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SYNTHESIS OF AQUEOUS CARICA PAPAYA LEAF BASED TiO_2 NANOPARTICLES IT'S ANTI-INFLAMMATORY, ANTI – CANCER ACTIVITY AGAINST MCF-7 CELL LINE

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ABSTRACT

Green synthesis is an easy, safe, affordable and environmentally beneficial method of creating nanoparticles. In this present study aqueous extract of Carica papaya leaves was used as a capping agent to prepare photosynthesized TiO_2 nanoparticles. The formation of TiO2 nanoparticle was confirmed by taking UV-Visible spectra which shows a maximum absorbance in the range of 314nm.The synthesized TiO_2 nanoparticles were characterized using XRD, SEM, SEM-EDAX to determine the size, surface morphology, elemental composition. The XRD data revealed that the nanoparticles prepared was to about 19.79nm in size, SEM images predicts that TiO_2 nanoparticles are spherical and EDAX spectra confirms the presence of Titanium and Oxygen. Anti-inflammatory activity reveals that biosynthesized TiO_2 nanoparticles exhibit good inhibitory activity than the standard used. The synthesized nanoparticles showed dose dependent cytotoxicity towards MCF-7 cell line and shows an IC50 value of 42.59 µg/ml.

Keywords: Carica papaya, Titanium dioxide, Anti-inflammatory, Cytotoxicity, MCF-7 Cell line

1. Introduction

Nanomaterials, which are defined as materials with dimensions less than 100 nm, are used in a wide range of fields, including medicine, biotechnology, microbiology, pharmaceutics, chemistry, engineering, low-cost catalysts, cytotoxicity research, and more. They also have special chemical, physical, electrical, and mechanical capabilities [1-3]. Physical and chemical approaches are used for the creation of nanomaterials because of their huge surface area. However, due to their detrimental effects on the environment, these techniques are not appropriate for use in biological or therapeutic settings. Consequently, because the green synthesis method is straightforward, economical, and environmentally benign, researchers are choosing it as a means of producing nanomaterials [4, 5]. An intriguing technique in material science is green synthesis [6–8].Titanium dioxide is a cheap, harmless and inert substance, its remarkable UV absorption capacity and high refractive index make it an intriguing white pigment and eco-friendly catalyst [9] .Titanium dioxide nanoparticles have drawn a lot of attention due to its remarkable properties like antibacterial, antifungal, UV-filtering properties, catalytic and photochemical activities [10, 11].Due to its exceptional physical stability and nontoxicity, Titanium dioxide has been embraced by many as a versatile metallic oxide among other metal or metal oxide nanoparticles. When compared to other antimicrobial agents, TiO2 has drawn a lot of interest because of its broad-spectrum antibacterial activity, stability, safety, and environmental friendliness. [12, 13].

In the present study TiO_2 nanoparticles are prepared using Carica papaya leaf extract and evaluating its anti-inflammatory and anticancer activity against MCF-7 cell line.

2. Materials and methods

The precursor, Titanium tetra chloride, was purchased from Sigma Aldrich in India, to prepare Titanium dioxide nanoparticles. Human breast cancer lines were used to test the cytotoxicity of TiO_2 nanoparticles and for anti-inflammatory activity RAW 264.7 cells were used which were purchased from NCCS, Pune.

2.1 Collection of Carica papaya leaves and making an aqueous extract

Papaya leaves free of disease were taken from Annai Velankanni College campus at Tholayavattam. To get rid of ant dust, the leaves are properly cleaned with running tap water and

then with distilled water. After being cleansed, 10 grams of Carica papaya leaves were crushed, and 50 milliliters of double- distilled water were added. Then the extract was boiled for about 20 minutes at 800˚C in a heated mantle. The extract was then drained through Whatmann no. 1 filter paper after being fully cooled to room temperature.

2.2 Preparation of TiO_2 nanoparticles

10 ml of plant extract was added at 25˚C while the mixture was being swirled for two hours using a magnetic stirrer to mix 20ml of 1mM Titanium tetra chloride in a conical flask. The extract's color changed from yellow to golden yellow after four hours, the appearance of golden yellow denotes the development of Titanium dioxide nanoparticles, which was further verified by UV- Vis spectroscopy. After that, the solution was filtered and dried at 110˚C for 5 hours. Then the dried samples was calcined in muffle furnace at 500˚C for about 2 hours. Then the sample is powdered and used for further analysis [14].

