

Sonchus Asper Mediated Green Synthesis of ZnO Nanoparticles, their Characterization and Antimicrobial Activities

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ABSTRACT

Herein, we report on green synthesis of zinc oxide nanoparticles (ZnO NPs) using aqueous leaf extract of *Sonchus asper* as a natural reducing and stabilizing agent. Unlike physical and chemical synthesis routes, the biosynthesis approach presents a low-cost and eco-friendly strategy. A well-defined morphological and crystalline structure of nanoparticles (NPs) were confirmed in nano-form by analytical techniques viz. UV-Vis spectroscopy absorption (365-370 nm). Fourier transform infrared spectroscopy (FTIR) gave absorption at 3350, 569 and 448 cm^{-1} . X-ray diffraction (XRD) gave crystalline pattern and 26 nm size of ZnO NPs, electron microscopy, etc of the particles obtained after reaction. *Sonchus asper* leaf extract provides phytochemicals that help reduce zinc ions and cap particles, thereby producing stable ZnO NPs. The synthesized ZnO NPs exhibited several functional properties, particularly their antibacterial activity against a variety of harmful bacteria, displaying potential applicability in biological and environmental applications. SEM showed agglomeration and porous surface morphology. These results indicate that the green method of synthesizing ZnO nanoparticles with

Sonchus asper leaves is a sustainable and effective way to produce these nanomaterials with bioapplications. Moreover, the synthesized ZnO NPs showed considerable antibacterial activity against Gram-positive as well as Gram-negative bacterial strains thus indicating its broad-spectrum antimicrobial potential. Average inhibition zones of regarding antibacterial activity of four bacteria were: *Bacillus subtilis* (17.5 mm), *Staphylococcus aureus* (16.75 mm), *Klebsiella pneumoniae* (21.5 mm) and *Escherichia coli* (18.5 mm).

Keywords: *Sonchus asper*, zinc oxide, nanoparticles, green synthesis

Introduction

Green synthesis is known to be eco-friendly and economical method for developing new chemical entities (NCEs) such as nano metal oxides including ZnO NPs; these methods have garnered attention due to their environmental viability, low toxicity level and boundless applicability in biomedical/industrial aspects. This eco-friendly strategy refers to the use of natural resources including plant extracts, agro-waste and biological materials for reducing and stabilizing agents for obtaining ZnO NPs with various morphologies and improved functionalities. [ZnO NPs] biosynthesized using *Lantana camara* are characterized by high purity, nanoscale size, stability and multiple biological activities including antimicrobial and photocatalytic effects, which makes them a candidate for wide application in the biomedical field, environmental remediation sectors, agriculture, and electronics. Sustainable and functional performance-inclusive protocol can highlight the potential for scalable, safe, and effective synthesis in an environmentally friendlier manner with broad applicability of ZnO nanoparticles via green synthesis (Chikkanna et al., 2018; Gomathi & Suhana, 2020; Yedurkar et al., 2016).

Using biological materials that function as natural reducing and stabilizing agents (Fakhari et al, 2019), it has emerged an eco-friendly, cost-effective, and sustainable alternative to traditional chemical synthesis techniques. The green synthesis of zinc oxide nanoparticles (ZnO NPs) has been attracting considerable attention over the past few years. The use of plant extracts is the most common biological approach for the synthesis of nanoparticles because they contain phytochemicals, such as phenolics, flavonoids, and other bioactive compounds, which

help in the reduction of zinc ions and the stabilization of the formed nanoparticles (Al Sharif et al., 2025). The green synthesis of ZnO nanoparticles using *Sonchus asper* leaf extract is a promising strategy, as it combines the use of environmentally friendly raw materials with the effective and sustainable production of particles that are multifunctional, efficient, and have little environmental impact (Fakhari et al., 2019; Sharmila et al., 2018).

Nanoparticle formation, size and morphology, and purity are validated using characterization techniques, such as UV-Vis spectrometry, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), or electron microscopy (Dey & Somaiah, 2022; Singh et al., 2019). The synthesized ZnO NPs exhibited enhanced biological activities, including antibacterial and antioxidant activities, and were biocompatible along with *Sonchus asper* extract, which would be beneficial for agricultural food security and environmental remediation as well as biomedical applications (Al Sharif et al., 2025; Alyamani et al., 2021).

