Zinc oxide from aloe vera extract: two-level factorial screening of biosynthesis parameters

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ABSTRACT

Zinc oxide (ZnO) was biosynthesised from aloe vera plant extract. The aloe vera plant extract was used as a reducing agent in biosynthesis process. Green synthesis method was proposed because it is cost effective and environmentally friendly. ZnO was characterised using SEM, EDX, FTIR, and XRD analyses. The antibacterial property was tested against Escherichia coli. The effects of aloe vera volume (2 – 50) mL, precursor concentration (0.001 – 0.300) M, reaction time (20 min – 48 h), and temperature of the reaction (26 – 200) °C on ZnO characteristics were investigated and screened using a two-level factorial method. Based on the observation and ANOVA analysis result, precursor concentration was the only significant parameter that affected the production of the ZnO nanoparticles (NPs). The EDX analysis proved the presence of ZnO while the SEM analysis confirmed the average size of ZnO particle size was in the range of (18 – 618) μm with a rod-shape appearance. The XRD analysis showed that the average crystallite size was 0.452 μm and it was in the hexagonal phase. It was also proven to have antibacterial property against E. coli.

1. Introduction

Nanomaterial research has been developing rapidly and has potential in various areas, including biomedical, magnetics sciences, biosensors, optoelectronics, and catalysis. In the past years, green synthesis of nanomaterials such as silver [1], zinc oxide [2], magnesium oxide [3], gold [4], cerium oxide [5], copper oxide [6] and titanium dioxide [7] has been conducted extensively due to simple work-up procedure, environmentally benign nature, reusable, low cost, and ease of isolation [8]. The biosynthesised NPs are also stable, capped by the biological compound, robust, and economical compared to other NPs produced by standard techniques [9].

ZnO is one of the most valuable nanomaterials and is potential to be used in the industry. Furthermore, ZnO has been recognized as safe to be used as a food additive by the Food and Drug Administration (FDA) [10]. ZnO possesses a wide bandgap yield (3.37 eV) and high excitation binding energy (60 meV) in which it absorbs a larger reaction of the UV spectrum and exhibits a greater photocatalytic performance than TiO₂ in the photodegradation of organic pollutants [11]. Physical, chemical, and biological methods have been used to synthesise ZnO particles. The synthesis route determines the properties of the produced NPs in terms of its crystal growth, morphology, size, size distribution, stability, and aggregation [12]. Due to the increasing popularity of biological methods, different sources like bacteria, fungus, algae, and plants have been used to produce ZnO NPs. Plant extract is used as an aid in the synthesis of NPs as it is cheap and safe to the environment. Various works on the use of plant extract to synthesise ZnO NPs have been reported, as listed in Table 1.

Aloe vera is a cactus-like plant since it is succulent, have thorns along the edge of its leaves, covered with wax, and contains a lot of water [26]. Aloe vera contains 99%–99.5% water and the remaining solid material contains over 75 active compounds including water- and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acids [27]. Aloe vera belongs to the lily family of Aloe barbadensis group and has 400 species [28]. Aloe vera is also well known for its medicinal properties and has been used as a soothing agent for burn and inflammation. Furthermore, the therapeutic properties of aloe vera have been employed in the commercial applications of

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Table 1. Research works using natural resources for the biosynthesis of ZnO NPs.

