Green Synthesis and Characterization of Zinc Oxide Nanoparticles using *Centella asiatica* Plant Extract

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Abstract

Green synthesis is an environmentally friendly way of producing nanoparticles that avoids the use of harmful chemicals, high temperatures, and expensive equipment in standard physical and chemical synthesis processes. In the current work, an environmentally friendly method was employed to produce zinc oxide nanoparticles (ZnO-NPs) using *Centella asiatica* leaf extract that act as a reducing agent. Fourier Transform Infrared Spectrometer (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), and Ultraviolet-Visible (UV–Vis) spectroscopy were used to characterise the synthesized ZnO-NPs. From FTIR, the peaks at wavenumber 650 cm⁻¹ and 415 cm⁻¹ represent Zn-O stretching. Through X-ray diffractograms, 11 peaks appear for ZnO-NPs, and the most intense peaks (100), (002) and (101) represent hexagonal quartzite structure. SEM images revealed the smallest size range of ZnO-NPs obtained is about 100 nm to 200 nm with mixtures of rod-like and spherical shapes. The Surface Plasmon Resonance (SPR) of ZnO-NPs exhibited at 375 nm. In conclusion, the green synthesis approach is quick, cheap, and environmentally friendly, and it produces stable ZnO-NPs comparable with chemical synthesis ZnO-NPs and has a variety of industrial and environmental applications.

Keywords Green synthesis, Centella asiatica, ZnO-NPs, nanoparticles

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1.0 INTRODUCTION

Nanotechnology is an interdisciplinary science that focuses on developing materials with nanoparticle sizes (1-100 nm) that can be used in various applications [1]. Nanoparticles have special chemical and physical properties because of their large surface area, scale, form, and surface morphology [2]. Among various metal oxide nanoparticles (MO-NPs) such as calcium oxide nanoparticles (CaO-NPs), titanium oxide nanoparticles (TiO₂-NPs), zinc oxide nanoparticles (ZnO-NPs) and magnesium oxide nanoparticles (MgO-NPs), ZnO-NPs have been widely explored due its wide applications in various field such as catalyst, anode materials in batteries and antibacterial agent [1-3].

ZnO has a high bandgap and binding energy of 3.3 eV and 60 meV, respectively. Due to these properties, ZnO-NPs are useful for optical coatings, solar cells, photocatalysts, lasers, and gas sensors [2,3]. Furthermore, ZnO is the second most abundant metal oxide after iron, and they are cheap, clean, and simple to prepare. Top-down approaches in synthesizing ZnO-NPs involve using chemical, physical and green approaches. Because of its low cost and fewer synthetic materials, green synthesis is an alternative method to replace the common chemical and physical approaches in synthesizing MO-NPs. Natural chemicals derived from plant sources have received much attention recently due to their ability to act as reducing and capping agents of NPs [4,5].

In this study, ZnO-NPs was synthesized using the co-precipitation method. The plant involved in this study is *C. asiatica* extract, also known as Gotu kola, a popular medicinal plant that has been used in Indian and Chinese medicinal systems for

thousands of years [6]. Several plants have been used in green synthesis of ZnO- NPs, such as *Nyctanthes arbor-tristis* [7] and *Euphorbia hirta* [8]. This study aimed to synthesize ZnO-NPs via green synthesis route using *C. Asiatica* plant extract and further characterize the obtained ZnO-NPs using Fourier Transform Infrared Spectrometer (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), Ultraviolet-Visible Spectrophotometer (UV-Vis) and Thermal Gravimetric Analysis (TGA) in selecting suitable calcination temperature.

2.0 EXPERIMENTAL

2.1 Preparation of Centella asiatica Plant Extract

Fresh *C. asiatica* plants were collected near Universiti Malaysia Terengganu in Kuala Nerus district. The plants were kept at room temperature, washed, dried and ground into a powder. 10 g of ground leaves was heated with 100 ml of distilled water and stirred using a magnetic stirrer for 1 hour. The mixture was filtered using Whatman's No. 1 filter paper. The obtained extract solution was used in synthesizing ZnO-NPs or kept at -4°C before subsequent use [9]. The extract was freshly used to avoid phytochemical degradation.

