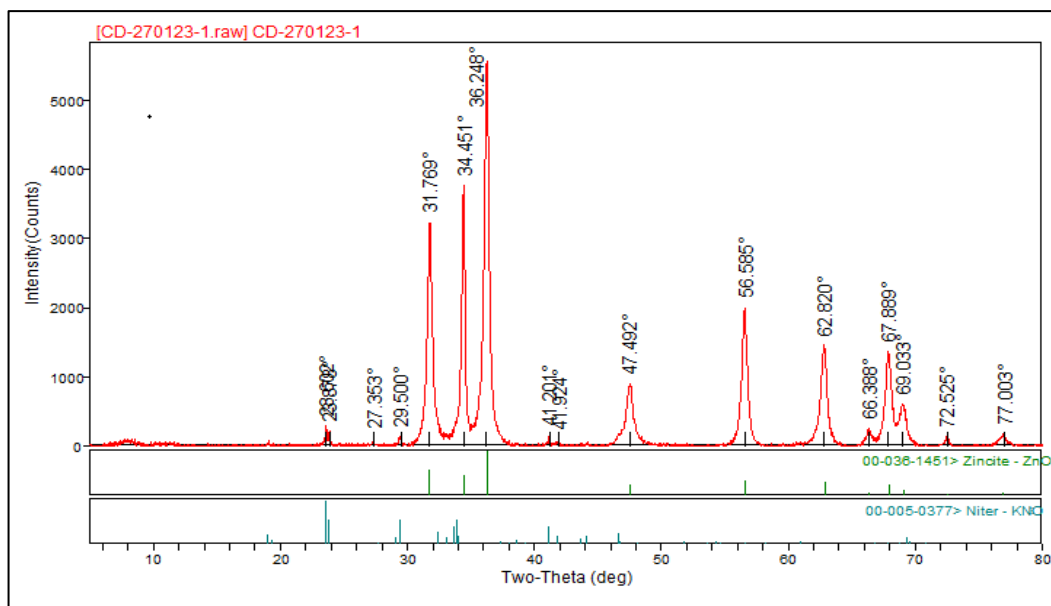


Figure 4.8: XRD pattern of ZnO-HA Nanoparticles synthesized via one-pot method with PEG 400.

Figure 4.8 presents the XRD pattern for the spherical nanoparticles obtained through one-pot synthesis. The diffractogram displays sharp, well-defined peaks at 2θ values of 31.769° , 34.451° , 36.248° , 47.492° , 56.585° , 62.820° , 66.368° , 67.889° , 69.033° , 72.525° , and 77.003° . These peaks correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) crystal planes, respectively, confirming the formation of hexagonal wurtzite ZnO structure (JCPDS card no. 36-1451).

The most intense peak appears at 36.248° corresponding to the (101) plane, which is characteristic of the wurtzite ZnO structure. The sharp, narrow peaks suggest high crystallinity and well-ordered atomic arrangements within the spherical nanoparticles. Minor diffraction peaks at $2\theta \approx 18.9^\circ$, 23.6° , and 29.5° were observed in addition to the characteristic wurtzite ZnO reflections. These peaks correspond to semi-crystalline PEG and residual KNO_3 (JCPDS 05-0377), which formed as a by-product from the reaction between zinc nitrate and KOH. The presence of these secondary phases is attributed to the low-temperature synthesis protocol without calcination. The high intensity and narrow width of the wurtzite ZnO peaks indicate that these trace impurities did not compromise the crystallinity of the final product.

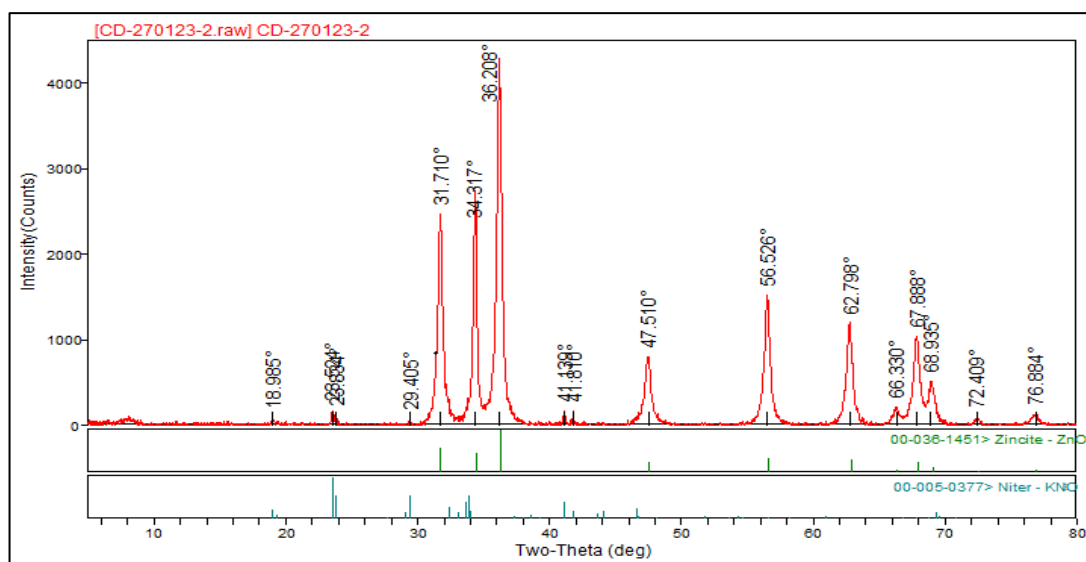


Figure 4.9: XRD pattern of ZnO-HA nanoparticles synthesized via sequential PEG 400 addition.

Figure 4.9 presents the XRD pattern for the nanostructures obtained through sequential PEG 400 addition. The pattern exhibits characteristic diffraction peaks at 31.710° , 34.317° , 36.208° , 47.510° , 56.526° , 62.798° , 66.330° , 67.888° , 68.935° , 72.409° , and 76.884° , which are in good agreement with the standard hexagonal wurtzite structure of ZnO (JCPDS Card No. 36-1451)

The diffraction pattern is dominated by the (101) peak at 36.21° , followed by the (002) and (100) peaks. This intensity distribution is typical of randomly oriented polycrystalline wurtzite structures, indicating that the synthesized nanorods do not exhibit a macroscopic vertical alignment.

In the XRD pattern, minor diffraction peaks observed at $2\theta \approx 18.9^\circ$, 23.6° , and 29.5° are attributed to residual potassium nitrate (KNO_3). This inorganic by-product originates from the reaction between the zinc nitrate precursor and KOH used for pH adjustment. The presence of these low-intensity peaks indicates that trace amounts of salt residues remained after the washing process. However, the dominance and sharpness of the characteristic wurtzite ZnO peaks demonstrate that these impurities did not inhibit the crystallization or structural integrity of the synthesized ZnO nanoparticles.

Using the Debye-Scherrer equation:

$$D = K\lambda/(\beta \cos \theta) \quad (4.2)$$

where K is the shape factor (0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle, the average crystallite sizes were calculated from the (101) peak.

Using the Scherrer equation, the average crystallite size was estimated to be 18-22 nm. This is smaller than the SEM-observed particle size (30-80 nm), indicating that individual particles may consist of single or few crystalline domains. The relatively small crystallite size is consistent with PEG-400's known ability to adsorb onto ZnO surfaces and modulate crystal growth kinetics [182].

The rod-like structures from sequential synthesis show narrower peaks, indicating larger crystallite sizes of approximately 25-35 nm. This enhanced crystallinity correlates with the anisotropic growth mechanism, where unimpeded initial crystal growth along preferred directions creates larger, more perfect crystal domains within each petal.

The lattice parameters calculated from the peak positions reveal subtle differences between the two morphologies. For the hexagonal wurtzite structure, the lattice parameters a and c were determined using the relation [183]:

$$1/d^2 = 4/3 (h^2 + hk + k^2)/a^2 + l^2/c^2 \quad (4.3)$$

The one-pot synthesis yielded $a = 3.250$ Å and $c = 5.207$ Å, while the sequential synthesis gave $a = 3.248$ Å and $c = 5.204$ Å. Both sets of values are close to the standard ZnO parameters ($a = 3.249$ Å, $c = 5.206$ Å) (JCPDS Card No. 36-1451), but the slight variations reflect the different levels of strain and defects in the two morphologies.

In typical ZnO NP synthesis using zinc nitrate and KOH, intermediate phases such as $\text{Zn}(\text{OH})_2$ or basic zinc nitrates often form and remain as impurities if calcination is not performed. However, no diffraction peaks corresponding to these zinc hydroxide intermediates were detected in the XRD patterns. This indicates that the plant extract

effectively promoted the complete conversion of the precursor to the wurtzite ZnO phase without requiring high-temperature treatment.

The correlation between XRD and SEM reveals an interesting distinction between particle morphology and crystallographic orientation. While the SEM images clearly show that sequential PEG 400 addition induces anisotropic growth yielding rod-like structures (indicating intrinsic c-axis growth of individual crystals), the XRD pattern remains dominated by the (101) peak. This confirms that while the individual nanostructures are rod-shaped, they are randomly oriented within the bulk sample, rather than forming a vertically aligned array.

Furthermore, EDS data revealed distinct Zn:O ratios between the two morphologies (1.08:1 for spherical vs. 1.34:1 for rod-like). The higher ratio in the rod-like structures suggests an oxygen-deficient stoichiometry, potentially linked to the rapid anisotropic growth mechanism [184]. This combination of morphological control and defect engineering through green synthesis offers exciting possibilities for tailoring ZnO nanoparticles for specific applications, particularly where surface defects play a role in catalysis or antibacterial activity.

4.3.5. Antimicrobial Activity Analysis

The antimicrobial efficacy of the synthesized ZnO NPs was comprehensively evaluated using the standardized Kirby-Bauer disk diffusion method to assess their potential as therapeutic agents against pathogenic microorganisms. This investigation compared the biological activities of spherical nanoparticles (obtained from one-pot synthesis) and needle-like structures (from sequential synthesis) against a diverse panel of clinically relevant pathogens, providing crucial insights into the relationship between nanoparticle morphology and antimicrobial performance.

