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Characterizations of ZnO Nanoparticles Green Synthesized using Flaxseeds Extract for Biomedical Applications

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Abstract

ZnO-NPs were synthesized chemically and using the green synthesis technique by flaxseeds *(Linum usitatissimum L.*), which uses water to extract flaxseeds in a biologically convenient, secure and affordable manner, under different conditions of shape of flaxseed, grinded and full seeds, different concentrations of the precursor, zinc nitrate hexahydrate, (7.43, 14.87 and 22.31 g) each per 50 mL of distilled water to obtain (0.5, 1 and 1.5 mol/L) concentrations and different pH 8, 10, 12 and 13 and studying its characterizations using X-Ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) to obtain the best ZnO-NPs size and morphological properties for biomedical uses.

Keywords: Zinc oxide nanoparticles (ZnO-NPs), Flaxseeds (*Linum usitatissimum L.*), Green synthesis

Introduction

Zinc oxide nanoparticles (ZnO-NPs) are the most significant among the metal oxide NPs because of their distinctive chemical and physical characteristics, which broaden their applications [1]. The presence of various metabolites, such as proteins, enzymes and other biomolecules that function as reducing, capping and stabilizing agents, is thought to be the cause of the biogenic synthesis of ZnO-NPs by biological entities. The released metabolites are associated with differences in ZnO-NP size, shape, dispersion and stability [2]. At the nanoscale, ZnO-NPs are capable of adsorption with relative ease, particularly when they are of a small size. Interestingly, ZnO-NPs can be used as additives in the food industry, particularly since the FDA (Food and Drug Administration) has deemed them safe [3]. Meaning that they can be applied to humans and animals without risk.

Zinc oxide is the most widely used nanomaterial in nanotechnology due to its outstanding properties. Enormous attention has arisen due to its unique physical properties consists of a wide energy band gap of 3.37 eV at ambient temperature and large binding energy of 60 meV [4], which give development to an extensive range of potential applications in many areas such as electronics, solar cells and biological applications. As biological applications of these novel nanoparticles continue to increase, concerns have been raised about the ability of zinc oxide nanoparticles (ZnO-NPs) in biological activity to treat wastewater. The biological activity of Zn ONPs was investigated through the antibacterial activity that identified the effect of ZnO NPs on the bacteria inhibition. The method used to prepare the ZnO-NPs also take an important part which is to reduce the by-product formation when applied in wastewater treatment. Thus, it is necessary to undertake careful antibacterial studies and the synthesis method used to prepare the Zn ONPs.

Biological Synthesis is a promising alternative beside has numerous advantages when compared with classical chemical protocols. This type of nanoparticles synthesis is more environmentally friendly and

gives researchers the chance to nanoparticles size and shape synthesis control, alongside without using toxic or highly cost organic solvents [5].

In biosynthesis by plants, these can decrease metal ions through terpenoids and flavonoids, alongside polysaccharides, besides it is believed that reduction can produce such plant nanoparticles [6]. Additionally, some plants are known to accumulate heavy metals and could detoxify such substances. There are numerous actions of metal tolerance in plants, for example, cell wall attachment, metal chelation and metal ions active transport into vacuoles [7]. This character has an attractive effect on researchers and can be exploited for the biosynthesis of nanoparticles of metals and metal oxides. It is possible to use plants that over-accumulate zinc as a precursor source for zinc oxide NPs formation [8].

ZnO-NPs biosynthesis using plants has been reported in *Physalis alkekengi L*. [9], *Sedum alfredii* [10], flowers of *Trifolium pretense* [11], *Pongamia pinnata* [12], *Cassia Auriculata* [13], beside *Plectranthus amboinicus's* extracts from leaf was performed [14]. It was noted that crystalline ZnO-NPs with a size of 72.5 nm were formed by *Physalis alkekengi* [9]. The plant can grow within soils rich in zinc content (50 - 5,000 mg/kg). Bladder cherry plants can take up zinc in their aerial parts and then aggregate it in their biomass. Findings of experiments by Qu *et al.* [9] showed that *Physalis alkekengi* can be used for zinc-contaminated soil remediation besides the nano-zinc oxide synthesis. However, flaxseeds (*Linum usitatissimum L.*), which is considered one of the oldest beneficial products that was used as a phytotherapy tool, was not much used to synthesis ZnO-NPs, and investigating the surface morphological, structural and optical properties differences between green synthesized ZnO-flaxseeds NPs, and chemical synthesized ZnO-NPs was not well studied. Hessien *et al.* [15]; Verrier *et al.* [16] studied the effects of the H changes on the formation of ZnO-NPs. However, the effect of precursor (for example: Zinc Nitrate hexahydrate) concentration used to synthesis ZnO-NPs on its morphological, structural and optical properties is not well investigated.

