International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 4S, 2025
https://www.theaspd.com/ijes.php

Citrus-Mediated Green Synthesis of Zinc Nanoparticles: A Comparative Study

Kanchan Yadav Arya[1], Sanyogita Shahi[2*]
[1,2*]Department of Science, Kalinga University, Raipur, Chhattisgarh, 492101
Corresponding author: [2*]drsanyogitashahi@gmail.com

ABSTRACT
Green chemistry-mediated synthesis of zinc nanoparticles (ZnNPs) represents a sustainable and efficacious alternative to conventional physical and chemical approaches. Citrus fruits, including Citrus sinensis (orange), Citrus limon (lemon), Citrus aurantiifolia (lime), and Citrus limetta (mosambi), are rich reservoirs of bioactive phytochemicals such as flavonoids, phenolics, citric acid, and ascorbic acid, which serve as pivotal reducing and stabilizing agents in the biogenic fabrication of nanoparticles. This review presents a comparative analysis of ZnNPs synthesized utilizing extracts from these four distinct citrus species, elucidating variations in their physicochemical attributes, including particle size, morphology, antioxidant capacity, and antimicrobial potential. Comprehensive characterization employed techniques such as UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM),[1] Dynamic Light Scattering (DLS), and Energy Dispersive X-ray (EDX) spectroscopy to rigorously evaluate nanoparticle characteristics. The findings indicate that Citrus limon and Citrus aurantiifolia extracts are particularly effective in generating smaller, more stable ZnNPs, while Citrus sinensis and Citrus limetta extracts yield nanoparticles exhibiting superior antioxidant efficacy. This study underscores the profound influence of diverse phytochemical compositions on the functional properties of biogenically synthesized ZnNPs and explores their promising applications across biomedicine, food packaging, agriculture, and environmental remediation.

Keywords: Zinc nanoparticles, Citrus fruits, green synthesis, Nanotechnology, Antioxidant activity, antimicrobial properties, Phytochemicals.

1. INTRODUCTION AND LITERATURE REVIEW
Nanotechnology stands as a rapidly advancing interdisciplinary field, offering extensive applications across medicine, agriculture, electronics, and environmental sciences. Within this domain, zinc nanoparticles (ZnNPs) have garnered significant research interest due to their distinctive physicochemical properties, which encompass potent antimicrobial, antioxidant, UV-blocking, and catalytic activities.
Traditional methods for nanoparticle synthesis often rely on harsh chemical routes or energy-intensive physical processes. These conventional approaches typically involve the use of toxic solvents, consume substantial energy, and generate hazardous by-products, posing considerable environmental and health risks. In response to these limitations, green synthesis has emerged as a superior, sustainable alternative. This methodology leverages plant extracts, offering a pathway for nanoparticle fabrication that is inherently biocompatible, cost-effective, and environmentally benign.
Citrus fruits, including *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus aurantiifolia* (lime), and *Citrus limetta* (mosambi), are exceptionally rich in diverse bioactive phytochemicals. These compounds, such as ascorbic acid, citric acid, flavonoids (e.g., hesperidin, naringenin), tannins, and polyphenols, are crucial in the green synthesis process, acting as both reducing agents for metal ion conversion and stabilizing agents to prevent nanoparticle aggregation.
This review systematically examines the comparative green synthesis and biological profiling of ZnNPs derived from these four citrus species. We collectively term this investigative area "Citrus Zincomics" to highlight the unique synergy between citrus phytochemistry and zinc nanoparticle characteristics. The analysis specifically emphasizes the underlying chemical interactions, diverse biological potential, and promising applications of these biogenically synthesized ZnNPs.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 4S, 2025
https://www.theaspd.com/ijes.php

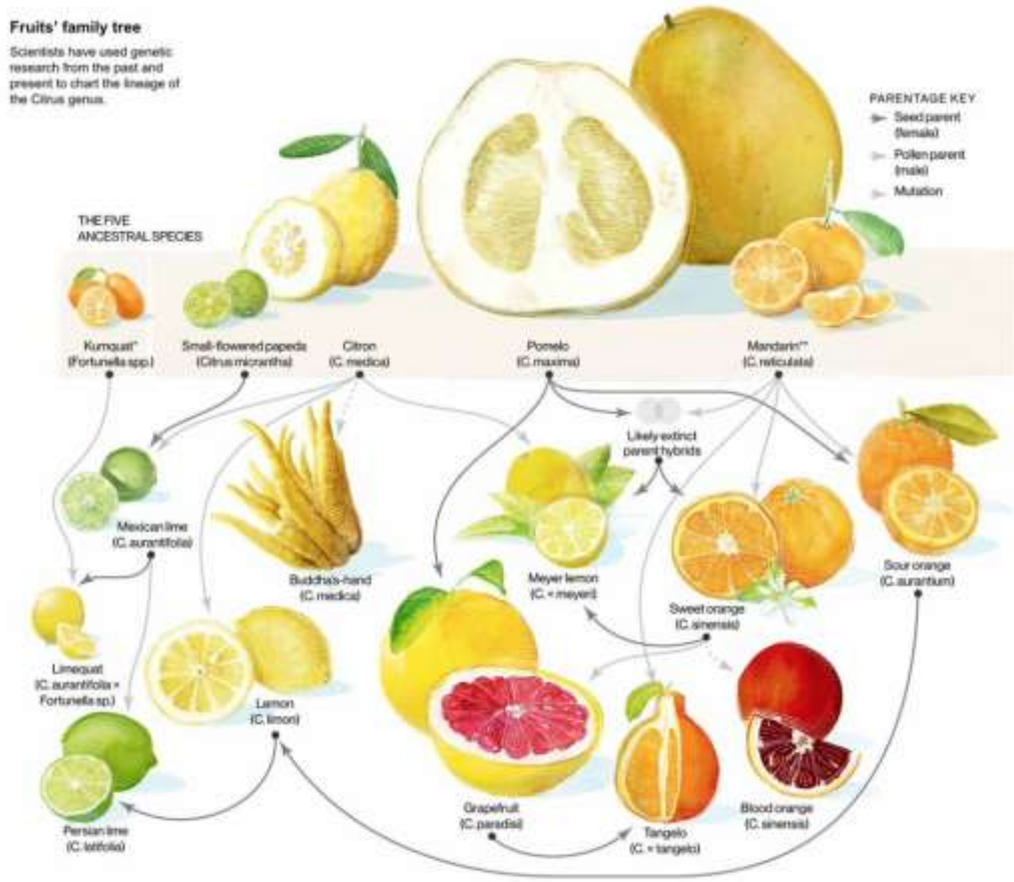
1.1. Green Synthesis of Zinc Nanoparticles

Nanotechnology, positioned at the forefront of material science and biomedical research, frequently involves the precise synthesis of metal nanoparticles. This is primarily driven by their unique size-dependent physicochemical properties. Historically, physical and chemical methods have been the mainstay for nanoparticle production. However, these conventional techniques are frequently associated with significant drawbacks, including high energy consumption, the generation of toxic by-products, and the necessity for non-biodegradable stabilizing agents.

