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RESEARCH PAPER

In Vitro Analysis: The Anticancer Activity of Zinc Oxide Nanoparticles from *Cinnamomum Verum*

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ABSTRACT

Zinc oxide (ZnO) has a wide range of applications. Green synthesis is an alternative to traditional physical and chemical methods of synthesis. Green nanoparticle synthesis is gaining popularity due to its low cost, reduced use of toxic chemicals, and broad antitumor activity. In this paper, we describe the synthesis of zinc oxide nanoparticles (ZnONPs) using different ways for synthesis and study the effect on different cancer cell lines. X-ray diffraction, and scanning electron microscope analysis were used to assess the purity, particle size, and morphological structure of synthesized ZnONPs. However, because nanoparticles have a small crystallite size, they appear to have uneven structures, such as spongy and flower-shaped particles. The nanoparticles obtained have good anticancer activity. This research will lead to the development of a new method of cost-effective synthesis and the reduction of chemical usage in future studies.

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INTRODUCTION

Nanotechnology is a cutting-edge branch of study that has the potential to transform several established disciplines. Due to their size and form, nanomaterials have attracted significant attention in both the fundamental and applied sciences. The new features of nano-sized semi-conductors have attracted a lot of attention in recent years due to their potential uses in optoelectronics. Zinc oxide nanoparticles (ZNPs) are flexible semi-conductors with notable UV-Visible (UV-Vis) optical transparency and luminous characteristics. Its high chemical and thermal stability [2] have made these nanoparticles increasingly significant in recent years. Sol-gel, hydrothermal, spray pyrolysis, microwave-assisted procedures, chemical vapor deposition, ultrasonic condition, and precipitation methods are only some of the methods established for the synthesis of ZNPs [3-6]. The use of poisonous and harmful chemicals in these sorts of preparations poses potential health dangers, in addition to the high energy costs. Green synthesis techniques, on the other hand, are gaining popularity since they are frequently single-step processes that are also environmentally friendly, safe, and economical [7-9]. Raveendran et al. [9] state that as compared to other physicochemical approaches, biosynthetic

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pathways yield nanoparticles with more precisely specified sizes and morphologies. Nanoparticle production and stabilization rely on natural substances found in biological systems, such as capping agents. According to the reviewed research, there are significant benefits to employing plants instead of other biological systems. Nanoparticles made using plant extracts have greater stability, and the plants themselves are readily available and safe to work with [10, 12].

Extracts from plants including Cassia fistula, Trifolium pratense, Ocimum basilicum, and others have been used to synthesize ZNPs in a green manner [13, 14].

The nobly scented Laurus nobilis L. has also been used to the creation of nanoparticles [15-17]. The additional reports [18-20] tested various medicinal characteristics of the plant extract.

The purpose of this research was to develop a stabilizing and reducing agent in the form of biogenic zinc oxide nanoparticles using *Cinnamomum verum* extract, and then to test the nanoparticles' in vitro anticancer activities.

MATERIAL AND METHODS

Preparation of Zinc oxide

We used all of the chemicals and materials exactly as they were given to us. The zinc acetate (M.W.183.48g/mol) supplied by Sigma was purchased. Local *Cinnamomum verum* leaf was collected, washed with distilled water, dried at room temperature in the shade, and stored in a sealed container for future use.

Approaches used for prepare Zinc Oxide nanoparticles

The zinc nanoparticles were prepared using two approaches. The first approach, in summary, involved mixing the zinc acetate salt with leaf extraction (where Ethanol was used for extraction) and placing the mixture in an autoclave for 30 minutes at 121 °C (250 °F) temperature and pressure of 15 psi [1] (103 kPa or 1.02 atm). After 30 minutes, the wight precipitant begins to develop, and the result is washed many times with cold water before being placed in a 450°C oven for 1-2 hours to dry and clean up any depress. This method will be referred as A1 from here on. Second approach, dissolve Zinc acetate in distal water on the stirrer plate until completely dissolved, then add 20 mL of leaf extract and keep the mixture on heat at 60°C with the stirrer for 4-5 hours before adding NAOH to raise the acidity of the mixture. Then, as previously described, white precipitate begins to form, which is isolated and washed with distal water many times. And this product call H1 here and after.

