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Lepidium Meyenii Walp (Maca) Roots Extract Assisted Green Synthesis of Zinc Nanoparticles and Their Antioxidant and Anticancer Activities

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Abstract

In this research paper, we looked at the synthesis and characterization of zinc nanoparticles using the green synthesis approach. The Brassicaceae family medicinal herb Lepidium meyenii Walp. was used in an attempt to make zinc nanoparticles. Zinc nanoparticles were made from zinc sulfate and an extract from Lepidium meyenii Walp. UV-VIS and FT-IR analyses were used to perform the optical characterization. The architecturally generated nanoparticles were characterized by X-RD, TEM, and SEM. The DPPH test was also used to measure antioxidant activity. At 100 µg/ml, the percentage inhibition values for ZnO-NPs, ascorbic acid, Lepidium meyenii Walp extract, and BHA were determined to be 86.62, 75.62, 71.75, and 59.35 µg/mL, respectively. The ability of the nanoparticle to prevent the growth of cancer cells in the large intestine has been found.

Keywords: *Antioxidant, Anticancer activity, Biosynthesis, Zinc nanoparticles, Lepidium meyenii Walp.*

1. INRODUCTION

Due to its applicability in a wide range of sectors, the emergence and development of nanotechnology has changed people's perceptions of what is possible for humans to accomplish through the manipulation of materials at the nanoscale. [1, 5]. Compared to their bulk counterparts, nanoparticles (NPs) have the unusual property of having a large surface to volume ratio, which makes them more appropriate choices in application-oriented performances [6]. The discipline of technology that deals with the study, application, and production of materials on a nanoscopic scale—typically between 1 and 100 nm—has recently come to be known as nanotechnology. These particles' main feature is their huge surface to volume ratio, which accounts for their widespread application in the fields of materials science and engineering, medicine, optics, biotechnology, micro-biology, electronics, and the environment [7, 8].

The Greek word from which the word "nano" originates means "too small" or "the tiniest thing infinitely [9, 10]. The ability of nanotechnology to produce and regulate materials at the nanoscale has recently made it the focus of intense research interest [11, 12]. It works with substances whose dimensions fall between 1 and 100 nm on the nanoscale [13, 14]. Because of their unique properties, such as their incredibly small size and higher surface area, nanomaterials are highly prized compared to their bulk counterparts [15, 16]. The main factor behind the extensive usage of nanoparticles in medicine is their ability to readily interact with cell membranes, receptors, proteins, and nucleic acids due to their size similarity feature (nanoscale range). Zinc oxide nanoparticles are among the most

widely used metal nanomaterials for a variety of biomedical applications [17].

Zinc oxide nanoparticles (ZnONPs) have gained recognition as a substitute photocatalyst due to their non-toxic nature, high catalytic activity, and low cost [18, 19]. Because of its low toxicity in vitro and in vivo, it has several applications in a variety of fields, including biology, agrochemicals, perfumes, dyes, petroleum, and medicines. In addition to these uses, ZnO-NPs have biological uses, such as anticancer applications [20], antibacterial [21], antioxidant [22], anti-inflammatory [23], drug delivery [24] and antifungal [25] applications because of its strong environmental resilience and great biocompatibility [26]

One such important metal oxide nanoparticle that is currently receiving attention is zinc oxide nanoparticles (ZONPs). Its distinctive properties, such as its wide bandgap, catalytic effectiveness, and benign nature, make it an effective tool for various applications. ZONPs are widely employed as semiconductors, adsorbents, photocatalysts, anti-microbials, drug-delivery agents, and self-cleaning agents. [27]. However, these methods typically involve the use of hazardous reducing agents and organic solvents, the majority of which are highly reactive and environmentally hazardous. ZnO nanoparticles have so been produced using a green synthesis approach in an effort to reduce their environmental impact. A technique called "green synthesis" uses plants and microbes to create nanoparticles with potential uses in biomedicine [28]. Medicinal plants have been used to treat illnesses since the beginning of human civilization [29]. According to estimates from the World Health Organization, more than 75% of people worldwide still receive their primary medical treatment from traditional healers using plant-derived remedies [30, 31].