2.2 Phytochemical Screening Test

Standard procedures were employed for preliminary phytochemical screening to determine the phytochemical compounds in the *C. asiatica* plants, as seen in **Table 1** [8,10].

| Tests | Method |
|-----------|---|
| Saponin | Plant extract (1 mL) was gradually mixed into distilled water (2 mL), followed by shaking the mixture. The development of foam confirms Saponin. |
| Alkaloid | A few Mayer's reagent drops were mixed with plant extract (0.5 mL). Colour changes and the formation of a brown precipitate showed the presence of alkaloid. |
| Protein | A few drops of strong nitric acid (HNO ₃) were mixed with plant extract (2 mL), which caused the solution to become yellow, indicating the presence of proteins. |
| Flavonoid | A few drops of sulphuric solid acid were mixed with plant extract (0.5 mL). The colour changes were noticed, and the orange colour shows the presence of flavonoids. |
| Phenol | Alcoholic Ferric Chloride (FeCl ₃) solution (4 drops) was applied to 2 mL leaf extract. The existence of phenolic chemical is confirmed by changes of bluish black in the solution. |
| Terpenoid | Plant extract (0.5 mL) was mixed with chloroform (2 mL), and then sulphuric acid, H_2SO_4 (3 mL) was carefully added. A coating of reddish-brown hue indicates the presence of terpenoids. |
| Steroid | A few drops of acetic anhydride were mixed with plant extract (0.5 mL). A few drops of strong sulphuric acid, H ₂ SO ₄ , were added to the test tube, and a brown ring was noticed at the intersection of two layers. The top layer's green hue shows a positive test for steroids. |

| Table 1 | Phytochemical | tests towards C. | asiatica p | lant extract. |
|-------------|------------------|------------------|------------|---------------|
| 1 4 5 1 5 1 | 1 11000100110001 | | adiation p | |

2.3 Green Synthesis of ZnO Nanoparticles using Centella asiatica extract

For the green synthesis procedures of ZnO-NPs, 2.1951 g and 4.3902 g of Zn(CH₃COO)₂.2H₂O were dissolved in 200 mL of distilled water to provide two different concentrations of 0.05 M and 0.1 M, respectively. The Zn(CH₃COO)₂.2H₂O solution was then gradually mixed with 25 mL of *C. asiatica* plant extract. This mixture was then heated for 2 hours at 80°C with constant stirring using a magnetic bar. To eliminate undesirable contaminants, the solution was centrifuged for 20 minutes at 8000 rpm, rinsed with distilled water, and washed with methanol. The precipitate was dried at 60°C overnight before being calcined at 800°C and 900°C for 5 hours [11].

2.4 Characterization Techniques

Thermogravimetric Analysis (TGA) of the precursor sample was carried out using Mettler Toledo TGA Disc 1, consisting of a precision balance and a sample pan. The pan is placed in a furnace and either heated or cooled during the experiment. The sample environment is regulated by a purge gas, which runs over the sample and escapes through an exhaust. Spectra for dried and powdered ZnO-NPs were acquired using a Shimadzu IRTracer-100 in the range of 4000 to 500 cm⁻¹ [7]. The Fourier Transform Infrared Spectrometer (FTIR) aids in the identification of distinct phytochemical substances involved in nanoparticle reduction and stabilization. ZnO-NPs samples were used for XRD analysis with Rigaku MiniFlex II at 1.5406 wavelengths at 40 kV and 40 mA with a divergence slit of 10 mm. Using 20/0 continuous scanning mode, XRD was carried out in the range of 20°-80° [7]. SEM creates images of a sample surface by scanning it with a concentrated stream of electrons. The structural and morphological confirmation of produced nanoparticles was examined using SEM examination. TESCAN VEGA and JEOL JSM-6610LV scanning electron microscope were used to examine morphological traits (SEM). For UV-visible spectroscopy, the calcined samples from each reaction were resuspended in an equivalent amount of sterile distilled water, and spectra scans were taken using a UV-Vis Spectrophotometer Shimadzu UV-Vis 1800 in the wavelength range of 300-800 nm. The absorption data were re-plotted [7].

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Tests

Plant extracts normally contain various phytochemical compounds. These compounds include alkaloids, saponins, phenolics, terpenoids, tannins, proteins, amino acids, polysaccharides, enzymes, and vitamins. Due to these compounds, plant extract can be employed as a potential alternative for the reducing agent and stabilizing agent in the green synthesis approach. **Table 2** shows the results of a phytochemical screening examination of pure aqueous plant extract of *C. asiatica* in three replicates. According to **Table 2**, the principal chemical constituents of the extracts derived from *C. asiatica* plant extracts include alkaloids, flavonoids, saponins, phenols, proteins, steroids and terpenoids. The phytochemical test result was similar to the previous study [6,10], with all positive results except for negative results for alkaloids.

