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Research Article

In Vitro Anticancer Activity of Biogenic Tio_2nps against Breast Cancer, Lungs Cancer and Prostate Cancer

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ABSTRACT

In this research, we report a novel plant-mediated method as for manufacture of titanium dioxide nanoparticles. Utilizing the titanium oxide derivative and a plant extract from the native medicinal herb Leucas cephalotes, the nanoparticles were effectively created. The reaction temperature was kept between 75°C and 80°C while 1M of TiO2 and the plant extract were being processed. The resulting white colored paste was completely parched, gathered, and packaged to more investigation. The absorption peak for titanium dioxide nanoparticles in the UV-Vis spectrometer was seen at 212 and 345 nm. The typical dimension of the nanoparticles was determined to be 38.99 nm from the XRD pattern. According to the findings, titanium and oxygen are composed with high energy signals of 61.27% and 23.16%, respectively. The Ti-O bonding absorption peak, as determined by FT-IR spectroscopic investigation, is situated at 586 cm1. SEM (scanning electron microscopy) was employed to confirm the spherical shape of the synthesized TiO2NPs. The creation of nanoTiO2-NPs is confirmed by the several characterization techniques used. The green synthesis-produced titanium dioxide nanoparticles could be used to treat a variety of malignancies. MTS and MTT assays were used to assess the anticancer activity of the biogenic titanium dioxide oxide nanoparticles. The IC50 values of the produced were 1.89, 2.00, 1.98, and 4.00 µM, respectively, against the MCF-7, HeLa, PC-3, and A549 nanoparticles cancer cell lines.

Keywords: Biogenic synthesis TiO2, Characterization, Anticancer activity

INTRODUCTION

An expanding discipline, nanotechnology is becoming more and more significant in all industries. Owing to its affordability and sustainability friendliness, biosynthesis of nanoparticles has recently attracted a lot of interest. tiny objects that operate as a single unit in terms of their properties and transport are called nanoparticles, which are 10-9 nm in size. Metallic nanoparticles have been made using a variety of techniques, but in general, they can be made using chemical, physical, and biological techniques [1, 2] Chemical and physical techniques typically cost a lot of money and result in adverse environmental effects.

One of the most crucial ingredients in toothpastes, toothpaste tubes, paints, plastics, papers, inks, and food colouring is titanium dioxide nanoparticles which are also, employed in cosmetic products medicines, and skin care products [3,4]. TiO_2 NPs also whiteness and opacity are provided to a multitude of things, like paints, resins, papers, inks, and tubes of toothpaste. The synthesis of nanoparticles using plants as catalysts has expanded significantly in recent years. However, the physical and electromagnetic properties of ZnO and TiO_2-NPs make them perfect for application in photo and sono-dynamic processes. [5] Many scholars have studied the production of titanium dioxide. Bacteria and plant extract were used in the investigation. Titanium oxide is an oxide of metal that displays n type wild band semiconductor properties as well as stable high photocatalytic activity and strong oxidising power.

According to the United Nations, titanium dioxide is very poorly soluble, thermally stable, and not regarded as harmful. Titanium is a sturdy metal that lusters and resists corrosion.

However, a lot of researchers haven't studied a lot of prospective plants that could be a biosynthesis reducing agent. The reducing agent utilized in this study to create TiO_2 nanoparticles is Leucas cephalotes, also known as dronapushpi. This particular leaf extract was chosen because of its phenolic makeup, which functions serving as a capping or lowering agent during the creation of nanoparticles. Making extract from plants, mixing it with the metallic solution, and creating biocompatible nanoparticles are

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the stages a component of the plant-based extraction method used to create nanoparticles. Then, using methods such as FTIR, SEM, TEM, XRD, and others, the nanoparticles were characterized; the specifics of these methods are covered in the session that follows. Leaf extracts of Catharanthus roseus and Bacillus subtilis were used to create the TiO_2 NPs, according to earlier reports by authors [6, 7].

MATERIALS AND METHODS

Collection of Plant Materials

The plant material was gathered from hills in the Uttarakhand area of Pauri Garhwal. Dr. Neelu Singh authenticated the plant as belonging to the Leucas Cephalotes (Dronapushpi) family of lamiaceae (Taxonomist). Jabalpur, Madhya Pradesh: National Tropical Research Institute of Forest. UK2023 is the species number.