Maca (*Lepidium meyenii Walp*., *Brassicaceae* family), is a well-known crop that is used extensively in Peru as a dietary supplement and medication [32, 33]. In 1992, the Food and Agriculture Organization declared maca to be a safe food [33]. Because of its many possible outcomes, it was then regarded as one of the top goods in the world health care market. The plant is widely used as a nutritional supplement around the world and is well known for its nutrient contents, which include minerals, proteins, carbohydrates, amino acids, sugars, and fatty acids. It is typically acquired in the form of capsules or powder [34]. *L. meyenii* root has traditionally been used as an active component in chocolate, coffee, and oils to increase libido, lessen fatigue, and improve sex and reproduction [34,35]. Numerous pharmacological effects have been reported, including those that are hepatoprotective, antifatigue, immune-modulatory, neuroprotective, aphrodisiac, antiproliferative, antioxidant, enhance memory and learning, antidepressant, antirheumatic, and protect against UV radiation [36]. Prostatic hyperplasia, osteoporosis, premenstrual pain, menopausal symptoms, chemical and physical stress responses, and mobility could all benefit from its application [34, 35]. Six classes comprise the primary bioactive components found in *L. meyenii*: fatty acids, alkaloids, polysaccharides, flavonols, macaene and macamides, glucosinolates and isothiocyanates, and thiohydantoins. [37]. In order to assess the biological properties of these samples as strong antioxidants and anticancer agents, we report here the preparation of Lepidium meyenii Walp aqueous extract and an environmentally friendly protocol for the synthesis of zinc nano-solution using this plant extract. Our goal is to identify alternative chemotherapeutic agents.

2. MARERIALS AND METHODS

2. 1. The Collection of *Lepidium Meyenii Walp*

Lepidium meyenii Walp samples were gathered from the Nasiriyia City, Thi-Qar, Iraq, local market. They were broken,

cleaned, and then ground with an electric grinder.

2. 2. Methods

2. 2.1. Preparation of *Lepidium Meyenii Walp* Extract

Lepidium meyenii Walp powder weighed five grams. 100 mL of distilled water was used to dissolve the powder, and it was then heated for 20 minutes at 50 °C. Whatmann No. 1 filter paper was used to filter the extract. After that, the filtrate was kept for later use in a tight-seal pack at or below 4 °C.

2. 2. 2. Synthesis of Zinc Oxide Nano-particles

A subsequent process was employed to prepare the zinc nano-solution. [38], made using *Lepidium meyenii Walp's* aqueous extract. A solution containing 1 mmol of zinc sulfate was made in deionized water. In a 500 mL conical flask, add 20 mL of the plant extract and 80 mL of the salt solution, stirring constantly at pH = 7 (neutral medium). It is held at room temperature in a magnetic stirrer for two hours. The mixture was exposed to a specific UV lamp with reduction influence effect for 15 minutes at a wavelength of (λ = 254 nm), using the methods described by Sharma *et al.* [39], and Devasenan *et al.*, [40]. An equimolar ratio of the produced zinc nano-solution was absorbed (1:1).

2. 3. Characterization of Zinc Oxide Nanoparticles

Using a UV-Vis spectrophotometer UV-1700 (Shimadzu, Tokyo, Japan) operating in the 250–750 nm scanning range, the produced ZnO NPs were examined. FTIR was used to characterize the synthesized ZnO NPs in order to identify the biomolecules that caused the ZnO NPs to decrease. The Shimadzu model, whose wavelength range was 400–4000 cm−1, was employed. The produced nanoparticles were analyzed using scanning electron microscopy (SEM, JEOL JSM-6490A) to determine their shape and chemical structure. The TEM method, or transmission electron micro-scope, offers crystallographic and morphological details about nanoparticles. JEM-HR-2100; JEOL, Japan was used to conduct the TEM analysis. The XRD X'PERT Powder Panalytical instrument was used to identify the crystal structure of the powder samples. During testing, 40 kV and 35 mA of voltage and current were applied, respectively.

2. 4. Potential Biological Characteristics

2. 4. 1. Antioxidant Activity Procedure

A colorimetric DPPH free radical assay was used to evaluate the antioxidant activity of the zinc nanoparticles and the aqueous extract of *Lepidium meyenii Walp*. To make a 0.1 mM DPPH solution, 0.0039 g of DPPH was dissolved in 100 ml of methanol. A solution containing 1.0 milliliter was combined with 2.0 milliliters of zinc nanoparticles in a range of concentrations (20–100 µg/mL). The absorbance of the samples (concentrations) under consideration was measured at 517 nm after thirty minutes. BHA (butylated hydroxyl anisol) and ascorbic acid were utilized as standards. With regard to radical scavenging activity, the sample with the greatest absorbance of the reacted mixture is indicated. [41]. Using the following formula, the sample's percentage of inhibition of free radicals was used to express the radical scavenging activity :

$$\% \text{ Inhibition of DPPH} = \frac{(Ac - As)}{Ac} \times 100$$

where Ac is the absorbance of the control (blank, without ZnO-NPs) and As is the absorbance in the presence of the ZnO-NPs.

