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RESEARCH ARTICLE

Comparative Evaluation of Biogenesis of ZnO Nanoparticles Using Leaf Extracts of *Bacopa monnieri, Acacia arabica* and *Catharanthus roseus*

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Abstract

Objective: Optimization of green synthesis of zinc oxide nanoparticles (ZnONPs) using leaf extracts of *Bacopa monnieri*, *Acacia arabica*, and *Catharanthus roseus*. **Method:** ZnONPs were synthesized through the co-precipitation method using leaf extracts of *B. monnieri, A. arabica, and C. roseus* separately, at pH 4, 6, 9, 11, and 13 on various reaction duration to optimize the biosynthesis. To validate the biogenesis of nanoparticles (NPs), the final product was collected as white-colored fine powder and analyzed by UV-visible spectroscopy, XRD, FESEM, and Zeta potential analyser. **Findings:** Hexagonal-shaped ZnONPs were synthesized with mean sizes of 33 nm, 35 nm, and 36 nm using leaf extracts of *B. monnieri*, *A. arabica*, and *C. roseus* respectively. Particles synthesized using *B. monnieri* leaf extract had sizes in the range of 40–60 nm, whereas particles synthesized using *A. arabica* and *C. roseus* leaf extracts had sizes in the range of 1-150 nm and 40–80 nm, respectively. It was also observed that the pH of the reaction mixture and reaction duration affect the biosynthesis of ZnONPs. At acidic pH (4 and 6), ZnONPs were not synthesized, but at basic pH (9, 11, and 13), ZnONPs were synthesized efficiently. An increase in the pH of the reaction mixture from 9 to 11 and 13 decreases the quantity of synthesized nano-powder. **Novelty:** The biosynthesis of ZnONPs using leaf extract of *B. monnieri* is reported for the first time, and comparatively analyzed with the optimization of ZnONPs biosynthesis using *A. arabica* and *C. roseus* leaf extracts.

Keywords: Green Approach; EcoFriendly; Biosynthesis; Optimization; Zinc Oxide Nanoparticles

1 Introduction

Zinc oxide nanoparticles (ZnONPs) have been widely used in various fields such as electronics, optoelectronics, biomedical, textile, cosmetics, water treatment, food packaging, and agriculture applications[1,2]. In chemical-based methods for the synthesis of ZnONPs typically involve the use of toxic chemicals, solvents, and

stabilizers, which are harmful for human health and the environment[3]. The release of hazardous byproducts during the synthesis process can increase environmental pollution. Physical methods, such as laser ablation and high-energy ball milling, and some chemical methods require high-energy input and sophisticated equipment, making them costlier and less accessible for large-scale production[4].The use of natural and eco-friendly materials in the synthesis of nanoparticles (NPs) is a better way to overcome the side effects of chemical and physical methods. The use of green materials can reduce the potential toxicity of NPs and minimize their negative environmental impact.

Several natural and eco-friendly materials have been used for the green synthesis of ZnONPs, such as plant extracts, bacteria, fungi, and algae[5]. One example of green synthesis of ZnONPs involved the use of plant extracts such as Aloe vera, *Moringa oleifera*, and *Azadirachta indica*[6]. These plant extracts contain various bioactive compounds such as flavonoids, terpenoids, and phenolics that act as reducing and stabilizing agents for the synthesis of ZnONPs[7]. Green synthesis of ZnONPs is a bottom-up strategy in which NPs can be synthesized by oxidation/reduction of metallic ions by organic moieties derived from biological resources[8].

Optimization of the green synthesis of ZnONPs involves the adjustment of various synthesis parameters such as the concentration of plant extract, pH, and reaction time. These parameters can affect the size, morphology, quantity, and stability of ZnONPs[9]. For example, increasing the concentration of the plant extract or zinc salt solution can increase the size of ZnONPs. On the other hand, increasing pH can decrease the size of ZnONPs. Optimization of these parameters can lead to the synthesis of ZnONPs with desired properties for specific applications[10,11].

