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Biosynthesis and In Vitro Antioxidant Activity of Zinc Oxide Nanoparticles with Turkish Oregano (*Origanum onites L.*) Extract

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ABSTRACT

The plant-mediated green synthesis of metallic nanoparticles (NPs) involves biomolecules with organic functional groups present in the plant. This study focuses on characterizing and assessing the antioxidant properties of zinc oxide nanoparticles (ZnO NPs) synthesized from Turkish Oregano (Origanum onites L.), a traditional endemic plant. The formation of biosynthesized O-ZnO NPs was indicated by a white-yellow solid precipitate and a visible color change. Key properties of ZnO NPs were determined using methods such as ultraviolet-visible spectroscopy (UV–Vis), scanning electron microscopy (SEM), Energy Dispersive X-ray analysis (EDX), and X-ray diffraction (XRD). The UV–Vis absorption peak for ZnO NPs was observed around 372 nm. SEM identified ZnO NPs as spherical and nano-sized, while EDX analysis indicated zinc as the primary constituent (50.69%) with strong peaks at 1 keV. XRD revealed an average crystallite size of 14.63 nm for ZnO NPs. Antioxidant activity was assessed through a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical quenching assay, demonstrating moderate antioxidant activity with a scavenging rate of 42.07%. In conclusion, the study affirms the successful synthesis of phyto-fabricated ZnO NPs with a reasonable antioxidant effect. Further study, the impressive antioxidant activity of ZnO NPs suggests their potential use in future nanotechnological applications.

Keywords:
Antioxidant effect, Green synthesis, Origanum onites L. extract, Zinc oxide nanoparticles

1. INTRODUCTION

Nanotechnology has rapidly evolved as a pivotal scientific domain, producing valuable nanoscale materials with diverse applications in science and technology (1). These applications span across healthcare, food, aerospace, pharmaceuticals, and cosmetics, employing nanoparticles like gold (AuNPs), silver (AgNPs), iron (FeNPs), aluminum (AlNPs), copper (CuNPs), and zinc oxide nanoparticles (ZnO NPs) due to advancements in industrial production at the nanoscale (2). Zinc oxide nanoparticles, a focus of intense research, exhibit unique physico-chemical properties, including a controllable small size (1 to 100 nm), high reactivity, functionalized structure, and a substantial surface area/mass ratio (3-4).

Nanoparticle synthesis has seen the evolution of three distinct methods: physical, chemical, and green synthesis, also known as biosynthesis. Physical methods demand expensive equipment, high temperatures, and high pressure, posing challenges in terms of resource requirements. Chemical synthesis, while effective, employs toxic chemicals with potential environmental and health risks (5).

Recognizing the drawbacks of physical and chemical synthesis, biosynthesis has gained prominence as an alternative method (6-7). Biosynthesis involves utilizing microorganisms and plants for nanoparticle synthesis, particularly in biomedical applications and is lauded for its cost-effectiveness, environmental friendliness, biocompatibility, and safety (6). The broader category of biosynthesis, termed green synthesis, facilitates the large-scale production of ZnO nanoparticles. Green synthesis, involving bacteria, fungi, algae, and plants, is preferred for its scalability and environmental benefits, eliminating the need for expensive and toxic chemicals (8). Nanoparticles synthesized through biomimetic approaches exhibit heightened catalytic activity, further emphasizing the advantages of the green synthesis approach (9). The involvement of natural strains and plant extracts in nanoparticle synthesis contributes to phyto-chemicals that serve as both reducing and capping/stabilizing agents (10).

The Origanum genus, part of the Lamiaceae family, holds significance in the food and pharmaceutical industries (11). Folk medicine commonly employs Origanum species for various ailments, including gastrointestinal disorders, diabetes, high cholesterol, leukemia, and bronchitis (12). Phyto-chemical investigations reveal that Origanum species are abundant in bioactive compounds like terpenoids, flavonoids, and steroids (13-14). Turkish Oregano oil is predominantly composed of carvacrol, thymol, γ-terpinene, p-cymene, α-terpinene, and α-pinene, with Turkish Oregano extract containing 12 identified phenolic compounds, primarily rosmarinic acid and acetin (15).

