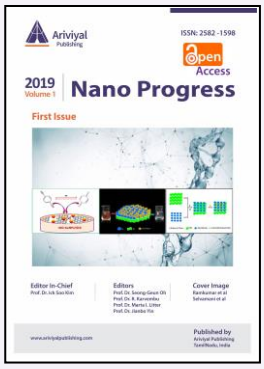
Nano Progress

Ariviyal Publishing

Research Article

DOI: 10.36686/Ariviyal.NP.2020.02.04.019

Nano Prog., (2020) 2(4), 53-57.



A Comparative Cytotoxicity Evaluation of ZnO Nanoparticles against THP-1 Cells

Niraj Kumari,[a] Priti Kumari,[a] Anal K. Jha[b] and Kamal Prasad*[c]

[a]*Aryabhatta Centre for Nanoscience and Nanotechnology, Aryabhatta Knowledge University, Patna, 800001, India*
[b]*University Department of Chemistry, T.M. Bhagalpur University, Bhagalpur, 812007, India*
[c]*University Department of Physics, T.M. Bhagalpur University, Bhagalpur, 812007, India*

***Corresponding author E-mail address**: prasad_k@tmbuniv.ac.in (K. Prasad)

ISSN: 2582-1598

Crossref

Publication details
Received: 25th October 2020
Revised: 20th November 2020
Accepted: 20th November 2020
Published: 30th November 2020

Abstract: Medicinal plants such as *Achyranthes aspera*, *Phyllanthus niruri*, and *Azadirachta indica* assisted fabrication of ZnO nanoparticles (ZnO NPs) are reported in the present work. Formations of ZnO NPs were ascertained from different characterizing tools such as X-ray diffraction, scanning, and transmission electron microscopy and UV-visible spectroscopy. A comparative study of the cytotoxic potentials of biosynthesized ZnO NPs against THP-1 cells, a human leukaemia cell lines has been undertaken. These ZnO NPs showed dose-dependent cytotoxicity against THP-1 cells by using WSA-1 assay. The results indicated that the biosynthesized especially *Azardica indica* mediated ZnO NPs could be utilized as a potential future therapeutic agent for cancer therapy.

Keywords: ZnO; nanomaterials; bionanotechnology; green synthesis; cytotoxicity; THP-1 cells

1. Introduction

Mother Nature has innumerable phytodiversity and provided everything to nurture its congeneric cohorts since the origin of life on this planet. Plants are the most valuable and essential for the survival of life of all organisms which provide us food, fruits, vegetables, fodder fuel, and rendering protection against different diseases. They also act as a great source of medicine and therapeutics even for life-threatening diseases and minimize the risks of side effects. Further, the utilization of medicinal plants and/or plants' parts (e.g., root, stem, barks, leaf, flower, fruit, and bulbs) affect human health/diseases because they contain the biologically active phytonutrients/chemicals, which possess antibacterial, antifungal, antiviral, antioxidant, anti-aging, anticancer, immuno-stimulating and detoxification properties. Among the phytochemicals present in them, the flavonoids are believed to have a varied group of hydroxylated polyphenolic phytonutrients with low molecular weight compounds having a benzo-γ-pyrone ring structure ubiquitous in higher to lower plants/plant parts which found as free forms, glycosides, aglycones and methylated derivatives. Their chemical behaviour relies upon the structure, degree of hydroxylation, polymerization and other substitutions, and conjugations. It is used as a medicine, foods, beverages, and an ingredient in supplements since ages that possess potential pharmacological properties. According to the FDA, all classes of flavonoids are used as pharmaceutical drugs in the management of chronic diseases. Among the flavonoids, quercetin (molecular formula $C_{15}H_{10}O_7$) is a polyphenolic compound found in medicinal plants such as *Azadirachta indica*, *Phyllanthus niruri*, and *Achyranthes aspera.* [1-15]

