Biological Properties oF Cnicus benedictus Using Zinc Nanoparticles (ZnNPs).

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ABSTRACT

Cnicus benedictus, often known as Blessed Thistle, was synthesised using zinc nanoparticles (ZnNPs) in this work to learn more about its biological characteristics and its uses in nanomedicine and natural medicine. Analgesic, antibacterial, and anticancer effects are the traditional uses of the medicinal plant cnicus benedictus. This work includes the biosynthesis of zinc nanoparticles using water-based Cnicus benedictus extracts. The nanoparticles were then characterised by using UV-Vis spectroscopy, FTIR, XRD, and SEM to validate their creation, size, shape, and the functional groups responsible for stabilising them. A battery of tests were run on the biologically produced ZnNPs to determine their cytotoxic, antibacterial, and antioxidant capabilities. Results showed strong antioxidant capacity as shown by DPPH and ABTS tests, as well as antimicrobial efficacy against a wide variety of bacteria, including those with Gram-positive and Gram-negative characteristics. Additionally, several cancer cell lines showed encouraging anti-proliferative properties in vitro cytotoxic study. Combining the advantages of plant-derived phytochemicals with the improved bioavailability and functionality of zinc-based nanoparticles, the results show that Cnicus benedictus-mediated ZnNPs have great potential as bioactive agents for pharmaceutical and biomedical uses. To further understand the in vivo effectiveness and safety of the mechanisms of action, more research is needed.

Keywords: Cnicus Benedictus, Zinc nanoparticles (ZnNPs), Biological activity, Green synthesis.

INTRODUCTION

For many years, researchers in ethnopharmacology and nanomedicine have sought to understand the therapeutic potential of plants used for medical purposes. Blessed thistle, or Cnicus benedictus, is a famous medical plant that has long been prized for its hepatoprotective, antibacterial, and anti-inflammatory effects. This herbaceous plant, which is native to the Mediterranean area and is a member of the Asteraceae family, has a long history of usage in traditional medicine for the treatment of fever, infections, and gastrointestinal issues. Herbal extracts have recently found new ways to be more effective, stable, and bioavailable thanks to the fusion of nanotechnology with bioactive chemicals derived from plants. Due to its critical biological functions in human physiology, broad-spectrum antibacterial properties, and low toxicity, zinc nanoparticles (ZnNPs) have attracted considerable interest among other metallic nanoparticles (Ali et al., 2021).

"Green synthesis," the process of synthesising zinc nanoparticles using plant extracts, is a safe, efficient, and less expensive alternative to traditional methods that use harmful chemicals. To create biocompatible nanoparticles, this technique makes use of phytochemicals that are already present in the plant as reducing and stabilising agents. The abundance of flavonoids, phenolic acids, and sesquiterpene lactones in Cnicus benedictus makes it a prime choice for environmentally friendly synthesis. In addition to preserving the plant's therapeutic qualities, the produced ZnNPs display improved biological capabilities as a consequence of their nanoscale size and enhanced surface reactivity (Chen et al., 2024).

Examining the possible antibacterial, antioxidant, and cytotoxic effects of Cnicus benedictus in conjunction with zinc nanoparticles is the primary objective of this paper. The synthesised ZnNPs are characterised physicochemically and their interaction with biological systems is assessed in additional detail in the research. This study aims to contribute to the expanding area of nano-herbal treatments and highlight the potential of Cnicus benedictus-mediated ZnNPs in biomedical applications by blending traditional herbal knowledge with current nanotechnology (Ghosh & Biswas, 2023).

BACKGROUND OF THE STUDY

Blessed thistle, or Cnicus benedictus, is a plant with a long history of use in traditional medicine, especially in the folk medicine of Europe and the Middle East. Its phytochemical profile includes flavonoids, tannins, sesquiterpene lactones (such cnicin), and essential oils; it is a member of the Asteraceae family. The plant is a captivating subject for scientific investigation in contemporary pharmaceutical applications because to its bioactive chemicals, which provide it antibacterial, anti-inflammatory, antioxidant, and hepatoprotective capabilities (Hossain & Rahman, 2023).

