Indian J. Applied & Pure Bio. Vol. 40(3), 2336-2342 (2025). **ISSN: 0970-2091**
A web of Science Journal

Evaluation of synergistic Antioxidant potential of Embelin-conjugated ZnO Nanoparticles

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Abstract

Embelin, a naturally occurring benzoquinone, exhibits strong antioxidant activity but is limited by poor solubility and stability. To overcome these challenges, embelin-conjugated zinc oxide nanoparticles (Emb-ZnO NPs) were synthesized and evaluated for antioxidant potential. Three *in vitro* assays DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and metal chelating activity were employed to assess their efficacy. The results revealed that Emb-ZnO NPs showed moderate radical scavenging activity with IC_{50} values of 607.58 µg/ml and stronger reducing power (750 µg/mL,). Additionally, the nanoconjugates demonstrated superior $Fe^{2}z$ chelation capacity (1172.13 µg/mL), indicating enhanced ability to prevent metal-catalyzed oxidative damage. The improved activity is attributed to the synergistic effect of embelin's bioactive functional groups and the large surface area of ZnO nanoparticles, which promote stability, dispersibility, and reactivity. These findings highlight Emb-ZnO NPs as promising antioxidant agents with potential biomedical applications, particularly in managing oxidative stress-related disorders.

Key words : Antioxidant activity, Embelin, ZnO Nanoparticles, Nano Conjugation.

The imbalance in the production of reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and hydrogen peroxide, is known as oxidative stress. The body's antioxidant defenses are essential in the development of cancer, inflammatory disorders, aging, and dementia[20]. This redox imbalance damages cells and molecules, which emphasizes how crucial it is to find potent antioxidants.

Embelin, a natural para-benzoquinone derived from *Embelia ribes*, is popularly known for its anti-inflammatory, antitumor, and cytoprotective activities, it is largely attributed

to its redox-active structure featuring phenolic hydroxyls and for a quinone moiety[17,19]. Its potential for single-electron transfer (SET) and hydrogen-atom transfer (HAT) mechanisms contributes to efficient radical scavenging, as demonstrated in pulse-radiolysis and biochemical studies[11]. Additionally, embelin is known to coordinate transition metals such as zinc (II), leveraging its hydroxy-quinone motif to form stable metal complexes that may suppress Fenton-mediated ROS generation[18].

However, the efficacy of embelin in biological systems, however, may be diminished by its hydrophobicity and poor water stability. Zinc oxide nanoparticles (ZnO NPs), which are prized for their advantageous surface chemistry, relative biocompatibility, and ease of synthesis including environmentally friendly methods utilizing plant extracts have got attention for integration with nanotechnology due to this constraint[1,5]. ZnO NPs produced using phytochemical-mediated techniques have demonstrated notable anti-inflammatory and antioxidant properties *in vitro*[15].

Combining embelin with ZnO NPs holds several advantages. ZnO's high surface area and surface hydroxyls support the functionalization of redox-active moieties, enhancing nanoparticle dispersion, stability, and surface reactivity[12]. Prior nanoformulation studies such as embelin-loaded N,O-carboxymethyl chitosan nanoparticles and embelin nanoliposomes demonstrated enhanced antioxidant performance in assays like DPPH scavenging compared to free embelin[2,10,13]. Chemically, the embelin–ZnO conjugate concept is attractive: embelin can coordinate Zn(II) without introducing redox cycling, while ZnO offers a stable inorganic support. The surface arrangement may enable cooperative radical quenching and electron transfer, potentially enhancing antioxidant efficacy beyond that of embelin or ZnO alone[7].

Applying this panel provides mechanistic insight DPPH captures radical-quenching activity, FRAP gauges reducing power under acidic conditions, and metal chelation probes capacity for inhibiting metal-driven ROS generation. Such a multi-assay approach aligns with contemporary best practices in antioxidant evaluation[14].

Given that phytochemical-functionalized ZnO NPs often exhibit superior radical-scavenging and reducing activity relative to uncapped ZnO [1,5], and that embelin nanoformulations have enhanced antioxidant outcomes compared to embelin alone[2]. embelin-conjugated ZnO NPs are hypothesized to show further improved antioxidant profiles.