In this investigation, the green synthesis of ZnO NPs was conducted by employing zinc acetate with the aqueous extract of Turkish Oregano (Origanum onites L.) serving as a dual role, acting as both a reducing and stabilizing agent. The structural characteristics of the synthesized ZnO NPs were assessed using X-ray diffraction (XRD). Scanning Electron Microscope (SEM) was employed to ascertain the morphologies of the ZnO NPs, and Energy Dispersive X-ray analysis (EDX) was utilized to identify their elemental components. Furthermore, the antioxidant activity of the ZnO NPs was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

2. MATERIALS AND METHODS

2.1. Preparation of Turkish Oregano *(Origanum onites L.)* extract

5 g of dried Turkish Oregano purchased from a local market was weighed and boiled in 100 mL of deionized water for 5 min. The extract was cooled to room temperature and filtered.

2.2. Biosynthesis of Zinc Oxide Nanoparticles

ZnO NPs were synthesized utilizing Turkish Oregano extract. In the process, a 50 mL solution of zinc acetate (0.02 M) was prepared with deionized water, mixed with 20 mL of Turkish Oregano extract, and the pH was regulated to 10 using 1 M NaOH (16). The reaction mixture, rendered white-yellow, underwent centrifugation with a magnetic stirrer at 80°C. The washing procedure was iterated three times using water and ethanol, and the ensuing precipitate was dried in an oven at 60°C overnight.

2.3. Characterization of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles underwent ultraviolet-visible spectral analysis (PG Instruments T60 UV Visible Spectrophotometer) within the 300-600 nm range. To assess morphology and elemental composition, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX) were employed. The structural features, including purity and crystal size, of the synthesized Turkish Oregano zinc oxide nanoparticles (O-ZnO NPs) were determined using the X-ray diffraction (XRD) method.

2.4. Antioxidant Activity of Zinc Oxide Nanoparticles

The antioxidant activity of ZnO NPs, synthesized through biosynthesis, was assessed using the DPPH test. Initially, 4 mg DPPH was dissolved in 100 mL methanol and stored at +4 °C. Subsequently, 500 µL of this DPPH solution was combined with 500 µL of ZnO NP samples, prepared in methanol at varying concentrations. The resultant mixture underwent a 30 -minute incubation on a shaker at 37°C (17). The absorbance of the samples at 517 nm was recorded to estimate the DPPH scavenging activity, calculated using a specific equation.

Scavenging Activity (%): (Abs Control – Abs Sample)/ Abs Control x100

Blank: Methanol

Control: 500 µL Methanol and 500 µL DPPH reagent instead of sample

3. RESULT AND DISCUSSION

3.1. Characterization of Zinc Oxide Nanoparticles

Various biomaterials can be employed in the green synthesis method to produce nanoparticles with diverse shapes, sizes, contents, and physicochemical properties. The synthesis process is influenced by factors such as reaction temperature, pH, incubation time, and concentration, leading to notable alterations in the shape, size, stability, and nucleation process of the synthesized NPs. Utilizing water as a solvent and the biocompatibility of phyto-nanotechnology contribute to the non-toxic nature of the synthesized NPs, expanding their applicability in biomedical and environmental domains (19, 20). The charge differences in natural phytochemicals play a role in shaping and yielding NPs, impacting binding abilities and metal ions during the synthesis process (21).

The addition of zinc acetate to Turkish Oregano aqueous extract resulted in a noticeable color change, indicating the formation of O-ZnO NPs with a white-yellow solid precipitate. The biosynthesized O-ZnO NPs were subjected to characterization and in vitro antioxidant activity assessment (Figure 1). Diverse techniques, including UV-Vis, SEM, EDX, and XRD, were utilized for the characterization of ZnO NPs, allowing the determination of sizes, shapes, distributions, surface morphologies, and surface areas of NPs (22). The UV-Vis spectrum of ZnO NPs synthesized through the green synthesis method from Origanum onites L. displayed an absorption

