Contents lists available at ScienceDirect

Journal of Saudi Chemical Society

journal homepage: www.ksu.edu.sa

Antitumor activity of zinc oxide nanoparticles fused with green extract of *Nigella sativa*

M. Fakhar-e-Alam [a], Irsa Amjad [a], Malik Saadullah [b], M. Tahir [a], M. Jawad [a], M. Asif [a], M. Atif [c], Susi Zara [d], Muhammad Rashad [b,d,*]

[a] Department of Physics, Government College University Faisalabad, Pakistan
[b] Department of Pharmaceutical Chemistry, Government College University Faisalabad, Pakistan
[c] Department of Physics and Astronomy, College of Science, King Saud University, Riyadh 11541, Saudi Arabia
[d] Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, via dei vestini 31, 66100 Chieti, Italy

ARTICLE INFO

Keywords:
Green synthesis
ZnO NPs
FTIR analysis
Antitumor/anticancer activity

ABSTRACT

Zinc oxide nanoparticles have attracted considerable attention as promising candidates in various fields, especially in the realm of medical science. In the present experimental study, the co-precipitation method was used to synthesize chemical and green ZnO NPs. Zinc nitrate Zn $(NO_3)_2$ served as the main precursor, while sodium hydroxide (NaOH) and *Nigella sativa* extract were used to synthesize chemical and green Zinc Oxide nanoparticle, respectively. Then, the chemical and green fabricated Zinc Oxide nanoparticles were subjected to annealing at 400 °C and 550 °C temperature for a period of 2 hours respectively. After final product obtained, various characterization techniques were employed to validate the crystal structure, morphology, optical properties, and functional groups (e.g., —OH stretching group, Zn—O mode, etc.) via XRD, SEM, UV–Vis, and FTIR analysis. The Scherer formula was applied to calculate the average crystalline size of green and chemical fabricated Zinc Oxide nanoparticles, which confirmed the presence of the hexagonal wurtzite phase. Moreover, the fabricated products were finally tested for anticancer activity using MTT assay by optimizing cellular absorption against concentration. The MTT assay confirmed significant antitumor/anticancer efficacy against HepG2 cancer cells for both green actively and chemical fabricated Zinc Oxide nanoparticles superficial. The current development of this novel experimental strategy is anticipated to make a substantial contribution in the field of biomedical sciences, particularly in anticancer activity.

1. Introduction

Green nanotechnology has developed an attractive focus due to its tremendous advantages such as environment-friendly, cost-effective and easily scale-up nature. Plants have been identified as an ideal source of materials among the different options accessible due to their bio-reducing and stabilization capabilities. A variety of nanomaterials in pure and hybrid forms with important properties, such as physical, chemical and biological effect and their optical behavior, make them suitable for biomedical applications. There are many physical, chemical and biological methods for the fabrication of nanoparticles. The most suitable and well-known methods are hydrothermal, sol–gel and co-precipitation [1–6].

In recent years, nanoparticles have gained much popularity [7–11] and have been effectively used in the delivery of therapeutic agents. In the last two decades, several NPs have been developed for the diagnosis and treatment of various diseases such as allergy, fever, diabetes, inflammation, cough, etc [12,13]. Zinc oxide nanoparticles (ZnO NPs) have a versatile property (like wide band gap, important physiochemical and biological), play an important role in biomedical application as well as in every field of life, for example, water purification systems such as degradation of organic impurities and removal of heavy metals, rubbers, cosmetics, inks, soaps, batteries, textiles and medicines [14–21]. ZnO NPs are excellent candidates due to their appealing characteristics, particularly in biomedical applications such as photodynamic therapy, drug delivery and nano-biosensors. In view of the importance of ZnO NPs, various attempts are made for synthesis, for which green synthesis is the most prominent method due to its eco-friendly nature and non-toxic approach [22–28].

In this study, green ZnO NPs were synthesized using the leaves of the

* Corresponding author at: Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, via dei vestini 31, 66100 Chieti, Italy.
E-mail address: muhammad.rashad@unich.it (M. Rashad).

https://doi.org/10.1016/j.jscs.2024.101814
Received 24 October 2023; Received in revised form 27 December 2023; Accepted 8 January 2024
Available online 13 January 2024

Nigella sativa seed extract, which has been reported in a number of medicinal properties, because it contains bioactive agents. Different research on this plant has shown that *Nigella sativa* is an effective anti-cancer, anti-inflammatory and anti-viral agent with significant therapeutic efficacy for a wide range of diseases. The research also supported that leaf extract of *Nigella sativa* have significant antioxidant activity. *Nigella sativa* seed is the most potent source of antioxidant property and antitumor which shows its ability to treat many health disorders including anti-cancer, anti-viral, anti-inflammatory as *Nigella sativa* extract has the maximum total flavonoid content and phenolic, highest ABTS assay activity, highest DPPH radical scavenging activity and highest antitumor capacity among four different crude methanolic extracts [29–34].

2. Experimental procedure

2.1. Materials

Nigella sativa seeds, $Zn(NO_3)_2.6H_2O$ (zinc nitrate hexahydrate), NaOH (sodium hydroxide), double distilled water, DMSO (dimethyl sulfoxide), FBS (fetal bovine serum) and DMEM (Dulbecco's Modified Eagle Medium) and antibiotics (penicillin + streptomycin) was purchased from Merck (US).