Studies have found that the prominent compounds that are responsible as reducing agents are phenols and flavonoids and could also stabilize metal and metal oxide NPs [8]. This is due to the existence of OH groups in both phenol and flavonoids that can reduce zinc acetate into ZnO NPs. Additionally, other functional groups such as C=O, C=O–C, and C=C of heterocyclic compounds might operate as stabilizers and reducing agents [8].

| Phytochemical | Test 1 | Test 2 | Test 3 |
|---------------|--------|--------|--------|
| Alkaloid | - | - | - |
| Flavonoid | + | + | + |
| Phenol | + | + | + |
| Protein | + | + | + |
| Saponin | + | + | + |
| Steroid | + | + | + |
| Terpenoid | + | + | + |

 Table 2
 Phytochemical test result towards C. asiatica plant extract.

note: (+) = Presence of active compound, (-) = No active compounds

3.2 Thermal Gravimetric Analysis

TGA analysis relies on reproducibility, hence two sets of ZnO samples were generated independently using the same procedure but at different concentrations. The TGA curves in **Figure 1** represent 2 curves of ZnO precursor at two different concentrations of precursors which are 0.05 M (red) and 0.1 M (black). Based on **Figure 1**, two weight losses are observed in the TGA curve. The first weight loss, approximately 5%, is observed at temperatures below 150°C and is attributed to the loss of residual water in the precursor followed by the second weight loss which starts at about 150°C, and the material undergoes a 45% weight

reduction progressively up to 600°C, but there is no plateau in this graph. This weight reduction is attributed to the decomposition of the precursor to form ZnO, accompanied by the production of heat. From this result, the calcination temperatures chosen were 800°C and 900°C since it appears that the formation of ZnO-NPs can be obtained at higher temperatures [12].



Figure 1 TGA graphes of ZnO precursor before calcination.

3.3 Fourier Transform Infrared Spectrometer (FTIR)

Figure 2 shows the FTIR spectra of *C. asiatica* plant extract and ZnO precursor before calcination for both concentrations at 0.05 M and 0. 1 M. Whereas, **Table 3** shows the corresponding band assignments for functional group representing O-H stretching at 3322.29 cm⁻¹, aromatic overtone from benzene ring at 2113.21 cm⁻¹, C=O stretching at 1636.87 cm⁻¹, -NH stretching at 1371.82 cm⁻¹, and C-H bending at 593.93 cm⁻¹. All samples have similar band assignments of functional groups which represent O-H stretching, aromatic overtone, C=O stretching, -NH stretching, C-O stretching, C-Cl stretching and Zn-O with slightly shifted wavenumber values [13]. These functional groups represent the phytochemicals compounds that can be found in the plant extract which are mixtures of flavonoids, phenols, and other compounds.

The initial shift in colour of the aqueous solution, from brown to dark brown, indicated the synthesis of ZnO-NPs has occurred, which were then further validated by FTIR spectra. All spectra had two tiny peaks at 3738.98 cm⁻¹ for 0.05 M at 800°C and 3743.90 cm⁻¹ for 0.05 M at 900°C, as can be seen in **Figure 3**. This owing to the vibration, which showed that the functional group of O-H stretching was stretching. Adsorbed water molecules, which are abundant in metal oxide samples, were responsible for the occurrence of this band [14]. Because of the presence of alkyl groups in *C. asiatica* leaf extract which can be seen from two peaks at 2370 cm⁻¹ to 2120 cm⁻¹ may belong to either C-H stretching or carbonyl. Between 1000 cm⁻¹ and 1300 cm⁻¹, the vibration mode C-O was found. There was a Zn-O stretching vibration present between 650 cm⁻¹ and 415 cm⁻¹. These findings indicate that phytochemicals from plant extract may contribute as a capping agent for ZnO-NPs [14-15].



Figure 2 FTIR spectra for (a) *C. asiatica* plant extract solution, (b) ZnO precursor at 0.05 M concentration and (c) ZnO precursor at 0.1 M concentration.

| Wavenumber (<i>cm</i> ^{−1}) Extract Solution | Wavenumber (<i>cm</i> ⁻¹) 0.05 M Pre-Calcined | Wavenumber (<i>cm</i> ⁻¹) 0.1 M Pre-Calcined |
|--|---|--|
| 3322.29 | 3277.66 | 3280.85 |
| 2113.21 | 2113.70 | 2115.48 |
| 1636.87 | 1561.52 | 1554.84 |
| 1371.82 | 1412.72 | 1412.99 |
| 593.93 | - | - |
| - | 1017.11 | 1016.13 |
| - | 607.23 | 612.94 |
| - | 440.48 | 486.77 |
| | Wavenumber (cm ⁻¹) Extract Solution 3322.29 2113.21 1636.87 1371.82 593.93 - - - - - - - - - - - - | Wavenumber (cm ⁻¹) Extract SolutionWavenumber (cm ⁻¹) 0.05 M Pre-Calcined3322.293277.662113.212113.701636.871561.521371.821412.72593.931017.11-607.23-440.48 |

Table 3 FTIR bands assignment for *C. asiatica* extract solution.



Figure 3 FTIR spectra of (a) 0.05 M at 800°C, (b) 0.05 M at 900°C, (c) 0.1 M at 800°C and (d) 0.1 M at 900°C.