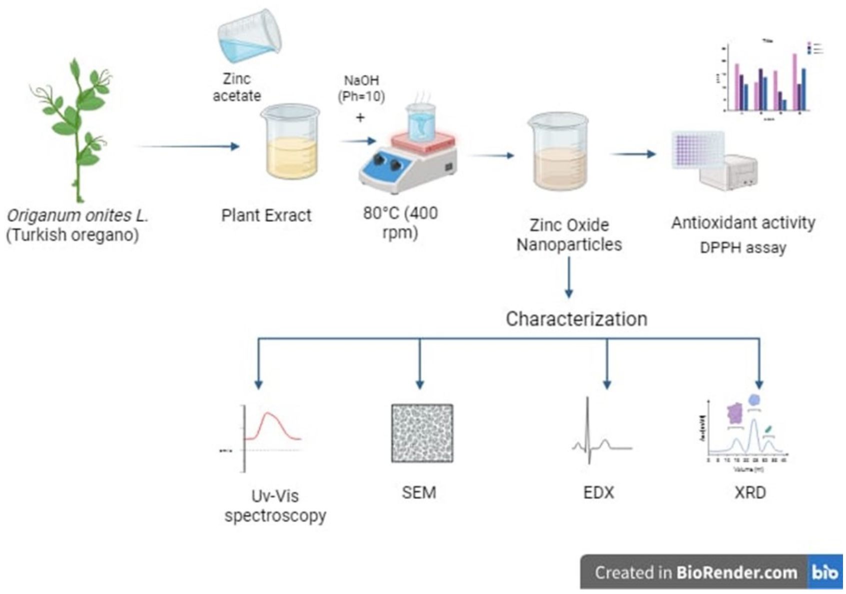


Figure 1. Biosynthesis of Turkish Oregano Zinc Oxide Nanoparticles (O-ZnO NPs)

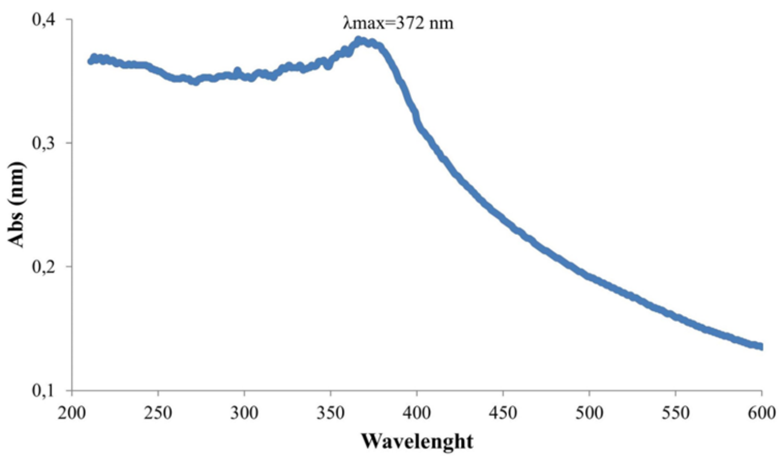
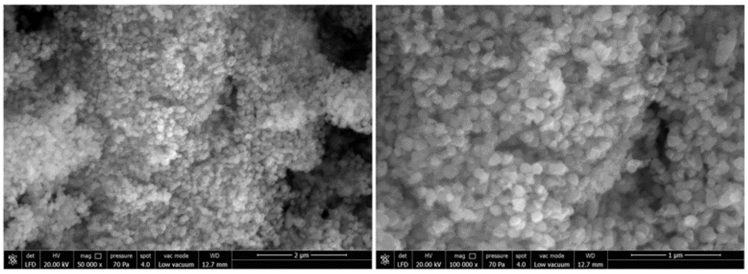


Figure 2. UV-visible spectrum of O-ZnO-NPs.



a b

Figure 3. SEM microscopy of O-ZnO nanoparticles at different magnifications: a) x 50 k, b) x 100 k

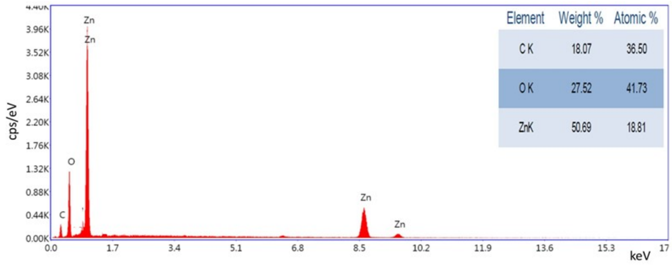


Figure 4. EDX spectrum of O-ZnO NPs.

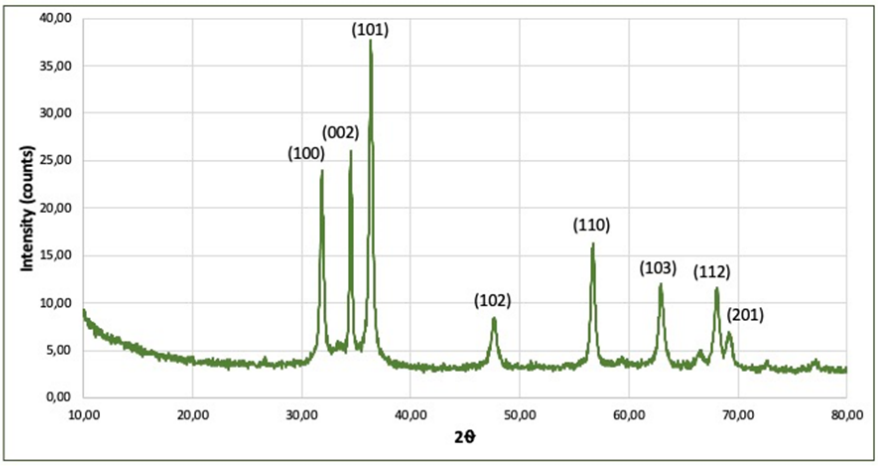


Figure 5. Peak values of the ZnO phase

peak at 372 nm (Figure 2).