2.2. Green synthesis approach

Frist of all took 400 g of *Nigella sativa* seed, then grinded into fine powder and 400 g powder of *Nigella sativa* seed dissolved into the 600 mL of ethanol at 100 °C for 24 h. After 24 h, removed the solution from hot plate, stabilized and then filtered it by using filter paper. A dark green extract of *Nigella sativa* seeds was obtained. The Green synthesis method was used to fabricate the zinc oxide nanoparticles (ZnO NPs). Frist of all, 0.1 M of $Zn(NO_2)_3.6H_2O$ were mixed in 100 mL of double distilled water and stirred for 30 min. In the next step, the extract of of *Nigella sativa seeds* were added dropwise into the zinc nitrate hexahydrate solution to get (11to12) pH and stirring continuously at 90 °C for 2 h. Finally, the dark green precipitates were obtained then centrifuge at 5000 rpm for 10 min, dried and grinded to make fine powder. At the end, the fine powder was annealed in muffle furnace at 400 °C for 2 h [35,36].

2.3. Chemical synthesis approach

A chemical method was used to fabricated the ZnO NPs in which 0.1 mL of $Zn(NO_2)_3.6H_2O$ was taken and dissolved in 100 mL double distilled water and continuous stirring for 30 min to obtained the homogeneous solution. After that, The NaOH (0.05 M) was added to the 50 mL of double distilled water and get the NaOH solution. In the next step, NaOH solution was added dropwise into the zinc nitrate hexahydrate solution to attained the (11–12) pH and continuous stirring at 90 °C for 2 h. Finally, the milky white precipitates were attained, centrifuge, dried and grinded to make fine powder. After getting fine powder was annealed in muffle furnace at 550 °C for 2 h [37].

2.4. Characterization of ZnO nanoparticles

The crystal structure and miller indices were investigated by using XRD ("D8 Advance Bruker, Germany" with λ = 0.154 nm of Cu Kα radiation). The SEM ("NOVA NANO SEM 30") was used to examined the surface morphology. The vibrational and stretching groups were identified by FTIR spectrometer ("Spectrum 2, Perkin Elmer"). The UV–Visible spectrometer ("Bio base double beam, China") was used to analyze the optical properties [27].

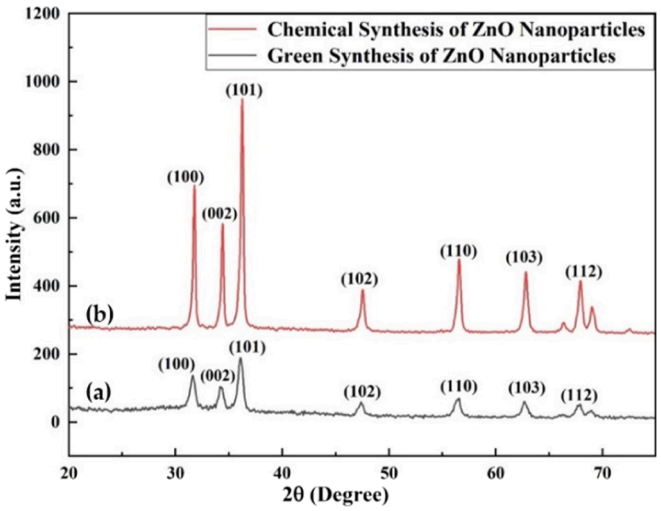

Fig. 1. Shows the XRD pattern of (a) Green synthesized Zinc Oxide nanoparticles at annealed temperature 400 °C (b) Chemical synthesized ZnO NPs at annealed temperature 550 °C.

2.5. Bioassay

2.5.1. Cell culturing

Human liver cancer (HepG-2) cells line was gifted from the Molecular and cell Biology laboratory at the Department of Zoology, Government college university Faisalabad. The HepG-2 cells (Liver cancer cell line) were seeded in 96 well plate in DMEM added with 10 %FBS, antibiotics and cells were incubated at 37° humidified environments with 5 % CO_2 and standard protocol were followed as per recommendation of laboratory of animal's/cells ethics committee, Department of microbiology, faculty of life sciences, GC University Faisalabad Pakistan [37].

2.5.2. Cell labeling with ZnO-NPs

The HepG-2 cells were treated with chemical and green ZnO NPs at various absorption ranging from 0 to 150 μg/mL for 24 h at 37 °C also added/maintained with 10 % FBS and 5 % CO_2. In the current study, 96 plates with 4 each well were organized in 10 columns. The last two columns were treated DMSO as a control/standard, while the first eight columns were filled with chemical and green ZnO NPs dispersion solution at various absorption ranging from 0 to 150 μg/mL. This cell line was treated to ZnO nanoparticles using the vitality of the cells was tested after 24 hours of incubation. The standard experimental procedures were used.

2.5.4. MTT assay

The 3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was used to evaluate/examine the in-vitro cytotoxicity of chemical and green ZnO NPs on liver cancer (HepG-2) cell lines. HepG-2 cells were grown/cultivated in 96 well plates and more incubated for 24 h in a humidified environment (37 °C). after that, cells were treated at various concentration nanoparticles (0–150 μg/mL) for 24 h to be treated. For further incubation at a humid environment (37 °C), 10 μL MTT reagent was added after 24 hours. Finally, the absorbance was calculated using micro-plate reader after added the DMSO (120 μL) to dissolve the formazan crystal [38–40].

