

Optimization of Plant-Mediated Green Synthesis of Zinc Oxide Nanoparticles Using Leaf Extracts of Different Plants.

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Abstract

Green synthesis of zinc oxide nanoparticles (ZnO NPs) has gained considerable attention as a simple, environmentally compatible approach that uses naturally occurring plant constituents during nanoparticle formation. The present study aimed to systematically optimize the synthesis conditions for plant-mediated ZnO NPs using aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua*. The effects of key synthesis parameters, including zinc precursor type, precursor concentration, pH, and reaction temperature, were systematically evaluated. Initially, three zinc salts, namely zinc acetate, zinc sulfate, and zinc nitrate, were screened for their suitability for ZnO NPs formation. Under the tested conditions, a detectable nanoparticle response was observed only with zinc acetate, whereas zinc sulfate and zinc nitrate did not show a detectable response. Subsequently, zinc acetate concentrations of 0.1, 0.5, and 1.0 M were evaluated, with 0.1 M exhibiting the most favorable response based on UV–visible spectroscopic analysis. Optimization of pH at 4, 6, and 8 identified pH 6 as the most favorable condition, while temperature screening at 20, 40, and 50°C indicated 20°C as the optimum temperature. Accordingly, the optimized conditions identified for ZnO NPs synthesis were zinc acetate as the precursor, a concentration of 0.1 M, pH 6, and a reaction temperature of 20°C. These findings demonstrate that systematic optimization can identify suitable conditions for the synthesis of plant-mediated ZnO NPs and that nanoparticle formation can be achieved under relatively mild reaction conditions. The study provides a useful foundation for further characterization and evaluation of the properties and potential applications of the synthesized ZnO NPs.

Keywords: Green synthesis; zinc oxide nanoparticles; optimization; *Cosmos sulphureus*; *Hamelia patens*; *Martynia annua*

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1. Introduction

Zinc oxide (ZnO) is an important semiconductor material with useful optical, electrical, photocatalytic, and surface properties. At the nanoscale, ZnO exhibits properties distinct from those of its bulk form. This is mainly due to its small particle size and high surface-to-volume ratio. Zinc oxide nanoparticles (ZnO NPs) have therefore been studied for many applications, including antimicrobial materials, photocatalysis, sensors, cosmetics, electronics, energy-related applications, and biomedical fields (Bandeira et al., 2020; Nguyen et al., 2022).

Various physical and chemical methods are available for synthesizing ZnO NPs. Although these methods can provide good control over nanoparticle properties, they may require high temperatures, high energy input, organic solvents, or other chemical reagents. Moreover, the environmental sustainability of nanoparticle-based technologies requires consideration of their potential

environmental fate, persistence, bioaccumulation, and ecological effects. These limitations have increased interest in greener methods of nanoparticle synthesis. Biological and plant-based methods are considered simple alternatives that can reduce reliance on additional chemical reagents (Bandeira et al., 2020; El-Gebaly et al., 2024; Geelani et al., 2026).

Plants are particularly useful for the green synthesis of nanoparticles. Plant extracts are easy to prepare and do not require maintaining microbial cultures. They also contain many naturally occurring compounds. These include phenolics, flavonoids, terpenoids, proteins, carbohydrates, alkaloids, organic acids, and other secondary metabolites. Such compounds can interact with metal ions and may help in nanoparticle formation and stabilization. Therefore, plant extracts can act as a natural source of compounds involved in different

stages of nanoparticle synthesis (Ahmed et al., 2017; Bandeira et al., 2020).

Green synthesis of ZnO NPs has been reported using different plant parts, including leaves, fruits, seeds, roots, flowers, and whole plants. The properties of the nanoparticles obtained in these studies are not always consistent. Differences can be observed in particle size, shape, crystallinity, surface properties, and optical characteristics. These differences may be related to the type and amount of phytochemicals present in the plant extract. The interaction between these compounds and the zinc precursor can also affect nanoparticle formation (Alamdari et al., 2020; Nguyen et al., 2022).

The formation of ZnO nanoparticles (ZnO NPs) using plant extracts involves several interconnected steps. Phytochemicals present in the extracts, particularly polyphenolic compounds such as flavonoids, tannins, anthocyanidins, and phenolic acids, can initially interact with Zn^{2+} ions through coordination or complexation. Functional groups such as hydroxyl ($-OH$) and carboxyl ($-COOH$) groups present in these compounds can facilitate the binding of Zn^{2+} ions and the formation of zinc-phytochemical complexes or intermediate precursor species. These intermediates may subsequently undergo hydrolysis, forming zinc hydroxide [$Zn(OH)_2$], which can dehydrate under suitable thermal treatment to form ZnO. The resulting ZnO nuclei then undergo nucleation and particle growth. At the same time, phytochemical constituents can adsorb onto the nanoparticle surface, acting as capping and stabilizing agents that control particle growth and limit aggregation. Since plant extracts contain a complex mixture of phytochemicals that may act synergistically, the precise mechanism of ZnO NP formation can vary with plant species, phytochemical composition, extraction conditions, and synthesis parameters (Hamed et al., 2023; Bandeira et al., 2020).

