



Competent antioxidant and antiglycation properties of zinc oxide nanoparticles (ZnO-NPs) phyto-fabricated from aqueous leaf extract of *Boerhaavia erecta* L.

Mahadevamurthy Murali¹ · Anjana Thampy² · Satish Anandan² · Mohammed Aiyaz³ · Natarajamurthy Shilpa⁴ · Sudarshana Brijesh Singh¹ · Hittanahallikoppal Gajendramurthy Gowtham⁵ · Abhilash Mavinakere Ramesh⁶ · Abbas Rahdar⁷ · George Z. Kyzas⁸

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Abstract

During the present century, plant-based zinc oxide nanoparticles (ZnO-NPs) are exploited extensively for their vast biological properties due to their unique characteristic features and eco-friendly nature. Diabetes is one of the fast-growing human diseases/abnormalities worldwide, and the need for new/ novel antiglycation products is the need of the hour. The study deals with the phyto-fabrication of ZnO-NPs from *Boerhaavia erecta*, a medicinally important plant, and to evaluate their antioxidant and antiglycation ability in vitro. UV-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) were used to characterize the phyto-fabricated ZnO-NPs. The characterization of nanoparticles revealed that the particles showed an absorption peak at 362 nm and band gap energy of 3.2 eV, approximately 20.55 nm in size, with a ZnO elemental purity of 96.61%. The synthesized particles were found agglomerated when observed under SEM, and the FT-IR studies proved that the phyto-constituents of the extract involved during the different stages (reduction, capping, and stabilization) of nanoparticles synthesis. The antioxidant and metal chelating activities confirmed that ZnO-NPs could inhibit the free radicals generated, which was dose-dependent with an IC_{50} value between 1.81 and 1.94 mg mL⁻¹, respectively. In addition, the phyto-fabricated nanoparticles blocked the formation of advanced glycation end products (AGEs) as noticed through inhibition of Amadori products, trapping of reactive dicarbonyl intermediate and breaking the cross-link of glycated protein. It was also noted that the phyto-fabricated ZnO-NPs significantly prevented the damage of red blood corpuscles (RBCs) induced by MGO. The present study's findings will provide an experimental basis for exploring ZnO-NPs in diabetes-related complications.

Keywords Amadori products · AGEs · Diabetes mellitus · Metal chelating · ZnO-NPs

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✉ Satish Anandan
satishanandan84@gmail.com

¹ Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

² Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar 563101, Karnataka, India

³ Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

⁴ Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

⁵ Department of PG Studies in Biotechnology, Nrupathunga University, Nrupathunga Road, Bangalore 560001, India

⁶ Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

⁷ Department of Physics, Faculty of Science, University of Zabol, Zabol 98613-35856, Iran

⁸ Department of Chemistry, International Hellenic University, Kavala, Greece

Introduction

The field of nanotechnology deals with the manipulation, production, and use of materials of ≥ 100 nm, which are synthesized either by top-down or bottom-up method irrespective of their synthesis mode (physical, chemical, or biological). These synthesized particles are grouped into organic or inorganic nanoparticles based on their nature of synthesis (Sharma et al. 2019). Nanoparticles have expressed significant advances in the present century owing to their wide range of applications in biomedical, sensors, antimicrobial, catalyst, and electronics, including agriculture (Basnet et al. 2018; Agarwal and Shanmugam 2020; Murali et al. 2021a). Both metal and metal oxide nanoparticles have been synthesized and employed in many industrial applications. However, zinc oxide nanoparticles have drawn the most attention because of their unique and superior biological properties, highlighting their use in the biomedical field. Zinc oxide is one of five zinc compounds that have been designated as “generally recognized as safe (GRAS)” materials by the Food and Drug Administration (FDA), USA (Gunalan et al. 2012; Anandan et al. 2019).

Among the different mode of synthesis, the biological route of ZnO nanoparticles synthesis by using plant extracts has been the study of interest as these are known to be highly effective in biomedical applications compared to other methods apart from reducing the risk of toxicity to the eco-system (Murali et al. 2021b). It is important to use plant resources for the synthesis of nanoparticles because they are known to possess various therapeutic compounds (such as alkaloids, terpenoids, phenols, and tannins) which have been exploited since ancient times as traditional medicine apart from the present-day application in industrial, pharmaceutical, and agricultural fields. During nanoparticle synthesis (zinc oxide), the metabolites of the plant act as reducing, capping, or stabilizing agents; as a result, biological qualities are better than those created through physical or chemical means (Stan et al. 2016; Kumar et al. 2020).

Glycation in humans has both physiological and pathophysiological significance that leads to diabetes condition and is one of the fast-growing diseases throughout the world, with an estimated number of victims to be raised to 642 million by 2040 (Ahmed 2005; International Diabetes Federation 2015). The process of sugar (carbohydrate) being covalently attached to a protein or lipid molecule without an enzyme is known as glycation. The glycation reaction is a slow process with the initial formation of Schiff's base followed by dehydration, fragmentation, oxidative modification, etc., resulting in the formation of a mixed product called advanced glycation end products (AGEs) (Ahmed 2005). It has been well documented that

the accumulation of these AGEs will result in secondary complications in the human body that result in oxidative stress, inflammation, etc., leading to neurodegenerative disorders (Singh et al. 2014; Lee et al. 2022). Numerous studies with various model systems have investigated the antiglycation effect of various chemical and natural antiglycation (Elosta et al. 2012). Various metal nanoparticles or their transformed forms (hybrids, composites, conjugates) are being explored as therapeutics for glycation-related disorders (Patil et al. 2022), but the gap in the effective management of AGEs is warranted. Recent studies have shown that the inhibition against AGE formation has led to the development of various AGEs inhibitors and AGE cross-link breakers from synthetic compounds and natural products. However, synthetic AGE inhibitors, along with their pharmacological efficiency, have adverse effects, expensive and are less available (Bolton et al. 2004; Anandan and Urooj 2019; Lee et al. 2022).