Flaxseed (*Linumusitatissimum L*), also known as flax plant, belongs to the *Flaxaceae* family. Because of its potential medicinal properties, flaxseed has received a lot of attention in the world of food and health research. Flaxseed also contains protein, soluble and insoluble fiber, short-chain poly-unsaturated fatty acids (omega-3 fatty acids), phytoestrogenic lignans and various antioxidants [14,17-19].

In addition to its nutritional benefits, flaxseed also has functional benefits such as: Antibacterial activity alongside ability to emulsify, bind water, in addition to change viscosity [20-22]. The bioactive constituents of flaxseed have many functions, including anti-inflammatory (decreased synthesis of the pro-inflammatory cytokines IL-6 and TNF-) beside anti-platelet (inhibition of the anticoagulant prostacyclin). Soluble flaxseed mucilage has antioxidant properties, optimizes digestive function, protects the liver, beside decreases the risk of cardiovascular disease. The antibacterial activity of flaxseed may be related to its long-chain un-saturated fatty acids, especially linoleic beside linoleic acids, lignans and phenolic acids. Most commonly usage of flaxseed is as a dietary supplement for lowering cholesterol and restoring ordinary alimentary canal function [23].

Materials and methods

The flax plant crop is harvested about 5 months after the date of planting the seeds, when the color of the flax plant capsule turns yellow. The process of harvesting flax plants is called "pulling" rather than harvesting because flax is literally pulled out of the ground instead of being cut. This is to maintain the length of the fibers inside the stem. To separate the seeds, which are needed for the experiment, from the straw, the roar process is done manually, as the straw is beaten at the capsule end on a wooden crawler or stone without damaging the straw. The seeds are collected and kept in containers under dry conditions. Flaxseeds were collected freshly from fields in Sharkia, Egypt. The chemical reagents like zinc acetate dihydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$] and sodium hydroxide (NaOH) were purchased from Teduh Sainstek Presources Company, Selangor Darul Ehsan, Malaysia.

Zinc acetate dihydrate is a precursor that used as a source of zinc ions (Zn^{2+}). The acetate groups in zinc acetate can provide a stabilizing effect during the nanoparticle synthesis process. They may help control the nucleation and growth of nanoparticles, influencing their size and morphology. Sodium hydroxide is a strong base that is used in to control pH.

Zinc acetate dihydrate is a precursor that used as a source of zinc ions (Zn^{2+}). The phytochemicals present in the Flaxseeds extract can reduce metal ions to form nanoparticles and also help in stabilizing the resulting nanoparticles. Flaxseeds extract contains compounds with antioxidant properties. These antioxidants can potentially contribute to the reduction of metal ions and prevent the oxidation of the synthesized nanoparticles. Sodium hydroxide is a strong base that is used in to control pH.

Preparation of flaxseed extract

Flaxseeds were first washed thoroughly with distilled water to remove any impurities. The seeds were then dried in an oven at 60 °C for 24 h. The 5.0 g of the dried Flaxseeds will be then mixed with 100 mL of distilled water in and stirred for 24 h at 40 °C temperature for complete extraction. Another 5.0 g were grinded before mixed with the water and stirred too. The 2 mixtures were labeled and then filtered through a cheese cloth then filter paper to obtain the flaxseed extract.

Synthesis of zinc oxide nanoparticles (ZnO-NPs)

A solution of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) concentration (0.5 mol/L) was prepared by dissolving (7.43 g) of it in 50 mL distilled water by stirring. Sodium hydroxide solution (NaOH) concentration (1 mol/L) was prepared by dissolving (4.0 g) of Sodium hydroxide to 100 mL of distilled water.

Chemical synthesis

Sodium hydroxide solution NaOH was added dropwise to zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) solution until pH reaches 8 - 10. The obtained precipitate will be washed several times with distilled water and ethanol to remove any impurities and dried in an oven at 60 °C for 7 - 8 h. The resulting powder will be put in Furnace to be calcinated under 450 °C temperature for an hour. The resulting powders was labeled (A1) and kept for further analysis.