To mitigate these limitations, green synthesis has advanced as a non-toxic, biocompatible, cost-effective, and environmentally conscious alternative. This approach mechanistically involves the bio-reduction of metal ions into zero-valent metal atoms using natural plant extracts. These extracts are rich in a diverse array of phytochemicals that concurrently fulfill dual roles: acting as reducing agents to facilitate nanoparticle formation and as capping agents to stabilize the nascent nanoparticles, thereby preventing their aggregation and ensuring colloidal stability.

1.2. Properties and Significance of Zinc Nanoparticles

Zinc nanoparticles (ZnNPs) have garnered substantial attention within materials science and nanotechnology due to their multifaceted properties. These include, but are not limited to, significant antimicrobial efficacy, robust antioxidant capabilities, potent catalytic activity, inherent biodegradability, and relatively low toxicity. Consequently, ZnNPs find diverse applications across various sectors, encompassing biomedicine, agriculture, cosmetics, environmental monitoring, and food packaging. It is crucial to note that the specific synthesis methodology profoundly influences the ultimate effectiveness and functional attributes of the nanoparticles. Green synthesis, in particular, offers an optimal approach for preserving their intrinsic functionalities while concurrently enhancing their biocompatibility for advanced applications.



1.3. Mechanism of Green Synthesis Employing Citrus Extracts

The fundamental principle underlying the green synthesis of ZnNPs using botanical extracts involves the reduction of metal ions to their zero-valent state. This core chemical transformation can be succinctly represented as:

Zn^{2+} + Phytochemicals→Zn0(Nanoparticles)+Oxidized Phytocompounds

Typically, zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) serves as the primary zinc precursor. An aqueous solution of this salt is augmented with a plant extract, which is intrinsically rich in various phytochemicals such as flavonoids, ascorbic acid, phenolic acids, and terpenoids. Under mild ambient conditions (typically 25–30°C and a pH range of 7–8), these phytochemicals act as reducing agents, facilitating the conversion of Zn^{2+} ions to nascent Zn0 atoms, which subsequently aggregate to form nanoparticles.

1.4. Key Phytochemicals and Their Roles in ZnNP Formation:

- **Ascorbic Acid:** Functions as a potent reducing agent, donating electrons to Zn^{2+} ions to form Zn^0, and concurrently imparts antioxidant protection to the synthesized nanoparticles.
- **Citric Acid:** Serves as a chelating agent for metal ions, which can influence reaction kinetics, and plays a crucial role in maintaining colloidal stability by adsorbing onto the nanoparticle surface.
- **Flavonoids & Polyphenols:** Exhibit dual functionality, acting both as effective reducing agents for Zn^{2+} and as essential capping agents, thereby stabilizing the newly formed nanoparticles and preventing agglomeration.
- **Tannins:** Contribute significantly to the structural integrity of the nanoparticles and are instrumental in preventing their undesirable agglomeration within the colloidal suspension.

The progress of nanoparticle formation is frequently evinced by a perceptible color change in the reaction mixture (e.g., from colorless to a yellowish or brown hue). This visual indicator is quantitatively corroborated by UV-Visible spectroscopy, where the appearance of a characteristic Surface Plasmon Resonance (SPR) peak, typically observed within the 300–380 nm range for ZnNPs, provides definitive spectroscopic confirmation of nanoparticle synthesis.

1.5. Advantages of Green Synthesis Over Conventional Methods

Green synthesis offers substantial advantages over traditional physical and chemical nanoparticle synthesis routes, establishing itself as a more sustainable and superior alternative.

a) Absence of Toxic By-products

Conventional chemical synthesis frequently necessitates the use of hazardous reagents (e.g., sodium borohydride, hydrazine) and noxious organic solvents, which invariably result in the generation of toxic by-products that pose considerable environmental and human health risks. Green synthesis, in stark contrast, bypasses these deleterious substances by utilizing water as a benign solvent and plant extracts as intrinsically safe reactants, thereby ensuring a clean and environmentally sustainable production process devoid of hazardous waste.

b) Enhanced Biocompatibility

The utilization of plant-derived capping agents in green synthesis imparts inherent biocompatibility to the resulting ZnNPs. This characteristic is paramount for their prospective applications in sensitive biological contexts such as drug delivery systems, wound healing formulations, and tissue engineering. Conversely, nanoparticles fabricated via chemical methods often mandate extensive and costly surface modifications to render them biologically compatible. Natural capping molecules, including ascorbic acid and various polyphenols, not only enhance cellular uptake but also significantly mitigate potential cytotoxicity.

c) Superior Stability via Natural Capping

A pervasive challenge in nanoparticle synthesis is aggregation, stemming from the high surface energy of individual particles, which diminishes their effective surface area and compromises their functional properties. In green synthesis, the phytochemical constituents effectively encapsulate the nanoparticles, conferring both electrostatic and steric stabilization. This natural capping mechanism robustly prevents particle aggregation, thereby enhancing their colloidal stability and extending their shelf life.

d) Economic Efficiency

The green synthesis approach significantly reduces overall operational and production costs by obviating the need for:

- Energy-intensive high-temperature or high-pressure reaction conditions.
- Expensive and often toxic chemical reagents.
- Sophisticated and high-maintenance laboratory equipment.

This inherent cost-effectiveness renders green synthesis a highly scalable solution, particularly well-suited for deployment in resource-limited settings or for large-scale industrial manufacturing applications.

e) Environmental Sustainability

As a water-based and plant-driven process, green synthesis inherently aligns with the twelve principles of green chemistry. This includes core tenets such as maximizing energy efficiency, utilizing renewable feedstocks, and designing for inherent degradation. Consequently, this methodology ensures a minimal carbon footprint, making a substantial contribution towards achieving global sustainable development goals (SDGs) across healthcare, agriculture, and advanced materials science.

