*International Journal of Latest Trends in Engineering and Technology*
Special Issue - International Conference on Nanotechnology: The Fruition of Science-2017, *pp.010-015*
*e-ISSN:2278-621X, p-ISSN: 2319-3778*

# EVALUATION OF IN-VITRO ANTICANCER AND ANTIOXIDANT ACTIVITY OF ZINC OXIDE NANOPARTICLE BY CHEMICAL AND GREEN METHOD

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**Abstract-In this exploration paper, dicussed about on the synthesis, characterisation, antioxidant and anticancer activity of ZnO nanoparticles by green and chemical technique. Nanotechnology is a creating interdisciplinary field of research scattering material science, bionanoscience, and innovation. Nanoparticles created by plants are more steady, and the rate of amalgamation is quicker than that on account of different creatures. This study demonstrates the bio blend of ZnO nanoparticle from Zerumbone separated from Zingiber Zerumbet through green science approach which is ecofriendly. Arrangement of zinc oxide nanoparticles have been portrayed by X-Ray diffraction (XRD), and transmission electron microscopy (TEM) examination. Useful gatherings and concoction creation of zinc oxide were additionally affirmed. Zerumbone intervened zinc oxide nanoparticles demonstrated viable cytotoxic impact against HT29 colon malignancy cell line with an IC50 estimation of 50 µg/ml/24 h by the MTT examine and free radical scavenging activity was determined by DPPH assay. These outcomes plainly bolster the advantages of utilizing organic strategy for blending zinc oxide nanoparticles with anticancer and antioxidant activity.**
**Keywords:Zerumbone, antioxidant, anticancer, Zingiber Zerumbet, DPPH assay.**

## INTRODUCTION

Nano-science depends on the control of individual particles and additionally atoms to create materials from them for working great underneath the sub-infinitesimal level. They include physical, concoction and organic learning at scales running between individual iotas and particles underneath the nanometer or up to 100 nm and rising as a standout amongst the most dynamic ranges of research in present day science.[1] The nanoparticles display totally new or enhanced properties in light of particular qualities, for example, size, appropriation and morphology.[2] Nanotechnology is a creating interdisciplinary field of research blending material science, bionanoscience, and technology.[3] Recent progress in the field of nanotechnology, especially the capacity to plan exceptionally requested nanoparticles of any size and shape, have prompted to the improvement of new biocidal operators. Nanomaterials are called "a ponder of cutting edge drug" [4] Zinc oxide (ZnO) is a wide band hole semiconductor with a vitality crevice of 3.37 eV at room temperature. It has been utilized impressively for its synergist, electrical, optoelectronic, and photochemical properties.[5,6] ZnO nanostructures have an incredible favorable position to apply to a reactant response handle because of their expansive surface territory and high reactant activity.[7,8] Since zinc oxide indicates diverse physical and substance properties relying on the morphology of nanostructures, different blend techniques as well as the physical and concoction properties of incorporated zinc oxide are to be researched as far as its morphology.[9] Z. zerumbet, generally known as the pinecone or cleanser ginger. [10] The rhizome of Z. zerumbet (RZZ), specifically, has been utilized customarily as natural pharmaceutical as a part of Asian, Indian, Chinese, and Arabic fables since old times. [11] Despite its normal uses as sustenance enhancing and hors d'oeuvre in Malays and Indian cooking styles, the rhizomes of Z. zerumbet, specifically, have likewise been utilized as a part of folkloric prescription as a cure for different ailments.[12]

Zerumbone is a monocyclic sesquiterpene that can be discovered bounteously in rhizomes especially from Zingiber zerumbet Smith Its molecular formula is $C_{15}H_{22}O$. Zerumbone is a nourishment phytochemical having awesome potential use in chemoprevention and chemotherapy techniques against malignancies. [13]. In light of the broad research done already, zerumbone has indicated exceptionally intense anticancer and antitumor movement Furthermore, it additionally restrained the development of human leukemia cell line (HL-60 cell) and human colon disease (HT-29) in vitro. Zerumbone went about as an exceptionally intense chemoprevention specialists against colon and skin cancer.[14]

Tumor is a class of maladies portrayed by crazy cell development. Colon malignancy frames when this uncontrolled cell development happens in the cells of the huge intestine.[15] The colon is another term for the internal organ, it is the most reduced part of the stomach related framework. Inside the colon, water and salt from strong squanders are separated before the waste travels through the rectum and ways out the body through the anus.[16] Colon disease is

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a dangerous tumor emerging from the inward mass of the internal organ. Colorectal disease is the third driving reason for malignancy in guys and fourth in females in the U.S. In this paper, we embrace a green science approach for the amalgamation of zinc oxide nanoparticles from plant concentrate and molecule's shape and size were recognized by the impact of different standard procedures. Anticancer movement of green orchestrated zinc oxide nanoparticles was resolved against HT29 colon growth cell lines. In view of our perception and investigation, zinc oxide nanoparticles have compelling anticancer action.

