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Study on the proapoptotic potential of Zinc oxide nanoparticles green synthesized from *Detarium microcarpum* methanol stembark extract on MCF-7 breast cancer cell lines *in vitro*

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Abstract

Breast cancer remains one of the leading health care concerns globally, with an estimated 2.3 million new cases and 670,000 deaths in 2022. This research studied the proapoptotic potential of Zinc oxide nanoparticles (DMZnONPs) green synthesized from *Detarium microcarpum* methanol stembark extract on MCF-7 breast cancer cell lines *in vitro.* The green synthesized DMZnONPs was characterized using UV-vis spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR) and atomic force microscopy (AFM). Preliminary antioxidant potential was assessed in vitro, using DPPH, H_2O_2 radical Scavenging assay and Reducing power assay. The proapoptotic potential against MCF-7 breast cancer cell line was also determined i*n vitro.* The DMZnONPs showed maximum absorption at a range between 340 and 380 nm confirming the synthesis of nanoparticles, while the AFM revealed an average particle size of around 89 nm. The results indicate a dose dependent antioxidant activity. The lower IC_{50} value in the DPPH and H_2O_2 of the standard BHT (98.61 and 97.55 µg/ml respectively) compared to the DMZnONPs (129.47 and 207.46 µg/ml) affirms higher scavenging potential of the standard. The DMZnONPs treatment significantly decreased the viability of MCF-7 cancer cells at higher concentrations (1250-5000 µg/mL) compared to untreated cells and other concentrations after 24 h and 72 h stand point. These findings demonstrate that DMZnONPs treatment acts as a highly effective, specific therapeutic agent, disrupting both the surface and internal structure of breast cancer cells and leading to programmed death

Keywords: Nanoparticles; Green synthesis; Breast cancer; DMBA; Oxidative stress

1 Introduction

Breast cancer which is predominant in females has become the most popular type of cancer globally, surpassing lung cancer [1]. The recent GLOBOCAN data discloses about 2.3 million new breast cancer cases and 670, 000 deaths in 2022, representing data for both sexes. The grossly inadequate public health response to this growing problem prompted the World Health Organization (WHO) to launch the Global Breast Cancer Initiative [2]. The initiative's goals are to reduce breast cancer mortality by encouragement timely diagnosis and adequate treatment and patient management. Significant progress has been made in the area of diagnosis and treatment of breast cancer, including surgical excision, targeted agents, hormone therapy, chemotherapy, and radiation [3], however, challenges, such as treatment resistance, recurrence, and adverse effects have more often than not characterized such medical interventions.

Traditional Health Practitioners have a history of employing *Detarium microparpum* stem bark and their derivatives in the treatment and management of diseases including cancer with appreciable outcomes (4,5,6). There is a recent surge in the standardizing herbal remedies in order to overcome the challenges associated with the use of medicinal plants in Breast Cancer treatment. Nanotechnology is an emerging alternative remedy with promising efficacy in circumventing the major downsides of the conventional breast cancer treatments [7]. The therapeutic advantage of green synthesized

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nanoparticles is due to their enhanced biocompatibility, reduced immunogenicity, and environmental sustainability, making them effective in enhancing transmembrane uptake, enhanced delivery and, and prevention of systemic toxicity, thus making green nanotechnology both promising and reliable breast cancer treatment option [8].

2 Materials and Methods

2.1 Chemicals and Reagents

The chemicals 2,2-diphenyl-2-picryl hydrazyl (DPPH), bovine serum albumin (BSA) Were purchased from (Sigma Aldrich, Germany). Butylated Hydroxytoluene (BHT), Potassium persulphate and potassium ferricyanate were purchased from Vinipul Inorganics Pvt. Ltd, Mumbai India. Other reagents and solvents were of analytical grade.

2.2 Plant Collection and Authentication

Fresh stem bark samples of *Detarium microcarpum* was collected from the wide forest of Kwambla, Hong Local Government Area, Adamawa State, Nigeria between October and December. The plant was authenticated at the Botany Department of Adamawa State University, Mubi, Nigeria and deposited at the University Herbarium been assigned a voucher specimen number, ADSUM/BOT/HAB/STB/0026.

2.3 Cell culture

Breast cancer cell line MCF-7 was obtained from the cell and tissue culture unit of the LUTH/CHEVRON Molecular biology research Laboratory, College of Medicine University of Lagos. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% 2 mM l-glutamine, and penicillin/streptomycin. The cells were constantly maintained under cell culture conditions of 37°C and 5% CO_2 in a humidified chamber.

2.4 Methods

2.4.1 Preparation of plant extract

The bark was washed properly with deionized water, shade dried, and then pulverized into coarse powder using mortar and pestle. *Detarium microcarpum* methanol extract was prepared using maceration method as described by Hussain *et al*., [9]. Briefly, the extraction was performed using methanol as solvent in which 200 g of the coarse powder is soaked in 1000 mL of methanol (99.8%) in a conical flask and then heated in a water bath under constant shaking at 45°C for 24 hours. The mixture was then be filtered using Whatman No 1 filter paper and the filtrate stored at 4°C until used.

2.5 Preparation of *Detarium microcarpum* mediated ZnO Nanoparticles

The step-by-step procedure outlined by Kyene *et al.*, [10] was adopted with slight modifications. Briefly, 9.15 g (0.05 mol) of zinc acetate was dissolved in 50 mL deionized water. The mixture was then heated to 60 °C on a hotplate with constant stirring. In addition, 25 mL of deionized water was used to dissolve 2.80 g potassium hydroxide in a 100 mL conical flask under the similar conditions as the precursor and added drop wise into the zinc acetate solution. The stirring continued for 1 hour at 60 °C until precipitate of ZnO is formed. Fifty milliliters (50 mL) of the prepared plant extract (10%) was then added gradually into the reaction medium while stirring at 20 °C for 3 h until the solution turns yellowish creamy. The mixture was then centrifuged at 4000 rpm for 10 min and the precipitate obtained washed with deionized water and dried under hot air. The prepared ZnO NPs was then stored in an airtight bottle for analysis.