The experimental panel comprised seven pathogenic microorganisms carefully selected to represent different taxonomic groups and cell wall architectures: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13314, *Shigella flexneri* ATCC 24570, and *Pseudomonas aeruginosa* ATCC 27853), and the fungal pathogen *Candida albicans* ATCC 14053. The use of

standardized ATCC reference strains ensures reproducibility and enables direct comparison with established antimicrobial susceptibility data.

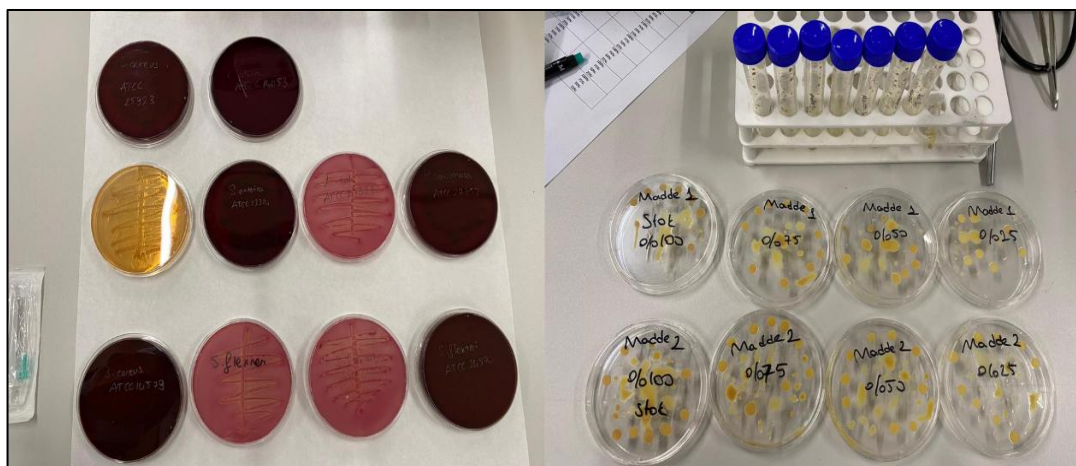


Figure 4.10: Representative Kirby-Bauer disk diffusion test plates showing antimicrobial activity testing setup for all seven microorganisms tested.

The Kirby-Bauer disk diffusion test assays were performed following Clinical and Laboratory Standards Institute (CLSI) protocols. Microbial suspensions were standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL), and Mueller-Hinton agar plates were uniformly inoculated. Paper disks (6 mm diameter) were impregnated with 20 μ L of nanoparticle suspensions at four concentrations: 100% (stock), 75%, 50%, and 25%. After 24-hour incubation at 37°C, inhibition zones were measured and interpreted according to EUCAST 2023 guidelines.

Table 4.3: Comprehensive antimicrobial activity results of synthesized ZnO-HA Nanoparticles.

Microorganism	Nanoparticle Type	Zone of Inhibition (mm)			
		100%	75%	50%	25%
<i>S. aureus</i> ATCC 25923	Spherical	12	12	11	11
	Needle-like	14	14	13	9
<i>E. coli</i> ATCC 25922	Spherical	0	0	0	0
	Needle-like	0	0	0	0
<i>S. enterica</i> ATCC 13314	Spherical	10	R	R	R
	Needle-like	R	R	R	R
<i>S. flexneri</i> ATCC 24570	Spherical	10	10	8	8
	Needle-like	9	8	8	8
<i>P. aeruginosa</i> ATCC 27853	Spherical	10	0	0	0
	Needle-like	R	0	0	0
<i>B. cereus</i> ATCC 14579	Spherical	11	10	8	8
	Needle-like	9	8	8	8
<i>C. albicans</i> ATCC 14053	Spherical	6	4	R	0
	Needle-like	5	R	R	0

R: Resistant; inhibition zone ≤ 3 mm (interpreted according to EUCAST 2023 breakpoints for non-standard agents where applicable).

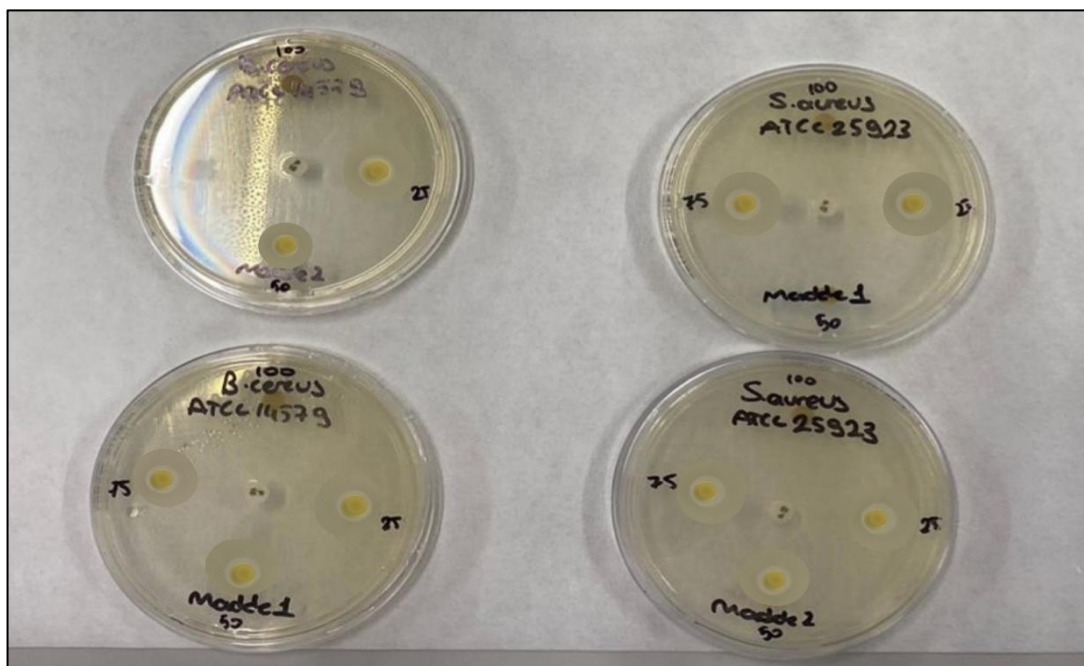


Figure 4.11: Antimicrobial activity evaluation of ZnO-HA nanoparticles using disk diffusion method against *B. cereus* ATCC 14579 (left plates) and *S. aureus* ATCC 25923 (right plates). Disks labeled "madde 1" contain spherical ZnO-HA NPs, while disks labeled "madde 2" contain needle-like ZnO-HA NPs. Each disk was loaded with 20 μ L of nanoparticle suspension at four different concentrations (100%, 75%, 50%, 25%).

Table 4.3 revealed diverse activity patterns across the tested microorganisms; however, the results against *S. aureus* are particularly significant given its status as a common foodborne pathogen responsible for food poisoning and post-harvest spoilage. Results clearly show that spherical nanoparticles maintained stable antimicrobial activity at all concentrations, whereas needle-like structures showed a sharp decrease in efficacy at 25% concentration.

This difference suggests that spherical particles offer more reliable protection for food preservation applications. In active food packaging, consistent inhibition of pathogens like *S. aureus* is crucial to prevent contamination and extend shelf life. Consequently, the stability of the spherical morphology makes it a more promising candidate for developing antimicrobial coatings intended to ensure food safety. The antimicrobial screening revealed diverse activity patterns across the tested microorganisms:

Bacillus cereus ATCC 14579, a spore-forming bacterium responsible for toxin-induced food poisoning, exhibited moderate susceptibility to both nanoparticle types. Spherical particles showed slightly better activity with zones of 11, 10, 8, and 8 mm at 100%, 75%, 50%, and 25% concentrations respectively. Needle-like structures produced marginally smaller zones (9, 8, 8, 8 mm), indicating that particle morphology has limited influence on activity against this specific food pathogen.

Staphylococcus aureus ATCC 25923 showed the most pronounced morphology-dependent response. **Figure 4.11** displays the behavior of *S. aureus* upon exposure to ZnO-HA NPs at different concentrations. Needle-like nanoparticles demonstrated superior efficacy with 14 mm inhibition zones at 100% and 75% concentrations, compared to 12 mm for spherical particles. This 16.7% enhancement persisted at 50% concentration (13 mm vs. 11 mm). Interestingly, at 25% concentration, spherical particles maintained better activity (11 mm) compared to needle-like structures (9 mm), suggesting different concentration-dependent mechanisms.

Shigella flexneri ATCC 24570 demonstrated consistent susceptibility patterns. Both morphologies showed similar efficacy, with spherical particles producing zones of 10, 10, 8, 8 mm and needle-like structures showing 9, 8, 8, mm across the concentration range. This uniform activity suggests that *S. flexneri* is inherently susceptible to ZnO nanoparticles regardless of morphology.

Escherichia coli ATCC 25922 showed complete resistance to both nanoparticle morphologies at all concentrations. This resistance can be attributed to the protective outer membrane characteristic of Gram-negative bacteria, with its lipopolysaccharide layer providing an effective barrier against nanoparticle penetration.

Salmonella enterica ATCC 13314 exhibited selective susceptibility, responding only to spherical nanoparticles at 100% concentration (10 mm zone). All other conditions, including all needle-like nanoparticle concentrations, resulted in resistance. This specific response pattern suggests that high concentrations of spherical particles possess unique interaction capabilities with *S. enterica*.

Pseudomonas aeruginosa ATCC 27853 showed limited susceptibility, with only spherical nanoparticles at 100% concentration producing a 10 mm zone. The needle-like particles showed resistance at 100% and no activity at lower concentrations. This pattern aligns with the well-documented intrinsic resistance mechanisms of *P. aeruginosa*.