2.3 Characterization of Titanium dioxide nanoparticles

The produced nanoparticles were examined using a Deep vision 2373 spectrophotometer in the range of 200-800nm. The sample's reproducibility for the absorption value was noticed [15] Utilizing Cu αk incident radiation, XRD was utilized to ascertain the nanoparticle size and type of TiO_2 nanoparticles. The analysis was conducted at 30 KV and 30 Ma current. SEM and EDAX analysis were used to examine the morphology and elemental composition respectively.

2.4 Anti-inflammatory activity

RAW 264.7 cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and 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% CO_2 incubator. The cells were grown to 60% confluency followed by activation with 1µL lipopolysaccharide (LPS: 1µg/ml). LPS stimulated RAW cells were exposed with different concentrations (25, 50, 100 µg/ml) of sample solution and incubated

for 24 hours. After incubation the anti- inflammatory assays were performed using the cell lysate.

Cyclooxygenase (COX) activity

100µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L, and haemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated

after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 200µL of 1% thiobarbiturate, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm [16].

Calculation

Percentage inhibition of the enzyme was calculated as,

% inhibition = (Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Lipoxygenase (LOX) activity

Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL). The LOX activity was monitored as an increase of absorbance at 234 nm (Shimadzu), which reflects the formation of 5-hydroxyeicosatetraenoic acid [17].

Calculation

Percentage inhibition of the enzyme was calculated as,

% inhibition = (Absorbance of control-Absorbance of test)/Absorbance of control) × 100

2.5 Invitro cytotoxic study (MTT assay)

MTT diagnosis helps in determining the percentage of cell viability by treating the live cells with biosynthesized nanoparticles and incubating them for 24 hours. Mitochondrial lactate dehydrogenase produced by viable cells reduces MTT to insoluble formazan crystals, which was further dissolved using a suitable solvent which forms purple colour, and the colour intensity is propotional to the number of viable cells and be measured spectrophotometrically at 570nm [18,19].

Maintenance of cell lines

The MCF-7 (Human breast cancer cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM media with NEAA mixture supplemented with 10% FBS along with 1% antibiotic – antimycotic solution in the atmosphere of 5% CO_2 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub- cultured for every 2 days. Passage No -41 was used for the present study.