1.6. Key Parameters Influencing Green Synthesis of ZnNPs

The characteristics of green-synthesized ZnNPs, including their size, morphology, and functional properties, are profoundly influenced by several critical synthesis parameters (Table: 1):

Table: 1

S.No.	**Parameter**	**Influence**
1.	Type of Citrus Extract	Distinct phytochemical compositions lead to varying reduction and stabilization rates.
2.	pH of the Reaction	A slightly basic pH (7–8) is empirically identified as optimal for efficient nanoparticle growth and stability.
3.	Temperature	Ambient temperatures are preferred to preserve the bioactivity of phytochemicals and minimize energy consumption.
4.	Concentration Ratio	A balanced ratio of plant extract to metal salt precursor is critical for controlling nucleation kinetics and optimizing nanoparticle yield.
5.	Reaction Time	Directly correlates with the extent of $Zn2+$ reduction and significantly determines the final size distribution of the nanoparticles.

By precisely modulating these variables, researchers can effectively control the morphological characteristics of the nanoparticles, which, in turn, directly dictates their biological activity and potential applicability in diverse fields.

1.7. Comparative Efficiency of Citrus Extracts in ZnNPs Synthesis

The varied phytochemical profiles inherent to different citrus fruits—specifically oranges, lemons, limes, and mosambi—exert distinct influences on the kinetics and outcomes of ZnNP synthesis. This differentiation is primarily attributed to varying concentrations of citric acid, ascorbic acid, and flavonoids within each fruit extract.

- **Lemon and Lime Extracts:** Characterized by higher levels of citric and ascorbic acids, these extracts typically facilitate the production of smaller and demonstrably more stable ZnNPs.
- **Orange and Mosambi Extracts:** These extracts tend to possess higher concentrations of flavonoids, which contribute to enhancing the inherent antioxidant activity of the synthesized nanoparticles.

These observed variations suggest the significant potential for developing synergistic strategies, possibly involving the co-utilization of mixed citrus extracts, to precisely tailor nanoparticle properties for specific functional requirements.

1.8. Applications of Green-Synthesized Zinc Nanoparticles

Green-synthesized ZnNPs demonstrate remarkable versatility and relevance across numerous sectors, owing to their unique properties and biocompatibility (Table 2).

Table: 2

S. No.	**Application**	**Mechanism**
1.	Antimicrobial Agent	Induces disruption of microbial cell membranes primarily through the generation of reactive oxygen species (ROS) and direct interaction.
2.	Antioxidant Therapy	Functions by effectively scavenging free radicals and modulating oxidative stress pathways, thereby mitigating cellular damage.
3.	Agricultural Fertilizer	Enhances essential nutrient uptake by plants and promotes robust crop growth and yield.
4.	**Sunscreen Formulations**	Exhibits significant UV-absorbing capacity, attributed to the surface interactions and light scattering properties of the nanoparticles.
5.	**Cancer Therapy (Research)**	Shows promising potential for targeted delivery of therapeutic agents and exhibiting intrinsic cytotoxicity towards malignant cells; currently under extensive investigation.

These diverse applications collectively underscore the broad utility and substantial potential of green-synthesized ZnNPs across various scientific and industrial domains.

1.9. Role of Citrus Phytochemicals in ZnNP Formation

The green synthesis of zinc nanoparticles (ZnNPs) is fundamentally dependent on the presence and intricate interactions of natural phytochemicals inherent to botanical sources. Within citrus fruits, these pivotal phytochemicals encompass a diverse range of compounds, including organic acids, various classes of flavonoids, polyphenols, and vitamins. These constituents collectively function as dual-purpose agents: serving as effective reducing agents for the metal ions and as crucial capping agents during the nascent stages of nanoparticle formation.[1] Beyond merely mediating the reduction of Zn^{2+} ions to elemental Zn0, these bioactive components exert a critical influence on the resultant nanoparticles' size, morphology, surface charge, and colloidal stability. The inherent phytochemical diversity across the investigated citrus species—namely *Citrus limon* (lemon), *Citrus aurantiifolia* (lime), *Citrus sinensis* (orange), and *Citrus limetta* (mosambi)—directly dictates the efficiency and ultimate quality of the ZnNP synthesis. This section comprehensively examines the distinct phytochemical profiles of these fruits and elucidates their individual and comparative contributions to nanoparticle generation.

1.10. Phytochemical Constituents of Citrus Fruits and Their Functions

Citrus fruits are renowned for their substantial concentrations of key bioactive compounds. These include: citric acid, ascorbic acid (Vitamin C), a variety of flavonoids (e.g., hesperidin, naringin, quercetin), phenolic compounds, and other organic acids. These constituents collectively act as potent bioreductants, facilitating the conversion of metal ions into neutral metal atoms (Zn0), while concurrently functioning as vital capping agents to stabilize the newly formed nanoparticles, thereby precluding aggregation and oxidative degradation.

(i) Citric Acid: Predominantly abundant in *Citrus limon* and *Citrus aurantiifolia*, this tricarboxylic acid serves as both a chelating agent and a primary reducing agent. It effectively binds to Zn^{2+} ions, reducing them under mildly alkaline conditions, and subsequently stabilizes the nascent particles through adsorption onto their surface, enhancing their colloidal integrity.

(ii) Ascorbic Acid (Vitamin C): A highly potent reducing agent found in significant quantities in *Citrus limon* and *Citrus sinensis*. Ascorbic acid accelerates the nucleation process by readily donating electrons to Zn2+ ions and plays a critical role in determining the final particle size by precisely controlling the rate of ion reduction.

(iii) Flavonoids: Compounds such as hesperidin, naringin, and quercetin are found in elevated concentrations in *Citrus sinensis* and *Citrus limetta*. These primarily function as efficient capping agents, profoundly influencing the antioxidant properties inherent to the biogenically synthesized ZnNPs.
(iv) Polyphenols and Phenolics: These compounds, widely distributed in *Citrus aurantiifolia* and *Citrus sinensis*, exhibit a dual mechanistic role. They actively participate in the reduction of zinc ions and concurrently impart enhanced biocompatibility and stability to the ZnNPs through their robust radical-scavenging capabilities and surface passivation effects.