One of the major challenges in plant-mediated nanoparticle synthesis is optimizing reaction conditions. Several factors can affect nanoparticle formation. These include the type and concentration of the zinc precursor, amount of plant extract, extract-to-metal ratio, pH, reaction time, and temperature. Changes in these conditions can affect the formation and growth of nanoparticles and may also influence their size, morphology, aggregation, crystallinity, and optical properties (Nguyen et al., 2022; Zelekew et al., 2023).

The type of zinc precursor is an important factor in this process. Different zinc salts can provide different conditions for the availability and interaction of Zn^{2+} ions with plant compounds. The concentration of the precursor is also important. A higher concentration of zinc ions does not always result in better nanoparticle formation. If the concentration of zinc ions is too high relative to the available phytochemicals, it may affect particle

growth and stabilization. Therefore, the precursor and its concentration need to be optimized for each plant-based synthesis system (Bandeira et al., 2020; Zelekew et al., 2023).

pH can also have a major effect on nanoparticle synthesis. It can change the ionization of phytochemicals and their interaction with zinc ions. It may also affect nucleation, particle growth, surface charge, and nanoparticle stability. The optimum pH is not necessarily the same for all plant extracts. Differences in plant species and phytochemical composition can lead to distinct responses under the same pH conditions (Nguyen et al., 2022).

Temperature is another important factor. An increase in temperature can speed up the reactions involved in nanoparticle formation. However, higher temperatures do not always produce better nanoparticles. Excessive heating may affect some plant compounds or increase particle growth and aggregation. Therefore, the most suitable temperature needs to be determined for each plant-extract system (Bandeira et al., 2020).

Although many studies have reported the green synthesis of ZnO NPs, the synthesis conditions vary considerably between studies. Different plant species, extraction methods, zinc salts, precursor concentrations, pH values, temperatures, and reaction times have been used. This makes it difficult to compare the results of different studies directly. In addition, many studies have focused on a single plant species. There is comparatively less information on the systematic comparison of different plant extracts under similar experimental conditions.

Such a comparison is important because different plants contain different combinations of phytochemicals. These differences may affect their ability to interact with zinc ions and support nanoparticle formation. Therefore, studying multiple plant species under identical experimental conditions can help identify suitable plant sources and reaction conditions for synthesizing ZnO NPs.

In the present study, aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua* were used to green-synthesize ZnO NPs. Different zinc precursors were first evaluated. Zinc acetate was then further optimized at different concentrations. The effects of pH and temperature on nanoparticle synthesis were also studied. UV-visible spectroscopy was used to compare the response obtained under different conditions. The main aim of the study was to identify suitable conditions for synthesizing ZnO NPs using these three plant extracts. The optimized conditions can provide a useful starting point for further characterization and evaluation of the synthesized nanoparticles.

2. Materials and methods

2.1 Plant material collection

Fresh, healthy, and disease-free leaves of *Cosmos sulphureus* Cav., *Hamelia patens* Jacq., and *Martynia annua* L. were collected for the present study. The leaves of *C. sulphureus* were collected from Jugalpura, Jaipur, Rajasthan, India. The leaves of *H. patens* were collected from Delhi Road, Amber, Jaipur, Rajasthan, India. The leaves of *M. annua* were collected from Delhi road, Amber, Jaipur, Rajasthan, India.

The freshly collected leaves were initially washed thoroughly under running tap water to remove adhering dust and other surface impurities. The leaves were subsequently immersed in sterile 0.9% (w/v) saline solution and rinsed several times with deionized distilled water to ensure thorough cleaning. Excess moisture was removed by gently blotting the leaves, after which they were shade-dried at room temperature for 8–10 days (Anuradha et al., 2015; Kamaraj et al., 2020).

To determine the moisture loss and dry weight of the plant material, 5 g of fresh leaves were weighed and placed in a laboratory cabinet dryer set to 40–50°C. The samples were weighed at regular intervals until a constant weight was achieved (Anuradha et al., 2015). The dried leaves were subsequently ground using a laboratory grinder (Gupta et al., 2024) and sieved through a 100-mesh sieve to obtain a fine and uniform powder (Suresh et al., 2015). The resulting powdered plant material was stored in airtight containers until further use.