PREPARATION OF PLANT EXTRACT

Getting The Extract From The Leaves Of The Leucas Cephalotes Plant

After being harvested from their natural habitat in Kotdwara, Leucas Cephalotes were washed with in distilled water to eliminate any remaining dust. After washing and shorting the leaves, they were dried in the shade for 3 to 4 days before being ground into a powder. A grinder was used to reduce the dried leaves to a fine powder, which was then kept for further use in the test (Leucas Cephalotes leaves powder, see in figure 1).

In a 1000 ml round- bottom flask, 8 gram of Leucas Cephalotes leaves powder was combined with 300 ml of deionized simmered for one hour in water at 85 to 90 C to extract the desired substance. After waiting 1 hour, the watery extract solution becomes a yellow, indicating that the extract had formed. After allowing the extract to cool to ambient temperature, it was filtered using Whatman no one filter paper. (Displayed in figure 2)

Tio_2 Nanoparticle Synthesis

Titanium di oxide nanoparticles were created using a solution of 1 M TiO_2 (80 ml of deionized water) and a stirrer. 20 ml of leaf extract (Leucas Cephatoltes) was included in the 1M TiO_2 the combination was a solution stirred working with a magnetic stirrer for two hours at 80 C. The hue of the solution changed from brown to whitish. (Shown in figure 3)

Following this, a solution containing nanoparticles was incubated for 24 hours before being centrifuged for 15 minutes at 1000 rpm to produce pilletes, which were then dried and stored for later research.

Figure 1: A photo of a Leucas Cephalotes plant and leaf powder.

Figure 2: Boiled leaves at Magnetic stirrer, Filteration of leaves extract, Leaf extract (*Leucas cephalotes*).

Figure 3: TiO_2 solution at magnetic stirrer, TiO_2 nanoparticles solution.

INVITRO CYTOTOXICITY STUDY

Cell Line Culture

PC-3 (Prostate Cancer Cell Line), Hela (Human Endometrial Cancer Cell Line), A459 (Human Lung Cancer Cell Line), and MCF-7 (Human Breast tumor) cells were used in this assay.

General Techniques

Cell growth inhibition MTS assay for pc-3 (prostate cancer)

MTS is a less hazardous alternative to MTT test, and the formazan generated from MTS is water soluble. The MTS experiment measures cell growth, viability, and cytotoxicity. The MTS assay process is based on viable mammalian cells reducing the MTS tertazolium to create a colored formazan dye that is lipophilic in cell culture conditions use a chemical.

Cell titer was ascertained using the cell titer 96 aqueous non-radioactive cell growth assay, as specified by the manufacturer (Promega, WI, USA). In a proliferation or cytotoxicity experiment, the amount of live cells may be measured calorimetrically using the Cell titer 96 aqueous one-solution assay. In this solution, both the MTS chemical and the PES electron-coupling reagent may be found. A coloured formazan derivative that is dissolved in tissue culture media is produced when cells bio-reduce the MTS chemical (3-(4, 5-dimethyl thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetrazolium). The cells were seeded at a density of 5,000 per well in growth media onto 96-well plates and allowed to adhere overnight. Subsequently, the cells were exposed to chalcones and their derivatives at the given doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg mL-1). Twenty microliters of cell Titer 96 aqueous solution were poured into each well after 72 hours of treatment. The plates were put back into the incubator and the lights were turned off for a period of two hours. Absorbance was determined at 490 nm against a 690 nm standard using a Spectra Max 340 microplate reader (Molecular Devices, USA). It was repeated three times to ensure accuracy. The cell viability was determined using the mentioned formula

Cell viability (%) = (AS/ AControl)

Where

AS denotes the absorbance of cells plates were inoculated with nanoparticles, A control is the absorbance of cells plates were inoculated only with broth culture.

MTS assay for HeLa cancer cell line

Method: Rather than treatment, HeLa breast cancer cells were moved to 96-well tissue culture seeded at a density of 5000 cell lines for 24 hours. After that, the medium was changed to a new medium containing different ratio of PTAE or metallic nanoparticles (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg/mL). The control was culture medium with no formulation development at all. The samples were removed after 72 hours at 37 ° C and 5% CO2, and the rinsed thoroughly twice with sterile PBS. Each well was given 20 l of MTS solution (0.5 mg/ml) and incubated at 37 C for four hours. Immediately after the removal of the medium, each well received 100 ml of DMSO to dissolve the formazan crystal formed from MTS. Cell viability was measured using only a Spectra Max 340 microplate reader (Molecular Devices, USA) at 490 nm and a reference at 690 nm.