Green synthesis using flaxseeds grinded powder and the whole seeds

The flaxseed extract of grinded powder was added dropwise to zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) solution under constant stirring at room temperature. Sodium hydroxide solution NaOH was added dropwise until pH reaches 8 - 10. The resulting mixture was stirred for 1 h at room temperature for complete reaction between the precursors and flaxseed extract. The mixture was then filtered through a filter paper to separate the ZnO-Flaxseeds NPs from unreacted precursors and flaxseed extract. The obtained precipitate will be washed several times with distilled water to remove any impurities and dried in an oven at 60 °C for 7 - 8 h. The resulting powder will be put in Furnace to be calcinated under 450 °C temperature for an hour. Same procedures were done using the full seeds of flaxseed. The 2 resulting powders were labeled (A2 for grinded seeds - A3 for the whole seeds), respectively and kept for further analysis to find out if the difference between the ZnO nanoparticles synthesized using flaxseeds grinded powder and the whole seeds and choose the most suitable for biomedical uses among both of them.

Green synthesis using flaxseeds whole seeds under different precursor concentrations

Same previous procedures were done using full seeds flax extract but under different concentrations of the precursor, zinc nitrate hexahydrate, (0.5, 1 and 1.5 mol/L). The 3 resulting powders were labeled (B1 - B2 - B3), respectively and kept for further analysis.

Green synthesis using flaxseeds whole seeds under different pH degrees

Same procedures were don using full seeds flax extract a concentration of the precursor, zinc nitrate hexahydrate (0.5 mol/L), but under different pH degrees of (8, 10, 12 and 13). The 4 resulting powders were labeled (C 1 - C 2 - C 3 - C4), respectively and kept for further analysis.

Results and discussion

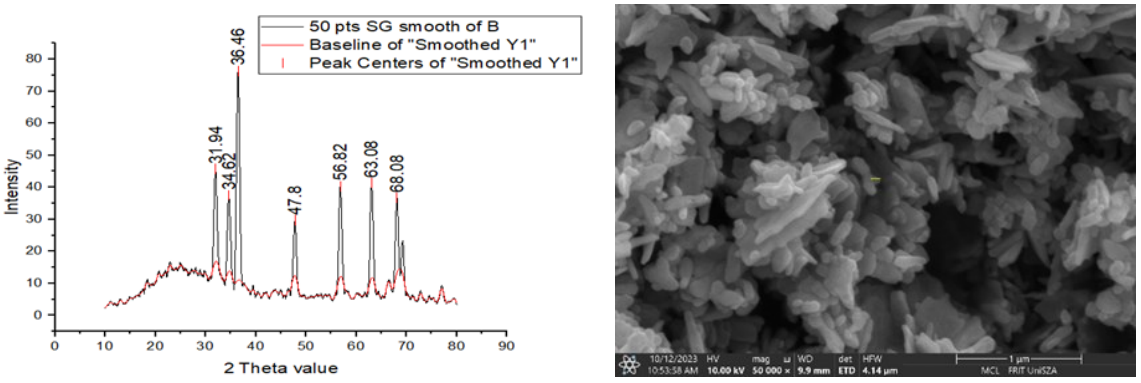
The structure of synthesized ZnO-NPs was characterized by X-Ray diffraction (XRD) at 2θ angle by Cu-Kα radiation operated at 45 kV and 40 mA. Debye-Scherrer Eq. (1) was used to calculate the crystalline size (D) [$D = 0.89\ \lambda/\beta\ Cos\theta$]. Where 'β' is FWHM (the full width half- maximum) of ZnO (101) line, 'λ' is the wavelength of (CuKa), 0.89 = Scherrer's constant, and 'θ' is the diffraction angle.The morphology of ZnO-NPs was studied by field emission scanning electron microscopy (FESM). The size of ZnO-NPs was detected using ImageJ software.

Characterization of synthesized zinc oxide nanoparticles (ZnO-NPs)

Chemical method synthesized zinc oxide nanoparticles (ZnO-NPs) (Sample A1)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 31.94, 34.62, 36.46, 47.8, 56.82, 63.08 and 68.08 ° obtained via XRD at 2θ angle corresponded to the intensity of 45.62, 37.43, 76.27, 29.655, 40.30, 41.22 and 36.70, respectively (**Figure 1(a)**). From the equation Debye-Scherrer equation, the crystalline size of ZnO NPs was calculated as 11.206 nm.