2. 4. 2. Maintenance of Cell Cultures

Ccolon cancer in humans Minimum essential media (MEM) supplemented with 10% fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin was used to sustain HCT-8 or [HRT-18] cells. Trypsin-EDTA was used to passage the cells, which were then reseeded at 50%

confluence twice a week and cultured at 37 °C [42].

2. 4. 3. Cytotoxicity Assays

On 96-well plates, the MTT cell viability assay was performed to ascertain the cytotoxic effect. [43]. 1 × 104 cells/well were used to seed the cell lines. Cells were treated with the tested drug after 24 hours or until a confluent monolayer was formed. Following a 72-hour treatment, the media was removed, 28 µL of a 2 mg/mL MTT solution was added, and the cells were incubated for 1.5 hours at 37 °C to determine the viability of the cells. Following the removal of the MTT solution, 130 µL of DMSO (dimethyl sulphoxide) was added to the wells to solubilize the residual crystals. This was followed by a 15-minute shake-free incubation period at 37 °C [44]. The assay was run in triplicate, and the absorbency was measured using a microplate reader at the test wavelength of 492 nm. The following formula was used to determine the percentage of cytotoxicity, or the inhibition rate of cell growth [45]:-

"Inhibition rate(IR) = A- B/A*100"

where A is the optical density of control, and B is the optical density of the samples . GraphPad Prism 6 was used to perform a statically analysis of the acquired data using an unpaired t-test [46]. The values were shown as the triple measurements' mean ± standard deviation [47].

3. RESULTS AND DISCUSSION

3.1. Morphology and Size

3.1.1. UV-Analysis

The produced ZnO nanoparticles' UV-Vis spectrophotometric analysis is displayed in Figure 1. According to the UV absorbance finding from earlier research, there are ZnO nanoparticles present when there is an absorbance peak at 350 nm. Vaishnav *et al*., 2017[48], reached a peak for zinc oxide nanoparticles at 351 nm.