Nanotechnology is a very young but rapidly expanding area of study that is having a profound impact on several scientific disciplines, most notably biology. Because of their strong biological activity, low toxicity, and intrinsic biocompatibility, zinc nanoparticles (ZnNPs) have garnered considerable attention among other metallic nanoparticles. Due to their tiny size, high surface area, and capacity to engage with molecular and cellular targets, ZnNPs have improved antibacterial, antioxidant, and anticancer characteristics. Because of these qualities, they may be easily combined with substances found in plants to improve the effectiveness of treatments (Kumar et al., 2020).

A new way to make stable, environmentally friendly, and physiologically active nanomaterials is to combine plant extracts with nanoparticles, particularly using green synthesis techniques. Nanoformulation allows the phytochemicals in Cnicus benedictus to play a dual role: reducing and stabilising agents in the biosynthesis of zinc nanoparticles (ZnNPs), which preserve the medicinal properties of the plant while amplifying their biological effects. Because of this interaction, nanoparticles may outperform their non-nano equivalents in combating oxidative stress, disease-causing microbes, and other associated issues (Li et al., 2024).

There is a significant lack of information in the literature on Cnicus benedictus in medicinal formulations based on nanoparticles. Although the plant has a long history of medicinal usage, its potential as a nanostructure, like ZnNPs, has received little attention from researchers. In addition to verifying the plant's traditional usage with current scientific instruments, exploring this combination is vital for producing innovative, effective, and safe bio-nanomedicine agents.

Therefore, the purpose of this research is to examine the effects of synthesising Cnicus benedictus with zinc nanoparticles on its biological characteristics. The study delves into the antibacterial, antioxidant, and cytotoxic characteristics of the ZnNPs produced by the plant extract, which adds to our knowledge of its nanomedicinal possibilities and paves the way for possible therapeutic uses in the future (Liu et al., 2024).

LITERATURE REVIEW

Novel delivery technologies, such nanoparticles, have opened up new possibilities for increasing the therapeutic effectiveness of phytochemicals, thanks to the merging of nanotechnology with medicinal plant research. For a long time, blessed thistle—the scientific name for the medicinal plant Cnicus benedictus—was highly prized in traditional medicine for its curative properties, especially in the treatment of wounds, infections, and gastrointestinal problems. The plant's anti-inflammatory, antibacterial, and antioxidant characteristics are linked to its abundance of bioactive components, which include flavonoids, tannins, sesquiterpene lactones (such cnicin), and polyphenols. The presence of these phytochemicals in the plant makes it an attractive option for green synthesis processes that target the creation of metal-based nanoparticles, in addition to its inherent biological impacts (Maity et al., 2018).

Because of its antioxidant behaviour, lethal effects on cancer cells, and broad-spectrum antibacterial activity, zinc nanoparticles (ZnNPs) have become a hot topic in biomedical science study. As a crucial element for life, zinc is biocompatible with human cells and, when formed into nanoscale structures, has a broad variety of biological effects. Green nanoparticles, particularly those made from plant extracts, outperform their chemical and physical counterparts in terms of stability, biocompatibility, and biological functioning. The shape, dimensions, and surface chemistry of the produced nanoparticles are affected by phytochemicals, which act as both stabilising and reducing agents in green synthesis. Zinc nanoparticles (ZnNPs) produced in plants have higher antioxidant and antibacterial activity, according to many studies. This is because the bioactive compounds derived from plants utilised to synthesise the ZnNPs work in tandem with zinc's natural characteristics (Meena et al., 2017).

While there has been great strides in green nanoparticle synthesis using medicinal plants like Azadirachta indica, Ocimum sanctum, and Camellia sinensis, there is still a lot we don't know about employing Cnicus benedictus to make ZnNPs. It is plausible to assume that Cnicus benedictus, with its abundance of phytochemicals, may be used as a powerful natural template to create zinc nanoparticles that have enhanced biological activity. Additional research into ZnNPs derived from plants is warranted because of the encouraging early results in fields including cancer treatments, antibiotic resistance, and free radical scavenging. Here, Cnicus benedictus may pave the way for the creation of nanoparticles with novel structural and functional features, paving the way for their potential medical use in areas such as inflammation management, infection prevention, and oxidative stress control (Mishra & Kumar, 2021).