The FESEM image demonstrated the formation of rode like shaped NPs with an average 190 nm size and rode like shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 1(b)**).



(a) (b)

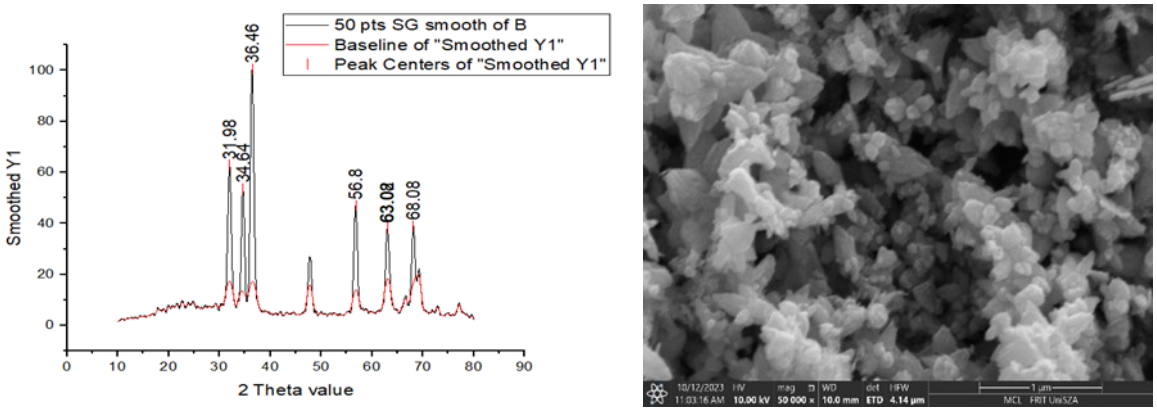
Figure 1 (a) XRD and (b) FESEM results for chemically synthesized zinc oxide nanoparticles (Sample A1).

Green synthesis using flaxseeds full seeds under different precursor concentrations

(1) Grinded seeds (Sample A2)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 31.98, 34.64, 36.46, 56.8, 63.02 and 68.08 ° obtained via XRD at 2θ angle corresponded to the intensity of 62.8, 53.5, 100.41, 46.98, 38.26 and 38.98 °, respectively (**Figure 2(a)**). From the equation Debye-Scherrer equation, the crystalline size of ZnO-NPs was calculated as 13.27 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with an average of 148 nm size and irregular shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 2(b)**).



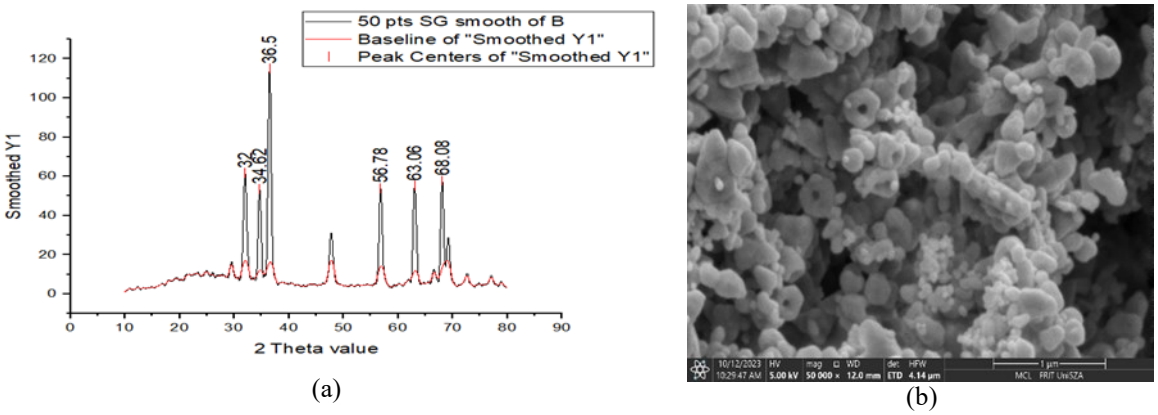
(a) (b)

Figure 2 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles (Sample A2).