2.6 Characterization of *Detarium microcarpum* ZnO Nanoparticles

To confirm the synthesis of nanoparticles, the green synthesized ZnO Nanoparticles were characterized by using different techniques. UV–visible spectrum was evaluated using UV–Visible spectrophotometer (Model Jenway 7315) and the spectrum recorded between 300 and 800 nm. Fourier Transform Infrared Spectroscopy (FTIR) was taken to assess the presence of various functional groups by using model L1600312 spectrum TWOLITA/ZnSe. Fourier transform infrared (FTIR) analysis was carried out with Fourier transform spectrometer at a frequency range of 4,000–500 cm^{-1}.

2.6.1 DPPH radical Scavenging Activity

The free radical scavenging activities of the *D. microcarpum* methanol stem bark Zinc oxide Nanoparticle was measured according to the method described by Tit and Bungau, [11]. Different concentrations of the test compounds (in methanol) were added at an equal volume (10 mL) to methanol solution of DPPH (400 µg/mL). The same concentrations of Butylated Hydroxytoluene (BHT) were used as the standard antioxidant. After 30 min at room temperature, the absorbance values were measured at 517 nm using a spectrophotometer (Jenway 7315). Methanol plus different concentration of plant extract solution was used as blank, while DPPH solution plus methanol as a control. IC_{50} values denote the concentration of the sample required to scavenge 50% of DPPH radicals. The percentage inhibition was calculated thus:

$$\%\text{Inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \text{ X } 100$$

Were Abs (control) being the absorbance without extract; Abs (sample) is Absorbance with extract or standard.

2.6.2 Total Reducing Power Assay

The total reducing power of the *D. microcarpum* methanol stem bark Zinc oxide Nanoparticle was measured using the potassium ferricyanide reduction method as described by Alavi and Karimi [12]. Varying concentration of *D. microcarpum* methanol stem bark Zinc oxide Nanoparticle and standard were taken in different test tubes. Then 2.5 mL of distilled water and 2.5 mL of potassium ferricyanide ($[K_3Fe_3(CN)_6]$ 1 %) solution was then added in all test tubes and mixed well. After incubation at 50 °C for 20 min, 2.5 mL of trichloro acetic acid (10% w/v) was added in all test tubes and centrifuged at 3000 rpm for 10 min. Afterwards, upper layer of solution (5 mL) was mixed with 5 mL distilled water. Then 1 mL of $FeCl_3$ was added to each test tube. Then from each test tube we collected 1 mL of solution and mixed it with 9 mL of distilled water. Then the solution was incubated at 35 °C for 10 min. The formation of perl perussian color was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increasing reducing power. Butylated Hydroxytoluene (BHT) was used as a standard. The analysis was performed in triplicate.

2.6.3 Hydrogen peroxide (H_2O_2) Scavenging Activity

This assay was done according to the method outlined by (Keser, *et al.*, [13]; Alara, *et al.*, [14]; Henry, *et al.*, [15]) with minor modification. Hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (pH 7.4). *D. microcarpum* methanol stem bark Zinc oxide Nanoparticle (50-400 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of the *D. microcarpum* methanol stem bark extract and its Zinc oxide Nanoparticles were calculated thus:

$$\%\ H_2O_2 \text{ Scavenged} = \frac{A\ control - A\ sample}{\text{Acontrol}}\ X\ 100$$

Where *A control* is the absorbance of the control and *A sample* is the absorbance in the presence of the sample extracts or standards.

2.7 *In vitro* Anticancer Studies

2.7.1 Preparation of Test Substances

D. microcarpum methanol stem bark Zinc oxide Nanoparticles (100 mg) was separately weighed into a 30 mL bottle and 100 µL of dimethysulfoxide (DMSO) overnight and afterwards made quantity sufficient to 10 mL with phosphate buffered saline (PBS) to prepare a 10,000 µg/mL. The solution was then filter-sterilized through a 0.22µm syringe filter (PES) and stored at 4°C until use. 5-fluorouracil (10000 µg/mL) was used as positive control.

2.7.2 Cell Culture and Conditions

Human breast Adenocarcinoma (MCF-7) cells were resuscitated from the cryo-storage of the cell culture unit of LUTH/CHEVRON Molecular biology research Laboratory, College of Medicine University of Lagos. Following the stabilization of the cells, they were propagated and maintained in complete growth medium, (Dulbecco's Modified Eagle Medium (DMEM-F12) supplemented with 10% FBS, and 1% Antibiotics/ Antimycotic) in a humidified 5% CO_2 incubator (Nuaire, USA) at 37°C. Depleted growth medium was replaced once weekly and cells at 80% confluence were sub cultured. Cell viability and concentration of cells were done by trypan blue exclusion assay and 90% live cells were considered viable for experiments.