RESULTS AND DISCUSSION

XRD analysis

X-Ray diffraction tests were used to measure the crystallinity and average particle size of

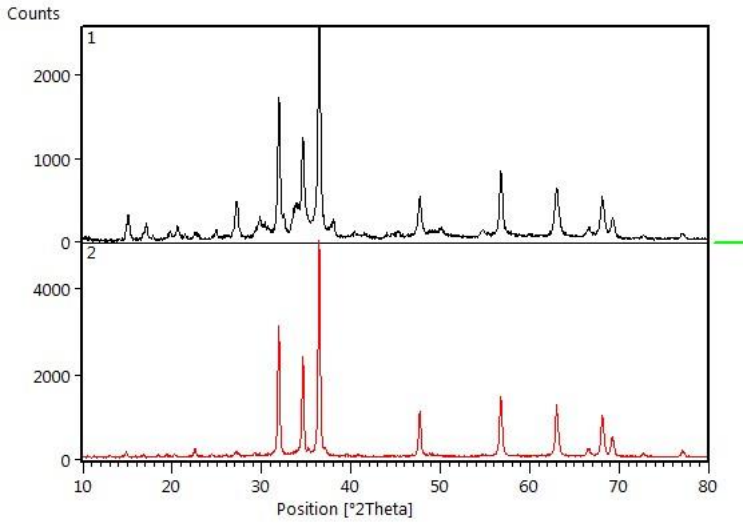

Fig. 1. XRD for Zinc nanoparticles for *Cinnamomum verum* leaf extract with ethanol. Top part showing the approach one (Autoclave) to prepare the nanoparticle and bottom part display the heat methods

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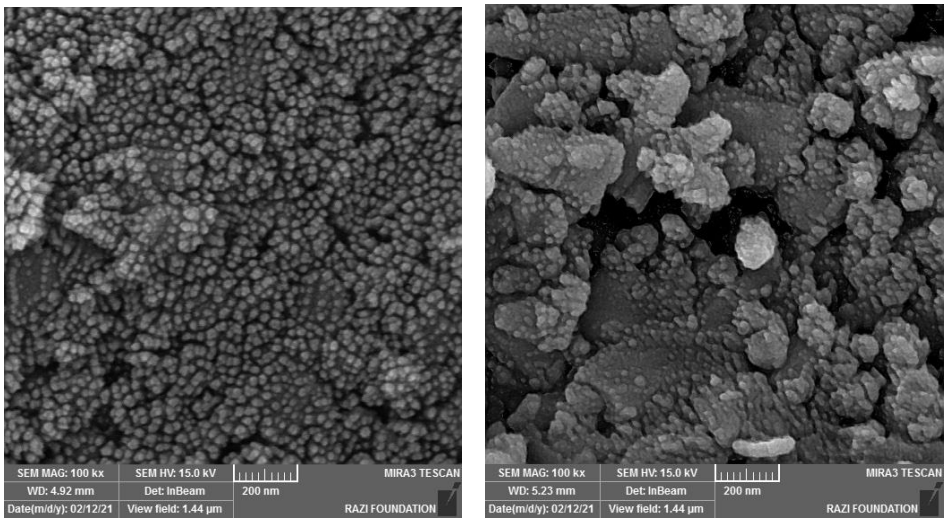

Fig. 2. FESEM for Zinc nanoparticles for *Cinnamomum verum* leaf extract with ethanol.

produced nanoparticles. The XRD pattern of zinc oxide nanoparticles may be seen here. XRD is one of the finest methods for characterizing produced Zinc Oxide nanoparticles. It defines the nanomaterial's purity and phase. XRD provides information on the diffraction angle, interlayer spacing, and, most importantly, crystallite size. Bruker D8 advance XRD was employed in our research. Fig. 1 depicts the XRD pattern of zinc oxide obtained using a chemical approach. The initial approach peaks were seen at 31.97, 33.77, 34.59, 36.46, 47.71, 56.78, 62.92, and 68.07, which correspond to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystalline planes of ZnO and were in good agreement with the JCPDS Card no. 01-079-0205. Additionally, the peaks for the second approach were 31.95, 34.61, 36.43, 36.46, 47.71, 56.74, 62.92, and 68.08. All of these peaks closely match the literature [8, 9], indicating that zinc oxide is formed. The average crystallite size of ZnO NPs estimated using Scherrer equation based on the whole width at half-maximum of the (101) diffraction plane was 29.61nm for autoclave approach and 35.53nm for heat approach (SFM). As the crystallinity diminishes, the wide peaks reflect the formation of smaller particles (Fig. 2).