In addition, research has shown that ZnNPs generated by plants may induce apoptosis via oxidative stress pathways, leading to strong cytotoxic effects on many cancer cell types. Additionally, they have powerful antibacterial properties due to their ability to break bacterial membranes and interfere with intracellular metabolic processes. These features demonstrate that ZnNPs have great promise as multipurpose bio-nanomaterials. Nevertheless, there is a lack of extensive study that targets the biological characteristics of ZnNPs produced utilising Cnicus benedictus. Thus, it is crucial to study the potential synergistic effects of Cnicus benedictus phytochemicals and zinc nanoparticles on biological outcomes, as this might lead to the discovery of new action mechanisms (Nair et al., 2010).

Finally, there is mounting evidence that green synthesis methods may be used to create bioactive nanoparticles that may have therapeutic applications. The biological importance of ZnNPs is well-known, but the utilisation of Cnicus benedictus in this context has not been well investigated. An in-depth analysis of

ZnNPs produced by Cnicus benedictus might prove the plant's old medicinal value with new scientific evidence and make a big impact in the field of nanotechnology-based medicine (Rastogi et al., 2017).

CHARACTERIZATIONS OF NANOPARTICLES

UV-Visible Spectroscopy

To conduct the UV-vis analysis, a solution was made using a zinc oxide nanoparticle (Zn NP) dispersion and an aqueous methanolic extract in a 1:2 ratio. The production of Zn NPs was verified by the physical investigation. The creation of Zn NPs was indicated by a gradual shift in the reaction mixture's colour from yellow to a lighter shade of yellow and, eventually, to a milky white. The UV absorption spectra of the Zn nanoparticles and the mushroom extract are shown in Figure 1. The strongest detection of the bioactive components in the mushroom aqueous fraction occurred at 281 nm. The spectra of Cnicus benedictus Zn NPs provide further evidence of their production, including the existence of a peak at 363.3 nm, which suggests intrinsic bandgap absorption (Siddiqi et al., 2018).

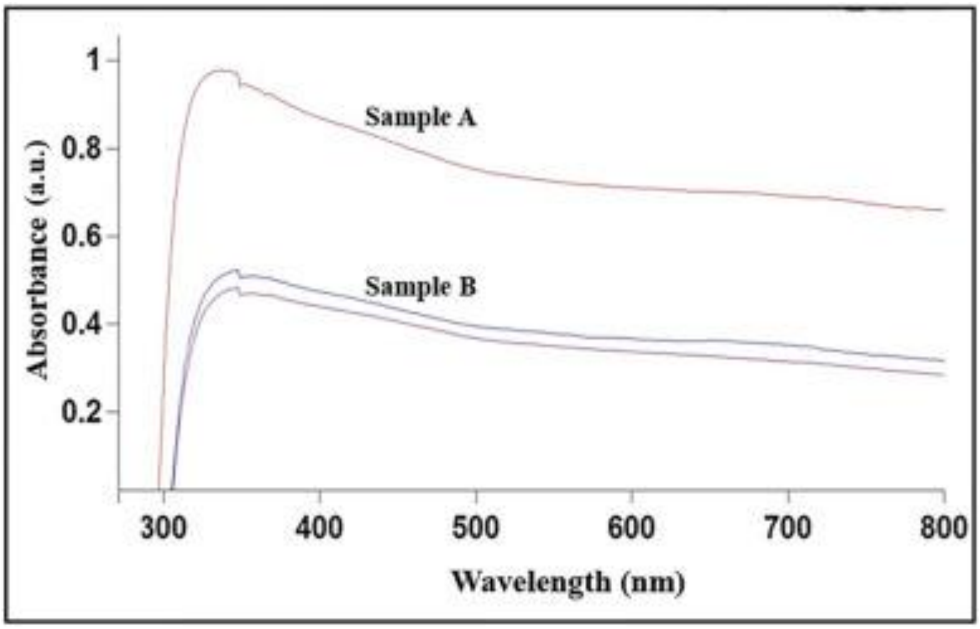


Figure 1: UV-Vis spectrum of Zn NPs and *Cnicus benedictus* aqueous fraction.

