Comparative antimicrobial and anticancer activity of biologically and chemically synthesized zinc oxide nanoparticles toward breast cancer cells

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ABSTRACT

Objective(s): This study was aimed to investigate the synthesis of novel zinc oxide (ZnO) nanoparticles (NPs) using Solanum trilobatum leaf extract as the reducing and capping agents, called green synthesized zinc oxide nanoparticles (GS-ZnONPs).

Materials and Methods: Chemically synthesized zinc oxide nanoparticles (CS-ZnONPs) were synthesized using precipitation method with zinc nitrates hexahydrate as reducing precursors. The synthesized GS- and CS-ZnONPs were examined and characterized using UV-visible spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron microscopy (SEM), Energy dispersive X-ray analysis (EDAX), and X-ray diffraction (XRD) analysis, respectively.

Results: GS-ZnONPs exhibited a higher zone of inhibition of 28.6 mm, 27.63 mm, and 29.33 mm for Bacillus subtilis, Escherichia coli, and Klebsiella pneumoniae, respectively compared to CS-ZnONPs. From the growth inhibition experiments with E. coli and Staphylococcus aureus, it was evident that GS-ZnONPs have exhibited higher growth inhibition as compared to CS-ZnONPs. The IC50 for CS-ZnONPs in MCF-7 cell line was found at 136.16 µg/mL and for GS-ZnONPs was found at 85.05 µg/mL. The proliferation of cancer cells were directly proportional to the concentration of NPs. As compared to CS-ZnONPs, GS-ZnONPs have exhibited higher cytotoxic effects on MCF-7 cell line.

Conclusion: It was concluded that GS-ZnONPs represented much enhanced anticancer and antibacterial activity compared to CS-ZnONPs.

Keywords: Antimicrobial, Breast cancer, Cytotoxicity, Zinc oxide nanoparticles

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INTRODUCTION
Nanotechnology researches are the priority field and the forefront of modern research in recent years in different sciences such as medicine, physics, chemistry, biology, etc [1, 2]. Nanomaterials with its unique physical and chemical properties supported by various applications by changing their size, distribution, and morphology [3]. Multiple methodologies were developed in this field to synthesize biocompatible NPs to meet the specific needs, mainly in the applications related to the medicinal field. Metal NPs have recently attracted significant attraction due to their particular morphologies which are account for numerous applications [4]. ZnO is a naturally existing metal in the earth crust as a non-toxic mineral. ZnO is a multifunctional element that exhibits new and improved properties by controlling their physical and chemical properties [5]. Nanomaterials based on ZnO exhibit various applications mainly as energy storage devices, optical devices, and nano-sensors [6-8]. The critical feature of the ZnONPs is that they are rich in hydroxyl groups which provide space for functionalization [9]. It also possesses less toxicity, high biodegradability which gain importance in biomedical applications [10]. ZnONPs can be synthesized with different mechanochemical, chemical and biological methods. The mechanochemical methods included laser ablation and high-energy ball milling techniques [11, 12]. Pyrolysis byproducts resulting from laser ablation method is a significant disadvantage of the laser ablation technique [13]. Besides, contamination from milling balls is the major disadvantage of the high-energy ball milling technique [14, 15]. Moreover, the chemical methods such as co-precipitation, sol-gel, and hydrothermal techniques have been used for the synthesis of ZnONPs [11, 12]. Trace impurity and time consuming are the drawbacks of co-precipitation technique [12]. Besides, shrinkage and cracking during drying and expensive raw materials are some of the disadvantages of sol–gel technique [16]. Moreover, slow reaction kinetics [17] and the need for expensive autoclaves [18] are the drawbacks of hydrothermal technique for preparation of NPs. Hence, there is a growing need for a simple, fast and eco-friendly method to synthesized ZnONPs. Plant extracts-based nanoparticle synthesis becomes more attractive as they eliminate the use of hazardous chemicals for the synthesis process. S. trilobatum Linn belongs to the Solanaceae family and is a prominent Indian medicinal plant. It is commonly known as ‘Thuthuvalai’ in Tamil. The leaves of the plants were utilized for curing a common cold, asthma, and tuberculosis [19]. Moreover, it was prescribed to various other diseases in Ayurveda and Siddha medicinal systems [19-23]. The phytochemical analysis of the plant has revealed the presence of alkaloids such as soladunalinidine, tomatidine [24], sobatum [19], β-solamarine, solasodine [25], and solaine. It possesses excellent antioxidant and hepatoprotective properties [26]. In this study, we have mainly focused on the optimization of both the chemical and green synthesized ZnONPs from the leaves of thorny creeper Solanum and its characterization and their application as an antimicrobial and anticancer agent.