It has been reported that NPs improved cell survival, inhibited the glycation and consequent fibrillation of particular proteins, and drastically reduced the total production of reactive oxygen species (ROS). In addition, NPs have proven to have strong antiglycation, ROS scavenging capacity, blood-brain barrier permeability, and the capacity to inhibit protein aggregation and secondary structural modifications (Ashraf et al. 2016; Anandan et al. 2019). However, there is no clinical evidence that glycation targeting utilizing NP-based drug delivery systems might be employed as a therapy for T2DM and lower the risk of associated neurodegenerative problems; therefore, the clinical usefulness of these NPs is yet unknown (Patil et al. 2022). A proper investigation of NPs with antiglycation capabilities could be a very efficient way to treat AGEs-related disease and its disorder. The recent rise in the application of nanoparticles in medicine and their effectiveness as antiglycation agents has resulted in the exploration of these NPs (both chemical and biological) with various manipulations in the synthesis process. Due to the scope of these nanoparticles, the study was designed to phyto-fabricate ZnO-NPs from the aqueous leaf extract of *Boerhaavia erecta* L. (based on its medicinal importance and antidiabetic properties) (Surange and Pendse 1972; Nisha et al. 2018) and to assess its significance in preventing the formation of AGEs and its byproducts *in vitro*.

Materials and methods

Collection of plant material and phyto-fabrication of ZnO-NPs

The phyto-fabrication of ZnO-NPs was achieved using *Boerhaavia erecta* L. leaves (healthy) gathered from

Manasagangotri, Mysuru, Karnataka, India (Murali et al. 2017) with minor changes. The collected fresh leaf material (25 g) was extracted with Milli-Q water [1:10 (w/v)] using a hand blender and subjected to filtration using Whatman No.1 filter paper. The aqueous leaf extract (ALE) (50 mL) was heated to ~80 °C over a magnetic stirrer, and upon reaching the said temperature, approximately 5 g of zinc nitrate hexahydrate was added with constant stirring and left until the solution became a paste/slurry. The resultant extract was moved to a silica crucible and calcinated at 300 °C for 2 h. The resulting powder (ZnO-NPs) was stored until further studies.

Physicochemical characterization of phyto-fabricated ZnO-NPs

By measuring the optical density between 200 and 800 nm and using Milli-Q water as a blank, the bio-reduction of ZnO was determined using a UV-Vis Spectrophotometer (Beckman Coulter-DU739, Germany) and band gap energy using Tauc's plot equation. The X-Ray diffraction (XRD) study was

performed with Miniflex II X-ray powder diffractometer using Cu k radiation as the energy source (recorded 20–80° at 2θ angles). The ZnO-NPs along with ALE were evaluated for their binding capacities between 4000 and 400 cm⁻¹ using Perkin Elmer Spectrum 1000. Using a HITACHI S-3400N (Japan) scanning electron microscope (EEM), the morphology of ZnO-NPs was captured. An energy dispersive spectroscopy (EDS) of the particles was studied to determine the elemental properties of the ZnO-NPs using a HITACHI (Noran Equipment 7, USA) machine connected to an SEM.

Antioxidant properties of phyto-fabricated ZnO-NPs

Using the DPPH method that Kumar et al. (2020) developed, the free radical scavenging activity of phyto-fabricated ZnO-NPs was evaluated. The DPPH solution (0.1 mM) containing various doses of ALE and ZnO-NPs (0.25, 0.5, 1, 1.5, and 2 mg) (sonicated) was incubated for 30 min at 37 ± 2 °C in the dark. The percentage of RSA in the tested samples was calculated using spectroscopic measurement of the incubated samples at 517 nm.

$$\text{Radical Scavenging Activity(\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test sample}}{\text{Absorbance of Control}} \times 100$$

Metal chelating properties of phyto-fabricated ZnO-NPs

The metal chelating (Fe²⁺) capabilities of ZnO-NPs were investigated according to Sajjad et al. (2021). Ferric chloride (2 mM) and ferrozine (5 mM) were added to 0.8 mL of each

of the different quantities of ALE and ZnO-NPs (sonicated) (0.25, 0.5, 1, 1.5, and 2 mg) and thoroughly mixed. The reaction combination was incubated at 37 °C for 10 min in the dark before its absorbance was read spectrophotometrically at 562 nm. The reaction mixtures with and without EDTA served as the positive and negative controls, respectively.

$$\text{Metal Chelating Ability(\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test sample}}{\text{Absorbance of Control}} \times 100$$

Effect of phyto-fabricated ZnO-NPs on advanced glycation end products (AGEs)

Hemoglobin-δ-gluconolactone assay

The hemoglobin-δ-Gluconolactone (δ-Glu) assay was carried out to assess the inhibitory effect of ZnO-NPs on protein glycation as per the protocol of Losso et al. (2011) with a few modifications. About 800 μL of fresh human blood samples along with 0.1 M PBS (0.16 mL, pH 7.4) containing δ-Glu (50 mM) and ZnO-NPs (0.5, 1.0 and 2 mg) for a final volume of 1 mL was mixed thoroughly and subjected for incubation for 16 h at 37 °C. The fresh blood samples with PBS containing 50 mM δ-Glu alone and aminoguanidine (AG) (10 mM, final concentration) served as respective control. Using a hemoglobin A1C

chromatographic-spectrophotometric ion-exchange kit, the incubated blood samples were then tested for the presence or absence of glycated hemoglobin (HbA1C) (Biosystems, India).

Protein glycation and aggregation by BSA-MGO model

Sample preparation

BSA (10 mg mL⁻¹) was incubated with methylglyoxal (MGO) (10 mM, final concentration) as a glyating agent in PBS (0.1 mM, pH 7.4) following the method of Prasanna and Saraswathi (2016) under sterile conditions. The phyto-fabricated ZnO-NPs (0, 0.5, 1.0, and 2 mg) was added to

the reaction mixture, mixed thoroughly, and subjected to incubation for 30 min. Only BSA was used as native and the reaction mixtures with and without AG (10 mM, final concentration) served as the positive and negative controls, respectively. The incubated samples were dialyzed (with PBS) to remove unbound MGO.

