

Green Synthesis and Characterization of Zinc Oxide Nanoparticles Using Mulberry Fruit and Their Antioxidant Activity

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Biosynthesis of metal-oxide nanoparticles using plant extracts has been attracting increasing interest. In this study, we focused on the green synthesis of zinc oxide (ZnO) nanomaterials using zinc acetate as a precursor and mulberry fruit extract as a green reducing agent and determined the antioxidant activity. Powder X-ray diffraction and UV-Vis and Fourier Transform Infra-Red (FT-IR) spectroscopy were used for structure elucidation and to determine the crystallinity of the synthesized product. The morphology of samples was determined using Scanning Electron Microscopy (SEM). Our results indicated the successful synthesis of ZnO nanoparticles. SEM findings revealed the nanoparticles to be spherical; they were found to agglomerate and showed a narrow space between particles, which could be indicative of improved activity. The antioxidant activity of ZnO nanoparticles was determined using a 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) free-radical scavenging assay taking into account time and concentration. Our results indicated that ZnO nanoparticles with mulberry fruit extract that were synthesized using green chemistry could effectively scavenge the free DPPH radicals, thereby confirming their superior antioxidant activity.

Keywords: antioxidant activities, green synthesis, mulberry fruit, nanoparticles, zinc oxide.

1. INTRODUCTION

Nanotechnology is one of the most rapidly developing fields of research in material science. The interest in this area has been constantly expanding as researchers delve deeper for further understanding of the synthesis of various nanoparticles.

Nanomaterials ranging from 1–100 nm are typically studied the most. The properties of nanomaterials include enhanced catalytic reactivity, thermal conductivity, non-linear optical performance, chemical stability, high surface energy owing to a large surface area, and a wide bandgap between the valence and conduction bands [1]. These nanoparticles can be generated using several metals, including titanium, silver, gold, zinc, copper, and iron. Nanoparticles have various applications in physics, chemistry, and material science.

Nanoparticles can be synthesized using physical, chemical, and green methods [2–4]. Physical methods, such as sputtering, milling, and nano-sphere lithography entail expensive equipment and the use of high temperatures and vacuum and rely on processes including pulsed laser deposition [5]. Chemical methods typically involve the use of toxic chemicals that are used to prepare microemulsions. These are environmental pollutants, and moreover, they can pose various health risks [6]. Additional capping and stabilizing agents are required for the synthesis of nanoparticles using physical and chemical processes [7–10].

Therefore, an environmentally friendly synthetic procedure is much needed to address these drawbacks. Biosynthesis is a much sought-after approach owing to the ease of synthesis and the use of low temperature [11]. Compared to physical and chemical processes, the synthesis of nanoparticles using green chemistry is associated with several advantages. Apart from being eco-friendly and inexpensive, chemicals having low toxicity profiles are used; consequently, this technique results in the generation of fewer toxic wastes and is effective in reducing environmental pollution [12, 13]. The synthesis of metal and metal oxide nanoparticles may involve the incorporation of plant, bacterial, fungal, algal, and microbial metabolites, as well as the extracts of certain fruits and vegetables. Microbial metabolites and plant extracts contain components that function as reducing agents and stabilizers.

Among metal oxide nanoparticles, ZnO has attracted increasing attention owing to its stimulating properties and high binding energy of 60 meV. Moreover, this metal oxide has been reported to have antibacterial, antifungal, and UV-blocking properties. It is a non-toxic semi-conductor with high transparency and good photocatalytic properties, all of which have led to its use in the preparation of nanoparticles with antibacterial, antimicrobial, catalytic, and optical properties [14, 15]. The green synthesis of ZnO nanoparticles is a simple, single-step, and an environmentally friendly procedure that does not use any hazardous chemicals. ZnO nanoparticles incorporating different plant extracts, including those of leaves [16–18], roots [19], rhizomes [20], fruits [21, 22], and flowers

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[23–25], have been synthesized. Mulberry is a plant belonging to the Moraceae family. Mulberry fruit is a rich source of sugars, organic acids, lipids, vitamins, pigments, non-pigment phenols, and Minerals Split. As a traditional Chinese medicine, Mulberry has the functions of nourishing the liver, benefitting the kidney, and regulating tune ear of Mulberry. Recent studies have also shown that mulberry plays a role in regulating and promoting immunity [26], promoting the growth of hematopoietic cells, preventing arteriosclerosis, resisting mutation, lowering blood glucose levels, resisting oxidation, delaying aging, and preventing viral infections. It has antioxidant effects owing to its radical-scavenging ability. Mulberry is well studied and is known to exert several biological activities owing to the presence of various phytochemicals. These bioactive components have been reported to exert immunoregulatory, antioxidant [27, 28] anti-cancer [29], anti-thrombotic [30], anti-obesity [31], and anti-inflammatory [32], and neuroprotective [33] effects.