MATERIALS AND METHODS
Synthesis of ZnO nanoparticles (ZnONPs)
Chemical synthesis–precipitation method
The utilized chemicals were of analytical grade obtained from Fisher chemical (India), Mumbai. Zinc nitrate hexahydrate (Zn(NO₃)₂ 6H₂O) and ammonium carbonate ((NH₄)₂CO₃) were used as a precursor and doping agent to synthesize ZnONPs via direct precipitation method [27].

Briefly, 1.0 M of zinc nitrate hexahydrate (100 mL) was slowly dropped on to vigorously stirred (400 rpm and 28 °C) solution with the molar ratio of 1:1.5 (1.0 mM of ammonium carbonate (150 mL)). The addition of zinc nitrate hexahydrate and ammonium carbonate solution resulted in the formation of white precipitate. The precipitate was collected through filtration using filter paper (Whatman® quantitative filter paper, ash less, Grade 41), and the filtrate was washed thrice with distilled water and 70% ethanol. The precipitate was collected and dried at 100 ºC for six hours to form ZnO precursor. The ZnO precursor was galvanized in the muffle furnace at the temperature of 550 ºC for 4 h [28].

Green Synthesis of ZnONPs using S. trilobatum
Collection of plant material
S. trilobatum leaves of the same age group of single inhabitants were collected from the Tamil Nadu medicinal plant farms, and herbal medicine corporation limited, Anna Hospital Campus, Chennai, Tamil Nadu. S. trilobatum leaves were utilized as the herbal source for green nanoparticle
synthesis. The Taxonomic identification of the collected plants was done at the Botanical Survey of India, Southern Regional Center, Coimbatore, Tamil Nadu. The collected leaves were transported to the laboratory; fresh and healthy leaves were separated, washed, and rinsed with tap water. The leaves of *S. trilobatum* were washed with distilled water to remove the dirt and grime and further washed with the Hiclean (Liquid soap) and rinsed thrice with deionized water. The leaves were blotted with blotting paper and shade dried at room temperature to preserve the phytochemicals for two weeks. Ensuring complete drying, the leaves were cut into tiny pieces and powdered finely by applying an electric blender, and the debris was sieved and stored in the airtight container, sieved and stored in the cool place of the airtight container until further studies [29, 30].

**Preparation of aqueous extract**

The leaf extract was obtained by boiling 20 g of the powdered leaf in 100 mL distilled water for 10 minutes. The extract was cooled, filtered, and stored in the dark (30 °C) using airtight containers until utilized for the study [31].

**Phyto-reduction method**

The green synthesis was carried out with different concentrations of plant extract, and the optimum process of the synthesis was reported below. To 50 mL of 1 M of Zn(NO3)26H2O, 25 mL of *S. trilobatum* extract was gently added dropwise under continuous stirring (250 rpm and 75 °C) for six hours to attain colloidal complexity. The mixture was stirred until a solid particle of light greenish-yellow color was formed. At this juncture, the precipitate was washed with deionized water, and then centrifugation at 12000 rpm for 15 min [32]. The pellet was dried in a hot air oven (60 °C) for 8 h to attain the green synthesized ZnONPs [33-36].