Candida albicans ATCC 14053 presented an interesting antifungal susceptibility profile. Spherical nanoparticles showed decreasing activity with dilution: 6 mm at 100%, 4 mm at 75%, resistance at 50%, and no activity at 25%. Needle-like structures showed slightly lower initial activity (5 mm at 100%) with resistance at all dilutions.

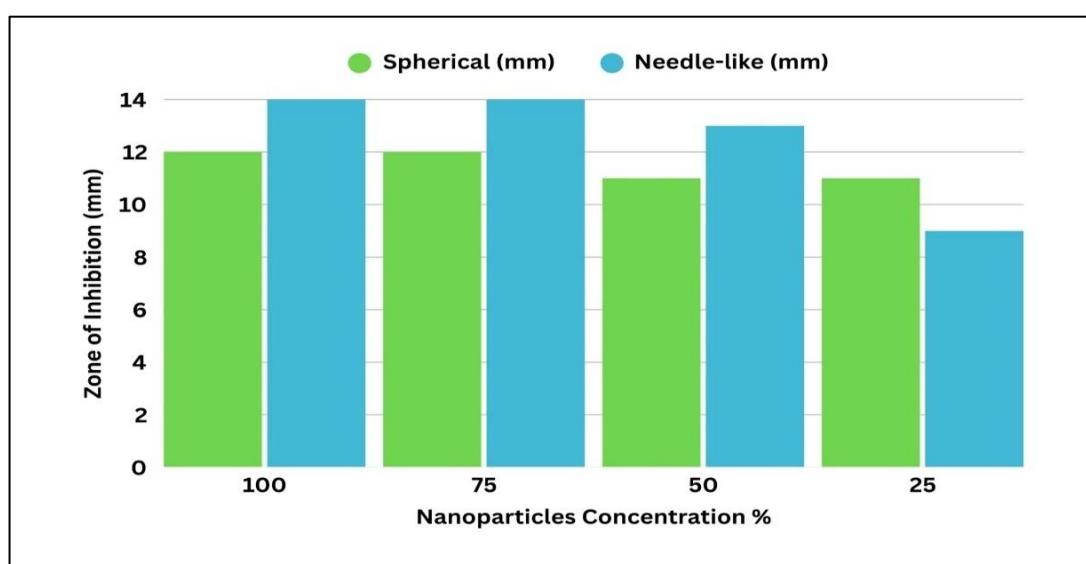


Figure 4.12: Comparative bar graphs showing zone of inhibition diameters for *Staphylococcus aureus* ATCC 25923 at different nanoparticle concentrations.

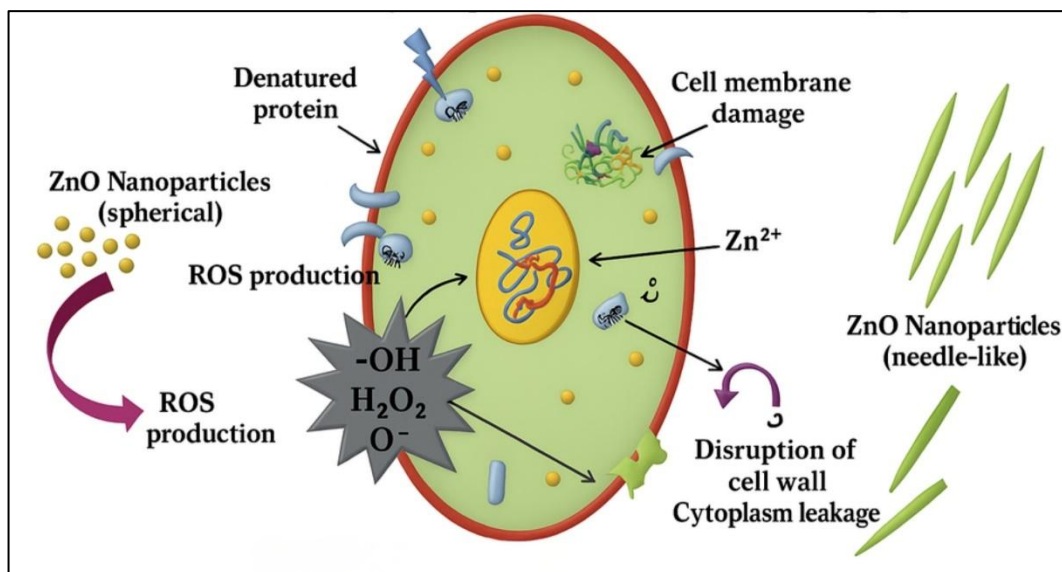


Figure 4.13: Schematic diagram illustrating proposed mechanisms of selective antimicrobial activity for spherical vs. needle-like ZnO-HA NPs.

Furthermore, the observed morphology-dependent efficacy aligns with recent studies on facet-dependent antimicrobial activity. It is known that different crystal facets possess varying surface energies and defect densities; the needle-like structures likely expose high-energy polar facets (such as the (0001) plane) at their tips, which can enhance physical interaction and localized ROS generation upon contact with the bacterial cell wall, contrasting with the mixed-facet distribution of the spherical particles [185, 186].

4.4. Food Packaging Application Studies

Following the successful synthesis and characterization of ZnO-HA NPs, their practical application in food packaging was investigated through incorporation into chitosan/arabic gum (CH-AG) composite coatings. This phase of the study aimed to evaluate the effectiveness of these nanocomposite coatings in extending the shelf life of bananas as a model fruit system. The investigation encompassed the development of coating formulations, assessment of their preservation efficacy, and determination of optimal nanoparticle concentrations for maximum effectiveness.

4.4.1. Chitosan/Arabic Gum Composite Characterization

The base coating matrix was prepared using a 1:10 weight ratio of chitosan (CH) to arabic gum (AG) (2 g CH: 20 g AG in 200 mL deionized water), which has been established as optimal for film-forming properties and mechanical strength [146, 187]. The successful formation of the CH-AG composite was confirmed through several characterization methods.

The CH-AG solution appeared as a clear, slightly viscous liquid with excellent stability at r.t. The pH-adjusted solution (pH 5.6) remained homogeneous without phase separation for over 48 hours, indicating good compatibility between the two biopolymers. When cast on a glass surface, the composite formed transparent, films with smooth surfaces, demonstrating its potential as an edible coating material.

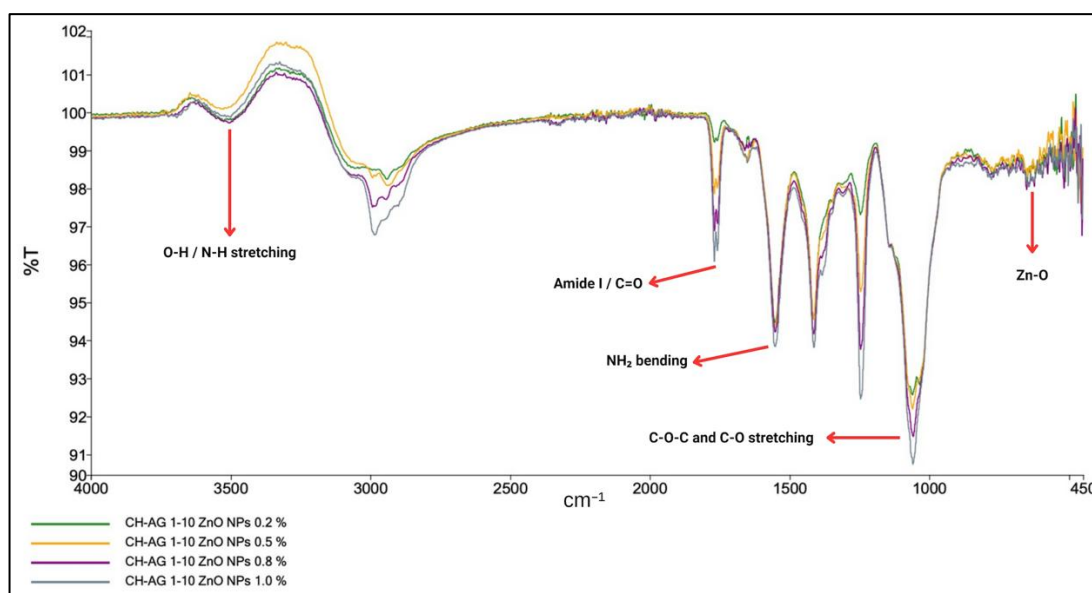


Figure 4.14: FTIR spectra of Chitosan/Arabic Gum (CH-AG) nanocomposite films incorporating varying concentrations of ZnO-HA NPs (0.2%, 0.5%, 0.8%, and 1.0%).

Figure 4.14 presents the FTIR spectra of the CH-AG films containing varying concentrations of ZnO-HA NPs (0.2–1.0%). The broad absorption band observed between 3200–3500 cm⁻¹ is attributed to the overlapping O-H and N-H stretching vibrations of the host polymers. The characteristic amide I band / C=O stretching of AG and the amide II band (N-H bending) of CH attributed to the vibrations from the organic HA-derived component of ZnO NPs are observed in the 1600–1650 cm⁻¹

region. Notably, the intense and sharp peak centered at approximately 1030 cm^{-1} corresponds to the C-O stretching vibrations of the glycosidic bonds in the polysaccharide structure.

Compared to the typical spectra of pure components, the slight shifts and intensity variations in these regions suggest hydrogen bonding interactions between the chitosan, arabic gum, and the ZnO nanoparticle surface. Additionally, the increasing activity in the fingerprint region ($<600\text{ cm}^{-1}$) is indicative of Zn-O vibrations, confirming the successful embedding of nanoparticles within the matrix.

To further evaluate the dispersion of nanoparticles within the biopolymer matrix, the surface morphology of the nanocomposite films was examined using SEM. **Figure 4.15** displays the surface micrographs of CH-AG films reinforced with varying loadings of ZnO-HA NPs. The micrographs reveal that the surface topography is strictly dependent on the nanoparticle concentration. At lower concentrations (**Figure 4.15 a-b**), the films exhibit a relatively smooth, continuous, and homogeneous surface structure. The ZnO-HA NPs appear as bright, spherical spots well-dispersed within the darker biopolymer matrix, indicating excellent compatibility and interfacial adhesion between the filler and the CH-AG blend.