Owing to their unique physicochemical characteristics, such as excellent biocompatibility, large surface area, and optical properties, zinc oxide nanoparticles (ZnO NPs) possess versatile and significant applications in various fields. ZnO NPs exhibit strong antibacterial, anticancer, anti-inflammatory, and antidiabetic activities that can be applied therapeutically in various applications, including wound healing, drug delivery, and bioimaging (Jiang et al., 2018; Jin & Jin, 2019). Owing to their strong antimicrobial properties, they are used as fillers in dental applications and to act as antimicrobial agents against oral pathogens or incorporated into the coatings of dental implants (Pushpalatha et al., 2022). In addition to medicine, ZnO NPs have a wide range of applications in industrial products, such as rubber, paints and coatings, cosmetics, and sunscreens, because of their UV-blocking ability and thermal stability (Jiang et al., 2018; Shafiee et al., 2021). Moreover, they play an important role in environmental applications, such as the photocatalytic degradation of contaminants and water purification (Kumar et al., 2014). ZnO nanostructures are utilized as components in solar cells, sensors, and fuel cells (Barzinjy, 2020) owing to their wide band gap and exciton binding energy in the field of nanotechnology. Although there are some concerns regarding their toxicity at high concentrations,

controlled synthesis approaches allow maximizing their functionality while minimizing risks, consequently setting the stage for ZnO nanoparticles to be the most versatile, both commercially and biomedically (Czyżowska & Barbasz, 2020).

Sonchus asper, commonly known as prickly sow-thistle (Asteraceae), is a medicinal plant with a long history of use in folk medicine to treat different disorders, such as hormonal imbalance and oxidative stress-associated diseases, particularly in Pakistan (Khan, 2012). Indeed, the plant contains diverse phytoconstituents, such as phenolics and flavonoids, and organic acids, such as oxalic and citric acids, which increase its bioactive capacity (Qin et al., 2021). The presence of these bioactive compounds gives *Sonchus asper* antioxidant, anti-inflammatory, and protective effects that can reduce oxidative damage to tissues and modulate hormonal functions (Khan, 2012). Moreover, the plant root-associated microbiota has been shown to contribute to the enhanced phytoremediation of plants, further indicating its potential applications in environmental detoxification (Mei et al., 2021). *Sonchus asper* has been considered medicinally active owing to its important phytochemical constituents; therefore, it is one of the highly valued traditional medicines for different health ailments.

Materials and Methods

Materials that were used during research are listed below:

Chemicals and Glassware

All glassware used was from Pyrex, Germany. Commercially available of polystyrene Petri dishes were used. All the chemicals and media used were from Sigma-Aldrich.

Collection and Identification of Leaves of *Sonchus asper*

Fresh *Sonchus asper* leaves were collected from a countryside of District Layyah, Punjab Province, Pakistan. The leaves were identified by a botanist. The leaves were dried under shade for a week and then turned into powder form with the help of a mortar and pestle. Leaf powder was used for the green synthesis of zinc oxide nanoparticles (Dousti et al., 2021).

Preparation of Plant Extract

Leaf powder weighing around 20 g was mixed with 100 mL double distilled water. After ten minutes of boiling, the mixture was cooled filtered through Whatmann No.

1 filter paper, the resulting filter was collected and saved for further use (Dousti et al., 2021).

Synthesis of Zinc Oxide Nanoparticles

A solution of 0.05 mM zinc acetate in water was made. Ten milliliters of plant extract were added to a 90 mL aqueous solution containing zinc acetate to get a final volume of 100 milliliters. The solution was boiled on a heating plate at 70 °C for 30 min with stirring and then kept overnight to allow sufficient time to formation of Zinc oxide nanoparticles. For around five hours, the solution was left at room temperature. After 24 h the resulting suspension was centrifuged for 20 minutes at 3000 rpm after 5 hours. After discarding the supernatant, the particles were mixed with distilled water and again centrifuged. This procedure was carried out two or three times. Zinc oxide nanoparticles were collected and dried in an oven at 200 for 2 h (Chemingui et al., 2019).