## II. Matreials & Methods

### 1.Plant Material:

Zingiber Zerumbet is normally known as Snap Ginger was gathered in the month of July from Kulasekaram, Kanyakumary District, Tamil Nadu.

### 2. Chemical synthesis of Zinc oxide nanoparticle

0.10M zinc acetatedihydrate including poly vinyl pyrrolidone (PVP) as structure directore was sonicated. 0.28M tetramethylammoniumhydroxide(TMAH) was gradually included. The mixture was separated and washed with distilled water and ethanol for three times. Ethanol was added to the got encourage and again sonicated. The ppt was got dried out at $500^0$ C for 4 hours

### 3.Green synthesis of Zinc oxide nanoparticles using Zerumbone from zingiber Zerumbet

To the 50 ml of zerumbone from Zingiber Zerumbet, Zinc acetic acid derivation was included and put under an attractive stirrer. Following 15 minutes NaOH was included till the PH 12 brought about a pale white fluid arrangement. This was then put in an attractive stirrer for 2 hours. The pale white ppt was then taken out and washed again and again with refined water took after by ethanol to get free polluting influences. At that point a pale white powder of MgO nanoparticle was gotten subsequent to drying at $600^0$ C at 3 hours.

### 3.AntiOxidant assay

**Free radical scavenging activity**

Bio and chemically synthesized ZnO were tested for the scavenging effect on DPPH radical. Different concentrations ZnO -NPs were added, in equal volume, to 0.1 mM metabolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature under shaking condition and the absorbance was recorded at 517 nm. The synthetic antioxidant butyl hydroxyl toluene (BHT) was used as positive control. All determinations were performed in triplicate. The DPPH radical scavenging activity (RSA) was expressed in percentage of inhibition using the following formula.

RSA = AControl-ASample/Acontrol **× 100**

### 4.Anticancer assay

The anticancer activity of samples on HT 29 cells were determined by the MTT assay Cells ($1 \times 10^5$/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % $CO_2$ incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 48hrs at 5 % $CO_2$ incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of HT 29 cells cells were expressed as the % cell viability, using the following formula:

**Calculation**

**% cell viability = A540 of treated cells / A540 of control cells × 100%**

## III.Result&Discussion

### 1)XRD

Figure1&2 demonstrates the X-Ray diffraction patterns of ZnO nanoparticle orchestrated by chemical and greensynthesis. From this figure the peaks from (100),(002),(101),(102),(110),(103),(200),(112),(201)planes in hexagonal sort of ZnO nanoparticle are in brilliant record with the estimations of standard card (2003 JCPDS NO=89-1397 universal place for Diffraction information). More over the broad peak at around $36^0$ as demonstrates of nanocrystaline nature of the ZnO stage. No other peaks are observerd. From the XRD peak the normal grains size was assessed utilizing Scherrer's condition is 46nm forchemical and 28nm for green strategy.

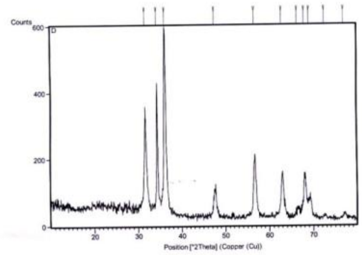


*Fig .1 X-Ray Diffraction of ZnO np by Chemical synthesis*

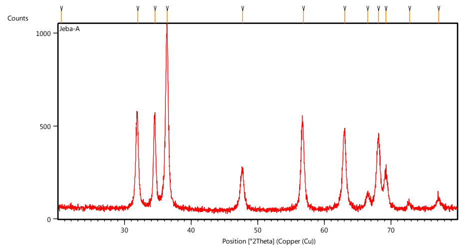
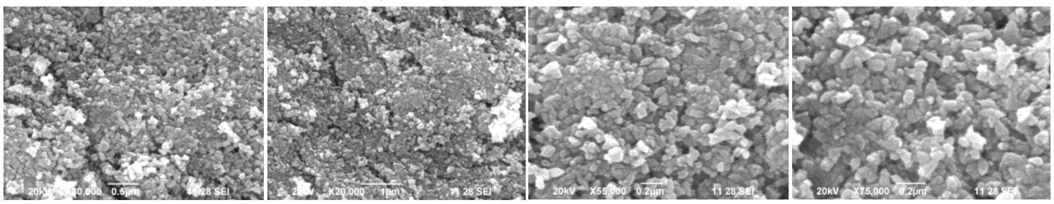