1.11. Comparative Phytochemical Profiles and Their Impact on ZnNP Synthesis

The distinct biochemical compositions of the various citrus fruits directly translate into differential efficiencies and characteristics during ZnNP synthesis (Table 3):

Table: 3

S.No.	Citrus Fruit	Key Phytochemicals	Primary Functions in ZnNPs Synthesis
1.	*Citrus limon* (Lemon)	Citric acid, ascorbic acid	Rapid reduction kinetics, conferring high colloidal stability and uniformity to the nanoparticles.
2.	*Citrus aurantiifolia* (Lime)	Citric acid, polyphenols	Effective size control, moderate reduction rates, resulting in stable particles.
3.	*Citrus sinensis* (Orange)	Flavonoids (hesperidin), phenolics	Impart enhanced antioxidant activity to ZnNPs, associated with slower nucleation.
4.	*Citrus limetta* (Mosambi)	Flavonoids, organic acids	Exhibits a mild reduction rate, leading to the formation of stable, albeit potentially larger, particles.

The elevated concentration of citric acid in *Citrus limon* and *Citrus aurantiifolia* renders their extracts particularly efficacious for the rapid reduction of Zn^{2+} ions, consequently yielding smaller and more monodispersed nanoparticles. Conversely, *Citrus sinensis* and *Citrus limetta*, characterized by their comparatively higher flavonoid and polyphenol content, tend to slow the reduction process. However, this often results in particles possessing enhanced antioxidant and other bioactive properties due to the nature of their capping agents.

1.12. Mechanistic Insights into Phytochemical-Mediated ZnNP Synthesis

The formation of ZnNPs via green chemistry is a complex, multi-stage interplay encompassing reduction, nucleation, and subsequent stabilization:
a) **Reduction:** In the initial stage, Zn^{2+} ions undergo reduction to Zn^0 atoms. This electron transfer is primarily mediated by reducing agents such as ascorbic acid and citric acid present in the extract. This step is pivotal as it dictates the rate of initial ZnNP nuclei formation.
b) **Nucleation:** As a critical concentration of Zn^0 atoms is achieved, they spontaneously coalesce into nascent clusters, forming stable nuclei. These nuclei serve as the foundational building blocks for the subsequent growth of the nanoparticles.
c) **Growth and Stabilization:** Following nucleation, the newly formed nanoparticles continue to grow. During this phase, flavonoids and polyphenols present in the extract adsorb onto the surface of the growing nanoparticles, effectively acting as capping agents. This capping mechanism is crucial for controlling their ultimate size and shape, preventing undesirable aggregation, and ensuring their long-term colloidal stability in solution.

1.13. Impact of Phytochemical Composition on Nanoparticle Morphology and Stability

The specific phytochemical composition of the citrus extract not only influences the kinetic rate of ZnNP formation but also significantly affects their resultant size, shape, and surface charge (zeta potential):

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 4S, 2025
https://www.theaspd.com/ijes.php

a) **ZnNPs from Lemon and Lime Extracts:** Characteristically, these nanoparticles are often smaller (ranging from 20–40 nm), predominantly spherical, and exhibit superior colloidal stability. This is attributed to the rapid reduction and high capping efficiency facilitated by their rich citric acid content.
b) **ZnNPs from Orange Extracts:** These nanoparticles tend to be slightly larger (typically 40–60 nm) but demonstrably possess higher antioxidant properties, largely owing to the predominant presence of hesperidin and other flavonoids as capping agents. They generally maintain a uniform spherical morphology.
c) **ZnNPs from Mosambi Extracts:** Synthesis kinetics with mosambi extracts are often slower, potentially leading to the formation of irregular or larger particles. Nevertheless, these nanoparticles typically demonstrate robust capping, which contributes to lower aggregation tendencies despite their varied morphology.

1.14. Influence on Biological Properties

The variability in the nature and abundance of capping agents, derived from different citrus sources, confers distinct biological activities upon the synthesized ZnNPs:
a) **Antioxidant Activity:** ZnNPs derived from orange and mosambi extracts consistently exhibit the strongest antioxidant capacities. This enhanced activity is directly correlated with their higher flavonoid and phenolic content, which are known for their radical-scavenging properties.
b) **Antimicrobial Activity:** ZnNPs synthesized using lemon and lime extracts typically demonstrate more pronounced antimicrobial efficacy. This is likely attributable to their smaller particle size and consequently larger surface area-to-volume ratio, which facilitates enhanced interaction with microbial membranes and augmented generation of reactive oxygen species (ROS).
c) **Stability in Suspension:** Lemon and lime-derived ZnNPs generally exhibit higher absolute zeta potential values. This indicates stronger electrostatic repulsion between particles, which is a key factor in preventing aggregation and maintaining long-term suspension stability.

2. METHODOLOGY

The comprehensive characterization of zinc nanoparticles (ZnNPs) is paramount for elucidating their physicochemical and structural properties, which directly govern their stability, reactivity, and biological efficacy. Given that green synthesis, particularly using diverse citrus extracts, yields nanoparticles with varying attributes influenced by phytochemical composition and synthesis parameters, a suite of advanced analytical techniques is essential for their thorough evaluation. The following methodologies are widely employed for the detailed assessment of green-synthesized ZnNPs.

2.1. Characterization Techniques (Table 4):

	Technique	**Purpose**
1.	UV-Visible Spectroscopy (UV-Vis)	Confirms nanoparticle formation through the detection of characteristic Surface Plasmon Resonance (SPR).
3.	Fourier Transform Infrared Spectroscopy (FTIR)	Identifies the functional groups from citrus extracts responsible for the reduction and stabilization of ZnNPs.
4.	X-Ray Diffraction (XRD)	Determines the crystalline structure and enables the calculation of average crystallite size using the Scherrer equation.
5.	Dynamic Light Scattering (DLS)	Analyzes hydrodynamic size distribution and measures zeta potential for assessing colloidal stability.
6.	Scanning Electron Microscopy (SEM)	Visualizes surface morphology, shape, size, and aggregation tendencies of the nanoparticles.
7.	Energy Dispersive X-ray Spectroscopy (EDX)	Confirms the elemental composition of the nanoparticles, specifically verifying the presence of zinc and identifying other constituent elements.

2.1.1. UV-Visible Spectroscopy

UV-Visible spectroscopy serves as a rapid and non-destructive technique for real-time monitoring of ZnNP synthesis. Nanoparticle formation is unequivocally confirmed by the emergence of a characteristic Surface Plasmon Resonance (SPR) peak, typically observed within the 300–400 nm range for ZnNPs. The precise position and intensity of this SPR band offer critical insights into the particle size, distribution, and overall concentration. A narrow and sharp peak typically signifies a uniform and smaller particle size distribution, whereas a broad or shifted peak may indicate polydispersity or particle aggregation.