The cell viability was measured by given formula.

$$\%Cell\ Viability = \frac{Mean\ Absorbance\ of\ treated\ cells}{Mean\ Absorbance\ of\ controal\ cells} \times 100\% \quad (1)$$

Table 1
Data about 2θ°, intensity, FWHM and Crystalline size of green ZnO NPs.

Sr#	2θ (degree)	Intensity (a. u.)	Crystalline size (nm)
1	31.74//0.033	135	37.214
2	34.40	110	31.9202
3	36.21	190	42.6134
4	47.51	60	38.8764
5	56.45	72	35.412
6	62.77	63	32.9461
7	67.97	52	80.4989

Table 2
Data about 2θ°, intensity, FWHM and crystalline size of Chemical ZnO NPs.

Sr#	2θ (degree)	Intensity (a. u.)	Crystalline size (nm)
1	31.74	460	69.9567
2	34.40	350	72.6723
3	36.21	770	57.7427
4	47.51	140	55.6148
5	56.45	240	53.7665
6	62.77	170	46.0406
7	67.97	150	64.6157

2.6. Statistical analysis

Using Graph Pad Prism software (version 8.0), we performed statistical analysis. Results of all experiments are presented as mean ± SD with *P < 0.05 considered statistically significant and **P < 0.01 considered highly statistically significant.

3. Results and discussion

3.1. X-ray diffraction (XRD) analysis

The crystal structure of green and chemical developed Zinc Oxide nanoparticles was investigated using X-ray diffraction (XRD). Fig. 1 depicted the XRD spectra of **(a)** Green ZnO NPs and **(b)** Chemical ZnO NPs. XRD analysis of green synthesized Zinc Oxide nanoparticles showed a hexagonal wurtzite structure, as well as chemical synthesized ZnO NPs which matched well with **JCPDS Card No: 36-1451.** The same pattern was observed in both conditions (green and chemical synthesis process), and some additional/extra peaks due to laboratory environment or air contamination are shown. In the green and chemical synthesis process, peaks appeared at (2θ) = 31.74°, 34.40°, 35.91°, 36.21°, 47.51°, 56.45°, 62.77°, and 67.97° with matching miller indices (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3) and (1 1 2). The average crystalline size calculated from the all peaks of green and chemical synthesized Zinc Oxide nanoparticles pattern is 43 nm and 60 nm respectively [41].

By using the "Debye Scherrer formula" to calculate the crystalline size of NPs:

$$C.S = \frac{k\lambda}{\beta Cos\theta} \quad (2)$$

Here, the crystalline size is represented by "C.S", the Scherrer constant is represented by "k = 0.9", the X-ray wavelength is given as "λ = 0.154 nm", and the Full width half maximum is denoted by "β".

Tables 1 and 2. Describe the 2θ (degree), intensity (a. u) and crystalline size (nm) of green and chemical synthesized Zinc Oxide nanoparticles samples. After data analysis it was inspected that the crystalline size of the green and chemical synthesized Zinc Oxide nanoparticles is 43 nm and 60 nm, respectively.

3.2. Fourier transformation infrared (FTIR) analysis

The various functional groups associated with the produced green and chemical ZnO NPs were determined using FTIR analysis. Fig. 2**(a)** depicted the FTIR spectra of green synthesized ZnO NPs. The —OH stretching vibrations, C—H stretching mode, OH bending in carboxylic acid, C═C band in aromatic ring, C═O mode in polyphenol, and C—N stretching in amide -I protein, C—O stretching of amino acid and metal–oxygen (Zn—O) vibration mode attached at wavelength 3511 cm^{-1}, 3052 cm^{-1}, 2922 cm^{-1}, 1491 cm^{-1}, 1520 cm^{-1}, 1077 cm^{-1}, 660 cm^{-1} respectively. The Zn—O stretching mode, as confirmed by the FTIR spectra of the chemical synthesized ZnO NPs, is indicated by a band found between 441 and 665 cm^{-1}, which is illustrated in Fig. 2**(b).** The peak at 1064 cm^{-1} is attributed to the stretching mode of the C—N bond of the amine group or the stretching vibration of the C—O bond of the alcohol group. The peaks at 1172 cm^{-1}, 1352 cm^{-1}, and 1375 cm^{-1} are