*Fig. 2X-Ray diffraction of ZnO np by green strategy utilizing Zerumbet*

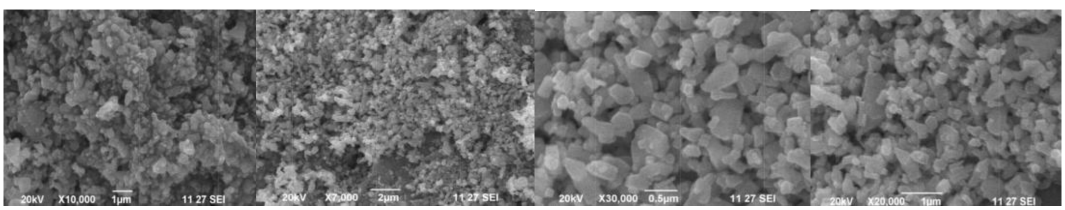
**2)SEM**

Fig 3 (A,B,C,D) and 4 (A,B,C,D) demonstrates the surface morphologies of the ZnO nanoparticles of various amplification for chemical and green synthesis. Pictures demonstrated that the circular molded individual particles and additionally number of totals. However the normal size of the chose singular particles are in the request of 89nm for chemical and 67 nm for green synthesis



3A 3B 3C 3D

*Fig . 3 (A,B,C,D) SEM pictures of ZnO np by Chemical synthesis*



4A 4B 4C 4D

*Fig. 4 (A,B,C,D) SEM pictures of ZnO np by green strategy utilizing Zerumbone*

**3.TEM**

Figure 5&6 demonstrates TEM picture and SAED patterns of ZnO np arranged by chemical and green synthesis. There is no arrangement of ring with spots affirmed that it has shapeless in nature in chemical synthesis. Nearness of the arrangement of rings containing spots in SAED affirmed ZnO nps in green synthesis have bigger grain measure. It indicates crystalline structure.

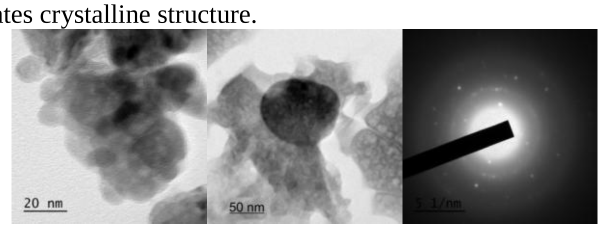


*Fig. 5 TEM pictures of ZnO np by synthetic technique*

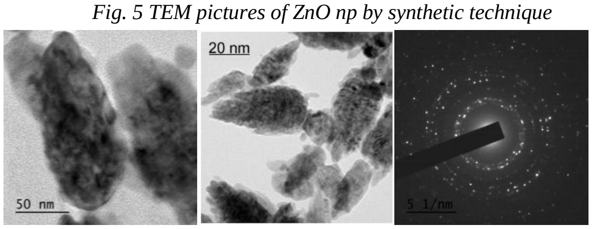


*Fig.6 TEM pictures of ZnO np by green strategy utilizing Zerumbone*

**4.FT-IR Spectroscopy**

FT-IR pattern of the synthesised nanoparticle were studied and it ranges from 400-4500 $cm^{-1}$ This fig 7 shows the FT-IR spectra of ZnO nanoparticle prepared for chemical method. Broad band near 3410 $cm^{-1}$ represents the hydrogen bonded –OH stretching vibration. The band at 2924 and 2854 $cm^{-1}$ represents C-H stretching of alkyl group. The strong asymmetric mode of vibration of C=O was observed at 1697 and 1581$cm^{-1}$. The peak at 1411$cm^{-1}$ denotes the symmetric stretching because the presence of C-O and C-O-C. Due to the carbonate moieties that are generally observed when FTIR are measured in air, a peak shown at 910$cm^{-1}$ .

