Green cardamom mediated phytosynthesis of ZnONPs and validation of its antibacterial and anticancerous potential

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Abstract

Different conventional methods for synthesizing zinc oxide nano-particles (ZnONPs) significantly involves physical and chemical routes. But, often these synthesis processes are quite expensive and also involve the profuse use of many hazardous reagents. To overcome these problems, we have employed a convenient single step phytosynthesis of ZnONPs by using abundantly available bioresource, green cardamom. Aqueous extract of green cardamom served the purpose of reducing agent in the formation of ZnONPs, as the extract contains a high amount of flavonoids which hold great reducing properties. Various characterization analyses such as XRD, UV–vis, FTIR, SEM has been done to confirm the formation of ZnONPs and their size and shape. Antibacterial activity of the green ZnONPs was identified against Gram-positive as well as Gram-negative bacterial strains. Significant growth inhibition was found against all. The further anti-cancerous potential of synthesized nanoparticles was also studied on hepatocellular cell lines showing its potential to be a trustworthy anti-cancerous agent.

1. Introduction

Nano-materials are drawing the attention of researchers due to their extensively small size and morphology [1] which has different and exclusive properties than the bulk one. Nanomaterials extended the frontier of the scientific boundaries. ZnONPs reported being used in various approaches including chemical and gas sensors [2], piezoelectric transducer [3], surface acoustic wave devices, etc [4].

ZnO (zinc oxide) has been found to be an excellent semiconductor as it has a direct gap near UV (ultra violet) [5]. This wide band gap gives the ZnO benefit of being used in the photonic applications in the UV range. ZnO being sensitive to a variety of gaseous substances makes it appropriate for gas sensing applications and it is also used in electrochemical sensor applications [6] based on its piezoelectric properties. The demand for ZnO in biological application increases depending on its biocompatibility [7] and non-toxic properties [8]. Moreover, ZnO is a stable non-hazardous material to be used in different scientific fields as announced by the US Food And Drug Administration (21CFR182.8991) [9]. These favorable properties of ZnO lead to the research of finding out more promising and user-friendly synthesis procedures.

Synthesis of ZnONPs gains a lot of attention in biomedical industries [4]. It has been reported to have an excellent inhibitory effect on high temperature and pressure-resistant bacterial spores [10] in addition to its promising effect against large categories of bacterial strains [11]. As per documented reports, zinc ions can inhibit proliferative and metastatic cancer cells by interacting with altered cell metabolism [12]. Cellular abnormalities or even cell death is a normal consequence resulting from disruption of redox (reduction–oxidation) balance in a cancer cell [13]. Elevation in reactive oxygen species (ROS) level can be a major cause for promoting redox imbalance leading to oxidative damage of the cell [14]. The lethal effect of ZnONPs to bacterial cells may result from the adherence of the mentioned nanoparticles to bacterial surface electrostatically in addition to the liberation of hydrogen peroxide due to the same
agent [15]. ZnONPs have several applications including wound dressing [16], food preservation [17], disinfecting agent, wastewater treatment [18], etc. Many established methods for synthesizing ZnONPs importantly includes chemical vapour deposition [19], sol-gel [20], precipitation [21] etc. All these methods require Application of high temperature, pressure along with usage of toxic and expensive chemicals, are reported to be important parameters in synthesizing ZnONPs via the mentioned methods [22]. To avoid these hazardous issues, green synthesis provides an efficient alternative method for producing well-defined nanoparticles which can be used in several applications [23]. Nanoparticles synthesis using plants, over-rided bacterial synthesis as, multiple purification steps required for this kind of synthesis is not easy [24]. Various plants as a whole or their several parts have been used till now for phytochemical synthesis such as Coriander sativum [25], Azadirachta indica [26], Ocimum sanctum [27], Cassia auriculata [28], etc.

Antibacterial activities of spices are well known and they are a natural source of antioxidants [29]. There are also several other properties of spices already reported, such as anti-inflammatory [30], anticarcinogenic [31], etc. Green cardamom is one of the spices which are used traditionally in cuisines [32] and India is world’s largest consumer market of cardamom [33]. It gained the title of ‘Queen of spices’ and mainly used in food as a flavoring agent due to its nice aroma [34]. Elettaria cardamomum belongs to Zingiberaceae family. It is also used to treat intestinal gas and digestive problems in traditional Chinese and Indian treatment [35]. Although the chemical composition of cardamom varies according to the region they grow and the age of the product [36], the principal compounds are 1,8-cineole (account for more than 50%) in addition with α-terpinyl acetate, camphor, borneol, α-terpineol, limonene, etc. [37]. Vitamin B, vitamin C, protein and iron content in cardamom are also very high along with low fat [38].