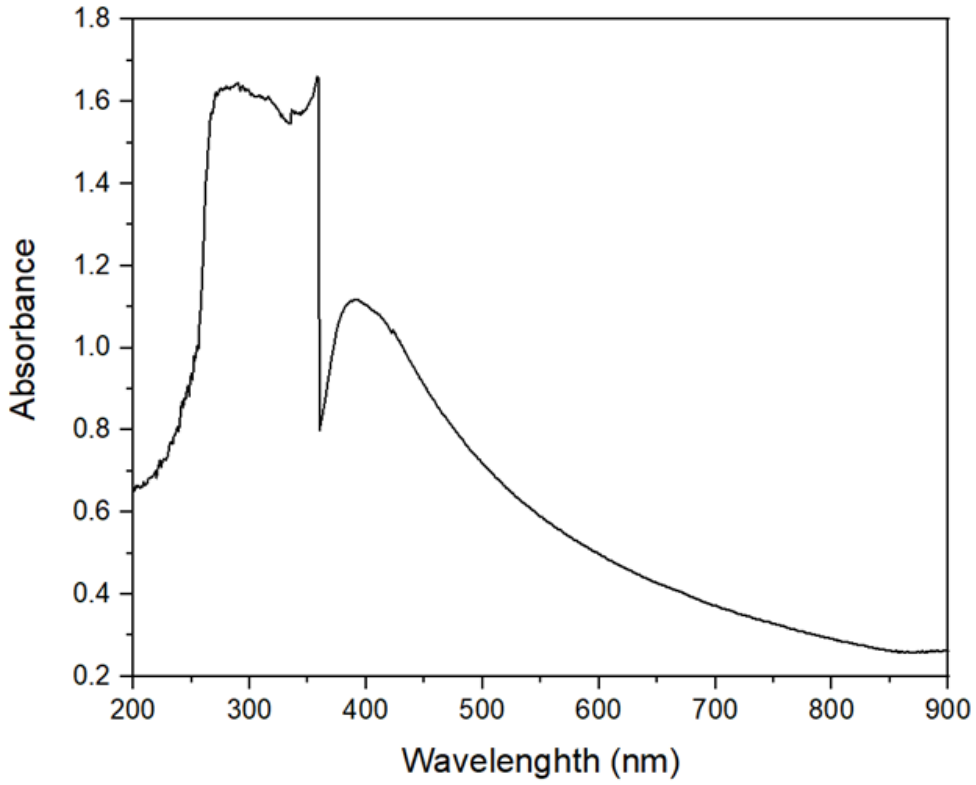

Figure 1. *UV-Visible spectra of the synthesized ZnO-NPs.*

3. 1. 2. FTIR Analysis

Figure 2 displays the FTIR data for ZnO-nanoparticles. ZnO nanoparticles were found to exhibit distinct bands at 3496 cm^{-1}, 2923 cm^{-1}, 1725 cm^{-1}, 1609 cm^{-1}, and 901 cm^{-1}, in that order. The polypeptide amide bond, OH stretching of the phenolic bond, and OH stretching of the carboxylic acid, respectively, may be the cause of the strongest absorption peaks at 1609 cm^{-1}, 3496 cm^{-1}, and 2923 cm^{-1}, respectively. The presence of C=O was the cause of the peak at 1725 cm^{-1}. Figure 2 revealed a peak attributed to ZnO stretching vibration at 901 cm^{-1} [49], verifying that *Lepidium meyenii Walp* extract is used as a reducing and capping agent during the synthesis of ZnO NPs.

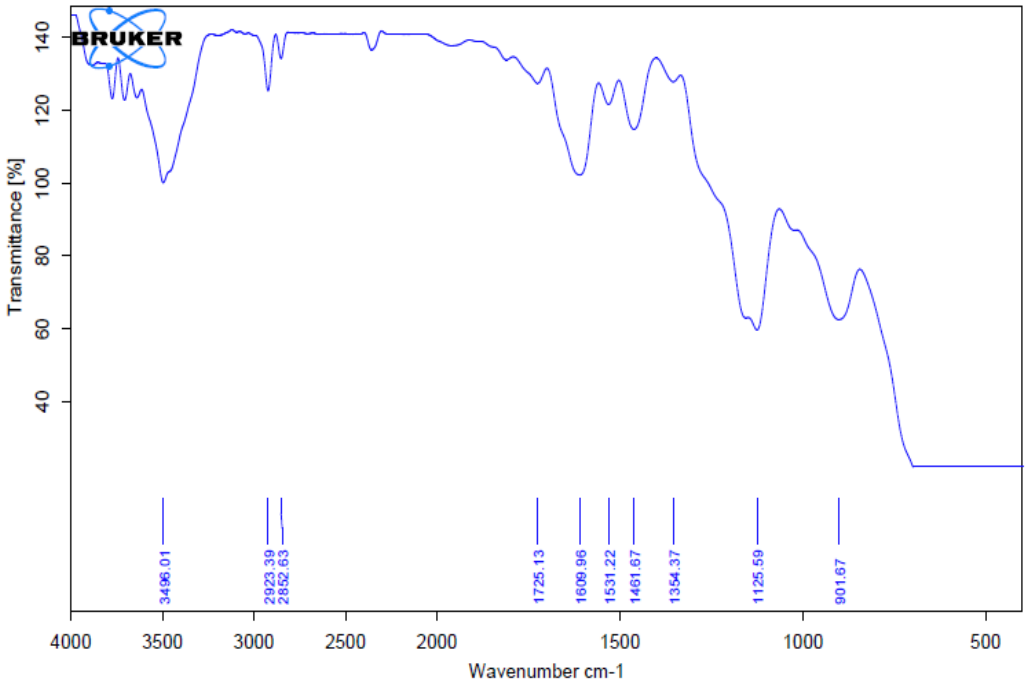

Figure 2. *FTIR spectrum of ZnO- NPs.*

3. 1. 3. Scanning Electron Microscope (SEM)

Scanning electron microscopy was used to analyze the zinc nanoparticles' size and surface morphology (SEM). The zinc nanoparticles produced by *Lepidium meyenii Walp's* plant extract are shown under scanning electron microscopy (Figure 3) and are acquired using the suggested bio-reduction approach. It was established that the zinc nanoparticles were spherical in form.

3. 1. 4.Transmission Electron Microscopy of ZnO Nanoparticles

ZnO nanoparticle TEM pictures are displayed in (Figure 4). The average nanoparticle size measured from TEM images was 30.83 nm, which was consistent with the results from XRD. The performance was used to examine the size, shape, and aggregation of the produced nanoparticles as well as the surface morphology of the metal nanoparticles. Dhabian and Jasim, 2021 [49] have described the similar process for characterizing nano-metals in order to gauge the morphological characteristics of the nanoparticles.