2.1.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis is indispensable for identifying the specific functional groups present in the precursor citrus extracts and, crucially, those adsorbed onto the surface of the synthesized nanoparticles. This technique elucidates which phytochemical constituents actively participate as reducing and capping agents. Common functional groups identified include:

- **O–H stretches** (characteristic of hydroxyl groups from flavonoids, polyphenols, and organic acids),
- **C=O stretches** (from carbonyl groups),
- **C–O stretches** (indicating alcohols and esters),
- **C=C stretches** (associated with aromatic compounds). Observed shifts or the disappearance of specific vibrational peaks post-synthesis provide conclusive evidence of the successful interaction between these functional groups and zinc ions during the nanoparticle formation process.

2.1.3. X-Ray Diffraction (XRD)

XRD is a definitive technique for confirming the crystalline nature of the synthesized ZnNPs. The diffraction patterns obtained are compared against standard zinc oxide (ZnO) crystalline phases, frequently confirming the wurtzite hexagonal structure. The distinct peak positions (2θ values) can be precisely indexed to specific crystallographic planes, such as (100), (002), and (101). Furthermore, the average crystallite size of the nanoparticles can be quantitatively determined using the Debye-Scherrer equation:

$$D = \beta \cos\theta K\lambda$$

where:

- D is the average crystallite size,
- K is the shape factor (typically 0.9 for spherical particles),
- λ is the wavelength of the X-rays (for Cu Kα radiation, $\lambda=1.5406$ A˚),
- β is the full width at half maximum (FWHM) of the diffraction peak in radians,
- θ is the Bragg angle.

2.1.4. Dynamic Light Scattering (DLS)

DLS is a powerful technique for characterizing the hydrodynamic diameter of ZnNPs in a colloidal suspension. It provides essential information regarding their dispersity and size distribution in their hydrated state. Crucially, DLS also quantifies the zeta potential, which directly reflects the electrical charge at the nanoparticle surface. The zeta potential is a key predictor of colloidal stability; nanoparticles with absolute zeta potential values greater than +30 mV or less than −30 mV are generally considered highly stable due to strong electrostatic repulsion, which effectively prevents aggregation. A smaller DLS size indicates well-dispersed particles, while a higher absolute zeta potential correlates with enhanced suspension stability, particularly vital for biomedical applications.

2.1.5. Scanning Electron Microscopy (SEM)

SEM is widely utilized for high-resolution imaging to elucidate the surface morphology and macroscopic structure of ZnNPs. SEM micrographs provide crucial visual information on:

- **Shape:** Identifying spherical, rod-like, or irregular morphologies.
- **Surface Texture:** Assessing whether surfaces are smooth or rough.
- **Aggregation/Clustering Tendencies:** Directly observing the extent of particle agglomeration. SEM allows for the direct visualization of nanoparticle size and dispersion, thereby complementing the data obtained from DLS measurements. These images are also invaluable for

understanding how various synthesis parameters and the specific phytochemical constituents influence the ultimate morphology of the nanoparticles.

2.1.6. Energy Dispersive X-ray (EDX) Spectroscopy

Often integrated with SEM, EDX spectroscopy is an indispensable tool for elemental analysis of the synthesized nanoparticles. It definitely confirms the presence of zinc within the synthesized materials and can also detect other constituent elements, such as oxygen (from ZnO) or residual organic compounds originating from the plant extract. A prominent zinc peak (typically around 1.0 keV) in the EDX spectrum provides robust confirmation of successful ZnNP synthesis. The absence of significant impurity peaks further indicates high purity, a desirable characteristic for applications in biomedical and environmental fields.

3. RESULTS AND DISCUSSION

This section presents a comparative profiling of zinc nanoparticles (ZnNPs) synthesized using extracts from various citrus fruits: orange (*Citrus sinensis*), lemon (*Citrus limon*), lime (*Citrus aurantiifolia*), and mosambi (*Citrus limetta*). The observed distinctions in their physicochemical properties, stability, and biological activities are attributed to the unique phytochemical profiles of each fruit, particularly their varying concentrations of flavonoids, citric acid, and ascorbic acid. These phytochemicals critically influence the nanoparticle formation kinetics, growth, and subsequent stabilization.

3.1. Comparative Profiling of Citrus-Derived ZnNPs

A summary of the distinct attributes for ZnNPs synthesized from each citrus extract is presented below (Table 5):

Parameter	Orange ZnNPs	Lemon ZnNPs	Lime ZnNPs	Mosambi ZnNPs
Size (nm)	20–50	10–30	15–35	25–60
Shape	Predominantly spherical	Spherical, uniform	Spherical, some agglomeration	Irregular
Zeta Potential	Moderate stability	High stability	High stability	Moderate stability
Antioxidant Activity	High	Moderate	Moderate	High
Antimicrobial Activity	Moderate	High	High	Low

3.1.1. Lemon and Lime Extracts: These extracts, notably rich in citric and ascorbic acids, consistently yielded smaller (10–35 nm), more uniformly spherical, and highly stable ZnNPs. Their high zeta potential values corroborate superior colloidal stability and reduced aggregation.[2] This enhanced stability, coupled with a larger effective surface area from smaller particle sizes, likely contributes to their pronounced antimicrobial efficacy through increased interaction with microbial membranes and efficient generation of reactive oxygen species (ROS).

3.1.2. Orange-Derived ZnNPs: Nanoparticles synthesized using orange extracts, characterized by higher concentrations of flavonoids (e.g., hesperidin), exhibited high antioxidant potential. While their particle size was slightly larger (20–50 nm), they generally maintained a uniform spherical morphology. This suggests that while flavonoids may lead to a slower nucleation rate, they provide effective capping that enhances antioxidant properties.

3.1.3. Mosambi ZnNPs: These nanoparticles displayed a tendency towards larger sizes (25–60 nm) and irregular shapes, indicative of slower or less controlled synthesis kinetics. This could be attributed to a relatively lower concentration of strong reducing agents in mosambi extract compared to lemon or lime. Despite a comparatively lower antimicrobial activity, their high antioxidant activity is noteworthy, likely stemming from the presence of other specific phenolic constituents acting as effective antioxidant capping agents.