2.2 Preparation of aqueous leaf extract

Aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua* were prepared separately from the previously dried and powdered plant material. For each extraction, 10 g of the respective leaf powder was accurately weighed and suspended in 100 mL of distilled water. The resulting suspensions were ultrasonicated at 40°C for 10 min to facilitate the extraction of phytochemicals and other bioactive constituents into the aqueous medium (Shahzad et al., 2022). After sonication, the extracts were allowed to cool to room temperature and subsequently filtered through Whatman No. 42 filter paper to remove insoluble plant residues (Ganaie et al., 2014). The resulting clear filtrates were collected separately and stored at 4°C until further use. Freshly prepared aqueous leaf extracts were used for the plant-mediated synthesis of ZnO nanoparticles, providing phytochemical constituents that may participate in Zn^{2+} complexation, nucleation, and subsequent stabilization of the synthesized nanoparticles.

2.3 Optimization of ZnO Nanoparticle Synthesis Conditions

Various synthesis parameters were systematically evaluated to optimize the green synthesis of ZnO nanoparticles. The parameters investigated included the type of zinc precursor, precursor concentration, pH of the reaction medium, and reaction temperature. Three zinc salts, zinc acetate, zinc

sulfate, and zinc nitrate, were initially screened to determine their suitability for ZnO NP formation. The concentration of the selected zinc precursor was subsequently optimized at three levels (0.1, 0.5, and 1.0 M). The effect of pH was evaluated at pH 4, 6, and 8, while the influence of temperature was assessed at 20, 40, and 50°C. The optimal conditions for each parameter were determined based on the observed ZnO NP formation and the UV–visible spectroscopic response.

2.4 Synthesis of ZnO Nanoparticles Under Optimized Conditions

ZnO nanoparticles were synthesized separately using the aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua*. Zinc acetate was used as the zinc precursor, and the required concentration of the precursor solution was prepared using distilled water. Subsequently, 10 mL of the respective plant leaf extract was added dropwise to 90 mL of the zinc precursor solution under continuous magnetic stirring. The reaction mixture was maintained under the respective optimized reaction conditions identified through the systematic optimization experiments described in Section 2.3 and stirred for 2 h.

Following the reaction, the synthesized nanoparticles were recovered by centrifugation at 10,000 rpm for 15 min, and the supernatant was discarded. The resulting pellet was washed three times with distilled water, with each washing cycle followed by centrifugation at 5,000 rpm for 5 min, to remove unreacted precursor and residual plant-derived constituents. The purified nanoparticle pellet was subsequently transferred to a porcelain crucible and subjected to thermal treatment in a muffle furnace. After cooling, the resulting ZnO nanoparticles were collected and preliminarily analyzed by UV–visible spectroscopy to confirm nanoparticle formation.

3. Results

3.1 Optimization of Conditions for Green Synthesis of Zinc Oxide Nanoparticles

Green synthesis of zinc oxide nanoparticles (ZnO NPs) was performed using aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua*. The synthesis conditions were systematically optimized by evaluating the effects of zinc precursor type, zinc precursor concentration, pH, and temperature. A visible change in the color of the reaction mixture was considered an initial indication of nanoparticle formation. In contrast, UV–visible spectroscopic analysis was used to evaluate and compare the synthesis under the different experimental conditions.

3.1.1 Effect of Zinc Precursor

Three zinc salts, namely zinc acetate, zinc sulfate, and zinc nitrate, were evaluated for their suitability for the plant-mediated synthesis of ZnO NPs. A distinct difference in nanoparticle formation was observed among the tested precursors. Under the

experimental conditions employed, zinc sulfate and zinc nitrate showed no detectable evidence of nanoparticle formation. In contrast, the reaction containing zinc acetate exhibited a visible change following the addition of the plant extract.

The comparatively favorable response observed with zinc acetate may be associated with differences in the chemical nature of the precursor and its interaction with phytochemical constituents present in the plant extracts. Plant-derived compounds such as phenolics, flavonoids, proteins, and other oxygen-containing biomolecules can interact with Zn^{2+} ions and participate in precursor complexation, nucleation, growth, and stabilization of the resulting nanoparticles. The nature of the counter-ion associated with Zn^{2+} may also influence the availability and behavior of the metal ions during the synthesis process. Thus, under the conditions investigated in the present study, zinc acetate was identified as the most suitable precursor and was selected for subsequent optimization experiments.