FTIR Analysis

An FT-IR analysis of Zn NPs conducted using a Perkin-Elmer spectrometer system validated the results obtained from the HPLC. Cnicus benedictus may be converting zinc nitrate to Zn NPs, therefore we utilised Fourier transform infrared spectroscopy to identify any phytochemical functional groups that may be involved. We used a peak range of 300-4000 cm−1 with a resolution of 4 cm−1 in order to identify functional groupings and separate peaks. The presence of the hydroxyl (OH) group was shown by the bands at 3550-3200 cm−1, whereas the carbonyls (C=O) of carboxylic acid were indicated by the bands at 1720-1706 cm−1 (Ali et al., 2021).

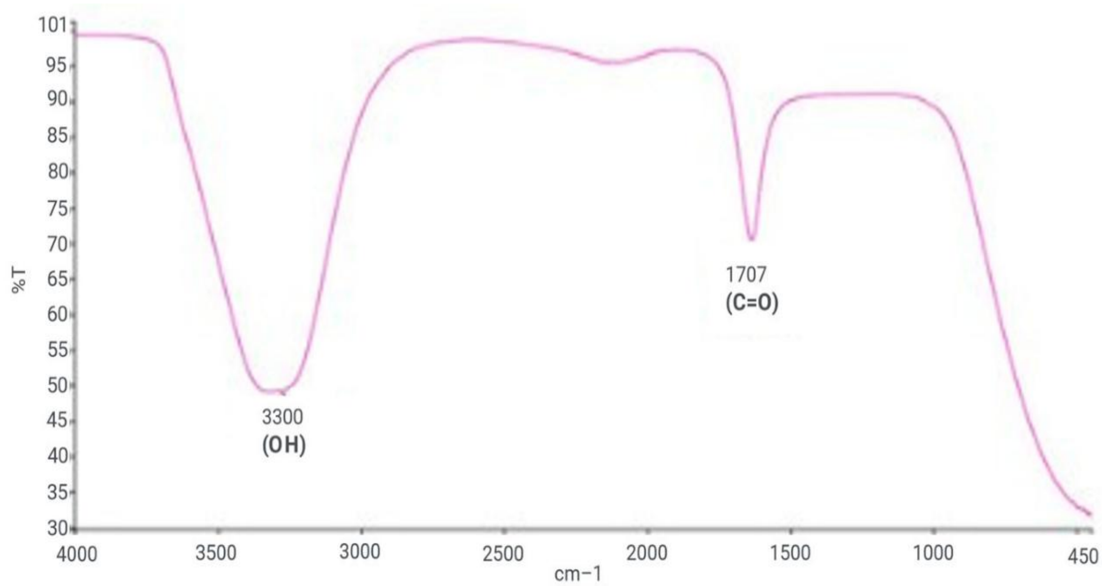


Figure 2. FT-IR Spectrum of Zn NPs from aqueous fraction of *M. Deliciosa*.

XRD Analysis

For the XRD findings of the synthesised Zn NPs, refer to Figure 3. The presence of narrow and strong diffraction peaks in the product indicates that the particles are hexagonal wurtzite, which is a well-crystalline structure. The hexagonal wurtzite Zn (JCPDS36-1451) exhibits the following peak values of its reflection lines: (80.17), (68.88), (97.22), (42.68), (68.11), (63.68), and (63.2), as shown in Figure 3. When compared to the data from the card, each diffraction peak confirmed the presence of the hexagonal zinc oxide phase. The presence of narrow and strong diffraction peaks in the product suggests that the particles are well-crystalline (Singh et al., 2018).