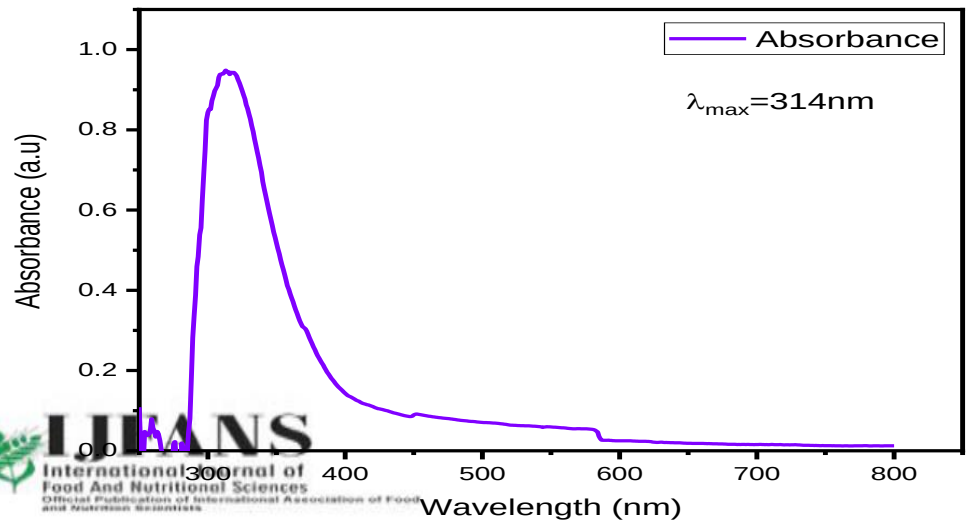
Cell viability assay

Seed 200µl cell suspension in a 96- well plate at required cell density (20,000 cells per well), and allow the cells to grow for about 24 hours. Then various concentrations of TiO_2 nanoparticles were added to cells in the plate and incubated again for 24 hours at 37^0C in a 5% CO_2 atmosphere. After incubation, the plates are taken out from the incubator and remove spent media and add 0.5mg/ml of MTT reagent to each well and incubate for 3 hours. Then the MTT reagent is removed and 100µl of DMSO solution is added to dissolve the purple formazan crystals. The absorbance is readed using an ELISA reader at 570 nm wavelength.

3. Results and discussion

3.1 UV-vis spectral analysis

The titanium dioxide nanoparticle prepared using titanium tetra chloride and Carica papaya leaf extract as capping agent shows a peak of λ_{max} 314nm with an absorbance of 0.94 a.u within the absorbance range of 200 -800nm shown in figure 1. The results of the current study are comparable with Mobeen Amanulla et al, with that on orange peel extract where the absorbance



peak was observed at 315nm [20].

Figure 1 UV-Visible spectra of TiO_2 nanoparticles

3.2 X- diffraction studies

The X- ray dlffractogram of biosynthesixed TiO2 nanoparticles showed diffraction peaks at 2θ i.e 28.04, 30.36, 40.29, 49.94, 66.16 which corresponds to (110) (121) (022) (212) (221) . The intense peak at 28.04 matches the (110) crystallogrphic plane of rutile form of TiO_2 nanoparticles showed that the formed TiO_2 nanoparticle is in rutile shape while comparing with the Joint Committee on powder Diffraction standards (JCPDS) data (File no 88-1175).

The average particle size was computed using Debye-Scherrer's formula

$$D = k\lambda/\beta\cos\theta$$

Where D is the average particle size in nm.

λ is the X-Ray wavelength (0.15406nm)

β is the full width at half maximum of the diffraction peak

K is an debye scherrer constant with value of 0.9 to1

θ is the Bragg diffracting angle.

The size of the synthesised Titanium dioxide nanoparticles from the XRD data was estimated as 19.79nm.

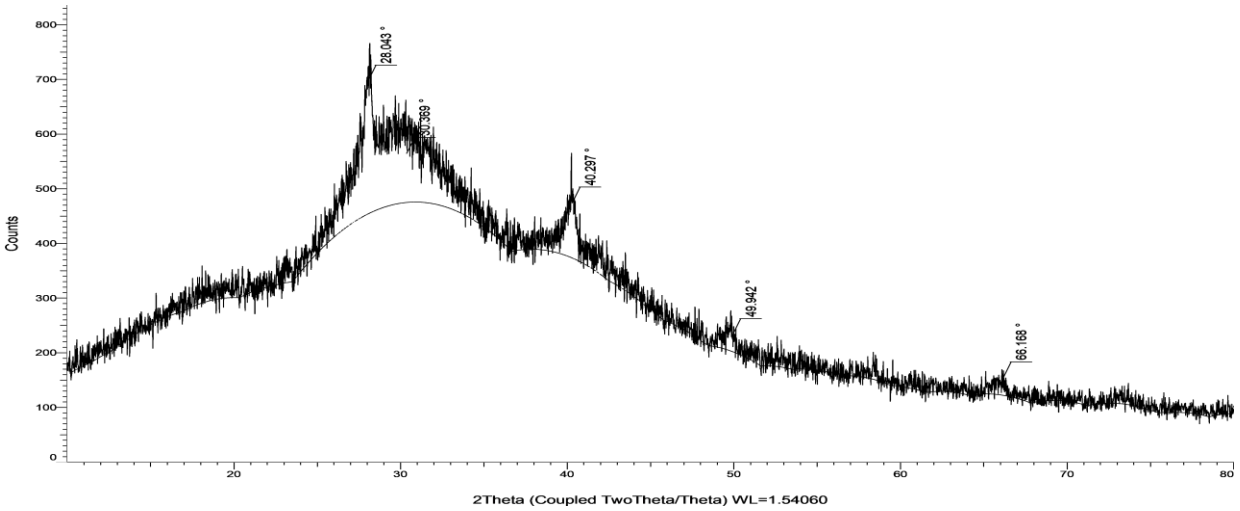
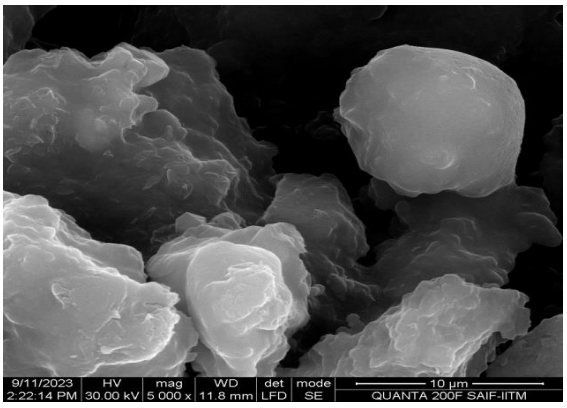