MTS Assay against A459 (Human Lung Cancer) Cell Line

Method: MTS is a less toxic alternative to MTT assay, and the formazon developed from MTS is water soluble. The MTS Assay is often used to evaluate cell proliferation, viability, and cytotoxicity. The MTS assay method is based on the inhibition of viable mammalian cells with MTS tetrazolium compound to produce a colored formazan dye that is soluble in cell culture medium. The cell growth inhibition assay was performed according to the manufacturer's instructions by using cell titer 96 aqueous non-radioactive cell proliferation assay (Promega, WI, USA). MTS compound and the electron coupling reagent phenazine ethosuphate are both present in one solution (PES). Cells bio reduce the MTS compound (3-(4, 5-dimethylthiazol-2-yl)-5-(-3-carboxymethoxyphenyl)-2(4-sulphonyl)-2H-tetrazolium) to a colored formazan product that is soluble in tissue culture medium. A459 (Human Lung Cancer) Cell Line was transferred 24 hours before treatment to 96-well tissue culture plates at a density of 5000 cells/well.

The medium was then replaced with a new medium containing PTAE or metallic nanoparticles at different doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg mL). The control group received culture medium that was devoid of any drug formulation. Following 72 hours of incubation at 37°C and 5% CO_2, the media was removed, and the rinsed thoroughly twice with sterile PBS. Each well received 20 l of MTS solution (0.5 µg/ml) and was incubated at 37 degree for 4 hours. Following the removal of the medium, each well received 100 ml of DMSO to solubilize the formazan crystal created from MTS. Cell viability was ascertained employing spectra at 490 nm and a citation at 690 nm with a Max 340 microplate reader (Molecular Devices, USA).

MTT Assay for MCF-7 HeLa cells

The MTT colorimetric assay (dimethylthiazol-diphenyltetrazolium bromide) helps determine mitochondrial viability and thus cell viability. A mitochondrial dehydrogenase enzyme in living cells gets converted yellow tetrazolium MTT salt to blue MTT formazan, which results in undamaged cells..

Method: MTT assay was conducted the cytotoxic nature of MNPs in MCF-7 (human breast cancer) cell lines. In 96-well plate, 5,000 MCF-7 cells were seeded and incubated for a 24-hour period. Cells were also treated with TiO2NPs at various doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg) and 72 hours incubation. After that, the cells were exposed for four hours to ten litres of freshly prepared yellow MTT reagents (0.5 µg/mL). Finally, 100 µL of dimethyl sulfoxide (DMSO) was introduced, and the violet formazan solution's UV absorbance at 570 nm was measured (Multimode reader, Tecan, Austria).

The cytotoxicity of produced TiONPs against HeLa cells was tested in vitro at various doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 g/ml). The cytotoxic effect of synthesised AgNPs, AuNPs, FeONPs, ZnONPs, and ZnONPs was greater at higher concentrations (11 to 100 µg/ml), but less at lower concentrations (0.41 to10 µg/ml).

Characterization Of Titanium Dioxide Nanoparticles

Analysis of UV Vis. Spectroscopy:

Nanoparticles generated at distinct temperatures and concentrations have varied absorption spectra allowed for the observation of the optical property of created TiO2 nanoparticles. To determine their particular characteristics, the produced TiO_2-NPs were subjected to a number of analytical methods. In order to determine the surface plasmon resonance (SPR) band, the optical characteristics were recorded using Shimadzu's UV-3900 UV-Vis spectrometer. Leucas cephalotes' surface plasmon resonance-induced absorption spectra are shown in Figure 4 at wavelengths of 212, 238, 305, 366, 379, and 553. The creation of titanium dioxide nanoparticles in the nanoparticle solution mixture is demonstrated by the absorbance peak.

Fourier transforms infrared spectroscopy

This characterization method generates infrared absorption spectra that show the internal chemical bonds of synthesized nanoparticles. FT-IR Spectrophotometer Shimadzu, I.R. Affinity 1A, Japan is used for this. In order to find any potential changes in functional group bonds during the reduction process, FTIR measurements were made on the produced zinc oxide nanoparticle and the extract of Leucas cephalotes. Figure 5, 6 illustrates the FTIR spectrum for extracts of Leucas cephalotes, which showed multiple distinct bands. The functional group associated with biologically produced nan materials are identified using FTIR spectrophotometry the band of O-H, C=O are mostly ascribed to the peaks. There are peaks at 3880 cm^{-1} 2895 cm^{-1} relating to C-H in the FTIR spectra and 1506 cm^{-1} related to show C=C band and other peaks 887 cm^{-1} and 586 cm^{-1} show Ti-O_2 bond.