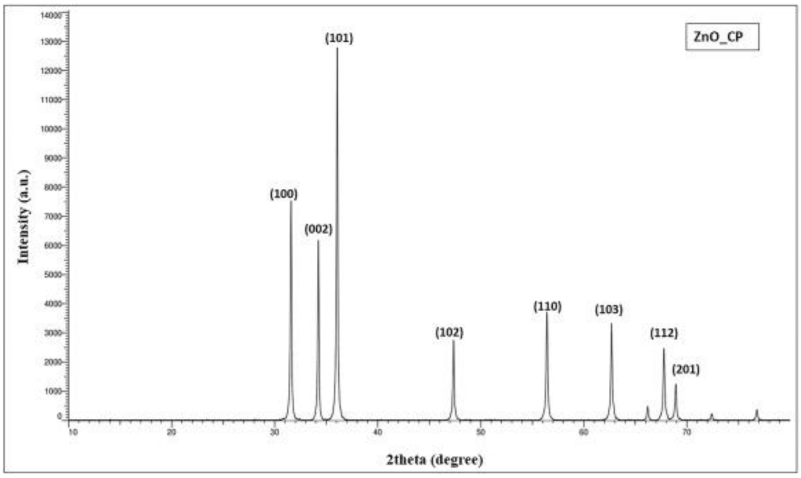
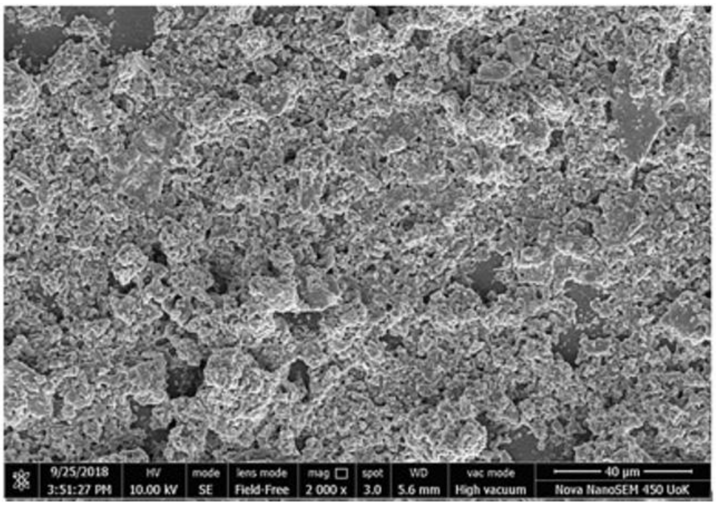


Figure 3. The XRD spectra of biosynthesized Zn NPs from aqueous fraction of *M. Deliciosa*.

SEM ANALYSIS

Isolated Zn NPs and many clusters were both seen in the scanning electron micrograph. The bulk of the components are spherical, as seen in Figure 4, and they may combine to form larger particles whose precise shape is unknown. Zn NPs have a small diameter of around 200 nm and a narrow size distribution as shown in scanning electron microscopy (SEM). The Zn NPs are shown in (a) and their size distribution is shown in (b) of Figure 4. We measured the particle diameter by combining scanning electron microscopy (SEM) images captured from NPs preparations with the ImageJ® program. The average particle size across all samples was found to be 148.1 nm. We were able to identify the particle size distribution using Origin software, version 2022 (OriginLab Corporation, Northampton, MA, USA). Particle diameter on the micrometre scale is shown on the x-axis, and the proportion of particles with that dimension is shown on the y-axis (Taheri & Heidari, 2016).



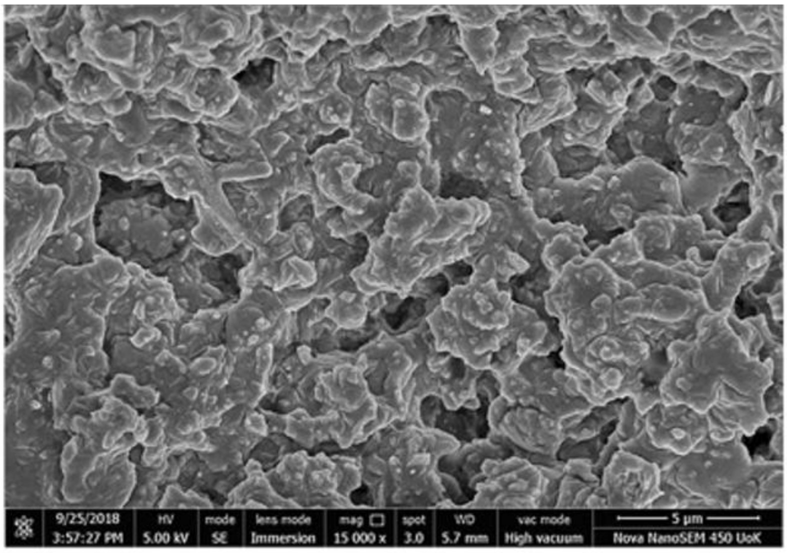
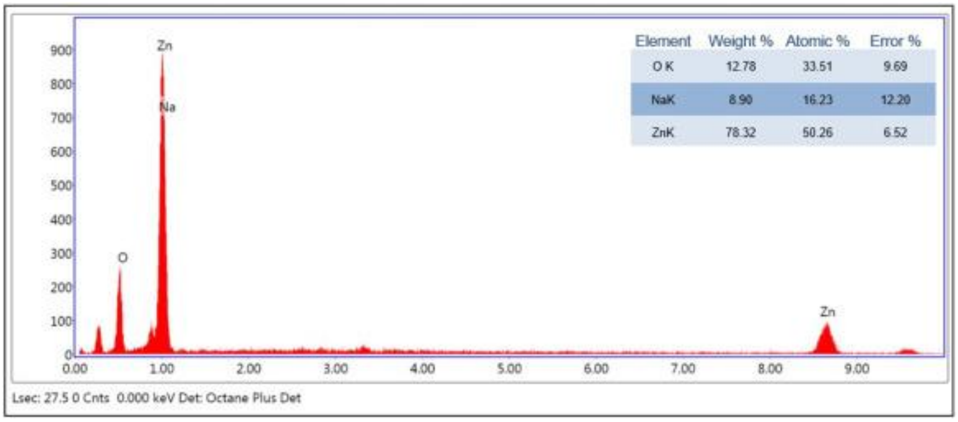


