

Green Synthesis of Zinc Oxide Nanoparticles From Mangifera Indica Seed Extract and Their Anticancer Activity against Skmel- 28 Cell Line

B.T.Delma¹, Dr.M.Anitha Malbi²

¹Research scholar (Reg.No:18123042032016), Department of Chemistry, Holy Cross College (Autonomous), Nagercoil, Affiliated to Manonmanium Sundaranar University, Tirunelveli.

²Assistant Professor, Department of Chemistry, Holy Cross College, Nagercoil.

Email: delma.chem5@gmail.com, anithamalbi@holycrossncl.edu.in

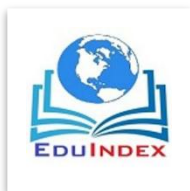
Abstract

This study describes the synthesis of zinc oxide nanoparticles by using Mangifera indicaseed extract as a reducing and capping agent. The synthesized nanoparticles were confirmed by UV-Vis spectroscopy and the average grain size of the nanoparticle is 34nm found by using XRD. FT-IR showed the presence of organic molecules capped in synthesized nanoparticles. The morphology of the nanoparticles was analyzed using SEM and the presence of zinc oxide nanoparticles was confirmed through elemental analysis. The synthesized ZnO nanoparticles have good anticancer activity against the SKMEL-28 cell line and showed IC₅₀ at 44.309. Then these nanoparticles were analyzed for apoptotic activity by acridine orange/ethidium bromide (AO/EB) double staining method showed chromatin condensation.

Keywords: Mangifera indica seed extract, Zinc oxide nanoparticles, Anti-cancer activity, Green synthesis

INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) have large bandwidth and high exciton binding energy and it showed good antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant and optic properties. So it has been used as nano-optical and nano-electrical devices, in food packaging and medicine as antimicrobial and antitumor agents in industries [1]. Instead of toxic chemicals plants, fungus, bacteria, and algae have been used as reducing and stabilizing agents [2]. Compare to microorganism the plants are easily available and more cost-effective [3]. Several plant extracts have been used for the preparation of zinc oxide nanoparticles [4-9] and the zinc oxide nanoparticles showed good anti-microbial activity against bacteria, fungi and also cytotoxicity against various cell lines. The chemical constituents proteins, amino acids, enzymes, vitamins, alkaloids, phenolics, saponins, tannins, and terpenoids present in plant extract can act as reducing and capping agent. ZnO nanoparticles have low toxicity. So it is listed



in “Generally Recognized as Safe” (GRAS) by the US Food and Drug Administration (21, CFR 182, 8991) [10]. Gold nanoparticles synthesized from *Mangifera indica* seed Aqueous extract was already reported and showed good antibacterial, anticancer and anti-angiogenic properties [11].

Cancer is the most dangerous disease and it is difficult to cure in the later stage. It can be treated using several methods like chemotherapy, surgery, and radiotherapy. In chemotherapy, the normal cells also damage along with cancer cells. So we need the drug which specific to affect the cancer cell without damage the normal cell-line [16]. Melanoma is one of the types of most harmful cancer. In the World 160,000 affected per year due to this type of cancer and leads to 48000 people died from them [13]. Recently several nanoparticles have wide applications in cancer therapy. From this, ZnO nanoparticle gives an appropriate biological response to the body [14]. In this study, we used the ethanolic extract of *Mangifera indica* as a reducing agent for the preparation of zinc oxide nanoparticles and characterized by UV-Vis spectrophotometer, FT-IR analysis, XRD and SEM-EDAX. The anti-cancer activity against the SKMEL-28 cell-line was analyzed by MTT assay. Then apoptotic activity determined by acridine orange/ethidium bromide (AO/EB) double staining method.

Materials and methods

Preparation of *Mangifera indica* seed extract

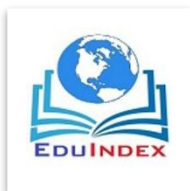
The *Mangifera indica* seeds were collected from Marthandam, India. The seeds were cut into small pieces and shade dried in 12-14 days. After it is powdered using the mechanical blender. Then the extract can be prepared by a Soxhlet apparatus using ethanol as solvent. The extract filtered through Whatman No.1 filter paper and stored at room temperature.