Figure 2 XRD pattern of TiO_2 nanoparticles synthesized from Carica papaya leaf extract

3.3 SEM analysis

SEM analysis provide informations that helps to recognize the shape ,size, surface arrangenent of phytosynthesized Titaniun dioxie nanoparticles.The SEM image of biosynthesised TiO_2 nanoparticles using carica papaya leaf extract at differerent magnification are shown below in the figure 3(a) & 3(b) in that images some of the particle shows spherical shape and some of them are gathered which looks like flakes. If the range of the nanoparticles reduced to smaller size,

results in enlarged surface area to volume ratio [21,22].



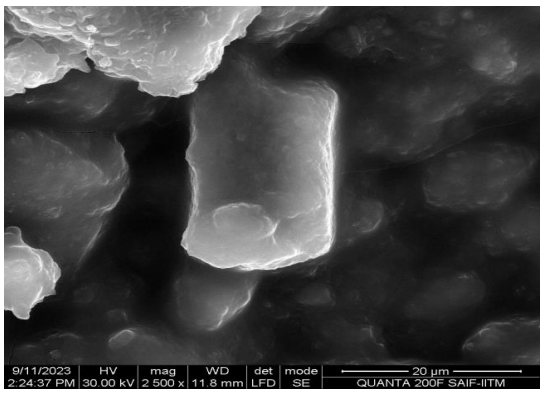
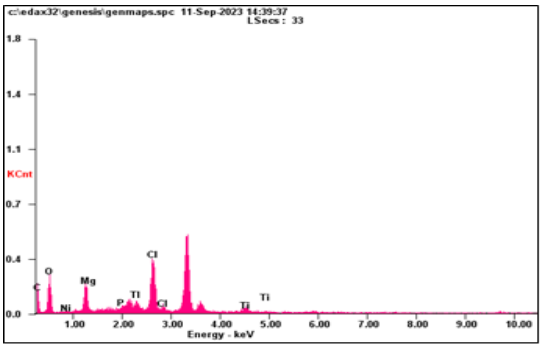


Fig 3(a) & 3(b) SEM images of TiO_2 nanoparticles synthesized from Carica papaya leaf aqueous extract at 10&20µm magnification.

3.4 SEM- EDAX analysis

SEM-EDAX examines the electron – sample interactions that takes place in an interaction volume that is usually 1to 5µm in depth, which is dependent on the sample density and energy of electrons. EDAX analysis provides information about the elemental composition present in the green synthesized TiO_2. The spectrum portrayed the peaks of various elements such as Titanium carbon, oxygen, Potassium, Magnesium and also their atomic and weight percentage. Vitamins and Minerals in Papaya leaves are the reason for the existence C and k peaks in EDAX spectra. The percentage of Ti and O in the EDAX spectra is 4.88, 3.85 for Ti and 39.08, 32.13 for oxygen at 5, 2µm magnification.



