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*Original Article*

# Zinc Oxide Nanoparticle Green Synthesis Using Black Cumin Seed Aqueous Extract: Its Characterization and *in vitro* Anti-Hyperglycaemic Properties

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## ABSTRACT

**Introduction:** α-Amylase and α-glucosidase are carbohydrate metabolizing enzymes that are known to be involved in postprandial hyperglycaemia in diabetic patients. **Objective:** In a bid to source potent inhibitors of these enzymes, the study synthesized zinc oxide nanoparticles and evaluated the *in vitro* anti-hyperglycaemic activity. **Materials and Methods:** The seed aqueous extract of black Cumin (BC) was used as a capping and/or reducing agent to synthesize ZnO NPs from zinc acetate precursor. The BC-ZnONPs were characterized by microscopy (FESEM and HRTEM) and spectroscopy (UV-Vis and EDX) methods. *In vitro* anti-hyperglycaemic evaluation was based on α-amylase and α-glucosidase inhibition assays. **Results:** The BC-ZnONPs showed a spherical-to-cubical shape with a 10-50 nm size range. The UV-Vis absorption peaks at 387 and 415 nm suggest the formation of biogenic ZnO NPs. The EDX spectrum revealed 68.92% and 27.49% weight compositions of Zn and O, respectively, to further substantiate ZnO nanoparticle synthesis. The BC-ZnONPs showed notable anti-hyperglycaemic properties with $IC_{50}$ of 87.72±5.13 and 124.21±15.20 µg/mL against α-amylase and α-glucosidase, respectively. **Conclusion:** Black Cumin seed extract was a useful biogenic material for synthesizing ZnO NPs. The BC-ZnONPs showed promising anti-hyperglycaemic properties based on the notable inhibitory activities against α-amylase and α-glucosidase enzymes. Future work may include evaluating the synergistic effects of black Cumin metabolites and ZnONPs, as well as determining the *in vivo* toxicity profile for safety considerations.

**Keywords:** Anti-Hyperglycaemia, Black Cumin, α-Amylase, α-Glucosidase, ZnO Nanoparticles

## INTRODUCTION

Green techniques have recently become the preferred synthesis method for nanoparticles (NPs) because of their relative safety, cost, and environmental friendliness[1]. Zinc oxide nanoparticles (ZnONPs) are the most promising nanomaterials in recent years because of their size-dependent characteristics and low toxicity that have been extensively used in various fields, including microelectronics, textiles, diagnostics, cosmetics, and medicine[2]. Nowadays, plant extract-mediated NPs have found useful applications in food, medicine, and agriculture. Plants contain metabolites and biomolecules with interesting functional groups, such as amine (NH), carbonyl (C=O), and hydroxyl (OH) that can react with metal ions to reduce them to nanoscale particles[3]. The seeds of *Nigella sativa* L. (Family: Ranunculaceae), commonly known as black Cumin, are used in Asian and African ethnomedicines to manage cancer, diabetes, and other chronic inflammatory diseases[4]. This study green-synthesized zinc oxide nanoparticles (ZnONPs) using black cumin seed extract (BC), characterized the BC-ZnONPs using microscopy and spectroscopy methods, and evaluated the anti-hyperglycaemic properties based on α-amylase and α-glucosidase inhibitions.

## MATERIAL AND METHODS

### Ethical approval

The study was approved by Walter Sisulu University – Research Ethics Committee on 10 August 2023, with the approval code, WSU/FNS-GREC/2023/03/22/G33.

### Plant material

Dry seeds of black Cumin were purchased from the traditional herbal market in Ile-Ife, Osun State, Nigeria (latitude 7°33′00″N and longitude 4°33′00″E) under the registered name of RC: 875523. The plant list catalog (http://www.theplantlist.org) confirmed the plant as kew-2381679.