**Characterization of ZnONPs**

The green synthesized and chemically synthesized ZnONPs were characterized using different techniques. For the UV–vis analysis, ZnO nano-suspension was sonicated for ten minutes, and the UV–vis spectra (Thermo Scientific, Mumbai) was recorded in the wavelength range of 200 to 800 nm [37]. The XRD studies of ZnONPs were carried out using X-ray diffractometers (Shimadzu, Kyoto, Japan) [30, 38-40]. The crystal lattice, phase structure, material identification, and the size of the ZnONPs were determined by XRD.

The surface morphology of the CS- & GS-ZnONPs was characterized by high-resolution SEM analysis (JSM-5600LV; JEOL, Tokyo, Japan). Moreover, the elemental compositions were characterized by EDAX analysis (S-3400N; Hitachi, Tokyo, Japan). Morphology and size of the sample were investigated using Hi-Resolution Transmission Electron Microscope (JEM-2100 Plus, JEOL, Tokyo, Japan).

**Time-dependent growth inhibition assay**

To measure and monitor the growth inhibition of *E. coli*, and *S. aureus*, new colonies were inoculated from Mueller Hinton Agar plates into 100 mL of Luria–Brentani (LB) broth (Hi-Media, Mumbai). The inoculated flasks were allowed to grow until the optical density at 600 nm reached the medium was raised to 0.1. Consequently, 2×108 CFU/mL was added to 100 mL of LB broth enhanced with 0, 0.5, 1, and 10 mM of ZnONPs and incubated in a temperature-controlled incubator cum rotary shaker (37 °C; 100 rpm). Correspondingly, the control broths of NPs were not added as the positive control. All the experiments were performed in duplicates. The optical density, which is directly proportional to the bacterial growth was determined at 600 nm using a UV–vis spectrophotometer (Thermo Scientific, Mumbai).

**Antimicrobial activity of ZnONPs**

The antimicrobial assay was carried out by the disk diffusion method for CS-ZnO, GS-ZnO, and *S. trilobatum* leaf extract. The cultures utilized for the study were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The received cultures were reviewed and subcultured twice before the study. Three different concentrations of nanoparticles/plant extract (50,100 and 200 mg/mL) were investigated against *B. subtilis, E. coli,* and *K. pneumoniae*. The 6 mm sterile disks (Hi-Media. Mumbai) was loaded with 20 μL of the sample and air-dried. For positive control of antibiotic disks, Cefotaxime (30 μg), Tetracycline (30 μg) and Ampicillin (10 μg) were used against *B. subtilis, E. coli,* and *K. pneumoniae*. The zones of inhibitions were measured using the Antibiotic Zone scale (Hi-media, Mumbai) for measuring inhibition zone size in millimeter (mm).
All the assays were carried out in triplicate.

**In vitro cytotoxicity assay**

Human breast carcinoma (MCF7 (ATCC® HTB-22™)) cells utilized for the study was from American Type Culture Collection (ATCC, USA). The cells were cultured in DMEM/F-12 (DMEM and Ham's F-12, Gibco®, Invitrogen Cell Culture, Sigma Aldrich, Mumbai) supplemented with fetal bovine serum (10 % FBS, Sigma Aldrich, Mumbai), penicillin (100 U/mL), and streptomycin (100 μg/mL) in a 5% CO2 incubator at 37 °C. *In vitro* cytotoxicity study was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [41]. Briefly, 96 well plates were used at a cell density of 1 × 10^6 cells per 100 µL. Different concentrations of GS- and CS-ZnONPs (0, 25, 62.5, 125, 250, 500, and 1000 μg/mL) were treated and incubated at 37 °C for 24 hours. The media without any NPs were used as negative control, and each concentration was assayed in triplicate. The following equation was utilized to calculate cell viability (percentage):

\[
\text{Cell viability (\%) = } \frac{\text{OD of control} - \text{OD of ZnO NP}}{\text{OD of Control}} \times 100
\]

IC50 values were determined using the nonlinear regression algorithm implemented by GraphPad.

The 50% growth inhibition concentration (IC50) was calculated by plotting the dose-response curve [42].