(2) The whole seeds without grinding (Sample A3)

The crystalline nature of ZnO-NPs was investigated by XRD method. The diffraction peaks of 32, 34.62, 36.5, 56.78, 63.06 and 68.08 ° obtained via XRD at 2θ angle corresponded to the intensity of 61.6, 53.38, 114.9, 53.8, 55 and 507.7, respectively (**Figure 3(a)**). From the equation Debye-Scherrer equation, the crystalline size of ZnO NPs was calculated as 10.00 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with an average 136 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 3(b)**).



(a) (b)

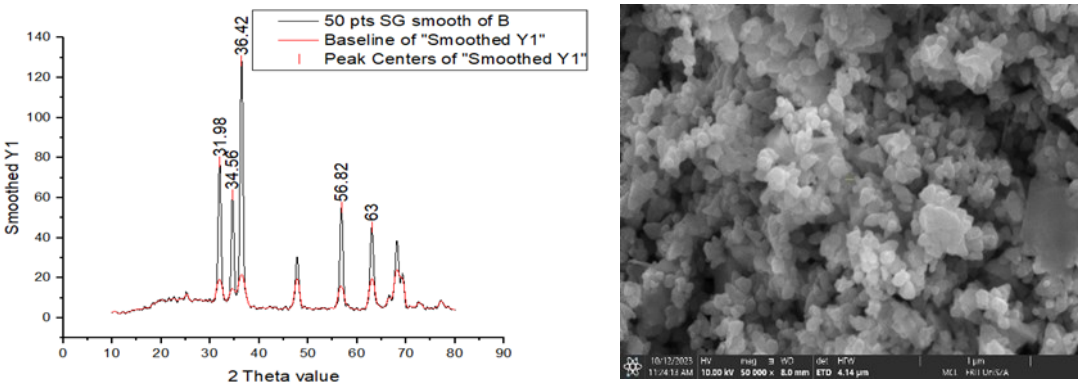
Figure 3 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles (Sample A3).

Green synthesis using flaxseeds the whole seeds under different precursor concentrations

(1) Concentration 0.5 mol/L (Sample B1)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 31.95, 34.56, 36.31, 56.82 and 63 ° obtained via XRD at 2θangle corresponded to the intensity of 77.57, 61.21, 118.09, 55.05 and 45.33, respectively (**Figure 4(a)**). From the equation Debye-Scherrer equation, the crystalline size average of ZnO NPs was calculated as 10.96 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with an average 129 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 4(b)**).



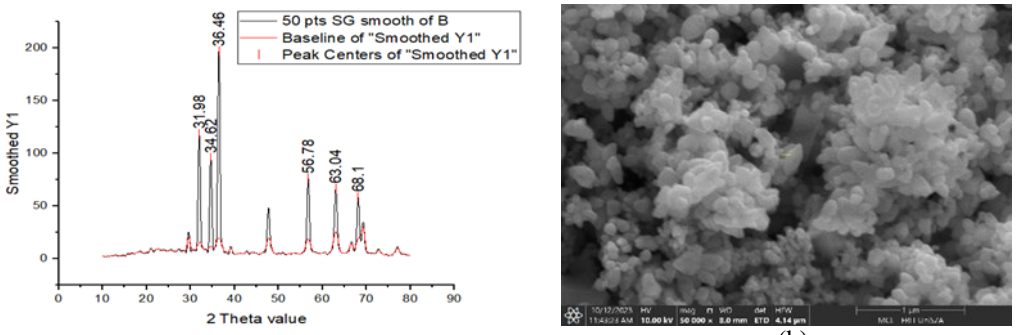
(a) (b)

Figure 4 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles under a concentration of 0.5 mol/L (Sample B1).

(2) Concentration 1 mol/L (Sample B2)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 31.98, 34.60, 36.46, 56.78, 63.04, and 68.1 ° obtained via XRD at 2θangle corresponded to the intensity of 118.44, 98.06, 196.96, 76.20, 66.20 and 58.59, respectively (**Figure 5(a)**). From the equation Debye-Scherrer equation, the crystalline size average of ZnO NPs was calculated as 14.19 nm.

The FSEM image demonstrated the formation of spherical shaped NPs with an average of 164 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 5(b)**).