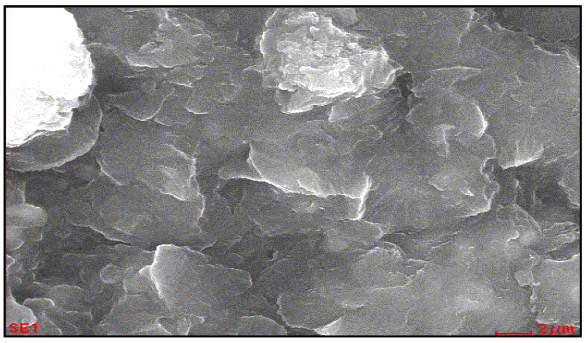
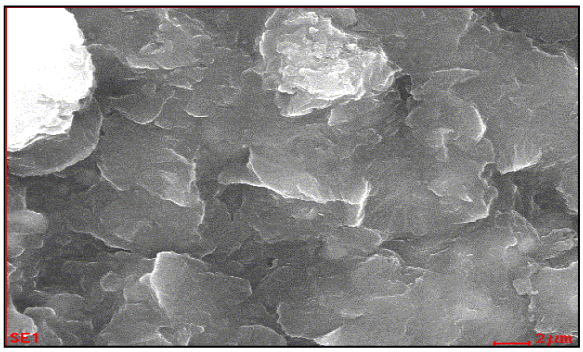


Figure 4(a) & 4(b) SEM –EDAX image of TiO_2 nanoparticle at 2µm magnification



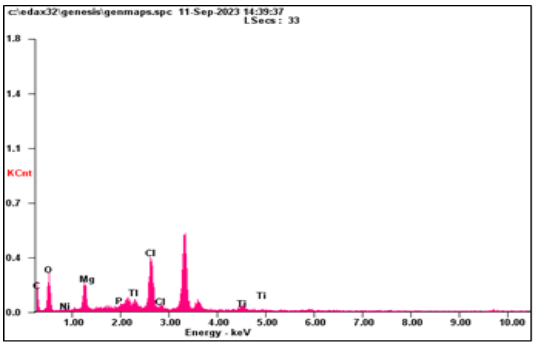


Fig 5(a) & 5(b) SEM –EDAX image of TiO_2 nanoparticle at 2µm magnification

3.5 Anti- inflammatory activity

Cyclooxygenase (COX) and Lipoxygenase (LOX) assay

Exaggerated immune reaction to viruses, damaged cells, irritants and damaging stimuli is what the living immune system displays as inflammation. COX and LOX enzyme plays an important role in inflammatory response [23]. LPS stimulated raw cells are treated with different concentration of TiO_2 nanoparticles shown in table1 and 2. The biosynthesized TiO_2 nanoparticles show the inhibition of COX-LOX enzyme in a concentration dependent manner. The inhibition of cox and lox enzyme elevated by increasing the concentration of TiO_2 nanoparticles Aspirin was used as a standard drug which shows an inhibitory activity of 73.98 % (Cox assay), 56.11% (Lox assay) and the synthesized TiO_2 nanoparticle (COX assay) shows 81.93% and for (LOX assay) at a concentration of 100µg/ml. Then the IC50 value was calculated and found to be 40.22µg/ml (COX assay) and 65.85µg/ml.The percentage of inhibition was noted higher for TiO_2 nanoparticles.TiO_2 nanoparticles inhibited both of the enzymes therefore it has the potential to treat inflammatory diseases and to be developed as anti-inflammatory drugs.

Sample Concentration (µg/ml)	OD at 632 nm	Percentage inhibition
Control	0.2275	
Aspirin (54%) 100-Standard	0.0592	73.98
25	0.1508	33.71
50	0.0758	66.68

100	0.0411	81.93

Table 1 The percentage inhibition of COX enzyme by TiO_2 nanoparticles.

Sample Concentration (µg/ml)	OD at 234 nm	Percentage inhibition
Control	0.0622	
Aspirin (54%) 100- Standard	0.0273	56.11
25	0.0425	31.67
50	0.0366	41.15
100	0.0208	66.55