In the present study, antioxidant activity of Emb-ZnO NPs assessed through DPPH assay, FRAP assay, and ferrous ion chelation assay offering insight for designing redox-active nanomaterials with therapeutic or functional applications.

Materials :

We synthesized embelin-conjugated ZnO nanoparticles (Emb-ZnO NPs) and published in our previous work[16] DPPH (2,2-diphenyl-1-picrylhydrazyl), analytical grade, Methanol (HPLC grade), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), L-ascorbic acid (for positive control in DPPH; optional), FRAP reagents, Acetate buffer (300

mM, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine; 10 mM in 40 mM HCl), $FeCl_3 \cdot 6H_3O$ (20 mM), $FeSO_4$ (or $FeCl_3$) solution (2 mM), Ferrozine (5 mM) (Carter, 1971), EDTA disodium salt (positive control) (Decker & Welch, 1990), Ultrapure water, Embelin-conjugated ZnO nanoparticles (Emb-ZnO NPs), UV–Vis spectrophotometer (190–800 nm) with 1 cm quartz cuvettes or 96-well microplate reader, Microcentrifuge tubes (1.5–2.0 mL), pipettes and tips, amber vials (for light-sensitive reagents), Vortex mixer, thermostated water bath/incubator (room temperature or 37 °C as needed), Analytical balance and glasswares.

Methodology :

DPPH Free-Radical Scavenging Assay :

Antiradical activity of Emb-ZnO NPs was measured by a decrease in absorbance at 517 nm of ethanol solution of 0.004% DPPH (1, 1-diphenyl-2-picrylhydrazyl)[24]. After 30 min incubation in dark, the decrease in absorbance in the presence of Emb-ZnO NPs at different concentrations was noted Ascorbic acid was used as a standard. The free radical inhibiting activity was calculated according to the formula Percentage Inhibition = ((Acontrol – Atest)/Acontrol × 100) where Acontrol is the absorbance of the control and Atest is the absorbance of the Emb-ZnO NPs. The IC50 value of the Emb-ZnO NPs concentration resulting in 50% inhibition was calculated using the inhibition curve[9].

Ferric-reducing Antioxidant Power (FRAP) Assay :

The Emb-ZnO NPs were assayed for Ferric reducing power according to the method of Benzie and Strain[6] with minor modifications at concentrations (250, 500, 750,1000 µg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.5) and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated for 20 min at 50° C. Trichloroacetic acid (2.5 ml, 10%) was added and centrifuged at 3000 rpm for 10 min. Supernatant (2.5 ml) was added to 2.5 ml distilled water and ferric chloride (0.5 ml, 0.1%) was added. The absorbance was evaluated at 700 nm against blank. The total reducing capacity was calculated from Quercetin standard curve and the results were expressed as equivalents of Quercetin.

Ferrous-Ion Chelation (FIC) Assay (Ferrozine Method) :

The chelation of ferrous ions by Emb-ZnO NPs was evaluated by the modified method of Dinis *et al.*[8]. Different concentrations of samples were added to a solution of $FeCl_2$ (2 mM). The reaction was initiated by the addition of ferrozine (5 mM, 0.2 ml). The mixture was incubated for 10 min at room temperature and the absorbance was evaluated at 562 nm. Metal chelating activity was evaluated according to the formula Percentage Inhibition = ((Acontrol – Atest)/Acontrol × 100) where Acontrol is the absorbance of the control and Atest is the absorbance of Emb-ZnO NPs. The IC_{50} value of the Emb-ZnO NPS concentration resulting in 50% inhibition was calculated using the inhibition curve.

DPPH Radical Scavenging Activity :

The antioxidant potential of embelin-conjugated ZnO nanoparticles (Emb-ZnO

NPs) was evaluated using the DPPH free radical scavenging assay. Emb-ZnO NPs exhibited a concentration-dependent increase in radical scavenging activity. Emb-ZnO NPs showed moderate scavenging with IC_{50} value of 607.58 µg/ml (Figure 1). The IC_{50} value of Emb-ZnO NPs was lower indicating improved free radical quenching ability. Asorbic acid was taken as standard drug and comparative values were depicted in Table-1.

