



Article

Bioactivity Assessment of Functionalized TiO₂ Powder with Dihydroquercetin

Valentina Nikšić ¹, Andrea Pirković ², Biljana Spremo-Potparević ³, Lada Živković ³, Dijana Topalović ³, Jovan M. Nedeljković ¹ and Vesna Lazić ^{1,*}

¹ Vinča Institute of Nuclear Sciences—National Institute of the Republic of Serbia, Centre of Excellence for Photoconversion, University of Belgrade, 11351 Belgrade, Serbia; valentina.niksic@vin.bg.ac.rs (V.N.); jovned@vin.bg.ac.rs (J.M.N.)

² Department for Biology of Reproduction, INEP Institute for Application of Nuclear Energy, University of Belgrade, 11080 Belgrade, Serbia; andrea.pirkovic@inep.co.rs

³ Department of Pathobiology, Faculty of Pharmacy, University of Belgrade, 11221 Belgrade, Serbia; bilja22@pharmacy.bg.ac.rs (B.S.-P.); lada@pharmacy.bg.ac.rs (L.Ž.)

* Correspondence: vesna.lazic@vin.bg.ac.rs

Abstract: Biological activities, including cell viability, oxidative stress, genotoxicity/antigenotoxicity, and antimicrobial activity, were evaluated for a visible-light-responsive TiO₂-based ICT complex with dihydroquercetin (DHQ) and compared with pristine TiO₂, its inorganic component. Pristine TiO₂ did not induce cytotoxicity in MRC-5 or HeLa cells within the tested concentration range (1–20 mg/mL), while TiO₂/DHQ displayed a significant reduction in cell viability in both cell lines at higher concentrations (≥ 10 mg/mL). The analysis of reactive oxygen species (ROS) production revealed that TiO₂/DHQ significantly reduced ROS levels in both cell types (MRC-5 and HeLa), with HeLa cells showing a more substantial reduction at lower concentrations. Genotoxicity assessment using the comet assay demonstrated that TiO₂ induced DNA damage in MRC-5 cells, while TiO₂/DHQ did not, indicating that DHQ mitigates the genotoxic potential of TiO₂. Furthermore, TiO₂/DHQ exhibited antigenotoxic effects by reducing H₂O₂-induced DNA damage in MRC-5 cells, supporting its protective role against oxidative stress. Preliminary antimicrobial tests revealed that TiO₂/DHQ exhibits antimicrobial activity against *E. coli* under visible-light excitation, while TiO₂ does not. These findings suggest that the TiO₂-based ICT complex with DHQ with enhanced antioxidant properties can potentially serve as a safe, non-toxic biocide agent.

Keywords: TiO₂; dihydroquercetin; interfacial charge transfer complex; cytotoxicity; antigenotoxicity; antimicrobial activity



Academic Editor: Antonella Piozzi

Received: 17 January 2025

Revised: 7 February 2025

Accepted: 10 February 2025

Published: 10 February 2025

Citation: Nikšić, V.; Pirković, A.; Spremo-Potparević, B.; Živković, L.; Topalović, D.; Nedeljković, J.M.; Lazić, V. Bioactivity Assessment of Functionalized TiO₂ Powder with Dihydroquercetin. *Int. J. Mol. Sci.* **2025**, *26*, 1475. <https://doi.org/10.3390/ijms26041475>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Titanium dioxide (TiO₂) is a widely studied, wide-bandgap (3.2 eV) photocatalyst [1], displaying antimicrobial activity when exposed to ultraviolet (UV) light. Photogenerated charge carriers (electrons and holes) lead to the formation of reactive oxygen species (superoxide anions and hydroxyl radicals, respectively) capable of effectively inactivating a broad range of microorganisms, including bacteria, viruses, and fungi [2]. Because of that, TiO₂ is a promising candidate for a variety of eco- and bio-related applications, such as wastewater and air purification [1], self-cleaning surfaces [3], medical devices [4,5], and food packaging [6]. For example, TiO₂ coatings can reduce the risk of healthcare-associated infections in hospitals [7], while wastewater treatment with TiO₂-based materials leads to