In Ayurveda, the utilization of medicinal plants as therapeutic agents is being practiced since ancient times. The traditional use of *A. indica*, *P. niruri*, and *A. aspera* leaves is to treat many chronic diseases especially skin-related ones like tetanus, urticaria, eczema, scrofula, erysipelas, early stages of leprosy, skin cancer, and other types of infections. Nowadays, considerable researches are being prospected worldwide to enhance the efficacy/medicinal properties of the plants and/or plants' parts by the use of bionanotechnology approaches. Besides, the synthesis of nanoparticles using medicinal plant extracts (in line with the RoHS legislation of EU) has been reported in the past. A vast review of literature on plant-mediated synthesis of different nanoparticles has emerged but quite a few of them have been able to ensure the candidate metabolites accountable for the synthetic methods. Once such candidate metabolites are identified towards the fabrication of intended nanoparticles, then plant tissue culture and/or downstream processing procedures are applied for the industrial production of nanoparticles.[5-10,15-26] The present investigation is an effort in this direction which is truly an eco-friendly bionanotechnology approach. Accordingly, this work reports the phytosynthesis of Zinc oxide nanoparticles (abbreviated hereafter as ZnO NPs) with the help of *A. indica*, *P. niruri*, as well as *A. aspera* and cytotoxicity assay against

THP-1 cells, a human monocytic leukemia cell lines. The prepared ZnO NPs have been characterized by standard techniques like X-ray diffraction, scanning and transmission electron microscopy, and UV-visible spectroscopy.

2. Experimental Section

2.1. Biosynthesis of ZnO NPs using A. indica, P. niruri and A. aspera

ZnO NPs were prepared by using ethanolic extracts of three plant leaves (*A. indica*, *P. niruri*, and *A. aspera*). A known weight (15 g each) of freshly collected, healthy leaves of these plants were taken and washed thoroughly and then dried and chopped into small pieces. Now, they were kept in a beaker (250 ml) containing 200 ml of Et-OH (50%) and the same were placed on a boiling water steam bath for 15–20 min until the colour of the solvents changed from light yellowish green to dark green. These solutions contain probable candidate flavonoids. They were cooled to room temperature, pressed, and firstly filtered using the serene cloth (sterile) and then through the Whatman filter paper. These solutions were considered as source extracts for later processes.

Thereafter, the source extract (50 ml) of each of the plant was mixed with sterile distilled water (50 ml). Now, the equal volume (20 ml) of 0.25 (M) aqueous $ZnCl_2$ solutions was added to each extracts maintaining the pH value to be 6, and it was heated until the appearance of starch like haziness in solution and the deposition of white mass at the bottom of each flask was noted. This was anticipated as the initiation of nano-transformation. These flasks were kept for incubation in the laboratory conditions for four hours till duck white fluffy mass settles at the bottom leaving transparent supernatants at the top and were get filtered for future studies.

2.2. Characterization of ZnO NPs

The formations of the single-phase compound were analyzed by the X-ray diffraction (XRD) technique. The XRD spectra were taken with an X-ray diffractometer (Bruker D8 Advance) at room temperature, using CuK_α radiation λ = 1.5406 Å over a wide range of Bragg angles 20° to 80°. Rietveld refinements on the XRD data have been taken into use to obtain the crystal structure and unit cell parameters using software (*FullProf*). The scanning (SEM) and transmission (TEM) electron micrographs of ZnO NPs were obtained, respectively using a computer-controlled scanning electron microscope (EVO 18 of Carl Zeiss Microscopy Ltd., UK) and high-resolution Bruker transmission electron microscope operated at an accelerated voltage 200 keV. The absorption spectra of all the specimens (ZnO NPs) were obtained by a computer-controlled UV-visible-NIR spectrophotometer (PerkinElmer, UK).

2.3. Cell viability assays

The cell viability assay was carried out with WST-1 (Cell proliferation reagent, Roche) reagent as per the instructions of manufacturer. The cells of THP-1 were harvested in 96-well plates and treated with different concentrations (0-25 µg/ml) of *P. niruri*, *A. aspera* and *A. indica* mediated ZnO NPs. These cultures were incubated at 37°C in an incubator for 24 h. Now, 10 µl of WST-1 reagent was added to each culture well which were further incubated at 37ºC for 4 h. The well of blank media was used as background control and the value of which was deducted from the absorbance values of the treated well culture cells. The absorbance values were determined in dual mode (A450 nm – A655 nm) using an *i*Mark ELISA plate reader (Biorad). The results shown in this work are the mean of three independent experiments. The values of IC50 were evaluated for the concentration of ZnO NPs at which cell viability was reduced to half of its original value.

