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Algae-Mediated Green Synthesis of Dextran-Coated Titanium Nanoparticles and Their Cytotoxic Potential Against MCF7 Breast Cancer Cells

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Abstract

Background: The green synthesis of nanoparticles through algae-mediated processes offers an eco-friendly, cost-effective, and scalable approach for producing nanomaterials with potential applications in cancer therapy. The present study investigated the algae-mediated green synthesis of dextran-coated titanium oxide nanoparticles (TiO_2NPs) and evaluated their cytotoxic effects against MCF-7 breast cancer cells.

Methods: *Chlorella vulgaris* was isolated and identified. The polymerase chain reaction (PCR)-amplification of the 18S ribosomal RNA gene was used to confirm the isolate. Dextran from *C. vulgaris* was used to prepare coated TiO_2NPs, characterized using three techniques. The cytotoxicity of the dextran-coated TiO_2NPs was evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay on MCF7- breast cancer cells at various concentrations (25, 50, and 75%) and exposure times (24, 48, and 72 hours). The bioactive compounds in the algal extract were also identified by gas chromatography-mass spectrometry (GC-MS).

Results*: Chlorella vulgaris* was successfully isolated as confirmed by the 345-bp PCR-amplified fragment. The characterization of the TiO_2NPs confirmed the successful nanoparticle formation. A cluster of nanocrystalline particles had an average diameter of 71.44 nm. Compositional analysis revealed 15.85% atomic percentage for titanium. The dextran-coated TiO2NPs exhibited an impressive cytotoxicity rate of up to 99% at optimal concentration (25%) and exposure time (48 hours). Additionally, GC-MS analysis identified bioactive compounds in the algal extract, such as fatty acids, which may contribute to the observed anticancer effects.

Conclusion: The study demonstrated the potential of algae-mediated TiO_2NPs in cancer co-therapy, enhancing treatment effectiveness and reducing the side effects of traditional therapies.

Keywords: Anaphalisoic acid, Capping, *Chlorella vulgaris*, Cytotoxicity, rRNA gene.

Introduction

Green synthesis has become a popular way to achieve the Sustainable Development Goals (SDGs) by 2030, and it demonstrates the biological method used to prepare nanomaterials (1). Several biological sources are well-known for the environmentally friendly production of nanoparticles (NPs). Bacteria, fungi, plants, and algae are among these biological sources (2). To generate and stabilize these nanoparticles, whole cells or an extracellular extract can be employed (1). Plant-based nanoparticle synthesis is known to be more stable than microbe-based synthesis. Phytochemical synthesis of NPs is simple, easy, cost-effective, and provides high yields while promoting sustainable development (3).

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Recently, microalgae have emerged as a safe source of metabolites that can contribute to producing various nanoparticles through cost-effective and non-hazardous alternatives to chemical synthesis. Interestingly, the emergence of global health risks has focused attention on these living organisms as they are increasingly used in medicine (4).

Algae-mediated synthesis has several advantages over plant-mediated processes, including the ease and speed of synthesis, which can be carried out at ambient pressure, temperature, and normal pH values in simple aqueous media. Additionally, scalability and ease of handling, natural availability in nature, low toxicity to workers and the environment, and the ability to produce various metal oxide nanoparticles are additional advantages. This discovery could greatly accelerate the development of innovative algal nanomaterials with novel properties and concepts. As a result, research into algae-based biosynthesis of metallic nanoparticles has led to the development of a new sector known as phyco-nanotechnology (1,5).

Cancer is regarded as the main cause of mortality in the global population as of 2019, surpassing other non-transmissible diseases. This approach is established according to the most recent report by the World Health Organization. Several cancer treatment methods have previously been confirmed, including surgery, chemotherapy, and radiotherapy. However, these strategies may not always be optimal for the patient's clinical situation, as each cancer subtype has unique characteristics specific to its organ of origin, complicating both diagnosis and treatment. Furthermore, numerous researchers have identified certain negative aspects of traditional therapy. A study reported that conventional medicines do not have a high impact in all cases since they can promote an unfavorable selection of the most resistant and ultimately aggressive subtypes (7). While this can lead to significant improvements in the patient's condition, it may quickly deteriorate due to the re-emergence of drug resistance. Additionally, a major drawback of current treatments is the frequent occurrence of adverse side effects, which directly affect the patient's quality of life (7).