2.7.3 Trypan Blue Exclusion Assay

The Trypan blue exclusion viability assay was carried out according to the method described by Fadeyi *et al.,* [16]. MCF-7 cells at confluence were brought into suspension by trypsinization. Growing cultures of MCF-7 of Passage 45 in T_{25} culture flask was brought out of the incubator and placed in a biosafety cabinet II (LABNET Nuaire, USA). Depleted growth medium was discarded into a split jar and excess medium washed off with PBS. A 1 mL aliquot of trypsin-EDTA was added into the flask just enough to cover the cell monolayer and incubated for 3 minutes to allow for the cells to detach. The cells were then washed off the floor of the flask with 4 mL of complete growth medium (CGM) to resuspend the cells and stop the activity of trypsin. The cell suspension was collected into a 15 mL centrifuge tube and centrifuged at 500rpm for 5minutes and the supernatant was discarded. The pellets were then resuspended with 5 mL CGM and a 10 µL aliquot was mixed with 10 µL trypan blue solution and immediately charged in an ethanol-wiped haemocytometer chamber. Live and dead cells were counted from each of the 4 corner squares and the center square box. The following equations were used to determine the percent cell viability and concentration of cells per mL of cell suspension.

$$\text{Percentage of viable cells} = \frac{No\ of\ viable\ cells}{Total\ No.of\ cells} \times 100$$

2.7.4 Cell Viability Assay

Confluent MCF-7 cells were brought to suspension by trypsinization at a seeding concentration of 5000 cells per well in a 96 well plate and were incubated overnight to allow for the adherence to the floor of the plates. Thereafter, the growth medium in the plates were replaced with *D. microcarpum* methanol stem bark Zinc oxide Nanoparticles diluted CGM in a 2-fold serial dilution (9.77 µg/mL - 5000 µg/mL) and incubated for 24 hours. The experiment was positively controlled with 5-flourouracil (5FU) as a standard drug of choice. Afterwards, 10 µL of Cell Counting Kit-8 (CCK-8) reagent was added to each well and incubated for 2 hours. Post incubation, the optical density (OD) was determined by measuring the absorbance at test wavelength of 450 nm and reference wavelength of 630nm on a microplate reader (Biotek 800 TS, USA). The half-maximal inhibitory concentration (IC_{50}) values were calculated as the concentration of extract resulting in 50% reduction of cell population relative to untreated cells as deduced from the equation below:

$$\%\ \text{Cell viability} = \frac{ODTest - ODBlank}{ODControl} \times 100$$

2.7.5 Morphology Assay

The morphology assay was conducted according to the method described by Domura *et al.,* [17]. Confluent MCF-7 cells were brought to suspension by trypsinization at a seeding concentration of 100,000 cells per well in a 24- well plate and incubated overnight to allow for the cells to attach to the floor of the plate. Afterwards, the growth medium was replaced with *D. microcarpum* methanol stem bark extract and its Zinc oxide Nanoparticles diluted CGM in a 2-fold serial dilution (9.77 µg/mL - 5000 µg/mL) and incubated for 24 hour or 48 hours. 5FU served as the positive standard drug of choice while cells with fresh CGM only served as the negative control.

Thereafter, the morphology of the treated cells compared with negative control was observed using inverted phase contrast microscope (Leica DMi1, Germany) with an overall magnification of X 100. Photographs were taken at 0 hours, 24 hours, and 48 hours.

3 Result and discussion

3.1 Characteristics of *Detarium microcarpum* Zinc Oxide Nanoparticles

The UV–vis spectra of the synthesized Zinc oxide nanoparticles shown on Figure 1. showed maximum absorption at a range between 340 and 380 nm. This confirms Zinc oxide nanoparticles synthesis from *Detarium microcarpum* stem bark extract. The conceivable theory behind this, is that electrons get excited from valence to conduction band. The earlier confirmed presence of phytochemicals such as alkaloids, flavonoids, terpenoids and tannins in *Detarium microcarpum* stem bark extract [18] reduced the Zn^{2+} ions to the zero valent Zn atoms, acting as bio reductants, stabilizing as well as capping agents in the formation of Zinc oxide nanoparticles. This finding is in consonance with other previous study [19].

The green synthesized zinc oxide nanoparticle (DMZnONPs) analyzed by AFM. Figure 2. shows the micrograph, 3D AFM image, profile and the Abbott-Firestone curve of DMZnONPs scanned in an area of 1.2 µm X 1.2 µm. Height minimum for DMZnONPs was 7.06 nm while the maximum stood at 146nm. The Abbott-Firestone curve, also material ratio curve generated from Atomic Force Microscopy (AFM) to characterize the surface of

nanoparticles as shown on Figure 2. reveals insights into the surface's roughness, including the distribution of peaks and valleys. It also provides the size distribution profile of synthesized Zinc Oxide nanoparticles depicting a narrow size distribution at room temperature. The average particle size was observed to be around 89 nm. These results also suggested that there is an absence of large aggregates, when these nanoparticles were dispersed in aqueous medium and its supports previous report [20]

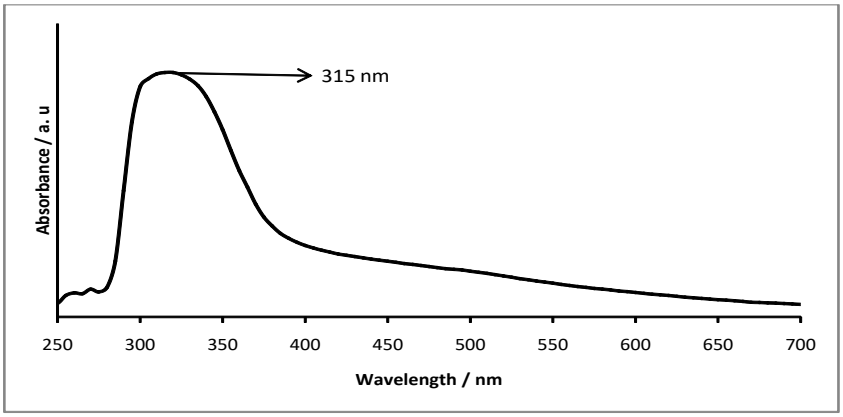

Figure 1 UV-vis Spectroscopic Analysis of Detarium microcarpum ZnO Nanoparticles.