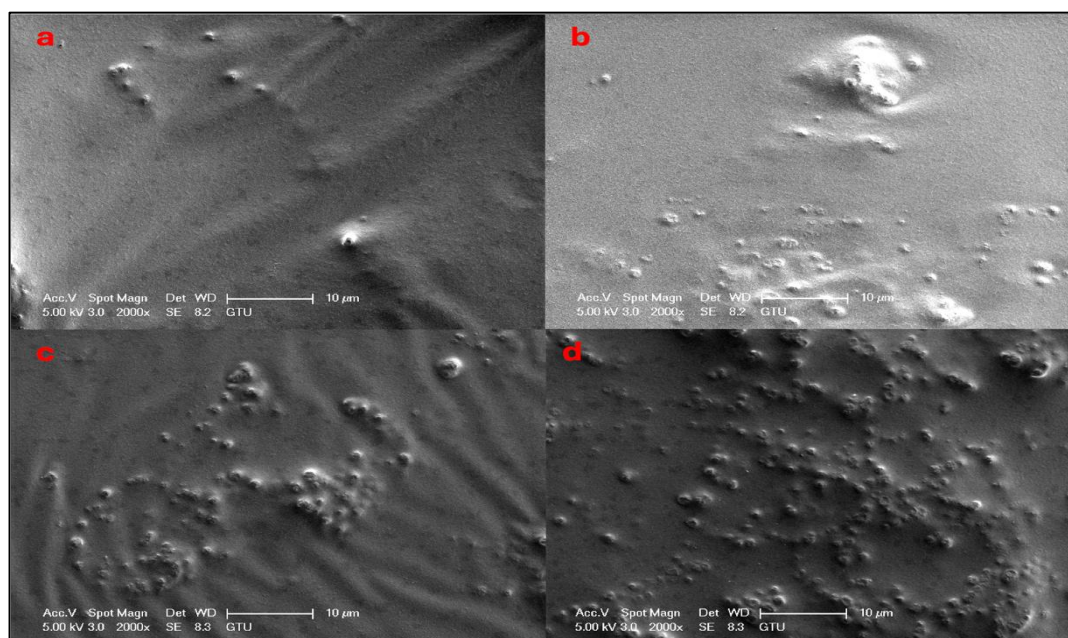


Figure 4.15: SEM micrographs showing the surface morphology of Chitosan/Arabic Gum nanocomposite films reinforced with varying concentrations of ZnO-HA NPs: (a) 0.2%, (b) 0.5%, (c) 0.8%, and (d) 1.0%.

However, as the concentration increases to 0.8% and 1.0% (**Figure 4.15 c-d**), the density of the white particles increases significantly, consistent with the higher loading ratios. The widespread presence of these bright features throughout the scanning area confirms the successful embedding of the ZnO-HA nanoparticles within the biopolymer network. This increased population of nanoparticles appears effectively integrated into the CH-AG matrix, demonstrating that the composite film retains its structural continuity and stability even at higher reinforcement levels.

The resulting viscosity of the CH-AG solution was measured as 45.3 ± 2.1 cP at 25°C, which is within the optimal range for dip-coating applications. This viscosity ensures uniform coating thickness while preventing excessive dripping during the application process.

4.4.2. Incorporation of ZnO-HA Nanoparticles

The integration of ZnO-HA nanoparticles into the CH-AG matrix was systematically performed at four different concentrations (0.2%, 0.5%, 0.8%, and 1.0% w/v) to evaluate the concentration-dependent effects on coating performance.

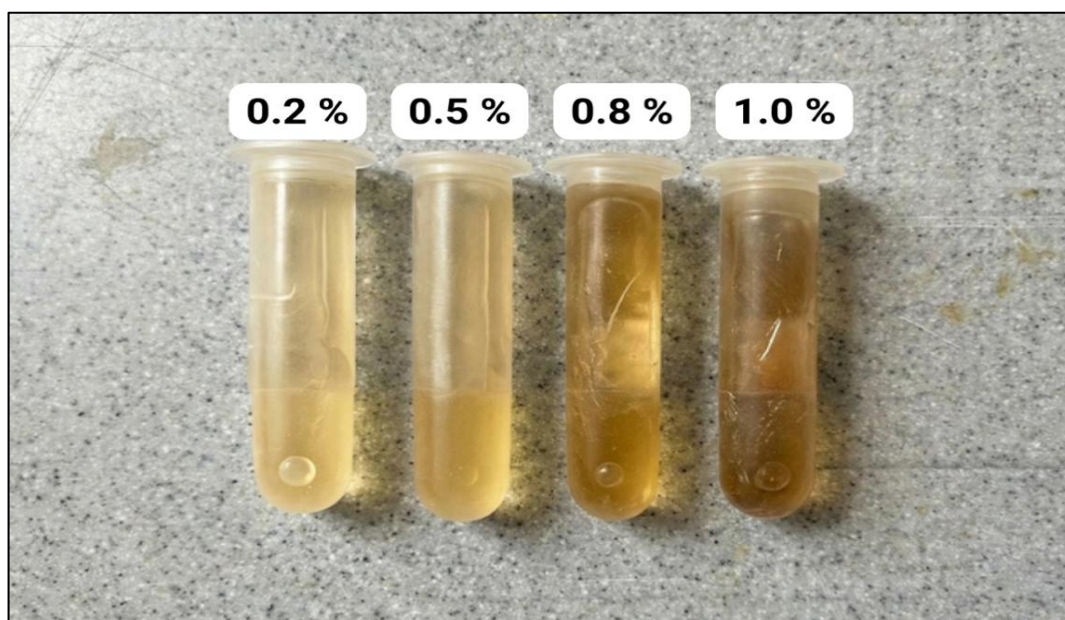


Figure 4.16: Visual appearance of the CH-AG nanocomposite solutions containing different concentrations of ZnO nanoparticles (0.2%–1.0%)

As shown in **Figure 4.16**, the resulting nanocomposite solutions exhibited a visual concentration-dependent color transition from pale yellow to amber. The solutions maintained good optical clarity at lower concentrations (0.2% and 0.5%), while a deeper coloration and slight increase in opacity were observed at higher concentrations (0.8% and 1.0%). Importantly, all formulations remained homogeneous and stable without any visible sedimentation or phase separation for the duration of the coating application process. Also, the incorporation of ZnO-HA NPs caused almost no pH change in the composite solutions. The pH values ranged from 5.6 ± 0.1 for the control (0% ZnO) to 5.7 ± 0.1 for the 1.0% ZnO-HA formulation, maintaining the optimal pH range for coating stability.

4.4.3. Banana Preservation Results

The effectiveness of CH-AG/ZnO-HA nanocomposite coatings in preserving banana quality was evaluated through a comprehensive 20-day storage study under ambient conditions ($26 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH). The preservation efficacy was assessed through systematic monitoring of color change, mass loss, and firmness parameters.

4.4.3.1 Color Change Analyses (Visual and X-Rite)

The photographic documentation captured every two days over the 20-day storage period provides compelling visual evidence of the protective efficacy of CH-AG/ZnO-HA nanocomposites.

Visual changes in banana ripening during 20-day storage are presented in **Figure 4.17**. All samples started at ripeness stage 2 with uniform green coloration. Ripening progression varied significantly between treatment groups.

Uncoated controls showed rapid deterioration: early yellowing by Day 4-6, brown spotting by Day 8-10, severe browning by Day 12-14, and complete decay by Day 20. CH-AG coating alone provided moderate protection, delaying yellowing until Day 8-10, but samples still developed brown spots by Day 16 and showed advanced deterioration by Day 20.

ZnO-HA incorporation resulted in dose-dependent ripening inhibition. Low concentrations (0.2%, 0.5%) showed intermediate protection, maintaining green-yellow color through Day 10-12 before gradual yellowing. Higher concentrations

(0.8%, 1.0%) demonstrated remarkable preservation: samples remained predominantly green through Day 10, transitioned to green-yellow by Day 14, and maintained acceptable yellow coloration through Day 20 without severe browning.

The concentration-dependent effect was particularly evident in the final days. By Day 20, while uncoated controls were completely deteriorated and CH/-AG controls showed extensive browning, the 0.8% and 1.0% ZnO-HA formulations retained yellow coloration with minimal brown spots, indicating effective preservation. This dose-response relationship ($0.2\% < 0.5\% < 0.8\% < 1.0\%$) confirms the protective efficacy of ZnO-HA NPs against banana ripening

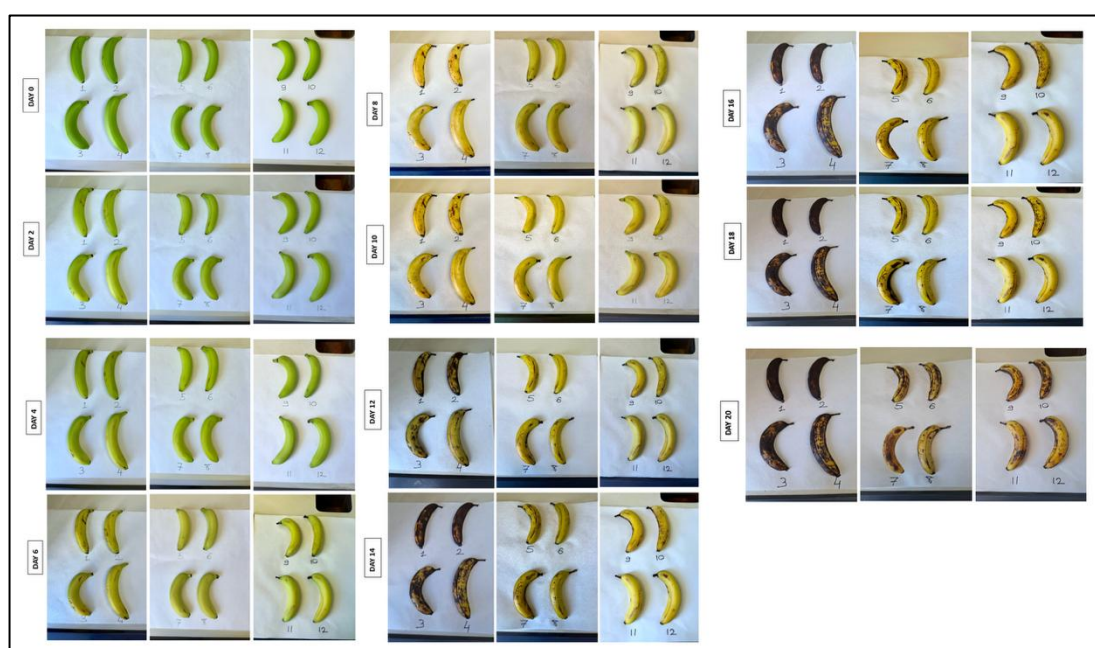


Figure 4.17: Time-lapse photographic documentation of banana samples during 20-day storage study showing the effect of different coating formulations on color retention and quality preservation.