**Analysis of DNA strand breakage and DNA damage**

The alkaline comet assay was performed as described elsewhere [43]. The slides were precoated with agarose (0.5%) and air-dried at room temperature. Initially, the cells were collected by trypsinization, washed, and re-suspended in ice-cold PBS.

The re-suspended cells (10 µL) with low melting point agarose (100 µL) were added as the second layer and stored in a lysis buffer (4°C) for one hour [44, 45]. After lysis, the slides were performed electrophoresis in yellow light to prevent any DNA damage [46].

The slides were stained with EtBr (20 µg/mL) and analyzed in a fluorescence microscope (Carl Zeiss Optical Fluorescence Microscope). The comet images were analysed using open cometimage analysis software [43].

**Statistical analysis**

All the data in the experiments were analysed and expressed as the mean and standard deviation (SD) of two independent experiments. The data were analysed by Student’s t-test or one-way analysis of variance using GraphPad Prism. A value of *p*<0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**UV–vis spectrophotometer**

The ZnONPs showed the characteristic UV-vis absorption spectra at 300–380 nm, which revealed the proof for the synthesis of ZnONPs [47]. The UV-vis spectrum of GS- and CS-ZnONPs are shown in Fig 1.

![Ultraviolet–Visible spectrum of chemically synthesized and green synthesized nanoparticles](image)

The spectrum shows a characteristic sharp escalation in the absorption points at 372 nm and 378 nm for CS- and GS-ZnONPs. The absorption peak between 300-380 nm can be apportioned to the absorption spectra of ZnONPs due to the electron shifts to the conduction band from the valence band [1, 48]. Lack of any other peak in the range endorses that synthesized items are ZnONPs only, and a definite shift in the consumption onset was discernible in ZnONPs was synthesized with *S. trilobatum*. The spectrum of ZnO metal NPs will exhibit the property of SPR, which can shift in the wavelength due to the particle size or capping of phytochemicals from plant extract [40, 49, 50]. As described by earlier researchers, the SPR pattern strongly depends on the particle size, the capping or the stabilizing molecules which cover the surface of the NPs and the dielectric constant of the medium [51, 52]. The increase in the band gap energy from the CS- and GS-ZnONPs revealed the change of crystal structure of synthesized ZnONPs, which was in line with the results of Nithya and Kalyanasundharam, 2019 [52]. The ZnONPs
synthesized using *Eryngium foetidum* L., which revealed a characteristic ZnONPs peak at about 343 nm [53]. Besides, as reported by Ishwarya et al. (2018), ZnONPs were synthesized using *Ulva lactuca* seaweed extract exhibited a characteristic UV-vis absorption spectrum at 325 nm [54], whereas ZnONPs obtained from the root extract of *Scutellaria baicalensis* exhibited the absorption peak at 360 nm [33].

**Scanning Electron Microscopy analysis (SEM)**

The morphology of the ZnONPs was studied by SEM which were recorded at 30 kV from samples covered with a gold thin film with high vacuum mode.

![SEM image](image)

**Fig 2.** SEM image of (a) chemically synthesized ZnO nanoparticles, (B) green synthesized ZnO nanoparticles

Fig 2 shows the morphology of the CS- and GS-ZnONPs. From Fig 2, it is observed that the NPs were amorphous, spherical shaped with various sizes. Fig 2(A) clearly shows that NPs are not aggregated, but due to electrostatic and the surface energy, it is likely together. Fig 2(B), SEM image clearly shows that the surface of the ZnONPs is capped with the phytochemicals from the plant extract, acting as the stabilizing agents. The phytochemicals from *S. trilobatum* were responsible for the synthesis and bioreduction of the ZnONPs. As reported by Amir and Kumar (2004), the leaf extract of *S. trilobatum* contains phytochemicals such as alkaloids, triterpenoids, phytosterols, and saponins [55]. These phytochemicals and other constituents from *S. trilobatum* were responsible for the bioreduction, which acted as a capping agent to prevent aggregation of the synthesized ZnONPs. The SEM images of our earlier research have also shown the green synthesis methods utilizing the leaf extracts of *Mukia maderaspatna* [56], *Chrysopogon zizanioides* [39], and *M. umbellatum* [40] that were responsible for bioreduction and also showed no aggregation. Also, other studies confirmed the synthesis of ZnONPs utilizing the extracts of *Pithecellobium dulce* [57], *Codonopsis lanceolat* [58], *Juglans regia* [51], and *Scutellaria baicalensis* [33].