Preparation of Zinc oxide nanoparticles from *Mangifera indica* seed extract

Zinc oxide nanoparticle is prepared using zinc nitrate solution [12] purchased from Merk, India. 1ml of the *Mangifera indica* seed extract is added dropwise into 10ml of 0.1M zinc nitrate solution. Then 1ml of NaOH solution is added. The mixture was under magnetic stirring at 60⁰ C. The colour of the solution turned to white. After 1 day it turns to yellow. Then it was centrifuged and washed with ethanol and dried in an oven at 60⁰ C. The dried powder is collected and stored in an airtight container.

Characterization of Zinc oxide nanoparticles

The formation of the zinc oxide nanoparticles was confirmed by UV-Vis spectrophotometer. The crystalline nature can be studied by XRD and also from XRD values the average grain size of the nanoparticles was calculated using the Scherrer equation. The surface morphology and components present in the nanoparticles were analyzed by SEM with EDAX. The chemical components present in the nanoparticles by FT-IR analysis.



Invitro Anti-proliferative effect determination by MTT assay

The SK-MEL-28 cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium Himedia. The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and an antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (Galaxy® 170 Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by an Inverted phase contrast microscope and followed by MTT assay method. Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10⁴ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

1 mg of ZnO nanoparticles were added to 1ml of DMEM and dissolved completely by cyclomixer. After that, the ZnO nanoparticle was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. After 24 hours the growth medium was removed, freshly prepared samples in 5% DMEM were five times serially diluted by two-fold dilution (6.25µg, 12.5µg, 25µg, 50µg, 100µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator.

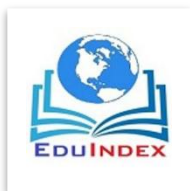
Anti-proliferative effect by Direct Microscopic observation:

The entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase-contrast tissue culture microscope (Labomed TCM-400 with MICAPSTM HD camera) and microscopic observation were recorded as images.

15mg of MTT (Himedia, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of the incubation period, the sample content in wells was removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and DMSO was added and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 570 nm.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$



Determination of Apoptosis by Acridine Orange (AO) And Ethidium Bromide (EB) Double Staining method:

DNA-binding dyes AO and EB (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells. AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into a double-stranded nucleic acid (DNA). EB is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA. After treatment with IC₅₀ concentrations of the test samples and 24 hr incubation at CO₂ incubator, cells were washed by cold PBS and then stained with a mixture of AO (100 µg/ml) and EB (100 µg/ml) at room temperature for 10min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in the blue filter of a fluorescent microscope (Olympus CKX41 with Optika Pro5 camera).

RESULTS AND DISCUSSION

UV-Visible spectral analysis

The formed nanoparticle was confirmed by UV-Vis spectrophotometer and operated in the range of 250nm to 600nm. The colour of the zinc oxide nanoparticles is changed from white to yellow [16] due to surface Plasmon resonance and the peak value is obtained at 325nm (Fig.1) which has similar to peak obtained using *Ulva lactuca* seaweed extract [21] *Cassia alata* extract [17] and *Scutellaria baicalensis* Root extract [20].

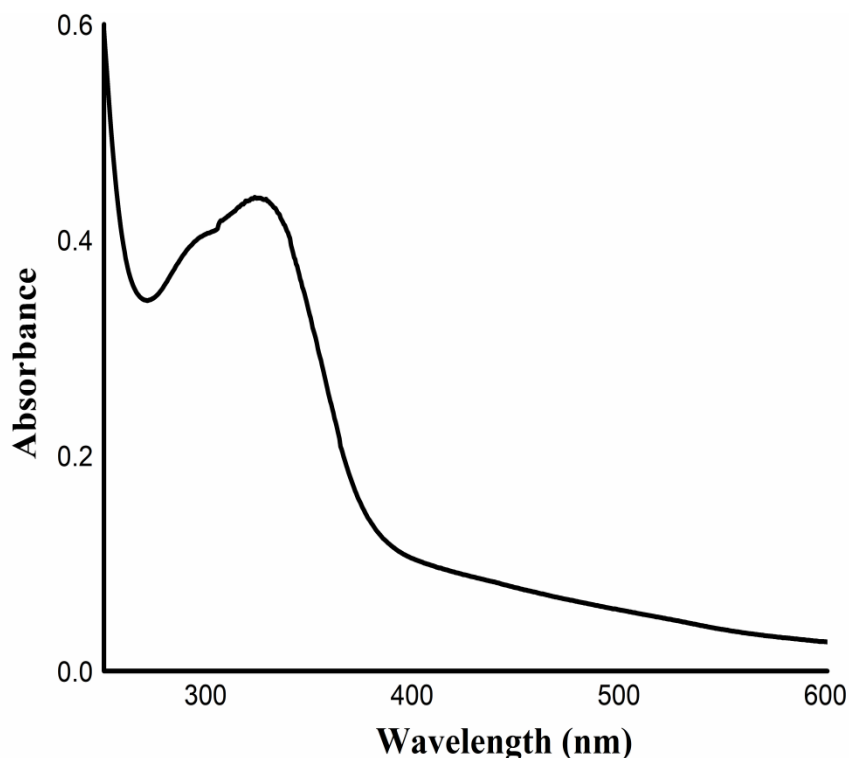
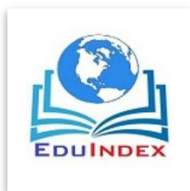