In the present study, three local plant species were selected (*B. monnieri, A. arabica*, and *C. roseus*) to pick their leaves for the synthesis of ZnONPs at variable pH (4, 6, 9, 11 and 13) and reaction duration to optimize the biosynthesis of ZnONPs. Final products were characterized through UV-visible spectroscopy, X-ray diffraction (XRD), Zeta potential analysis, and Field emission scanning electron microscopy (FESEM) to confirm the shape, size, and stability of the NPs. The study clarified the potential of leaf extract of *B. monnieri* in the synthesis of ZnONPs and its comparative analysis with the biosynthesis of ZnONPs by using leaf extracts of *A. arabica and C. roseus*.

2 Methodology

2.1 Preparation of plant leaf extract

Fresh leaves of all the selected plants (*B. monnieri, A. arabica*, and *C. roseus*) were collected from the Herbal Garden of the Amity University, Gurugram, Haryana, India (Figure 1 a, b, c). All the dirt, debris, or foreign matter was removed from the plant leaves by washing them with clean water. 5 gm leaves of each were chopped into the pieces and heated up with 100 ml deionized water at 55°C for 20 minutes, separately. The extracts were filtered through Whatman filter paper Grade-1 to remove impurities or plant solids and were stored in clean containers at 4°C.

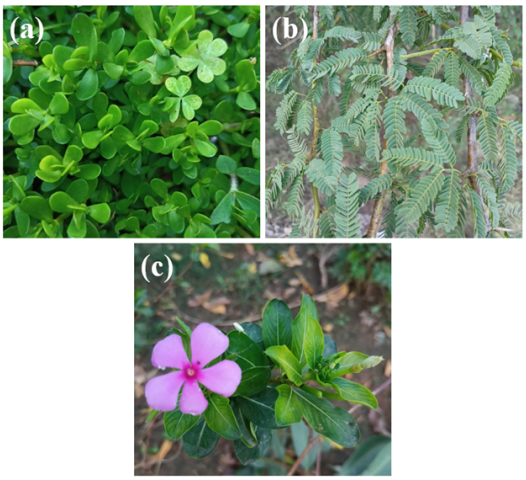

Fig 1. Leaves were collected from the plants (a) B. monnieri, (b) A. arabica, and (c) C. roseus at Herbal Garden of Amity University Haryana

2.2 Optimization of biosynthesis of ZnONPs

0.05 M zinc acetate dehydrate (Qualigens) was added to 50 ml of deionized water while it was being stirred by a magnetic stirrer. After 15 minutes, 1 ml of leaf extract was added to the mixture and left for a variable time while stirring (repeat the same reaction with time intervals of 15, 30, 45, and 60 minutes separately). 50 ml of 0.1 M NaOH (Qualigens) was added drop by drop to the reaction mixture. After 30 minutes of continuous stirring, whitish to light yellow color precipitate formation was initiated. The reaction mixture was placed in the oven for two hours at 100°C, and then allowed to cool down the reaction mixture till reached to the room temperature, gently stirred the solution and let the precipitate settle. The precipitate was collected using a glass pipette and then centrifuged that for 30 minutes at 3000 rpm. Washed the pellet two times with distilled water and finally with 100% ethanol (4000 rpm for 10 minutes). The pellet was dried in the oven overnight at 50°C then ground into fine powder by using a mortar pestle. The fine powder was put in the muffle furnace for calcination at 450°C for two hours. The powder was gathered and stored in airtight jars for further use. The same protocol was repeated with all three plant leaf extracts (*B. monnieri, A. arabica*, and *C. roseus*) at variable pH 4, 6, 9, 11 and 13, separately.

2.3 Characterization of synthesized product

The characterization of the final product involved the use of various techniques to determine their physicochemical properties such as size, shape, elements and surface charges. The sophisticated analytical techniques used for the characterization of ZnONPs include UV-visible spectroscopy, XRD, FESEM, and Zeta potential analysis.

2.3.1 UV-visible spectroscopy analysis

The well-dispersed synthesized fine powder in deionized water was subjected to analysis in a UV-visible spectrophotometer (Shimazdu, UV-1900i). The Surface Plasmon Resonance (SPR) peak of the sample within the predetermined range 355-380 can confirm the presence of ZnONPs. The Tauc's plot method was used for the calculation of band gap energy[12].