3.1.2 Effect of Zinc Acetate Concentration

Following the selection of zinc acetate as the precursor, its concentration was optimized at 0.1, 0.5, and 1.0 M. The UV–visible spectra demonstrated differences in the optical response of the reaction mixtures at the tested concentrations. Among the concentrations evaluated, 0.1 M zinc acetate exhibited the most favorable UV–visible response, indicating comparatively efficient nanoparticle formation under the experimental conditions.

The enhanced response at lower precursor concentrations may be due to a more favorable balance between available Zn^{2+} ions and the phytochemical constituents in the plant extract. At higher precursor concentrations, the amount of Zn^{2+} may exceed the effective binding or stabilizing capacity of the available phytochemicals, potentially affecting nucleation and subsequent particle growth. Excess precursor may also promote uncontrolled growth or aggregation, which can influence the optical response of the synthesized nanoparticles. Therefore, 0.1 M zinc acetate was selected as the optimum precursor concentration for subsequent experiments.

The corresponding reaction mixtures obtained during the ionic strength optimization are shown in Figure 5.

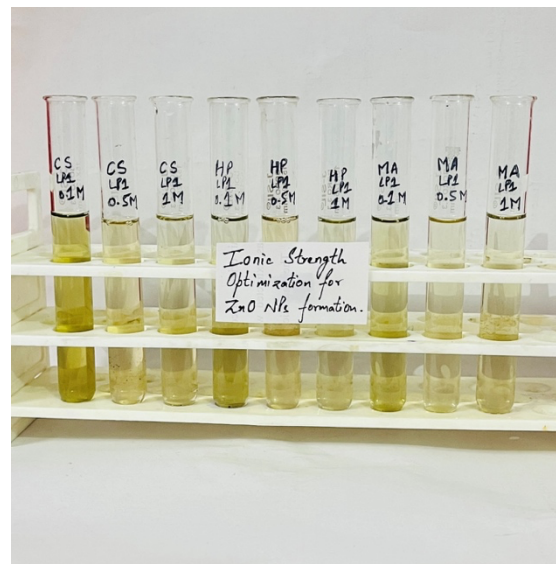


Figure 1. Visual appearance of reaction mixtures obtained during ionic strength optimization of ZnO nanoparticle synthesis using leaf extracts of *Cosmos sulphureus* (CSLP1), *Hamelia patens* (HPLP1), and *Martynia annua* (MALP1) at different ionic strengths.

3.1.3 Effect of pH

The influence of pH on ZnO NP synthesis was investigated at pH 4, 6, and 8. A distinct variation in the UV–visible response was observed at the different pH conditions. Among the tested values, pH 6 exhibited the most favorable response, indicating comparatively enhanced nanoparticle formation under the conditions employed.

The effect of pH may be attributed to its influence on Zn^{2+} speciation, hydrolysis, phytochemical ionization, metal–phytochemical interactions, nucleation, and nanoparticle stabilization. Changes in pH can alter the availability of functional groups in plant-derived compounds and, consequently, affect their interactions with Zn^{2+} ions. In the present study, the comparatively higher UV–visible response at pH 6 suggests that this condition provided a more favorable environment for the formation and stabilization of ZnO NPs using the selected plant extracts. The lower responses observed at pH 4 and 8 indicate that these conditions were comparatively less favorable under the experimental conditions employed. Accordingly, pH 4 was selected as the optimum pH.

The corresponding reaction mixtures obtained during pH optimization are shown in Figure 6.

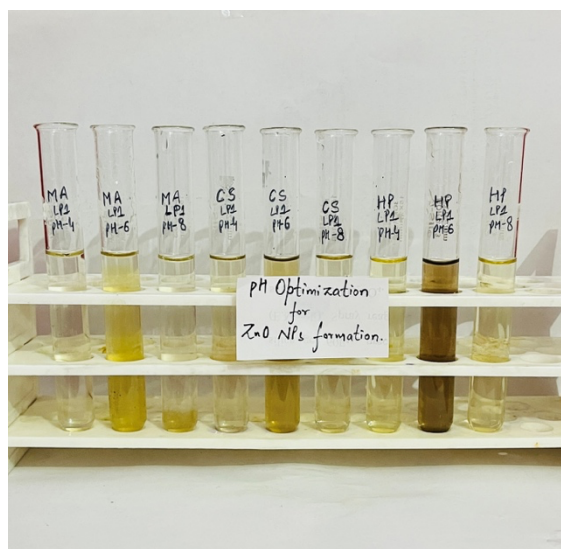


Figure 2. Visual appearance of reaction mixtures obtained during pH optimization of ZnO nanoparticle synthesis using leaf extracts of *Cosmos sulphureus* (CSLP1), *Hamelia patens* (HPLP1), and *Martynia annua* (MALP1) at different pH levels.