Fig.1 UV-Vis spectrum of ZnO nanoparticles



FT-IR analysis

The stretching vibration of the aromatic ring appears at 1449 cm^{-1} and O-H scissoring deformation of an aromatic ring is observed at 1326 cm^{-1} . The band at 1360 cm^{-1} is attributed to C-H stretching. The characteristic bands of C-O aromatic stretching and C-O-C asymmetric stretching are observed at 1207 cm^{-1} and 1180 cm^{-1} respectively. O-C-C stretching is observed by a characteristic band at 1043 cm^{-1} . The vibrational bands observed at 838 cm^{-1} represents the C-O stretching of alcohols. These observed peaks are mainly contributed by the flavanoids, terpenoids and tannins present in the seed extract. The seeds of *Mangifera Indica* are rich in polyphenols (79%) such as gallic acid, gallotannins, ellagic acid, Xanthones (mangiferin), methyl gallate, digallic acid, α -gallotannin and β -glucogallin[5]. FTIR analysis is used to detect the functional groups that are present in the synthesis of nanoparticles, molecules that are involved as capping and reducing agent in synthesizing ZnO nanoparticles. The % of transmittance for varying wavelengths is shown in Fig.2. From FTIR result it can be observed that the Metal oxides give absorption in the fingerprint region that is below 1000 cm^{-1} arising from interatomic vibrations. The soluble elements present in plant extract could have acted as a coating agent preventing the aggregate of Nanoparticles in solution and playing a significant role in their synthesis and structure creation [27].

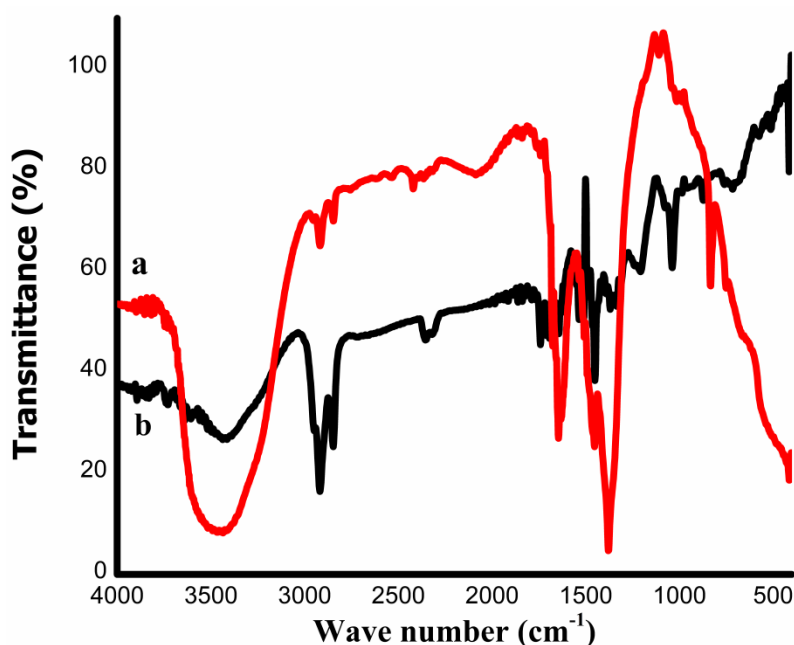
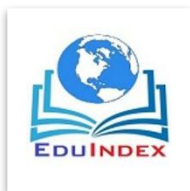