2.3.2 X-ray diffraction (XRD) analysis

XRD analysis determines the nature of the crystalline structure of the particles[13]. Dry powder was sonicated in deionized water to homogenize the mixture, and subjected to analysis under an X-ray diffraction analyzer (Rigaku, Ultima IV). The size of synthesized crystals was calculated by the Scherrer equation.

2.3.3. Field emission scanning electron microscopy (FESEM)

FESEM provides information about the size and shape of the synthesized NPs[14]. Selected dry samples were analyzed under the electron microscope (Zeiss, EVO40). The pictures were analyzed through ImageJ software to calculate the size of the particles.

2.3.4 Zeta Potential Analysis

Zeta potential analysis provides information about the charges over the particles' surface, which is directly related to the stability (dispersity) of the NPs in the medium[15]. Well-dispersed sample analyzed in the Zeta potential analyzer (Malvern, Nano ZS). The zeta potential spectra gave the results in mV.

3 Result and discussion

3.1 Optimization of biosynthesis of ZnONPs

During the processing according to the methodology, it was observed that the precipitate did not form at acidic pH 4 and 6 while formed at the selected basic pH values (9, 11 and 13) by using all three plant leaf extracts (*B. monnieri, A. arabica*, and *C. roseus*). The pH also affects the quantity of the final product as it was observed that when the pH increased from 9 to 11 and 13, the quantity of the final product decreased (Table 1). When increased the duration between the third and fourth steps (after the addition of leaf extract), the precipitate formation was reduced according to the increment in the time interval. More than 30-minutes interval between these stages reduced the precipitate formation. Maximum precipitate was formed when reduce the time interval for the reaction mixture to 15 minutes.

Much research has been undertaken to investigate optimization strategies for increasing the production of NPs to acquire commercial acceptability[16,17]. Changes in the reaction parameters such as metal ion concentration, pH, temperature, and reaction duration can enhance and regulate the size, shape, and yield of NPs[18]. Mohd Yusof et al[14] reported the optimization of the biosynthesis of ZnONPs by using the supernatant of *L. plantarum* TA4 with independent parameters like substrate

Table 1. The dry weight of the final product (fine powder) was obtained by biosynthesis using leaf extracts of *B. monnieri, A. arabica,* and *C. roseus* at pH 9, 11 and 13

Name of Plant	pH 9	pH 11	pH 13
B. monnieri	460 mg	310 mg	240 mg
A. arabica	550 mg	400 mg	150 mg
C. roseus	490 mg	310 mg	240 mg

concentration, supernatant concentration and pH. Response surface methodology (RSM) has recently been used to optimize various biotechnological processes. RSM works by examining the relationships between the parameters and output to discover the ideal circumstances after running numerous experiments [14]. In a previous study, the influence of sonication amplitude and duration on the hydrodynamic size of biosynthesized gold nanoparticles has been investigated by using RSM [19]. An aqueous extract of the *Ocimum lamifolium (O. lamifolium)* plant was used to biosynthesize ZnONPs, for such purpose randomized response surface methodology (RSM) was utilized to optimize the effect of zinc acetate precursor, temperature, and reaction duration [20].

3.2 Characterization of the synthesized product

3.2.1 UV- visible spectroscopy analysis

The biosynthesis of ZnONPs by using all three selected plant leaf extracts at pH 9, 11 and 13, was initially confirmed by the UV- visible spectroscopy analysis by obtaining the ideal Surface Plasmon Resonance (SPR) peak between the 355-380 nm wavelengths. SPR peak of biosynthesized ZnONPs using leaf extract of *B. monnieri* at pH 9, 11 and 13 observed at 366 nm, 374 nm and 373.5 nm respectively (Figure 2 a). ZnONPs biosynthesized at similar pH through *A. arabica* gave SPR peak at 368.5 nm, 371 nm, 377 nm (Figure 2 b) and through *C. roseus* observed the peak at 368.5 nm, 371 nm, 375 nm (Figure 2 c), respectively. Table 2 represents the band gap energy calculated by Tauc's plot method, of all the analyzed samples under the UV-visible spectroscopy. Previously reported that the biosynthesis of ZnONPs initially confirmed by UV-visible spectra at 370.5 nm [21]. Many other past studies are supporting the current results [22–24].