| Source | Precursor | Colour | Size (nm) | Shape | Functional group |
|--------|-----------|--------|-----------|-------|------------------|
| **Coriaria japonica** Aobl. leaf extract | Zinc acetate | White | n/a | Flakes | 3407, 2926, 1639, 1449, and 1040 cm$^{-1}$ ($\text{O-H}$, $\text{C=H}$, $\text{C-C}$, and $\text{C-O}$ of polyphenols and some aromatic compounds) 1321–1202 cm$^{-1}$ ($\text{C-O}$ stretch of phenolic compounds) 3407–3388, 2926–2923, 1639–1585, and 1040–1052 cm$^{-1}$ (phenolic compounds) 853 cm$^{-1}$ (ZnO) Bacillus cereus [13] Klebsiella pneumonia Escherichia coli Micrococcus luteus Salmonella typhi Vibrio cholerae |
| **Jasminum glandulosum** flower extract | Zinc gluconate hydrate | White | 2-4 | Spherical | 3273.12 cm$^{-1}$ ($\text{O-H}$ stretching vibration) 2942.29 cm$^{-1}$ ($\text{C-H}$ stretching) 2830.04 cm$^{-1}$ (carbonyl group) 1467.43 and 1031.62 cm$^{-1}$ ($\text{C-H}$ bending) 745.54 and 779.45 cm$^{-1}$ (ZnO) Escherichia coli Enterococcus faecium |
| **Trifolium pratense flower extract** | Zinc oxide | 60–70 | n/a | Spherical | 3245 and 1599 cm$^{-1}$ (hydroxyl group $\text{O-H}$) 1383 and 1076 cm$^{-1}$ ($\text{C-O}$ and $\text{C-O-C}$ stretching modes) 2168 cm$^{-1}$ ($\text{C=C}$ stretching) 515 cm$^{-1}$ (ZnO stretching vibration) Staphylococcus aureus Escherichia coli Pseudomonas aeruginosa |
| **Nyctanthes arbortristis flower extract** | Zinc acetate | White | 12–32 | Spherical and oval | 3340.6 and 3258.2 cm$^{-1}$ ($\text{H}$ bonded $\text{OH}$ stretch and $\text{N-H}$ stretch) 1641.1 cm$^{-1}$ (stretching bands of $\text{C-O}$ functional groups) 1456.7 cm$^{-1}$ (amine –$\text{NH}$ vibration stretch in protein amide linkages) 1362.5 cm$^{-1}$ (aromatic amines) 1040 and 1026.8 cm$^{-1}$ ($\text{C-N}$ stretch of aliphatic amines) 746.25 cm$^{-1}$ (alkanes) 620.65 cm$^{-1}$ (alkynes) n/a [16] |
| **Lactobacillus plantarum** VITES07 | Zinc sulfate | White | 7–19 | Spherical | 3441 cm$^{-1}$ (symmetric stretching mode of water molecules) 1631, 1400, 1068 cm$^{-1}$ (bending vibrational mode of water molecules) 528 cm$^{-1}$ (ZnO) n/a [17] |
| **Palm pollen** | Zinc acetate | White | n/a | Spherical | 3349–2925 cm$^{-1}$ (primary amine, $\text{OH}$ stretching of alcohols and $\text{CH}$ stretching vibrations of alkanes) 1642 cm$^{-1}$ (stretching vibration of $\text{NH}_2\text{C-O}$) 1569 and 1433 cm$^{-1}$ (stretching vibration of $\text{COO}$ groups in the amino acid) 600–400 cm$^{-1}$ (Zn-O) n/a [18] |
| **Eichhornia crassipes leaf extract** (Aquatic weed) | Zinc nitrate | White | 32 | Spherical | 340 cm$^{-1}$ (bending vibrational mode of water molecules) 2923, 1639 | n/a [19] |
| **Rosa canina** (Rosehip) | Zinc nitrate | n/a | <50 | Spherical | 470 and 433 cm$^{-1}$ (Zn-O) 1107, 1049, and 1117 cm$^{-1}$ ($\text{C-O}$ stretching mode of esters) 2855 and 2924 cm$^{-1}$ ($\text{C-H}$ stretching) 1742 and 1741 cm$^{-1}$ ($\text{C-O}$ of esters) Staphylococcus aureus Listeria monocytogenes Escherichia coli [20] |
| **Anchusa italica flower extract** | Zinc acetate | White | 7.6–14.3S | Hexagonal | 3422 cm$^{-1}$ (stretching vibration of $\text{O-H}$) 2943 cm$^{-1}$ (stretching vibration of $\text{C-H}$) 1727 and 1390 cm$^{-1}$ ($\text{C-O}$ stretching vibrations) 1451 and 1271 cm$^{-1}$ ($\text{O-H}$ and $\text{C-OH}$) 435 and 420 cm$^{-1}$ (stretching vibrations of zinc oxide) Staphylococcus aureus Escherichia coli Salmonella typhimurium [21] |
| **Zingiber officinale** (Dry ginger rhizome) | Zinc carbonate | Yellow | 23–26 | Spherical | 865 cm$^{-1}$ (CH) 1105 cm$^{-1}$ (CH$_3$) 1192, 1495, and 2926 cm$^{-1}$ (CH$_2$-OCH$_3$/CH$_2$-CH$_3$) 3445 and 3836 cm$^{-1}$ (OH) Klebsiella pneumoniae Staphylococcus aureus Candida albicans Penicillium notatum [22] |
| **Cuminum cyminum** (Cumin) | Zinc nitrate | N/a | 7 | Spherical or oval | n/a Bacillus subtilis Listeria monocytogenes Enterococcus faecalis Pseudomonas aeruginosa Escherichia coli Klebsiella pneumoniae [10] |
| **Nephetium lappaceum L.** (Kambutan peel extract) | Zinc nitrate | n/a | 25–40 | Spherical | 3439 cm$^{-1}$ ($\text{O-H}$ stretching vibration) 1629 cm$^{-1}$ ($\text{H-O-H}$ bending vibration) 1383 cm$^{-1}$ ($\text{C-}$ and $\text{C-O-C}$ stretching modes) 446 cm$^{-1}$ (Zn-O stretching vibration) n/a [23] |
| **Azadirachta indica** (Neem) leaf extract, (curry leaf) | Zinc acetate | White | 9.6–25.5 | Spherical | 400–600 cm$^{-1}$ (ZnO) 2987 and 3197 cm$^{-1}$ (amide linkage) 1081 and 1036 cm$^{-1}$ ($\text{C-N}$ stretching vibrations) 713 and 844 cm$^{-1}$ ($\text{C-H}$ stretching of alkanes, $\text{C-H}$ (aromatics), $\text{C-C}$ (alkynes), and $\text{O-H}$ stretching of intramolecular H-bond, $\text{C-O}$, and $\text{C-C}$ stretching of alkanes) Staphylococcus aureus Streptococcus pyogenes Escherichia coli [24] |