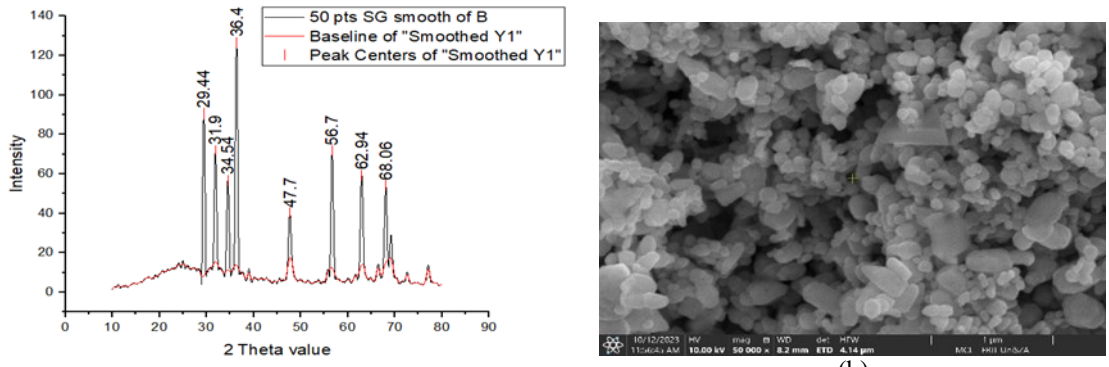
(a) (b)

Figure 5 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles under a concentration of 1 mol/L (Sample B2).

(3) Concentration 1.5 mol/L (Sample B3)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 29.44, 31.9, 34.54, 36.31, 47.7, 56.64, 62.94 and 65.06 ° obtained via XRD at 2θangle corresponded to the intensity of 90.39, 71.71, 56.67, 127.82, 39.97, 71.98, 59.17 and 54.02, respectively (**Figure 6(a)**). From the equation Debye-Scherrer equation, the crystalline size average of ZnO NPs was calculated as 14.19 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with an average 170 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 6(b)**).



(a) (b)

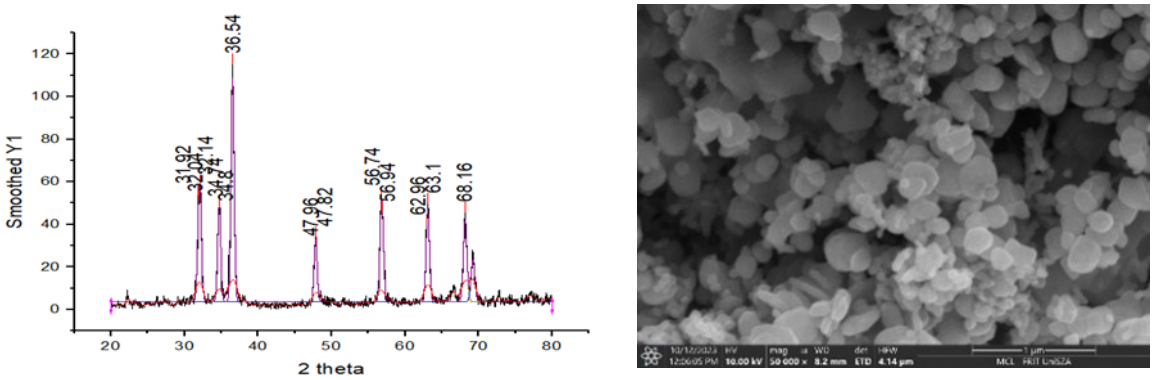
Figure 6 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles under a concentration of 1.5 mol/L (Sample B3).

Green synthesis using flaxseeds full seeds under different pH degrees

(1) pH 8 (Sample C1)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 32.15, 34.79, 36.63, 47.89, 56.95, 63.19, 66.79, 68.30, 69.39, 72.86 and 77.28 ° obtained via XRD at 2θangle corresponded to the crystal planes of 100, 002, 101, 102, 110, 013, 200, 112, 201, 004 and 202, respectively (**Figure 7(a)**). From the equation Debye-Scherrer Eq. (1), the crystalline size of ZnO NPs was calculated as 17.56 nm

The FESEM image demonstrated the formation of spherical shaped NPs with a 23 - 55 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 7(b)**).