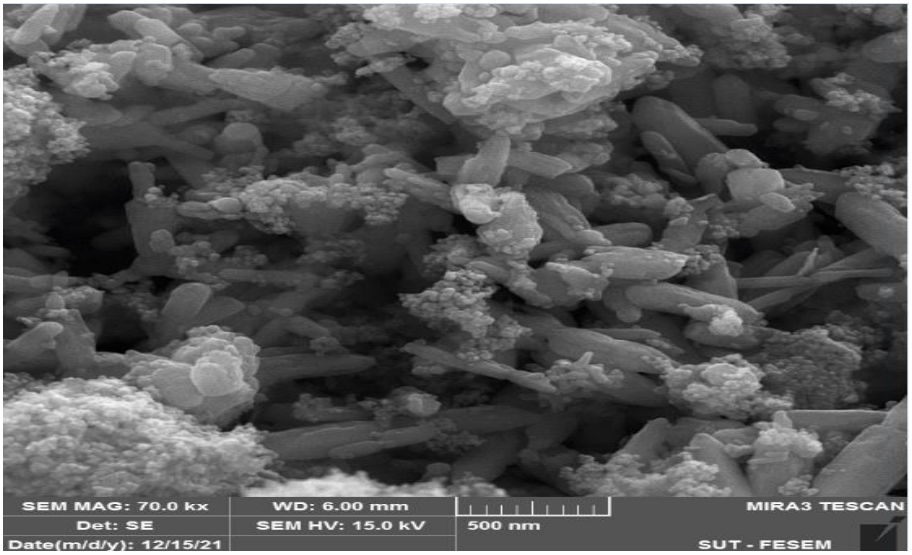

Figure 3. *Scanning electron microscopy images of ZnO nanoparticles.*

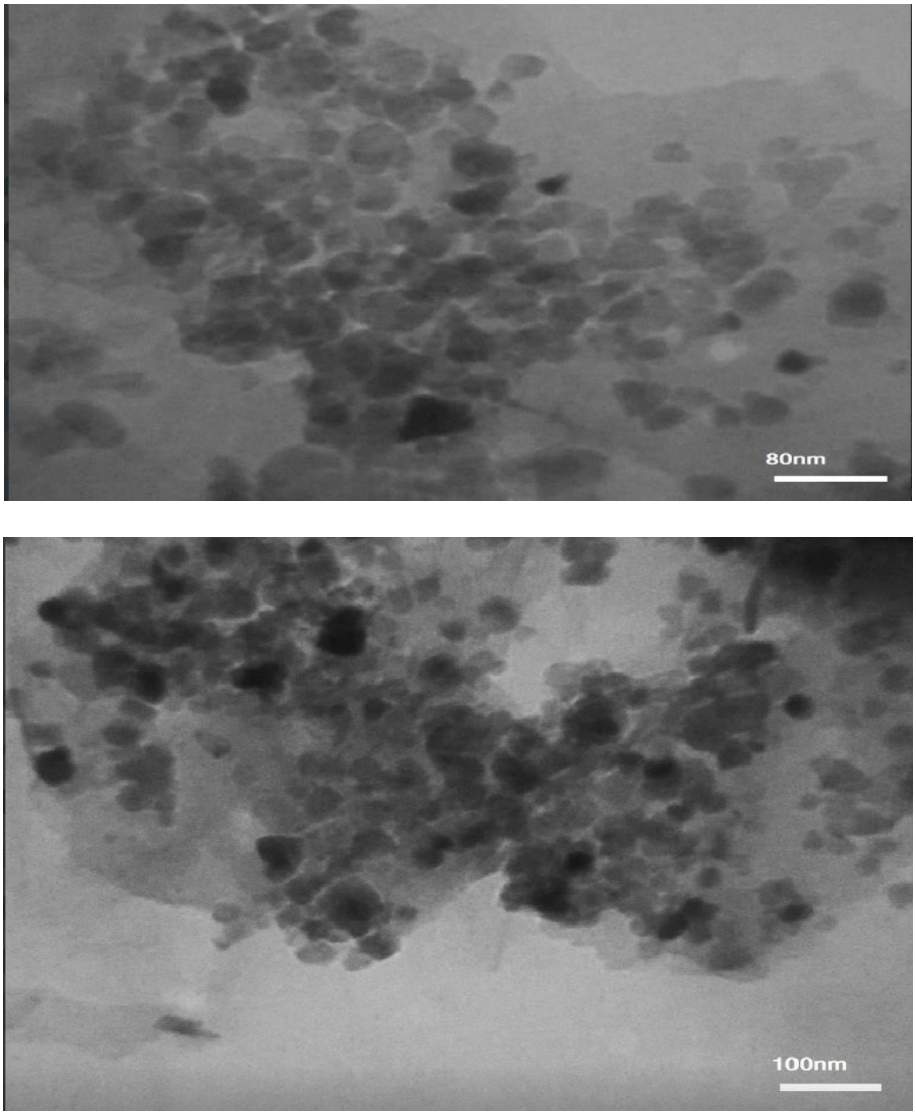

Figure 4. *TEM screened micrographs of zinc nanoparticles prepared by extract Lepidium meyenii Walp at a magnification of 80 nm and 100 nm.*

3. 1. 5. X-Ray Diffraction Pattern of ZnO (XRD)

ZnO NPs are evenly distributed and have a powdered form, according to X-ray diffraction (XRD) results. ZnO NPs' XRD pattern revealed the following unique diffraction peaks at 2θ values indexed to 100, 002, 101, 102, 110, 103, 200, 112 and 201, respectively: 32.23º, 34.77 º, 35.14 º, 36.57º, 47.72º, 56.87º, 63.22º, 66.62º, 68.16º and 69.37º. The hexagonal (wurtzite) structure of ZnO, which has a preferred orientation along the (101) plane, is represented by these peaks in Figure 5. The computed lattice parameters, "a" and "c," were 3.201 A and 5.540 A, respectively, in agreement with previous research [50]. The ZnONPs produced using Aspalathus linearis flower extract fitted well with the lattice parameters "c/a" ratio of 1.730.

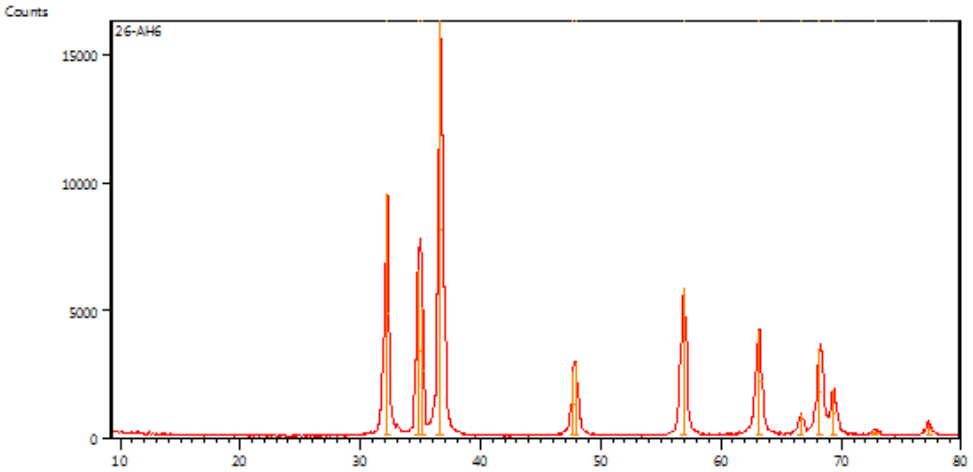

Figure 5. *X-ray diffraction of the synthesized ZnO-NPs.*

The ZnO-HPNs sample that was generated had a crystallite size of 25.74 nm, which was determined using the Debye-Scherrer formula. (Scherrer 1918; [52] Sornalatha *et al*. 2014[53]): D = (0.89 λ) / (β cos θ).

3. 2. Antioxidant Activity of ZnO-NPs