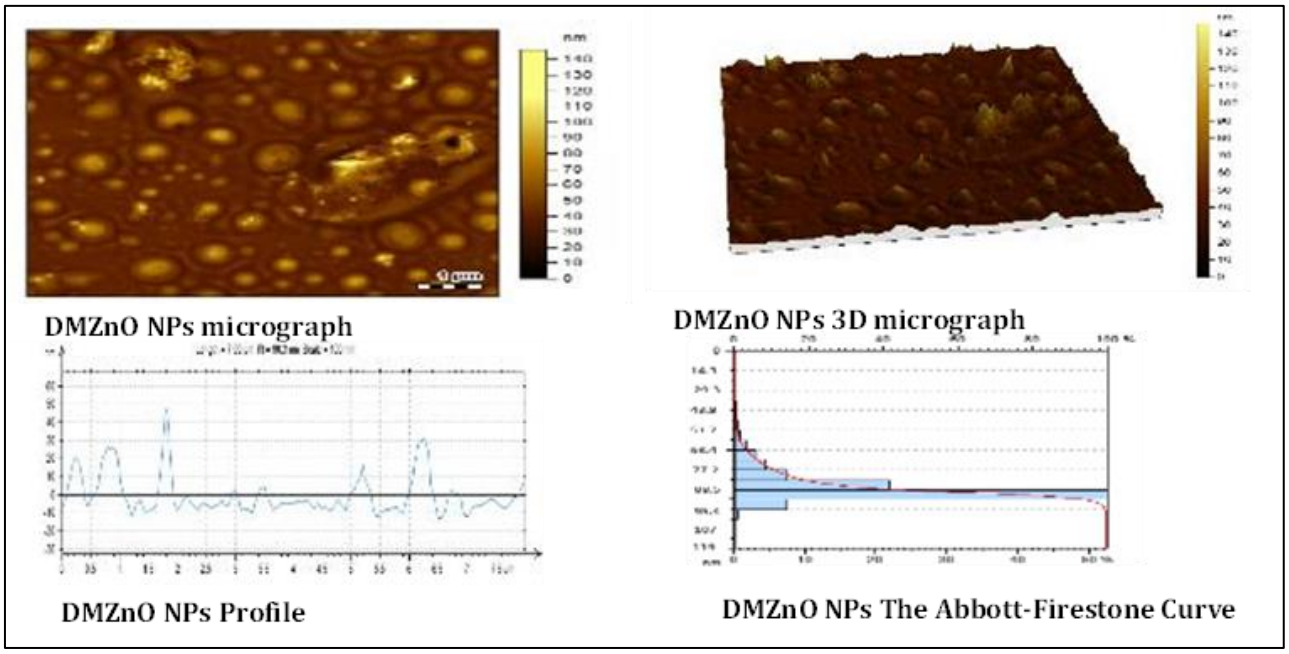

Figure 2 Atomic force microscopy (AFM) micrograph (top left), 3 D AFM image (top right), profile (down left) and the Abbott-Firestone of green synthesized *Zinc Oxide Nanoparticles* by *Detarium microcarpum* stem bark.

The FTIR spectrum for the synthesized Zinc Oxide nanoparticles is shown on Figure 3. with spectra in the range of 500–4000 cm^{-1}. The synthesized Zinc Oxide nanoparticles, showed peak around 3321.42, which leads to the presence of OH stretching vibrations. Another peak around 1551.12 corresponds to the C– –C stretch in aromatic rings and C– –O stretch in polyphenolic compounds. Another peak at 835.42 and 432.05 corresponds to the presence of C–N stretch of aliphatic amines and = C–H bending of alkene respectively. These peaks and corresponding functional groups suggested the presence of different phytochemicals such as phenolics, alkaloids, flavonoids, tannins which where earlier identified from *Detarium microcarpum* methanol stem bark extract. These compounds interact with zinc ions by donating atoms and subsequent adsorption on the surface of metals, which is established by a decrease in peak intensities of bands observed in the synthesized Zinc Oxide nanoparticles [21,22].

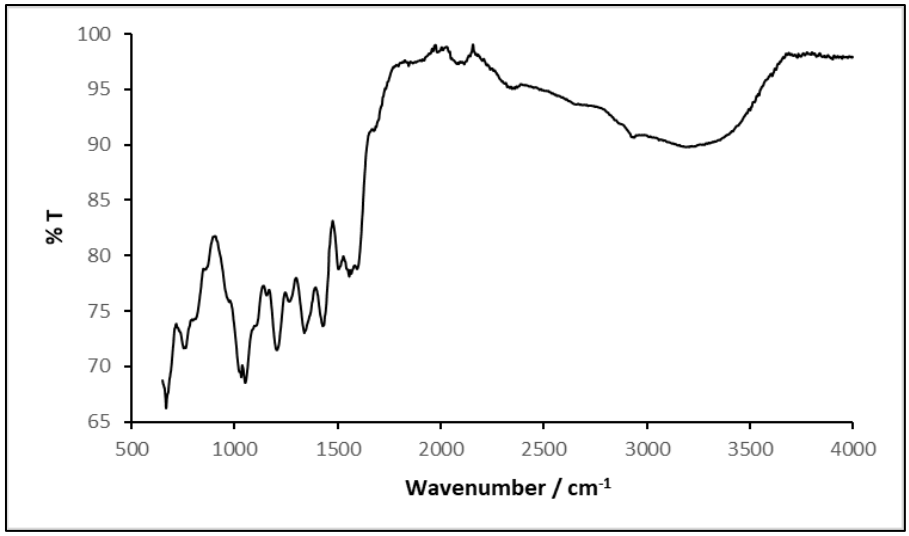

Figure 3 FTIR Spectrum of *Detarium microcarpum* Zinc Oxide Nanoparticles**.**

3.2 *In vitro* Antioxidant Studies

The ability of *Detarium microcarpum* Zinc Oxide nanoparticle to reduce DPPH free radical compared to standard ascorbic acid is illustrated on Table 1 The DPPH free radical has been extensively used to investigate the antioxidant potency of inorganic compounds and nanomaterials [23]. The prepared deep violet DPPH solution undergoes chemical change to pale yellow upon the addition of DMZnONPs, which generally indicates the antioxidant capacity of ZnO-NPs. The results indicate a dose dependent manner of activity. The lower IC_{50} value of the standard BHT (98.61 µg/ml) compared to the DMZnONPs (129.47 µg/ml) affirms higher scavenging potential of the standard. The smaller particle size and larger surface area to volume ratio of the green synthesized nanoparticle is responsible for the observed activity due to domination of materials surface atoms [24]. These results are in line with previous studies [25] which reported lower IC_{50} values of BHT than green synthesized Zinc oxide nanoparticle.