Thioflavin-T (ThT) assay and fluorescence microscopy

Thioflavin-T, a marker for amyloid cross β -structure, was used to determine the inhibitory activity of phyto-fabricated ZnO-NPs for β -aggregation. The glycated and control MGO-BSA samples were incubated for 60 min with ThT (32 μ M). The incubated sample was subjected to fluorescence measurements by their characteristic excitation (435 nm) and emission (485 nm) fluorescence wavelengths. The aggregation of AGEs formation was detected by fluorescence microscopy (LeVine 1999). The dyed samples (10 μ L) were put on glass slides and a fluorescence microscope was used to capture the pictures.

Protective effect of ZnO-NPs on red blood cells

A healthy individual blood sample was taken (2 mL) in citrate-containing centrifuge tubes and centrifuged (1000 rpm, 20 min) to remove the white blood corpuscles and other plasma proteins (Prasanna and Saraswathi 2016). The pellet was washed three times with phosphate buffer saline (PBS), and MGO (5 μ M, final concentration) was added with or without ZnO-NPs (2 mg mL⁻¹) and allowed to react for 60 min at 37 °C. After incubation, the samples were centrifuged and the pellet (RBCs) was repeatedly washed with PBS before fixing with 2.5% glutaraldehyde for 30 min. The fixed samples were centrifuged (1000 rpm for 30 min), and the washing step was repeated to remove glutaraldehyde adhered to RBCs. The obtained suspension (5 μ L) of RBCs was placed on an aluminum foil and dried aseptically overnight, which was later observed under SEM after gold coating.

GK-peptide ribose assay

The G.K. peptide-ribose experiment was carried out using the method outlined in Nagaraj et al. (1996). Under sterile conditions, a reaction mixture including 0.5 M sodium phosphate buffer (pH 7.4), G.K. peptide (80 mg mL⁻¹), and ribose (0.1 M), with or without ZnO-NPs (2 mg), was made in an amount of around 1 mL. The resulting sample was kept at 37 °C for roughly 24 h, and each sample was examined for its unique fluorescence at 340 nm (excitation) and 420 nm (emission) after incubation. The positive and negative controls were the reaction mixtures containing or omitting AM (10 mM).

Statistical analysis

Each experiment was run three times, and the mean results were subjected to a one-way ANOVA using SPSS Inc. 16.0. The magnitude of the F value ($p \leq 0.05$) and Tukey's HSD test were used to differentiate the significant effects of the treatments.

Results and discussion

Physicochemical characterization of phyto-fabricated ZnO-NPs

The physicochemical characteristics of the ZnO-NPs were evaluated by various techniques to confirm the size, morphology and elements of the said particles. The UV-visible study of the ZnO-NPs revealed an absorption peak at 362 nm (Fig. 1A), which falls in long wave (UV-A region: 315–400 nm) of the spectra with a band gap energy of 3.2 eV (Fig. 1B). The spectral outcome confirms that the particle possesses high optical absorbance, which is characteristic of ZnO and is also considered one of the main reasons for their enhanced biological applications (Sirelkhatim et al. 2015; Udayashankar et al. 2021; Ramesh et al. 2022). Well-defined narrow peaks were observed during the XRD studies of the ZnO-NPs at Bragg's angle (2θ) = 31.78, 34.45 and 36.28 that correspond to the planes with miller indices (100), (002) and (101) (Fig. 1C) and are considered to be obtained of products of pure and high crystalline nature with hexagonal wurtzite (JCPDS File No. 01-079-2205) (Venu Gopal and Kamila 2017). The average size of the phyto-fabricated particles was 20.55 nm, which was calculated with Scherrer's formula (Supplementary Table 1). The XRD results of the study are in agreement with Nagajyothi et al. (2015) and Dinga et al. (2022), wherein the planes that correspond to the miller indices at (100), (002), and (101) indicate the pure and crystalline nature in the ZnO-NPs. FT-IR spectra of ALE and the phyto-fabricated ZnO-NPs were comparatively evaluated to identify the functional groups that contributed to metal oxide (ZnO) bonding during the study (Fig. 1D). Likewise, in the ALE of *B. erecta*, spectrum bands were observed at 3288.24 cm⁻¹ [alcohol/phenol (O–H)], 2974.95 cm⁻¹ [aliphatic stretch (C–H)], 1731.78 cm⁻¹ [aldehyde aliphatic (C=O)], 1628.00 cm⁻¹ [(CO–NH) amide stretch], 1546.16 cm⁻¹ (NH bend), 1371.19 cm⁻¹ [alkane group (C–H)], 1240.08 cm⁻¹ [nitro compound group (N–O)], and 1014.89 cm⁻¹ [aliphatic amine group (C–N)]. Besides, from the FT-IR studies of the ZnO-NPs, it was noted that a shift or change in the intensity peak position directly correlates to the interaction of functional groups with the nanoparticles through the donation of electrons (capping, reducing, or stabilizing) that reduces the zinc