![EDAX spectrum](image)

**Energy dispersive analysis spectroscopy (EDAX)**

Fig 3 (A) shows the EDAX of CS-ZnONPs which discloses the presence of Zn and O that indicate the activity of pure ZnONPs. Tentatively, the stoichiometric mass percent of Zn and O was 59.8 and 25.56%, respectively. The sharpness of the peak corresponds to the elemental composition CS-ZnONPs. Fig 3 (B) shows the EDAX of GS-ZnONPs, which reveals the presence of Zn, O, and other Phyto-constituents confirming the purity of the synthesized ZnONPs.

![TEM images](image)

**Fig 4.** (A) TEM images of chemically synthesized ZnO nanoparticles, (B) TEM images of green synthesized ZnO nanoparticles

The contamination-free nanoparticle unveils the promising antimicrobial and antibiofilm activity.

Preferably, the anticipated stoichiometric mass percent of Zn and O was 80.3 and 19.7%, correspondingly. The GS-ZnONPs showed the elemental composition of Zn–76.47%, O–20.53%, and the other components were K–3.00%.
Transmission electron microscopy (TEM)

Fig 4(A) shows the TEM image of the CS-ZnONPs. It is observed that CS-ZnONPs have uniform size shape with different morphology. Fig 4 (B) shows the TEM image of GS-ZnONPs which resemble a diffuse wave leaving from the crystal lattice plane. Fig 5 (A) shows the particle size distribution of CS-ZnONPs. The particle size and the distribution of the ZnONPs were determined using ImageJ Software.

Fig 6(A) represents the XRD pattern of CS-ZnONPs. The peak intensity, full width half maximum, position, and width were analyzed from the XRD spectrum. The diffraction peaks were positioned at 31.89º, 34.58º, 36.38º, 47.70º, 56.69º, 62.99º, 66.49º, 68.12º and 69.19º which have been indexed as phases of ZnONPs.

Table 1. Antimicrobial activity of various ZnO nanoparticles and S. trilobatum leaf extract at different concentrations against B. subtilis

<table>
<thead>
<tr>
<th>Type</th>
<th>Zone of inhibition (mm)</th>
<th>Standard Antibiotic</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50(mg/mL)</td>
<td>100(mg/mL)</td>
</tr>
<tr>
<td>CS-ZnO</td>
<td>15 ± 4.522</td>
<td>18 ± 1.2</td>
</tr>
<tr>
<td>GS-ZnO</td>
<td>19.6 ± 6.65</td>
<td>25.3 ± 2.081</td>
</tr>
<tr>
<td>Solanum trilobatum leaf extract</td>
<td>20.6 ± 7.57</td>
<td>19.66 ± 3.78</td>
</tr>
</tbody>
</table>

a The zone of inhibition represented has excluding the standard disk diameter of 6 mm
b The values represented are mean ± SD of three replicates.
# The standard antibiotic utilized is Cefotaxime 30 µg (Hi-Media Laboratories, Mumbai, India)

Table 2. Antimicrobial activity of various ZnO nanoparticles and S. trilobatum leaf extract at different concentrations against E. coli