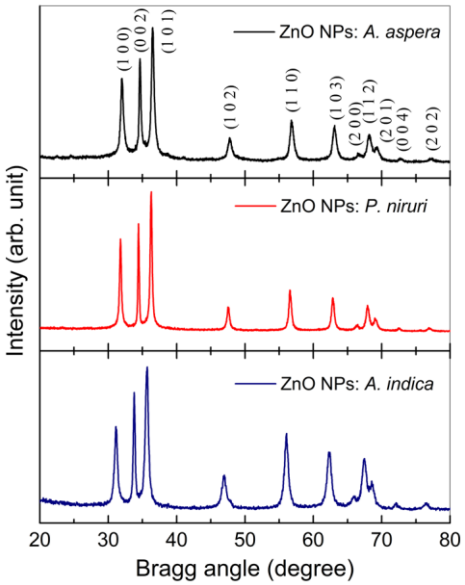


Fig. 1. X-ray diffraction patterns of ZnO NPs synthesized from the ethanolic extracts of *Achyranthes aspera*, *Phyllanthus niruri*, and *Azadirachta indica* leaves.

3. Results and Discussions

Fig. 1 illustrates the indexed room temperature XRD profiles of all the synthesized ZnO NPs. The eleven Bragg reflections (2ϑ) of different lattice planes of Wurtzite hexagonal structure were labelled with (100), (002), (101), (102), (110) (103), (200), (112), (201), (004), and (202) in ZnO NPs. Besides, the presence of broadened clear-cut XRD peaks of all the prepared nanoparticles is primarily because of the formations of small-sized particles which are semi-crystalline, and the absence of extra diffraction peaks suggested that the formations of ZnO NPs are free from any kind of impurities. The unit cell structures and lattice parameters as obtained from the XRD analyses have been listed in Table 1, which are consistent with the standard literature PDF #89-0510 for ZnO NPs.[26-28] A slight difference in the cell parameters of bulk and nanoparticles has been observed in all the cases. Consequently, a lowering in unit cell volumes from bulk ZnO to ZnO NPs has been noticed which is due to the downsizing effect (nano-regime). The average particle sizes (*D*) and lattice strain ($\Delta\xi/\xi$) of all the prepared nanoparticles were approximated by evaluating the XRD peaks' broadening, utilizing Williamson and Hall method:

$$B\cos\theta = \frac{k\lambda}{D} + 2\frac{\Delta\xi}{\xi}\sin\theta$$

Table 1. Lattice parameters of ZnO NPs synthesized using *A. indica*, *P. niruri* and *A. aspera* leaves' extracts.

Parameters	ZnO NPs from *A. aspera*	ZnO NPs from *P. niruri*	ZnO NPs from *A. indica*
Unit cell structure	Hexagonal (*P6/mmm*)	Hexagonal (*P6/mmm*)	Hexagonal (*P6/mmm*)
Lattice parameter			
a (Å)	3.2544	3.2538	3.2530
c (Å)	5.2123	5.2120	5.2119

When $B\cos\theta$ *vs.* $\sin\theta$ are plotted one gets a straight line having the slope $2\Delta\xi/\xi$ with the intercept of $k\lambda/D$. Here *k* is a constant whose value is nearly equal to unity which ranges between 0.8 and 1.39. A Lorentzian model:

$$I = I_o + (2A/\pi)[w/\{4(\theta-\theta_c)^2 + w^2\}]$$

here *A*, *w*, and ϑ_c are respectively the area, width, and centre of the curve; was used to obtain the value of *B*. The average particle sizes, as well as lattice strains of ZnO NPs, were estimated to be ~8 nm and ~0.004 using the linear least-square fitting of *B*cosϑ-sinϑ data. Such a small value of lattice strain, in all the cases of ZnO NPs, could be due to the nano-synthetic protocol as adopted is considered as natural one in which the constraint imposed during the formations of nanoparticles is notably small compared to other techniques. Hence, the plant-mediated synthetic procedure can produce ZnO NPs, which might have been resulted due to the activities of different plant metabolites as well as the availability of various phytochemicals especially the flavonoid, quercetin present in the leaves parenchyma of *Achyranthes aspera*, *Phyllanthus niruri*, and *Azadirachta indica* that played important role in the synthesis of ZnO NPs. The ethanolic extract of these plants is rich in a variety of flavonoids which contains –OH groups able to release protons under oxidizing environment to reduce the bulk into ZnO NPs. Besides, it was observed that the values of lattice parameters (LP) follow the sequence (Table 1): $LP_{A.\ aspera} > LP_{P.\ niruri} > LP_{A.\ indica}$, which suggested that the candidate metabolites present in *A. indica* leaves are much stronger compared to *A. aspera* as well as *P. niruri* leaves that allowed the nano-transformations to occur in a way that it could synthesize still smaller nanoparticles.

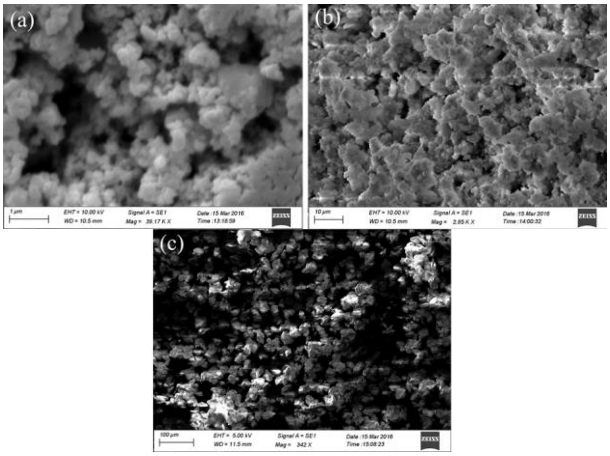


Fig. 2. SEM micrographs of ZnO NPs synthesized from the alcoholic extracts of (a) *Achyranthes aspera*, (b) *Phyllanthus niruri*, and (c) *Azadirachta indica* leaves.

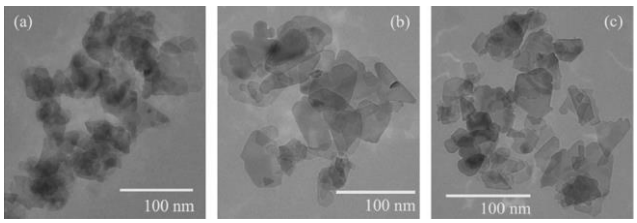


Fig. 3. TEM micrographs of ZnO NPs synthesized from the alcoholic extracts of (a) *Achyranthes aspera*, (b) *Phyllanthus niruri*, and (c) *Azadirachta indica* leaves.

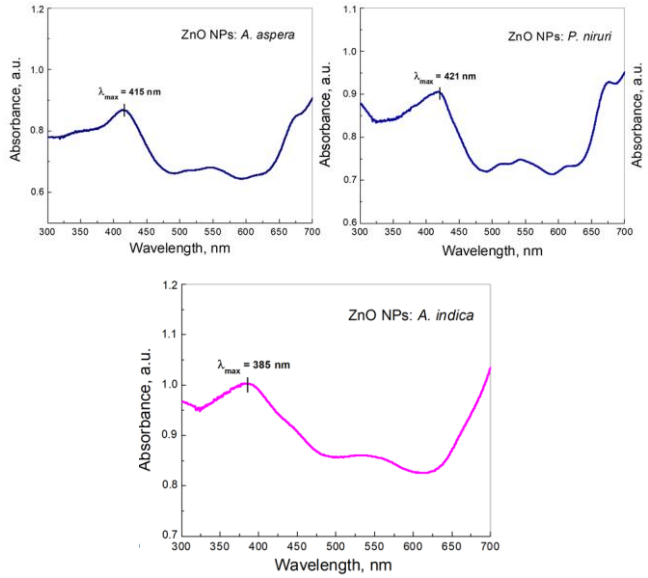


Fig. 4. UV-Vis absorption spectra of Ag and ZnO nanoparticles obtained using BPSE. Inset sows the synthesized solutions of Ag and ZnO nanoparticles.