the photocatalytic degradation of organic pollutants and the inactivation of pathogens [8]. However, excitation by high-energy photons (UV light) limits any photo-driven application of TiO_2 , including antimicrobial. So, an extension of TiO_2 's response in the visible spectral range (Vis) is crucial from a technological point of view, particularly in indoor and low-light-intensity environments. The most recent promising strategy to obtain visible-light-responsive TiO_2 -based composites is the formation of interfacial charge transfer (ICT) complexes upon coordination of organic molecules [9], preferably benzene derivatives, to the TiO_2 surface [10]. The functionalization of the TiO_2 surface with properly chosen non-absorbing in Vis organic molecules leads to red-shifted TiO_2 absorption, enabling its photoactivity under visible-light excitation [11]. Recent studies have demonstrated the successful formation of TiO_2 -based ICT complexes with various ligand molecules (catechol [10,12–14], salicylic acid [10,14,15], and phenol [16–19] derivatives). ICT complexes with catecholate- and salicylate-type ligands are stable when exposed to light and display enhanced photocatalytic abilities [13,14,20–23]. On the other hand, when phenol and its derivatives are organic components of the ICT complex, their degradation [16–19] occurs under Vis light [24,25].

However, TiO_2 particulates can pass into an organism via oral, inhalation, and dermal exposure routes and accumulate in various tissues. Previous research on human lung cell systems (A549 epithelial cells, macrophages differentiated from THP-1, and A549/differentiated THP-1 cocultures) indicated that the morphological and interface properties of TiO_2 crucially affect its toxicity [26]. Also, exposure to TiO_2 NPs in human embryonic lung cells resulted in cytotoxicity and genotoxicity after 24 h of exposure, suggesting that exposure to TiO_2 NPs may lead to undesired health effects and bioaccumulation [27].

In the most recent studies, high-biological-value compounds originating from plants, such as flavonoids, have gained attention since, on the one hand, they can coordinate on metal-oxide surfaces and modulate their optical properties and, on the other hand, they can diminish the undesired toxic effect of inorganic particulates [28,29], which increases with decreasing particle size. However, studies concerning the use of bioactive compounds to facilitate the formation of ICT complexes and take advantage of their enhanced optical properties in photocatalytic bactericidal activity [30], as well as to evaluate toxicity using either in vitro [31,32] or in vivo [33,34] experiments upon particle functionalization, are still scarce.

Dihydroquercetin (DHQ), a naturally occurring flavonoid, is an efficient scavenger of reactive oxygen species (ROS) capable of protecting cells from oxidative stress, a crucial factor in various diseases and aging processes [35]. Because of this, DHQ has a wide range of therapeutic effects, including anticancer, anti-inflammatory, antigenotoxic, anti-Alzheimer, antiangiogenic, and diuretic properties, as well as being effective in the prevention of diabetes and some cardiovascular diseases [36,37].

Very recently, we prepared a TiO_2 -based ICT complex with DHQ (TiO_2/DHQ), performing thorough optical characterization supported with density functional theory (DFT) calculations and indirect electron paramagnetic resonance (EPR) spectroscopy techniques to detect and identify transient species upon photoexcitation [29]. As an extension, to follow the safer-by-design approach, we evaluated the cytotoxic and genotoxic potential of TiO_2/DHQ in human fetal lung fibroblasts MRC-5 in vitro, a cell type often used in cytocompatibility studies [38]. Also, knowing that TiO_2/DHQ displays extended absorption in the Vis spectral range, the antimicrobial efficiency of TiO_2/DHQ against *E. coli* was tested using solely Vis light to estimate the possibility of its safe application in antimicrobial treatments.

2. Results

2.1. Basic Properties of ICT Complex Between TiO_2 and DHQ

The composition of the TiO_2 /DHQ sample was determined by analyzing the TG curve (Figure S1 in Supplementary Materials). The gradual decrease in weight of ~1% is due to the removal of adsorbed water. Then, melting, followed by the decomposition of DHQ, takes place in the temperature range of 230–400 °C, and finally, at the high-temperature end of the TG curve, the residual mass of ~95% corresponds to the remaining TiO_2 , indicating that the DHQ content in TiO_2 /DHQ is ~4%.

A prerequisite for understanding the toxicological and antimicrobial behavior of functionalized TiO_2 powder with DHQ lies in its optical properties and surface structure, i.e., the coordination of DHQ to the TiO_2 surface. Figure 1 shows the optical absorption of pristine TiO_2 powder (Degussa P25) and the corresponding ICT complex with DHQ, obtained by Kubelka–Munk transformations of diffuse reflection spectroscopy (DRS) data. Also, images of pristine TiO_2 and TiO_2 /DHQ powders and the spectral profile of excitation light from the light source used in antimicrobial testing are included in Figure 1. The significant red shift in the absorption onset in TiO_2 /DHQ (~600 nm) is a consequence of the formation of the ICT complex and is consistent with the reported literature data for TiO_2 -based ICT complexes with catecholate-type ligands [10–14].