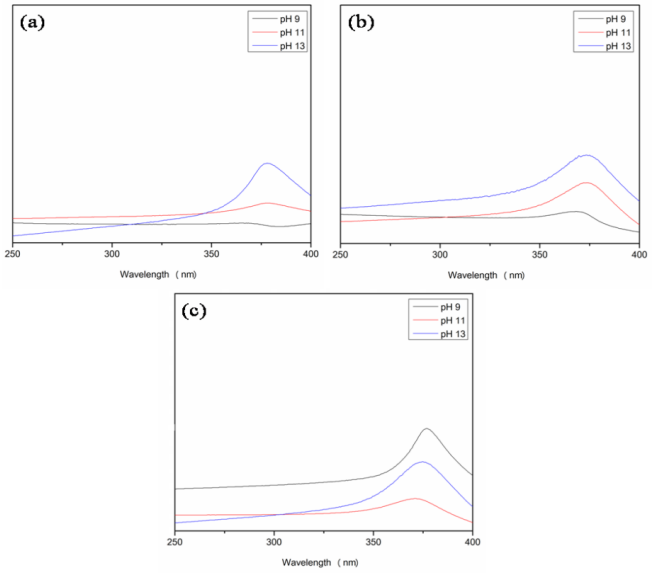

Fig 2. The UV-visible SPR spectral peak of biosynthesized fine powder by using *B. monnieri* (a), *A. arabica* (b), and *C. roseus* (c) leaf extracts at pH 9, pH 11 and pH 13

3.2.2 X-ray diffraction (XRD) analysis

The observed diffraction peaks 31.76°, 34.4°, 36.22°, 47.6°, 56.58°, 62.9° and 6.39° were corresponding to the Miller indices (100), (002), (101), (102), (110), (103), and (200) respectively. XRD analysis confirms the presence of zinc oxide with hexagonal

wurtzite structure (JCPDS card no: 36-1451) in the samples synthesized at various pH 9, 11 and 13 by using leaf extracts of *B. monnieri, A. arabica*, and *C. roseus* (Figure 3 a, b, c, d, f, g, h, i), except one sample synthesized at pH 11 by using leaf extract of *A. arabica* (Figure 3 e). All the data analyzed through Scherrer equation $B = k \lambda / \cos \theta$, where B is the crystallite size, K is the Scherrer constant, λ is the wavelength of the X-ray used and β is the full width at half maximum (FWHM) of the diffraction peak. The Scherrer equation calculates the estimated size of crystals present in the samples. Comparatively, ZnONPs synthesized at ordinary pH 9 have smaller crystal sizes than ZnONPs synthesized at pH 11 and 13 (Table 2). In a previous study, the presence of ZnO and its crystalline structure was confirmed by using XRD data [25]. The work reported by Hashem et al [26] and Kiani et al [27],also supports the current results. Samples synthesized at pH 9 were subjected to further characterization under the FESEM and zeta potential analyzer because of the smaller size of crystals present in the samples.

Table 2. The band gap energy of biosynthesized ZnONPs using leaf extracts of *B. monnieri*, A. arabica, and C. roseus at pH 9, 11, and 13 by UV-visible spectroscopy and the mean size of crystals present in the samples analyzed through XRD by applying Scherrer equation

Name of plant	pH	Band gap energy(by UV-visible spectrophotometer analysis)	The mean size of crystals(by XRD analysis)
B. monnieri	9	3.39 eV	28.06 nm
	11	3.32 eV	33.73 nm
	13	3.32 eV	32.32 nm
A. arabica	9	3.36 eV	18.65 nm
	11	3.34 eV	19.62 nm
	13	3.29 eV	30.52 nm
C. roseus	9	3.36 eV	16.71 nm
	11	3.34 eV	18.82 nm
	13	3.31 eV	26.36 nm