The SEM micrographs of ZnO NPs biosynthesized utilizing ethanol-mediated extracts of leaves of *Achyranthes aspera*, *Phyllanthus niruri*, and *Azadirachta indica* have been presented in Fig. 2(a-c). All the SEM micrographs indicate the formations of very small-sized ZnO NPs in the little nm range. Also, it is seen that quite a few particles get agglomerated which could be because of the larger surface area that allowed the formations of litter bigger particles.

With the aim to verify the SEM results, TEM micrographs of ZnO NPs formed using ethanol-mediated *Achyranthes aspera*, *Phyllanthus niruri*, and *Azadirachta indica* leaves extracts, were obtained. Fig. 3(a-c) illustrates the TEM micrographs of ZnO NPs which ensure the formations of thin nanosheets like structure, that are irregular in shapes having sizes ranged approximately between 10 and 40 nm, were observed in the case of ZnO NPs. The differences in the sizes of biosynthesized nanoparticles may because the nanoparticles have been formed at different times and agglomerations took place. This could be due to the high surface energy. Further, it was observed that the particle sizes (L) follow a similar trend as seen in the case of the lattice parameter *i.e.* $L_{A.\ aspera} > P_{P.\ niruri} > P_{A.\ indica}$. Besides, it is seen that the average (apparent) particle sizes estimated using Williamson and Hall method (XRD results) to be in very good concurrence with the sizes estimated by SEM as well as TEM results.

Fig. 4 depicts the UV-Vis spectra of ZnO NPs. The surface plasmon resonance (λ_{max}) was observed, respectively at 415, 421, and 385 nm for the ZnO NPs being formed using the ethanolic extracts of

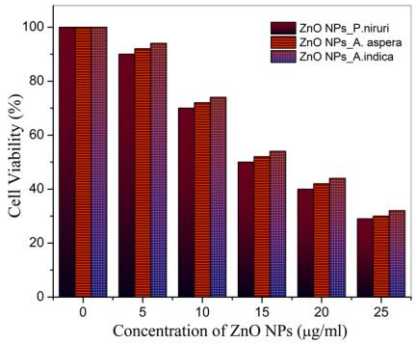


Fig. 5. Dose dependent cytotoxicity assay in THP-1 cells after treatment with *P. niruri, A. aspera*, and *A. indica* mediated ZnO NPs.

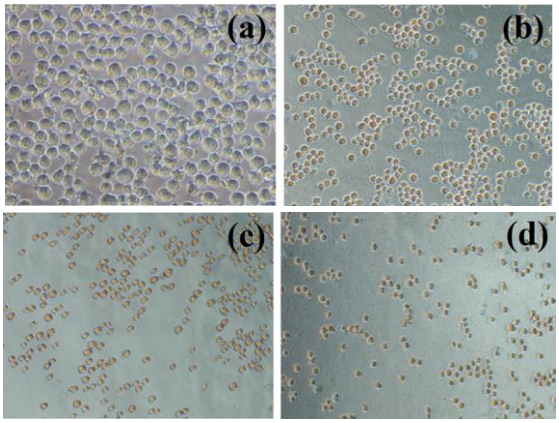


Fig. 6. In vitro cytotoxicity effect of SNPs on THP-1 cell lines showing (a) untreated cells with (b) *P. niruri*, (c) *A. aspera* and (d) *A. indica* mediated ZnO NPs at a concentration of 25 µg/ml.

and is completing the reactions within four hours. Besides, the plasmon bands are having the tendency to be broadened having an absorption tail in the higher wavelength side that may happen because of the size distribution of the particles. This supports the distribution of particle sizes as observed in the TEM micrographs (Fig. 3).