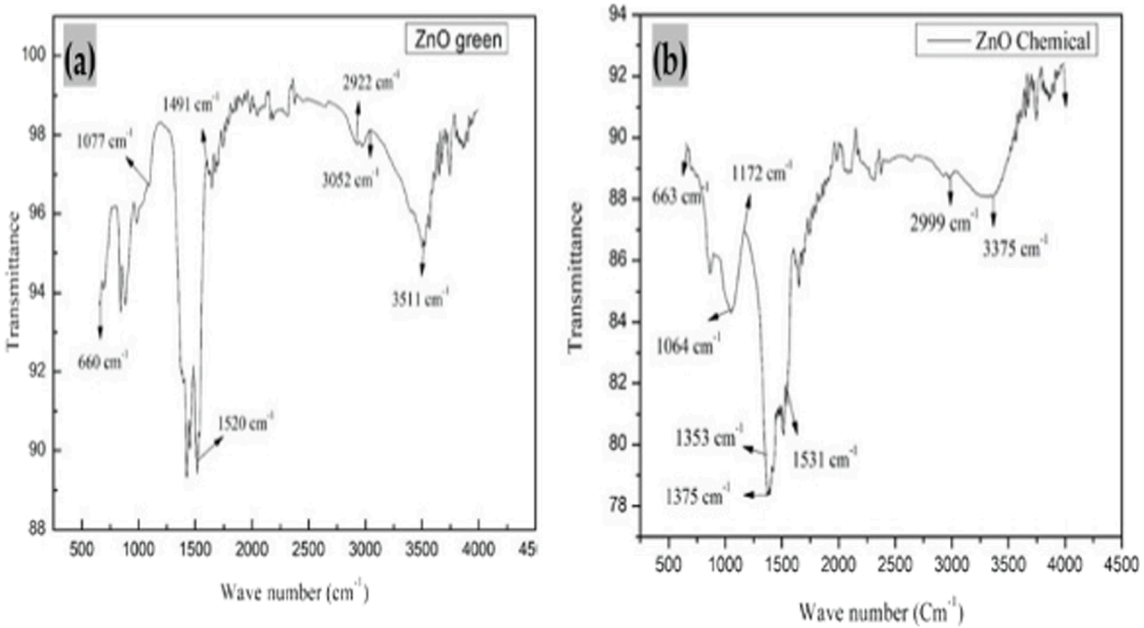

Fig. 2. Shows the FTIR Spectra of (a) Green synthesized ZnO NPs at annealed temperature 400 °C (b) Chemical synthesized ZnO NPs at annealed temperature 550 °C.

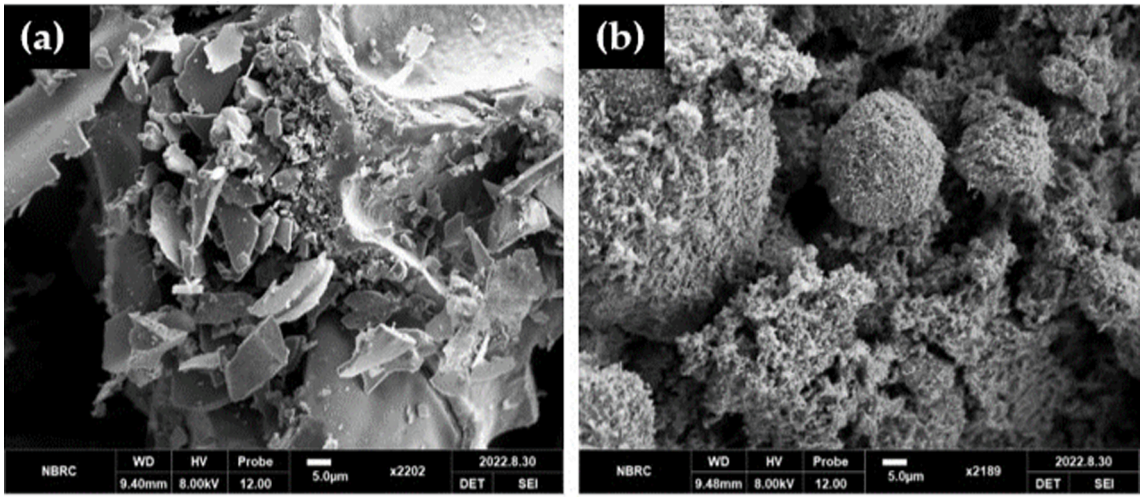

Fig. 3. Shows the SEM analysis of (a) Green synthesized ZnO NPs at annealed temperature 400 °C (b) Chemical synthesized ZnO NPs at annealed temperature 550 °C.

