

## ORIGINAL RESEARCH ARTICLE

# Therapeutic potential of green formulation of *Silybum marianum*-based zinc nanoparticles on cervical cancer

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## Abstract

*Silybum marianum* possesses numerous therapeutic properties and has the potential to inhibit the spread of cancer. Research indicates that this plant can prevent the proliferation of cancer cells. It is likely that *S. marianum* could enhance the efficacy of chemotherapy. In addition, the adverse effects of current treatments could potentially be alleviated through the use of this plant. While the U.S. Food and Drug Administration has not officially approved *S. marianum* for carcinoma treatment, it shows promise in treating various types of cancer such as skin, small intestine, blood, cervical, breast, and prostate cancers. The primary focus of research is the exploration of metallic nanoparticles (NPs) formulated with medicinal plants. In this study, we successfully synthesized *S. marianum*-based zinc NPs (Zn-NPs). Various characterization techniques such as transmission electron microscopy, field-emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FT-IR), and ultraviolet–visible spectroscopy were employed. The DPPH inhibition efficiency was determined using DPPH method, while the MTT assay was utilized to assess the cytotoxic effects of the Zn-NPs on HT-3, LM-MEL-41, C-33 A, and SiHa cells. The findings from the FE-SEM indicated that the NPs appeared spherical with some degree of aggregation. Furthermore, the FT-IR analysis identified peaks at 472 and 518  $\text{cm}^{-1}$ , which can be attributed to Zn-O bond. The  $\text{IC}_{50}$  values of the Zn NPs against HT-3, LM-MEL-41, C-33 A, and SiHa cervical cancer cells were 161, 125, 155, and 108  $\mu\text{g/mL}$ , respectively. In conclusion, these findings showed that *S. marianum*-based Zn NPs possess potential benefits in treating cervical carcinoma.

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**Keywords:** Zinc nanoparticles; Anti-cervical cancer; Antioxidant; *Silybum marianum*

## 1. Introduction

*Silybum marianum*, a flowering herb belonging to the chicory family, is commonly utilized as an herbal remedy in Mediterranean regions. Silymarin, the active component of *S. marianum*, is frequently prescribed by physicians for the treatment of liver ailments. Studies have indicated that this plant may also have potential benefits in lowering cholesterol levels and controlling type 2 diabetes. Numerous research studies have highlighted the therapeutic properties of *S. marianum*. The majority of clinical studies on silymarin focused on researching its use in treating liver diseases such as hepatitis, cirrhosis, and poisoning caused by the *Amanita*, a toxic mushroom.

While there have been no reports suggesting silymarin use as a cure for carcinoma treatment, two articles have demonstrated its use as a supplementary medication for individuals with cancer.<sup>1-3</sup> In a particular instance, a patient with leukemia was forced to stop receiving the concurrent anti-cancer treatment of mercaptopurine and methotrexate due to elevated level of liver enzymes, but he was prescribed silymarin at a dosage of 800 mg/day and throughout the 120-day treatment period with silymarin, the patient did not need to halt taking the anti-cancer medications.<sup>1</sup> A 52-year-old male patient with liver cancer was successfully treated with 450 mg of daily silymarin, evidenced by a range of tests which confirmed that the administration of silymarin alone resulted in the complete cure of the cancer, obviating the need for taking any anti-cancer medications.<sup>2</sup>

Most laboratory studies conducted on both human and animal cells focused on investigating the silymarin impact, whether in the form of a purified silybinin or total extract derived from the extract.<sup>3-7</sup> However, the specific characteristics of the other compounds present in the total extract of silymarin have not yet been fully clarified. Several studies have reported the beneficial impact of silibinin and silymarin on animal and cell models of tumors in colon, lung, breast, prostate, and ovary, as well as histiocytic lymphoma and leukemia.<sup>8-12</sup> Furthermore, in animal models of cancer, these compounds have revealed promising effects on colon, bladder, and skin cancers.<sup>12-15</sup> In the aforementioned studies, total and purified compounds have demonstrated the ability to inhibit the growth of cancerous tissues or cells. However, it has been noted that in small intestine cancer, this herbal remedy was ineffective.<sup>12-14</sup>