In this study, our target was to synthesize ZnONPs biogenically, using cardamom (green), followed by characterization of synthesized nanoparticle, as this was not reported yet, as per our knowledge. The next part of our study includes antibacterial and anticancer potential determination of this biogenically synthesized ZnONPs along with the mechanism underlying these cellular events.

2. Experiments

2.1. Materials

Zinc acetate dihydrate ([Zn(CH₃CO₂)₂].2H₂O) and sodium hydroxide (NaOH) were bought commercially from Sigma Aldrich Chemicals. Bacterial culture media and MTT reagents were from Merck Ltd and SRL Pvt. Ltd, India (Mumbai) respectively. Reagents used here were not subjected to purification as they were of analytical grade. Deionised (Millipore) water of 18 MΩ cm resistivities was used throughout the study.

Fresh green cardamoms were purchased from a local grocery shop (Kolkata). All glassware was thoroughly washed before performing each experiment and preventive measures have been taken to avoid contamination.

2.2. Strains of bacteria

Bacterial strains for this study include, Enterococcus faecalis 441, Staphylococcus aureus 740 and Pseudomonas aeruginosa 1688, Escherichia coli 443.

Microbial Type Culture Collection (MTCC), IMTECH, India (Chandigarh), supplied all used bacterial strains. All strains were maintained throughout the study by performing subculture once per month and stock cultures were maintained at −20 °C (degree centigrade).

2.3. Methods

2.3.1. Green cardamom extract preparation

Freshly collected green cardamoms were washed several times to remove all impurities using deionized water and kept for air-drying until fully dried. This spice was used as a whole without removing any part for extract preparation. The extract was prepared according to the standard protocol [40] with little modification. 5 g (gram) of whole green cardamoms were boiled at 80 °C, using deionized H₂O (water) [100 ml (millilitre)] for 20 min. Filter paper (Whatman) was used for purification of resultant extract at room temperature. Freshly prepared filtered extract was used for each and every study.

2.3.2. Zinc acetate solution preparation

2.2 g of powdered ([Zn(CH₃CO₂)₂].2H₂O) was dissolved in 50 ml deionized H₂O and stirred on a magnetic stirrer until fully dissolved to prepare 0.2 M (molar) zinc acetate solution.

2.3.3. ZnONPs formation

50 ml of prepared green cardamom extract was poured drop-by-drop into [Zn(CH₃CO₂)₂].2H₂O (50 ml) and the solution was continuously stirred, followed by adding drop-wise addition 10 ml of 1 M sodium hydroxide solution. A yellowish-white precipitate was obtained immediately after adding NaOH solution [41].
2.3.4. ZnONPs purification
Following nanoparticle synthesis, only the precipitate was washed repeatedly (3 times) using analytical grade ethanol by centrifugation at 5 000 rpm (rotation per minute) for 10 min and supernatant was precisely discarded. Next, the precipitate was thoroughly dried in a vacuum. Finally purified green synthesized ZnONPs were obtained by calcination of the dried sample at 400 °C in muffle furnace [42].

2.4. Characterisation of prepared ZnONPs
Powder x-ray diffraction (XRD) performed using x-ray powder Diffractometer model D8, Bruker AXS, Wisconsin, USA was implemented to investigate the crystal morphology of synthesized ZnONPs [43]. As a target, Cu-Kα was used with a wavelength of 1.5418 Å (angstrom) within the 2θ (theta) range of 20–80° and 1 s (second) /step speed of scan were maintained while operating at 35 kV (kilovolt). An Ultraviolet-visible spectrophotometer (Bio-tek) was used in a range of 250–800 nm (nanometer) to observe the absorbance spectra of the synthesized particles [44]. Fourier transform infrared spectroscopy (FTIR) was performed by FTIR-8400 s, Shimadzu [400 to 4000 cm (centimetre) -1 ] to find the probable functional group of significant biomolecules, the spice extract contains [45]. Zetasizer (NANO ZS90, Malvern Instruments Ltd, UK) was used to perform DLS (Dynamic Light Scattering) to investigate the stability and particle size distribution of synthesized nanoparticles [46]. Synthesized nanoparticles size and morphology were further assessed by field Emission Scanning Electron Microscope (FE-SEM) [47] with the help of INSPECTF50 (FEI, Netherland).