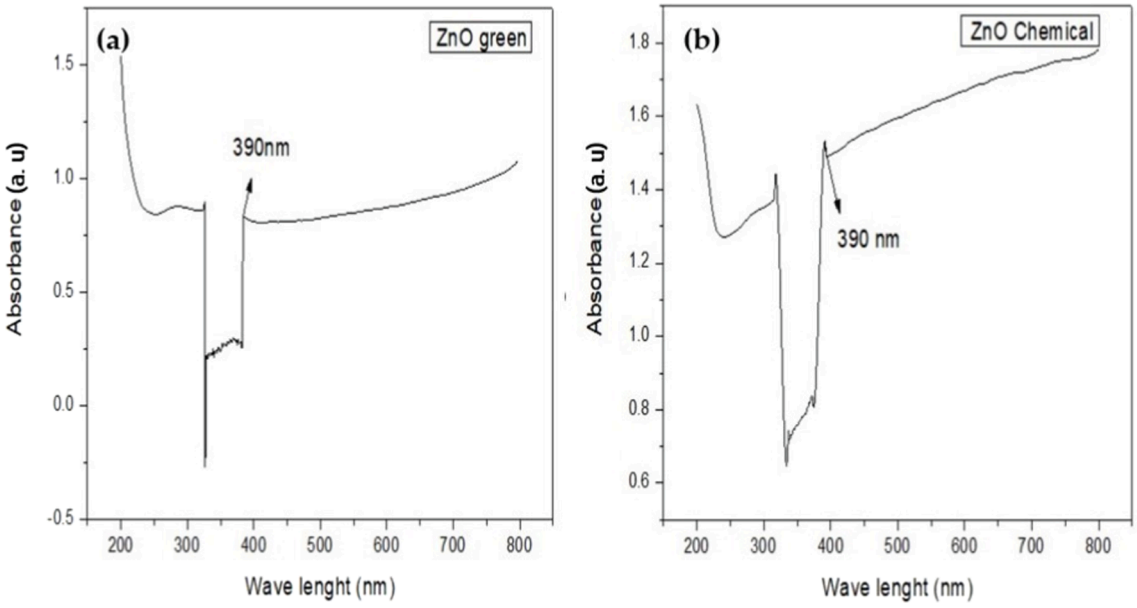

Fig. 4. Shows the UV–Visible analysis of (a) Green synthesized ZnO NPs at annealed temperature 400 °C (b) Chemical synthesized ZnO NPs at annealed temperature 550 °C.

attributed to bending or vibration within the primary and secondary alcohol planes, respectively. The vibrational modes of aromatic nitric compound and alkyls are identified at a peak at 1531 cm^{-1}. The peaks at 2999 cm^{-1} and 3375 cm^{-1} are attributed to the stretching vibration of hydroxyl group [42–44].

3.3. Scanning electron microscopy

The surface morphology of green and chemical produced ZnO NPs was studied using SEM analysis. which are shown in Fig. 3**(a, b).** A significant difference was observed between both green and chemical synthesized Zinc Oxide nanoparticles due to the difference in annealing temperature at 400 and 550 °C. SEM micrographs showed aggregates, irregular small and large grains, smooth, uniform and non-uniform surface morphology. Morphology of green and chemical zinc oxide powders, producing the same image at 5 μm magnification level. Finally, the grain size of green is smaller than that of chemical ZnO NPs [45].

3.4. UV–Visible spectroscopy

UV–Visible spectra of the green and chemical synthesized ZnO NPs, are illustrated in Fig. 4**(a, b).** There are some variations in the UV–visible spectra of green and chemical synthesized Zinc Oxide nanoparticles but the absorption peaks of both samples were found at similar wavelength 390 nm. This analysis was performed at room temperature, green and chemical synthesized ZnO NPs showed significant absorption due to high exciton binding energy. The obtained spectra were non-ideal geometric. This error graph is caused by contamination or lack of instruments [46].

3.5. Antitumor/anticancer activity

The results of the cytotoxicity analysis revealed that both chemical and green ZnO nanoparticles (NPs) exhibited toxicity towards HepG-2 cells, which is shown in the Fig. 5**.** The HepG-2 cells were treated at varying concentrations (0, 20, 40, 60, 80, 100 and 120 μg/mL) of

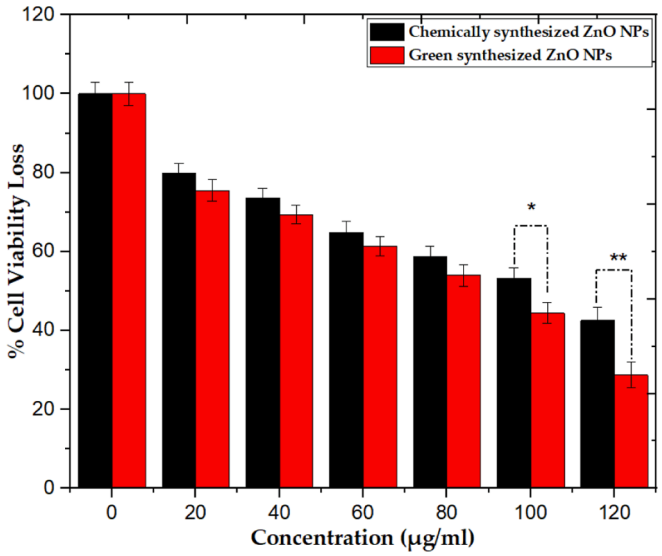

Fig. 5. Shows the antitumor activity of Chemical and Green synthesized Zinc Oxide nanoparticles against HepG-2 Cell, where *P is P < 0.05 and **P is P < 0.01.

chemical and green ZnO NPs. After 24 hours of treatment, it was noticed that the green ZnO NPs shown a higher % cell viability in HepG-2 cells compared to the chemical ZnO NPs. Moreover, the maximum % cell viability loss was observed in the case of green Zinc Oxide nanoparticles (ZnO NPs) at 120 µg/mL concentration [35,36].

It can also be noted that in the green ZnO NPs, Healthy cells exhibit persistent behavior, maintaining at least 80 % cell viability even with increased green synthesis of ZnO NPs [47]. Similarly, the % cell viability of HepG-2 cells decreased to less than 15 %. This distinctive behavior clearly exhibits that healthy cells will persist unaffected, while HepG-2 cells are significantly affected. Chemical ZnO NPS showed a significant decrease in %cell viability for both normal healthy cells and HepG-2 cells.