Extensive findings have also suggested that silymarin can effectively alleviate the adverse reactions attributable to anti-cancer medications, while also enhancing their therapeutic benefits. In addition, silibinin and silychristin have been found to shield monkey kidney cells from the toxic effects of vincristine and cisplatin.<sup>16,17</sup> The daily silibinin administration at a dosage of 200 mg/kg in rats effectively protected against the development of renal toxicity induced by cisplatin.<sup>18</sup> A study demonstrated that silibinin enhanced the cisplatin and doxorubicin efficacy in targeting breast cancer cells.<sup>15</sup> Furthermore, silibinin has been found to enhance the chemotherapy-resistant prostate cancer cells sensitivity to anticancer medications.<sup>19</sup> It has been suggested that silymarin might hinder the proliferation of cancer cells by virtue of its anti-inflammatory characteristics or its impact on blood vessel formation in cancerous tissue.<sup>18-20</sup> This phenomenon has been witnessed in various types of cancer cells, including

those found in the prostate, breast, ovaries, lungs, and leukemia.<sup>5,6,11,20</sup> Silymarin has also been found to hinder the development of cancer caused by exposure to ultraviolet (UV) rays. A previous study has reported that silymarin effectively hampered the proliferation and growth of skin carcinoma cells triggered by UV radiation but was not able to prevent UV-triggered cancer initiation, suggesting silymarin can only impede cancer progression but not prevent its initial onset.<sup>13</sup>

The discovery and advancement of cancer treatment options in modern times have been greatly influenced by the field of nanotechnology.<sup>21-23</sup> Nanotechnology enables the development and fabrication of materials with unique properties and characteristics, with sizes ranging from 1 to 100 nm.<sup>23-25</sup> At present, a wide array of nanoparticles (NPs) are synthesizable through chemical or physical means. However, the utilization of toxic and hazardous chemicals in this process, along with the subsequent environmental harm, has raised significant concerns.<sup>23-28</sup> Given that, metal oxide NPs have garnered increasing attention in the realm of medical and biological applications. Nevertheless, the conventional chemical methods employed in their synthesis often leave behind toxic reactive substances, rendering the resulting NPs unsuitable for medical purposes. As a result, the utilization of the green method for NP synthesis has emerged as a distinctive focus in various studies.<sup>25-28</sup>

Plant extracts stand as a promising option in the synthesis of metal oxide NPs, offering a viable alternative to traditional methods. The versatility and numerous applications of these NPs in medicine, industry, and agriculture are vast and varied, making them highly sought after by researchers.<sup>29,30</sup> The unique properties of NPs have captured the interest of many scientists in medical and pharmaceutical uses, further highlighting their significance.<sup>27-30</sup> Employing plants in the synthesis of these NPs on a bigger scale can yield additional advantages by virtue of their capability in absorbing metal ions and reducing their levels.<sup>22-25</sup>

In this study, we adopted a green synthesis method to generate *S. marianum*-based zinc NPs (Zn-NPs) for the first time. In addition, we investigated the cytotoxic effects of these NPs on cervical cancer cells such as HT-3, LM-MEL-41, C-33 A, and SiHa.

## 2. Materials and methods

### 2.1. Green synthesis of Zn-NPs

Initially, 15 g of the *S. marianum* plant material was boiled with 300 mL of distilled water for a duration of 10 min. Following the cooling and filtration process, 20 mL of the resulting extract were combined with 60 mL of 0.04 M

sodium metavanadate solution. As the reaction proceeded at 40°C, yellow precipitates began to form. Afterward, centrifugation was conducted at 12,000 rpm for 10 min to isolate the Zn-NPs. Subsequently, the NPs were subjected to three rounds of washing and centrifugation. No alterations in color or physical state were detected during the chemical characterization and assessment of bioactivity for the Zn-NPs.