<table>
<thead>
<tr>
<th>Type</th>
<th>Zone of inhibition (mm)</th>
<th>Standard Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50(mg/mL)</td>
<td>100(mg/mL)</td>
</tr>
<tr>
<td>CS-ZnO</td>
<td>20 ± 4.35</td>
<td>23 ± 2.64</td>
</tr>
<tr>
<td>GS-ZnO</td>
<td>25.33 ± 1.15</td>
<td>25.6 ± 2.309</td>
</tr>
<tr>
<td>Solanum trilobatum leaf extract</td>
<td>14.6 ± 1.53</td>
<td>21.6 ± 0.577</td>
</tr>
</tbody>
</table>

a The zone of inhibition represented has excluding the standard disk diameter of 6 mm
b The value represented are mean ± SD of three replicates.
# The Standard Antibiotic utilized is Tetracycline 30mcg (Hi-Media Laboratories, Mumbai, India)

The diffraction peaks associated with the impurities were not perceived in the XRD pattern. The line enlargement detected in the diffraction peaks was a typical observation, which proves that the synthesized materials are in the nanometer range. A similar observation was reported elsewhere [59]. There is no evidence of bulk residue materials, and other contamination in the CS-ZnONPs [60].

As reported by Talam et al., (2013), ZnONPs have exhibited the diffraction peaks located at 31.84°, 34.52°, 36.33°, 47.63°, 56.71°, 62.96°, 68.13°, and 69.18° have been strongly indexed as hexagonal quartzite phase of ZnO [61]. XRD analysis confirmed that CS-ZnONPs were small spherical shaped, crystalline in nature and were in good agreement with the characteristics of nano-sized particles as reported in the literature [62-64]. Fig 6 (B) represents the XRD pattern of the GS-ZnONPs. The leading diffraction peaks detected in the peaks matched to the Bragg reflections with 2θ values of 28.28°, 31.70°, 34.40°, 36.20°, 46.51°, 47.43°, 56.51°, 62.90°, and 66.49° which was related to the lattice plane of (1 0 0), (0 0 2), (1 0 1), (1 0 3), (1 1 0), (1 0 3), (1 1 2), (2 0 1), (0 0 4) suggesting the face-centered cubic crystal structure of the NPs. Our results were in line with the results of researchers reported earlier [35, 60, 65].

**Antimicrobial activity**

The antimicrobial activity of the CS- and GS-ZnONPs was investigated with three different concentrations (50, 100 and 200 mg/mL) against *B. subtilis*, *E. coli*, and *K. pneumoniae*. *B. subtilis* is Gram-positive, aerobic, rod-shaped bacteria, whereas *E. coli* and *K. pneumoniae* are Gram-negative, facultative anaerobic, rod-shaped bacteria. Table 1 shows the antimicrobial activity of CS- and GS-ZnONPs, and *Solanum trilobatum* leaf extract at different concentrations against *B. subtilis*. The GS-ZnONPs exhibited the higher zone of inhibition of 25.3±2.081 and 28.6±2.52 mm at 100 and 200 mg/mL, whereas the standard antibiotic Cefotaxime (30 µg) exhibited 21.46±1.32 mm. The presence of the inhibition zone clearly indicated that the *B. subtilis* cell wall membrane was disturbed and ZnONPs penetrated the cell and ultimately led to cell death. Table 2 shows the antimicrobial activity of CS- and GS-ZnONPs, and *S. trilobatum* leaf extract at different concentrations against *E. coli*. It is observed that GS-ZnONPs exhibited a higher zone of inhibition at all the concentrations.

The higher zone of inhibition of 27.63±0.577 mm at 200 mg/mL, whereas the standard antibiotic Tetracycline (30 µg) exhibited the zone of inhibition of 17.9±0.25 mm. From the morphology of nanoparticles, it was found that the GS-ZnONPs were spherical shaped, and the size of NPs were also smaller as compared to CS-ZnONPs.

It can be interpreted as the smaller NPs can easily penetrate the bacterial cell wall and show enhanced antimicrobial activity.