The specific phytochemical composition of each citrus fruit plays a direct and discernible role in tailoring the final properties of the green-synthesized ZnNPs. This comparative analysis highlights that different citrus sources can be strategically chosen to optimize ZnNP characteristics for targeted applications.

4. CONCLUSION

This study unequivocally demonstrates the viability and advantages of the green synthesis approach for producing zinc nanoparticles (ZnNPs) using diverse citrus fruit extracts, offering a sustainable, biocompatible, and cost-effective alternative to conventional methods. Through a comparative profiling of ZnNPs derived from *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus aurantiifolia* (lime), and *Citrus limetta* (mosambi), it has been clearly established that the phytochemical composition of each citrus species profoundly influences the physicochemical and biological characteristics of the synthesized nanoparticles.

Specifically, lemon and lime extracts, owing to their rich content of citric and ascorbic acids, were found to be highly effective in facilitating the formation of smaller, more stable ZnNPs with superior antimicrobial activity. In contrast, orange and mosambi extracts, characterized by a higher abundance of flavonoids and phenolic compounds, yielded ZnNPs exhibiting stronger antioxidant properties, albeit with generally larger particle sizes and varied morphologies.

This comparative analysis underscores that the choice of citrus extract is a critical determinant in tailoring the specific attributes of green-synthesized ZnNPs. The observed variations highlight the significant role of natural reducing and capping agents, such as organic acids, flavonoids, and polyphenols, in dictating nanoparticle morphology, colloidal stability, and functional efficacy. The inherent simplicity and bioactivity of this plant-mediated synthesis route open considerable avenues for future research. This includes exploring synergistic effects from mixed citrus extracts, optimizing synthesis parameters for precise control over nanoparticle properties, and developing novel applications in areas such as advanced biomedicine, smart food packaging, sustainable agriculture, and environmental remediation.

5. REFERENCES

1. Ali, S., Rasul, A., Shah, P. A., Al-Rawi, M. B., & Naseem, M. (2024). Green synthesis of antipathogenic particles by utilizing the citrus plant waste and their application in medicated fabrics. *Journal of Industrial Textiles*, *54*, 15280837241275203.
2. Ara, C., Andleeb, S., Ali, S., Majeed, B., Iqbal, A., Arshad, M., ... & Muzamil, A. (2023). Protective potential of fresh orange juice against zinc oxide nanoparticles-induced trans-placental and trans-generational toxicity in mice. *Food Science & Nutrition*, *11*(9), 5114-5128.
3. Aziz, T. H., & Hasan, A. Y. (2024). The Biological Activity of Green Synthesis ZnO nanoparticles by Citrus Sinensis Fruit Peels against Staphylococcus aureus Isolated from Skin Infections: The Biological Activity of Green Synthesis ZnO nanoparticles by Citrus Sinensis Fruit Peels against Staphylococcus aureus Isolated from Skin Infections. *Academic Science Journal*, *2*(3), 186-204.
4. Batterjee MG, Nabi A, Kamli MR, Alzahrani KA, Danish EY, Malik MA. Green Hydrothermal Synthesis of Zinc Oxide Nanoparticles for UV-Light-Induced Photocatalytic Degradation of Ciprofloxacin Antibiotic in an Aqueous Environment. *Catalysts*. 2022; 12(11):1347. https://doi.org/10.3390/catal12111347
5. Bhat RS, Al-Dbass AM, Khayyat AIA, Al-Daihan S. Utilizing Biomolecule-Rich Citrus Fruit Waste as a Medium for the Eco-Friendly Preparation of Silver Nanoparticles with Antimicrobial Properties. *Inorganics*. 2024; 12(7):180. https://doi.org/10.3390/inorganics12070180
6. Chander, S., Yadav, S., Gupta, A., & Luhach, N. (2023). Sequestration of Ni (II), Pb (II), and Zn (II) utilizing biogenic synthesized Fe3O4/CLPC NCs and modified Fe3O4/CLPC@ CS NCs: Process optimization, simulation modeling, and feasibility study. *Environmental Science and Pollution Research*, *30*(53), 114056-114077.
7. Dalal, U., Gupta, V. K., Reddy, S. N., & Navani, N. K. (2020). Eradication of water borne pathogens using novel green nano Ag-biocomposite of Citrus Limetta peels. *Journal of Environmental Chemical Engineering*, *8*(2), 103534.
8. Devi, K. G., Dhanemozhi, A. C., & Priya, L. S. (2023). Green synthesis of Zinc oxide nanoparticles using lemon extract for waste water treatment. *Materials Today: Proceedings*.