A helpful reagent to utilize is DPPH, which may also be used as a substrate to assess the antioxidant activity of phenolic compounds by scavenging free radicals. Regardless of any enzymatic activity, a decrease in DPPH absorption indicates the extracts' ability to scavenge free radicals. This technique can be used to test the antiradical potency of an antioxidant by measuring the DPPH absorbance decline at 517 nm. Certain chemicals (flavonoids or phenolic compounds) have strong anti-free radical properties, and the presence of hydroxyl groups in the plant may have an impact on these properties. Because of their strong antioxidant content, these compounds are effective antiviral treatments for bacterial and fungal infections [54]. ZnO-NPs showed significantly more inhibitory activity in the DPPH scavenging assay than did the standard, which was ascorbic acid and BHA. Using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical, ZnO-NPs and a methanolic extract of *Lepidium meyenii Walp* were evaluated for their antioxidant qualities. Zinc nanoparticles were demonstrated to have a higher antioxidant capacity than *Lepidium meyenii Walp* extract. The activity of treated zinc particles was significantly higher. The highest recorded radical scavenging activity of 86.62% was observed in zinc derived from *Lepidium meyenii Walp* extract at a concentration of 100 g/mL. According to Figure 6, the antioxidant activity of the aqueous extract was 75.62%, while that of the normal

ascorbic acid was 71.76% and that of BHA was 59.35%. Antioxidants are microscopic substances that have the ability to scavenge reactive oxygen species (ROS) by halting an oxidative chain reaction [55, 56].