Fig.2 FT-IR spectrum of a) ZnO nanoparticles and b) Mangifera indica seed extract



XRD Analysis

XRD pattern (Fig.2) confirms the crystalline nature of the nanoparticle. The 2θ values 31.83, 34.43, 36.26, 47.81, 56.62, 62.88, 68.05 which has a closeness to values obtained from *Ocimum basilicum* L. var. *purpurascens* leaf extract [18] and *Cinnamomum Tamala* leaf extract [4] which has hexagonal wurtzite structure. The XRD pattern indicates the complete formation of the nanoparticle and doesn't notice other elements in the compound. The peak 36.26 denotes polycrystalline nature of the nanoparticles [19]. The average grain size of the nanoparticle is 34nm which was found by using the Debye-Scherrer formula.

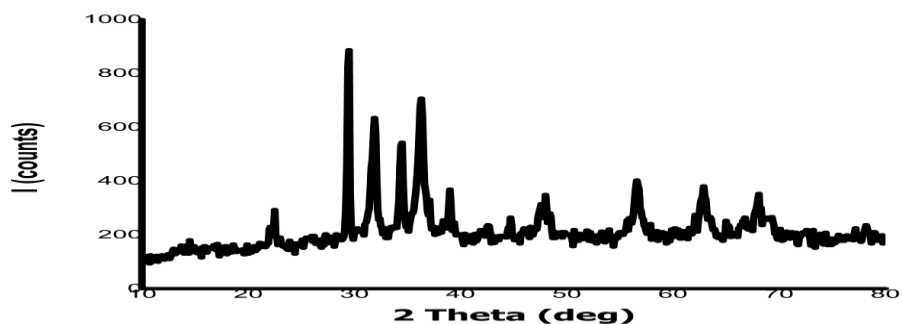


Fig.2. XRD Pattern of ZnO nanoparticles

SEM-EDAX analysis

SEM image (Fig.3) shows the morphology of the nanoparticle and it describes singlespherical particle can contain several agglomerated particles [22].

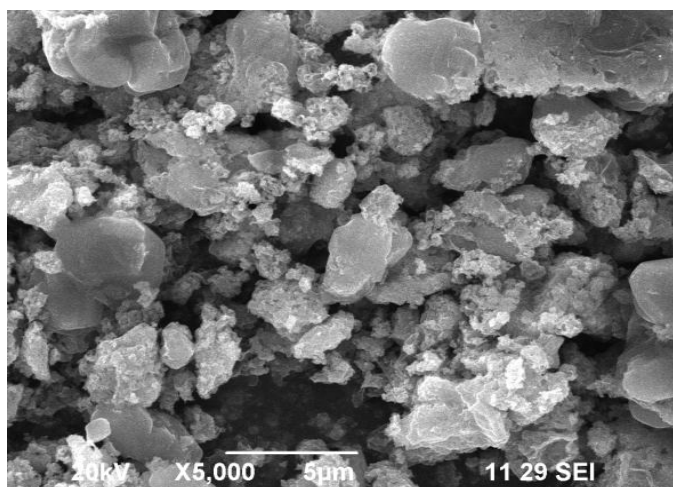
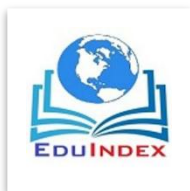


Fig.3 SEM image of Zinc oxide nanoparticles



EDAX spectrum shows the elemental composition of ZnO nanoparticles. It reveals that no impurities present in the compound. The weight percentage of Zinc and oxygen are 54.37 and 45.63 respectively.