9. Dulta, K., Koşarsoy Ağçeli, G., Thakur, A., Singh, S., Chauhan, P., & Chauhan, P. K. (2022). Development of alginate-chitosan based coating enriched with ZnO nanoparticles for increasing the shelf life of orange fruits (Citrus sinensis L.). *Journal of Polymers and the Environment*, *30*(8), 3293-3306.
10. Dulta, K., Koşarsoy Ağçeli, G., Thakur, A., Singh, S., Chauhan, P., & Chauhan, P. K. (2022). Development of alginate-chitosan based coating enriched with ZnO nanoparticles for increasing the shelf life of orange fruits (Citrus sinensis L.). *Journal of Polymers and the Environment*, *30*(8), 3293-3306.
11. G. K, P., P. A, P., Ramani, M., S, A., B. M, N., G. M, K., ... & H. M, S. (2019). Comparison of antimicrobial, antioxidant and anticancer activities of ZnO nanoparticles prepared by lemon juice and citric acid fueled solution combustion synthesis. *BioNanoScience*, *9*, 799-812.
12. Gao, Y., Xu, D., Ren, D., Zeng, K., & Wu, X. (2020). Green synthesis of zinc oxide nanoparticles using Citrus sinensis peel extract and application to strawberry preservation: A comparison study. *Lwt*, *126*, 109297.
13. Gaur, J., Kumar, S., Pal, M., Kaur, H., Rana, R. K., Bala, K., ... & Rajesh, C. (2023). Bio-functionalized, elongated hexagonal bi-pyramidal, citrus limetta/ZnO nanostructures as potential photocatalytic and seed germinating agents. *Functional Composites and Structures*, *5*(3), 035003.
14. Genaidy, E. A., Abd-Alhamid, N., Hassan, H. S., Hassan, A. M., & Hagagg, L. F. (2020). Effect of foliar application of boron trioxide and zinc oxide nanoparticles on leaves chemical composition, yield and fruit quality of Olea europaea L. cv. Picual. *Bulletin of the National Research Centre*, *44*, 1-12.
15. Ghosh, D. K., Kokane, S., Kumar, P., Ozcan, A., Warghane, A., Motghare, M., ... & Sharma, A. K. (2018). Antimicrobial nano-zinc oxide-2S albumin protein formulation significantly inhibits growth of "Candidatus Liberibacter asiaticus" in planta. *PLoS One*, *13*(10), e0204702.
16. Gowthambabu, V., Balamurugan, A., Satheeshkumar, S., & Kanmani, S. S. (2021). ZnO nanoparticles as efficient sunlight driven photocatalyst prepared by solution combustion method involved lime juice as biofuel. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *258*, 119857.
17. Hinge, S. P., & Pandit, A. B. (2017). Solar-assisted synthesis of ZnO nanoparticles using lime juice: a green approach. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *8*(4), 045006.
18. Hossain, A., Abdallah, Y., Ali, M. A., Masum, M. M. I., Li, B., Sun, G., ... & An, Q. (2019). Lemon-fruit-based green synthesis of zinc oxide nanoparticles and titanium dioxide nanoparticles against soft rot bacterial pathogen Dickeya dadantii. *Biomolecules*, *9*(12), 863.
19. Hussien, N. A., Khalil, M. A. E. F., Schagerl, M., & Ali, S. S. (2025). Green Synthesis of Zinc Oxide Nanoparticles as a Promising Nanomedicine Approach for Anticancer, Antibacterial, and Anti-Inflammatory Therapies. *International Journal of Nanomedicine*, 4299-4317.
20. Jayabal, R., Madhu, S., Devarajan, Y., & Domian, C. S. (2025). *A Comparative Study of Combustion and Performance in Diesel Engines Fueled by Mosambi Peel Biodiesel-Butylated Hydroxytoluene Nanoparticles Blend* (No. 2024-01-5258). SAE Technical Paper.
21. Jha, A. K., Kumar, V., & Prasad, K. (2011). Biosynthesis of metal and oxide nanoparticles using orange juice. *Journal of Bionanoscience*, *5*(2), 162-166.
22. Kem, A., Ansari, M. R., Prathap, P., Jayasimhadri, M., & Peta, K. R. (2022). Eco-friendly green synthesis of stable ZnO nanoparticles using citrus limon: X-ray diffraction analysis and optical properties. *Physica Scripta*, *97*(8), 085814.
23. KHAN, M. A., KHAN, M., NAEEM, R., & HAFEEZ, M. (2024). Synergistic effect of citrus sinensis (orange) and Aloe barbadensis (Aloe vera) extracts on biosynthesis and antimicrobial activities of zinc oxide nanoparticles. *Pak. J. Bot*, *56*(4), 1373-1378.
24. Khanal, D. P., Aryal, S., & Aryal, S. (2020). Characterization, and Anti-microbial Properties of Zinc Oxide Nanoparticles Synthesized Using Citrus sinensis (L.) Osbeck Peel Extract. *bioRxiv*, 2020-10.
25. Khane, Y., Benouis, K., Albukhaty, S., Sulaiman, G. M., Abomughaid, M. M., Al Ali, A., ... & Dizge, N. (2022). Green synthesis of silver nanoparticles using aqueous Citrus limon zest extract:

Characterization and evaluation of their antioxidant and antimicrobial properties. *Nanomaterials*, *12*(12), 2013.

26. Khodaparast, M., Rajabi, A. M., & Mohammadi, M. (2021). Mechanical properties of silty clay soil treated with a mixture of lime and zinc oxide nanoparticles. *Construction and Building Materials*, *281*, 122548.
27. Kumar, K. J., Basumatary, S., Daimari, J., Mondal, A., & Deka, A. K. (2024). Photocatalytic degradation of methylene blue with polyphenol rich Citrus limetta (peel waste) facilitated ZnO nanoparticles under sunlight. *Reaction Kinetics, Mechanisms and Catalysis*, *137*(1), 483-503.
28. Lamichhane, Y. R., Parajuli, K., & Pradhanang, S. S. (2024). Preparation of zinc oxide nanoparticles using Citrus sinensis fruit peel aqueous extract and its antimicrobial study. *Scientific World*, *17*(17), 99-105.
29. Li, Z., & Sun, Z. (2024). Fabrication of Nickel/Zinc Oxide Nanocomposites from Citrus sinensis Extract Prompts Apoptosis Through Impeding JAK/STAT3 Signaling in Gastric Cancer. *Applied Biochemistry and Biotechnology*, *196*(6), 3534-3552.
30. Luque, P. A., Soto-Robles, C. A., Nava, O., Gomez-Gutierrez, C. M., Castro-Beltran, A., Garrafa-Galvez, H. E., ... & Olivas, A. (2018). Green synthesis of zinc oxide nanoparticles using Citrus sinensis extract. *Journal of Materials Science: Materials in Electronics*, *29*, 9764-9770.
31. Mazhar, T., Shrivastava, V., & Tomar, R. S. (2021). Biogenic Synthesis of Bi-Metallic (Zn-Cu) Nanoparticles by Leaf Extract of Citrus Limon and Evaluation of its Antibiofilm Activity Against E. Coli. *Biomedical and Pharmacology Journal*, *14*(4), 2017-2028.
32. Metwally, R. S., MH Kheiralla, Z., M Ashour, S., & S Zaki, S. (2022). Anticandidal activity of green synthesized zinc oxide nanoparticles using lemon peel extract. *Journal of Scientific Research in Science*, *39*(2), 62-81.
33. Molla, A., Hoque, M. M., Richi, F. T., Kabir, M. H., Alam, S., Ahmed, N. U., Emon, N. U., Shao, C., Zeng, C., Wang, S., Geng, P., & Mamun, A. A. (2025). Biosynthesis and Characterization of ZnO Nanoparticles Using *Citrus reticulata* Peel Followed by Photocatalytic, Antibacterial, and Antioxidative Nanotherapeutic Attributes Assessment Supported by Computer Simulation. *International journal of nanomedicine*, *20*, 4803–4829. https://doi.org/10.2147/IJN.S493905
34. Montaño-Herrera, A., Santiago-Saenz, Y. O., López-Palestina, C. U., Cadenas-Pliego, G., Pinedo-Guerrero, Z. H., Hernández-Fuentes, A. D., & Pinedo-Espinoza, J. M. (2022). Effects of Edaphic Fertilization and Foliar Application of Se and Zn Nanoparticles on Yield and Bioactive Compounds in Malus domestica L. *Horticulturae*, *8*(6), 542.
35. Muthu, R. N. (2024). Synthesis of Zinc Oxide Nanoparticles by Citrus Limon as an Effective Photoanode for Dye-Sensitized Solar Cells. *Physical Chemistry Research*, *12*(2), 383-392.
36. Nabi, G., Majid, A., Riaz, A., Alharbi, T., Kamran, M. A., & Al-Habardi, M. (2021). Green synthesis of spherical TiO2 nanoparticles using Citrus Limetta extract: Excellent photocatalytic water decontamination agent for RhB dye. *Inorganic Chemistry Communications*, *129*, 108618.
37. Osman, D. A. M., Nur, O., & Mustafa, M. A. (2020). Reduction of energy consumption in cement industry using zinc oxide nanoparticles. *Journal of Materials in Civil Engineering*, *32*(6), 04020124.
38. Pagar, K., Chavan, K., Kasav, S., Basnet, P., Rahdar, A., Kataria, N., ... & Ghotekar, S. (2023). Bio-inspired synthesis of CdO nanoparticles using Citrus limetta peel extract and their diverse biomedical applications. *Journal of Drug Delivery Science and Technology*, *82*, 104373.
39. Patil, S., Hosapete, S., Irkal, S., & Rajput, S. (2019). Synthesis and characterization of zirconium nano particles from orange juice and orange peel. *Journal of Applied Chemistry (IOSR-JAC,)*, *12*(7).
40. Rafique, S., Bashir, S., Akram, R., Jawaid, S., Bashir, M., Aftab, A., ... & Awan, S. U. (2023). In vitro anticancer activity and comparative green synthesis of ZnO/Ag nanoparticles by moringa oleifera, mentha piperita, and citrus lemon. *Ceramics International*, *49*(4), 5613-5620.
41. Rajeswari, M., Rao, N. N., Agarwal, T., & Kavyasree, S. (2022). Green Synthesis of Zinc Oxide Nanoparticles Using Citrus Sinensis (Orange) Peel Extract for Achieving Ultraviolet Blocking Properties. In *Biobased Materials: Recent Developments and Industrial Applications* (pp. 275-285). Singapore: Springer Nature Singapore.