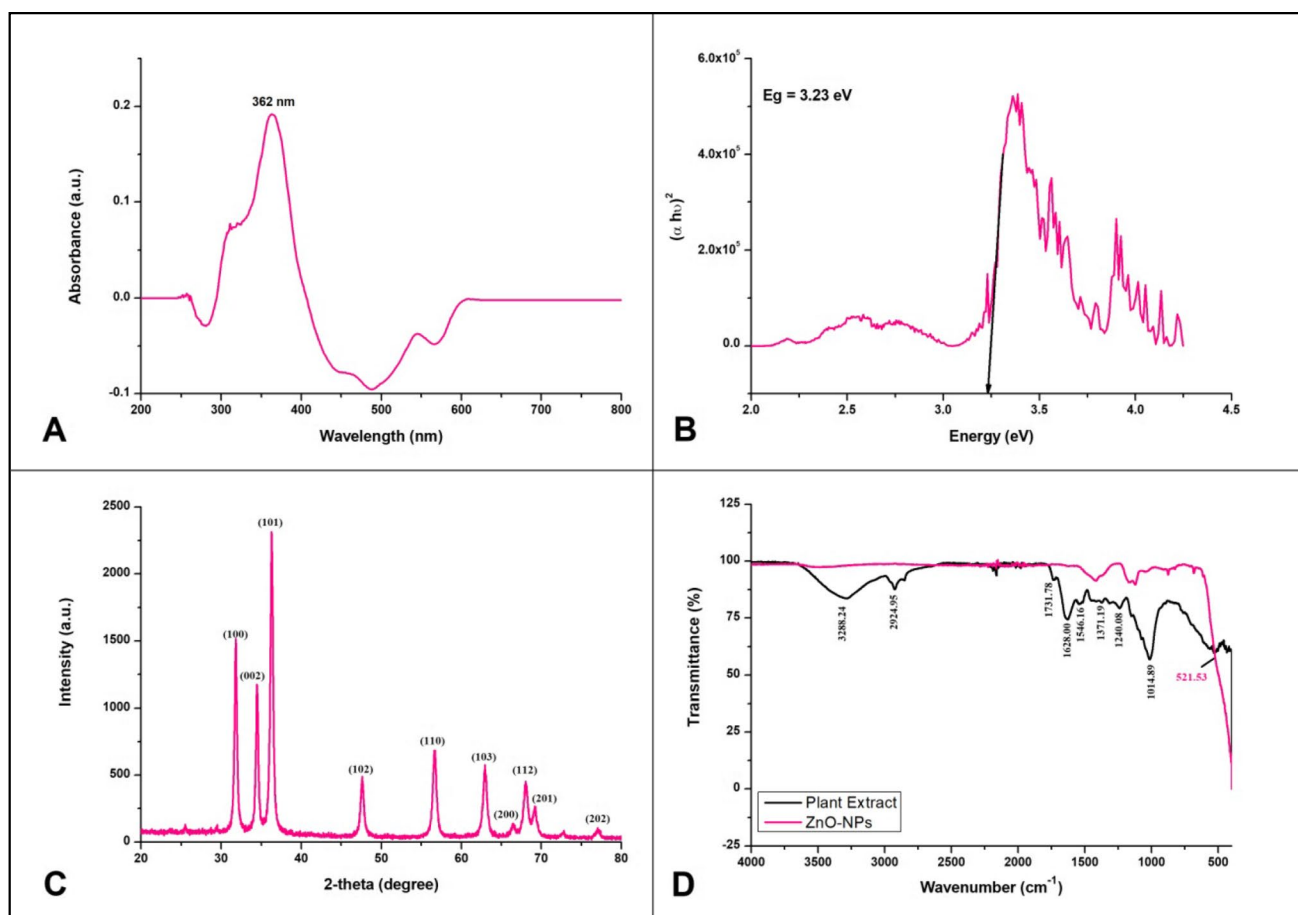


Fig. 1 Physicochemical characteristic features of phyto-fabricated ZnO-NPs. **A** UV-visible spectroscopy, **B** band gap energy, **C** XRD, **D** FT-IR

ions from Zn^{2+} to Zn^{+1} and finally leading to Zn^0 (Alamdari et al. 2020). Additionally, a spectral band was observed at 521.53 cm^{-1} during the study that agrees with the findings of Udhayan et al. (2021), wherein the spectral band observed between 600 and 450 cm^{-1} is designated for the presence of the Zn–O bond. The morphology of the ZnO-NPs evaluated through SEM studies revealed that the particles were agglomerated (Fig. 2A). According to Zhang et al. (2002), the agglomeration was mainly due to the polarity and electrostatic attraction of ZnO-NPs. Numerous other researchers' observations support the study's results wherein ZnO-NP agglomeration was observed during the green-route synthesis (Udayashankar et al. 2021; Murali et al. 2021b). Besides, high-quality ZnO-NPs were identified through quantitative and qualitative elemental analysis using EDS (Fig. 2B), which demonstrated a high zinc content of 63.53% and the presence of oxygen at 33.08%. Similarly, high amounts of ZnO have been observed when analyzed through EDS in the ZnO-NPs synthesized using the plant extracts (Ali et al. 2021; Ramesh et al. 2022).

Antioxidant properties of phyto-fabricated ZnO-NPs

The free RSA evaluated for ALE and phyto-fabricated ZnO-NPs are depicted in Table 1. The study's results noted that the phyto-fabricated nanoparticles showed an RSA of 63.38% at 2 mg mL^{-1} , which was dose-dependent with an IC_{50} of 1.62 mg mL^{-1} . Similarly, in the above-said concentrations of the ALE of *B. erecta*, a maximum of 29.26% RSA was noticed. The study's findings are in line with many other researchers wherein concentration-dependent antioxidant potential was noticed in ZnO-NPs synthesized using plants irrespective of their parts used (Stan et al. 2016; Basnet et al. 2018; Murali et al. 2021b). The enhanced antioxidant ability of the nanoparticles phyto-fabricated using the plant source is ascribed to the presence of Zn ions in the structure or the capacity of the particles to transfer an electron from an oxygen atom (Khan et al. 2019; Alamdari et al. 2020). Besides, the literature suggests that the capping of phyto-constituents on the surface of nanoparticles helps in the donation of extra