Mulberry fruit extract was used as a reducing material in the green synthesis of silver (Ag), copper (Cu), and palladium (Pd) nanoparticles (NPs) for the preparation of antibacterial nanocellulose films [34].

Phenolic compounds possess antioxidant properties and are useful in the reduction of metal ions. Additionally, high levels of proteins, lipids, and amino acids stabilize nanoparticles and inhibit their agglomeration. Thus, these compounds have been widely used for the photosynthesis of gold [35], zero-valent iron [36], and silver [37] nanoparticles using green chemistry and a time-efficient heating process using microwave treatment. No external chemical stabilizing, capping, or complexing agents are used. The mulberry paper from tube sedge is coated with the ZnO obtained directly by calcination to investigate their antifungal properties [38]. Using a simple TEA-assisted microwave hydrothermal method, [39] ZnO nanoparticles containing mulberry-fruit extract were synthesized on a large-scale and were found to exhibit potent antibacterial effects. To the best of our knowledge, the use of mulberry fruit for the green synthesis of ZnO has not been reported in the literature. Moreover, there are no previously published studies on the antioxidant properties of ZnO nanoparticles containing the fruit extract of mulberry.

In this study, we used a green chemistry approach to synthesize ZnO nanoparticles containing the fruit extract of mulberry. The formation of nanoparticles was confirmed using X-ray diffraction (XRD), and Fourier-Transform infrared (FT-IR) spectrometry. The Morphology of ZnO nanoparticles was characterized using scanning electron microscopy (SEM). Finally, the antioxidant activity of the ZnO nanoparticles was tested using 1-diphenyl-2-picrylhydrazyl (DPPH) in terms of nanoparticle concentration and time interval individually. The DPPH scavenging activity was determined using UV-Vis spectrophotometry.

2. EXPERIMENTAL DETAILS

2.1. Materials

Mulberry fruits were bought from China (Ningxia) (Fig. 1). Zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$) dehydrates with

99% purity were used as the precursors. Deionized water was used for all reactions.



Fig. 1. Mulberry fruit

2.1.1. Extract preparation

Mulberry fruits were first washed several times with double-distilled (D.D.) water to remove dust particles from the surface of the leaves. To prepare the leaf extract, 50 g of Mulberry fruit was mixed with 200 mL of D.D. water and magnetically stirred for 3 h in a water bath maintained at 90 °C. The resultant solution was filtered through Whatman No. 1 filter paper. The filtrate was used for the green synthesis of ZnO nanoparticles from Mulberry fruit.

2.1.2. Synthesis of zinc oxide nanoparticles

To prepare ZnO nanoparticles, 0.5 M zinc acetate was mixed with 100 mL of the plant extract and stirred vigorously for 3 h at 90 °C. The resultant precipitate was allowed to settle for 24 h, separated from the reaction solution by centrifugation at 7000 rpm for 10 min, and repeatedly washed with deionized water to remove the impurities. Lastly, the product was dried in an oven at 80 °C. The powdered product was then subjected to calcination in a muffle furnace at 600 °C for 2 h to get a pale white powder. The fine yellowish powder of ZnO nanoparticles with Mulberry fruit extract was stored until further use in characterization studies.

2.1.3. Characterization

X-ray diffraction (Philips, X'pert, Pro-MPD, Cu-K α 40 kV, 20 mA) was used to evaluate the phase purity and the structural properties of the synthesized ZnO nanoparticles. It was operated at 40 kV and 30 mA, with 2°/min (scanning rate), 0.02 (sample interval), and range from 20° to 80°. The pattern was recorded using Cu-K α radiation at 1.54060 Å.

Scanning rate of 2°/min and a sample interval of 0.02 were employed in the 2 h range from 20° to 80°. The pattern was recorded by Cu K α radiation with about 1.54060 Å.