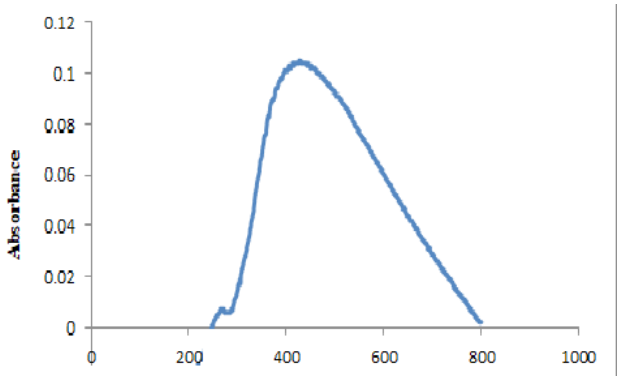

Figure 4: UV- Visible spectra of TiO_2 nanoparticles using plant extract of Leucas cephalotes.

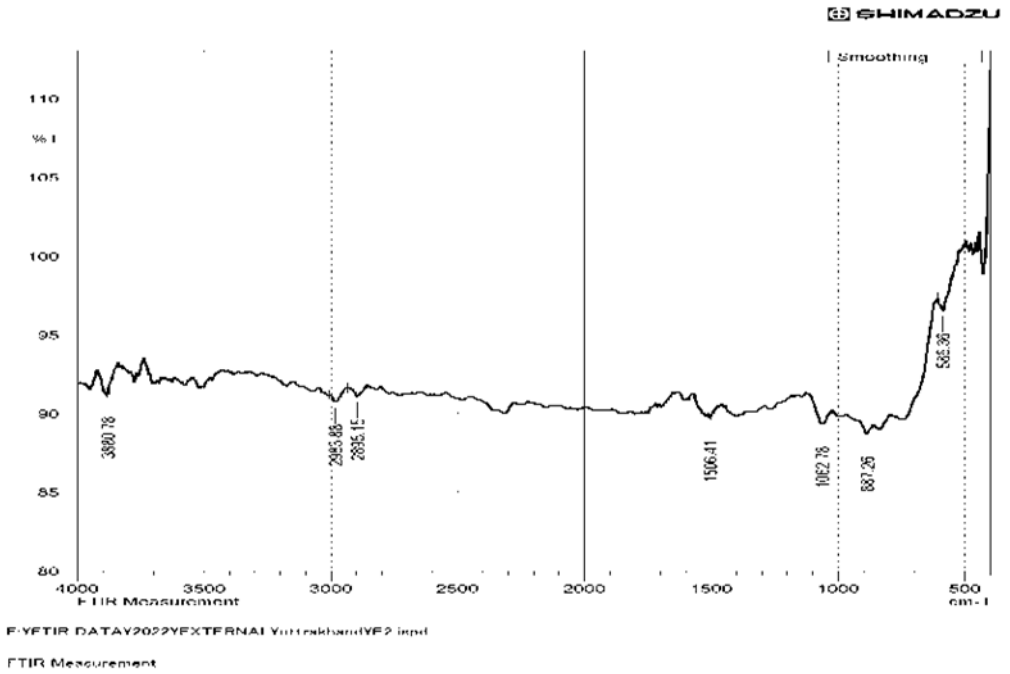

Figure 5: FTIR spectra of titanium dioxide nanoparticles using leaf extract of Leucas cephalotes.