Scanning electron microscope (FESEM) and energy dispersive spectra (EDS) analysis

The synthesized nanoparticles' structure and shape are verified by FESEM analysis. Particles unmistakably constitute the sphere's structure. The overall images showing the obtained particles have structural nanoparticles that resemble

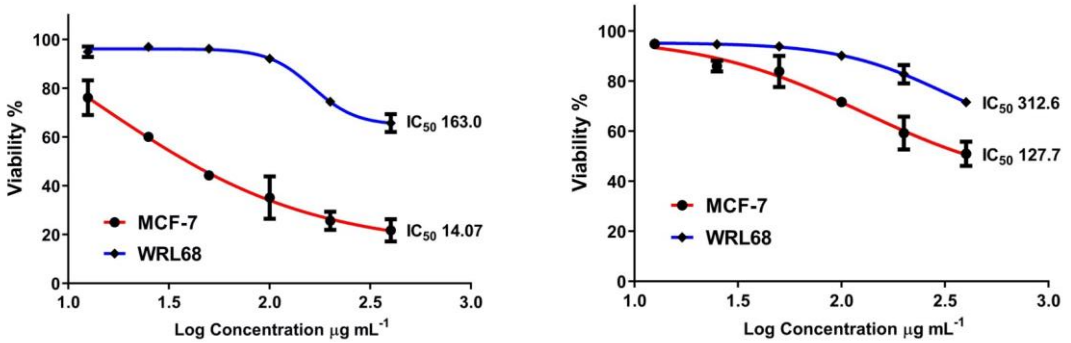

Fig. 3. The MTT assay for Zinc nanoparticles. Left side: the cytotoxicity activity that produce with first approach, the right side: the cytotoxicity activity that produce with second approach

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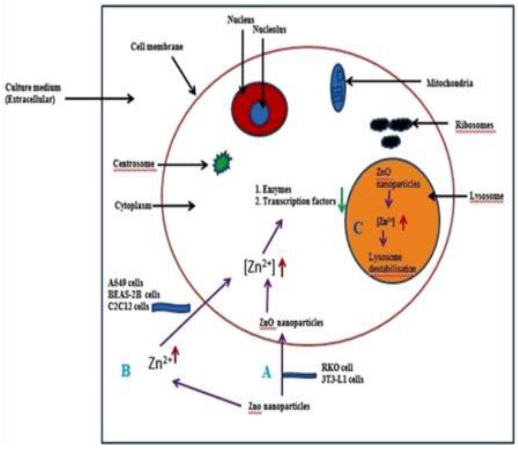

Fig. 4. The toxicity of ZnO nanoparticles in cells is explained by three different a general mechanism. (Modified [28])

spongy or flower-like structures. The next photos show particles with irregular shapes, such as ovals and flowers. Typically, zinc nanoparticles (GFM) range in size from 18-25 nm. If the particles are dispersed evenly, we can use morphological analysis to acquire a more accurate sense of their size.

Particle agglomeration, like that seen in biologically synthesized ZnO nanoparticles [34], was observed to occur when using natural particles as reducing agents. This strongly corroborates the idea that nanoparticles of pure zinc oxide may be formed. These results confirm that Zinc nanoparticles can be obtain from both approaches.

Cytotoxicity for Zinc nanoparticles prepare with Autoclave

Two cancer cell lines, one for breast (MCF-7) and the other for hepatic (WRL68), were employed to investigate the anticancer activity of zinc nanoparticles. As it showing in Fig. 3 the Zinc nanoparticles showed potential effect on MCF-1 compare with WRL68.

How ZnO nanoparticles cause cell line toxicity and what it means

In three distinct ways, toxicity from ZnO nanoparticles can be caused by their unique method of action across various cell types[21]. Fig. 4 displays an increase in intracellular [Zn2] levels as a result of the dissolution of extracellular ZnO nanoparticles[22]. When the intracellular [Zn2] level is raised, the activity of Zn-dependent enzymes and transcription factors is lowered. There is a possibility that BeAS-2B and C2C12 cells are influenced by this process[23]. ZnO nanoparticles dissolve inside of cells, inhibiting the activity of enzymes and transcription factors that rely on it[24]. Some cell types, including RKO and 3T3-L1, may go through this. The pH of the lysosomes is lowered as a result of the dissolution of ZnO nanoparticles[25]. Lysosomal instability occurs when the pH is lowered, which has a negative effect on protein-digesting lysosomal enzymes, and when intra-lysosomal [Zn2] levels are increased[26, 27].

CONCLUSION

With these techniques, it is possible to synthesis ZnO NP with excellent purity and crystal structure from *Cinnamomum verum* leaf. Furthermore, based on the foregoing in vitro investigation, shows potent anticancer activity. The anticancer impact is stronger in MCF-7 cell lines than in WRL68 cell lines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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