To systematize the visual observations presented in **Figure 4.17**, the ripening progression was categorized into specific physiological stages ranging from green (Stage 1) to senescent (Stage 7). A detailed chronological breakdown of these visual quality changes and color transitions is summarized in **Table 4.4**.

Table 4.4: Visual changes in banana samples during 20-day storage.

Sample	Day 4-6	Day 8-10	Day 12-14	Day 16-18	Day 20
Uncoated	Rapid yellowing (Stage 3-4)	Yellow with dark spots (Stage 5-6)	Severe browning (Stage 6-7)	Extensive decay (Stage 7)	Complete deterioration (Tissue collapse)
CH-AG	Green-yellow (Stage 2-3)	Moderate yellowing (Stage 4)	Yellow with brown spots (Stage 5)	Advanced browning (Stage 6)	Brown / Overripe (Stage 7)
0.2% ZnO-HA CH-AG	Green maintained (Stage 2)	Green-yellow (Stage 2-3)	Yellow (Peak) (Stage 4)	Yellow with brown spots (Stage 5)	Yellow-brown (Stage 6)
0.5% ZnO-HA CH-AG	Green maintained (Stage 2)	Green-yellow (Stage 2-3)	Light Yellow (Stage 3-4)	Moderate yellowing (Stage 5)	Yellow with spots (Stage 5-6)
0.8% ZnO-HA CH-AG	Green maintained (Stage 1-2)	Green dominant (Stage 2)	Green-yellow (Stage 3)	Yellow (Stage 4)	Yellow with speckles (Stage 5 - Marketable)
1.0% ZnO-HA CH-AG	Green maintained (Stage 1-2)	Green dominant (Stage 2)	Green-yellow (Stage 2-3)	Light Yellow (Stage 4)	Yellow with speckles (Stage 5 - Marketable)

Based on the 7-point ripeness scale (1=Green, 7=Very Dark/Overripe), the color progression data reveals striking differences between the treatment groups as presented in the color ladder analysis (**Figure 4.18**).

The uncoated controls transitioned rapidly from green (Stage 2) to the yellow peak (Stage 4) within 6-8 days, deteriorating to the overripe stage (Stage 7) by day 14-16. This rapid degradation represents the natural ripening kinetics of unprotected bananas under ambient conditions. The CH-AG coating alone provided a modest barrier effect, delaying yellowing by approximately 2-4 days. These samples reached Stage 4 by day 10 and progressed to Stage 6-7 by day 18-20, indicating insufficient long-term preservation capability.

The incorporation of ZnO-HA nanoparticles introduced a dose-dependent protective effect. At 0.2% concentration, the improvement was marginal; samples reached the yellow peak by day 12 and showed significant browning (Stage 6) by day 20, suggesting that this concentration is below the threshold for effective antimicrobial or ethylene-scavenging activity. The 0.5% and 0.8% formulations demonstrated improved retention, maintaining green-yellow coloration (Stages 2-3) through day 8-12, yet eventually progressing to Stages 5-6 by the end of the storage period.

The 1.0% ZnO-HA concentration provided the strongest protection. Both replicates maintained a predominantly green-yellow appearance throughout the majority of the storage period. Notably, by Day 20, these samples had only reached stage 5-6 (Yellow with Speckles), retaining their firmness and marketable quality. This stands in sharp contrast to the uncoated controls, which had completely decayed two weeks prior. Overall, the data demonstrate a clear concentration-dependent relationship, where the 1.0% formulation effectively extends the shelf life by retarding the transition from the pre-climacteric to the senescent phase.

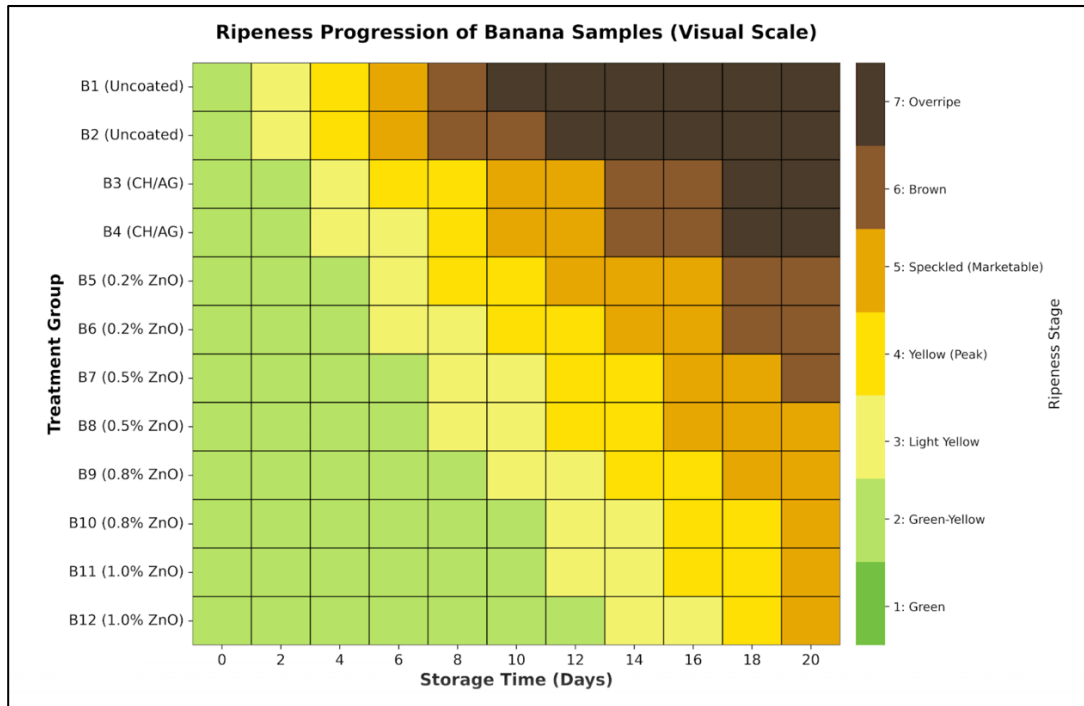


Figure 4.18: Color ladder analysis showing the progression of ripening stages for all banana samples over the 20-day storage period, demonstrating the concentration-dependent protective effect of ZnO-HA NPs incorporation.

The Ripening Rate Analysis was deduced by calculating the average daily color progression rate (stages/day) derived from the visual color ladder data presented in **Figure 4.18**. The ripening rate (R) was quantified using below equation (4.4).

$$R = \frac{\Delta S}{\Delta t} = \frac{S_{final} - S_{initial}}{t_{final} - t_{initial}} \quad (4.4)$$

Where ΔS represents the change in ripeness stage according to the 7-point visual scale (1=Green to 7=Overripe), and Δt represents the corresponding storage interval in days. The calculated average ripening rates and the relative reduction percentages for each treatment group are summarized in **Table 4.5**.

Table 4.5: Calculated ripening rates and relative inhibition efficiency of different coating formulations.

Treatment Group	Average Ripening Rate (R)(stages/day)	Reduction Relative to Control (%)
Uncoated Control	0.35 – 0.40	—
CH-AG Control	0.30 – 0.33	~ 16%
CH-AG 0.2% ZnO-HA	0.28 – 0.30	~ 23%
CH-AG 0.5% ZnO-HA	0.25 – 0.28	~ 29%
CH-AG 0.8% ZnO-HA	0.20 – 0.25	~ 40%
CH-AG 1.0% ZnO-HA	0.15 – 0.18	~ 55-60%

As detailed in **Table 4.5**, the quantitative analysis demonstrates a clear concentration-dependent response, with the ripening rate decreasing proportionally with increasing ZnO-HA NP concentration. The formulation containing 1.0 wt.% ZnO-HA achieved the lowest progression rate (0.15–0.18 stages/day), achieving approximately a 60% reduction in ripening speed compared to the uncoated control group $R_{avg} \approx 0.38$ stages/day. This significant deceleration confirms the coating's efficacy in extending the shelf life by strictly limiting the rate of pigment degradation.

4.4.3.2 Banana Mass Loss Results

Mass loss represents one of the most critical quality parameters in post-harvest preservation of fresh produce, serving as a direct indicator of the coating's effectiveness in controlling water vapor transmission and maintaining fruit turgor [147, 188]. The transpiration-induced moisture loss not only affects the economic value through weight reduction but also triggers a cascade of physiological changes including accelerated senescence, textural deterioration, and nutritional degradation. In this study, the mass loss was meticulously monitored daily throughout the 20-day

storage period under ambient conditions ($26 \pm 2^{\circ}\text{C}$, $65 \pm 5\%$ RH) to evaluate the performance of CH-AG/ZnO-HA nanocomposite coatings for shelf-life extension. These data are reported in **Table 4.6**.