3.4 Ultraviolet-Visible Spectroscopy (UV-Vis)

As demonstrated in **Figure 4**, all of the calcined ZnO-NPs show high absorption maximum ranging from 374 nm to 394 nm. Surface plasmon resonance (SPR) of ZnO-NPs calcined at the temperatures of 800°C and 900°C exhibited at 375 nm for both concentrations 0.05 M and 0.1 M, respectively. While SPR for ZnO-NPs calcined at 900°C exhibited at 381.50 nm for concentration 0.1 M and 384 nm for concentration 0.05 M. These findings are in agreement with the previous study which indicated the UV-Vis spectra of ZnO-NPs absorb between 350 nm until 380 nm [15].

Furthermore, it is known that ZnO-NPs with absorption at a greater wavelength in the UV-visible spectrum have bigger particle sizes. These results supported the presence of ZnO-NPs since the absorption bands obtained were remarkably comparable to previous studies that the observed peaks may be attributed to the intrinsic absorption of ZnO-NPs [16] and can be in relation with the band gap properties of ZnO [17].



Figure 4 UV-Visible spectra of ZnO-NPs calcined at 800°C and 900°C.

3.5 X-ray Diffraction (XRD)

Figure 5 depicts the XRD diffractograms of commercial ZnO and various ZnO-NPs calcined at 800°C and 900°C. The ZnO-NPs found were hexagonal wurtzite in structure, according to Joint Committee on Powder Diffraction Standards (JCPDS) card No.89-1397. All of the characteristic peaks found for ZnO-NPs were in good agreement with those obtained from JCPDS card No.89-1397, a=3.253 nm, c=5.213 nm. Sharp and prominent peaks can be seen at the diffractograms and match the JCPDS.

The (100), (002), and (101) planes corresponded to the three most intense peaks at corresponding 2theta (2) of 31.718°, 34.381° and 36.186° respectively. For ZnO-NPs, the optimal orientation matched the (101) plane. Their findings confirmed that the pure ZnO-NPs have been obtained for all samples and at both calcination temperatures as can be seen in XRD diffractograms. ZnO calcined at 800°C and 900°C had different values for average crystallite size, which were 33.37 nm (0.05 M at 800°C), 45 nm (0.1 M at 800°C), and 34.85 nm (0.05 M at 900°C), 46.53 nm (0.1 M at 900°C). The estimation of the crystallite size was calculated using the common Debye-Scherrer's formula [13,18].

Debye-Scherrer's formula:

$$\mathsf{D} = \frac{K\lambda}{\beta coscos\,\theta}$$

where D is the crystal size, λ is the wavelength of the X-ray, θ is the Bragg's angle in radians, and β is the full width at half maximum of the peak in radians.



Figure 5 XRD diffractograms of ZnO: (a) commercial, (b) 0.05 M at 800°C, (c) 0.05 M at 900°C, (d) 0.1 M at 800°C and (e) 0.1 M at 900°C.

3.6 Scanning Electron Microscope (SEM)

Figure 6 shows SEM images of ZnO-NPs calcined at 800°C and 900°C for both precursor concentrations at 0.05 M and 0.1 M. From the images, it can be seen that there are mixtures of spherical and rod-like shapes for all samples. An abundance of rod shapes can be seen at a calcination of 900°C from 0.1 M concentration. The smallest size range for all samples is about 100 nm to 200 nm. In addition, rod-like structures can normally obtained by facile and physical methods but in this study, it can be obtained through green synthesis using a co-precipitation approach. Furthermore, for various applications such as drug delivery systems and electronic devices, a rod-shaped nanostructure is considered the most preferred when compared to others. This preference arises from its one-dimensional nature, which contributes to enhanced stability and efficiency [19-21].





Figure 6 SEM images of a) 0.05 M at 800°C, b) 0.05 M at 900°C, c) 0.1 M at 800°C, and d) 0.1 M at 900°C at a magnification of 5000X.

4.0 CONCLUSION

ZnO-NPS has been successfully synthesized using *C. asiatica* plant extract. The process is a simple, one-step approach for the production of ZnO-NPs. Phytochemical compounds that exist in the plant extract act as both the reducing and stabilizing agents as can be seen from FTIR spectra. The average size obtained for the nanoparticle was calculated to be 46.53 nm, 45.0 nm, 34.85 nm, and 33.37 nm for 0.1 M (900°C), 0.1 M (800°C), 0.05 M (900°C), and 0.05 M (800°C) respectively, using XRD diffractograms data with highly crystalline features. Through SEM observation, the rod-like structure can be seen for the sample using a starting material concentration of 0.1 M at calcination temperatures of 800°C and 900°C with the smallest size range of about 100 to 200 nm. These findings show that a green synthesis approach using plant extract can be an alternative to a low-cost, straightforward, and environmentally friendly method of producing ZnO-NPs.

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