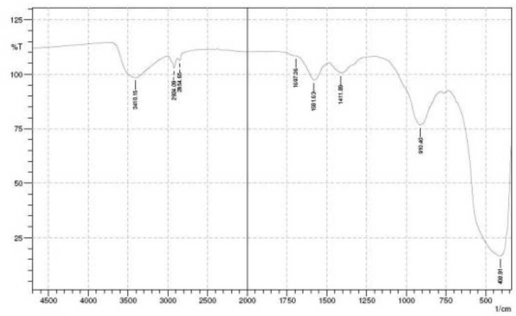



*Fig .7 FT-IR spectra of ZnO by Chemical synthesis*

This fig shows the FTIR spectra of ZnO nanoparticle prepared using Zerumbone from Zingiber zerumbet was acquired in the range of 450-4000 cm-[1] by Bruker Alpha T (Made in Germany) machine. There was a peak at 3693&3421$cm^{-1}$ showed OH stretching. N-H stretching vibration of secondary amine denoted at 2470$cm^{-1}$. A peak at 1448$cm^{-1}$ showed aromatic ring. C-N stretching vibration amine observed at 1033cm-1. There was a peak at 866&686cm-1 explained aromatic $CH_2$ group. ZnO np identified at 464$cm^{-1}$

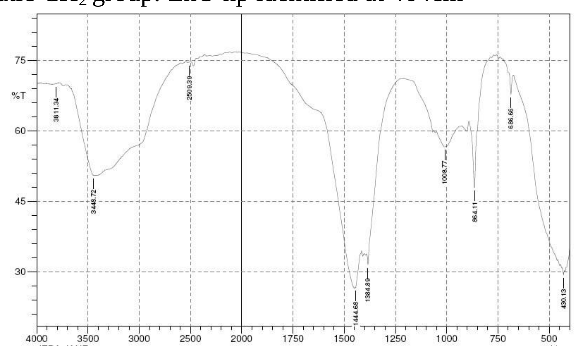



*Fig . 8 FT-IR spectra of ZnO np by green strategy utilizing Zerumbone*

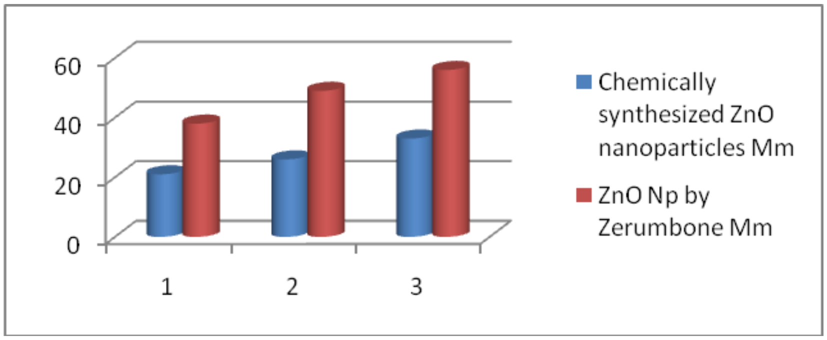
**5.Antioxidant activity**

Plants contain specific metabolites that are acknowledged to perform a range of purposeful activities. The reducing activity of ZnO nanoparticles synthesized from chemical and green method was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Percent of inhibition of DPPH radical scavenging activity was presented on Table 1 and Graph 1. Biologically synthesized nanoparticles were found to be potent free radical scavenger when compared to chemical method. The average percentage inhibition of ZnO by green method compared to that of chemically synthesized ZnO nanoparticles (33% and 56%) used in this study and the activity increased with increasing concentrations of green synthesised ZnO nanoparticles. This antioxidant activity may be due to the capping constituents present in plant extract and present on metal surface.

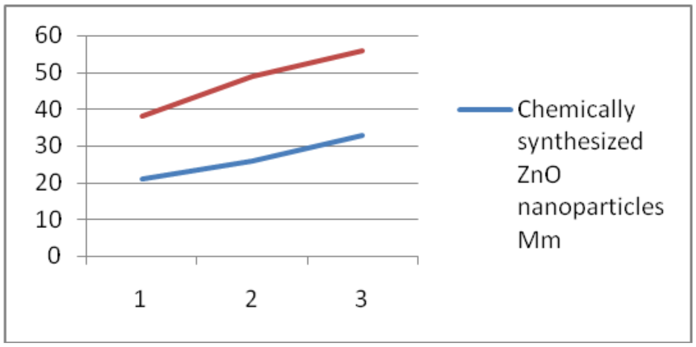
*Table 1. Antioxidant activity of ZnO ny chemical and green method*

| Sl.No | Concentrations (µL) | Chemically synthesized ZnO nanoparticles Mm | ZnO Np by Zerumbone Mm |
|---|---|---|---|
| 1 | 50 | 21 | 38 |
| 2 | 100 | 26 | 49 |
| 3 | 150 | 33 | 56 |

*Bar Diagram 1 antioxidant of ZnOnp by Chemical and Green method*




Graph 1. Anti oxidant activity of ZnO np by chemical and green method




**4.Cytotoxicity**

ZnO np incorporated by compound and green strategy brought on measurements subordinate executing of the colon disease cells Figure 9 &10 , demonstrating $IC_{50}$s of 50 µg/ml for green method. Cisplatin, as a positive control, additionally indicated dosage subordinate cytotoxicity on colon tumor cells. In this study green combined ZnO nanoparticle demonstrated incredible critical impact when contrasted with chemically synthesied MgO nanoparticles.