### Green synthesis of ZnONPs using black cumin seed extract (BC)

The green synthesis method by Naseer et al.[5] was followed for the development of BC-ZnONPs. About 15 mg of BC was dissolved in 5 mL of distilled water and was added to 100 mL of 0.2 M zinc acetate dihydrate solution (4.3898 g in 100 mL distilled water) at 100 °C and 250 rpm for 4 h. The solution was centrifuged at 10,000 rpm for 10 min, washed with distilled water, centrifuged again, and oven-dried at 60 °C, affording BC-ZnONPs.

### Characterization of black Cumin extract-mediated zinc oxide nanoparticles (BC-ZnONPs)

The surface morphology of the BC-ZnONPs was recorded using field emission scanning electron microscopy (FESEM) on the FESEM instrument (German manufacturer Carl Zeiss; model: SIGMA-0261). A high-resolution transmission electron microscope (model name FEI TECNAI

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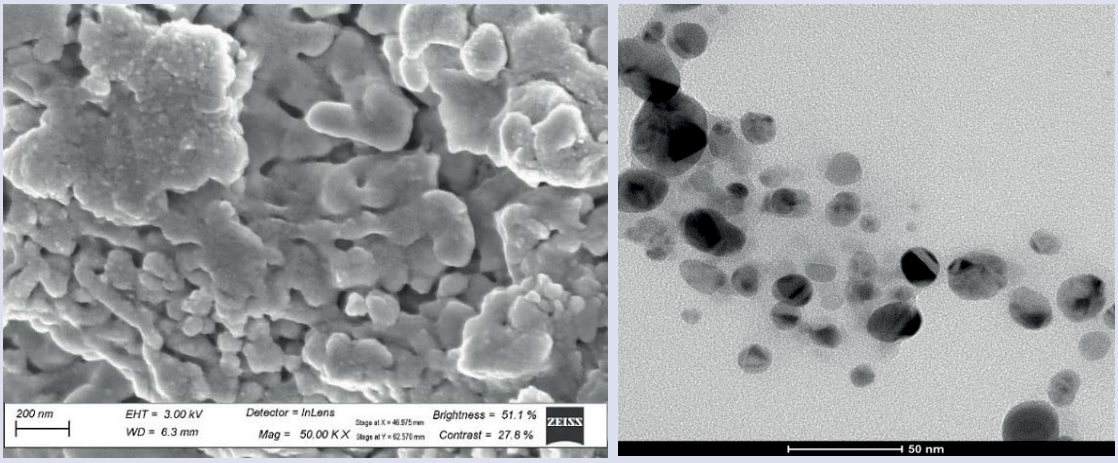
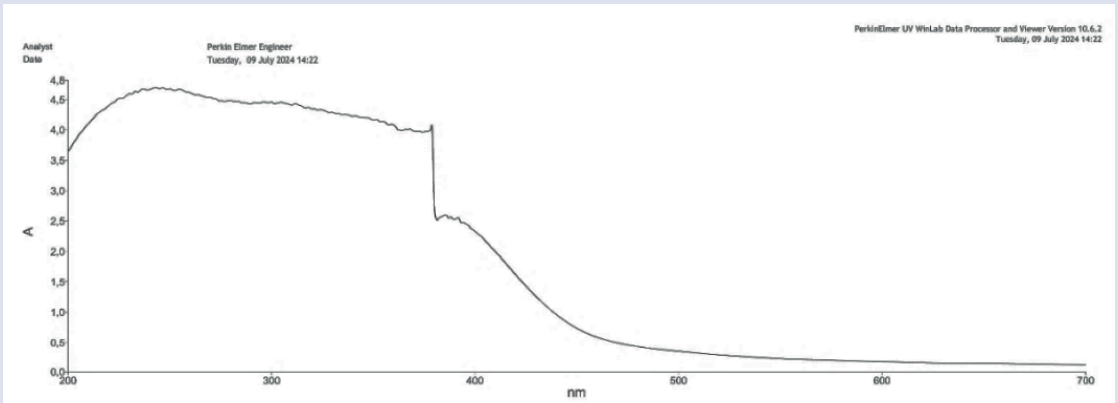
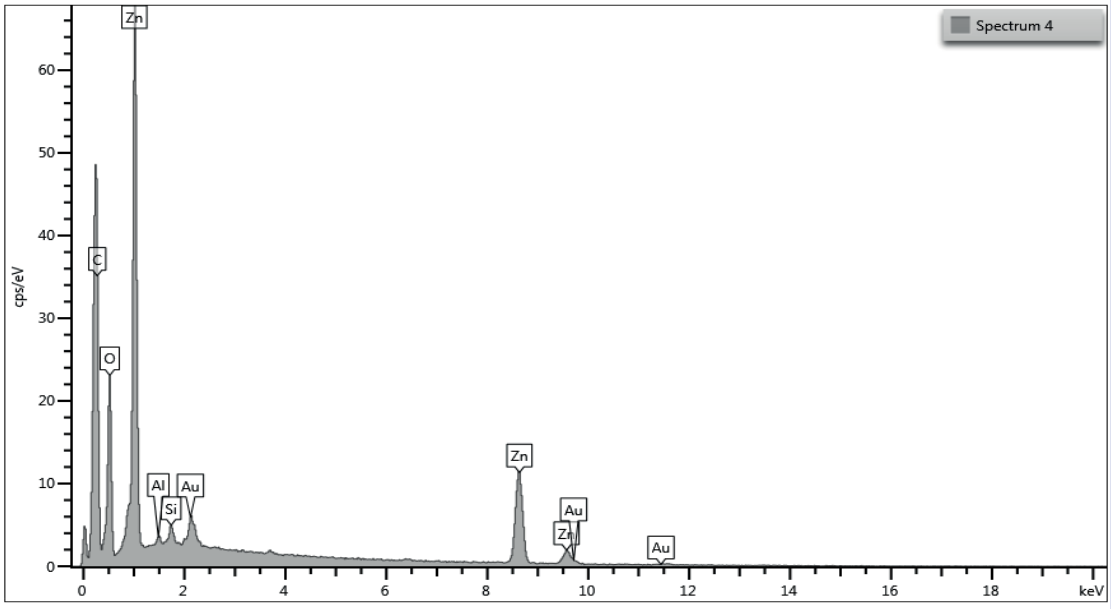