Table 2 The percentage inhibition of LOX enzyme by TiO_2 nanoparticles

3.6 Anti- cancer activity of biosynthesized TiO_2 nanoparticles

Cytotoxicity of biosynthesized TiO_2 was examined by MTT assay against MCF-7 cell line and this assay was performed to confirm the capability of Titanium dioxide nanoparticles as a curative agent for breast cancers in human. Different concentrations 12.5, 25 50, 100, 200µg/ml of biosynthesized TiO_2 nanoparticles was added to Human breast cancer cell line and incubated for about 24 hours. Camptothecin 20 uM were used as a positive control (standard drug) and at this concentration the percentage of live cell was monitored as 54.29% for Human Breast cancer cell line. After adding Titanium dioxide nanoparticle to Human breast cancer cell line the percentage of viable cells becomes 47.61% % at a concentration of 50µg/ml and it decreases by increasing the concentration and it becomes 3.12 % at concentration of 200µg/ml. From this

assay, Cytotoxicity of bio fabricated TiO_2 nanoparticles against MCF-7 cells are decided as concentration dependent. The IC50 value was calculated using the following equation Y=Mx+C and studied as 42.59 µg/ml. The presence of capping elements from the plant extract on the synthesized TiO_2 nanoparticles was likely the cause of their cytotoxicity. Additonally, the plant extract may have supplied excess electrons to the TiO_2 nanoparticles, which may have encouraged the accumulation of reactive oxygen species on the surface of MCF-7 cell line [24].

Culture Condition	% Cell viability ± SD
Untreated	100±0.19
Camptothecin	54.29±0.66
TiNPs-12.5ug	82.40±1.65
TiNPs-25ug	68.26±0.24
TiNPs-50ug	47.61±1.35
TiNPs-100ug	25.24±1.34
TiNPs-200ug	3.12±1.17
IC50 – 42.59µg/ml	

Table 3 Percentage of cell viability of TiO_2 nanoparticles against MCF-7 cell line.