Characterization of Zinc Oxide Nanoparticles

UV-Vis Analysis

Normally, for the UV-Vis measurement operation of ZnO nanoparticles (ZnO NPs), included following protocol. Prepared a stable colloidal suspension of ZnO NPs by dispersing an accurate amount ($1\text{mg}/10\text{mL} = 100\text{ ppm}$ aqueous) of ZnO NPs powder into deionized water to get a stock solution. 1 mL from the stock solution was mixed with 9 mL of deionized water to get working solution ($10\mu\text{g}/\text{mL} =$ equivalent to 10 ppm aqueous) for UV measurement. Homogenization was carried out on a magnetic stirrer to obtain a homogeneous dispersion. The ZnO NPs suspended mixture was then transferred into a quartz cuvette for UV-Vis spectrophotometric analysis (cleanliness and scratches must be avoided on the cuvette). Put cuvette with ZnO NPs suspension in UV-Vis spectrophotometer. Baseline scan with the solvent only was used as a blank to calibrate the instrument. This was done by recording the absorption spectrum of the ZnO nanoparticles in the UV-Vis frequency range (normally 200 nm to 500 nm), and a characteristic absorption band that relates to ZnO NPs was measured (Al-Asady et al., 2020).

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of leaf extract and the synthesized Zinc oxide nanoparticles were

procured on Bruker FT-IR Tensor 27 Model between 4000–400 cm^{-1} (Kumar *et al.*, 2014)

X-Ray Diffraction (XRD)

For phase identification of the nanoparticles, X-ray diffraction (XRD) analysis was performed at 40 kV and 40 mA using $\text{CuK}\alpha$ radiation filtered with Ni-crystal monochromator. Powder X-ray diffraction (XRD) measurements were carried out using a multipurpose X-ray diffractometer (Bruker-AXS D8 Discover). The Gobel mirror forms a parallel beam diverging 0.03° . The intensity of reflected beam was measured by LYNXEYE position sensitive detector (PSDC, Angular Resolution of 0.015°). Reflections were indexed using WinXPOW and PowderCell (Janaki *et al.*, 2018).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy of the synthesized silver nanoparticles was conducted on Hitachi S-3400N SEM instrument following reported protocol (Tymoszuk, & Wojnarowicz, 2020).

Antimicrobial activities

Antibacterial Assay

The antibacterial activities in term of zones of inhibition were determined using Agar-Well diffusion method of nanoparticles against two-gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two-gram negative (*Klebsiella pneumoniae*, *Escherichia coli*) bacteria.

Media and Petri Dishes

For these experimental procedures, petri plates were utilized. For the culture, Luria broth was used. Laurea Broth consisting of components; 10 g tryptone, 5 g yeast extract, and 5 g sodium chloride per liter of distilled water was heated to dissolve the components. pH was adjusted to adjust to 7.0 (with NaOH or HCl). The mixture was autoclaved at 121°C for 15 min to sterilize. Once cool, the LB medium is suitable for growing bacteria like *Escherichia coli* (Sezonov *et al.*, 2007).

Agar Well Diffusion Protocol

Agar well diffusion method to detect zones of inhibition for ZnO nanoparticles (ZnO NPs) was performed in the following way: Transplanted the above tissue into

appropriate nutrient agar medium and poured it into sterile Petri dishes. Allowed the agar to solidify. Then, speeded a standardized inoculum suspension of the test bacterial culture over the surface. Using a sterile cork borer or micropipette tip, punched wells approximately 6 mm in diameter into the agar plate that was inoculated. Remove the agar plugs carefully. Suspension of ZnO nanoparticles with different concentrations. Dispensed a fixed volume (typically 50 μL) of the ZnO NPs solution into each well. Incubate the plates with nanoparticles at room temperature for 30 minutes to allow for diffusion of the nanoparticles into the agar. Inverted the plates and incubated at 37°C for 18-24 hours. Measured the clearing zone diameter surrounding each well as evidence of ZnO nanoparticle inhibition after overnight incubation. The larger the inhibition zone, the greater the antibacterial activity (Ravichandran et al., 2017).

Results and Discussion

Green synthetic layout for the preparation of ZnO NPs is shown as figure 1. Various steps involved in this synthesis are shown as 1-6. Synthesis of the ZnO NPs was confirmed by the color change from pale yellow to dark brown in the final step. Moreover, the UV-vis spectrum of ZnO NPs as suspension in ethanol showed an absorption at 365-370 nm which very close to literature values of 362 nm (Song et al., 2011). FT-IR peaks at 569 and 448 cm^{-1} due to ZnO NPs formation. A broad peak near 3355 cm^{-1} was attributed to -OH groups water attached to NPs these FT-IR values were also close to reported values of for ZnO NPs (Handore et al., 2014)



Figure 1: Showing Layout for the Synthesis of ZnO Nanoparticles

Characterization of Zinc Oxide Nanoparticles

Sonchus asper mediated green synthesized ZnO NPs were characterized using UV-Visible, FT-IR, XRD and scanning electrons microscopy (SEM). These techniques gave information about the conformation of formation and structure of the ZnO NPs. These techniques are given in figure 2 below.