Fig. 2 SEM image (A) and EDS spectra (B) of phyto-fabricated ZnO-NPs

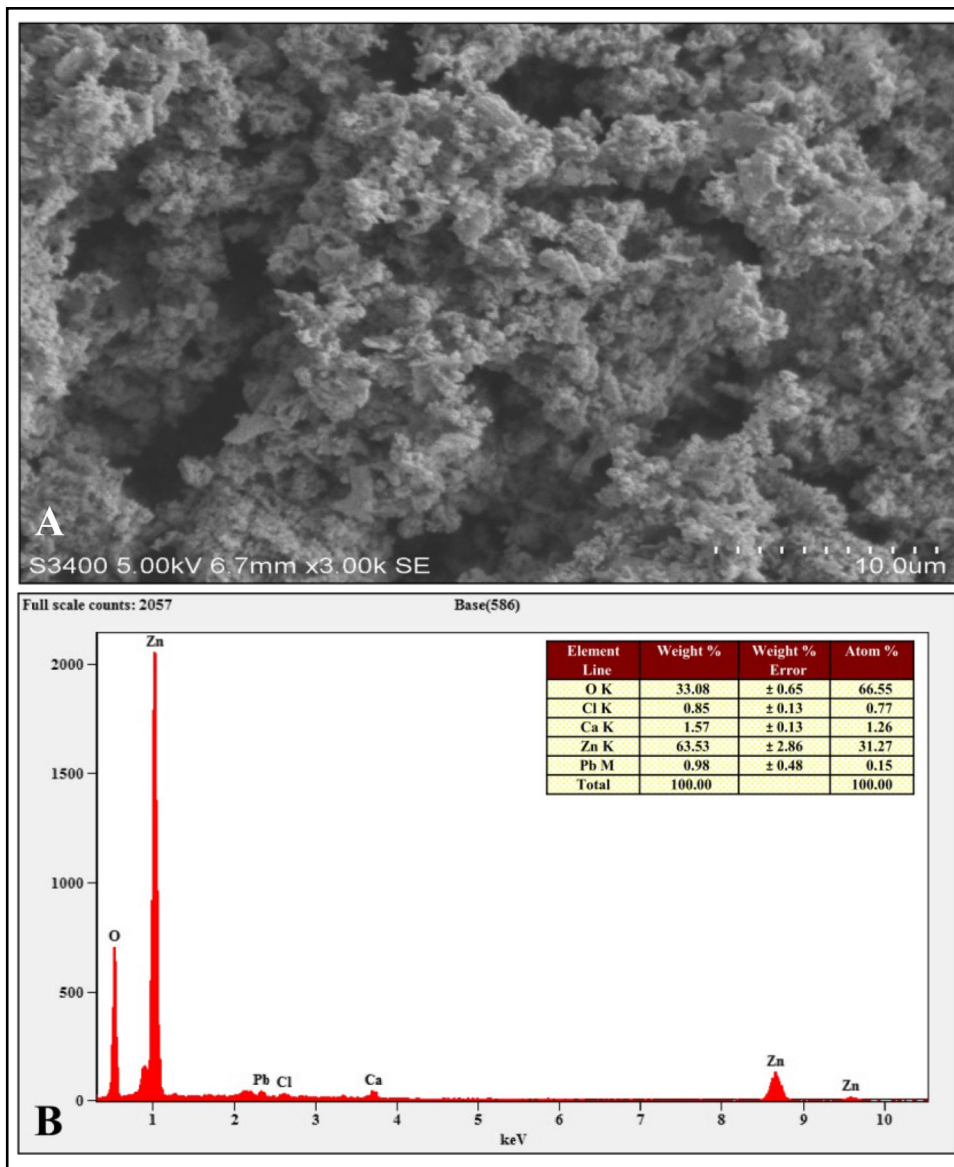


Table 1 Antioxidant and metal chelating properties of phyto-fabricated ZnO-NPs

Concentration (mg mL ⁻¹)	Antioxidant (%)	Metal chelating (%)
0.25	08.06 ± 0.45 ^c	05.51 ± 0.39 ^c
0.5	25.65 ± 0.43 ^d	21.50 ± 0.40 ^d
1.0	48.29 ± 0.39 ^c	36.86 ± 0.60 ^c
1.5	59.56 ± 0.62 ^b	51.39 ± 0.34 ^b
2	63.38 ± 0.54 ^a	57.32 ± 0.61 ^a

Values are the mean of triplicates and ± indicate SE. Means followed by the same letter(s) within the column are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

electrons on a nitrogen atom, thereby ensuring the creation of a stable DPPH molecule which results in their better antioxidant potential (Murali et al. 2022).

Metal chelating properties of phyto-fabricated ZnO-NPs

The evaluation of phyto-fabricated ZnO-NPs for the metal chelating properties indicated that the synthesized nanoparticles had the potential to reduce the concentration of metal catalyzing transition, i.e., from ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) in oxidative degradation which is in agreement with the findings of Sajjad et al. (2021). There was an increase in the metal chelating properties from 5.51% to 57.32%, which was dose-dependent (from 0.25 to 2 mg mL⁻¹) (Table 1). The IC_{50} value of the particles' metal chelating ability was noted at 1.69 mg mL⁻¹. The increase in the metal chelation properties compared to ALE is allied to the capping of phyto-constituents during fabrication (Arunmugam et al. 2021). According to the reports of Younus and

Anwar (2016), the property of bioactive constituents to chelate metal ions is believed to prevent the formation of AGEs. It has been well noted that ZnO-NPs effectively blocked the formation of ferrous and ferrozine complexes in our study, indicating that they had a chelating effect on the ferrous ion prior to the combination with ferrozine being formed and antioxidant ability is believed to be one of the main variables that determine the metal ion chelation from our ZnO-NPs (Soren et al. 2018; Khan et al. 2019).

Effect of phyto-fabricated ZnO-NPs on advanced glycation end products (AGEs)

Hemoglobin- δ -gluconolactone assay

It has been well noted from the literature that an increase in the hemoglobin level in RBCs results in a significant increase in the HbA1c level (Anandan et al. 2019). Similarly, during the study, the increase or decrease in the HbA1c level in the Amadori product (resulted in RBCs reacting with δ -gluconolactone) upon treatment with different concentrations of ZnO-NPs was measured using ion-exchange HPLC (BIOSYSTEMS). From the results of the HDG assay, the HbA1c level was higher in the HDG reaction mixture alone compared to non-glycated ones (fresh blood). In addition, it was noted that the glycated hemoglobin (HbA1c) level decreased in the presence of nanoparticles which was concentration-dependent after the incubation period (Fig. 3). Due to ZnO-NPs' ability as an antioxidant, this observation can be explained by the fact that they have a greater

impact on the suppression of the production of early AGEs, because, during the Amadori glycation process, the generation of free radicals is noticed (Ahmad et al. 2011; Prasanna et al. 2018). Hence, it may be accomplished that during the glycation process, our ZnO-NPs might have impacted on inhibiting the glycated analogue of hemoglobin as observed from the results of the present study.