3.1.4 Effect of Temperature

The effect of temperature on ZnO NP synthesis was evaluated at 20, 40, and 50 °C. The UV–visible spectra showed variations in nanoparticle formation as the temperature changed. Among the tested temperatures, 20°C exhibited the most favorable UV–visible response.

Temperature can influence reaction kinetics, Zn^{2+} –phytochemical interactions, nucleation, particle growth, and the stability of phytochemical constituents involved in the synthesis process. Although an increase in temperature may accelerate reaction kinetics, excessive or prolonged thermal exposure can also alter phytochemical constituents and influence particle growth and aggregation. In the present study, increasing the temperature from 20 to 40 and 50°C did not improve the UV–visible response. Therefore, 20°C was selected as the optimum temperature for the subsequent synthesis of ZnO NPs.

The corresponding reaction mixtures obtained during temperature optimization are shown in Figure 7.

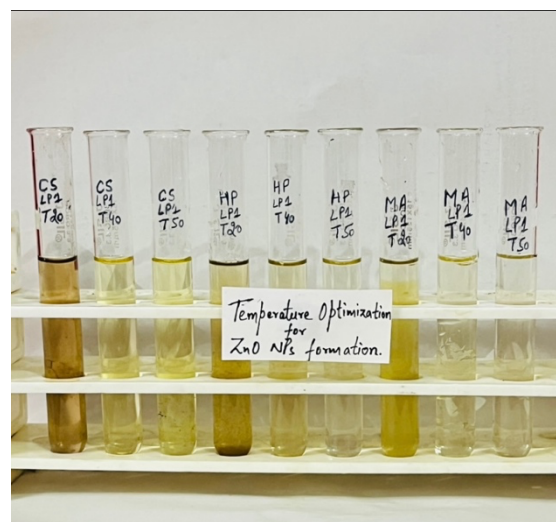


Figure 3. Visual appearance of reaction mixtures obtained during temperature optimization of ZnO nanoparticle synthesis using leaf extracts of *Cosmos sulphureus* (CSLP1), *Hamelia patens* (HPLP1), and *Martynia annua* (MALP1) at different reaction temperatures.

3.2 Optimized Conditions for ZnO NP Synthesis

The optimization experiments demonstrated that the type and concentration of the zinc precursor, pH, and reaction temperature influenced the synthesis of ZnO NPs. Under the conditions investigated, zinc acetate was the most suitable zinc precursor, while a concentration of 0.1 M, pH 6, and a temperature of 20°C produced the most favorable UV–visible response. These conditions were therefore selected as the optimized parameters for the subsequent synthesis and characterization of ZnO NPs using the selected plant leaf extracts. The optimized conditions are summarized in Table 1.

Table 1. Optimization of synthesis parameters for green synthesis of ZnO nanoparticles

Optimization parameter	Conditions tested	UV–Vis response	Selected condition
Zinc precursor	Zinc acetate	Nanoparticle formation observed	Zinc acetate
	Zinc sulphate	No detectable nanoparticle formation	

	Zinc nitrate	No detectable nanoparticle formation	
Zinc acetate concentration	0.1 M	Favorable response	0.1 M
	0.5 M	Lower response	
	1.0 M	Lower response	
pH	4	Favorable response	
	6	Lower response	pH 6
	8	Lower response	
Temperature	20°C	Favorable response	20°C
	40°C	Lower response	
	50°C	Lower response	

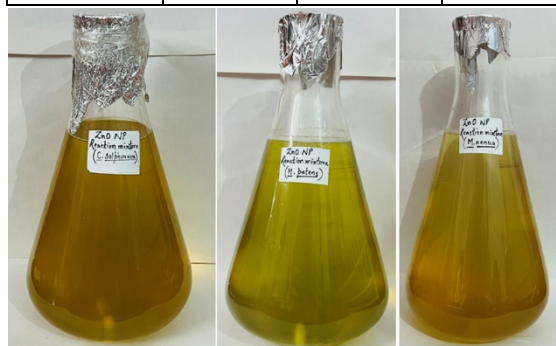


Figure 4. Visual appearance of the final reaction mixtures obtained during green synthesis of ZnO nanoparticles using aqueous leaf extracts of (a) *Cosmos sulphureus*, (b) *Hamelia patens*, and (c) *Martynia annua*.