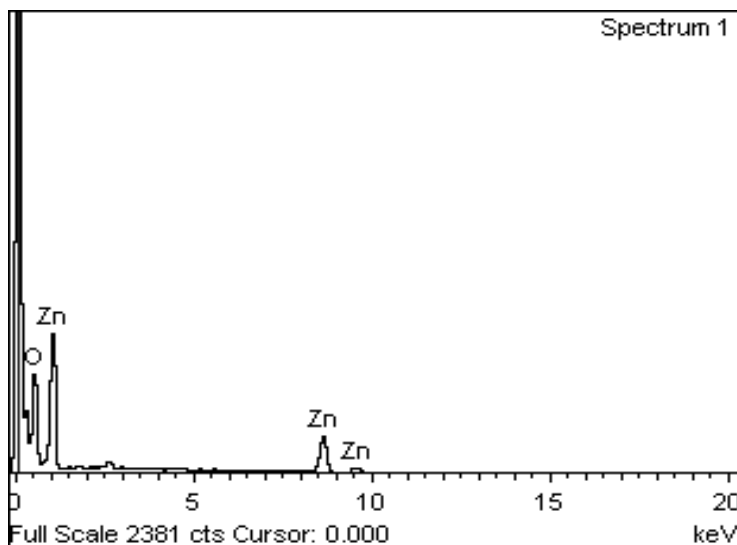


Fig.4. EDAX spectrum of Zinc oxide nanoparticles

Anti-cancer and apoptotic activity

ZnO nanoparticles are a good anticancer agent which was proved by many in-vitro studies and it was selectively killing the cancer cells. In physiological condition, ZnO nanoparticles carry a positive charge, because it has an isoelectric point at 9-10. The cancer cells have negative phospholipids. So electrostatic interaction is happening in between ZnO nanoparticles and cancer cells. This will promote the anticancer activity of the nanoparticles [25]. The anticancer activity of the nanoparticles (Fig.5) is dose-dependent. 6.25, 12.5, 25, 50, 100 $\mu\text{g/ml}$ of ZnO nanoparticles was treated with SKMEL-28 cancer cell by using MTT assay. The concentration decreases the cell viability is increases and the nanoparticles have the IC_{50} value at 44.309 $\mu\text{g/ml}$. The nanoparticles produce reactive oxygen species (ROS). This ROS can damage the mitochondrial enzyme and change the mitochondria membrane permeability (MMP). This will also change the morphology of the cancer cells through nuclear fragmentation, nuclear condensation, blebbing of the cell membrane [23]. The ROS promotes damage of the DNA cells resulting in arrested cell cycle and cell death [24]. In AO/EB double staining method (Fig.6) the green colour shows living cells and orange colour denotes treated cells and this can be identified by chromatin condensation [15, 26]. This result shows early and late apoptotic nature of ZnO nanoparticles.

MTT-Assay (SKMEL cell line)

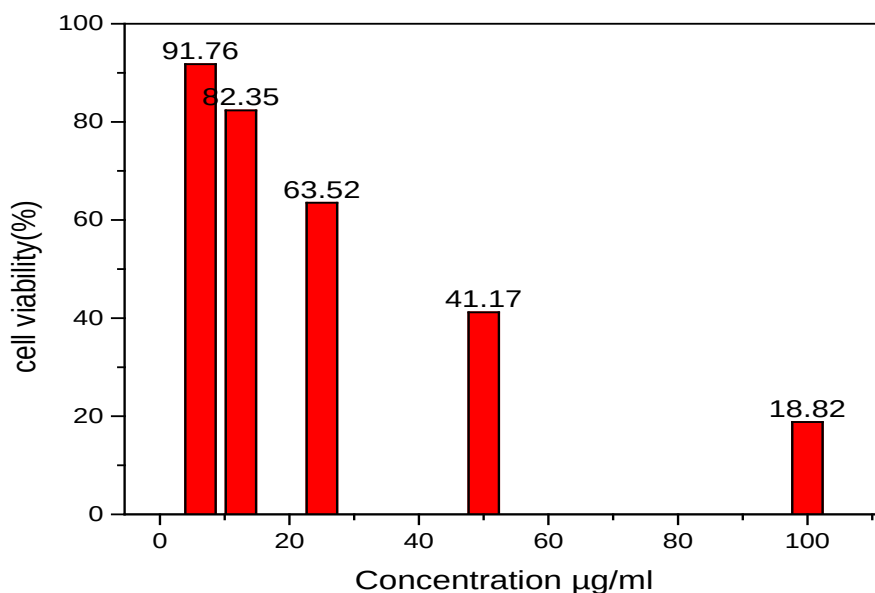


Fig. 5 Graphical representation of the cytotoxic effect of ZnO nanoparticles with different concentration