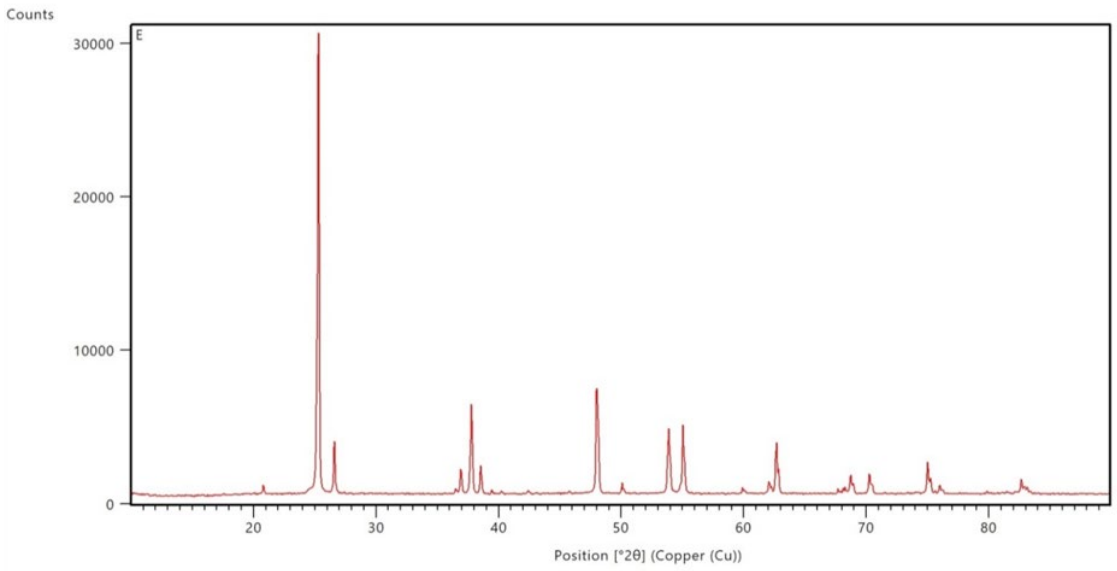

Figure 6: Illustrates the XRD spectrum of the titanium dioxide nanoparticles that were created utilising leucas cephalotes.

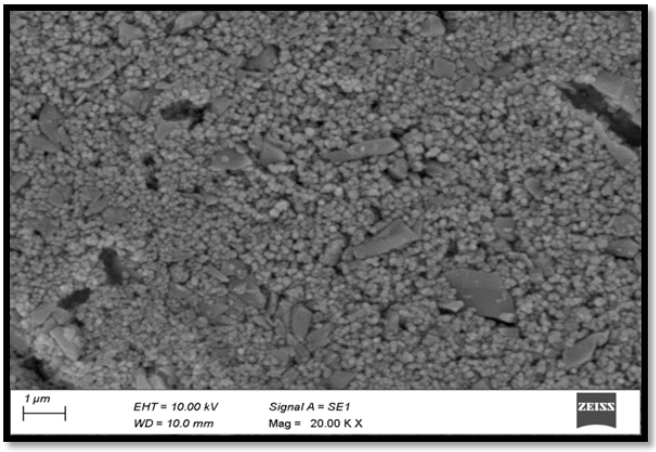

Figure 7: Titanium dioxide nanoparticle SEM results utilising Leucascephalotes plant extract (a) SEM analysis with 25 KX (b) SEM analysis with 20 KX.

Analysis of XRD

At 25, 36, 37, 74, 47, 97, 55, 12, and 62°, green synthesized TiO2 nanoparticles were seen in XRD patterns. At 65, 75, 06°, the 101, 004, 20105, 204, and 215 peaks can be found. Titanium dioxide nanoparticles created synthetically have crystalline anatase structures. These results were confirmed in conformity with JCPDS Nos. 21-1272 [8], which is the Joint Committee on Power Diffraction Standards.

Scherrer's equation (D = K/cos) was used to determine the created nanoparticles' size. The peak's line width at half its optimum height (FWHM), the diffracting angle, and the length of an x-ray photons (CuK = 0.15406 nm). The size of synthetic nanoparticles' crystals, on average, is 38.99 nm.

Scanning electron microscopy with Energy dispersive x-ray

SEM (JEOL JEM 2100, Japan) was used to observe the form and

morphology. Based on the surface investigation, measurements from SEM images were used to perform topographical analysis. Leucas cephalotes leaf extract is used to make titanium dioxide synthetically. The smooth, spherical TiO2 NPs that were created were in good condition. These results similar to Babu et al and Ganesan et al [9] [10]. The photographs of the produced nanoparticles at different magnifications (shown in figure 7) clearly demonstrate their physical form. The EDX analysis investigation establishes confirming that the particles are metallic TiO2-NPs and are crystalline in character (Figure 8). Ti (61.27%) and the weight percentages of oxide (23.16%) and other elements (16.00%) were measured by EDAX.