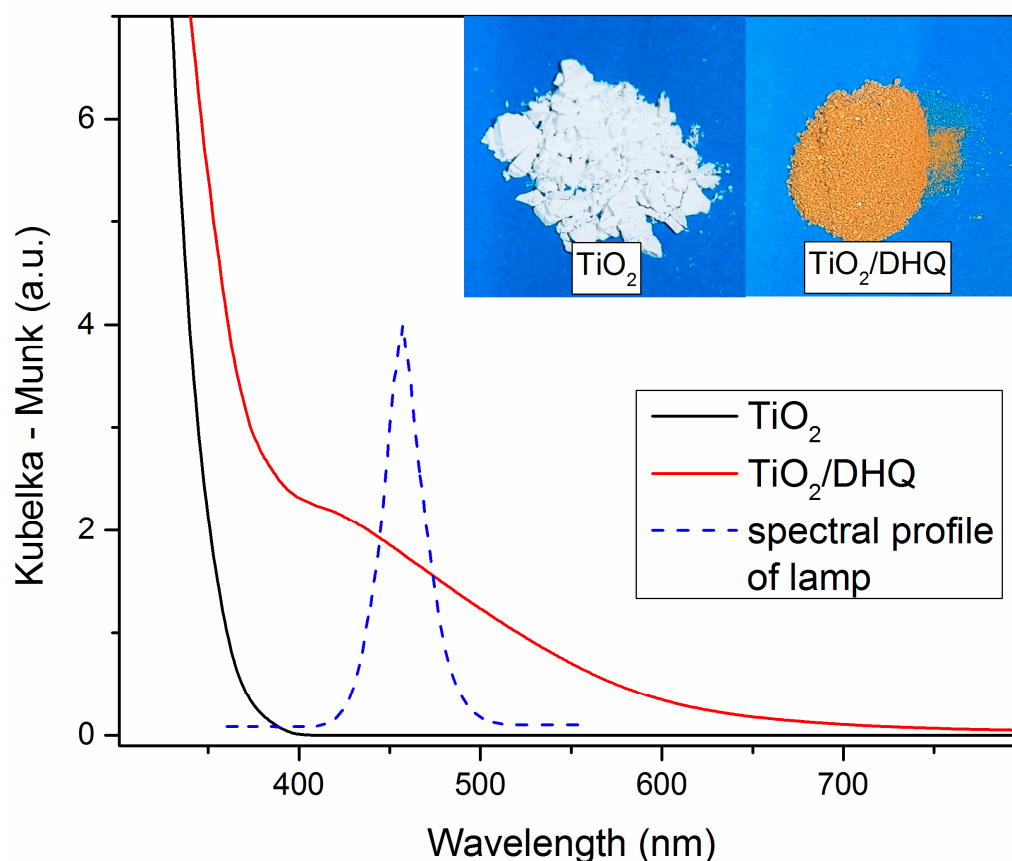


Figure 1. Kubelka–Munk transformations of DRS data for the pristine TiO_2 (Aeroxide® P25) powder and TiO_2 -based ICT complex with DHQ. Also, images of TiO_2 and TiO_2 /DHQ and the spectral profile of emitted light used in antimicrobial tests are included.

2.2. Cytotoxicity

The evaluation of the effects of 24 h incubation with pristine TiO_2 on cell viability in MRC-5 cells (Figure 2A) and HeLa cells (Figure 2B) showed that TiO_2 , in the investigated concentration range (1–20 mg/mL), did not induce cytotoxic effects. Moreover,

a slight increase in the percentage of viable non-malignant MRC-5 cells was observed compared to the control sample, while in the case of HeLa cancer cells, there was no significant difference versus the non-treated control. The concentration-dependent cell viability in MRC-5 and HeLa cells after 24 h of incubation with TiO_2 /DHQ is presented in Figures 2C and 2D, respectively. The results indicate that the exposure of MRC-5 cells to the released material from TiO_2 /DHQ leads to significant cytotoxic effects at concentrations above 10 mg/mL compared to the control (sample treated only with medium). However, the observed percentage reduction in viable cells after the treatment with 10 and 20 mg/mL of TiO_2 /DHQ was quite small (7.0% and 21.6% viability reduction, respectively). Similarly, the exposure of HeLa cells to the released material from TiO_2 /DHQ over 24 h displayed concentration-dependent cytotoxicity (Figure 2D) and a significant reduction in viability at a 10 mg/mL concentration or higher (compared to the control). The most pronounced decrease in viability (25%) was observed for the highest concentration of TiO_2 /DHQ (20 mg/mL).