(continued on next page)
pharmaceutical, food, and cosmetics [29]. The extract of aloe vera plant has been used for the synthesis of gold [4], silver [30], copper oxide [31], indium oxide [32], titanium dioxide [33], cerium oxide [34], and tin oxide [35].

Engineers and scientists generally employed one-factor-at-a-time (OFAT) experiments that vary from one factor or parameter to the next with constant variables. However, statistically designed experiments with several different factors are more effective when two or more factors are investigated at the same time [36]. The advantage of factorial design becomes more pronounced as more factors are added. For example, with three factors, the factorial design requires only eight runs (in the form of a cube) versus sixteen for an OFAT experiment with an equivalent power [37]. Moreover, factorial design offers two additional advantages over OFAT in terms of broader inductive basis and revealing the interactions of factors. In this study, two-level factorial screening approach was used to discover the significant parameters in obtaining ZnO via biosynthesis. Two-level factorial design is one of the components in response surface methodology using Design Expert software, which is used to estimate the first-order effect and also could be used for optimization, the next level in evaluating this experiment.

2. Materials and method

Zinc nitrate used as the precursor was purchased from QRec and was used without treatment. The aloe vera leaves were collected locally from Johor, Malaysia.

2.1. Preparation of aloe vera plant extract

The method was adapted from Ali, Dwivedi, and Azam [38]. The aloe vera extract was prepared by finely cutting the aloe vera leaf. Twenty grams of aloe vera was mixed with 100 mL of distilled water (1:5) and stirred at 60 °C for 10 min. Next, the mixture was cooled at room temperature and filtered using vacuum filtration. Finally, the extract was stored at 4 °C until further use.

2.2. Biosynthesis of ZnO using aloe vera extract

The biosynthesis of ZnO was conducted by applying a screening step using two-level factorial experimental design with the aid of Design Expert software. The purpose of the screening steps was to determine the parameters that influenced the production of the nanoparticles. Four parameters were studied, namely aloe vera volume, reaction time (stirring time), precursor concentration, and temperature. Table 2 shows the parameters and their ranges applied in this work. The aloe vera plant extract was added with zinc nitrate as the precursor with a total volume of 200 mL. The precipitate obtained was dried at 80 °C Figure 1 shows the whole process of ZnO biosynthesis.