2.5. Antibacterial study of ZnONPs
2.5.1. Minimum Inhibitory concentration (MIC) determination
Prepared ZnONPs were vigorously ultrasonicated by using the ultrasonicator bath to prepare a uniform dispersion before each experiment. All bacterial strains were freshly cultured one day before the experiment into the liquid culture. The graduated concentration of prepared ZnONPs was added to multiple 1 ml sterile Mueller Hinton Broth, followed by inoculation of 10 μl (microlitre) bacterial culture, containing $2.5 \times 10^5$ CFU (colony forming unit) ml -1 bacteria. This experimental set-up was used for four different bacterial strains. These inoculated mixtures were incubated for 24 h (hours) at 37 °C using a shaker-incubator. The turbidity of vials containing bacteria was used for measuring the Minimum Inhibitory Concentration (MIC) [48].

2.5.2. Minimum bactericidal concentration (MBC) determination
Sterile Mueller Hinton agar plates were prepared using the same concentrations of ZnONPs which were used for MIC study. 10 μl bacterial cultures were spread on these solid media plates by a glass spreader and incubation was given at 37 °C for 24 h using an incubator. This total experiment was coordinated in a bio-safety cabinet. The lowest concentration required for total killing of bacteria was accepted as MBC concentration [49].

2.5.3. Level of tolerance
The Tolerance level of the bacterial strains, used in this study was estimated by the help of MIC and MBC value, by the following formula [50]:

$$\text{MBC/MIC} = \text{Tolerance level} \quad (1)$$

2.5.4. Agar well diffusion method
The above-mentioned strains were inoculated in 1 ml freshly prepared Mueller Hinton Broth and incubation at 37 °C was given for 24 h in an incubator. Next day, turbidity of the grown cultures was adjusted to $10^8$ CFU/mL according to Mac Farland standards (0.5) using sterile Mueller Hinton Broth. 100 μl of these bacterial suspensions were spread over solid media (Mueller Hinton Agar) by using a glass spreader and 0.563 cm² diameter wells were punched with a well cutter at equal distance. These wells were filled with 70 μl of green ZnONPs dispersion which contains nanoparticles at its MBC concentrations. Inhibitory-zone diameters, surrounding wells were determined from plate followed by incubation for 24 h [51].

2.5.5. Growth inhibition kinetics
A set of ten glass containers with sterilized Muller Hinton Broth was used to conduct this experiment. Green ZnONPs were added to 4 of these containers using their MIC values. Fresh bacterial cultures of E. coli, P. aeruginosa, S. aureus and E. Faecalis were added to 8 containers, 4 of which were pre-loaded with green ZnONPs. From the remaining two containers, one was used as blank (having only sterile broth) and the other contains sterile distilled water along with sterile broth, which was used as a solvent for green nanoparticle synthesis. These containers were then kept in a shaker incubator at 37 °C. The turbidity of the contents of each container was measured spectroscopically at 530 nm, at an interval of one hour, starting from the zero hours (just before incubation). The obtained values were used to plot the growth curve for each strain of bacteria used [32].
2.5.6. Viability assay of bacterial cells

The viability of bacterial cells was analysed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) following a standard protocol [53]. Cell viability determination was done after treating the cells for 24 h with various concentrations of ZnONPs at 37 °C. After incubating the bacterial cells, they were centrifuged for 10 min (minute) at 1400 rpm, maintaining a constant temperature of 4 °C. Bacterial cells were collected finally, and washed 3 times using sterile phosphate-buffered saline (PBS, pH = 7.4). Next, replacing the medium with fresh culture media containing the MTT reagent [0.5 mg (milligram).ml⁻¹] was done followed by incubation for 3 h at 37 °C. Then, HCl (hydrochloric acid) -isopropanol solution was added and incubation at room temperature was finally given for 15 min to form MTT formazan product. These solutions were used to measure the absorbance at 570 nm (Shimadzu UV–Vis 1800 spectrophotometer).