Titanium nanoparticles (TiNPs) have also been investigated as a cancer treatment. The study by Khorasaninejad *et al*. (8) examined the combination of TiNPs and anticancer drugs. It demonstrated that it could be used as an effective medication delivery mechanism for chemotherapy. For a long time, TiO_2 was known as "the environmental white knight" due to its low toxicity, biocompatibility, and inertness. The predicted fatal dosage at a 50% TiO_2 concentration is greater than 10 g/kg (9).

The present study explored an extracellular solution of *Chlorellavulgaris* as a safe medium to synthesize dextran-coated titanium oxide nanoparticles (TiO_2NPs) and evaluated their cytotoxic potential against breast cancer cells (MCF-7).

Materials and Methods

Isolation, identification, and cultivation of the algal isolate

The green alga *Chlorella vulgaris* was isolated from a water stream at Mustansiriyah University, Baghdad, Iraq.Serial dilutions of the obtained water sample were made and streaked onto solid Chu-10 agar plates (HI Media, India). Taxonomic standards were applied to identify the isolated microalga (10). To cultivate algal biomass, an illuminated incubator (Han Yang, Korea) was utilized with a light intensity of around 200 μE/m²/s and a temperature of 26±2 °C to grow and sustain the isolate in liquid Chu-10 medium. The culturing was initially done in a conical flask and eventually poured into a beaker containing 4 L of medium. The biomass was harvested, washed with sterilized distilled water, and dried for use in subsequent experiments.

Molecular identification of the isolated microalga

The 18S ribosomal RNA gene (NCBI accession number: KU720636.1) was chosen as the target region to confirm the presence of the eukaryotic genome. The primers 18SCH

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were designed using the Primer 3 Plus program. The designed forward oligonucleotide primer sequence is 5′-AGACGAACTACTGCGAAAGC-3′, while the reverse oligonucleotide primer sequence is 5′-CCACCCATAGAATCAAGAAA-3′. DNA was extracted from fresh microalgal biomass using the Presto™ 96-Well Plant Genomic DNA Extraction kit (Gene aid, Taiwan), following the manufacturer's protocol. The polymerase chain reaction (PCR) was carried out with Taq PCR Master Mix (Intron, Korea). PCR conditions were 3 min at 95°Cas the initial denaturation step, then 35 cycles, including denaturation for 45 sec at 95 °C, annealing for 1min at 55°C, and extension for 1min at 72 °C. Finally, an extension step for 7 min at 72 °C. The produced amplicon was separated through electrophoresis using 1% agarose gel stained with ethidium bromide and observed on a UV transilluminator. The sizes of amplified products were compared with the 100 bp DNA ladder to determine the size of these products.

Algae-mediated synthesis of titanium dioxide nanoparticles

To synthesizetheTiO$_2$NPs, water collected from the microalga *C. vulgaris* was heated to 60 °C and filtered. A 5 mg/mL titanium dioxide (TiO$_2$) solution (Sigma Aldrich, China) was prepared as a precursor for titanium nanoparticles. According to the manufacturer's instructions, TiO$_2$ has the following properties: molar mass (79.87 g/mol), density (4.2 g/cm^3), and average size (550nm). The two solutions were combined dropwise at a volume ratio of 5:25 mL of algal filtrate to the bulk solution. Half gram of dextran (Spectrum Chemica, USA) was added to the premix, and the pH was set to 9. The final mixture was swirled at 300 rpm and 70 °C. Finally, the colour and constancy of the reaction solution were monitored until they changed, confirming the successful synthesis of NPs.