4. Conclusion

Zinc Oxide nanoparticles (ZnO NPs) were fabricated via co precipitation method by considering green and chemical routs procedure. XRD results revealed hexagonal wurtzite phase structure and the 43 nm and 60 nm crystalline size of green and chemical fabricated Zinc Oxide nanoparticles. The presence of various functional groups was analyzed using FTIR analysis, which are attached to spectra of ZnO NPs. Surface morphology was studied by SEM analysis and UV–Visible spectroscopy results give significant absorption peaks at 390 nm wavelength of green and chemical fabricated ZnO NPs. In vitro MTT assay of green and chemical fabricated ZnO NPs showed significant antitumor/anticancer activity towards HepG-2 cells. These results exposed that green fabricated ZnO NPs showed better, safer and much facile than chemical fabricated Zinc Oxide nanoparticles against tumor. Hence, ZnO NPs were synthesized using *Nigella sativa* seeds extract could be therapeutic source for treatment of tumor/cancer. This study could play a dynamic role in nanotechnology, nanoscience especially in Nano medicine in near future. Primarily level toxicity related study towards cancerous model experiment has been conducted but further examination by multiple analytic techniques will be down before in vivo study and applied based work. To the best of our knowledge, the novelty of the research is that this is the first study to demonstrate the antitumor/anticancer efficacy of *Nigella Sativa* ZnO NPs. We further recommend in vivo anticancer studies of *Nigella Sativa* ZnO NPs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Researchers Supporting Project No. (RSP2023R397), King Saud University, Riyadh, Saudi Arabia.

References

[1] F. Happé, A. Ronald, The 'Fractionable Autism Triad': a review of evidence from behavioural, genetic, cognitive and neural research, Neuropsychol. Rev. 18 (4) (2008) 287–304.
[2] E.B. Buynak, R.R. Roehm, A.A. Tytell, A.U. Bertland II, G.P. Lampson, M. R. Hilleman, Vaccine against human hepatitis B, J. Am. Med. Assoc. 235 (26) (1976) 2832–2834.
[3] M.M. Amer, M.A. Aziz, W.S. Shehab, M.H. Abdellattif, S.M. Mouneir, Recent advances in chemistry and pharmacological aspects of 2-pyridone scaffolds, J. Saudi Chem. Soc. 25 (6) (2021) 101259.
[4] G.A. Naikoo, M. Mustaqeem, I.U. Hassan, T. Awan, F. Arshad, H. Salim, A. Qurashi, Bioinspired and green synthesis of nanoparticles from plant extracts with antiviral and antimicrobial properties: a critical review, J. Saudi Chem. Soc. 25 (9) (2021) 101304.
[5] A. Patel, Benign vs malignant tumors, JAMA Oncol. 6 (9) (2020).
[6] P.-H. Yin, X. Liu, Y.-Y. Qiu, J.-F. Cai, J.-M. Qin, H.-R. Zhu, Q. Li, Anti-tumor activity and apoptosis-regulation mechanisms of bufalin in various cancers: new hope for cancer patients, Asian Pac. J. Cancer Prev. 13 (11) (2012) 5339–5343.
[7] A. Nazir, S. Latif, S.F. Adil, M. Kuniyil, M. Imran, M.R. Hatshan, F. Kanwal, B. Shaik, Photocatalytic degradation of cefixime trihydrate by bismuth ferrite nanoparticles, Materials 15 (1) (2022) 213.
[8] E.M. Almutairi, M.A. Ghanem, A. Al-Warthan, M. Kuniyil, S.F. Adil, Hydrazine high-performance oxidation and sensing using a copper oxide nanosheet electrocatalyst prepared via a foam-surfactant dual template, Nanomaterials 13 (1) (2023) 129.
[9] J.P. Shubha, H.S. Savitha, R.C. Patil, M.E. Assal, M.R. Shaik, M. Kuniyil, O. Alduhaish, N. Dubasi, S. Farooq Adil, A green approach for the degradation of toxic textile dyes by nickel oxide (NiO-SD) NPs: Photocatalytic and kinetic approach, J. King Saud Univ.-Sci. 35 (7) (2023) 102784.
[10] M. Khan, S.F. Adil, M.E. Assal, A.I. Alharthi, M.R. Shaik, M. Kuniyil, A. Al-Warthan, A. Khan, Z. Nawaz, H. Shaikh, M.R.H. Siddiqui, Solventless mechanochemical fabrication of ZnO–$MnCO_3$/N-doped graphene nanocomposite: efficacious and recoverable catalyst for selective aerobic dehydrogenation of alcohols under alkali-free conditions, Catalysts 11 (7) (2021) 760.
[11] M. Kuniyil, J.V. Shanmukha Kumar, S. Farooq Adil, M.E. Assal, M. Rafi Shaik, M. Khan, A. Al-Warthan, M. Rafiq, H. Siddiqui, Production of biodiesel from waste cooking oil using ZnCuO/N-doped graphene nanocomposite as an efficient heterogeneous catalyst, Arab. J. Chem. (2021) 102982.
[12] A. Panacek, M. Kolar, R. Vecerova, R. Prucek, J. Soukupova, V. Krystof, P. Hamal, R. Zboril, L. Kvitek, Antifungal activity of silver nanoparticles against *Candida* spp. Biomaterials 30 (2009) 6333–6340.
[13] P.L. Nadworny, J. Wang, E.E. Tredget, R.E. Burrell, Anti-inflammatory activity of nanocrystalline silver in a porcine contact dermatitis mode, Nanomed.: Nanotechnol. Biol. Med. 4 (2008) 241–251.
[14] H.A. Akram, M. Imran, S. Latif, M.R. Hatshan, M. Khan, A. Abuhagr, K.M. Alotaibi, S.F. Adil, Bi3+/Ce3+ doped ZnO nanoparticles with enhanced photocatalytic and dielectric properties, J. Saudi Chem. Soc. 27 (1) (2023) 101567.
[15] O. Muktaridha, M. Adlim, S. Suhendrayatna, I. Ismail, Highly reusable chitosan-stabilized Fe-ZnO immobilized onto fiberglass cloth and the photocatalytic degradation properties in batch and loop reactors, J. Saudi Chem. Soc. 26 (3) (2022) 101452.
[16] A. Kamal, M.H. Saleem, H. Alshaya, M.K. Okla, H.J. Chaudhary, M.F.H. Munis, Ball-milled synthesis of maize biochar-ZnO nanocomposite (MB-ZnO) and estimation of its photocatalytic ability against different organic and inorganic pollutants, J. Saudi Chem. Soc. 26 (3) (2022) 101445.
[17] Z. Alhalili, C. Romdhani, H. Chemingui, M. Smiri, Removal of dithioterethiol (DTT) from water by membranes of cellulose acetate (AC) and AC doped ZnO and TiO2 nanoparticles, J. Saudi Chem. Soc. 25 (8) (2021) 101282.
[18] A.B. Lavand, Y.S. Malghe, Visible light photocatalytic degradation of 4-chloro-phenol using C/ZnO/CdS nanocomposite, J. Saudi Chem. Soc. 19 (5) (2015) 471–478.
[19] Y. Zhang, T.R. Nayak, H. Hong, W. Cai, Biomedical applications of zinc oxide nanomaterials, Curr. Mol. Med. 13 (10) (2013) 1633–1645.
[20] I.S. Tania, M. Ali, Coating of ZnO nanoparticle on cotton fabric to create a functional textile with enhanced mechanical properties, Polymers 13 (16) (2021) 2701.
[21] I.S. Tania, M. Ali, M. Akter, Fabrication, characterization, and utilization of ZnO nanoparticles for stain release, bacterial resistance, and UV protection on cotton fabric, J. Eng. Fibers Fabrics 17 (2022) 15589250221136378.