Particle size analysis by Zetasizer

The synthetic molecules' typical size Titanium dioxide nanoparticles were 1.62 nm and 0.210 PDI value. The obtained single peak indicates that the quality of the synthesized TIO2-NPs was good (figure 9). PDI values suggest that the synthesized silver nanoparticles are monodispersed in nature

Zeta potential analysis

The charge stability of Titanium dioxide nanoparticles can be assessed using the Zeta potential. The zeta potential represents the charge density on the surface. In the synthesis of TiO_2-NPs by leucas cephalotes measured the zeta potential of nanoparticles as -17.8 mV (figure 10).

ANTICANCER RESULTS

TiONPs Cytotoxicity study against PC-3 (Prostate Cancer) cells line

Leaf extracts of L. cehalotes plant were used to create titanium

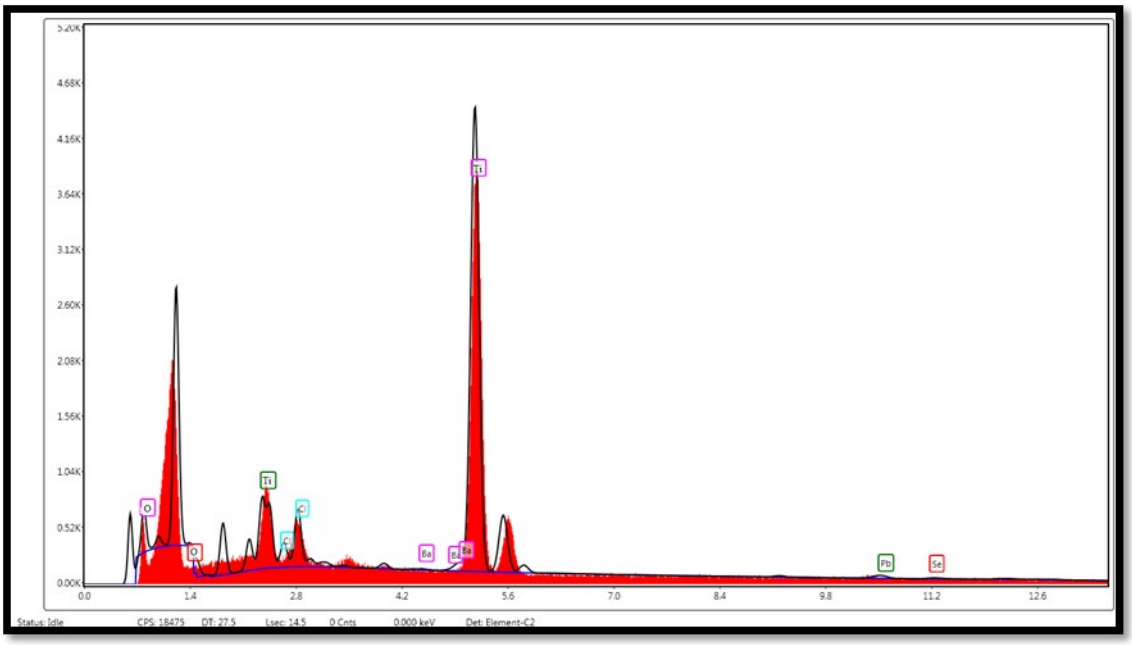

Figure 8: Show represent the EDAX image of Titanium dioxide nanoparticles using leaf extract of Leucas cephalotes.