Table 2. Minimum and maximum values for each factor.

| Factor  | Parameter                     | Unit   | Type    | Minimum | Maximum |
|---------|-------------------------------|--------|---------|---------|---------|
| A       | Aloe vera volume              | mL     | Numerical | 2.00    | 50.00   |
| B       | Reaction time                 | h      | Numerical | 0.3330  | 48.00   |
| C       | Precursor concentration       | M      | Numerical | 0.0010  | 0.3000  |
| D       | Temperature                   | °C     | Numerical | 23.00   | 200.00  |

Figure 1. Process flow of ZnO biosynthesis.
2.3. Characterisation of ZnO

The characterisation of ZnO was carried out using SEM, EDX, FTIR, and XRD analyses. The produced ZnO was also tested for antibacterial property.

2.3.1. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) analyses

The size and morphology of ZnO were examined using scanning electron microscopy (SEM) analysis and the elementary determination was carried out using energy-dispersive X-ray spectroscopy analysis (U1510, Hitachi) with an acceleration voltage of 15 kV.

2.3.2. Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR analysis was conducted by using Perkin Elmer FTIR Spectrometer 100 via the KBr method to determine the functional groups in the sample.

2.3.3. X-ray diffraction (XRD) analysis

X-ray diffraction analysis (Bruker D8) was used for identification, purity, and quantitative analyses of various forms of ZnO particles in the 2θ range from 20° to 80° and operated at 40 mA and 45 kV.

2.3.4. Antibacterial test

The biosynthesised ZnO was tested against E. coli via disk diffusion technique. The pure cultures of E. coli were swabbed uniformly on individual plates using sterile cotton swabs. Analysis of the inhibition zone was carried out after 24 h incubation at 37°C.

3. Results and discussion

3.1. Two-level factorial screening for biosynthesis of ZnO

Table 3 shows the results of the experiments for the two-level factorial screening for the biosynthesis of ZnO. The experimental runs 1, 2, 3, 5, 6, 7, 10, and 12 did not produce any ZnO NPs. The mixture of aloe vera extract and zinc nitrate precursor was dried out without producing any NPs. Meanwhile, the experimental runs 4, 8, 9, 11, 13, 14, 15, and 16 produced ZnO NPs from 4.00 to 5.47 g. The mixtures of aloe vera and zinc nitrate precursor formed white or brown slurry after 2 or 3 days of drying and fully dried after 5 days.

Table 4 lists the summary of analysis of variance (ANOVA) for the factors in the biosynthesis of ZnO. Precursor concentration shows an effect on NPs production. At low precursor concentration, no reaction occurred. The model $R^2$ of 0.9809 and the P-value less than 0.05 indicates that the model terms are significant. The half-normal plot and the Pareto chart (Figure 2a and b) show that the precursor concentration gave the most significant effect in the biosynthesis of ZnO.

3.2. Characterisation of ZnO

3.2.1. Morphology and elemental analysis of ZnO

The SEM images of ZnO are shown in Figures 3a and 3b. The images showed that the materials are flaky and in rod shape, however, other studies using other plants produced a spherical or oval shape [10, 14, 17, 18, 23, 24]. Figure 4 shows the particle size distribution in the range of 18–618 μm with the mean size of 240.167 μm. Analysis via EDX confirmed the presence of Zn element (40.47%), O (47.93%), and N (11.61%) as shown in Figure 5.
3.2.2. FTIR analysis

In FTIR spectrum (Figure 6a), the peak observed between 3283 and 3466 cm\(^{-1}\) is attributed to the stretching vibration of the hydroxyl group and the peak appeared at 1637 cm\(^{-1}\) is attributed to the amino groups present in alcohol, phenol, and amines in the aloe vera extract involved in the NPs synthesis [33]. This observation is similar to the data reported by Medda [39]. Meanwhile, the peak observed around 1000 cm\(^{-1}\) and below is ascribed to the existence of metal oxide (Figure 6b). The ZnO was formed from aloe vera leaf extract where the free carboxylic and the amino group of the plant extract acted as both reducing and capping agent [40]. Although in the biosynthesis, the standard mechanism of NPs is yet to be formed, the result from FTIR analysis suggests that the biological molecules may have a double function in the ZnO formation and stability in the medium [18]. The possible mechanism of interaction between Zn ions with plant phytochemical is the repetition of redox reaction during glycolysis process that involves the conversion of carbohydrate to energy. A large amount of H\(^+\) ions are produced along with the adenosine triphosphate (ATP) production. ATP is an energy carrier and nicotinamide adenine dinucleotide (NAD\(^+\)) is a coenzyme found in all living cells. NAD\(^+\) is a strong oxidizing agent that accepts electron from