## 2.2. DPPH assay

The DPPH inhibition efficiency – a measure of antioxidant activity – was determined using DPPH method. To carry out the test, 1 mL of 0.2 mM DPPH was combined with 6 mL of NPs. After an incubation period of 0.5 h, the absorbance of the NPs was measured at 517 nm. The radical absorption percentage was calculated using the following equation:<sup>31</sup>

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (\text{I})$$

Where  $A_0$  represents the control absorption and  $A_1$  represents the sample absorption.

## 2.3. MTT assay

To evaluate the anti-cancer property of Zn-NPs, we utilized several cervical cancer cell lines including HT-3, LM-MEL-41, C-33 A, and SiHa. In addition, we investigated the impact of the synthesized Zn-NPs on normal human umbilical vein endothelial cells (HUVECs).

Before conducting any experiments, the significance of maintaining the cell line culture was recognized, and a suitable culture medium was selected. Once the cell confluence reached approximately 80%, the cells were carefully rinsed with new media and subsequently subjected to centrifugation. The cells were then combined with a 5 mL culture medium and placed under suitable conditions. The live cells were counted manually with the use of an inverted microscope, and the cell culture was also checked for any contaminations. Following this, the cells were rinsed with phosphate-buffered saline and then detached from the plate using trypsin. The cell counting was then repeated using trypan blue dye. After a day of incubation, the synthesized Zn-NPs were combined with the cells at several dilutions (1 – 1000 µg/mL). Subsequently, the mixture was exposed to 20 µL of MTT dye and placed in an incubator for 4 h to generate purple-colored formazan crystals. The crystals were then dissolved using 200 µL of dimethylsulfoxide. The absorbance was assessed using an ELISA reader, and the cell viability percentage was calculated using the formula below:<sup>32</sup>

$$\text{Cell viability (\%)} = \frac{\text{Sample A}}{\text{Control A}} \times 100 \quad (\text{II})$$

Where sample A represents sample absorbance, and control A represents control absorbance.

## 2.4. Statistical analysis

The experiment was conducted multiple times, with each substance, dilution, and incubation time being tested independently 5 times. Moreover, each test was performed at least three times to ensure accuracy. For data analysis, two-way analysis of variance (ANOVA) was employed. In addition, one-way ANOVA with *post-hoc* Duncan's method was conducted. The significance level was set at  $P \leq 0.01$ .

## 3. Results and discussion

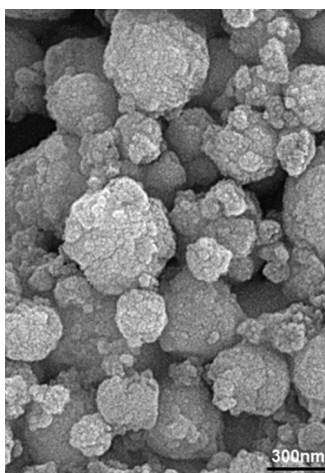
Field-emission scanning electron microscopy (FE-SEM) imaging is a suitable approach for examining the structure of nanomaterials. This technique involves scanning the surface using a beam of electrons with a precise energy range.<sup>31,32</sup> Figure 1 displays the FE-SEM images of Zn-NPs. According to the results, the NPs exhibited a spherical morphology and tended to aggregate. The aggregation of Zn-NPs was primarily attributed to the plant extract used as the synthesis reagent to reduce metal salts.<sup>21-24</sup> These NPs had an average size of <24.11 nm. Previous research has documented a size range of 20 – 90 nm for the green-synthesized Zn-NPs.<sup>23-27</sup>

Figure 2 displays the transmission electron microscopy (TEM) image of Zn-NPs. Similar to the FE-SEM images, the TEM image shows the spherical shape of the NPs, which had a tendency to aggregate.