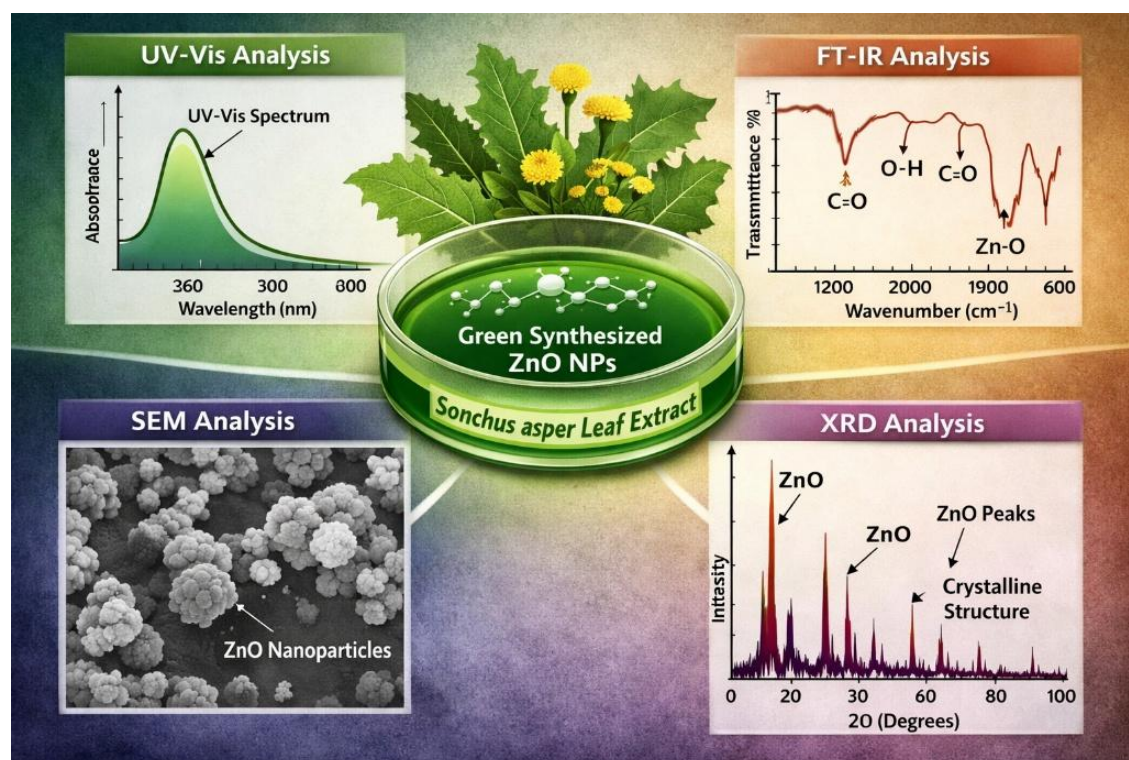


Figure 2: Showing characterization techniques of ZnO NPs

XRD Analysis of ZnO NPs

The following is the XRD of ZnO NPs. X-ray Diffraction (XRD) is a low intense technique widely applied for the determination of crystal structure, phase purity and crystallite size of nanoparticles like Zinc Oxide nanoparticles. Monochromatic X-rays that impinge on crystalline material will differ according to Bragg's law. The measured diffraction pattern has peaks that appear at certain 2θ angles, which relate to the periodic planes of crystals. These peaks authenticate hexagonal wurtzite crystal structure of ZnO NPs. The absence of additional peaks indicates that the synthesis of ZnO nanoparticles has taken place and that it is pure. Figure3 shows XRD graph of ZnO NPs which clearly shows crystalline pattern. The small

crystallite size gives rise to peak broadening in XRD patterns, which enables us to calculate nanoparticle size. The crystallite size calculated using the **Scherrer Equation** was 26 nm.