[22] A.R. Malik, S. Sharif, F. Shaheen, M. Khalid, Y. Iqbal, A. Faisal, M.H. Aziz, M. Atif, S. Ahmad, M. Fakhar-e-Alam, N. Hossain, H. Ahmad, T. Botmart, Green synthesis of RGO-ZnO mediated *Ocimum basilicum* leaves extract nanocomposite for antioxidant, antibacterial, antidiabetic and photocatalytic activity, J. Saudi Chem. Soc. 26 (2) (2022) 101438.
[23] S. Faisal, H. Jan, S.A. Shah, S. Shah, A. Khan, M.T. Akbar, M. Rizwan, F. Jan, N. A. Wajidullah, Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: their characterizations and biological and environmental applications, ACS Omega 6 (14) (2021) 9709–9722.
[24] N.M. Salem, A.M. Awwad, Green synthesis and characterization of ZnO nanoparticles using *Solanum rantonnetii* leaves aqueous extract and antifungal activity evaluation, Chem. Int. 8 (1) (2022) 12–17.
[25] S. Abel, J.L. Tesfaye, R. Shanmugam, L.P. Dwarampudi, G. Lamessa, N. Nagaprasad, M. Benti, R. Krishnaraj, Green synthesis and characterizations of zinc oxide (ZnO) nanoparticles using aqueous leaf extracts of coffee (*Coffea arabica*) and its application in environmental toxicity reduction, J. Nanomater. 2021 (2021) 1–6.
[26] S. Zeghoud, H. Hemmami, B.B. Seghir, I.B. Amor, I. Kouadri, A. Rebiai, M. Messaoudi, S. Ahmed, P. Pohl, J. Simal-Gandara, A review on biogenic green synthesis of ZnO nanoparticles by plant biomass and their applications, Mater. Today Commun. (2022) 104747.
[27] P. Manimegalai, K. Selvam, S. Loganathan, D. Kirubakaran, M.S. Shivakumar, M. Govindasamy, U. Rajaji, A.A.A. Bahajjaj, Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous leaf extract of *Hardwickia binata*: their characterizations and biological applications, Biomass Convers. Biorefin. (2023) 1–16.
[28] S. Faisal, H. Jan, S. Shah, S. Shah, A. Khan, M. Akbar, M. Rizwan, F. Jan, N. Akhtar, A. Khattak, Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: their characterizations and biological and environmental applications, ACS Omega 6 (14) (2021) 9709–9722.
[29] B.H. Ali, G. Blunden, Pharmacological and toxicological properties of *Nigella sativa*, Phytother. Res. 17 (4) (2003) 299–305.
[30] A. Ahmad, A. Husain, M. Mujeeb, S.A. Khan, A.K. Najmi, N.A. Siddique, Z. A. Damanhouri, F. Anwar, A review on therapeutic potential of *Nigella sativa*: a miracle herb, Asian Pac. J. Trop. Biomed. 3 (5) (2013) 337–352.
[31] C. Nergiz, S. Ötleş, Chemical composition of *Nigella sativa* L. seeds, Food Chem. 48 (3) (1993) 259–261.
[32] A.u.-H. Gilani, Q. Jabeen, M.A.U. Khan, A review of medicinal uses and pharmacological activities of *Nigella sativa*, Pak. J. Biol. Sci. 7 (4) (2004) 441–451.
[33] P.M. Paarakh, *Nigella sativa* Linn.–A comprehensive review (2010).
[34] N. Sharma, D. Ahirwar, D. Jhade, S. Gupta, Medicinal and phamacological potential of *Nigella sativa*: a review, Ethnobot. Leaflets 2009 (7) (2009) 11.
[35] M. Fakhar-e-Alam, Z. Shafiq, A. Mahmood, M. Atif, H. Anwar, A. Hanif, N. Yaqub, W.A. Farooq, A. Fatehmulla, S. Ahmad, A.E.E. Abd Elgawad, K.S. Alimgeer, T. N. Gia, H. Ahmed, Assessment of green and chemical synthesized copper oxide nanoparticles against hepatocellular carcinoma, J. King Saud Univ.-Sci. 33 (8) (2021) 101669.