2.5.7. Intracellular ROS generation

2,7-dichlorofluorescein di-acetate (DCFH₂-DA) was used to measure generated ROS within bacterial cells following the reported protocol [53]. After passive entry of DCFH₂-DA into the cells, it forms 2,7-dichlorofluorescein (DCF) which is a good fluorescent compound by reacting with produced ROS. After treatment with ZnONPs, bacterial cells were kept at overnight incubation and then subjected to PBS wash. Then, 30 min of incubation was given at 37 °C after adding the required quantity of DCFH₂-DA. Finally, with the help of a fluorescence microscope, bacterial cells were observed.

2.5.8. Study of bacterial cells by SEM (scanning electron microscope)

The degree of damage to the bacterial cells, post-treatment, by the green synthesized ZnONPs was analysed by SEM. Samples subjected to SEM study were prepared following overnight incubation of both the treated and control bacterial cells at 37 °C [12]. Centrifugation (4 min, 4000 rpm) of 1 ml of these incubated cultures (both control and treated) was performed to obtain the pellet. After washing the pellet three times with sterile and filtered PBS containing 2% glutaraldehyde, the bacterial cells were subjected to dehydration using serially diluted ethanol. Bacterial cells were then fixed on cover slips using the drop-casting method and were finally dried by placing under laminar airflow. SEM analysis was then performed followed by gold coating and placing on carbon tape (FEI, INSPECT F50, Netherlands).

2.6. Anticancer study of ZnONPs

2.6.1. Measurement of intracellular ROS

Normally, the DCFDA enters the cell and reacts with the reactive oxygen to give a green fluorescent colour compound dichlorofluorescein (DCF). Briefly, a stock solution of DCFDA (10 mM) was prepared in methanol and was further diluted with PBS to a working concentration of 100 μM. Hep G2 cells were treated with ZnONPs at LD₅₀ (lethal dose) for 12 h at 37 °C and washed with 1 × PBS (ice-cold). Next, the content was subjected to incubation after adding 100 μM of DCFDA for 30 min in the dark at 37 °C [32, 33]. The incubated Hep G2 (hepatoma G2) cells were spectroscopically (Hitachi, Japan) analysed to determine the intensity of fluorescence besides, evaluation by fluorescence microscope (Leica, Japan) using 485 nm as excitation and 520 nm as emission wavelengths.

2.6.2. Cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to perform an assay to determine the viability of Hep G2 cells after exposure to various concentrations of green ZnONPs [53]. Briefly, 96-well plates containing green ZnONPs at different concentrations like 20, 40, 60, 80, 100 μg/ml were prepared, with which 1 × 10⁴ cells (approximately) were added in each of the wells. An extra well containing no green ZnONPs but only the same amount of cells like other wells was used as control. These samples were then incubated for 24 h at 37 °C [5% CO₂ (carbon-di-oxide)] [54]. Next, another incubation at 37 °C (5% CO₂) for 4 h was followed after adding 10 μL MTT solution using a stock concentration of 1 mg ml⁻¹. Then, these cells were washed using 1 × PBS (phosphate-buffered saline) and MTT solubilising buffer was used to dissolve the formed formazan crystals. The absorbance of these samples including the control was measured at 570 nm. The result was evaluated after comparing the data obtained from the control and test samples.

2.6.3. Study of nuclear morphology (Apoptotic) by DAPI (4′,6-diamidino-2-phenylindole) staining

The Nuclear morphology of HepG2 cells was envisaged with the help of DAPI staining. The cells were After exposure of green ZnONPs (80 μg ml⁻¹ and 100 μg ml⁻¹) for 12 h, Afterwards, the cells were washed with 1 × PBS thrice and were then stained with DAPI. Ultimately the nuclear morphology of the hepatocellular cancer cells was studied using a fluorescence microscope.
3. Result and discussion