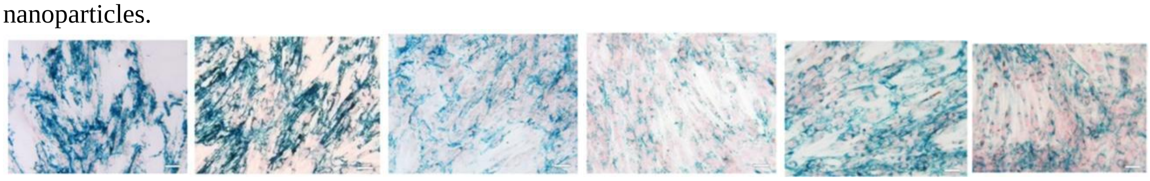


*Fig. 9 Anticancer activity of ZnO np by chemical method*

*Table 2. Anticancer activity of ZnO np by chemical method*

| S.No | Concentration µg/ml | Dilution | Absorbance 540nm | % cell Viability |
|---|---|---|---|---|
| 1 | 200 | Neat | 0.00 | 0.00 |
| 2 | 100 | 1:1 | 0.09 | 10.02 |
| 3 | 50 | 1:2 | 0.11 | 18.64 |
| 4 | 25 | 1:4 | 0.23 | 22.0 |
| 5 | 12.5 | 1:8 | 0.12 | 28.0 |
| 6 | Control | - | 0.43 | |

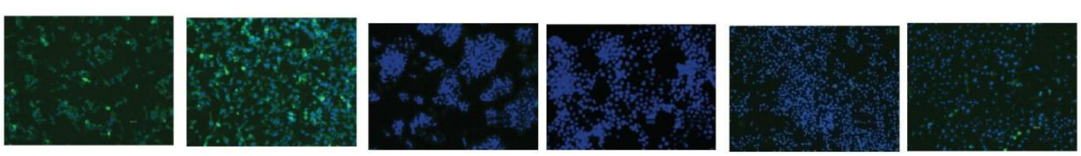


*Fig. 10Anticancer activity of ZnO np by green method*

*Table 3. Anticancer activity of ZnO np by chemical method*

| S.No | Concentration µg/ml | Dilution | Absorbance 540nm | % cell Viability |
|---|---|---|---|---|
| 1 | 200 | Neat | 0.11 | 39.00 |
| 2 | 100 | 1:1 | 0.21 | 46.02 |
| 3 | 50 | 1:2 | 0.34 | 52.64 |

| | | | | |
|---|---|---|---|---|
| **4** | **25** | **1:4** | **0.49** | **74.0** |
| **5** | **12.5** | **1:8** | **0.52** | **78.0** |
| **6** | **Control** | **-** | **0.53** | |


**Summary**

ZnONPs have been properly characterized by XRD, TEM , FTIR analysis. The obtained results confirmed that the ZnO np were crystalline in nature and the morphological studies reveal the spherical shape of ZnO np with size ranging from ---- nm. FTIR analysis was confirmed to identify possible presence of functional groups that might have contributed to the process of bio-reduction and stabilization of Zn ion to green synthesized ZnO np. The natural combination of ZnO nanoparticles utilizing plant extract gives an ecological well disposed, straightforward and proficient course for amalgamation of nanoparticles. The utilization of plant concentrates dodges the use of destructive and poisonous lessening and settling specialists than concoction strategies. Plant intervened ZnO nps indicated best anticancer action. ZnO np were successfully synthesized in a chemical and green approach using Zerumbone as a reducing and capping agents. Further, green ZnO np showed enhanced anticancer and antioxidant activity than chemical ZnO np. Finally, cost effectiveness, biocompatibility, and predictability to modify these ZnONPs make them viable choice in future biomedical applications particularly in cancer. Further, trails are needed to confirm these preliminary results in future nano toxicology research to establish better models to assess the long-term effects.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