The decrement of H_2O_2 in an incubation system containing H_2O_2 and DMZnONPs or BHT was valued spectrophotometrically as shown on Table 2. The green synthesized Zinc oxide nanoparticle IC_{50} (207.46 µg/ml) and the BHT IC_{50} value (97.55 µg/ml), depicts higher scavenging potential for BHT. The reducing power assay which is based on reaction of DMZnONPs or BHT with potassium ferricyanide (Fe^{3+}) to produce potassium ferrocyanide (Fe^{2+}), subsequently reacting with ferric chloride ($FeCl_3$) to form ferric–ferrous complex that absorbs maximally at 700nm is shown in Table 2. It depicts a dose dependent manner of activity where higher absorbance means higher reducing power. The lowest concentration assayed shows absorbance of 0.17 ± 0.007 and 0.55 ± 0.003 for the DMZnONPs and BHT respectively while the highest concentration produces absorbance of 0.92 ± 0.008 and 0.92 ± 0.010.

Table 1 Percentage (%) DPPH Radical Scavenging Activities of *Detarium microcarpum* Methanol Extract and *Detarium microcarpum* ZnO Nanoparticles**.**

Concentration (µg/ml)	DMZnONp	BHT
20	16.22 ± 0.64[a]	31.53 ± 0.64
40	17.72 ± 0.37[a]	38.44 ± 0.37
80	46.25 ± 0.74	53.15 ± 1.10
160	65.06 ± 0.64	71.47 ± 0.37
320	70.27 ± 0.64[a]	80.18 ± 0.64
IC_{50} (µg/ml)	129.47	98.61

Results were expressed as mean ± S.D. Mean[a] significantly different (P ≤ 0.05) from standard BHT at the same concentration.

Table 2 Percentage (%) H_2O_2 Scavenging Activities of *Detarium microcarpum* Methanol Extract and *Detarium microcarpum* ZnO Nanoparticles.

Concentration (µg/ml)	DMZnONp	BHT
20	1.49 ± 0.11[a]	22.57 ± 0.19
40	4.81 ± 0.21[a]	46.19 ± 0.19
80	36.22 ± 0.49[a]	53.46 ± 0.21
160	48.91 ± 0.28[a]	67.10 ± 0.11
320	77.69 ± 0.37[a]	86.79 ± 0.47
IC_{50} (µg/ml)	207.46	97.55

Results were expressed as mean ± S.D. Mean[a] significantly different (P ≤ 0.05) from standard BHT at the same concentration.

Table 3 Ferric Reducing Antioxidant Power of *Detarium microcarpum* Methanol Extract and *Detarium microcarpum* ZnO Nanoparticles.

Concentration (µg/ml)	DMZnONp	BHT
20	0.17 ± 0.007[a]	0.55 ± 0.003
40	0.34 ± 0.003[a]	0.64 ± 0.003
80	0.70 ± 0.005[a]	0.85 ± 0.005
160	0.85 ± 0.003	0.91 ± 0.003
320	0.92 ± 0.008	0.92 ± 0.010

Results (absorbances) were expressed as mean ± S.D. Mean[a] significantly different (P ≤ 0.05) from standard BHT at the same concentration.

3.3 *In vitro* Anti-Breast Cancer Studies

3.3.1 Cell Viability Assay

The cytotoxic potential of DMZnONPs on breast cancer cells was assessed by an MTT assay. The cells were treated with different concentrations (9.77-5000 µg/ml) DMZnONPs and assessed at 24h and 72 hours' time interval. The DMZnONPs treatment significantly decreased the viability of MCF-7 cancer cells at higher concentrations (1250-5000 µg/mL) compared to untreated cells and other concentrations after 24 h and 72 h stand point (Figure 4 & 5). The percentage of cell viability reduced gradually with an increase in the concentration. The IC_{50} of DMZnONPs at 24 h standpoint (113.7 µg/mL) and 72 h standpoint (70.48 µg/mL) indicate significant decrease in MCF-7 cells viability with treatment duration. Cytotoxicity of DMZnONPs is hence typically achieved by promoting apoptosis through generation of reactive oxygen species (ROS) and caspase activation, with efficacy strongly tied to the size and surface chemistry of the test compound (Gavanji *et al*., 2023)