3.1. Physical characterisations of phytofabricated ZnONPs

3.1.1. X-ray diffraction analysis
Sharp diffraction peaks observed in the case of the synthesised ZnONPs sample indicate a decent crystallinity of the sample and effect of environmental conditions maintained during the synthesis procedure is shown in Figure 1. Distinct peaks corresponding to $2\theta$ value was observed in 31.94, 34.59, 36.48, 47.74, 56.71, 63.16, 66.57, 68.09 and 69.36. The characteristic peaks correspond to (hkl) values of $(100), (002), (101), (102), (110), (103), (200), (112)$ and $(201)$. These plane values are matched with the hexagonal Wurtzite structure of ZnO [55] according to the Joint Committee on Powder Diffraction Studies Standards (JCPDS card number #82–1042 and 5–n0664). The absence of any other peak confirmed the purity of the synthesized sample. The size (average) of nanoparticle was calculated with the help of the highest peak by Debye–Scherer equation [43].

$$D = 0.9\lambda/\beta \cos\theta$$

($\lambda$, the wavelength is 1.5418 Å; $\theta$, Bragg’s diffraction angle and $\beta$, the full width at half maximum).

3.1.2. UV–vis spectroscopy analysis
The characteristic peak of green ZnONPs was observed around 350 nm which is shown in figure 2. A high value of excitation binding energy was responsible for the obtained peak at room temperature [44]. This result is in accordance with previously reported results [57] which confirms the formation of ZnONPs.

3.1.3. FTIR analysis
FTIR spectrum shown in figure 3, is of synthesized green ZnONPs. The purity of synthesized nanoparticle was evaluated and the presence of various phytochemicals was identified through this study. These phytochemicals are responsible for the stabilization of nanoparticles by creating interaction with the surface of nanoparticles. ZnO absorption band was obtained within the region 4000-500 cm$^{-1}$ [58]. The stretching frequency of Zn–O bond accounts for the peak near 450 cm$^{-1}$, confirming the presence of ZnO and M–O vibrational band [59]. –OH stretching of intramolecular hydrogen bond along with C–C stretching and C=O of alkanes are represented by the peak at 840 cm$^{-1}$. The Band at 3376 cm$^{-1}$ expresses the amide linkage between amino acid residues and the presence of atmospheric CO$_2$ [58].

3.1.4. Analysis of Zeta potential and average particle size distribution
Table 1 represents the result obtained from the Dynamic Light Scattering (DLS) study which was conducted to measure the size (average) and zeta-potential of the ZnONPs (green synthesized). The nature of the ZnONPs was found to be homogeneous and the average diameter was 177 d.nm along with a Polydispersive index (P.D.I) of 0.32 by interpretation of the obtained curve. The stability and conductivity of the ZnONPs were found to be quite impressive due to the presence of a moderately high zeta potential of 11.87 mV [46].

Figure 1. XRD of green synthesized ZnONPs.
3.1.5. FE-SEM analyses

FE-SEM study (figure 4) was done to expose the morphological characteristics of the green synthesized ZnONPs. A rod-shaped [60] and identical ordination was observed in obtained micrograph images. These results are in agreement with many other green synthesized ZnONPs [61].

3.2. Significant antibacterial activity of green synthesized ZnONPs

3.2.1. Inhibitory effect of green ZnONPs obtained from the Broth Dilution test

Gram-positive bacterial strains *S. aureus* and *E. faecalis* along with Gram-negative bacterial strains *E. coli* and *P. aeruginosa* were taken to explore the antibacterial potential of the synthesized ZnONPs. All the MIC values against each bacterial strain are depicted in table 2 and figure 5.

**Figure 2.** Graphical representation of the spectral pattern of green synthesized ZnONPs using the ultra violet range.

**Figure 3.** Spectral characterization of green synthesized ZnONPs using infra-red range.

**Table 1.** DLS, PDI, Zeta potential of green synthesized ZnONPs from green cardamom.

| Sample                        | DLS size (d.nm) | PDI       | Zeta potential (mV) |
|-------------------------------|-----------------|-----------|---------------------|
| ZnONPs from green cardamom    | 177 ± 2.3       | 0.32 ± 0.068 | 11.87 ± 1.7        |
Figure 4. Scanning microscopic image of ZnONPs synthesized from green cardamom.

Figure 5. Antibacterial effect of green synthesized ZnONPs on different bacterial strains.