Characterization of titanium dioxide nanoparticles

Ultraviolet (UV)-visible spectroscopy was performed on bio-synthesized TiO$_2$NPs using a spectrophotometer (Shimadzu, Japan). The average diameter, configuration, and shape of the synthesized nano fluid were determined using an atomic force microscopy (AFM) analyzer (AA-3000, USA). Furthermore, the elements present in the NPs were determined by energy dispersive X-ray (EDS) using Quantax 400, USA, were compared with the pre-treated TiO$_2$ solution.

Evaluation of cellular toxicity of alga-mediated synthesized titanium dioxide nanoparticles

To determine the antiproliferative activity of the three solutions, which included algal extract, TiO$_2$, and biosynthesized TiO$_2$NPs, a breast cancer cell (MCF7) was used as a model through the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (11). The cell line was obtained from the Centre for Biotechnology Research at Al-Nahraine University in Baghdad. The test solutions were diluted three times to make 25, 50, and 75%. These diluents were applied to the designated cell line at three exposure time points: 24, 48, and 72 hours. The absorbance readings were utilized to monitor the anti-proliferative potential of the solutions employed during the biosynthetic process. Starting with the algal extract and progressing to biosynthesized NPs, the optimal concentration and treatment period were determined to obtain the best antiproliferative results. After assessing the cell's vitality, the cytotoxicity % was computed for the treated cells and compared to untreated cells as a control, using the equation below:

$$Cell\ Cytotoxicity\ \% = 100 - Cell\ Viability$$

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Determination of bioactive compounds in algal extract

Gas chromatography-mass spectroscopy (GC-MS) analysis was performed on the algal concentrate to identify the primary phytochemicals that comprise it, particularly those that supported the combination of the NPs and anticancer activities. After injecting 5 µL of algal concentrate, a column was used to determine the following qualities: Inactive cap 1MS; 30m of 0.25 mm ID; plus 0.25 m of film thickness. Then, a high temperature of 280 °C was applied. The whole cycle was depicted using post-run programming equipment (Shimadzu, Japan; 12). Finally, the phytochemicals were identified by comparing their masses to the NIST library search and reference norms.

Statistical analysis

The recorded data, which included absorbance values of untreated (control) and treated cells with test solutions at various intervals, were statistically analyzed using an unpaired t-test in GraphPad Prism 6. All experiments in this study were independently replicated at least three times. The absorbance results were presented as the mean ± SD of three measurements, with $p < 0.05$ indicating statistical significance (13).

Results

Characteristics of the isolated micro algal isolate

In this study, the isolated algal strain was identified morphologically, and the photomicrograph is presented in Figure 1a. The isolated green alga is a unicellular organism, characterized by a ball shape with a diameter between 2 and 10 µm. It comprises different constituents, including cell walls and cytoplasmic organelles, such as chloroplasts. The green alga *Chlorella* sp. is considered one of the most significant micro algae genera. It belongs to the Chlorophyceae class, the Chlorococcales order, and the Chlorellaceae family. Furthermore, the results of the PCR amplicon confirmed the morphological identification of the isolated *Chlorella* sp. Primers specific to the 18S rRNA gene were used to amplify the target region from the genome of the isolated green alga, yielding 345 bp fragments, which were visualized on electrophoretic gel (Fig. 1b).