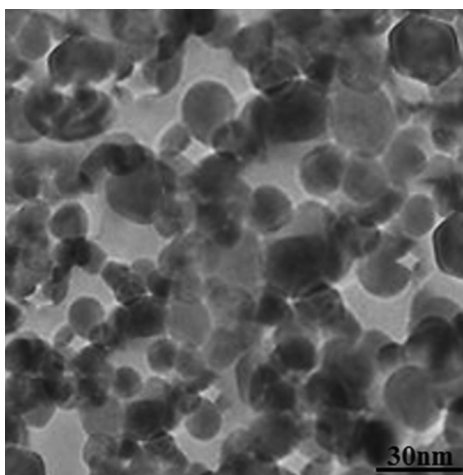
Figure 3 displays the Fourier-transform infrared (FT-IR) spectrum of Zn-NPs. Based on a comparison between this spectrum and another from a prior investigation, the peaks observed at 472 and 518  $\text{cm}^{-1}$  can be attributed to Zn-O bond. The presence of organic compounds associated with the surface of Zn-NPs is confirmed by the occurrence of other peaks in various regions (1384 – 1631  $\text{cm}^{-1}$ ) or bands at wavenumbers 1128, 2956, and 3441  $\text{cm}^{-1}$ . These compounds have a functional role in the green synthesis of metallic NPs.

Figure 4 illustrates the spectrum of Zn-NPs in the UV-visible spectroscopy range. Based on the results, the peak observed at 306 nm relates to the surface plasmon resonance of Zn-NPs. This phenomenon occurs when electrons on a metallic surface are excited by light.

Colorimetric assays were conducted on NPs derived from extracts to verify the existence of antioxidant compounds on the NPs and assess their toxicity toward different cancer cells. Results from the DPPH method revealed a proportional rise in inhibition (%) with an increase in NPs concentration. The highest antioxidant



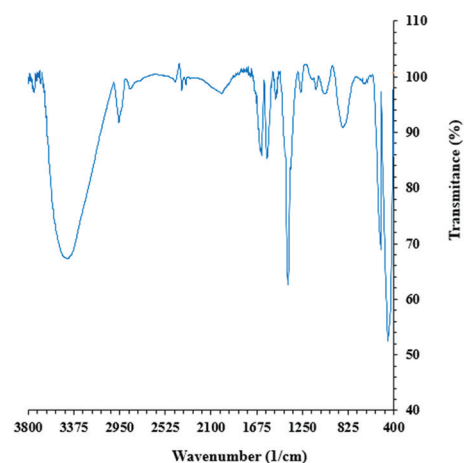
**Figure 1.** Field-emission scanning electron microscopy image of zinc-nanoparticles (20.00 kx × magnification).



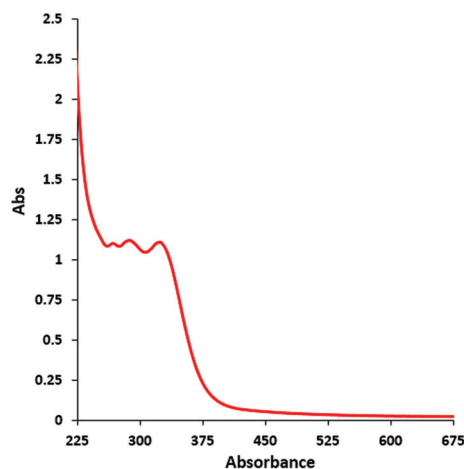
**Figure 2.** Transmission electron microscopy image of zinc-nanoparticles (50.00 kx × magnification).

activity recorded in this study was 1000  $\mu\text{g/mL}$ , reaching an inhibition (%) of around 100%. The NPs exhibited a half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) of 78  $\mu\text{g/mL}$  as an antioxidant. The antioxidant assay results are shown in Figure 5. These findings align with previous studies, which demonstrated that the combination of metallic MPs with medicinal plant extracts yields potent antioxidant effects comparable to butylated hydroxytoluene.<sup>31,32</sup> It has been documented that the green-formulated NPs  $\text{IC}_{50}$  falls in 50 – 300  $\mu\text{g/mL}$ .<sup>32</sup>