Protein glycation and aggregation by BSA-MGO model

BSA-MGO glycation model determines the antiglycation property of ZnO-NPs towards MGO mediated glycation, and the results are represented in Fig. 4A. The results specify that phyto-fabricated ZnO-NPs could inhibit the MGO mediated glycation of albumin, as evidenced by low fluorescence intensity. The results might corroborate that the ZnO-NPs blocked the development of AGEs products derived from methylglyoxal in a BSA-MGO model and inhibited the conversion of dicarbonyl intermediates to AGEs (Prasanna et al. 2018). Our findings further reveal that ZnO-NPs can interact with carbonyl groups of reducing sugars, Amadori products, and dicarbonyl intermediates, inhibiting them from converting to AGEs. Dicarbonyl intermediates like MGO have risen in popularity as mediators of AGEs synthesis and are known to generate glycosylamine protein cross-links when they react with lysine, arginine, and cysteine residues in proteins (Monnier 2003). However, there is no significant difference when comparing the intensity of fluorescence in BSA incubated with ZnO-NPs and AG to that in native BSA protein.

Further, utilizing amyloid-specific Th-T, which specifically binds to fibrous structure, it was able to observe

Fig. 3 Effect of phyto-fabricated ZnO-NPs on hemoglobin- δ -gluconolactone

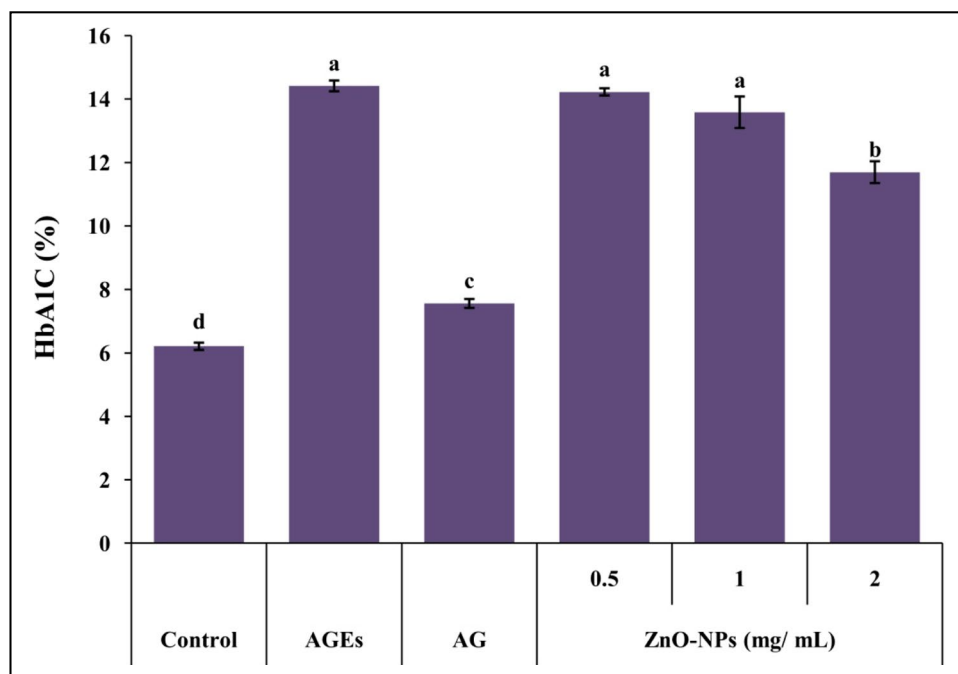
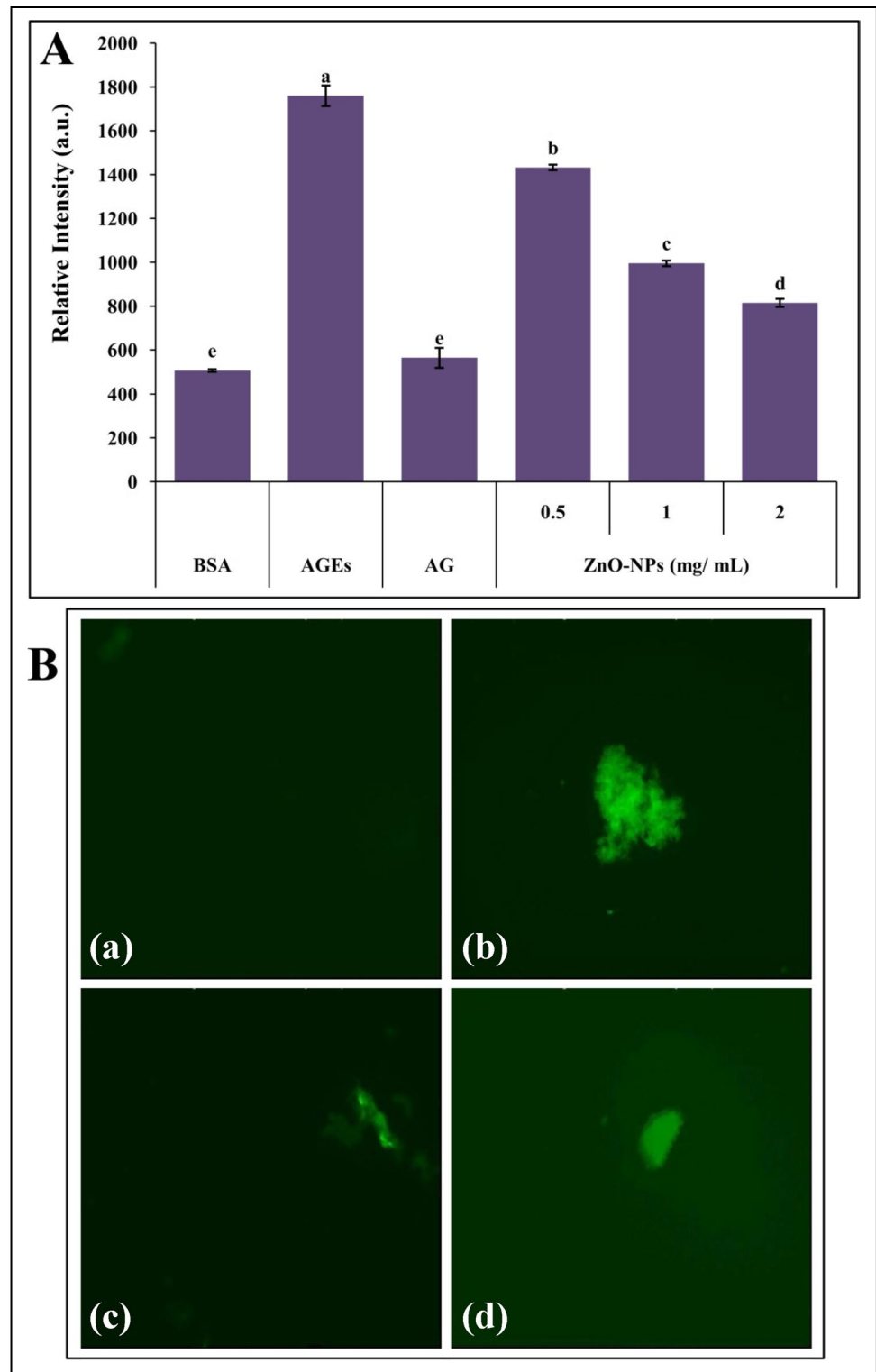