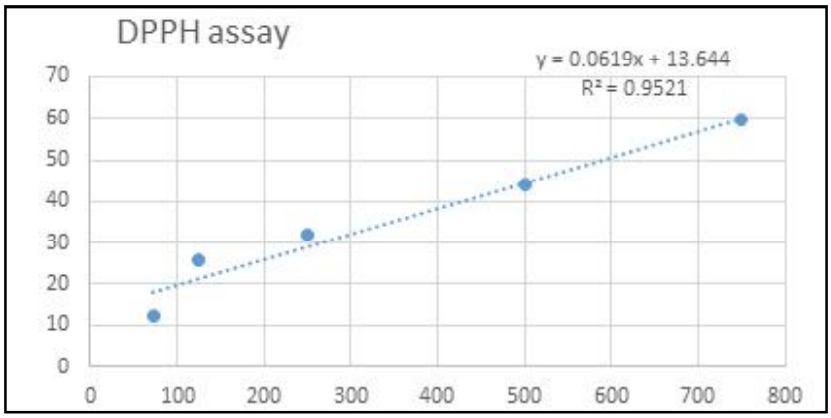

Figure 1. Radical scavenging activity of Emb-ZnO NPs

Ferric Reducing Antioxidant Power (FRAP):

The reducing ability of the samples was assessed through the FRAP assay. Emb-ZnO NPs demonstrated strong ferric ion reducing power in a dose-dependent manner. At 750 µg/mL, the reducing activity of Emb-ZnO NPs was considerably better. Quercetin was taken as standard and the standard curve is represented in Figure 2. The absorbance values at 593 nm increased proportionally with sample concentration, reflecting enhanced electron-donating ability.

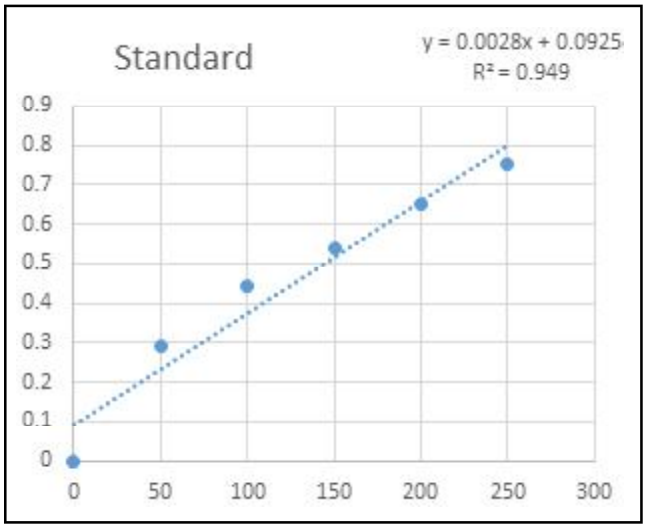

Figure 2. Standard graph of Quercetin

Ferrous ion chelation activity :

The metal-chelating capacity of the formulations was determined using the ferrozine assay. Emb-ZnO NPs showed significant Fe^{2+} chelation even at lower concentrations. With increasing concentrations, the chelation percentage rose steadily, with Emb-ZnO NPs reaching near-complete inhibition of the ferrozine–Fe^{2+} complex formation at 1172.12 µg/mL (Figure 3). EDTA was taken as standard drug. The IC_{50} values further confirmed the stronger chelating ability of Emb-ZnO NPs compared to the other test groups. Overall, Emb-ZnO NPs exhibited a synergistic enhancement in radical scavenging, reducing capacity, and metal ion chelation.