ROS play a pivotal role in the pathogenesis of various diseases, such as degenerative conditions like cancer and cardiovascular issues [57, 58]. ROS are essential to the pathophysiology of many diseases, including degenerative illnesses like cancer and heart problems [59].

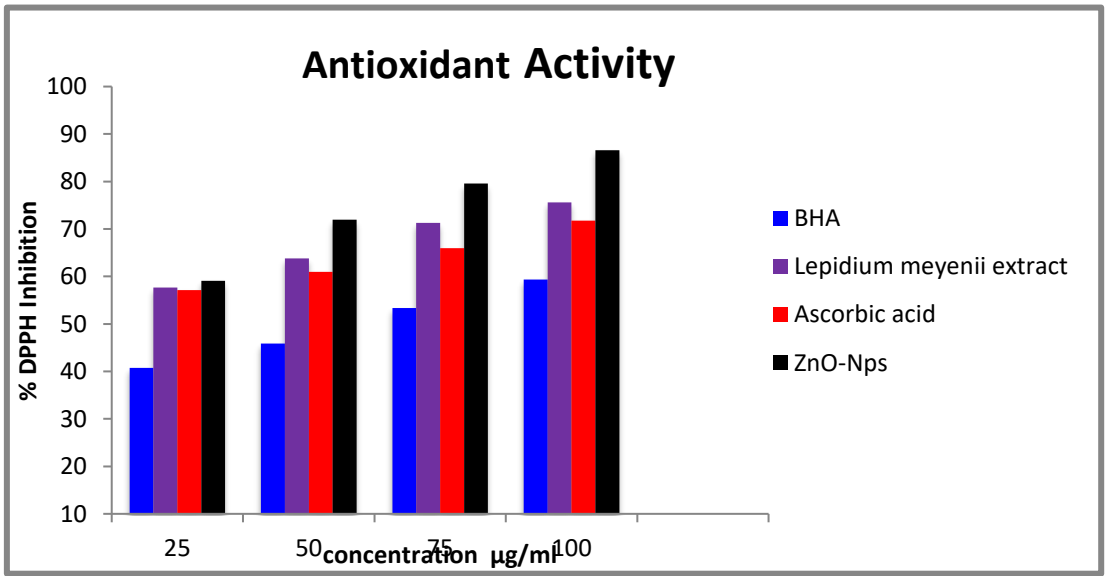

Figure 6. *The DPPH free radical scavenging activity of ZnO-NPs at different concentration when compared to the standard (ascorbic acid, BHA) and extract Lepidium meyenii Walp.*

3.3. Anticancer Activity of ZnO-NPs

The risk that ZnO-NPs posed to HCT-8 cells was examined. Examined was the ZnO-NPs' ability to halt the HCT-8 cell line's proliferation, which demonstrated their antiproliferative activity. This study showed that ZnO-NPs exhibit cytotoxic activity against the HCT-8 cell line, with different results observed at different concentrations. When the concentration of CuONPs was raised from 31.2 to 1000 g/ml, the rate of inhibition rose from 1.62% to 52.52%. The HCT-8 cell line exhibited the maximum rate of inhibition at 1000 g/ml, at 52.25%, during a 72-hour treatment period. As seen in Figures 7–8 (a–b). According to the findings of the current research, ZNO-nanoparticles show a significant cytotoxic potential against HCT-8 cell lines. The interaction between biosynthesized nanoparticles and the bioactive chemicals attached to their surface may give rise to such potential activity [49]. However, scientists found that the anticancer activity of biosynthesized ZnO-NPs was dose-dependent, indicating that a higher concentration of ZnO-NPs enhances its potency against cancer cells. Effective cancer treatment has been made possible by the intriguing research opportunity presented by the use of nanoparticles in targeted drug delivery. Drug dosages for treatment and associated adverse effects will be decreased through targeted drug delivery to malignant cells. Compared to other nanoparticles, ZnO NPs' low toxicity and biodegradable characteristics have led to a rise in their use in the delivery of cancer drugs [26].