The functional groups of ZnO nanoparticles were determined using FTIR spectrometry spanning a frequency range of 4000 cm^{-1} to 400 cm^{-1} . The surface morphology of the nanoparticles was determined using SEM. The UV-Vis spectra of the prepared nanoparticles were obtained using spectrophotometry (Lambda 750 UV-VIS-NIR, PerkinElmer, USA) at a wavelength ranging from 200–700 nm.

2.1.4. Antioxidant activity

The antioxidant activity of ZnO nanoparticles was determined using Brand Williams's method and 1,1-

diphenyl-2-picrylhydrazyl (DPPH) assay based on the radical-scavenging properties [40].

Briefly, 3.2 mL (0.14 μM) of DPPH was first evaluated by storing in the dark for up to 60 min to verify its stability, based on the change in color and absorbance at 517 nm. Then, 20, 30, 50, and 100 mg of ZnO nanoparticles were each mixed with 3.20 mL (0.14 μM) of DPPH, sonicated, and kept in the dark for 30 min. A gradual color change was observed in the presence of ZnO.

To determine the antioxidant activity as a function of time, 100 mg of ZnO nanoparticles was mixed with 3.2 mL of DPPH and incubated for 15, 30, 45, 60, and 90 min. DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [1 - A_s/A_c] \times 100, \quad (1)$$

where A_c and A_s are the intensity of peak at 517 nm for the control (DPPH) and supernatant DPPH solvent respectively.

3. RESULTS AND DISCUSSION

In this study, phytochemicals from mulberry fruit were used as reducing and stabilizing agents for the synthesis of ZnO nanoparticles. The dominant phenolic constituents of mulberry fruits comprise two phenolic acids, four anthocyanins, and four flavonols, all of which possess excellent antioxidant properties [41].

Thus, these compounds were responsible for the reduction of Zn^{2+} ions in an aqueous solution and stabilizing the Zn atoms. Polyphenols are also known for their anticancer effects [42, 43].

The possible chemical mechanism for the synthesis of Mulberry/ZnO- nanoparticles is presented in Fig. 2.

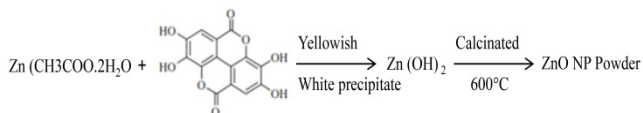


Fig. 2. Possible mechanism of the formation of ZnO nanoparticles containing mulberry fruit extracts

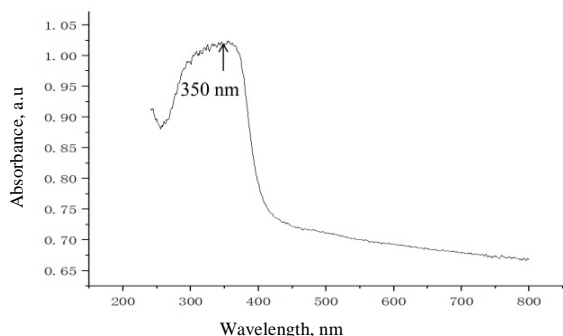


Fig. 3. UV-absorption spectra of ZnO nanoparticles

Due to the high temperature of calcination (600 $^{\circ}\text{C}$), this complex undergoes direct decomposition and conduct to synthesis of zinc oxide nanostructures. In addition, the Mulberry fruit extract has important anti-oxidant potential due to the presence of polyphenol compounds and present easy electron losing capacity, which contributes to the stabilization of positively charged Zn complex ions.

3.1. UV-visible spectrum analysis

The first indication of the formation of ZnO nanoparticles has been established by using UV-Vis spectrophotometry. The absorption spectrum of ZnO prepared using mulberry fruit extract is shown in Fig. 3. A strong broad absorption band can be seen around 350 nm, which is indicative of the formation of ZnO nanoparticles. This intense absorption band can be attributed to the surface plasmon resonance of the formed ZnO nanoparticles. Additionally, this broad peak extending from 300 to 380 suggests that the particle size distribution is large.