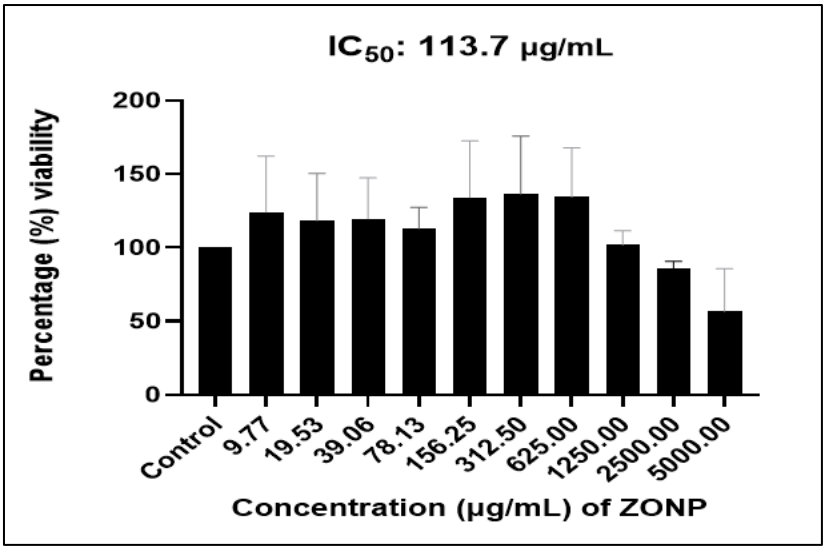

Figure 4 Cell Viability of Human Breast Adenocarcinoma (MCF-7) Cells Treated with Zinc Oxide (ZnO) Nanoparticles (ZONP) after 24 hours

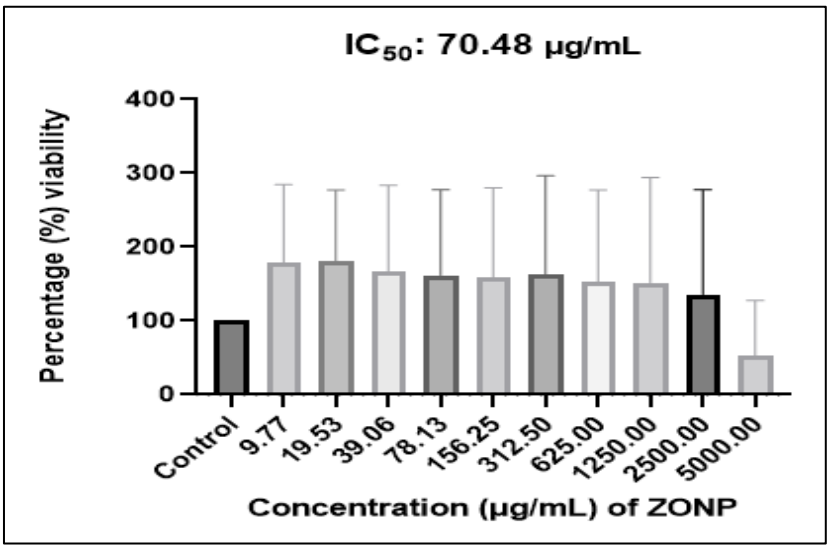

Figure 5 Cell Viability of Human Breast Adenocarcinoma (MCF-7) Cells Treated with Zinc Oxide (Zno) Nanoparticles (ZONP) After 72 Hours

3.4 The Effect of DMZnONPs on cell morphology of MCF-7 cancer cells

The cell morphological analysis of DMZnONPs treated breast cancer cells was observed in an inverted phase-contrast microscope. The micrograph of the MCF-7 cells treated with varying concentration of the test compound and 5-fluorouracil standard (312.48 – 5000 µg/ml) indicated significant changes in the normal morphology of untreated MCF-7 cells characteristic of apoptotic cells, such as cell shrinkage and reduced cell density (Figure 6-15) after 24 h. The increased morphological alterations observed at 72 h stand point also indicates improved prognosis with time almost at all concentrations of the test compound. MCF-7 cancer cells treated with the higher concentrations (1250 – 5000 µg/ml) also displayed other types of morphological changes such as rounded up cells that shrink and lose contact with neighboring cells, indicating a drastic decline in cell viability. Previous studies on the mechanism by which NPs exert cytotoxic effect asserts that the extremely small (<100 nm) Nanocomposites are coopted through endocytosis or macropinocytosis, where they accumulate in the endosomes and multivesicular bodies (Varma *et al.*, 2022). Once inside, they cause dysfunction to cellular organelles, reactive oxygen species (ROS) generation, which leads to mitochondrial membrane potential loss and the activation of caspase-3 and 7 pathways.

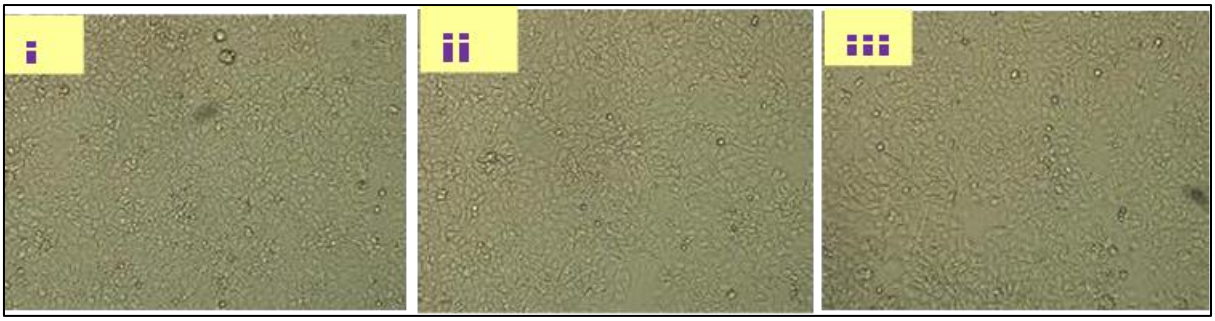

Figure 6 Morphological Changes and Viability Of MCF-7 Cells Treated With 312.48 µg/Ml After 24 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

Figure 7 Morphological Changes and Viability Of MCF-7 Cells Treated with 312.48 µg/Ml After 72 Hours (I) Untreated MCF-7 Cells , (ii) DMZnONPs, and (iii) 5FU