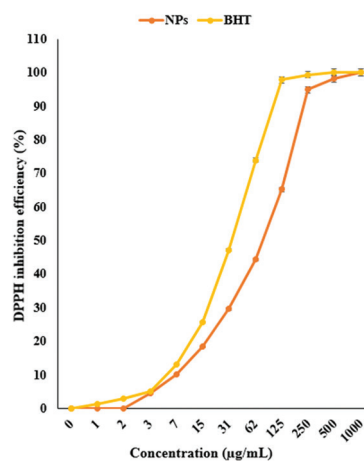
Antioxidants eliminate free radicals through the following mechanisms: (1) elimination of peroxidation initiator species, (2) chelation of metal ions, (3) peroxide formation prevention by quenching  $\text{O}_2^-$ , and (4)  $\text{O}_2$  concentration reduction.<sup>32</sup> The most effective antioxidant molecules



**Figure 3.** Fourier-transform infrared spectrum of zinc-nanoparticles



**Figure 4.** Ultraviolet-visible spectrum of zinc-nanoparticles



**Figure 5.** Antioxidant activities of zinc-nanoparticles and butylated hydroxytoluene

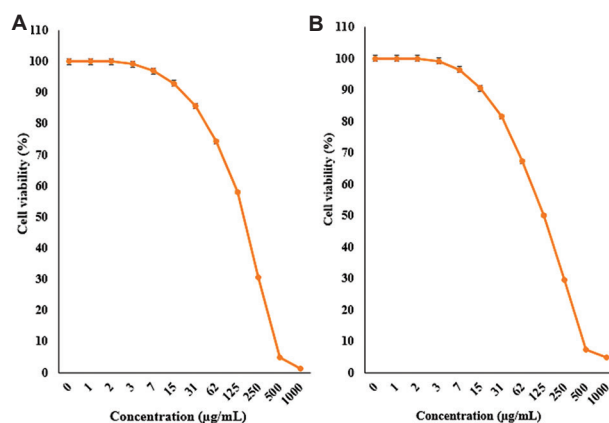


possess certain characteristics: (1) addition of H to the free radicals side chains, (2) disruption of the free radicals chain reactions, and (3) presence of phenolic or aromatic rings.<sup>33</sup> Previous research has revealed the antioxidant effectiveness of natural compounds and their NPs.<sup>33</sup>

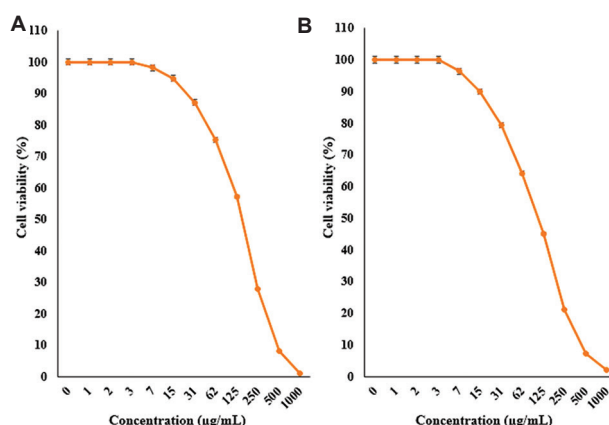
NP-mediated therapy represents the most effective approach for treating cancer. While encapsulating therapeutic agents for healing purposes, NPs have the ability to selectively target specific tumor tissues or patient cells. Numerous strategies involving NPs have been developed, but the discussion surrounding tumor heterogeneity remains significant for nanotechnologists and clinicians aiming to advance targeted drug delivery to cancer cells.<sup>22-24</sup> Another topic of discussion in the treatment of carcinoma involves the utilization of modern NPs on a single platform. This approach aims to enhance efficacy, safety, biocompatibility, and specificity while reducing toxicity and overcoming the limitations of conventional chemotherapy. However, it is crucial to identify and address the challenges and limitations associated with the use of NPs in carcinoma treatment.<sup>24-27</sup> Manufacturing and regulatory challenges, variability in NPs, improved permeability and retention effect, restricted carrying capacity, and physiological obstacles are all factors to consider.