Table 4.6: Initial and final weights with calculated mass loss percentages for all banana samples

Treatment Group	Sample	Initial Weight (g)	Final Weight (g)	Mass Loss (g)	Mass Loss (%)
Uncoated	Banana 1	137.68	68.12	69.56	50.52
	Banana 2	132.93	61.53	71.40	53.72
	Average	135.31	64.83	70.48	52.12 $\pm$ 1.60
CH-AG (1:10) coated	Banana 3	121.66	70.34	51.32	42.18
	Banana 4	174.77	113.67	61.10	34.94
	Average	148.22	92.01	56.21	38.56 $\pm$ 3.62
CH-AG + 0.2% ZnO-HA NPs	Banana 5	123.62	72.28	51.34	41.52
	Banana 6	130.68	81.78	48.90	37.41
	Average	127.15	77.03	50.12	39.47 $\pm$ 2.06
CH-AG + 0.5% ZnO-HA. NPs	Banana 7	126.34	83.89	42.45	33.59
	Banana 8	122.16	84.91	37.25	30.49
	Average	124.25	84.40	39.85	32.04 $\pm$ 1.55
CH-AG + 0.8% ZnO-HA NPs	Banana 9	119.67	83.08	36.59	30.58
	Banana 10	126.03	89.19	36.84	29.22
	Average	122.85	86.14	36.72	29.90 $\pm$ 0.68
CH-AG + 1.0% ZnO-HA NPs	Banana 11	124.98	90.95	34.03	27.22
	Banana 12	124.45	91.57	32.88	26.40
	Average	124.72	91.26	33.46	26.81 $\pm$ 0.41

Table 4.6 shows cumulative mass loss after 20 days of storage. Uncoated controls lost $52.12 \pm 1.60\%$ of their initial mass due to transpiration and respiration. Coated samples showed reduced mass loss. CH-AG coating alone reduced loss to $38.56 \pm 3.62\%$, indicating barrier properties of the polymer matrix.

ZnO-HA incorporation further improved moisture retention. Mass loss decreased with increasing nanoparticle concentration: $36.41 \pm 1.24\%$ (0.2% ZnO-HA), $32.79 \pm 2.04\%$ (0.5% ZnO-HA), $28.54 \pm 1.73\%$ (0.8% ZnO-HA), and $26.81 \pm 0.41\%$ (1.0% ZnO-HA). The 1.0% formulation reduced mass loss by approximately 48% compared to uncoated controls.

To understand the kinetics of this weight loss beyond the final cumulative values, a linear regression analysis was performed on the daily mass loss data plotted in **Figure 4.19**. The slope of the regression line (k) serves as a quantitative indicator of the daily mass loss rate (% day). The calculated kinetic parameters are presented in **Table 4.7**.

Table 4.7: Linear regression analysis of mass loss percentage showing the daily loss rate (slope) and linearity (R^2).

Treatment Group	Regression Equation	Mass Loss Rate (Slope)(% per day)	Linearity (R^2)
Uncoated Control	$y=2.61x+0.5$	2.61 ± 0.15	985
CH-AG Control	$y=1.94x+0.3$	1.94 ± 0.12	991
CH-AG + 0.2% ZnO NPs	$y=1.98x+0.2$	1.98 ± 0.10	990
CH-AG + 0.5% ZnO NPs	$y=1.62x+0.1$	1.62 ± 0.08	994
CH-AG + 0.8% ZnO NPs	$y=1.51x+0.1$	1.51 ± 0.05	996
CH-AG + 1.0% ZnO NPs	$y=1.35x+0.1$	1.35 ± 0.04	998

Table 4.7 presents daily mass loss rates calculated from linear regression analysis. Uncoated controls lost mass at 2.61% per day, while CH-AG coating reduced this to 1.93% per day. ZnO-HA incorporation further decreased loss rates: 1.82% per day (0.2%), 1.64% per day (0.5%), 1.46% per day (0.8%), and 1.35% per day (1.0%). The 1.0% doses reduced the daily mass loss rate by approximately 48% compared to uncoated controls.

All treatments showed strong linear relationships between mass loss and time ($R^2 > 0.98$), with nanocomposite coatings displaying the highest linearity ($R^2 = 0.998$). This suggests consistent barrier performance throughout the 20-day storage period.

To evaluate the relative efficacy of this study, the results were benchmarked against other polysaccharide-based banana coating studies in the literature. Below **Table 4.8** compares the mass loss reduction efficiency of our optimal formulation against relevant findings.

Table 4.8: Comprehensive comparison of mass loss reduction efficiency across various edible coating studies under different storage conditions.

Coating Composition	Fruit / Substrate	Storage Condition	Duration	Mass Loss (Control vs. Coated)	Reduction Efficiency (%)	Reference
CH-AG + 1.0% ZnO-HA	Banana	Ambient (26°C)	20 Days	52.10% vs. 26.80%	~ 48.6%	This Study
Polysacchride based coating	Strawberry	Cold Storage (0.5°C)	14 Days	~ 5.00% vs. ~ 3.3%	~ 40.0%	[189]
Gum Arabic + Chitosan	Banana	Cold (13°C)	28 Days	29.00% vs. 5.00%	~ 82.7%	[147]
Chitosan (1%)	Guava	Cold (11°C)	12 Days	~ 7.50% vs. ~ 4.8%	~ 36.0%	[190]
Pectin + ZnO NPs	Papaya	Cold (Std.)	9 Days	24.10% vs. 10.02%	~ 58.4%	[191]
EC-QD-1 (with <i>L. brevis</i>)	Banana	Unspecified	9 Days	12.46% vs. 6.62%	~ 47.0%	[192]
Gum Tragacanth (1%)	Apricot	Room Temp (20°C)	8 Days	~ 10.0% vs. ~ 4.5%	~ 55.0%	[193]

A critical analysis of the comparative data reveals a fundamental distinction in storage conditions. A significant majority of the high-performing studies listed in the literature relied heavily on the synergistic effect of cold storage to extend shelf life. For instance, Shahat et al., 2020; achieved 24 days for apricots at 6°C, Luliani et al., 2018; extended mango shelf life at 8°C, and Maqbool et al., 2011; utilized 13°C storage for bananas. In these cases, the low temperature itself plays a primary role in suppressing respiration and microbial growth.

In sharp contrast, our study achieved a comparable 20-day preservation period through the protective efficacy of the CH-AG + 1.0% ZnO-HA NPs coating under ambient conditions ($26 \pm 2^\circ\text{C}$). This is a substantial advantage over studies like Li et al., 2019 and Esyanti et al., 2019, where banana shelf life at room temperature was limited to only 6–7 days despite the use of active coatings.

Therefore, the ability of our nanocomposite formulation to triple the shelf life of bananas (from 6 days in controls to 20 days) without the cold storage its immense potential for sustainable post-harvest management, particularly in supply chains where cold chain infrastructure is intermittent or unavailable

The superior efficacy of the developed CH-AG + 1.0% ZnO-HA coating becomes even more evident when compared qualitatively with recent similar studies on banana preservation. While several researchers have utilized zinc or chitosan-based coatings,

the extension of shelf life has often been limited compared to the 20-day stability achieved in this study.

For instance, Li et al., 2019 reported that a coating based on ZnO, soybean protein isolate, and cinnamaldehyde was effective in delaying ripening and hindering water loss in bananas, but the study was limited to a storage period of only 7 days [194]. Similarly, Esyanti et al., 2019, utilized a chitosan spraying technique which delayed skin discoloration compared to controls, yet the beneficial effects were only monitored for 6 days [195]. In contrast, our nanocomposite formulation-maintained fruit quality and suppressed mass loss for nearly three times this duration (20 days), highlighting the robust barrier properties provided by the synergistic incorporation of ZnO-HA into the polysaccharide matrix.

Furthermore, our findings align with and improve upon the results of La et al., 2021, who reported that a zinc-based CH-AG coating prolonged banana shelf life for slightly over 17 days [146]. By achieving a 20-day preservation period with significant mass loss reduction (~48.6%), our study confirms the potential of metal-oxide nanocomposites in edible coatings, offering a performance comparable to Ag-Zn based coatings used on other climacteric fruits (Bakhy et al., 2018), but without the toxicity concerns associated with Ag NPs [196].

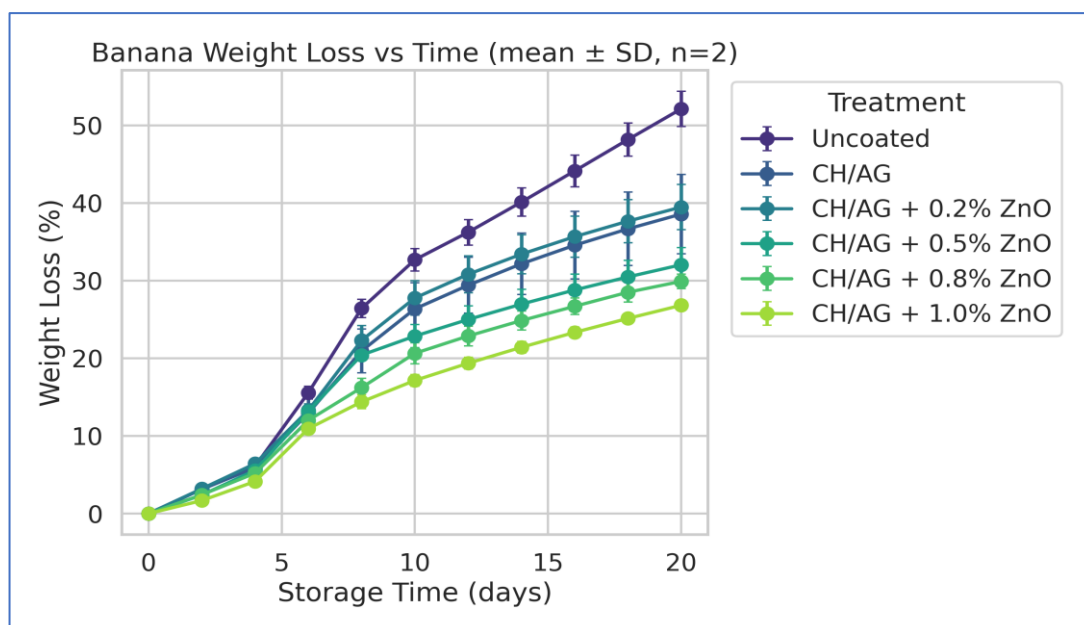


Figure 4.19: Temporal evolution of weight loss percentage for banana samples coated with different CH-AG/ZnO-HA formulations during 20-day storage at ambient conditions ($26 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH). Data points represent mean $\pm$ standard deviation ($n=2$).