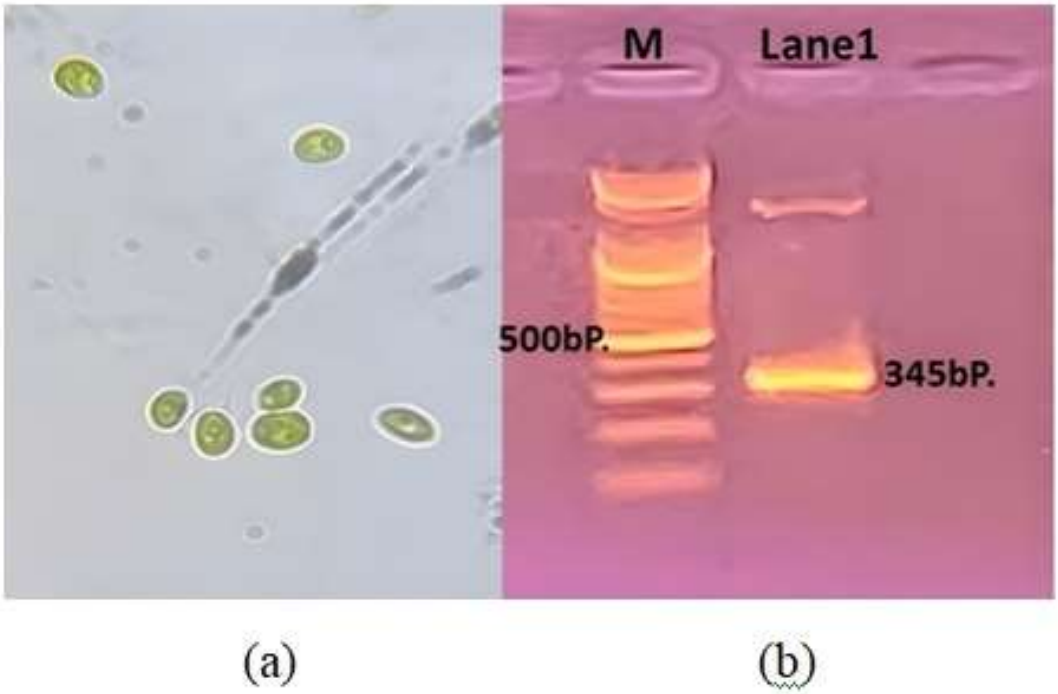

Fig. 1. (a) Microscopic photograph of the isolated *Chlorella* species (40X); (b) The amplified 18Sfragment (345bp) from *Chlorella* species on agarose gel electrophoresis. Lane 1: amplified fragment; Lane M:100 bp DNA ladder.

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Characteristics of alga-mediated titanium dioxide nanoparticles

The UV-Vis absorption spectrum of biosynthesized TiO_2NPs is shown in Figure 2. Other characteristic features of algae-mediated TiO_2NPs, such as the average diameter, roughness average, and root mean square were determined by AFM. All these characteristics are displayed in Table 1.According to EDS analytical data, titanium (4.45 keV) and oxygen (0.6 keV) occupied peak positions in bulk and synthesized TiO_2NPs. This validated the presence of elemental titanium in the sample prepared with *Chlorella*. Energy dispersive X-ray compositional study of TiO_2 particles revealed discrete peaks for titanium, aluminum, and oxygen. The atomic percentages were 17.85 for titanium and 82.15 for oxygen. Meanwhile, for alga-mediated synthesized NPs, the atomic percentages were 15.58 for titanium and 84.42 for oxygen (Table 2). Furthermore, other elements were discovered with titanium and oxygen at minor atomic percentages compared to titanium. The elements were Na, P, Al, and C. These elements could be derived from chemical components in the *Chlorella*sp. water extract used in the NP synthesis process.

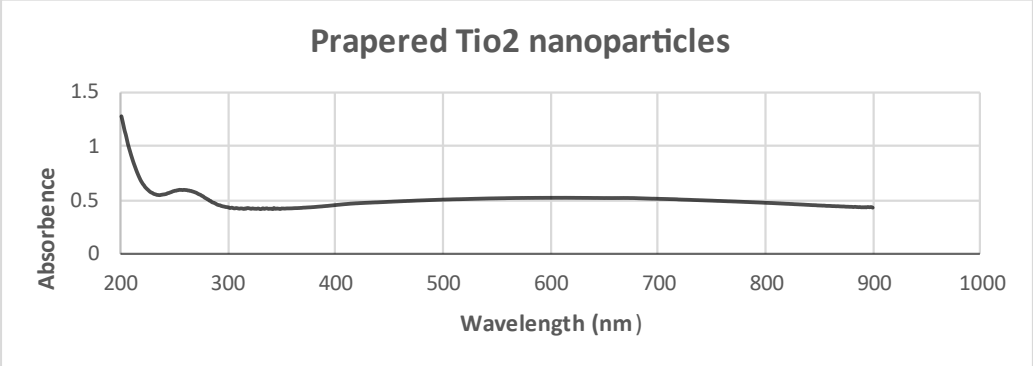

Fig. 2. The ultraviolet absorbance pattern of dextran-coatedtitanium dioxide nanoparticles synthesized using *Chlorella vulgaris.*