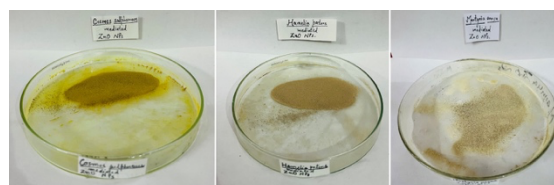


Figure 5. Visual appearance of the recovered ZnO nanoparticle powders synthesized using aqueous leaf extracts of (a) *Cosmos sulphureus*, (b) *Hamelia patens*, and (c) *Martynia annua*.

4. Discussion

Green synthesis provides a simple and environmentally friendly approach for the preparation of metal oxide nanoparticles. Plant extracts contain a complex mixture of naturally occurring compounds that can interact with metal ions and participate in nanoparticle formation. Phenolics, flavonoids, proteins, sugars, terpenoids, organic acids, and other phytochemicals may contribute to metal-ion complexation, nucleation, particle growth, and stabilization. The composition and abundance of these compounds can vary considerably among plant species, making the outcome of plant-mediated nanoparticle synthesis dependent on the biological source and reaction environment (Bandeira et al., 2020; Basnet and Chanu, 2018; Saraf et al., 2026). The complexity of plant extracts also makes it difficult to assign a single mechanism to ZnO nanoparticle formation, and several possible pathways have been proposed in the literature (Bandeira et al., 2020; Saraf et al., 2026).

In the present study, aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua* were investigated for the green synthesis of ZnO nanoparticles. The influence of precursor type, precursor concentration, pH, and temperature was systematically evaluated. The variation in the response observed under these conditions indicates that nanoparticle formation is governed by the combined effects of precursor chemistry and the phytochemical environment of the plant extracts.

4.1 Effect of zinc salt

The precursor is an important component of a plant-mediated synthesis system because it provides the zinc species involved in the subsequent formation of ZnO. Differences in precursor chemistry can influence the availability and behavior of zinc ions, as well as their interactions with the extract's phytochemical constituents. Consequently, the choice of zinc precursor can affect the formation and properties of biologically synthesized ZnO nanoparticles (Bandeira et al., 2020).

The more favorable response observed with zinc acetate in the present study may be attributed to its interaction with the phytochemical constituents present in the leaf extracts. Plant-derived compounds containing hydroxyl, carbonyl, carboxyl, and other oxygen-containing functional groups can coordinate with Zn^{2+} ions and participate

in the formation of zinc-containing intermediate species. These interactions may subsequently influence nucleation and particle growth, while some of the same phytochemicals may associate with the particle surface, contributing to stabilization (Bandeira et al., 2020; Basnet and Chanu, 2018).

The precursor-dependent behavior observed in the present investigation is consistent with previous studies demonstrating that the zinc source and other reaction parameters can influence plant-mediated ZnO synthesis. Bandeira et al. (2020) reviewed different biological routes for ZnO synthesis and emphasized the influence of synthesis methodology and precursor chemistry on nanoparticle formation. Similarly, Oluwaniyi and Oyewo (2025) used zinc acetate as a precursor for the synthesis of ZnO nanoparticles using *Dennettia tripetala* extracts. They investigated the effects of precursor concentration, pH, and temperature on the synthesis process.

It is important, however, that the absence of a detectable response with zinc sulfate and zinc nitrate in the present study should not be interpreted as evidence that these salts are generally unsuitable for ZnO nanoparticle synthesis. Different zinc precursors have been employed successfully in biological synthesis systems, and their performance can vary with plant species, phytochemical composition, precursor concentration, pH, temperature, extract-to-precursor ratio, reaction time, and post-synthesis treatment (Bandeira et al., 2020). Therefore, the precursor-dependent behavior observed here should be considered specific to the experimental conditions used in the present study.

4.2 Effect of zinc acetate concentration

Precursor concentration can influence nanoparticle formation by controlling the amount of Zn^{2+} available to interact with the phytochemical constituents in the plant extract. An appropriate balance between zinc species and phytochemical constituents may be important for controlled nucleation, particle growth, and surface stabilization (Bandeira et al., 2020; Oluwaniyi and Oyewo, 2025).

At relatively high precursor concentrations, the amount of Zn^{2+} available in the reaction mixture may become disproportionate to the concentration of phytochemical constituents that can coordinate or stabilize the developing particles. Such an imbalance may influence nucleation and subsequent particle growth and could increase particle–particle interactions or aggregation. Changes in particle size and aggregation can, in turn, affect the optical response of nanoparticle suspensions. These concentration-dependent effects have also been considered in optimization studies of plant-mediated ZnO synthesis (Oluwaniyi and Oyewo, 2025).

The findings of Oluwaniyi and Oyewo (2025) are particularly relevant because their study

systematically examined metal-ion concentration during ZnO nanoparticle synthesis using *D. tripetala* extracts. Their results demonstrated that increasing precursor concentration did not necessarily yield a proportional improvement in the synthesis response, indicating that precursor concentration should be optimized rather than increased.