Notably, distinct UV-vis peaks were reported in studies using different biomaterials and synthesis methods for ZnO NPs, such as grape seed (350 nm) and Origanum majorana leaf extract (379.75 nm) (18, 23). The UV-vis band of ZnO NPs containing Calendula officinalis flower extract exhibited a peak at 355 nm using the green synthesis method (17). SEM images (Figure 3) revealed nano-sized particles of O-ZnO NPs, primarily spherical and irregularly shaped, similar to findings in various biosynthetic ZnO NP studies (16, 17, 24, 25). EDX analysis (Figure 4) determined the bio-composition of O-ZnO NPs, with sharp zinc peaks at 1 keV constituting 50.69% of the composition, and carbon (18.07%) and oxygen (27.52%) attributed to the bioorganic component of the extract. Comparable elemental impurities of plant-derived carbon and oxygen were reported in other studies (26, 27). XRD data (Figure 5) allowed the determination of purity, crystalline size, geometry, orientation, and phases, aligning diffraction patterns with the Joint Committee on Powder Diffraction Standards (JCPDS) standard crystallographic database for structural information (28). From Figure 5, sharp diffraction peaks are observed located at at 31.850, 34.50, 36.350, 47.760, 56.680, 62.930, 68.160 and 69.510 corresponding to (100), (002), (101), (102), (110), (103) and (112), respectively. The data corresponding to the hexagonal zinc phase of ZnO are consistent with literature values (JCPDS card no.36-1451). Crystal size measurement for the synthesized ZnO nanoparticles was calculated using Scherrer's formula and the average crystal size of O-ZnO NP was found to be 14.63 nm (18).

3.2. Antioxidant Potential Evaluation

Antioxidants play a crucial role in the body's defense against reactive oxygen (ROS) and reactive nitrogen species (RNS) by scavenging or regulating their formation (29). Plant extract-derived antioxidant compounds are highly sought after for their efficacy in controlling ROS-mediated pathogenesis of degenerative diseases (30). The in vitro antioxidant capacity of compounds is often determined using methods like DPPH, where green-synthesized ZnO NPs act as scavengers for free radicals released into the environment. DPPH, a dark purple radical, exhibits maximum absorbance at 517 nm, and its color lightens to a reduced state (light yellow) in the

Figure 6. Radical scavenging activity of O-ZnO-NPs against Ascorbic acid

presence of antioxidants (31). The percentage radical scavenging (RSA) activity of biosynthesized O-ZnO NPs, presented in Figure 6, ranged from 30.17% to 42.07% at concentrations of 50–4000 µg/mL. In a different study, ZnO NPs synthesized via Calendula officinalis flower extract inhibited DPPH free radicals by 33.49% at a concentration of 1000 µg/mL (17). Rehman et al. (30) reported the highest radical scavenging activity of ZnO-NPs at 200 µg/mL to be 25.12 ± 1.48 µg AAE/mg. An evaluation of the antioxidant potential of green-synthesized ZnO-NPs from an endemic plant showed 5% to 59% radical scavenging activity (32). Additionally, Dianati et al. found that ZnO NPs synthesized via curcumin exhibited free radical scavenging activity of 18.06% at a concentration of 500 µg/mL (33).

4. CONCLUSION

The study showed that Turkish Oregano extract was used to synthesize ZnO NPs in a cost-effective, straightforward, and eco-friendly manner. Green synthesized O-ZnO NPs were characterized by UV–Vis spectroscopy, SEM–EDX, and XRD techniques. The green synthesized O-ZnO NPs exhibited remarkable radical scavenging activity. The adoption of biogenic methods, aligning with the principles of green chemistry, in the biosynthesis process is noteworthy for its utilization of eco-friendly, non-toxic, and safe materials. With the rapid progression of nanotechnology from laboratory settings to large-scale industrial production, nanomaterials have found widespread application. Furthermore, the study showcased dose-dependent antioxidant activity, reinforcing the potential use of ZnO-NPs as effective antioxidants for future applications.

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