Figure 6. Well diffusion experiment of green ZnONPs with positive (Ampicillin used as an antibiotic) and negative (Sterilised distilled water) control. (a) S. aureus and (b) P. aeruginosa.

| Bacterial strain | MIC value ($\mu$g/ml) | MBC value ($\mu$g/ml) |
|------------------|------------------------|-----------------------|
| E. faecalis      | 27.0 ± 1.5             | 103.4 ± 1.2           |
| S. aureus        | 25.8 ± 1.4             | 101.2 ± 1.7           |
| P. aeruginosa    | 41.0 ± 1.0             | 155.80 ± 2.1          |
| E. coli          | 37.6 ± 1.5             | 112.8 ± 1.8           |

Table 2. MIC and MBC values of E. faecalis, S. aureus, P. aeruginosa and E. coli.
MBC was determined by culturing each bacterial strain in the sterile nutrient agar plate with the MIC dilutions obtained [49]. At which dose there is the complete destruction of bacteria and no further growth was observed, that concentration considered being the MBC value for that particular bacterial strain. MBC values determined for E. coli, P. aeruginosa, S. aureus, and E. faecalis are listed in Table 2. From this result, it can be concluded that bacterial growth inhibition is directly proportional to the increasing concentration of green synthesized ZnONPs. Besides, the result also indicates, Gram-positive strains of bacteria (used in this study) were more susceptible to green synthesized ZnONPs compared to the Gram-negative strains (used in this study). The Difference in cell wall chemistry of Gram-positive and negative strains of bacteria might be the contributory factor for easy membrane penetration of ZnONPs leading to higher susceptibility of Gram-positive strains.

3.2.2. Potent bactericidal activity of synthesized ZnONPs
Tolerance level exhibits the discrimination between bacteriostatic agents and bactericidal agents. This level is determined by MIC and MBC value for a particular strain. Bacteriostatic agents account for the inhibition of bacterial growth which can be re-established whereas bactericidal agents account for total bacterial killing which can’t be reversed. When the ratio of MBC/MIC is equal to or more than 16 the agent is considered to be a bacteriostatic and when the ratio is equal to or less than 4, the agent is considered to be a bactericidal agent. In our

![Figure 7](image1.png)

*Figure 7. Time-dependent bacterial growth dynamics. (GZnONPS = Green-synthesized ZnO nanoparticles).*

![Figure 8](image2.png)

*Figure 8. Concentration-dependent bacterial cell viability determination.*
study, this ratio for all the four test organisms including *E. faecalis*, *S. aureas*, *P. aeruginosa* and *E. coli* was less than 4 respectively. So our green cardamom mediated synthesized ZnONPs exhibit excellent bactericidal activity [50].

3.2.3. Confirmation of selective bactericidal activity by agar well diffusion method
Each bacterial strain showed different zone of inhibition surrounding the well when they were treated with their corresponding MBC concentration of synthesized ZnONPs. This elaborates on a comparative analysis of the effect of our synthesized nanoparticles on different bacterial strains. This study also approves the ZnONPs to be a potent therapeutic agent against various pathogenic bacterial strains.

3.2.4. Growth curve analysis
Bacterial growth pattern as observed (at 530 nm) in Figure 6, for four different strains of bacteria after plotting the curves, indicates, high inhibition potential of green ZnONPs against each type of test organism used for this
study. It is worth mentioning, with time advancement, growth inhibition reached a steady-state for all the four different test organisms which were treated with green ZnONPs using their respective MICs and finally, no further increase of growth in relation to time was observed.

3.2.5. Treatment of E. coli, S. aureus, B. subtilis and P. aeruginosa with ZnONPs significantly diminishes the bacterial cell viability

ZnONPs is responsible for diminishing the bacterial cell survivability at their respective MBC conc. depending on time as depicted in figure 7. The concentration-dependent bacterial cell viability is also shown in Figure 8. This may be due to the ingressation of ZnONPs into the bacterial cells that can hinder the growth of the bacteria and acts as an ideal bactericidal agent.

3.2.6. Mechanisms behind the bactericidal activity of functionalized ZnONPs

The phytofabricated ZnONPs had the capability to induce intracellular ROS generation resulting in the death of the bacterial cells. In order to envisage the mechanisms behind the bactericidal activities of ZnONPs, the ROS generated intracellularly within bacterial cells was determined by employing DCF-DA which acts as an intracellular indicator for ROS, which is depicted in figure 9. The results indicate that the ZnONPs treated pathogenic bacterial cells augment the intracellular ROS formation, possibly resulting in bacterial cell death.
Enhanced intracellular generation of ROS contributes to bacterial cell membrane damage, in addition to disrupting electron transport chain integrity and detonation of genetic material [62].