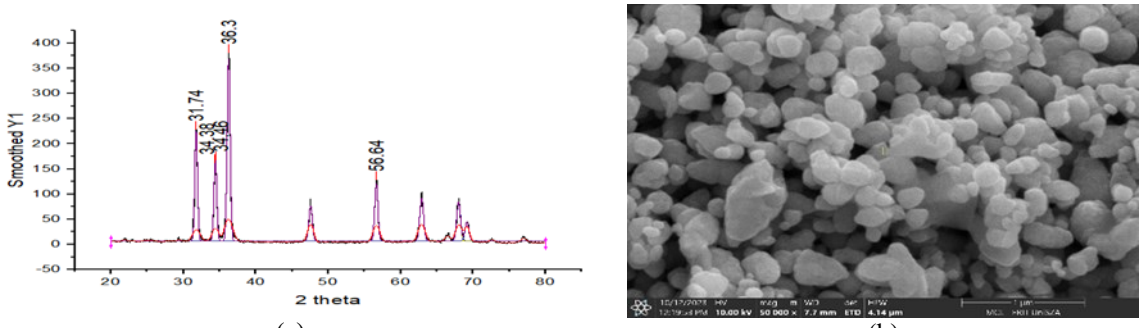
(a) (b)

Figure 7 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles at pH 8 (Sample C1).

(2) pH 10 (Sample C2)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 32.15, 34.79, 36.63, 47.89, 56.95, 63.19, 66.79, 68.30, 69.39, 72.86 and 77.28 ° obtained via XRD at 2θangle corresponded to the crystal planes of 100, 002, 101, 102, 110, 013, 200, 112, 201, 004 and 202, respectively (**Figure 8(a)**). From the equation Debye-Scherrer Eq. (1), the crystalline size of ZnO NPs was calculated as 17.56 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with a 23 - 55 nm size range and spherical shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 8(b)**).



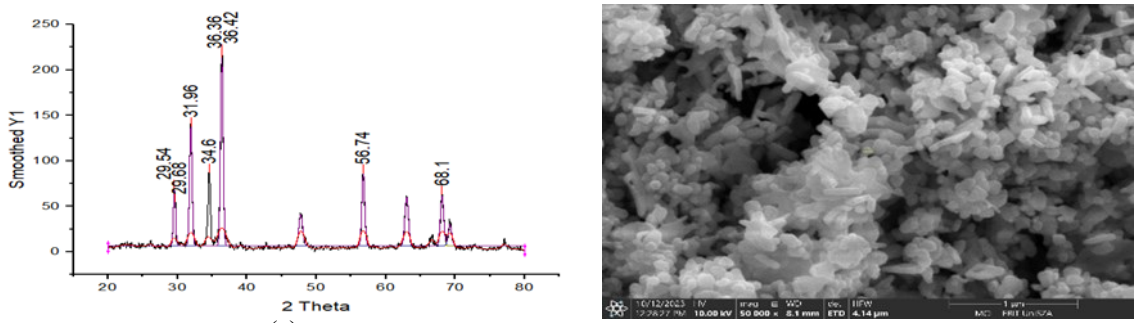
(a) (b)

Figure 8 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles at pH 10 (Sample C2).

(3) pH 12 (Sample C3)

The crystalline nature of ZnO NPs was investigated by XRD method. The diffraction peaks of 32.15, 34.79, 36.63, 47.89, 56.95, 63.19, 66.79, 68.30, 69.39, 72.86 and 77.28 ° obtained via XRD at 2θangle corresponded to the crystal planes of 100, 002, 101, 102, 110, 013, 200, 112, 201, 004 and 202, respectively (**Figure 9(a)**). From the equation Debye-Scherrer Eq. (1), the crystalline size of ZnO NPs was calculated as 17.56 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with a 23 - 55 nm size range and semi rode like shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 9(b)**).