**(A)** **(B)**

**Figure 1.** FESEM (**A**) and HRTEM (**B**) imaging of BC-ZnONPs




**(A)**




**(B)**

**Figure 2.** UV-Vis absorption spectrum (**A**) and Energy dispersive X-ray spectrum (**B**) of BC-ZnONPs

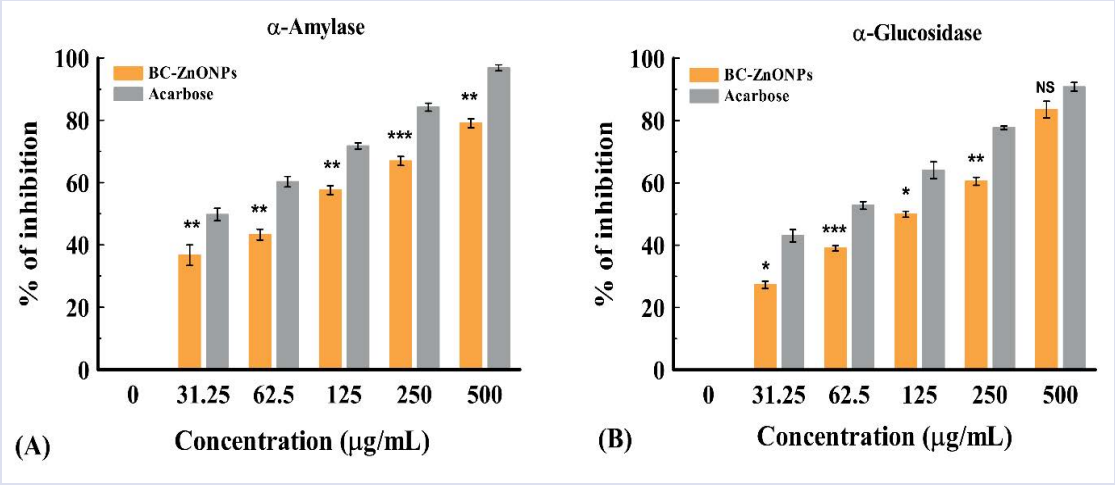



**Figure 3.** α-Amylase and α-Glucosidase mean percentage inhibition by BC-ZnONPs. [Data expressed as mean ± S.D. *p<0.05; **p<0.01; ***p<0.001; ns=non-significant when compared with standard].

G2 F300) running at an accelerating voltage of 300 kV was used to determine the size and shape of the NPs. The UV-Vis spectrum of absorption for BC-ZnONPs and the weight percentage of elements making up the NPs were obtained using energy dispersive X-ray spectroscopy (EDX) performed on a Scanning Electron Microscope (JEOL JSM-IT100InTouchScopeTM, Tokyo, Japan) equipped with Oxford-EDX software.

## *In vitro* anti-hyperglycaemic activities of black Cumin extract-based ZnONPs

### *α-Amylase inhibition*

The modified procedure by Wickramaratne et al.[6] was used. An equal volume (250 µL) of α-amylase (from *Aspergillus oryzae*) enzyme solution (2 U/mL) and 5% (*v*/*v*) DMSO (20 µL/well) of BC-ZnONPs were mixed in a test tube in triplicates (n=3), and incubated at 25 ºC for 10 min. Afterward, 1% starch solution (250 µL) was added to the mixture to initiate the reaction, and the resultant solution was further incubated for 10 min at 25 ºC. The reaction was put to a halt by adding 1 mL of 3,5-dinitrosalicylic acid (DNSA) reagent, and the mixture was subsequently boiled in a water bath for 10 min. After cooling, 3 mL of distilled water was added, and absorbance was read at 540 nm. Acarbose (500-3.13 µg/mL) and 5% (*v*/*v*) DMSO (20 µL/well) served as the positive (standard) and the negative controls, respectively. The enzyme inhibition percentage was calculated using Equation 1.

$$\%\ Inhibition = \frac{ABScontrol - ABSsample}{ABScontrol} x100 \quad (1)$$

### *α-Glucosidase inhibition*

The α-glucosidase inhibition of BC-ZnONPs was determined, as described by Rengasamy et al.[7]. Briefly, in 96-well microplates, equal volumes (20 µL) of BC-ZnONPs were dissolved in 5% (*v*/*v*) DMSO (20 µL/well) at different concentrations (500-3.13 µg/mL), and yeast α-glucosidase (0.1 U/mL) in phosphate buffer (0.1 M, pH 6.8) was mixed in three replicates (n=3). Thereafter, 40 µL of the substrate [0.375 mM p-nitrophenyl-a-D-glucopyranoside (pNPG) in 0.1 M phosphate buffer at pH 6.8] was added to the mixture to initiate the reaction. The reaction mixture was incubated at 37 ºC for 40 min. Subsequently, after the incubation period, 80 µL of 0.2 M sodium carbonate in potassium phosphate buffer (0.1 M, pH 6.8) was added to each well to halt the reaction. Acarbose (500-3.13 µg/mL) and 5% (*v*/*v*) DMSO (20 µL/well) served as the positive (standard) and the negative controls, respectively.Acarbose was used as the standard drug, while absorbance was measured at 405 nm. The enzyme inhibition percentage was calculated using Equation 1. The concentration that showed 50% inhibition of each enzyme represents the $IC_{50}$.