[36] A.S. Abdelsattar, A.G. Kamel, A.H. Hussein, M. Azzam, S. Makky, N. Rezk, K. Essam, M.M. Agwa, A. El-Shibiny, The promising antibacterial and anticancer activity of green synthesized zinc nanoparticles in combination with silver and gold nanoparticles, J. Inorg. Organomet. Polym Mater. 33 (7) (2023) 1868–1881.
[37] M.A. Kareem, I.T. Bello, H.A. Shittu, P. Sivaprakash, O. Adedokun, S. Arumugam, Synthesis, characterization, and photocatalytic application of silver doped zinc oxide nanoparticles, Clean. Mater. 3 (2022) 100041.
[38] S. Naz, A. Gul, M. Zia, R. Javed, Synthesis, biomedical applications, and toxicity of CuO nanoparticles, Appl. Microbiol. Biotechnol. 107 (4) (2023) 1039–1061.
[39] M. Fakhar-e-Alam, S. Kishwar, M. Siddique, M. Atif, O. Nur, M. Willander, The photodynamic effect of ZnO nanorods and their ligands with different photosensitizers, Rev. Nanosci. Nanotechnol. 1 (1) (2012) 40–51.
[40] M.W. Akram, F. Raziq, M. Fakhar-e-Alam, M.H. Aziz, K.S. Alimgeer, M. Atif, M. Amir, A. Hanif, W. Aslam Farooq, Tailoring of Au-TiO2 nanoparticles conjugated with doxorubicin for their synergistic response and photodynamic therapy applications, J. Photochem. Photobiol. A: Chem. 384 (2019) 112040.
[41] S. Iqbal, M. Fakhar-e-Alam, M. Atif, N. Ahmed, A. ul-Ahmad, N. Amin, R. A. Alghamdi, A. Hanif, W.A. Farooq, Empirical modeling of Zn/ZnO nanoparticles decorated/conjugated with Fotolon (chlorine e6) based photodynamic therapy towards liver cancer treatment, Micromachines 10 (1) (2019) 60.
[42] Y.T. Prabhu, K.V. Rao, V.S.S. Kumar, B.S. Kumari, Synthesis of ZnO nanoparticles by a novel surfactant assisted amine combustion method, Adv. Nanopart. 2 (01) (2013) 45–50.
[43] M. Fakhar-e-Alam, S. Saddique, N. Hossain, A. Shahzad, I. Ullah, A. Sohail, M.J. I. Khan, M. Saadullah, Synthesis, characterization, and application of $BaTiO_3$ nanoparticles for anti-cancer activity, J. Clust. Sci. 34 (4) (2023) 1745–1755.
[44] N. Jayarambabu, B.S. Kumari, K.V. Rao, Y. Prabhu, Germination and growth characteristics of mungbean seeds (*Vigna radiata* L.) affected by synthesized zinc oxide nanoparticles, Int. J. Curr. Eng. Technol. 4 (5) (2014) 3411–3416.
[45] S. Joghee, P. Ganeshan, A. Vincent, S.I. Hong, Ecofriendly biosynthesis of zinc oxide and magnesium oxide particles from medicinal plant *Pisonia grandis R.Br.* leaf extract and their antimicrobial activity, BioNanoScience 9 (1) (2019) 141–154.
[46] M. Fakhar-e-Alam, M.W. Akram, S. Iqbal, K.S. Alimgeer, M. Atif, K. Sultana, M. Willander, Z.M. Wang, Empirical modeling of physiochemical immune response of multilayer zinc oxide nanomaterials under UV exposure to melanoma and foreskin fibroblasts, Sci. Rep. 7 (1) (2017) 46603.
[47] J.F. Islas, E. Acosta, G. Zuca, J.L. Delgado-Gallegos, M.G. Moreno-Treviño, B. Escalante, J.E. Moreno-Cuevas, An overview of Neem (*Azadirachta indica*) and its potential impact on health, J. Funct. Foods 74 (2020) 104171.