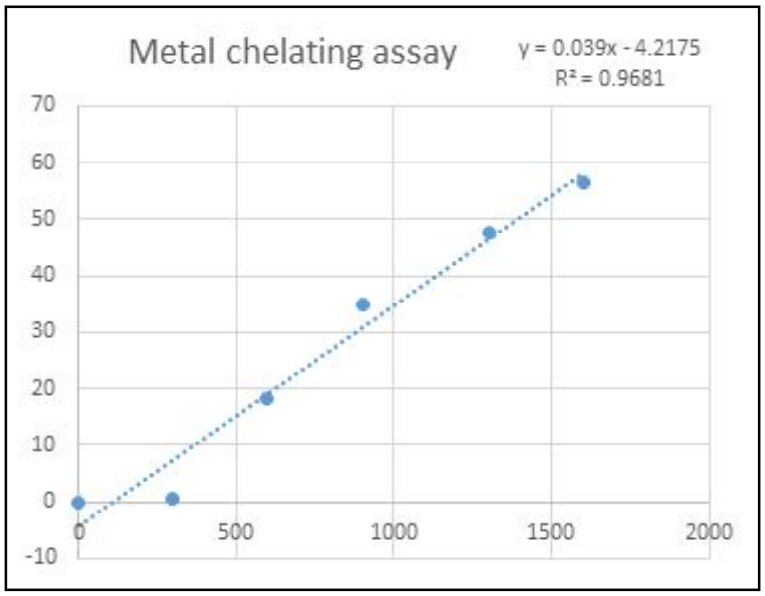

Figure 3. Ferrous Ion Chelation Activity of Emb-ZnO NPs

Table-1. Comparative IC_{50} values of standard and Emb-ZnO NPs

Activity	EMB-ZnO NPs IC_{50} (µg/mL)	Standard IC_{50} (µg/mL)
DPPH	607.58 ± 2.23	124.87
FRAP	750 ± 0.036	109.21
Metal chelating assay	1172.139 ± 8.71	111.93

The present study evaluated the antioxidant potential of embelin-conjugated ZnO nanoparticles (Emb-ZnO NPs) using three complementary assays: DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), and metal chelating activity. In the DPPH assay, Emb-ZnO NPs exhibited concentration-dependent scavenging activity with significantly lower IC_{50} values. This enhanced radical quenching ability can be attributed to the combined action of embelin, a naturally occurring benzoquinone with potent

antioxidant properties[21], and the large reactive surface area of ZnO nanoparticles[22]. Conjugation appears to stabilize embelin on the nanoparticle surface, thereby improving its radical interaction efficiency and preventing self-degradation, a limitation observed when using free phytochemicals alone. The FRAP assay further confirmed this synergistic effect, as Emb-ZnO NPs demonstrated stronger electron-donating ability. The ability to reduce Fe^{3+} to Fe^{2+} is an essential indicator of antioxidant potential, reflecting capacity to donate electrons and terminate oxidative chain reactions[6]. The high FRAP values of Emb-ZnO NPs suggest that conjugation not only preserved but also enhanced the redox activity of embelin, likely due to increased stability, dispersibility, and effective surface presentation of active groups. This finding is consistent with reports that plant-compound-functionalized nanoparticles show superior antioxidant activities compared to their unconjugated counterparts[3]. The metal chelating assay also revealed significant improvements with Emb-ZnO NPs. Free Fe^{2+} catalyzes Fenton-type reactions, generating highly reactive hydroxyl radicals, thereby amplifying oxidative stress[4]. Effective chelation of Fe^{2+} interrupts this process. Embelin possesses intrinsic metal-binding capacity, but when immobilized on ZnO nanoparticles, its accessibility and density appear enhanced, leading to superior chelating performance. This observation aligns with earlier findings where nanoparticle conjugation amplified the bioactivity of phenolic and quinone-based molecules by optimizing their orientation and stability[23].

In conclusion, this study establishes embelin-conjugated ZnO nanoparticles as potent antioxidant agent. The conjugation approach not only preserved the intrinsic activity of embelin but also amplified its functional potential through nanoparticle-assisted stabilization and synergism. Future work should focus on elucidating the mechanistic basis of this enhancement through molecular and cellular studies, as well as evaluating biocompatibility and *in vivo* antioxidant effects to validate their therapeutic potential.

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