## Statistical analysis

Data were expressed as the mean ± standard deviation (SD) of three replicate values (n = 3). Statistical analysis of the α-amylase and α-glucosidase anti-hyperglycaemic data was based on One-way analysis of variance (ANOVA), using the KyPlot program (version 5.0). This was followed by the student's t-test where p<0.05 (*), p<0.1 (**) and p<0.001 (***) were significant.

# RESULTS AND DISCUSSION

## Characterization of black Cumin extract-based zinc oxide nanoparticles (BC-ZnONPs)

### *Microscopy – FESEM and HRTEM*

The FESEM imaging revealed spherical-to-cubical BC-ZnONPs, with an average size range of 40–80 nm[8]. Further evaluation by the HRTEM imaging (Figure 1A and 1B) revealed that the majority of the BC-ZnONPs are spherical with a 10–50 nm size range. This outcome agrees with the report by Rajput et al.[9].

### *UV-Vis and EDX spectroscopy*

The UV-Vis spectrum (Figure 2A and 2B) of the NPs showed absorption peaks at 208–238 nm, which correspond to zinc acetate dihydrate's starting material (zinc precursor) that was used for the nano-synthesis[10]. Furthermore, the absorption peaks at 387 and 415 nm were attributed to the distinctive ZnONP peaks, suggesting that ZnONPs were successfully synthesized[11]. The EDX spectrum showed Zn and O as the abundant elements in the NPs, with 68.92 and 27.49 % weight composition, respectively[12]. The characteristic Zn peaks in the EDX spectrum at 1.11, 8.55, and 9.23 keV can be attributed to the surface plasmon resonance effect, which is a characteristic of ZnO nanostructures[13].

## *In vitro* anti-hyperglycaemic activities of BC-ZnONPs

A novel strategy to lower postprandial hyperglycaemia and delay the onset of diabetes is to inhibit the activity of the enzymes that break down carbohydrates, specifically α-amylase and α-glucosidase[14]. With

biogenic ZnONPs garnering a lot of interest recently, the ability of black Cumin seed extract to inhibit these enzymes was evaluated, and the result is presented in Figures 3A and B. The BC-ZnONPs inhibited α-amylase and α-glucosidase significantly ($p<0.05$) in a dose-dependent manner. At 500 µg/mL, the BC-ZnONPs exhibited up to 85% inhibition of α-glucosidase, which was comparable to acarbose, the standard anti-hyperglycaemic drug. The NPs exhibited an $IC_{50}$ of 87.72±5.13 µg/mL against α-amylase, which was significantly lower in activity compared to acarbose ($IC_{50}$ = 40.80±5.86 µg/mL). Additionally, it inhibited α-glucosidase enzyme with an $IC_{50}$ of 124.21±15.20 µg/mL, which was also significantly lower in activity than acarbose at an $IC_{50}$ of 37.55±0.05 µg/mL. Studies have shown the inhibitory effect of ZnONPs against α-amylase and α-glucosidase[15]. In summary, the study findings have shown the notable anti-hyperglycaemic activity of BC-ZnONPs, and its prospect as an inhibitor of α-amylase and α-glucosidase enzymes for effective control of high blood sugar.

## CONCLUSION

This study utilized an eco-friendly green synthesis method to develop black Cumin seed extract-mediated ZnONPs. Evidence for the spherical-to-cubical nanostructure, 10-50 nm size range, and the elemental Zn and O percentage composition in the BC-ZnONPs was presented through FESEM, HRTEM, UV-Vis, and EDX analyses. The biogenic NPs were a promising anti-hyperglycaemic agent, inhibiting the activities of α-amylase and α-glucosidase enzymes *in vitro*. Future work may consider evaluating the *in vivo* anti-hyperglycaemic activity of BC-ZnONPs, the possible synergistic effect of black Cumin metabolites and ZnONPs, and the level of toxicity of BC-ZnONPs for safety considerations.

## ACKNOWLEDGMENTS

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