![Figure 2. (a) Half-normal plot and (b) Pareto chart of the response.](image)

![Figure 3. SEM images of ZnO at (a) 35× and (b) 1000× magnification levels.](image)

![Figure 4. Particle size distribution of ZnO.](image)
other molecules resulting in the reduction process producing NADH that can donate electrons. Thus, this redox reaction was kept repeating in a cycle and this led to the transformation of Zn ions to Zn$^0$.

\[
\begin{align*}
\text{Zn(NO}_3\text{)}_2 & \rightarrow \text{Zn}^{2+} + \text{NO}_3^{-} \quad (1) \\
2\text{NAD}^+ + 2e^- & \rightarrow 2\text{NAD} \quad (2) \\
2\text{NAD}^+ 2\text{H}^+ & \rightarrow 2\text{NADH} + 2e^- \quad (3) \\
\text{Zn}^{2+} + 2e^- & \rightarrow \text{Zn}^0 \quad (4)
\end{align*}
\]

3.2.3. Compound identification and crystallinity

XRD analysis is used to identify the crystalline structure of the biosynthesised ZnO particles. The XRD pattern shown in Figure 7 indicates the production of ZnO particle at diffracted intensities ranging from 20° to 80°. The peak at 2θ = 31.999°, 34.480°, 36.855°, 47.481°, 56.355°, 63.579°, 65.598°, 69.148°, 75.892°, and 82.951° are assigned to the (0 1 0), (0 0 2), (0 1 1), (1 1 0), (0 1 3), (0 2 0), (0 2 1), (0 2 2), and (0 2 3) reflection lines of hexagonal ZnO particles, respectively. The

![Figure 5. EDX spectrum of biosynthesised ZnO.](image)

![Figure 6. FTIR spectra of (a) pure aloe vera plant extract and (b) biosynthesised ZnO NPs.](image)

![Figure 7. XRD spectrum of biosynthesised ZnO.](image)
calculated average crystallite size was 0.452 μm. The intensity of the resulting ZnO particles was quite small due to the biological synthesis adopted to prepare the NP [17].

3.2.4. Antibacterial activity
After 24 h of incubation, the active inhibition zone measured was around 1.325 mm² (see Figure 8). The existence of inhibition zone clearly indicates the involvement of membrane disruption and leads to the death of pathogens. ZnO is a well-known antibacterial agent and effective at very low concentration of bacteria as confirmed by previous studies [40]. In addition, the small size of the biosynthesised ZnO provides a large surface area and this leads to more contact between the NPs and the bacterial cells [11]. From the result, the biosynthesised ZnO from aloe vera plant extract has been proven to have antibacterial property. This is in accordance with Gunalan, Sivaraj, and Venkatesh [41] which demonstrated that the biosynthesised NPs at various concentrations of 2–12 mM reacted against bacteria such as Staphylococcus aureus, Serratia marcescens, Proteus mirabilis, and Citrobacter freundii and as well as fungi like Aspergillus flavus, Aspergillus nidulans, Trichoderma harzianum, and Rhizopus stolonifera.

4. Conclusion
In this study, we have reported a green approach for ZnO synthesis using aloe vera plant extract. The method is simple, inexpensive, and eco-friendly without using any toxic chemicals. The result from two-level factorial screening shows that precursor concentration was a significant factor in the synthesis of ZnO. The EDX analysis confirmed the presence of ZnO while the SEM analysis showed the average size of ZnO particle size was in the range of (18–618) μm with a rod-shape appearance. The XRD analysis showed that the average crystallite size was 0.452 μm and it was in the hexagonal phase. It was also proven that the produced ZnO possessed antibacterial property against E. coli bacteria.

Declarations

Author contribution statement
Nurul I Rasli: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Hatijah Basri, Zawati Harun: Contributed reagents, materials, analysis tools or data.

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Competing interest statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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