The concentration effect may also be related to the balance between nucleation and particle growth. Changes in precursor availability can influence the number of nuclei formed and the amount of zinc-containing material available for subsequent growth. Consequently, the relationship between precursor concentration and nanoparticle formation may not be linear (Bandeira et al., 2020). The favorable response observed at the selected concentration in the present study may therefore reflect a more suitable balance between zinc availability and the extracts' phytochemical constituents.

4.3 Effect of pH

pH is an important parameter in plant-mediated ZnO synthesis because it can influence Zn^{2+} speciation, hydrolysis, the ionization state of phytochemical functional groups, metal–phytochemical interactions, nucleation, particle growth, and surface stabilization (Bandeira et al., 2020; Basnet and Chanu, 2018). Changes in pH can alter the availability of functional groups in plant-derived compounds and, consequently, their ability to coordinate with zinc-containing species.

The pH-dependent response observed in the present study may therefore be associated with changes in the chemical interactions between Zn^{2+} and the phytochemicals present in the extracts. Functional groups such as hydroxyl and carboxyl groups can exhibit pH-dependent behavior, which may alter their interaction with metal ions. Changes in pH can also influence hydrolysis and the formation of zinc-containing intermediate species involved in subsequent ZnO formation (Bandeira et al., 2020). Previous studies have demonstrated that pH can significantly influence plant-mediated ZnO synthesis. Oluwaniyi and Oyewo (2025), for example, systematically investigated the effect of pH during the synthesis of ZnO nanoparticles using *D. tripetala* extracts and demonstrated that the synthesis response varied with pH. However, the optimum pH is not necessarily identical among different plant-mediated systems. This variation can be attributed to differences in phytochemical composition, extraction conditions, precursor chemistry, precursor concentration, temperature, and reaction time (Bandeira et al., 2020).

Thus, the favorable synthesis observed at pH 6 in the present study should be considered a system-specific finding rather than a universal optimum for ZnO nanoparticle synthesis. The enhanced response at this mildly acidic pH may be attributed to a suitable chemical environment that facilitates interactions between zinc species and phytochemical

constituents, thereby favoring nanoparticle formation. However, the precise molecular mechanisms underlying this pH-dependent behavior cannot be conclusively established from UV–visible analysis alone.

4.4 Effect of temperature

Temperature can influence the kinetics of reactions involved in nanoparticle synthesis and can consequently affect nucleation, particle growth, aggregation, and stabilization. It may also influence the interaction between zinc ions and phytochemical constituents present in the plant extract (Bandeira et al., 2020).

Although increasing temperature generally accelerates reaction kinetics, faster reaction rates do not necessarily result in a more favorable nanoparticle product. Temperature changes can alter the relative rates of nucleation and particle growth. They may also influence the stability or availability of phytochemical constituents involved in metal-ion coordination and surface stabilization. Consequently, the effect of temperature is dependent on the specific plant extract and precursor system.

Bala et al. investigated temperature-dependent ZnO nanoparticle synthesis using *Hibiscus sabdariffa* leaf extract and demonstrated that temperature influenced nanoparticle formation and particle growth. The synthesized material was evaluated using UV–visible spectroscopy, FTIR, and XRD, highlighting the importance of complementary characterization when assessing temperature-dependent synthesis. Oluwaniyi and Oyewo (2025) likewise reported a temperature-dependent effect in the synthesis of ZnO nanoparticles using *D. tripetala* extracts, further supporting the importance of temperature optimization in plant-mediated systems. The fact that elevated temperature did not provide a more favorable response in the present study is therefore noteworthy. It suggests that the plant extracts were capable of supporting the synthesis under relatively mild conditions without additional heating. From a green-synthesis perspective, such a condition may be advantageous because it can reduce external energy requirements and simplify the synthesis process. However, the effect of temperature on particle size, morphology, crystallinity, and surface chemistry should be assessed through complementary characterization rather than inferred solely from UV–visible measurements.

4.5 Role of plant phytochemicals and proposed mechanism

The successful incorporation of the three leaf extracts into ZnO nanoparticle synthesis may be attributed to their diverse phytochemical composition. Plant extracts contain numerous classes of compounds that can interact with metal ions and nanoparticle surfaces. Depending on their chemical characteristics, these compounds may participate in metal-ion coordination, precursor

transformation, nucleation, particle growth, and surface stabilization (Bandeira et al., 2020; Basnet and Chanu, 2018).