Fig. 4 Inhibitory effect of phyto-fabricated ZnO-NPs on β -aggregation of BSA glycation induced by MGO (A) and Fluorescence microscopic images of thioflavin T binding to amyloid fibrils (B). (a) Native BSA; (b) glycated (BSA + MGO); (c) standard (BSA + MGO + AG); (d) nano-particle (BSA + MGO + ZnO-NPs)



the degree of β -amyloid cross-structure in albumin (see Fig. 4B). Since the Th-T fluorescence intensity was higher in glycated albumin than in albumin without MGO, it seems that protein glycation eventually caused the formation of amyloid structure in BSA. Th-T is a dye that reacts

with the fibrillar structure of proteins to increase their fluorescence but is weakly fluorescent in its native/free form. Emission of high Th-T fluorescence indicates that glycation protein induced the formation of the fibrillar state in protein (Biancalana and Koide 2010). This observation can

be explained due to the existence of antioxidant properties of ZnO-NPs that might have helped in the inhibitory activity at different stages of protein glycation, interrupting the cascade of events and preventing glycation.

Protective effect of ZnO-NPs on red blood cells

The abnormal function of RBCs is disrupted by high levels of MGO, leading to acanthocyte formation (Awasthi et al. 2015). In our study, in the absence of MGO, RBCs had normal biconcave morphology (Fig. 5A), while the same was completely lost, distorted, and ruptured in the presence of MGO (Fig. 5B). It was noted that RBCs incubated with AG (Fig. 5C) and ZnO-NPs (Fig. 5D) exhibited a protective effect against MGO-induced damage. The disruption of RBCs causes the oxygen transport protein hemoglobin to lose function, resulting in oxidative stress. It is important to note that AGEs inhibition and dicarbonyl scavenging reduce oxidative stress and stop proteins from binding to the AGEs receptor (RAGE) (Prasanna et al. 2018). RBCs lose their normal form

due to hyperglycemia, and the presence of dicarbonyl intermediates like MGO, but when treated with ZnO-NPs, the morphological abnormalities brought on by MGO were stopped, and the results were comparable to AG. Similar observations have also been noticed in the RBCs upon incubation with the nanoparticles (Anandan et al. 2019).

GK-peptide ribose assay

As AGEs are also directly correlated to protein-crosslinking, a study was conducted to know whether the phyto-fabricated ZnO-NPs can break the cross-linking by GK-peptide–ribose assay. The literature reported that co-incubating GK peptide with ribose led to the rise in protein glycation end products due to protein cross-linking, also known as the Maillard reaction product (Rahbar et al. 2000). Similarly, in the present study, incubation of phyto-fabricated ZnO-NPs (2 mg) along with GK peptide and ribose, a decreased fluorescence intensity was noted, which is directly attributed to the inhibition of protein cross-linking or AGEs formation (Fig. 6).