42. Rashid, M. U., Shah, S. J., Attacha, S., Khan, L., Saeed, J., Shah, S. T., & Mohamed, H. I. (2024). Green synthesis and characterization of zinc oxide nanoparticles using Citrus limetta peels extract and their antibacterial activity against brown and soft rot pathogens and antioxidant potential. *Waste and Biomass Valorization*, *15*(6), 3351-3366.
43. Reyes-Gracia, A., Alvarado, J. A., Pérez-Cuapio, R., & Juárez, H. (2023). Comparison from lemon juice and N-dipentene ZnO nanoparticles green synthesis: Influence of byproducts in morphology and size. *Materials Science and Engineering: B*, *290*, 116335.
44. Saini, K., Gupta, K., Shekhawat, K. S., & Mathur, J. (2024). Exploration of the catalytic, anti-bacterial, and anti-oxidant capabilities of environmentally conscious zinc oxide nanoparticles synthesized using Citrus sinensis. *Journal of the Indian Chemical Society*, *101*(9), 101232.
45. Saini, R. K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K. G. M., & Keum, Y. S. (2022). Bioactive Compounds of Citrus Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. *Antioxidants (Basel, Switzerland)*, *11*(2), 239. https://doi.org/10.3390/antiox11020239
46. Sakthivel, S., Dhanapal, A. R., Paulraj, L. P., Gurusamy, A., Venkidasamy, B., Thiruvengadam, M., ... & Burkov, P. (2022). Antibacterial activity of seed aqueous extract of Citrus limon (L.) mediated synthesis ZnO NPs: An impact on Zebrafish (Danio rerio) caudal fin development. *Heliyon*, *8*(9).
47. Samanta, D., Basnet, P., Chanu, T. I., & Chatterjee, S. (2020). Biomolecule assisted morphology-controllable synthesis of Zinc Sulphide nanomaterials for efficient photocatalytic activity under solar irradiation. *Journal of Alloys and Compounds*, *844*, 155810.
48. Samanta, D., Chanu, T. I., & Chatterjee, S. (2017). Citrus limetta juice as capping agent in hydrothermal synthesis of ZnS nanosphere for photocatalytic activity. *Materials Research Bulletin*, *88*, 85-90.
49. Samanta, D., Chanu, T. I., & Chatterjee, S. (2017). Citrus limetta juice as capping agent in hydrothermal synthesis of ZnS nanosphere for photocatalytic activity. *Materials Research Bulletin*, *88*, 85-90.
50. Shahi S., Singh S. K. (2023), Biosynthesis Of Nanoparticles Using Milk Oligosaccharides, Journal of Advanced Zoology, Volume 44, Issue 5, Pages: 805-811, https://doi.org/10.53555/jaz.v44i5.3241
51. Sher, A., Khalil, A. T., Dogan, N., Ayaz, M., & Ahmad, K. (2024). Valorization and repurposing of Citrus limetta fruit waste for fabrication of multifunctional AgNPs and their diverse nanomedicinal applications. *Applied Biochemistry and Biotechnology*, *196*(4), 2067-2085.
52. Thenmozhi, J. R., Shahanaz, S., & Manuel, S. G. (2023). Green synthesis of ZnO nanoparticles using peels of Citrus limetta and evaluation of their antibacterial activity. *Journal of Applied Pharmaceutical Research*, *11*(4), 27-34.
53. Waiba, S., Sharma, M. L., & Sapkota, K. P. (2024). Remarkable photocatalytic performance of zinc oxide nanoparticles prepared through green synthetic method by using Citrus limon dry peel extract. *Scientific World*, *17*(17), 123-131.
54. Weldeslase, M. G., Benti, N. E., Desta, M. A., & Mekonnen, Y. S. (2023). Maximizing biodiesel production from waste cooking oil with lime-based zinc-doped CaO using response surface methodology. *Scientific Reports*, *13*(1), 4430.
55. Wirunchit, S., Sakulpeeb, N., Chutipaijit, S., Nukaew, J., & Koetniyom, W. (2024). GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES FROM LEMON PEEL EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY. *Suranaree Journal of Science & Technology*, *31*(3).