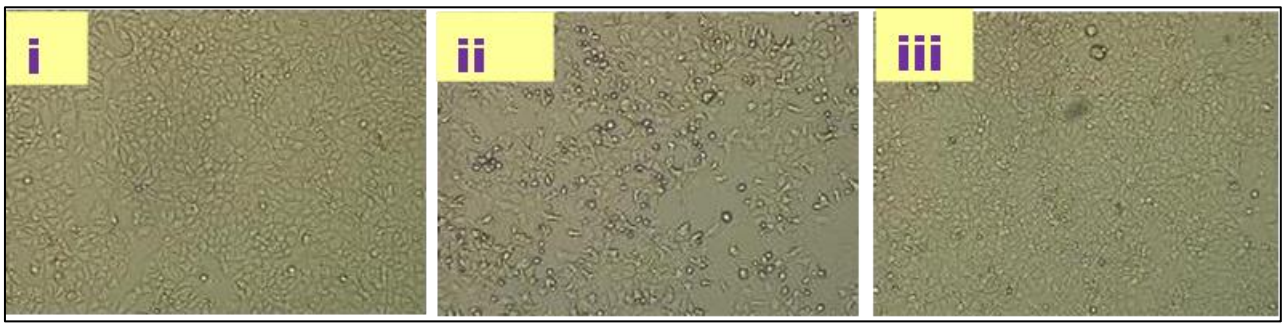

Figure 8 Morphological changes and viability of MCF-7 cells treated with 625 µg/Ml After 24 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

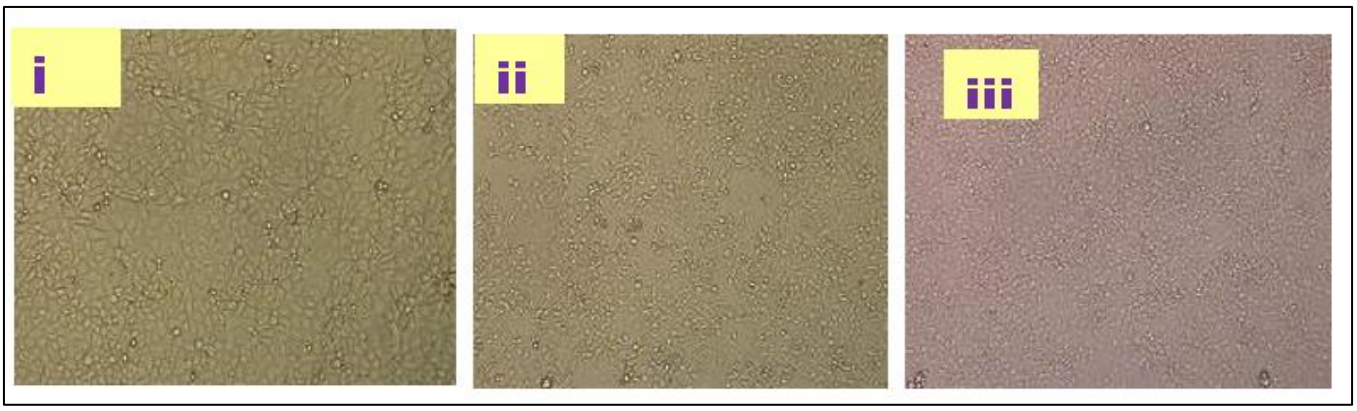

Figure 9 Morphological Changes and Viability Of MCF-7 Cells Treated With 625 µg/Ml After 72 Hours (I) Untreated MCF-7 Cells , (ii) DMZnONPs, and (iii) 5FU

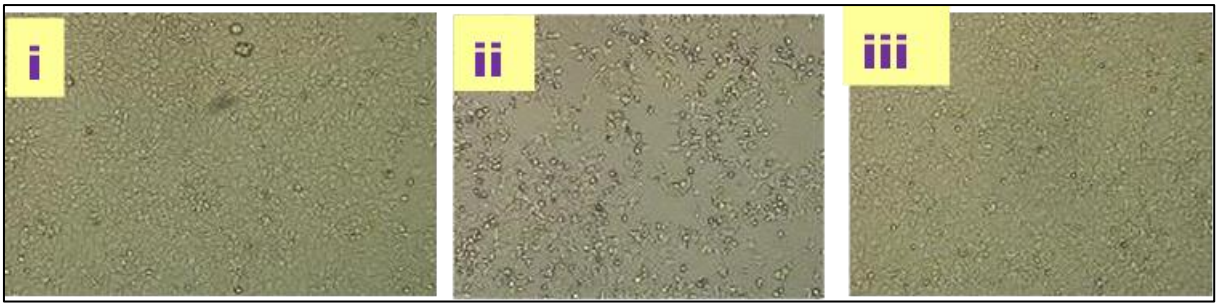

Figure 10 Morphological Changes and Viability Of MCF-7 Cells Treated With 1250 µg/Ml After 24 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

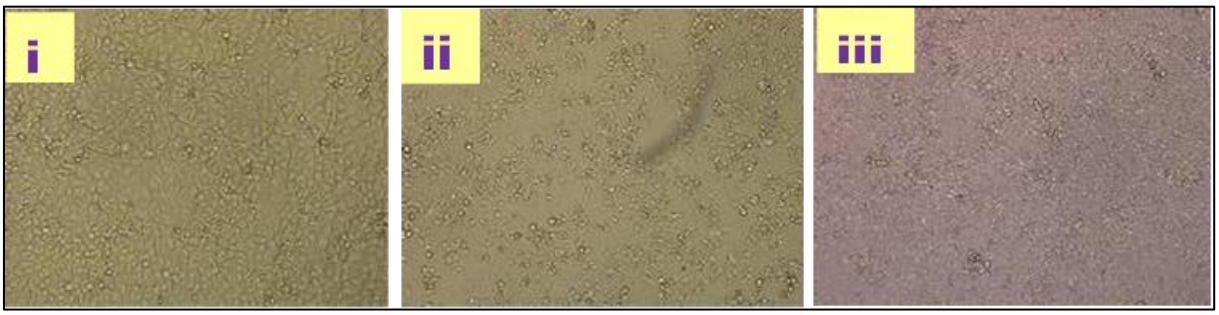

Figure 11 Morphological Changes and Viability Of MCF-7 Cells Treated With 1250 µg/Ml After 72 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