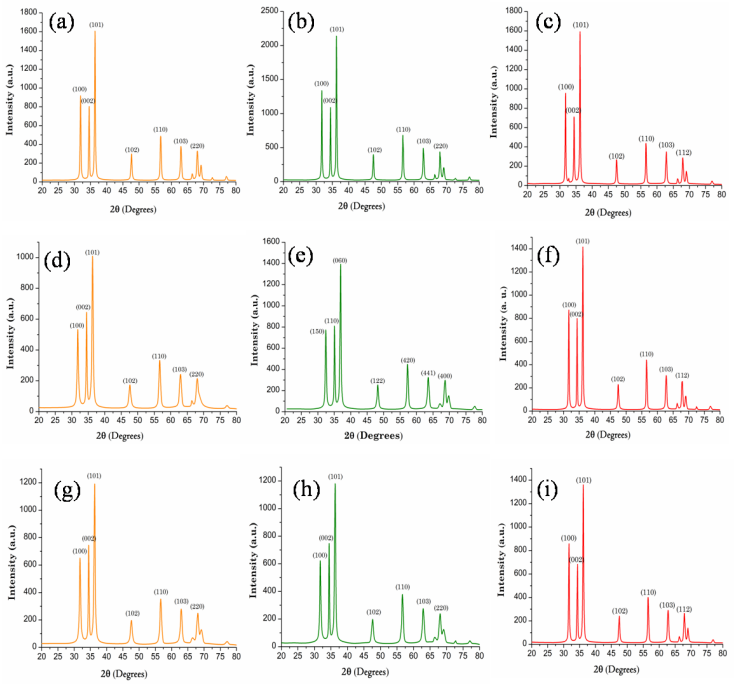

Fig 3. The spectrum of XRD shows the largest peak corresponding to the greatest number of crystals. Particles synthesized using leaf extract of *B. monnieri* at pH 9 (a), pH 11 (b), and pH 13 (c); similarly synthesized particles using leaf extract of *A. arabica* at pH 9 (d), pH 11 (e), and pH 13 (f); And synthesized using leaf extract of *C. roseus* at pH 9 (g), pH 11 (h), and pH 13 (i).

3.2.3 Field emission scanning electron microscopy (FESEM)

Three samples synthesized at pH 9 by using leaf extracts of *B. monnieri*, *A. arabica*, and *C. roseus* were analyzed under the FESEM (Figure 4 a, b and c, respectively). ZnONPs were synthesized using leaf extracts of *B. monnieri*, *A. arabica* and *C. roseus* with mean sizes of 33 nm, 35 nm and 36 nm respectively. Maximum NPs were synthesized between the size ranges of 20-40 nm with hexagonal shape. Comparatively, it was observed the particles synthesized by using *B. monnieri* leaf extract had a minimum size range between 40-60 nm while particles synthesized using leaf extracts of A. arabica and C. roseus were in 1-150 nm and 40-80 nm sizes respectively. NPs were agglomerated in condition due to electrostatic interaction in between. According to the previous investigation, the shape of biologically generated ZnONPs using SEM revealed nonspherical in shape[28]. Hayat et al[29] reported that rod-shaped ZnONPs were synthesized using leaf extract of *A. arabica* having diameter of 16–20 nm. The FESEM results are comparable to the other previous reports[30,31].

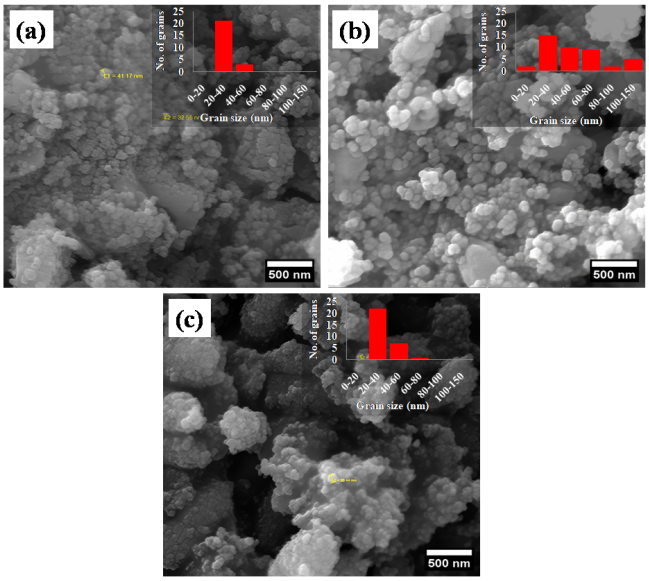

Fig 4. FESEM micrograph of biosynthesized ZnONPs using leaf extracts of (a) *B. monnieri*, (b) *A. arabica*, and (c) *C. roseus* at the resolution scale 500 nm. The included bar diagram represents the size range of synthesized particles.