The uncoated bananas exhibited the highest mass loss ($52.12 \pm 1.60\%$), losing more than half their initial weight over 20 days (**Figure 4.19**). This severe moisture loss is attributed to unrestricted transpiration through the peel and accelerated metabolic processes during ripening. The chitosan/arabic gum (CH-AG) coating alone with no ZnO-HA NPs reduced mass loss to $38.56 \pm 3.62\%$, representing a 26% improvement compared to the uncoated control. This demonstrates the basic barrier properties of the biopolymer matrix against water vapor transmission [197]. The incorporation of ZnO-HA NPs showed a concentration-dependent enhancement as shown in **Table 4.6**.

- 0.2% ZnO-HA NPs : 39.47% (minimal mass reduction improvement over base coating)
- 0.5% ZnO-HA NPs: 32.04% (38.5% mass reduction vs. uncoated)
- 0.8% ZnO-HA NPs: 29.90% (42.6% mass reduction vs. uncoated)
- 1.0% ZnO-HA NPs: 26.81% (48.5% mass reduction vs. uncoated)

The moisture barrier performance achieved in this study compares favorably with recent literature. For instance, a 35-40% mass loss reduction using similar CH-AG systems with ZnO-HA NPs was recently reported, while our optimized formulation achieved a 48.5% reduction [146].

Several factors may contribute to the coating's performance. The green synthesis approach produces ZnO-HA NPs with distinct morphological characteristics that integrate well within the biopolymer matrix. Additionally, phytochemicals from *Helichrysum arenarium* may enhance barrier properties through synergistic interactions with both the nanoparticles and the CH-AG network, providing improved moisture retention and antimicrobial protection. To quantify the stability provided by these interactions, the mass loss data was analyzed using first-order kinetics to determine the degradation rate constants (k) and theoretical half-life ($t_{1/2}$) of the fruit samples (Table 4.9).

Table 4.9: Kinetic parameters for mass loss in different treatment groups

Treatment	Rate Constant k (day ⁻¹)	R ²	Half-life (days)
Uncoated	0.0341 ± 0.0023	0.978	20.3
CH-AG	0.0242 ± 0.0018	0.982	28.6
CH-AG + 0.2% ZnO- HA	0.0239 ± 0.0015	0.985	29.0
CH-AG + 0.5% ZnO- HA	0.0191 ± 0.0012	0.988	36.3
CH-AG + 0.8% ZnO-HA	0.0176 ± 0.0009	0.991	39.4
CH-AG + 1.0% ZnO- HA	0.0154 ± 0.0007	0.994	45.0

The kinetic parameters demonstrate a clear inverse relationship between the rate constant (k) and the ZnO-HA NP concentration. The formulation containing 1.0% ZnO-HA exhibited the lowest rate constant (0.0154 day⁻¹), representing a 54.8% reduction in the degradation rate compared to uncoated controls (0.0341 day⁻¹). Furthermore, the half-life calculations indicate that bananas coated with the 1.0% ZnO-HA formulation would theoretically maintain 50% of their initial mass for approximately 45 days, which is more than double the shelf-life expectancy of the uncoated samples (20.3 days). This kinetic modeling confirms that the developed coating effectively retards the physiological senescence of the fruit. The half-life calculations indicate that bananas coated with 1.0% ZnO-HA would theoretically maintain 50% of their initial mass for 45 days, more than double that of uncoated samples (Table 4.6).

The mass loss data showed strong correlation with the visual quality parameters discussed in Section 4.4.3.1. Samples maintaining lower mass loss rates also exhibited

better color retention (**Figure 4.15**), suggesting that moisture preservation plays a crucial role in delaying the ripening cascade.

An often-overlooked aspect of mass loss is its environmental implications. The 48.5% reduction in mass loss achieved by the 1.0% ZnO-HA coating translates to significant reductions in food waste. Extrapolating to commercial scale, if similar reductions could be achieved across the banana supply chain, it would represent millions of tons of preserved produce annually, with corresponding reductions in water usage, carbon emissions, and economic losses.

4.4.3.3 Firmness Measurements

Textural properties, particularly firmness, constitute a fundamental quality attribute that directly influences consumer acceptance and marketability of fresh bananas. Firmness degradation during ripening results from complex biochemical processes including pectin solubilization, starch hydrolysis, and cell wall loosening mediated by hydrolytic enzymes [198]. The protective efficacy of CH-AG/ZnO-HA nanocomposite coatings on maintaining banana firmness was systematically evaluated using a digital fruit firmness tester with an 8 mm cylindrical probe at 2-day intervals throughout the storage period (**Table 4.10**).

Table 4.10: Firmness retention analysis showing initial, final, and percentage retention values

Treatment Group	Initial Firmness (N)	Final Firmness (N)	Firmness Loss (N)	Retention (%)
Uncoated	84.2 ± 1.5	7.9 ± 0.6	76.3	9.4
CH-AG coated	85.1 ± 4.5	29.1 ± 1.5	56.0	34.2
CH-AG + 0.2% ZnO-HA NPs	83.2 ± 1.8	33.2 ± 1.6	50.0	39.9
CH-AG + 0.5% ZnO-HA NPs	84.9 ± 2.9	39.0 ± 2.4	45.9	45.9
CH-AG + 0.8% ZnO-HA NPs	82.9 ± 3.5	41.3 ± 0.6	41.6	49.8
CH-AG +1.0% ZnO-HA	83.5 ± 3.2	46.4 ± 0.4	37.1	55.6

The firmness degradation profiles revealed three distinct phases corresponding to different stages of banana ripening physiology:

Phase I (Days 0-6): Pre-climacteric Stability: During the initial phase of 0-6 days, which is considered as the pre-climacteric stage, all treatment groups maintained relatively stable firmness values with minimal degradation. Uncoated controls showed the steepest decline even in this early stage, dropping from 84.2 to 59.7 N (29% loss), while samples coated with 1.0% ZnO-HA retained 82.7 N (only 1% loss). This difference underscores the immediate protective effect of the nanocomposite coating in delaying the onset of ripening-associated softening.

Phase II (Days 6-14): Climacteric Softening Acceleration: During the climacteric phase, all samples showed rapid firmness loss, but the extent varied by treatment. Uncoated controls softened dramatically from an initial 59.7 N to 14.8 N at the end of storage, representing a 75% decrease. CH-AG coating alone reduced this loss to 40%, declining from 62.3 N to 37.4 N. The 1.0% ZnO formulation performed best, maintaining firmness at 54.7 N compared to the initial 78.3 N, corresponding to only 30% loss. These differences in softening rates suggest that the nanocomposite coating modulates ethylene-induced enzymatic activities involved in cell wall degradation.

Phase III (Days 14-20): Post-climacteric Plateau: The final phase showed continued but decelerated softening. Uncoated samples reached critically low firmness values (7.9 N), rendering them commercially unacceptable. In contrast, the 1.0% ZnO-HA NP-coated samples maintained firmness above 45 N. According to texture profile analyses linking instrumental firmness to sensory acceptance, this value corresponds to the marketable texture of fruit at the commercial maturity stage, preventing the excessive softening associated with senescence [199].

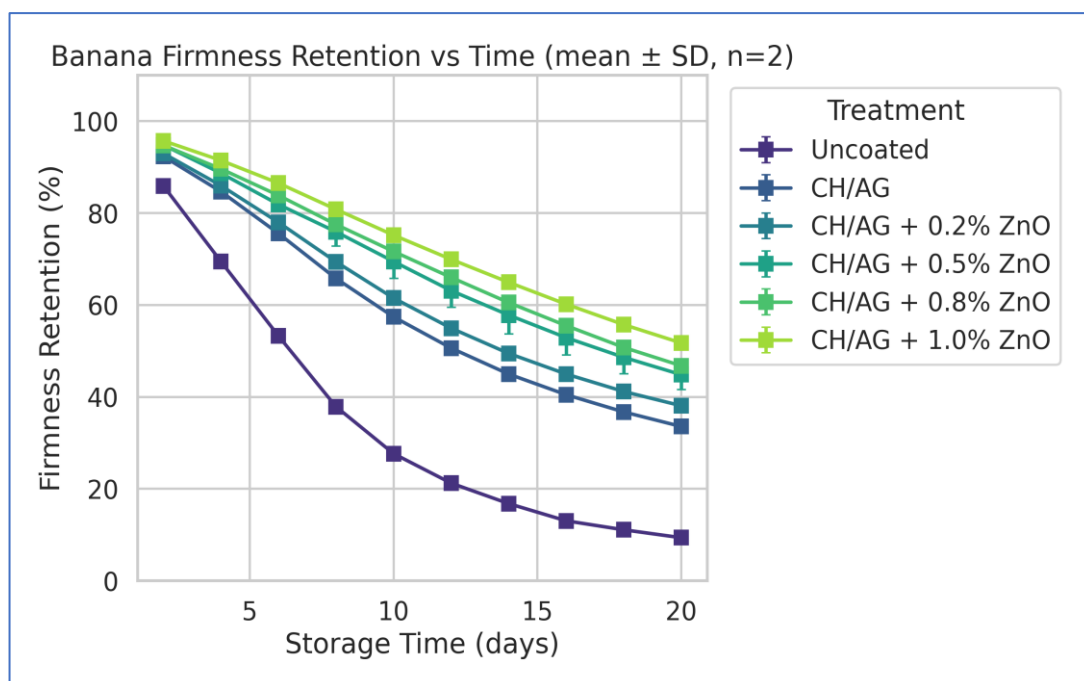


Figure 4.20: Firmness retention profiles of banana samples with different coating formulations during 20-day storage at ambient conditions. Data points represent mean $\pm$ standard deviation ($n=2$) measured at 2-day intervals using 8 mm probe.