A plausible mechanism for plant-mediated ZnO formation involves the initial coordination or complexation of Zn^{2+} ions with phytochemical constituents containing suitable functional groups. Such interactions may result in zinc–phytochemical complexes or other zinc-containing intermediate species. Subsequent hydrolysis can yield zinc hydroxide $[\text{Zn}(\text{OH})_2]$, which, under appropriate thermal treatment, dehydrates to form ZnO. Meanwhile, phytochemical constituents may become associated with the developing nanoparticle surface, thereby contributing to capping and stabilization. Because plant extracts contain numerous compounds that can participate simultaneously in these processes, the precise molecular pathway may differ among plant species and synthesis conditions (Happy et al., 2019; Bandeira et al., 2020; Alamdari et al., 2020; Hamed et al., 2023).

This proposed mechanism is consistent with previous studies in which plant-derived functional groups were associated with the synthesis and stabilization of ZnO nanoparticles. Jagdish and Nehra (2022), for example, synthesized ZnO nanoparticles using *Bryophyllum pinnatum* leaf extract and employed FTIR, XRD, SEM, EDX, HR-TEM, and zeta-potential analyses to characterize the synthesized material. Their FTIR findings supported the presence of phytochemical functional groups, while complementary techniques provided evidence of crystallinity, morphology, elemental composition, and particle size.

The role of phytochemicals should, however, be described carefully. ZnO formation differs from the synthesis of metallic nanoparticles in which metal ions are reduced to their zero-valent state. ZnO is a metal oxide, and therefore its formation should not be described simply as the reduction of Zn^{2+} to metallic Zn. A more appropriate interpretation involves the coordination of Zn^{2+} with plant constituents, the formation and transformation of zinc-containing intermediates, the development of the ZnO phase, and subsequent stabilization by plant-derived compounds (Bandeira et al., 2020).

Overall, the findings indicate that plant-mediated ZnO synthesis is a multifactorial process in which precursor chemistry, precursor concentration, pH, temperature, and the phytochemical environment of the extract can interact to influence nanoparticle formation. The differences observed under the tested conditions emphasize the importance of optimizing the synthesis system for a particular plant extract rather than directly adopting conditions reported for another biological source. The optimized conditions identified in the present study, therefore, provide a basis for subsequent synthesis and detailed

characterization of ZnO nanoparticles obtained from *C. sulphureus*, *H. patens*, and *M. annua* leaf extracts.

5. Conclusion

The present study demonstrated the feasibility of green synthesis of zinc oxide nanoparticles (ZnO NPs) using aqueous leaf extracts of *Cosmos sulphureus*, *Hamelia patens*, and *Martynia annua*. A systematic optimization approach was employed to evaluate the influence of zinc precursor type, precursor concentration, pH, and temperature on nanoparticle formation. Among the zinc salts evaluated, zinc acetate showed a favorable response under the tested conditions, whereas no detectable nanoparticle formation was observed with zinc sulfate and zinc nitrate. Further optimization using zinc acetate identified a precursor concentration of 0.1 M, pH 6, and 20°C as the conditions yielding the most favorable response, as determined by UV–visible analysis.

The favorable response at 20°C indicates that additional heating was not required under the experimental conditions, suggesting the potential for a relatively simple, lower-energy synthesis approach. The results further demonstrate that synthesis conditions can substantially influence the formation of plant-mediated ZnO NPs and that optimizing them is important for achieving a consistent and favorable synthesis outcome.

Nevertheless, UV–visible spectroscopy provides preliminary evidence of nanoparticle formation and cannot, by itself, conclusively establish the crystalline phase, morphology, particle size, or elemental composition of the synthesized material. Therefore, further characterization using techniques such as FTIR, XRD, SEM/TEM, and EDX is necessary for comprehensive confirmation and physicochemical evaluation of the ZnO NPs.

Overall, the study provides a comparative, systematic basis for plant-mediated ZnO NP synthesis across three plant species and identifies suitable synthesis conditions for further investigation. The optimized nanoparticles may subsequently be evaluated for potential antimicrobial, photocatalytic, environmental, and other biological applications, thereby providing opportunities for future studies on the functional properties and practical applications of plant-mediated ZnO NPs.

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Author contributions:

Arindam Saraf: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, and Writing – original draft.

Syed Burhan Geelani: Investigation, Data curation, Formal analysis, and Resources.

Prasanta Das: Supervision, Validation, and Writing – review and editing.

Anuradha Jayaraman: Supervision, Validation, and Writing – review and editing.

Conflict of interest

The authors declare that they have no conflict of interest concerning the research, authorship, or publication of this article.

Data Availability Statement

All data generated and analyzed during this study are included in this published article and its supplementary information.

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