3.2. XRD

The biosynthesis and the crystallinity of the synthesized samples were analyzed using XRD [44]. The XRD peaks of the synthesized ZnO nanoparticles are shown in Fig. 4. The XRD pattern of ZnO shows the main diffraction peaks that appeared at the following diffraction angles (2θ): 31.63, 34.3, 36.01, 47.3, 56.4, 62.6, 67.6, and 69.03 corresponding to lattice planes (h, l, k) of (100), (002), (101), (102), (110), (103), (112) and (201), respectively. These observations confirmed the crystalline Wurtzite ZnO structure of the nanoparticles. Moreover, the strong and narrow diffraction peaks that were obtained are indicative of a good degree of crystallinity of the nanoparticles. The absence of other diffraction peaks arising due to the presence of impurities and intermediate materials indicated the high purity of the synthesized ZnO nanoparticles. The crystallite size of the nanoparticles was calculated using the Debye-Scherrer formula [45]. The average crystallite size was determined to be 25.2 nm based on the most intense peak corresponding to the (101) plane located at 36.01. Comparable crystalline size dimensions of about 30 and 35.41 nm have been obtained using *Cassia auriculata* leaf extract [46] and *Gynostemma* plant [47] extract, respectively.

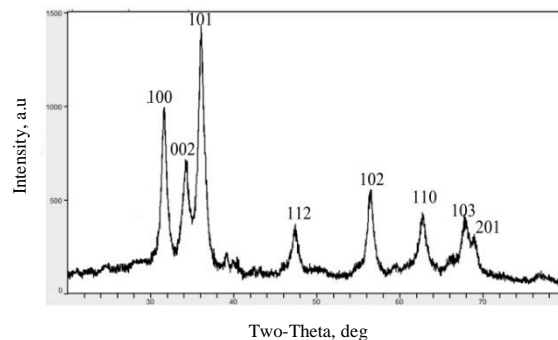


Fig. 4. XRD pattern of ZnO nanoparticles

3.3. FT-IR spectroscopy

Fig. 5 shows the FT-IR spectra of ZnO nanoparticles obtained at a resolution of 4000–500 cm^{-1} . The presence of ZnO nanoparticles is established by the absorption peak at 460.1 cm^{-1} , which corresponds to the stretching vibrations of ZnO [48]. Weaker bands observed at around 962.24, 1045.99, and 1551.34 cm^{-1} are assigned to the alkane (C-H), alcohol (C-O), and alkane di-substitutions (C-C), respectively. The presence of these peaks explains the successful role of phytochemicals as capping and stabilization agents for ZnO nanoparticles.

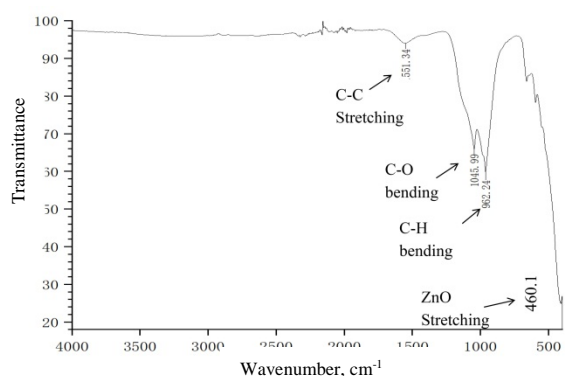


Fig. 5. FT-IR spectra of the synthesized ZnO nanoparticles

3.4. SEM

SEM was used to determine the surface morphology of the ZnO nanoparticles. Fig. 6 shows that most of the nanoparticles are spherical with an average size of about 252 nm as we can see from red dotted circle in Fig. 6 c.

In addition, it is also shown that the ZnO nanoparticles enclose a narrow space between particles, which could be an indication of expected improved antioxidant activity.

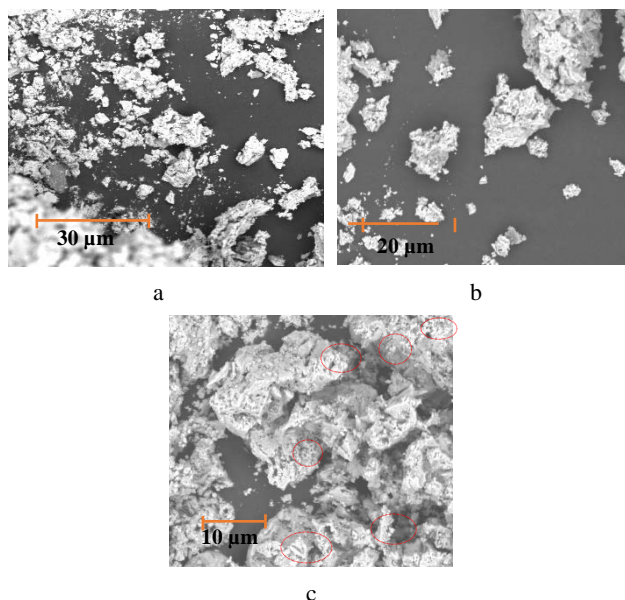


Fig. 6. SEM images of ZnO nanoparticles at different magnification: a – 30 μm; b – 20 μm; c – 10 μm