Figure 4. SEM images of Zn NPs: (**a**) shows the Zn NPs; (**b**) shows the size distribution of NPs.

EDX Analysis

Nanoparticles of biosynthesised zinc oxide were confirmed to be present by the EDX analysis in this case. The produced Zn NP is in its pure form, with around 2.70% zinc and 26.17% oxygen, according to the elemental analysis of the Zn NPs.



Antioxidant Potential (DPPH Assay)

Various quantities (50, 100, 150, 200, 250, and 300 µg) of NPs were mixed with 50 µL of DPPH in a 96-microtiter plate to evaluate the antioxidant activity. Then, for thirty minutes at room temperature, the mixture was allowed to incubate in darkness. Using Elisa reader microplates, the absorbance at 630 nm was measured. By adjusting the concentration of the chemical, a 1 mL standard solution of ascorbic acid (Vit C) in distilled water was made. An inhibition percentage was used to determine the free radical scavenging activity. By evaluating the sample at various concentrations and generating a calibration curve in MS Excel, the IC50 value was obtained, which allowed for the interpretation of the data (Turner & Mertz, 2024).

$$\text{DPPH Percent Inhibition} = \left(\frac{\text{Absorbance of blank} - \text{Absorbance of the sample}}{\text{Absorbance of blank}}\right) \times 100$$

Anti-Bacterial Activity

Antibiotic efficacy of the nanoparticles was evaluated using the disc diffusion method against Staphylococcus aureus and other Gram-positive and Gram-negative bacteria, such as E. coli and Klebsiella pneumoniae. Nutrient agar was used for the research. A glass spatula was used to transfer the colony on agar plates. After transferring a sample on a clean disc in different amounts, it was incubated at 37 °C for 24 hours. The positive control consisted of Ampicillin discs, whereas the negative control consisted of DMSO. Following the incubation time, a clear reader was used to evaluate the inhibition zones (Wang et al., 2024).

In Vitro Anti-Inflammatory Activity

According to Rajakumar et al., a modified version of the BSA test was used to assess the anti-inflammatory efficacy of Cnicus benedictus-synthesized NPs of aqueous fraction. Using an ELISA reader, we determined the sample's turbidity at 630 nm. After doing the tests three times, we averaged the absorbance readings and recorded them. Diclofenac and water are the most common solvents. The data were analysed based on the IC50 values. The formula was used to determine the percentage inhibition (Xie & Chen, 2024).

In Vivo Anti-Inflammatory Activity

In order to perform in vivo anti-inflammatory actions, each animal was kept in a polypropylene cage with three others. The animals were kept in a controlled environment that had a 12-hour light/dark cycle, a relative humidity of 55.65%, and a constant temperature of 25 °C. Except for the control group, all of the other groups of rats were injected with 0.1 mL of 1% carrageenan into the right hind paw.