3.2.7. ZnONPs mediated effect on bacterial morphology
After analysing the SEM images (figure 10) it can easily be concluded that the membrane was severely damaged in the case of ZnONPs treated bacterial cells resulting in loss of morphological integrity of the cells. On the other hand, membrane integrity was intact in the case of control cells. Bacterial cell death was an obvious consequence as a result of membrane destruction and perforations leading to the expulsion of cytoplasmic contents [63].

3.3. Anticancer activity of ZnONPs
3.3.1. Intracellular Reactive oxygen species generation induced by the treatment of ZnONPs
The reactive oxygen species is analysed with the dual aid of fluorescence microscopy and Spectrofluorometer by employing 2′,7′ dichlorofluorescein di-acetate (DCF-DA) as a probe [64]. The generation of ROS was
evaluated in case of hepatocellular carcinoma cells, Hep G2 with ZnONPs at concentrations of 80 and 100 μM for 12 h. The fluorescence microscopic images clearly depict the occurrence of enhanced green fluorescence intensity in the case of treated Hep G2 cell lines [65] after 12 h (figure 11).

3.3.2. Result of MTT assay
Cytotoxicity (in-vitro) of green ZnONPs was determined using the HepG2 cell line. MTT assay was done following exposure of cells to various concentrations of ZnONPs (0–120 μg ml⁻¹) for a time span of 24 h. It was observed that ZnONPs depicted a dose-dependent decrease in cell survivability. The LD₅₀ of ZnONPs was calculated to be 46 ± 3.2 μM. Thus, we can conclude that the synthesized phytofabricated ZnONPs could exert encouraging anticancer activity as exhibited in figure 12.

3.3.3. The biogenic ZnONPs was able to induce apoptosis in hepatocellular cells
The nuclear morphology study of the HepG2 cells clearly depicts that the untreated cells showed the absence of any nuclear anomalies whereas in treated cells distinct polynuclear fragmentation and nucleus shrinking were observed (figure 13(a)). The subsequent quantitative analysis confirmed the prevalence of 48% and 78% apoptotic cells in HepG2 cells treated with 80 μg ml⁻¹ and 100 μg ml⁻¹ of ZnONPs respectively as depicted in figure 13(b). Hence our results clearly depict that these biogenic ZnONPs could successfully induce apoptosis that in turn resulted in the death of the hepatocellular cancer cells.

4. Conclusion
In this study, we approached phytochemical synthesis of ZnONPs from green cardamom, which is a natural source and abundantly available. So, this synthesis procedure is cost-effective, less tedious, non-hazardous and eco-friendly along with the potential to synthesize stable nanoparticles. The various phytochemicals in accordance with flavonoids present in the green source might be responsible for stabilization and capping of ZnONPs. DLS confirmed particle size and Zeta potential, the stability of green ZnONPs. As per the x-ray diffraction study, these bio-synthesized nanoparticles are having a nice crystalline structure. Our synthesized ZnONPs also showed an excellent bacterial susceptibility against different types (Gram-positive and negative) of pathogenic bacterial strains, which is quite appreciable and lucrative in the field of microbiology. Our results clearly depict, biogenic ZnONPs are responsible for initiating ROS generated oxidative damage both in the case of prokaryotic and eukaryotic cells. The generated reactive oxygen or superoxide has the potential to directly interact with the cell metabolism in order to generate the hydroxyl radicals, which is responsible for damaging the DNA, lipid, and proteins. Our results clearly suggest that these phytofabricated ZnONPs can be considered as an effective antibacterial agents susceptible to both Gram-positive (S. aureus, B. subtilis) and Gram-negative (E. coli, P. aeruginosa) strains.

In this study, with ZnONPs, the probable reason behind the bacterial cell wall destruction might be the disruption in the steps of signaling cascades that leads to the improper synthesis of the cell wall or fragile polymer unit assembly resulting from enhanced ROS production [62] in bacterial strains of both types (Gram-positive and Gram-negative). Furthermore, the death of cancer cells (hepatocellular) from exposure to green ZnONPs was a result of enhanced cellular ROS production. This study can conclusively suggest, phytofabricated ZnONPs can emerge as a dual ameliorative agent against pathogenic bacterial strains and hepatocellular cancer cells.

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