Table1. Atomic force microscopy analysis of dextran-coated titanium dioxide nanoparticles synthesized using *Chlorella vulgaris.*

Parameter	Value (nm)
Sa roughness average	3.66
Square root mean square	4.23
Average diameter	71.44

Table 2. Energy dispersive X-ray analysis for bulk titanium dioxide (TiO_2) and dextran-coatedtitanium dioxide nanoparticles (TiO_2NPs) synthesized using *Chlorella vulgaris.*

Type	Spectrum 2Element	Line Type	Weight%	Weight% Sigma	Atomic %
Bulk TiO_2	O	K series	60.85	0.54	82.15
	Ti	K series	39.42	0.54	17.85
	Total	-	100.00	-	100.00
Synthesized TiO_2NPs	C	K series	23.98	0.41	35.90
	O	K series	46.41	0.55	84.42
	Na	K series	6.65	0.15	5.20
	Ti	K series	35.59	0.55	15.58
	Al	K series	0.92	0.07	0.62
	P	K series	1.75	0.12	1.02
	Total	-	100.00	-	100.00

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Cytotoxicity of alga-mediated synthesized titanium dioxide nanoparticles

For the MTT test, a decrease in the absorbance value indicates a strong antiproliferative effect. In the current study, the antiproliferative effect of the three test solutions compared with the absorbance of control has been shown (Fig. 3). After 24 hours of treatment, the minimum absorbance value with cytotoxicity percentage was 97% for the algal extract dilution of 25% and all test TiO_2 dilutions. These assays revealed that algae-mediated TiO_2NPs exhibited the highest cytotoxicity on MCF7 cells, reaching 99%. As a result, no significant differences were observed between the bulk titania dilutions after 24 hours of treatment, nor between the algal extract dilutions after 72 hours. Significant differences were observed between the dilutions of all other test solutions after various application times. The cytotoxic ability was increased for all test solutions, beginning with the highest concentration and progressing to the lowest concentration.

Phytochemical constituents of algal extract

A total of nineteen compounds were detected in the algal extract (Table 3). The most common phytochemicals found were fatty acids and their esters, such as hexadecanoic acid, octadecenoic acid, and oleic acid. These compounds were found at higher percentages (64.07%) in their ester forms and (9.94%) in their acid forms than other chemicals.

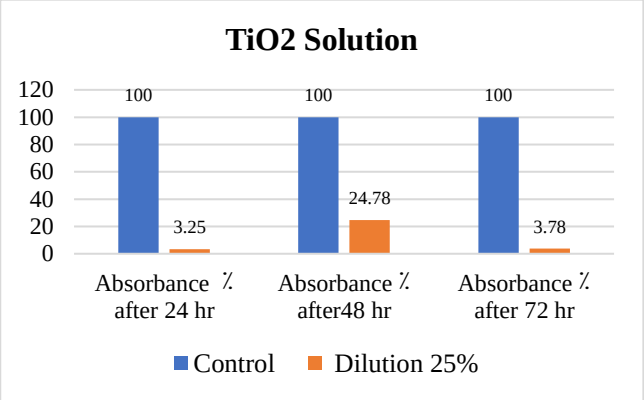

Fig. 3. % Absorbance values of 25% alga-mediated titanium dioxide nanoparticles (TiO_2NPs) on MCF7 cells at different exposure times.

Table 3. Phytochemicals with their area percentages and retention times, detected in *Chlorella* alga through gas chromatography-mass spectroscopy analysis.