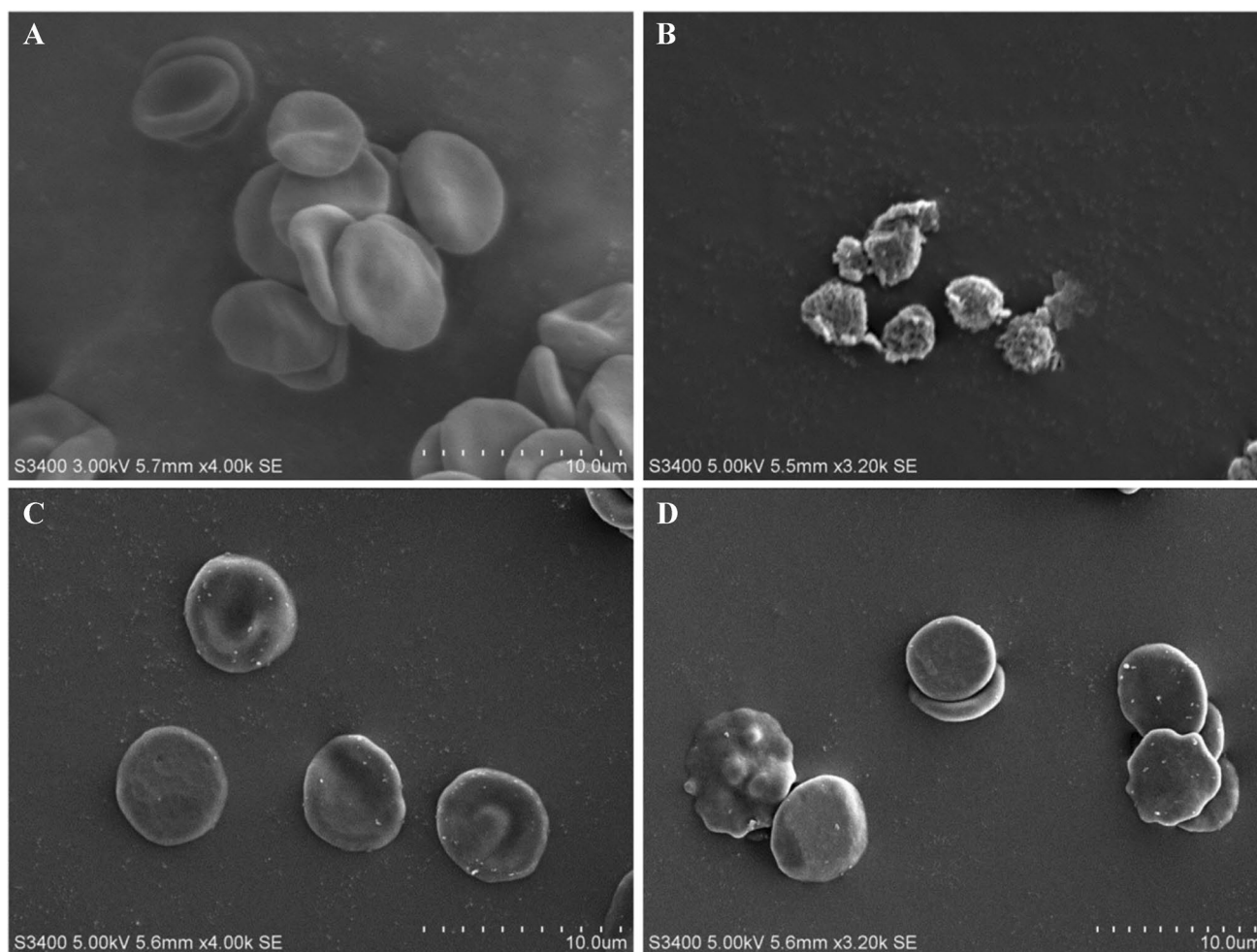
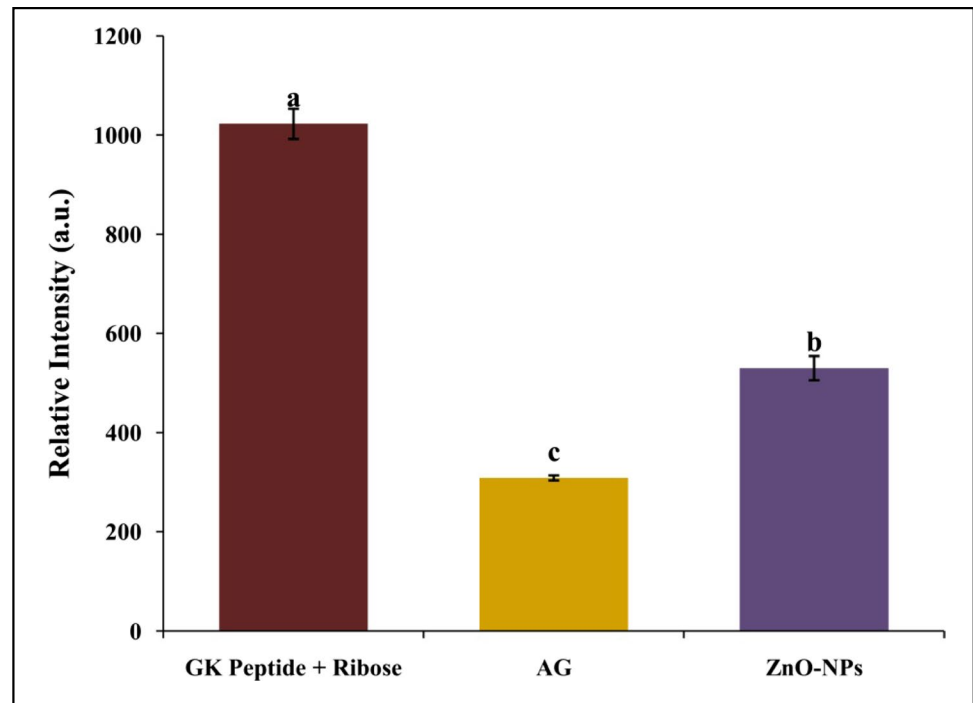


Fig. 5 Protective effect of phyto-fabricated ZnO-NPs on RBC against MGO-induced damage

Fig. 6 Inhibitory effect of phyto-fabricated ZnO-NPs on last-stage of glycation by using GK-peptide ribose assay



It was also noted that the co-incubation of AG with GK peptide + ribose and ZnO-NPs with GK peptide + ribose resulted in the inhibition of 84.8% and 60.92% of AGEs formation, respectively. The results are confirmed with the study of Sengani and Rajeswari (2017), wherein the gold nano-supplements were found to have a strong antioxidant effect, lower blood glucose levels, and prevent the interaction between methylglyoxal (MGO) and lysine.

Conclusions

The present study is the first report on the phyto-fabrication of ZnO-NPs from the medicinally important plant *Boerhaavia erecta*. The phyto-fabricated nanoparticles showed a band gap energy of 3.2 eV with a particle size of 20.55 nm. From the XRD results, it was noted that the particles were crystalline with a hexagonal wurtzite shape. The ZnO-NPs were agglomerated as observed in SEM and EDS confirmed that the phyto-fabricated particles were of high purity (96.61%). Besides, FT-IR proved that the phyto-constituents of the plant extract were involved during the fabrication of nanoparticles resulting in the formation of a metal oxide bond. It was noted that the phyto-fabricated ZnO-NPs possessed efficient antioxidant and metal chelating properties with an IC_{50} value of $\sim 1.6 \text{ mg mL}^{-1}$. Further, during the antiglycation studies, it was observed that the ZnO-NPs were effective in all the stages of evaluation, as noted through the prevention of the advanced

glycation end products (AGEs) and formation of Amadori products, trapping the reactive dicarbonyl intermediate and breaking the cross-link of glycated protein along with offering damage prevention in RBCs induced by MGO. The study's findings will provide an experimental basis for exploring ZnO-NPs from *B. erecta* in diabetes-related complications.

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