Table 3 shows the antimicrobial activity of CS- and GS-ZnONPs, and *S. trilobatum* leaf extract at different concentrations against *K. pneumoniae*. From the results, it is observed that GS-ZnONPs exhibited the higher zone of inhibition of 29.33±
0.816 mm at 200 mg/mL, whereas *S. trilobatum* leaf extract exhibited the higher zone of inhibition at lower concentrations. As reported by Elkady et al. (2015), ZnONPs at 30 mg/mL exhibited the inhibition zone of 23 mm against *B. subtilis*, and 32 mm against *E. coli* [66]. Rad et al., (2019) reported that ZnONPs from leaf extract of *Mentha pulegium* exhibited the maximum zone of inhibition at the concentration of 200 μg/mL [67].

The mechanism of antimicrobial property is related to the bactericidal effect of ZnO which prevents the bacterial growth wither by cell wall injury, pore development, seepage of ions and composites and cellular materials outside the membrane and causes the cell death. Our antimicrobial results were in line with the results of Darvishi *et al.*, (2019) that reported green synthesized ZnONPs from walnut leaves with an antibacterial effect as compared to chemically synthesized ZnONPs [51].

**Time-dependent growth inhibition assay**

Time-dependent growth inhibition of *E. coli* with CS- and GS-ZnONPs is shown in Fig 7. The control cells without any NPs showed a higher optical density as compared to the CS- and GS-ZnONPs. The increase in the concentration of CS- and/or GS-ZnONPs led to a decrease in the growth inhibition of *E. coli*. Even for 0.5 mM of CS- and GS-ZnONPs, the growth inhibition could be easily observed within 2 to 4 hr after the addition of NPs. The growth was declined entirely as compared to control within 10 hrs. From the results, it can be concluded that GS-ZnONPs exhibited higher growth inhibition as compared to CS-ZnONPs. Various researchers have reported that ZnONPs initially penetrated the cell membrane by damaging the membrane by direct or electrostatic interaction, thus induced the release of H2O2 [68, 69]. The released H2O2 initiated the production of reactive oxygen species, which caused alteration in the cellular internalization [70]. Time-dependent growth inhibition of *S. aureus*, with chemical and green synthesized ZnONPs is shown in Fig 8. The control cells without any NPs showed the higher optical density as compared to the CS- and GS-ZnONPs.

It could be seen that smaller ZnONPs has a much better antibacterial activity. Similar to the growth inhibition study of *E. coli*, the *S. aureus* encompassed the disruption of the cell membrane with a high rate of exponentiation of reactive oxygen species and eventually resulted in the death of microbes.

Kadiyala *et al.*, (2018) carried out an extensive study on antimicrobial activity of ZnONPs against methicillin-resistant *S. aureus* and concluded that oxidative stress and dissolution of ZnO were adequate for the killing of methicillin-resistant *S. aureus* [71].
Anticancer Activity of ZnONPs

The anticancer activity of both CS- and GS-ZnONPs was investigated toward MCF-7 cells using MTT assay. Here untreated cells were utilized as a negative control. Fig 9(A) shows the inhibition curve for CS-ZnONPs. Fig 9(B) indicates the inhibition curve for GS-ZnONPs toward MCF-7 cells. This curve was obtained by plotting cell survival (percentage) versus drug concentration (µg/mL), and the cell growth inhibition (IC50) was calculated from curves. The rate constants and the fitted curve exhibited the dose-dependent decrease in cellular survival, and the curve exhibited the sigmoidal ailment that was characteristic of a competitive inhibitor. The IC50 for CS-ZnONPs was found at 136.16 µg/mL and for GS-ZnONPs was found at 85.05 µg/mL. The cytotoxic effects of GS-ZnONPs in MCF-7 cell lines were treated with concentrations of 0, 25, 62.5, 125, 250, 500, 750, and 1000 µg/mL and found the decreasing percentage of cell viability of 100, 85.21, 67.38, 42.03, 13.69, 2.01, 1.3, 1.01, respectively as shown in Fig 10.

Fig 10. Cytotoxic effects of CS-ZnO and GS-ZnO on human breast cancer cell line (MCF-7). Each value represented are mean ± SD of three independent experiments (N=3).