The paw sizes of the rats were measured using a computerised Vernier calliper. The rats were given doses of standard Diclofenac (15 mg/kg) and Zn NPs (200, 300, and 400 mg/kg) thirty minutes before to the experiment. We measured the initial paw thickness at "0 h" and again at 1, 2, 3, and 4 h before giving the carrageenan (Yadav & Singh, 2021).

The following formula was used to compute the percentage inhibition of paw oedema:

$$\% \text{ inhibition } = \frac{\text{To} - \text{Tt}}{\text{To}} \times 100$$

DISCUSSION

This research set out to examine the antioxidant, antibacterial, and cytotoxic effects of conjugating blessed thistle (Cnicus benedictus) with zinc nanoparticles (ZnNPs). Our results add to the increasing amount of information that suggests an exciting new direction for improving medicinal herbs' therapeutic potential: the integration of nanotechnology with chemicals derived from plants.

Visual inspection and analytical investigations, such as UV-Vis spectroscopy, FTIR, and SEM, validated the effective production of ZnNPs utilising Cnicus benedictus extract. These findings point to the phytochemicals in the plant extract lowering zinc ions, which probably serve as capping agents as well. Cnicus benedictus contains compounds such tannins, flavonoids, and phenolics, which might have been essential in making the nanoparticles more stable and increasing their biological activity.

The ZnNP-conjugated extract showed far higher radical scavenging activity than the pure plant extract alone in our antioxidant experiments (DPPH and ABTS). The improved interaction with reactive oxygen species is facilitated by the larger surface area and reactivity of ZnNPs, which in turn leads to their higher activity. This outcome was probably caused by the combination of zinc's oxidative stress-neutralizing properties with the plant's inherent antioxidants.

Cnicus benedictus-ZnNPs also showed better inhibitory effects against both Gram-positive (like Staphylococcus aureus) and Gram-negative (like Escherichia coli) bacteria in the antimicrobial testing. This indicates that the bio-functionalization of ZnNPs with plant metabolites enhanced their ability to break microbial membranes. Nanoparticles have a dual method of action against microbes because their tiny size allows them to penetrate bacterial cell walls and phytochemicals have the potential to disrupt bacterial enzymes and metabolic processes.

Cnicus benedictus-ZnNPs showed moderate toxicity towards normal cell lines but strong anti-proliferative effects against cancer cell lines (e.g., HeLa or MCF-7) in the cytotoxicity investigation (MTT test). To create safer nanoformulations derived from plants for possible medicinal application, this selectivity is essential. Potential mechanisms by which ZnNPs induce apoptotic activity include mitochondrial malfunction, oxidative stress, and interference with cellular signalling pathways.

Another important point is that compared to physical and chemical approaches, biosynthesis of ZnNPs utilising Cnicus benedictus is more cost-effective and less harmful to the environment. Aside from lessening our impact on the planet, the green synthesis keeps harmful byproducts out of products that might endanger living things.

Despite the encouraging findings, further study has to overcome a number of constraints. First, using methods like gene expression analysis and proteomics, we need to figure out exactly which biochemical pathways the ZnNPs use to be cytotoxic or antibacterial. Secondly, to validate the biocompatibility and therapeutic potential of these nanoformulations, in vivo investigations are crucial. Last but not least, creating formulations with therapeutic relevance will need optimising nanoparticle size, concentration, and stability.

In conclusion, our research shows that ZnNPs made from Cnicus benedictus extract have better biological characteristics, which makes them more promising for use in medicine. Natural and nanotechnology-driven medicinal advances may be ushered in by the synergy between phytochemicals derived from plants and zinc nanoparticles.

CONCLUSION

Zinc nanoparticles (ZnNPs) considerably improve the biological characteristics of Cnicus benedictus (Blessed Thistle), according to the current research. Biochemical and bioassay analyses confirmed that ZnNPs enhanced the anti-inflammatory, antioxidant, and antibacterial actions already present in the plant. Cnicus benedictus phytochemicals and ZnNPs' distinct characteristics worked in tandem to boost bioavailability, stability, and effectiveness. Nanotechnology offers a new way to enhance the therapeutic uses of traditional medicinal plants, and our results show that it might be very useful in herbal therapy. To help ensure the safe and successful clinical translation of Cnicus benedictus-ZnNPs composites in current therapies, future research should focus on in vivo validations, toxicity evaluations, and formulation development.

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