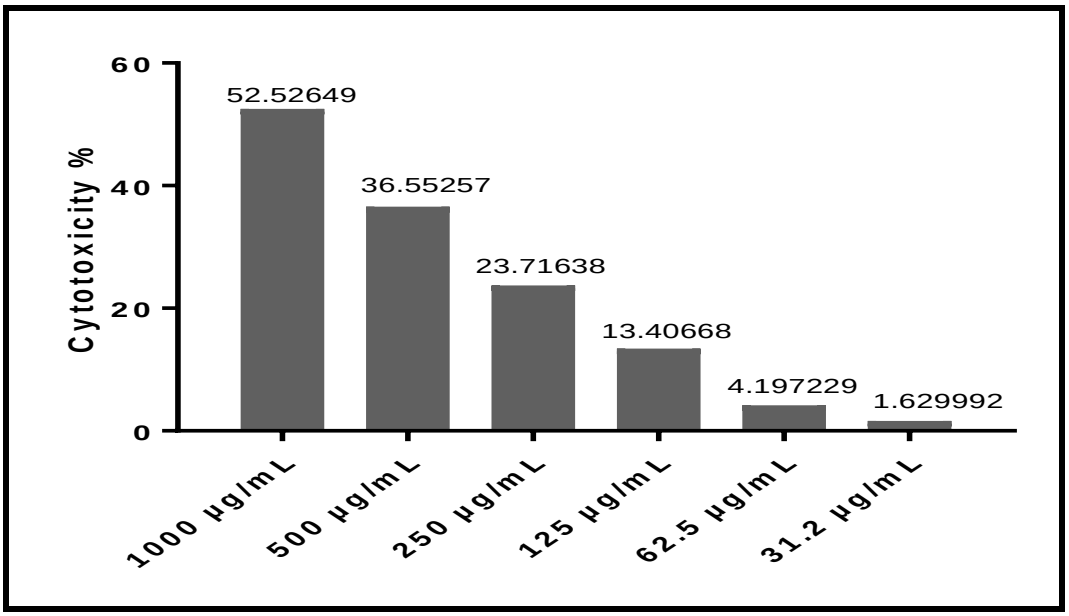

Figure 7. *Cytotoxic effect of Zinc nanoplarticles on HCT-8 cell.*

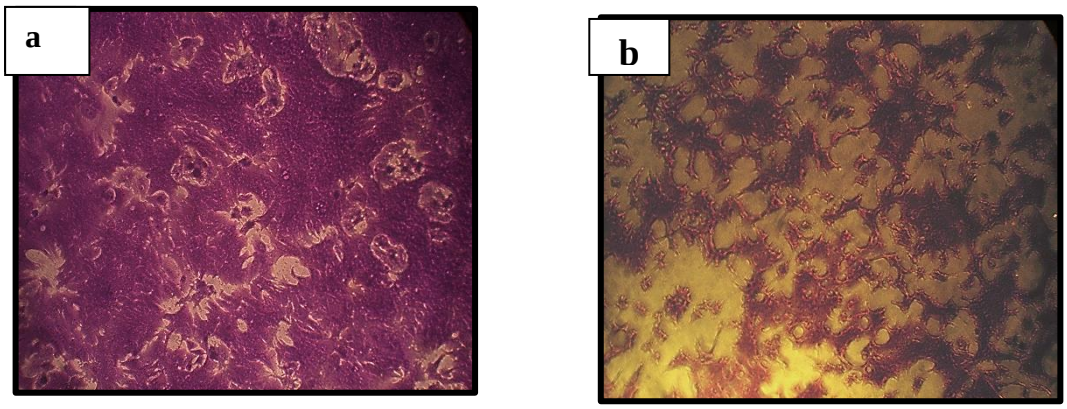

Figure 8. *(a) Control HCT-8 cells untreated under inverted microscope (X10), (b) Treated under inverted microscope (X10).*

4. CONCLUSION

In conclusion, *Lepidium meyenii Walp* extract was used to create ZnO NPs with an average size of 30.83 nm and spherical form. FTIR, TEM, XRDUV–vis spectroscopy, and SEM were used to analyze the ZnO NPs. Green produced ZnO NPs showed cytotoxicity against the HCT-8 cell line and strong antioxidant activity, suggesting that they could be used in medical applications.

DECLERATION

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The author do not receive any funding from an external source.

Availability of Data and Material

All data generated or analyzed during this study are included in this article.

Competing Interests

The author declare that there is no conflict of interest associated with this publication. The author has read and agreed to the publication of the manuscript.

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