The cell viability test is the most important method for the analysis of cytotoxicity which determine the cellular response to different nanoparticles and provide cell information *i.e.* cell survival, death and metabolic activities. THP-1 cells, a human monocytic leukaemia cell line, is a cancer of the blood cells that occurs due to rapid growth of abnormal cells in bone marrow and blood which interfere normal blood cells.[27-28] In this experiment, the THP-1 cells have been treated with various concentrations (0–25 µg/ml) of *P. niruri*, *A. aspera* and *A. indica* mediated ZnO NPs, which were found to be able to reduce the viability of human monocytic leukemia cell line *i.e.* THP-1 cells in a dose-dependent manner. After 24 h of treatment, ZnO NPs have been found cytotoxic to THP-1 cells at different concentrations (Fig. 5), the cellular morphologies of treated cell lines (THP-1 cells) showed cytotoxicity effect which undergo significant cell damage and the cell death was observed more at the concentration of 25 µg/ml of *P. niruri*, *A. aspera*, and *A. indica* mediated ZnO NPs which shown in (Fig. 6) with compared to untreated cell. The half of the maximum inhibitory concentration (IC50) has been estimated as the ZnO NPs concentration required for inhibition of the cells' growth in culture by 50% in comparison to the untreated cells. The concentration of *P. niruri*, *A. aspera*, and *A. indica*, and mediated ZnO NPs was 15±0.2, 15±0.8, 15±1.5 µg/ml at which viability of THP-1 cells decreased to be 50% and denoted as IC50. Seemingly, the action of ZnO NPs depends on the shape, size, dose, time, type of cells and condition of media. The cytotoxic potential of *P. niruri*, *A. aspera*, and *A. indica* mediated ZnO NPs on THP-1 cells could be considered due to the three major facts (i) generation of reactive oxygen species (ROS) and oxidative stress, (ii) DNA damage and apoptosis and (iii) disequilibrium created due to zinc-mediated protein activity.[27,28,29-33] Therefore, the ZnO NPs biosynthesized and stabilized from the metabolite rich sources (*P. niruri*, *A. aspera*, and *A. indica*) are encircled with many active, therapeutically significant phytochemicals like flavonoid, adding to their biocompatibility and nontoxicity.

4. Conclusions

The present biosynthetic method is a simple, cost-effective, eco-friendly, high yield, totally green, and handy protocol capable of synthesizing ZnO NPs which might have accomplished due to the activities of phytochemicals available in *Phyllanthus niruri*, *Achyranthes aspera*, as well as *Azardica indica* leaves, in the form of primary as well as secondary metabolites. The cytotoxicity effects of biosynthesized ZnO NPs especially *Azardica indica* mediated one, were found quite encouraging against THP-1 cells (a human leukemia cell line) and showed the morphological changes with a significant delay in the growth of a cell, cell shrinkage, cytoplasmic condensation, and membrane blebbing of the treated cells. Hence, biosynthesized especially *Azardica indica* mediated ZnO NPs could be utilized as a potential future therapeutic agent for cancer therapy.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1 Jha A.K.; Prasad K.; Prasad K.; Kulkarni A.R. Plant System: Nature's Nanofactory. *Colloids Surf. B: Biointerfaces*, 2009, **73**, 219–223. [CrossRef]
2 Jha A.K.; Kumar V.; Prasad K. Biosynthesis of Metal and Oxide Nanoparticles using Orange Juice. *J. Bionanosci.*, 2011, **5**, 162–166. [CrossRef]
3 Saleem A.M.; Prabhavathi G.; Karunanithy M.; Ayeshamariam A.; Jayachandran M. Green Synthesis of Nanoparticle by Plant Extracts - A New Aproach in Nanoscience. *J. Bionanosci.*, 2018, **12**, 401–407. [CrossRef]
4 Tripathy A.; Raichur A.M.; Chandrasekaran N.; Prathna T.C.; Mukherjee A. Process Variables in Biomimetic Synthesis of Silver Nanoparticles by Aqueous Extract of *Azadirachta indica* (Neem) Leaves. *J. Nanopart. Res.*, 2010, **12**, 237–246. [CrossRef]
5 Namratha N.; Monica P.V. Synthesis of Silver Nanoparticles using *Azadirachta indica* (Neem) Extract and Usage in Water Purification. *Asian J. Pharm. Tech.*, 2013, **3**, 170-174. [Link]
6 Bhattacharyya K.G.; Sharma A. Adsorption of Pb(II) from Aqueous Solution by *Azadirachta Indica* (Neem) Leaf Powder. *J. Hazardous Mater. B*, 2004, **113**, 97–109. [CrossRef]
7 Kasthuri J.; Kathiravan K.; Rajendiran N. Phyllanthin-Assisted Biosynthesis of Silver and Gold Nanoparticles: A Novel Biological Approach. *J. Nanopart. Res.*, 2009, **11**, 1075–1085. [CrossRef]