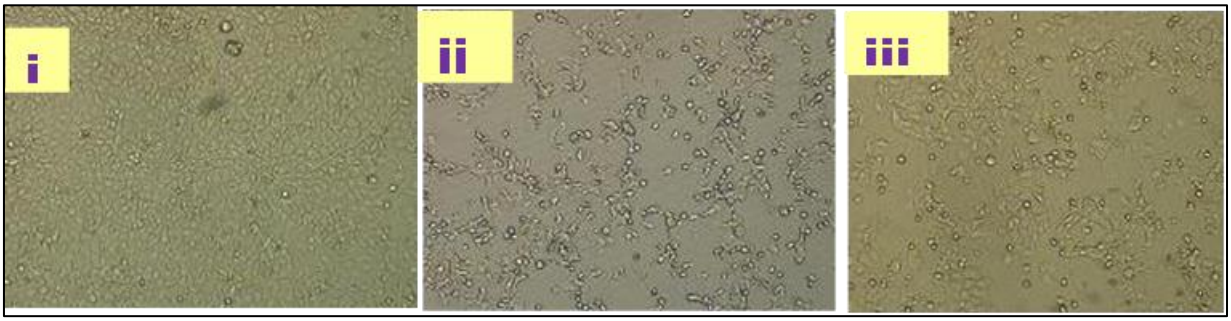

Figure 12 Morphological Changes and Viability Of MCF-7 Cells Treated with 2500 µg/Ml After 24 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

Figure 13 Morphological Changes and Viability Of MCF-7 Cells Treated with 2500 µg/Ml After 72 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

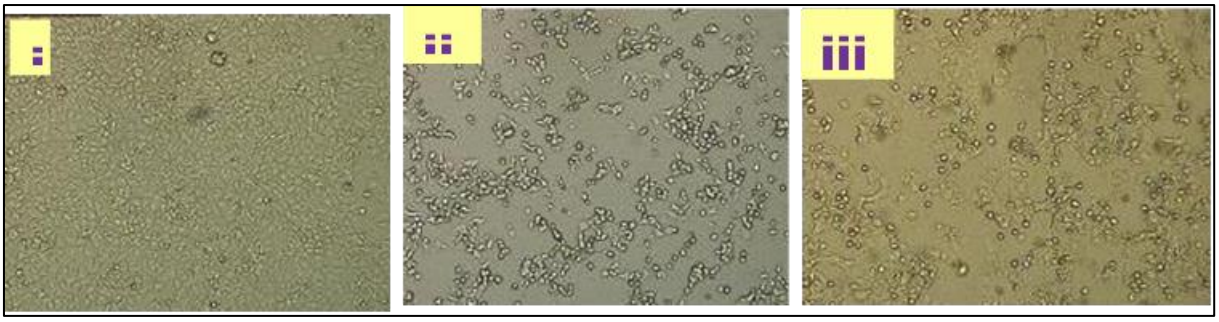

Figure 14 Morphological Changes and Viability Of MCF-7 Cells Treated with 5000 µg/Ml After 24 Hours (I) Untreated MCF-7 Cells, (ii) DMZnONPs, and (iii) 5FU

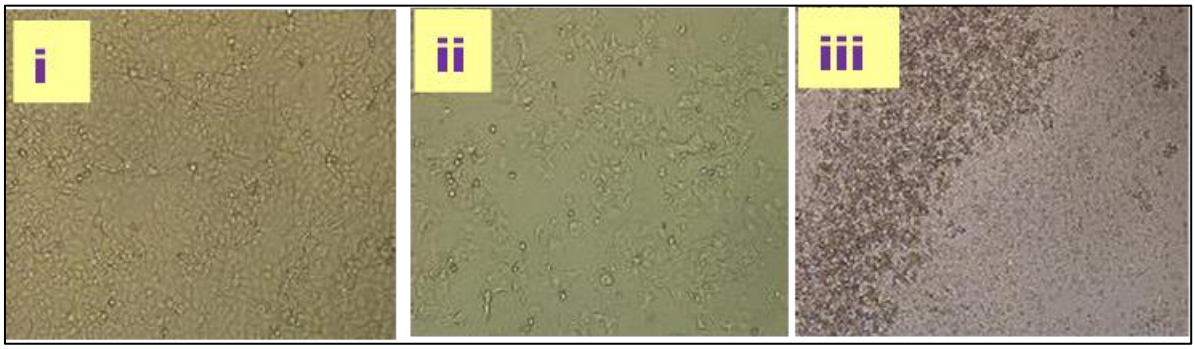

Figure 15 Morphological Changes and Viability of MCF-7 Cells Treated with 5000 µg/Ml After 72 Hours (I) Untreated MCF-7 Cells , (ii) DMZnONPs, and (iii) 5FU

4 Conclusion

Detarium microcarpum methanol stembark extract contains an array of bioactive components capable of serving as bioreductants and capping agents in the synthesis of zinc oxide nanoparticles. The characteristic small size (majority of which are 89 nm) green synthesized ZnO nanoparticles in this study optimized the ability of participating bioactive components to mediate biological oxidation *in vitro*. Furthermore, the green synthesized zinc oxide nanoparticle demonstrated ability to inhibit growth and induce apoptosis in MCF-7 cells *in vitro.* The morphological findings in this study demonstrate that DMZnONPs treatment acts as a highly effective, specific therapeutic agent, disrupting both the surface and internal structure of breast cancer cells and leading to programmed death.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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