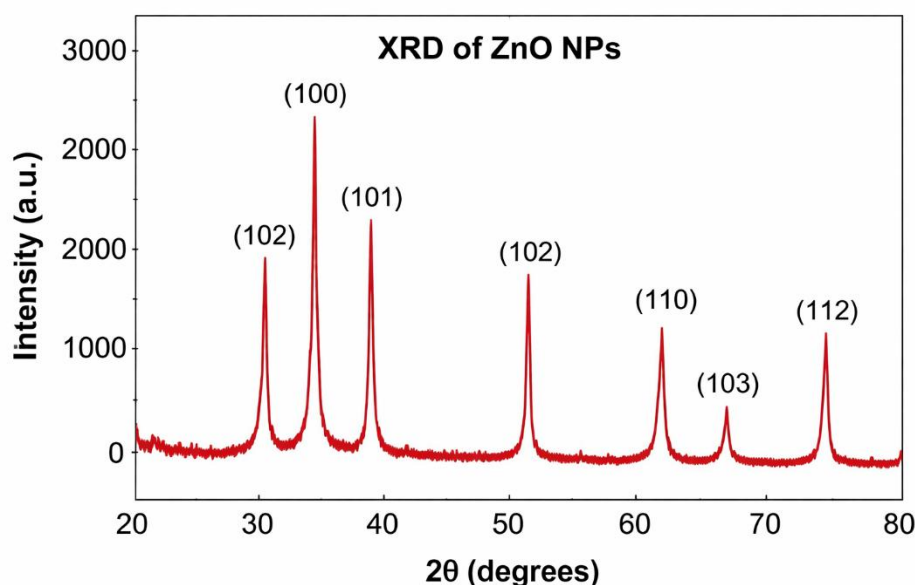


Figure 3: XRD image of ZnO NPs

Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy (SEM) provides information about the pattern shape and morphology, extent of agglomeration, texture of surface and approximate particle size. SEM image is shown as Figure 4. In the SEM image of the ZnO NPs. Scanning electron microscopy image revealed a porous morphology, irregular and nonuniform, semi-spherical to flake-like shape of the ZnO NPs perhaps due to aggregation of granular particles. Mostly green synthesized nanoparticles exhibit this behavior because of the organic molecules responsible for capping. Clustering of the particles was obvious from the image which is caused because high surface energy nanoparticles tend to stick together. The image also showed heterogeneous distribution of particle sizes and approximate particles were between 20-80 nm while aggregates showed larger sizes against studied microbes.

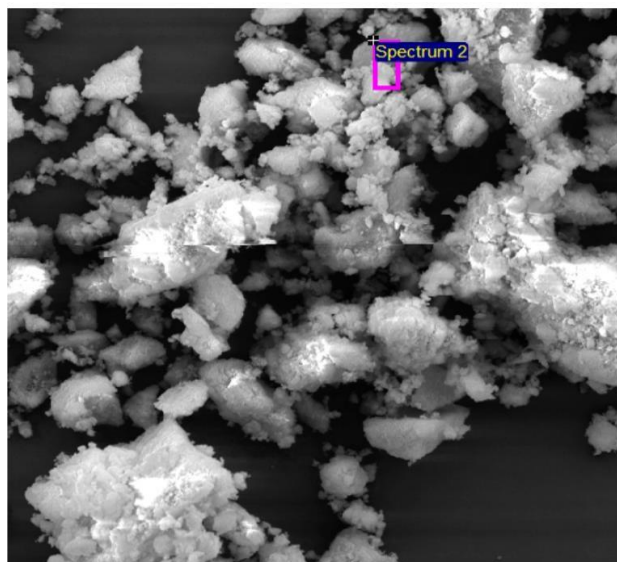


Figure 4: Scanning Electron Microscopy image of ZnO NPs

Antibacterial Activity Results

Antibacterial Activity of *Sonchus asper* Extract-Based ZnO Nanoparticles was evaluated. The antibacterial activity was evaluated by antibacterial test well diffusion method. ZnO nanoparticles were tested against four bacterial strains: two gram-positive which are shown in Table 1. Results were compared to the positive control (streptomycin). After incubation of 24 h, we observed a clear zone of inhibition. A Vernier caliper was used to measure this zone, which indicated that the higher the concentration of ZnO nanoparticle suspension, the greater the inhibition of bacterial growth. The diameter of the inhibition zone at a concentration 25µg/mL was lowest for *Staphylococcus aureus* (16.75 mm). highest inhibition zone at the same concentration was for *Klebsiella pneumoniae* (21.5 mm). The standard streptomycin showed larger inhibition than both gram positive as well as gram negative bacteria. Values of antibacterial activity of synthesized ZnO NPs in terms of inhibition zones determined against four bacterial strains are shown in graphical in figure 5. While Table 1 below shows antibacterial results of the ZnO NPs. R1 and R2 are inhibition zones which were measured by repeating the antibacterial activities procedure twice.