Peak No	Phytochemical name	Retention time (min)	Area (%)
1.	Hexadecane	46.963	1.07
2.	Celidoniol, deoxy	54.005	3.72
3.	Bis (2-ethylhexyl) phthalate	54.891	7.84
4.	Hexadecanoic acid	56.337	5.02
5.	Hexadecanoic acid, ethyl ester	57.039	2.05
6.	Carbonic acid, decyl undecyl ester	57.262	1.42
7.	Oleic Acid	59.789	0.16
8.	Heneicosane	60.400	2.61
9.	Anaphalisoic acid	61.195	0.67
10.	Iron, tricarbonyl	62.200	3.61
11.	Iron, tricarbonyl-2,3,4,5-tetrahydroxy-2,4-cyclopentadien-1-one]-	62.400	2.13
12.	9-Octadecenoic acid	63.178	0.86
13.	Oleic Acid	64.852	0.60
14.	Iron,tricarbonyl[(2,3,4,5-.eta.)- 2,3,4,5-tetrahydroxy-2,4-cyclopentadien-1-one]	65.715	1.88
15.	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	67.990	18.10
16.	9-Octadecenoic acid, methyl ester	68.259	2.01
17.	Hexanedioic acid, bis (2-ethylhexyl) ester	68.847	40.94
18.	Anaphalisoic acid [5,9,13-trimethyleicos-5-en-17,18-diol-24-oic acid	69.413	1.42
19.	9-octadecenoic acid	-	3.90

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Discussion

The present study investigated the algae-mediated green synthesis of dextran-coated TiO_2NPs using *C. vulgaris* and their cytotoxic potential against MCF7 breast cancer cells. The findings align with the increasing interest in eco-friendly and sustainable nanoparticle synthesis, particularly for biomedical applications. The use of microalgae for nanoparticle synthesis offers a cost-effective, non-toxic alternative to chemical methods, while also providing scalability and simplicity, making it a highly promising approach in nanotechnology. In the present study, *Chlorella vulgaris* was successfully isolated and identified with PCR. The nuclear ribosomal DNA is one of the most conserved genes in living organisms. In eukaryotic organisms, the ribosomal cluster, which includes 18S rDNA, 5.8S rDNA, and 28S rDNA, is considered a single transcript and is distinguished from the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions (14). Primers for the 18S rDNA gene region are "universal" and produce amplicons that contain the most variable region of the target gene, such as the V4 region of the 18S rRNA gene. Several investigations have proposed excellent universal primers for 18S rRNA (15).

The synthesis of TiO_2NPs using *C. vulgaris* as a biological agent demonstrated successful nanoparticle formation, as evidenced by a colour change, UV-Vis spectroscopy, and AFM analysis. The colour change is thought to be the result of a phenomenon known as surface resonance (16), which was detected as a preliminary indicator of metal ion reduction to nano-ions. The presence of dextran in the combination may have contributed to or been the primary cause of the change in stability, as the final product appeared as a colloidal solution. Dextran is a hydrophilic, nontoxic; branching polysaccharide known for its high biocompatibility. It has also been utilized to coat several metallic NPs (17,18).The spectroscopic and compositional analysis confirmed the presence of elemental titanium and oxygen, with minor contributions from other elements such as sodium, aluminum, and phosphorus, which likely originated from the algal extract. The presence of these elements indicates the participation of algal-derived compounds in the nanoparticle formation and stabilization processes, highlighting the role of biomolecules in facilitating the synthesis. The peak of the UV-Vis absorption spectrum for synthesized TiO_2NPsthat corresponds to the phytochemicals is slightly diminished and shifted to the right. This pattern is assumed to be due to alterations in poly hydroxyl composites involved in bio-reduction and the encapsulation of the generated nanoparticles (19). Titanium oxide nanoparticles absorb light at wavelengths ranging from 275 to 405 nm, which is consistent with the absorption pattern of titanium oxide (8).