3.5. Antioxidant activity

The antioxidant test of green biosynthesized ZnO nanoparticles using Mulberry extract was measured by DPPH analyzes in order to evaluate its radical scavenging activity. The solution has been kept undisturbed during two hours in order to check the stability of its. However, in the presence of ZnO nanoparticles, the solution color was gradually changed to pale yellow. The absorption intensity confirmed by the UV-Vis was localized at 517 nm, which indicates an antioxidant activity of ZnO nanoparticles. It can be seen from Fig. 7 a that the increasing of ZnO amount from 10 mg to 100 mg the absorbance localized at 517 nm decreases in intensity. This finding indicates that an increase in free radical scavenging activity with the increase in the concentration of ZnO nanoparticles.

Our results are in accordance with ZnO nanoparticles bioprepared using *Albizia lebeck* [49] and *Croton bonplandianum* [50], and which have been proved dependence between ZnO concentration and antioxidant activity. In addition, the peak gradually decreases with time (Fig. 7 b). The diminution of peaks is an evidence of free radical scavenging activity of ZnO nanoparticles. ZnO nanoparticles exhibited up to 66 % of free radical scavenging capacity. It is interesting to mention that referring to several studies, green synthesis can improve antioxidant activity of ZnO nanoparticles compared to that prepared by conventional chemical methods. And that thanks to several compounds such as: polyphenols, flavonoids, proteins, and fatty acids which are associated with ZnO nanoparticles [51].

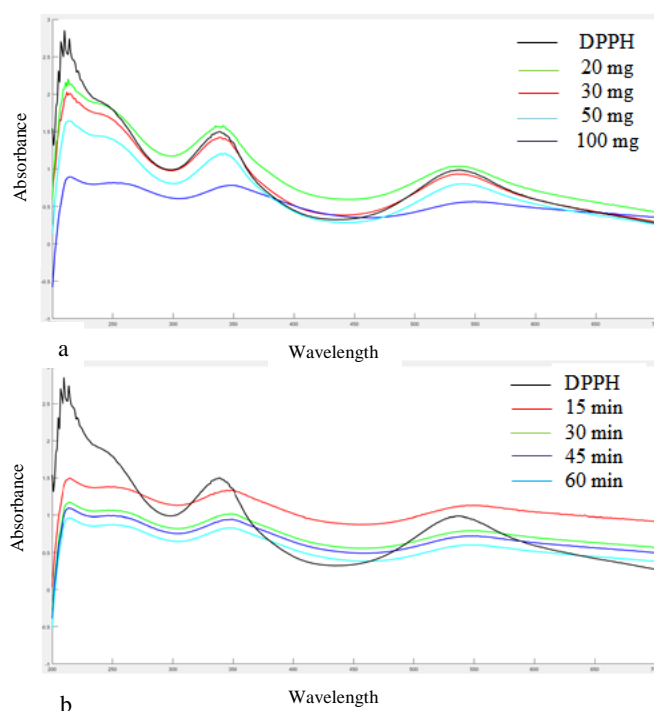


Fig. 7. a – antioxidant activity of ZnO nanoparticles at different concentrations; b – antioxidant activity of ZnO nanoparticles at different time intervals

4. CONCLUSIONS

In this study, ZnO nanoparticles were synthesized successfully using Mulberry fruit extract as reducing and capping agent with a green, efficient and economic biological process. The structural properties, dimensions, and morphology of the synthesized ZnO NPs were characterized using UV-Vis and FT-IR spectroscopy, as well as XRD, and SEM. The antioxidant activity of ZnO nanoparticles was determined using a DPPH free-radical scavenging assay and studied with respect to time and nanoparticle concentration. It was confirmed the formation of pure ZnO nanoparticles with hexagonal wurtzite structure and average crystallite size of 25.2 nm. SEM images showed the formation of spherical nanoparticles and UV-Vis spectrum ZnO nanoparticles exhibited characteristic absorption peak at 350 nm. The biosynthesized ZnO nanoparticles showed good antioxidant activity and which can be effectively utilized for several potential applications.

These finding suggested that ZnO nanoparticles prepared by green route can be effective and have potential for medical applications.

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