8 Dhale D.A.; Bhoi S. Pharmacognostic Characterization and Phytochemical Screening of *Achyranthes aspera* Linn. *Curr. Agri. Res*., 2013, **1**, 51–57. [CrossRef]
9 Raji R. *Achyranthus aspera* - Medicinal Plant: A Review. *Int. J. Pharm. Bio. Sci*., 2013, **4**, 719–724. [Link]
10 Ali S.S.; Kasoju N.; Luthra A.; Singh A.; Sharanabasava H.; Sahu A.; Bora U. Indian Medicinal Herbs as Sources of Antioxidants. *Food Res. Internat*., 2008, **41**, 1–15. [CrossRef]
11 Harish R.; Shivanandappa T. Antioxidant Activity and Hepatoprotective Potential of *Phyllanthus niruri*. *Food Chem*., 2006, **95**, 180–185. [CrossRef]
12 Ekaete D.; Umoh U.; Akpabio D.; Udo I.E. Phytochemical Screening and Nutrient Analysis of *Phyllanthus amarus*. *Asian J. Plant Sci. Res*., 2013, **3**, 116-122. [CrossRef]
13 Ahmed S.; Ahmad S.M.; Swami B.L.; Ikram S. Green Synthesis of Silver Nanoparticles using *Azadirachta indica* Aqueous Leaf Extract. *J. Rad. Res. Appl. Sci*., 2016, **9**, 1-7. [CrossRef]
14 Prasad K.; Jha A.K. ZnO Nanoparticles: Synthesis and Adsorption Study. *Nat. Sci*., 2009, **1**, 129–135. [CrossRef]
15 Jerome P.; Sharfudeen Y. A.; Babu S. G. Controlled Green Synthesis of Polymer Functionalized Zinc Oxide Nanoparticles. *Green Rep*., 2020, **1**, 16-20. [CrossRef]
16 Asharani P.V.; Mun G.L.K.; Hande M.P.; Valiyaveettil S. Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. *ACS Nano*, 2009, **3**, 279–290. [CrossRef]
17 Sukirtha R.; Priyanka K.M.; Antony J.J.; Kamalakkannan S.; Thangam R.; Gunasekaran P.; Krishnan M.; Achiraman S. Cytotoxic Effect of Green Synthesized Silver Nanoparticles using *Melia azedarach* against in Vitro Hela Cell Lines and Lymphoma Mice Model. *Process Biochem*., 2012, **47**, 273–279. [CrossRef]
18 Subramanian V.; Suja S. Green Synthesis of Silver Nanoparticles using *Coleus amboinicus* Lour, Antioxitant Activity and Invitro Cytotoxicity against Ehrlich's Ascite Carcinoma. *J. Pharm. Res*., 2012, **5**, 1268–1272. [CrossRef]
19 Sanpui P.; Chattopadhyay A.; Ghosh S.S. Induction of Apoptosis In Cancer Cells at Low Silver Nanoparticle Concentrations using Chitosan Nanocarrier. *ACS Appl. Mater. Interfaces*, 2011, **3**, 218–228. [CrossRef]
20 Rajeshkumar S. Anticancer Activity of Eco-Friendly Gold Nanoparticles against Lung and Liver Cancer Cells. *J. Genetic Eng. Biotechnol*., 2016, **14**, 195–202. [CrossRef]
21 Safaepour M.; Shahverdi A.R.; Shahverdi H.R.; Khorramizadeh M.R.; Gohari A.R. Green Synthesis of Small Silver Nanoparticles using Geraniol and its Cytotoxicity against Fibrosarcoma–Wehi 164. *Avicenna J. Med. Biotechnol*., 2009, **1**, 111–115. [Link]
22 Jha A.K.; Prasad K. Facile Green Synthesis of Metal and Oxide Nanoparticles using *Papaya* Juice. *J. Bionanosci*., 2015, **9**, 311–314. [CrossRef]