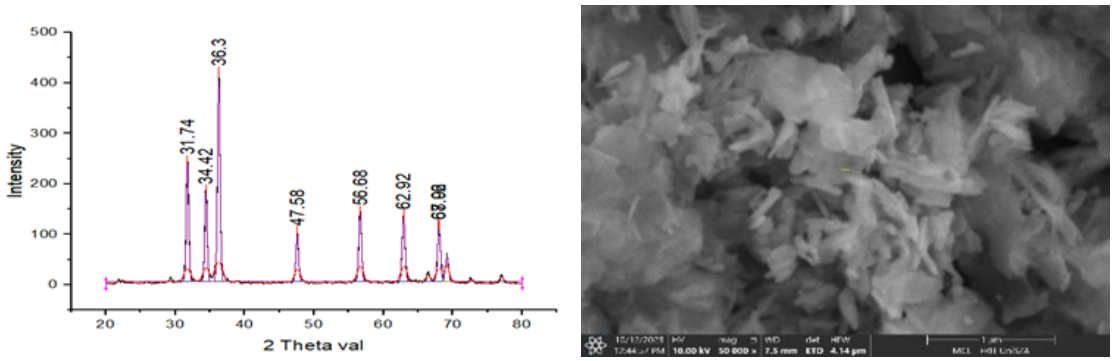
(a) (b)

Figure 9 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles at pH 12 (Sample C3)

(4) pH 13 (Sample C4)

The crystalline nature of ZnO-NPs was investigated by XRD method. The diffraction peaks of 32.15, 34.79, 36.63, 47.89, 56.95, 63.19, 66.79, 68.30, 69.39, 72.86 and 77.28 ° obtained via XRD at 2θangle corresponded to the crystal planes of 100, 002, 101, 102, 110, 013, 200, 112, 201, 004 and 202, respectively (**Figure 10(a)**). From the equation Debye-Scherrer Eq. (1), the crystalline size of ZnO NPs was calculated as 17.56 nm.

The FESEM image demonstrated the formation of spherical shaped NPs with a 23 - 55 nm size range and spikes shape. The NPs were agglomerated to form cluster-like structure in the sample (**Figure 10(b)**).



(a) (b)

Figure 10 (a) XRD and (b) FESEM results for green synthesized zinc oxide nanoparticles at pH 13 (Sample C4).

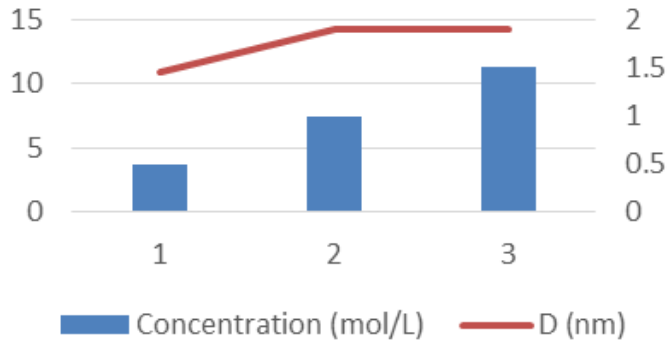


Figure 11 Relationship between concentration of $Zn(NO_3)_2 \cdot 6H_2O$ and size of ZnO-NPs.

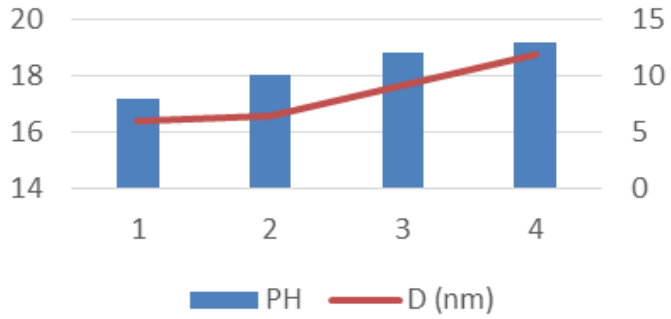


Figure 12 Relationship between pH of $Zn(NO_3)_2 \cdot 6H_2O$ and size of ZnO-NPs.

Conclusions

As a conclusion, using the whole seed flaxseed extract for synthesis ZnO-NPs have a smaller size of nanoparticles than using grinded flaxseed extract or chemical methods, in addition to their spherical morphology and shape, which are perfect for biomedical applications. Under pH 8–10, the higher the concentration of precursor zinc nitrate hexahydrate (Zn(NO3)2·6H2O), the greater the increase in the resulting size of ZnO-NPs (**Figure 11**). Under 0.5 mol/L concentration of precursor zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) the more the pH increases leads to increase in resulting size of ZnO-NPs (**Figure 12**). To use ZnO-NPs for biomedical applications, the spherical morphological shape and smallest nanoparticles size is synthesized using the whole seeds flaxseeds extract and 0.5 mol/L concentration of precursor zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) under pH 8 - 10.

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