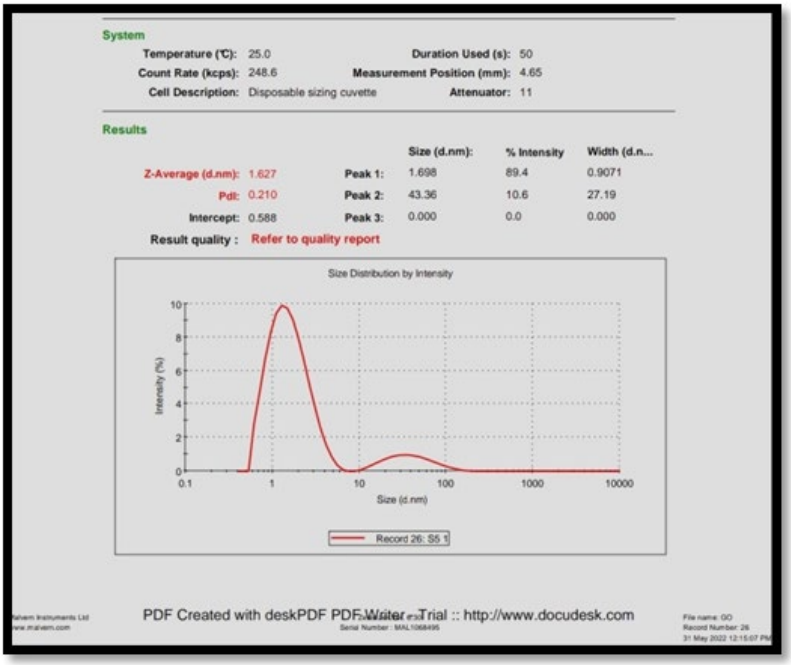

Figure 9: Particles size distribution of TiO2-NPs solution synthesized by Leucas cephalotes.

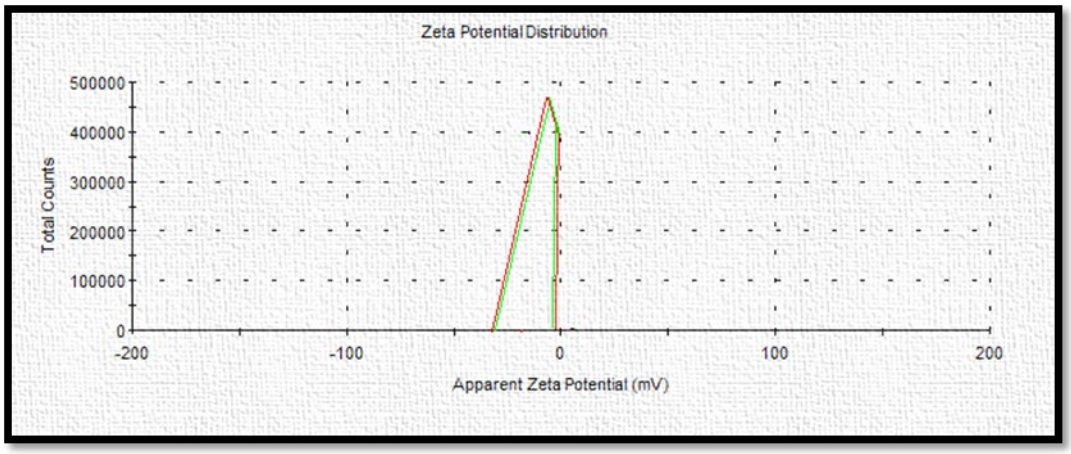

Figure 10: Zeta potential curve of Tio2-NPs solution synthesized by Leucas cephalotes.

dioxide nanoparticles. Titanium dioxide nanoparticles have historically been shown to be therapeutic in human lungs, breast, prostate, and cancer cell lines, but not normal cell lines. Titanium dioxide nanoparticles inhibited PC-3 cancer proliferation in a concentration-dependent manner. TiONPs have determined IC50 values of, 1.98 µM.

Cytotoxicity study of TiO2NPs against HeLa cancer cells line

Titanium dioxide nanoparticles made from the leaves of Leucs cephlotes. HeLa cancer growth in a concentration-dependent manner. Titanium dioxide nanoparticles have previously been reported to exhibit anti-cancer capabilities against a wide range of cancer cells. The inhibitory concentration (IC50) of TiO2 NPs on HeLa cancer cells was discovered. Its relative IC50 values were 2.0µM, correspondingly. The IC50 analysis shows were tested against cell lines and shown a potent anticancer effects at a concentration of 100 µg/ml

Cytotoxicity study of TiO2NPs against A459 (Human Lung Cancer)

Leaf extracts of Leucs cephlotes was used to make titanium dioxide nanoparticles. Synthesized TiO2NPs were tested in vitro against A459 at various dosages (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg/ml) (Human Lung Cancer). The cytotoxic effect of the TiO2NPs generated was larger at higher concentrations (11 to 100 µg/ml) but decreased at lower concentrations (0.41 to 10 µg/ml). Titanium dioxide nanoparticles revealed significant anticancer efficacy and inhibitory concentration levels in A459 cells (Human Lung Cancer). Cell viability was, 4.00 µM for titanium dioxide nanoparticles with IC50 values in the micro molar range, respectively.