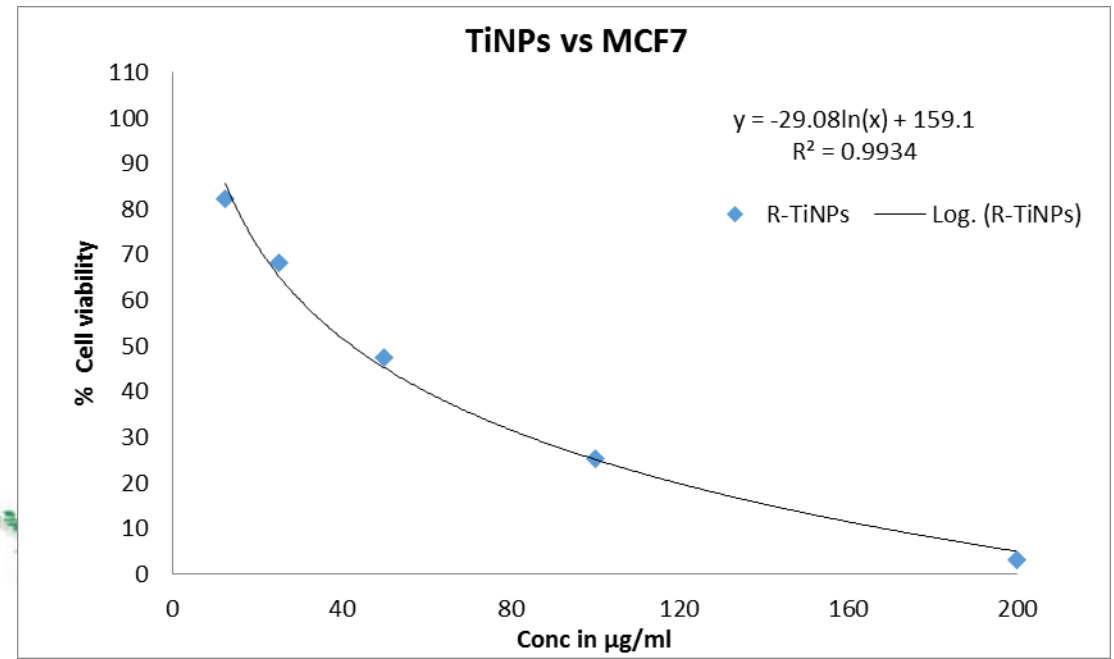
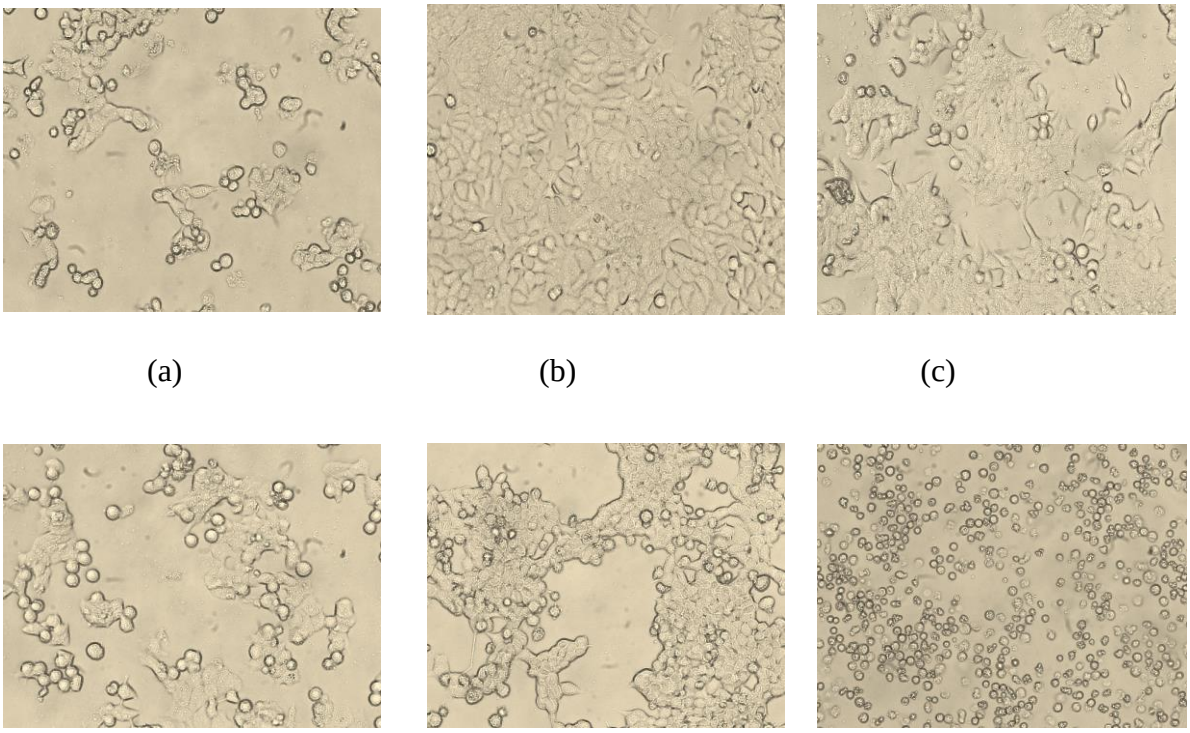


Figure 6 Graphical representation of MTT assay



(a) (b) (c)

(d) (e) (f)

Figure 7 Morphological changes takes place on MCF-7 cell line while adding (a) Standard, TiO_2 nanoparticles at different concentrations (b) 12.5µg/ml (c) 25µg/ml (c) 50µg/ml (d) 100µg/ml (e) 200 µg/ml.

Conclusion

By employing Carica papaya leaf extract as a capping agent, Titanium tetrachloride was converted to titanium dioxide nanoparticles by a green method of synthesis. The formation of nanoparticles is predicted by the solution's color shift from yellow to a light orange-yellow, which is then verified by studying the nanoparticles using a variety of techniques, including UV-Visible, XRD, FT-TR, and SEM with EDAX.The highest absorption is seen in the UV spectra at 314 nm. The XRD data indicated that the nanoparticle's was 19.74 nm in size and structure was

routile. The spherical nature of TiO2 and the presence of Ti and O are determined by SEM with EDAX. Anti-inflammatory activity revealed that the TiO_2 nanoparticles inhibits COX and LOX enzyme responsible for inflammation. The MTT assay results suggest that TiO_2 nanoparticles was effectively cytotoxic as well as anti- cancer in nature with low IC50 value on human breast cancer cells after the incubation of 24 hours. Hence the synthesized TiO_2 nanoparticles show safe and effective therapeutic applications.

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