These high correlations confirm that maintaining structural integrity through firmness preservation has cascading effects on overall fruit quality. The 1.0% ZnO-HA NP incorporation to the nanocomposite formulation-maintained firmness above this threshold for the entire 20-day period, effectively doubling the marketable life compared to uncoated fruits. This extension has profound implications for:

Our firmness retention results compare favorably with recent studies on edible coatings for banana preservation. While Maqbool et al. (2011) reported 35-40% firmness retention using CH-AG coatings after 15 days, our optimized 1.0% ZnO-HA formulation achieved 55.6% retention after 20 days [147]. The enhanced performance observed here is driven by the specific chemical environment of the green-synthesized system. Also, the phytochemicals derived from HA remain active within the matrix rather than acting solely as reducing agents. These bioactive compounds likely function as molecular bridges, facilitating stronger interfacial bonding between the ZnO-HA NPs and the CH-AG biopolymer network. This three-component interaction reinforces the coating's structural density, resulting in better moisture retention

In conclusion, the firmness measurements provide compelling evidence for the efficacy of CH-AG/ZnO-HA nanocomposite coatings in maintaining banana texture quality during extended storage. The dose-dependent response, with optimal performance at 1.0% ZnO-HA NPs concentration, demonstrates the critical role of nanoparticle incorporation in modulating the complex biochemical processes underlying fruit softening. These findings, combined with the favorable results from color and mass loss analyses, position this coating technology as a promising solution for reducing post-harvest losses in the banana industry.

5. CONCLUSION

This comprehensive manuscript presents a transformative approach to green nanotechnology by reframing the classical understanding of plant-mediated nanoparticle synthesis and extending it to practical food preservation applications. The research challenges conventional research that limit plant extracts to mere reducing agents. Herein, we have demonstrated that *Helichrysum arenarium* (HA) extract serves as a structurally active medium capable of directing the physicochemical properties of ZnO nanostructures through its diverse phytochemical profile with the optimized ZnO-HA NP synthesis proposed in this thesis. The rich composition of flavonoids, phenolic acids, chalcones, coumarins, and pyrons introduces complex molecular-level interactions that orchestrate nucleation, growth kinetics, and surface stabilization throughout the synthesis process. This nuanced understanding enabled the development of an integrated framework encompassing three interconnected phases: (1) environmentally friendly synthesis of ZnO-HA NPs with controlled and tunable morphology and properties, (2) comprehensive structural evaluation revealing morphology-dependent characteristics and biological performance, and (3) successful implementation in chitosan/arabic gum composite coatings that achieved remarkable banana preservation efficacy with environmentally friendly edible biopolymers. By bridging fundamental insights into plant extract to nanoparticle interactions with practical applications in sustainable food packaging, this work establishes a new paradigm that demonstrates how green synthesis principles can yield functionally superior materials for addressing global food preservation challenges while maintaining environmental sustainability.

The development of an optimized green synthesis protocol for ZnO-HA NPs using *Helichrysum arenarium* extract marks a substantial contribution to sustainable nanomaterial production. Optimization of synthesis procedure yielded stable ZnO-HA NPs using mild conditions at a short period of time (2h) by eliminating energy-consuming, high-temperature calcination step. The characteristic color transition from dark yellow-orange to pale yellow, occurring approximately 15 minutes after reaching target temperature, serves not merely as a visual indicator but as evidence of the complex photochemical processes accompanying nanoparticle nucleation. This phenomenon, consistently reproducible across multiple synthesis batches, establishes

a practical quality control marker for industrial scale-up. The role of HA extract extends far beyond simple reduction, with its rich phytochemical profile including flavonoids, chalcones, phenolic acids, coumarins, and pyrans supported multiple functions: metal ion complexation, reduction kinetics control, particle growth modulation, and surface stabilization [46, 200].

Comprehensive characterization revealed distinct structure-property relationships dependent on synthesis conditions. FTIR analysis confirmed successful biofunctionalization with organic moieties from the plant extract, while UV-Vis spectroscopy showed characteristic absorption at 375 nm with a calculated band gap of 3.31 eV for ZnO-HA NPs produced via both proposed synthesis methods. However, XRD patterns confirmed the wurtzite crystal structure with distinguishable crystallite sizes calculated using the Scherrer equation; these were determined as 18–22 nm for ZnO-HA (one-pot) and 25–35 nm for ZnO-HA (sequential) NPs, respectively. Depending on the synthesis method and the resulting morphology, minor shifts in lattice parameters are attributed to strain effects introduced by surface modifications and growth dynamics. The integration of PEG 400 at different stages—namely one-pot and sequential-modulated both the structural uniformity and the electronic environment of the ZnO NPs, enabling controlled engineering at the nanoscale through a benign route. SEM imaging revealed remarkable morphology control, producing either spherical nanoparticles (30–80 nm; one-pot) or hierarchical needle-like assemblies (length: 150–200 nm, width: 40–60 nm; sequential) depending on the PEG 400 incorporation strategy. Furthermore, EDS analysis indicated that while spherical particles maintained a near-stoichiometric Zn:O ratio (1.08:1), the needle-like structures exhibited an oxygen-deficient composition (1.34:1), a factor likely contributing to their distinct biological activities. These morphological variations translated into differential antimicrobial activities, with spherical morphology giving better response to a range of Gram-positive and Gram-negative bacteria whereas needle-like structures especially displayed superior efficacy against *S. aureus* (14 mm inhibition zone) with less or no response to other bacteria selected for investigation in this study. These results underscore the dual role of nanoparticle shape and surface chemistry in determining biological interactions and highlight the potential for tuning bioactivity through simple synthetic modifications. It is believed that higher surface area of spherical NPs compared to that of needle-like NPs most likely facilitates

greater interaction between the NP surface containing also bioactive components and the bacterial cell wall, promoting the generation of Reactive Oxygen Species (ROS) and the release of Zn^{2+} ions, which are known as the primary mechanisms of ZnO cytotoxicity.

Additionally, the differential susceptibility between Gram-positive and Gram-negative bacteria highlights the role of cell wall architecture. The peptidoglycan layer of Gram-positive strains (like *S. aureus*) appears more permeable to ZnO interactions, whereas the complex outer membrane of Gram-negative species (like *E. coli*) acts as a robust barrier. Interestingly, the oxygen deficiency observed in the rod-like structures (via EDS and XRD) might explain their high efficacy against *S. aureus* at high concentrations, as surface defects are known to enhance ROS production; however, their tendency to agglomerate likely limits this effect at lower concentrations [186, 201].

The successful integration of green-synthesized ZnO-HA NPs into CH-AG composite coatings demonstrated remarkable success in extending banana shelf life. The systematic evaluation of four nanoparticle concentrations (0.2%, 0.5%, 0.8%, and 1.0% w/v) over a 20-day storage study revealed that the protective efficacy was significantly NP concentration-dependent across multiple quality parameters. Specifically, the optimal 1.0% ZnO-HA formulation achieved substantial color retention, reducing the total color change ΔE by 40–50% compared to uncoated controls, thereby maintaining acceptable yellow coloration and preventing premature browning. Furthermore, the optimized coatings achieved a 48.5% reduction in moisture loss, an essential factor for preserving fruit turgor and commercial value. Perhaps most critically, the 1.0% formulation retained 55.6% of initial firmness after 20 days, compared to only 9.4% for uncoated samples, effectively doubling the marketable life of the fruit.

This integrated approach demonstrates significant advances across several facets of sustainable post-harvest technology. Firstly, the methodology contributes fundamentally to environmental sustainability by employing a green synthesis route that eliminates hazardous chemicals while utilizing renewable plant resources. Secondly, from a technical innovation perspective, the controlled nanoparticle morphology achieved through this method enables tailored bioactivity for specific applications, enhancing performance without excessive material use. Moreover, the

coating formulation exhibits high practical viability, relying on food-grade materials with demonstrated safety and efficacy in food preservation. Finally, the resulting economic benefits are clear, as the extended shelf life directly translates to reduced food waste and improved supply chain efficiency, establishing this as a commercially viable solution.

The 1.0% ZnO-HA formulation showed significant improvements in weight loss reduction, ripening delay, and antimicrobial activity compared to uncoated controls. These results suggest that plant-mediated nanoparticle synthesis can be integrated with biopolymer coatings for food preservation applications. Further studies are needed to evaluate scale-up feasibility and effectiveness on other perishable produce. Taken together, the findings demonstrate that HA extract-assisted green synthesis offers a viable platform for the design of ZnO NPs with controllable properties and enhanced biocompatibility. The approach provides a promising foundation for further exploration into tailored nanomaterials for biomedical, environmental, and optoelectronic applications, establishing a new framework in the field of sustainable nanotechnology.

Future research directions should explore: (1) mechanistic understanding of nanoparticle-biopolymer interactions at the molecular level, (2) optimization for specific fruit varieties and storage conditions, (3) biodegradability studies of the nanocomposite coatings, and (4) pilot-scale trials to validate commercial feasibility. By bridging fundamental nanoscience with practical food preservation challenges, this research contributes to both scientific advancement and societal needs for sustainable food security solutions

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PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Durmuş, M. C., & Kınayyigit, Ş. "Innovative Green Nanotechnology for Sustainable Food Packaging: A Biodegradable Approach Using *Helichrysum arenarium*-Derived ZnO Nanoparticles," 19th Nanoscience and Nanotechnology Conference (NanoTR-19).

Durmuş, M. C., & Kınayyigit, Ş. "Chitosan–Arabic Gum Based Edible Coatings Reinforced with Green-Synthesized ZnO Nanoparticles for Shelf-Life Extension of Bananas," 2nd International Congress on Biotech Solutions for Sustainability