3.2.4 Zeta Potential Analysis

The zeta potential of nano-powder synthesized at pH 9 by using leaf extracts of *B. monnieri* (Figure 5 a), *A. arabica* (Figure 5 b), and *C. roseus* (Figure 5 c) was observed (0.825), (-9.65), and (-7.54) mV respectively. Comparatively, it was also observed that the biosynthesized ZnONPs using *B. monnieri* leaf extract have positive charges on their surface while ZnONPs biosynthesized by using leaf extracts of *A. arabica* and *C. roseus* have negative charges. The similar charges in the particle's surface repel each other, which is good for the stability of NPs in the medium and particles will aggregate less[32]. Adebayo-Tayo et al[33] reported that the green synthesis of ZnONPs using leaf extract of *Senna alata* showed 0.595 polydispersity indexes. According to another report, the zeta potential of green synthesized ZnONPs was -18.4 mV[34].The charge on the particles should be between -30 to +30 mV. If particles have more charges on their surfaces (between the described ranges of charges) will have more stability in the medium[35].

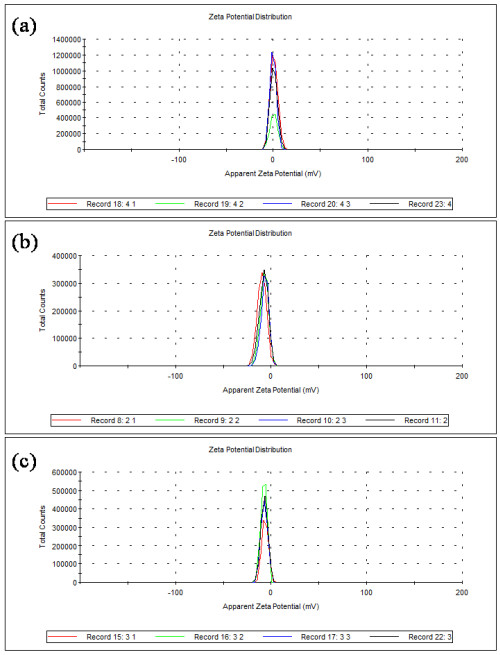

Fig 5. The above three graphs represent the zeta potential of samples synthesized at pH 9 by using leaf extracts of *B. monnieri* (a), *A. arabica* (b), and *C. roseus* (c)

4 Conclusion

It is concluded that the leaf extracts of B. monnieri, A. arabica, and C. roseus can work as natural capping and reducing agents. It is the first time that hexagonal-shaped ZnONPs with a mean size of 33 nm have been effectively biosynthesized by utilizing *B. monnieri* leaf extract. ZnONPs synthesized using A. arabica and C. roseus leaf extracts had larger mean sizes (35 nm and 36 nm, respectively). ZnONPs synthesized through *B. monnieri* leaf extract also have a compact size range with compared to the other two. During the biosynthesis process, the pH of the reaction mixture and reaction duration affects the production of NPs as well as their size. It was also observed that an increase in the pH of the reaction mixture decreases the quantity of the final product. Maximum nano-powder was synthesized at standard pH 9. The increment in standard pH of the reactions did not show any positive effect on the biosynthesis of ZnONPs while acidic pH inhibited the biosynthesis of ZnONPs. NPs synthesized using A. arabica and C. roseus leaf extracts were more stable in the medium because they had higher surface charges than NPs synthesized using *B. monnieri* leaf extract. The green synthesis of ZnONPs offers a promising and environment-friendly approach to the production of nano-material. By the utilization of natural extracts, this method reduces the need for harmful chemicals and energy-intensive processes typically associated with conventional synthesis techniques. The use of green synthesis also aligns with the principles of sustainable development and green chemistry. However, the biological process is slower than the chemical and physical methods for the synthesis of NPs. Continuous research and development in this field is essential to improve the efficiency of NPs biosynthesis.

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