The cytotoxic evaluation of the synthesized TiO_2NPs revealed their significant antiproliferative effect against MCF7 breast cancer cells, which is consistent with other studies (20). The MTT assay results indicated that algae-mediated TiO_2NPs exhibited the highest cytotoxicity, reaching 99% after 72 hours of exposure. Interestingly, there were no significant differences in cytotoxicity between bulk titania and TiO_2NPs after 24 hours of treatment, suggesting that the nanoparticles required a longer duration to manifest their full antiproliferative potential. At low concentrations, the produced NPs in the current investigation had strong antiproliferative effects on the test cells. High concentrations of TiO_2NPs have been shown to cause harmful consequences in living organisms, including humans. However, compared to physical and chemical approaches, green synthesis of TiO_2NPs provides the advantage of delayed ion release into the colloidal mix (21).Furthermore, the cytotoxicity of the algal extract alone was substantial, demonstrating the inherent bioactivity of *C. vulgaris* and its potential

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contribution to the observed anticancer effects.

The overall finding of this study is that the cytotoxicity on MCF-7 increased exponentially when titania particles were reduced and capped with the active metabolites found in the microalga *Chlorella vulgaris*. After 48 hours of treatment with 25% TiNPs, MCF-7 demonstrated 99% cytotoxicity. The results may establish a basic dilution and treatment duration for future research to improve the utilization of these nanoparticles in cancer co-therapy. Regarding the exposure period, some studies have demonstrated that longer exposure periods to anti-cancer drugs resulted in greater anticancer effects (22). Bulk solution's decreased cytotoxicity and low toxicity compared to nanoparticles can be attributed to its structural and physical features, including low phase stability and large band gap (23).The highest cytotoxicity observed in synthesized NPs could be due to the presence of dextran-coated material. Several studies have shown that polymers could improve drug stability and modify delivery targets. This allows for a consistent dosage at the lesion site and accelerates drug extravasation into the tumour system, reducing adverse effects (24). Furthermore, in the study conducted by Medhat *et al*. (2017), the nano-conjugate comprised of dextran and gold NPs was evaluated as a tumour therapeutic agent in a mouse model (25). This conjugation was discovered to improve kidney and liver functions, increase liver antioxidants, improve the expression level of the β-lymphocyte gene, and result in positive expression of the liver p53 protein.

The phytochemical analysis of the algal extract revealed a diverse array of bioactive compounds, with fatty acids and their esters, such as hexadecanoic acid and oleic acid, being the most abundant. These compounds are well known for their biological activities, including anti-inflammatory and anticancer properties, which may have contributed to the cytotoxic effects observed in this study. The combination of these bioactive molecules with TiO_2NPs likely enhanced the anticancer efficacy, suggesting a synergistic effect between the algal metabolites and the nanoparticles. Hexadecanoic acid has been reported to reduce Bcl-2 expression, while oleic acid inhibits breast cancer cell growth by inducing apoptosis, autophagy, and cell cycle arrest (27). Heneicosane is a green substrate for silver nanoparticles, showing antimicrobial and anticancer activity. Anaphalisoic and sesterterpene carboxylic acids were also identified in algal extracts (29).Since the previous findings, no new identifications of this metabolite have been reported in plants or algae based on current discoveries and compared to published mass statistics. This is the first time anaphalisoic acid has been identified in an algal extract. Sesterterpenes and sesterterpenoids, found in fungi, marine organisms, and plants, exhibit cytotoxic, anti-inflammatory, antibacterial, and potential anticancer activities (30).

These findings highlight the potential of algae-mediated nanoparticle synthesis as a novel and eco-friendly approach for developing anticancer agents. The use of *C. vulgaris* offers an efficient platform for nanoparticle production while simultaneously leveraging the bioactive compounds present in the algal extract. Future research should focus on elucidating the exact mechanisms underlying the cytotoxic effects of the synthesized TiO_2NPs and their interactions with cancer cells. Additionally, exploring the therapeutic efficacy of these nanoparticles in in vivo cancer models could pave the way for their potential application in clinical cancer therapy (31).

This study underscores the significance of algae-mediated synthesis of TiO_2NPs as a sustainable and effective strategy for nanoparticle production with potential applications in cancer treatment. The combination of green synthesis principles with advanced nanotechnology offers a promising pathway toward achieving the Sustainable Development Goals while contributing to the development of novel, eco-friendly anticancer therapies.

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Conflicts of interest
The authors claim to have no conflicting interests.

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