The CS- and GS-ZnONPs exhibited dose-dependent inhibition against the proliferation of cancer cells. In the present study, as compared to CS-ZnONPs, the GS-ZnONPs exhibited higher cytotoxic effects on MCF-7 cell lines. Shobha et al., (2019) reported that ZnONPs initiated the cytotoxicity toward the MB–231 cells through the oxidative pressure by means of reactive oxygen species age [72]. The cytotoxicity study with pomegranate peel mediated synthesized ZnONPs were studied on CCD112 (colon cell line), while anticancer effects were evaluated on HCT116 (colorectal cancer cell line). The ZnONPs revealed the discriminatory toxicity toward colon cancer cells (HCT116) and proved non-toxic to normal cells (CCD112) [73]. The ZnONPs showed a discerning anticancer effect and showed no cytotoxicity in healthy cells. The ZnONPs induced the death of malignant tumor cells [34, 51, 74].

Analysis of DNA strand breakage and DNA damage

The comet assay was performed to evaluate the DNA damage on MCF-7 cells treated with 125, 250, and 500 µg/mL of CS- and GS-ZnONPs. The DNA damage (%) evaluated by comet assay on MCF-7 cells is shown in table 4. The nuclear morphology of the treated and untreated cells were utilized to calculate the DNA damage. The control cells showed only 3% of DNA damage, whereas the cells treated with 500 µg/mL exhibited the DNA damage of 48.11 and 57.2% for CS- and GS-ZnONPs. Similarly, herbal-mediated synthesized ZnONPs demonstrated the 7% DNA damage for the cells treated with 30 µg/mL of ZnONPs [75].

Table 4. Mean percentage of DNA damage by the comet assay in MCF-7 cells treated with different concentrations of chemically synthesized (CS-ZnO) and green synthesized (GC-ZnO) nanoparticles. Each value represented is mean ± SD of three independent experiments (N=3). A total of 100 nucleoids were analysed for each group to obtain % DNA in tail

<table>
<thead>
<tr>
<th>Concentration</th>
<th>CS-ZnO</th>
<th>GS-ZnO</th>
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<tbody>
<tr>
<td>Negative Control</td>
<td>3.12±0.69</td>
<td>3.12±0.69</td>
</tr>
<tr>
<td>125 µg/mL</td>
<td>18.11±3.87</td>
<td>29.4±2.19</td>
</tr>
<tr>
<td>250 µg/mL</td>
<td>31.55±6.17</td>
<td>46.87±5.68</td>
</tr>
<tr>
<td>500 µg/mL</td>
<td>48.11±9.21</td>
<td>57.2±4.17</td>
</tr>
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</table>

CONCLUSION

In this study, the ZnONPs were fabricated by both precipitation process and green synthesis method. The characterization studies demonstrated the successful synthesis of ZnONPs, and the XRD pattern endorsed the wurtzite structure. The EDAX analysis established the purity of ZnONPs. It was established that the purity of GS-Zn was 59.82%, and O was 25.56 %. In case of CS-ZnONPs, the purity of Zn was 76.47%, and O was 20.53%. The morphology of the synthesized
ZnONPs was found to be spherical with an average particle size of 34.75 nm in the CS-ZnO method, and 39.14 nm in the GS-ZnONPs. The internal structure of ZnONPs was found by TEM analysis. The cytotoxic effects of ZnONPs toward MCF-7 cells were evaluated in various concentrations and exhibited dose-dependent inhibition against the proliferation of malignancy of cancer cells. In the present study, as compared to CS-ZnONPs, the GS-ZnONPs exhibited higher cytotoxic effects toward MCF-7 cells. The antimicrobial analysis was performed to find the effectiveness of ZnONPs against pathogens such as E. coli, K. pneumoniae, and B. subtilis which were majorly causing infections in the urinary catheters. The enhanced antimicrobial activity of GS-ZnONPs obtained from S. trilobatum leaf extract were shown compared to CS-ZnONPs. It was concluded that GS-ZnONPs represented much enhanced anticancer and antibacterial activity compared to CS-ZnONPs.

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