23 Azam A.; Arham S.A.; Oves M.; Khan M.S.; Habib S.S.; Memic A. Antimicrobial Activity of Metal Oxide Nanoparticles against Gram-Positive and Gram-Negative Bacteria: A Comparative Study. *Int. J. Nanomed*., 2012, **7**, 6003–6009. [CrossRef]
24 Aziz N.; Faraz M.; Pandey R.; Sakir M.; Fatma T.; Varma A.; Barman I.; Prasad R. Facile Algae Derived Route to Biogenic Silver Nanoparticles: Synthesis, Antibacterial and Photocatalytic Properties. *Langmuir*, 2015, **31**, 11605–11612. [CrossRef]
25 Birla S.S.; Tiwari V.V.; Gade A.K.; Ingle A.P.; Yadav A.P.; Rai M.K. Fabrication of Silver Nanoparticles by *Phoma glomerata* and its Combined Effect against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Lett. Appl. Microbiol*., 2009, **1**, 2173-2179. [CrossRef]
26 Jha A.K.; Prasad K. Green Synthesis and Antimicrobial Activity of Silver Nanoparticles onto Cotton Fabrics: An Amenable Option for Textile Industries. *Adv. Mater. Lett*., 2016, **7**, 42-46. [CrossRef]
27 Kumari P.; Kumari N.; Singh K.P.; Prasad K. *Cymbopogon flexuosus* Leaves Mediated Synthesis of ZnO Nanoparticles: Cytotoxicity Assay against THP-1 Human Leukemia Cell Line. *J. Bionanosci*., 2018, **12**, 683–688. [CrossRef]
28 Jha A.K.; Kumari N.; Kumari P.; Prasad K. Phytochemical Synthesis of ZnO Nanoparticles: Antimicrobial and Anticancer Activity. *J. Bionanosci.*, 2018, **12**, 836–841. [CrossRef]
29 Fatima F.; Bajpai P.; Pathak N.; Singh S.; Priya S.; Verma S.R. Antimicrobial and Immunomodulatory Efficacy of Extracellularly Synthesized Silver and Gold Nanoparticles by a Novel Phosphate Solubilizing Fungus *Bipolaris Tetramera*. *BMC Microbiol*., 2015, **15**, 52-61. [CrossRef]
30 Kumari N.; Kumari P.; Jha A.K.; Prasad K. Enhanced Antimicrobial Activity in Biosynthesized ZnO Nanoparticles. *AIP Conf. Proc*., 2018, **1953**, 030054-4. [CrossRef]
31 Lokina S.; Stephen A.; Kaviyarasan V.; Arulvasu C.; Narayanan V. Cytotoxicity and Antimicrobial Studies of Silver Nanoparticles Synthesized using *Psidium guajava* L. Extract. *Synth. Reactivity Inorg., Metal-Organic, Nano-Metal Chem.*, 2015, **45**, 426-432. [CrossRef]
32 Jacob S.J.P.; Finub J. S.; Narayanan A. Synthesis of Silver Nanoparticles using *Piper longum* Leaf Extracts and its Cytotoxic Activity against Hep-2 Cell Line. *Colloid Surf. B: Biointerfaces*, 2012, **91**, 212–214. [CrossRef]
33 Ramar M.; Manikandan B.; Marimuthu P.N.; Raman T.; Mahalingam A.; Subramanian P. Synthesis of Silver Nanoparticles using *Solanum trilobatum* Fruits Extract and its Antibacterial, Cytotoxic Activity against Human Breast Cancer Cell Line MCF 7. *Spectrochim. Acta A*, 2014, **140**, 223–228. [CrossRef]