Cytotoxicity study of TiO2NPs against MCF-7 cancer cells line

Leaf extracts of Abides pindrow royle, Leucs cephlotes, and Ajuga macrosperma were used to make titanium dioxide nanoparticles. After creation, TiO2NPs were tested in vitro against MCF-7 at various doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg/ml). TiO2NPs exhibit a substantial anticancer effect against MCF-7 cells at varied dosages, according to the cytotoxic experiment. When the concentration of FeONPs was increased, cell death in MCF-7 cells increased dramatically. In this study, extracts of A. pindow royle, L. cephalote, and A. macrosperma were used to create highly effective TiO2NPs with improved action against cancer cells and IC50 values in the micromolar range of 1.89 µM, respectively.

Cancer cell cytotoxicity

Commercial anti-cancer medications cisplatin and doxorubicin were used as the standard of comparison for the cytotoxicity of the produced chalcones and their derivatives toward cancer cells. Using the PC-3 (Prostate Cancer Cell Line), Hela, A459 (Human Lung Cancer Cell Line), and MCF-7 cells (Human Breast Cancer), preliminary screening using the MTT assay revealed that all of the complexes could suppress cell viability with IC50 values in the micromolar range of Cisplatin 13 M and Doxorubicin 4.1 M being used as standards. Synthesized TiO2NPs had IC50 values of 1.89, 2.00, 1.98, and 4.00 µM against the MCF-7 HeLa, PC-3, and A549 cancer cell lines, respectively.

CONCLUSION

Creating titanium dioxide nanoparticles using the plant leaf extract (Leucas cephalotes). The green synthesis process was discovered to be environmentally beneficial also may be accomplished by utilizing less chemical than the standard approaches used to make the nanoparticles. Wet chemical synthesis was used to produce different sizes of nano titanium dioxide; the average size was 38.99 nm. The TiO2 Purity and composition of the structure's size and structure were determined by EDX investigations, are confirmed by SEM examination. The size and shape of the produced nanoparticles were determined via xrd analysis. While FT-IR spectroscopy was used to look at the stretching and bonding. Moreover, TiONPs, showed enhanced cytotoxic effect against MCF-7(breast cancer) and prostate cancer in comparision to Hela and A549 lungs cancer.

DISCLOSURE OF CONFLICTING INTEREST

Alleged conflicts of interest are not present for any of the authors. Additionally, they affirm that this paper has not already been published or accepted by another journal**.**

REFERENCES

1. Kharissova, OV, Dias HR, Kharisov BI, Pérez BO, Pérez VMJ. The greener synthesis of nanoparticles. Trends in biotechnology. 2013; 31(4): 240-248.
2. Mirzayi, Behruz, Ali Nematollahzadeh, Somaye Seraj. Synthesis and characterization of magnetic maghemite/catecholamine core/shell nanoparticles. Powder Technology. 2015; 270: 185-191.
3. Gélis C, Girard S, Mavon A, Delverdier M, Paillous N, Vicendo P. Assessment of the skin photoprotective capacities of an organo

mineral broad spectrum sunblock on two ex vivo skin models. Photodermatology, photoimmunology & photomedicine. 2003; 19(5): 242-253.

4. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer research. 2009; 69(22): 8784-8789.

5. Kim D, Yu MK, Lee TS, Park JJ, Jeong YY, et al. Amphiphilic polymer-coated hybrid nanoparticles as CT/MRI dual contrast agents. Nanotechnology. 2011; 22(15): 155101.

6. Roopan SM, Bharathi A, Prabhakarn A, Rahuman AA, Velayutham K. Efficient phyto-synthesis and structural characterization of rutile TiO_2 nanoparticles using Annona squamosa peel extract. Spectrochim Acta A Mol Biomol Spectrosc. 2012; 98: 86-90.

7. Kirthi, A. Vishnu Biosynthesis of titanium dioxide nanoparticles using bacterium Bacillus subtilis. Materials Letters. 2011; 65(17-18) 2745-2747.

8. Masuda, Yoshitake, and Kazumi Kato. Synthesis and phase transformation of TiO2 nano-crystals in aqueous solutions. Journal of the Ceramic Society of Japan. 2009; 117(1363): 373-376.

9. Sunny, Nisha Elizabeth Green synthesis of titanium dioxide nanoparticles using plant biomass and their applications-A review. Chemosphere. 2022; 134612.

10. Aravind M, Amalanathan M, Mary M. Synthesis of TiO2 nanoparticles by chemical and green synthesis methods and their multifaceted properties. SN Applied Sciences. 2021; 3(4) 1-10.