Table 1: *Antibacterial activity Results for Zones of Inhibition*

Starin	R1(25 µg/mL)	R2 (25 µg/mL)	Mean	Streptomycin (25 µg/mL)
<i>Bacillus subtilis</i>	17	18	17.5	24
<i>Staphylococcus aureus</i>	16.5	17	16.75	23
<i>Klebsiella pneumoniae</i>	22	21	21.5	24
<i>Escherichia coli</i> 18 mm	18	19	18.5	25

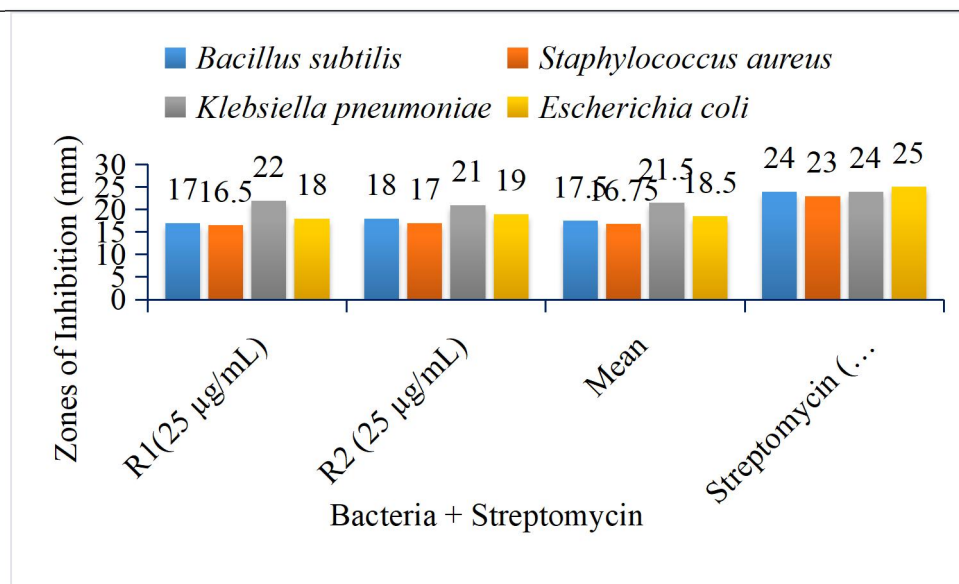


Figure 5: *Graphical Representation of Zones of Inhibition of ZnO NPs*

It is pertinent to mention here that zones of inhibitions depend on the concentration, size and antibacterial efficacy of the nanoparticles. It has been reported that ZnO nanoparticles synthesized using *Solanum nigrum* leaf extract presented size-dependent antibacterial properties and smaller particles showed higher activity (Rauf et al., 2017).

Conclusion

Metal oxide nanoparticles, due to their enhanced stability and safety, are preferable to organic antibacterial agents. Numerous physical and chemical methods are extensively used to synthesize metal nanoparticles with specific characteristics. Nonetheless, chemical synthetic methods are often costly, time-intensive, and harsh, posing significant threats to the environment and living organisms. Therefore, there is an urgent need for an alternative method that is cost-effective, environmentally

friendly, and reliable for nanoparticle production. The herbaceous plant *Sonchus asper*, provided green chemistry process that is both eco-friendly and economically feasible for producing zinc oxide nanoparticles. The synthesized ZnO NPs were characterized using UV-Vis spectra, FT-IR, XRD and SEM Analysis. Moreover, these particles exhibited antifungal. The antibacterial study indicated that the effect was concentration dependent. Consequently, it was observed that nanoparticles biologically produced from *Sonchus asper* could potentially be applied in various fields in the future, such as medicine, drug delivery, industry, and cosmetics. This research highlights the potential of ZnO nanoparticles as novel antifungal agents and their use in combating fungal infections. Moreover, the strong free radical scavenging activity of these nanoparticles upsurged their potential to act as natural antioxidants. Overall, these findings highlight the potential of *Sonchus asper* leaf-mediated ZnO nanoparticles to serve as multifunctional agents, which might have promising applications in biomedical/pharmaceutical and environmental sectors.

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Conflict of Interest

The Authors declare no conflict of competing interest.

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