The Zn-NPs utilized in treating tumors serve both therapeutic and diagnostic purposes. These Zn-NPs act as carriers for the targeted delivery of chemotherapeutic agents and as enhancers for photodynamic and radiation therapies.<sup>25-28</sup> In the past few years, there has been a growing interest in the Zn-NPs use for diagnostic and research purposes, particularly for their potential as anti-cancer agents. Research has shown that iron NPs also exhibit anti-angiogenic and anti-cancer properties.<sup>28-30</sup> Previous studies have highlighted the numerous side effects of chemotherapy in patients, underscoring the need for advancements in technology to minimize these effects. Various studies are exploring the formulation of NPs as a viable option for developing drugs that can selectively target cancer cells.<sup>26-28</sup> Previous research findings suggested that the introduction of Zn-NPs can trigger apoptosis and enhance the susceptibility of carcinoma cells. Zn-NPs induce alterations in cell morphology, decrease cell lifespan and metabolic activity, and elevate oxidative stress levels, leading to mitochondrial impairment and increased production of reactive oxygen species. Ultimately, this cascade of events culminates in DNA damage.<sup>21-25</sup> The cellular uptake of Zn-NPs primarily happens through endocytosis. Rather than directly treating cells with Zn-NPs, researchers have demonstrated the utilization of carrier molecules to transport zinc to cancer cells.<sup>23-27</sup> Along with the advancements of medical technologies, there has

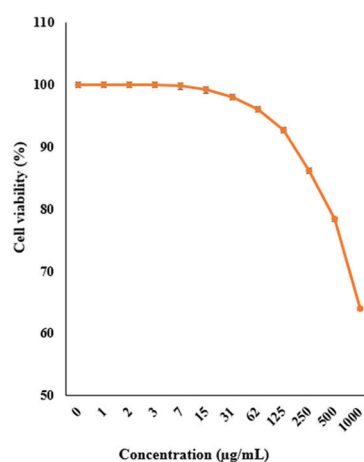
been a notable rise in inclination toward utilizing Zn-NPs as a therapeutic substitute or enhancement for existing



**Figure 6.** Anti-cancer efficacy of zinc-nanoparticles on LM-MEL-41 (A) and HT-3 cells (B)



**Figure 7.** Anti-cancer efficacy of zinc-nanoparticles on SiHa (A) and C-33 A cells (B)



**Figure 8.** Cytotoxic effect of zinc-nanoparticles on human umbilical vein endothelial cells

treatment methods. The field of nanobiotechnology is currently witnessing remarkable advancements exemplified by the utilization of NPs for the development of effective and innovative medical treatments and diagnostics.<sup>27-30</sup>

In the current study, Zn-NPs were utilized to treat cervical cancer cells and HUVECs at different concentrations. Even at a concentration of 1000 µg/mL, the Zn-NPs did not reach the IC<sub>50</sub> threshold (Figures 6-8). The Zn-NPs exhibited IC<sub>50</sub> values of 161, 125, 155, and 108 µg/mL when tested against HT-3, LM-MEL-41, C-33 A, and SiHa cell lines, as shown in Figures 6-8.

#### 4. Conclusion

The green formulation of Zn-NPs was successfully produced using a plant extract. The FT-IR analysis identified peaks at 472 and 518 cm<sup>-1</sup>, which can be attributed to Zn-O bond. According to the FE-SEM analysis results, the Zn-NPs exhibited a spherical morphology along with some degree of aggregation. The IC<sub>50</sub> values of the Zn-NPs against HT-3, LM-MEL-41, C-33 A, and SiHa cervical cancer cells were found to be 161, 125, 155, and 108 µg/mL, respectively. These findings provide evidence that the Zn-NPs possess potential benefits in treating cervical carcinoma.

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#### Conflict of interest

The authors declare no conflict of interest.

#### Author contributions

*Conceptualization:* All authors

*Investigation:* All authors

*Writing – original draft:* All authors

*Writing – review & editing:* All authors

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Availability of data

Data are available from the corresponding author upon reasonable request.

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