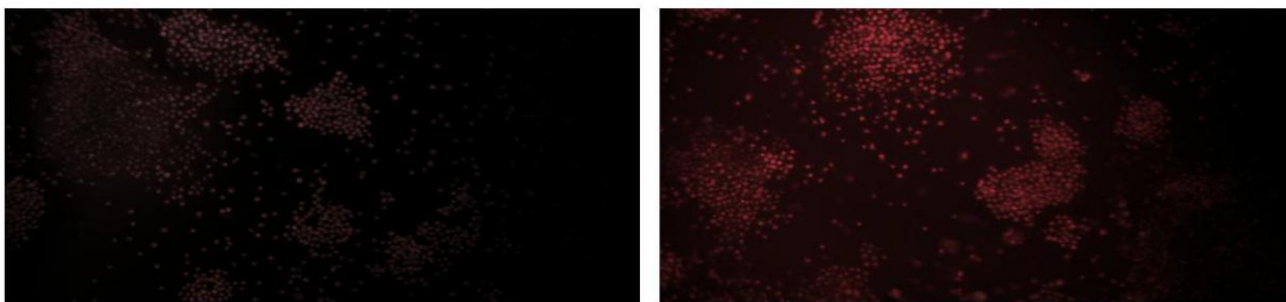
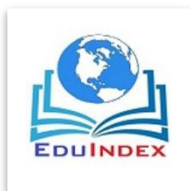


Fig.6. AO/EB staining of SKMEL-28 cells. Control- Live green cells and Treated cells Orange are apoptotic bodies.

CONCLUSION

In this study, we reported that ethanolic extract of *Mangifera indica* seed is a good source for the preparation of Zinc oxide nanoparticles. This method is eco-friendly and cost-effective. ZnO nanoparticles are confirmed by using UV-Visible spectrophotometer. FT-IR denotes the plant particles capped in the nanoparticles. XRD shows the particles are crystalline



nature and average grain size of the nanoparticle is 34nm. SEM image confirms the particles are spherical agglomerated particles and EDAX reveals that completion of the reaction and no impurities or other compounds present in the ZnO nanoparticles. ZnO nanoparticles are good anti-cancer agents against SKMEL -28 cell line and give the IC₅₀ value at 44.309µg/ml. The apoptotic activity of the nanoparticle determines the morphological change in the cell and also chromatin condensation. This confirms the early and late apoptotic activity of the ZnO nanoparticles.

REFERENCES

1. Król, P., Pomastowski, Rafińska, K., Railean-Plugaru, V., Buszewski, B., (2017). Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism., *Advances in Colloid and Interface Science* 249 37–52.
2. Happy Agarwal, Venkat Kumar, S., Rajeshkumar, S., (2017). A review on green synthesis of zinc oxide nanoparticles –An eco-friendly approach, *Resource-Efficient Technologies* 3, 406–413.
3. Xianchun Zhu, Kavitha Pathakoti, Huey-Min Hwang., (2019), Green synthesis of titanium dioxide and zinc oxide nanoparticles and their usage for antimicrobial applications and environmental remediation, chapter 10, 223-263.
4. Mehrdad Khatami, Hajar Q. Alijani, Hossein Heli, Iraj Sharifi., (2018), Rectangular shaped zinc oxide nanoparticles: Green synthesis by Stevia and its biomedical efficiency., *Ceramics International*, 44(13), 15596-15602.
5. Amit Singha, b, Neelama, b, Mahima Kaushika., (2019), Physicochemical investigations of zinc oxide nanoparticles synthesized from *Azadirachta Indica* (Neem) leaf extract and their interaction with Calf-Thymus DNA., *Results in Physics* 13, 102168.
6. Khurshed Ali, Sourabh Dwivedi, Ameer Azam, Quaiser Saquib, Mansour S. Al-Said, Abdulaziz A. Alkhedhairy, Javed Musarrat., (2016), Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates., *Journal of Colloid and Interface Science* 472, 145–156.
7. Elahe Darvishi, Danial Kahrizi, Elham Arkan., (2019), Comparison of different properties of zinc oxide nanoparticles synthesized by the green (using *Juglans regia* L. leaf extract) and chemical methods., *Journal of Molecular Liquids* 286, 110831.
8. S.R. Senthilkumar, T. Sivakumar., (2014), Green Tea (*Camellia Sinensis*) Mediated Synthesis of Zinc Oxide (ZnO) Nanoparticles And Studies On Their Antimicrobial Activities., *International Journal of Pharmacy and Pharmaceutical Sciences*., ISSN-0975-1491., 6 Issue 6.
9. Pragati Jamdagni, Poonam Khatri, J.S. Rana., (2016), Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity., *Journal of King Saud University – Science*., 30(2), 168-175.