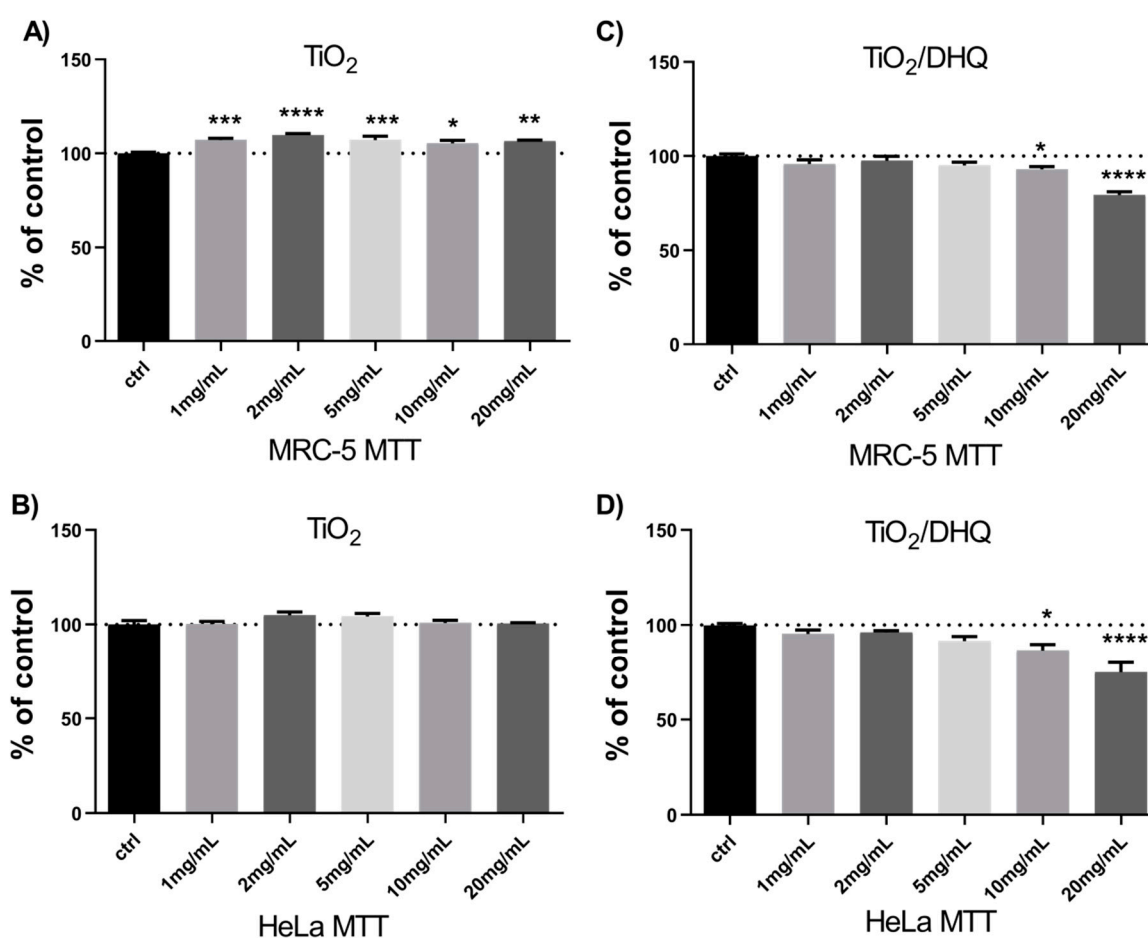


Figure 2. (A,B): Cytotoxicity of TiO_2 at a range of concentrations (1, 2, 5, 10, and 20 mg/mL) determined by MTT assay in MRC-5 cells (A) and HeLa cells (B), expressed as a percentage out of the control. (C,D): Cytotoxic effect of TiO_2 /DHQ at a range of concentrations (1, 2, 5, 10, and 20 mg/mL) in MRC-5 cells (C) and HeLa cells (D), determined by MTT assay. The data are expressed as mean + SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ versus control (by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post hoc test).

Figure 3 shows the production of ROS in MRC-5 and HeLa cells as a function of the concentration of pristine TiO_2 and the TiO_2 -based ICT complex with DHQ after the 24 h incubation period. The analysis of the obtained results indicated a decrease in ROS production in non-malignant MRC-5 cells with the increase in the pristine TiO_2 concentra-

tion compared to the control (Figure 3A), with a pronounced effect for the highest TiO_2 concentration (≥ 5 mg/mL). In the case of HeLa cancer cells, none of the applied TiO_2 concentrations influenced ROS production compared to the control (Figure 3B). On the other hand, in both cell lines, MRC-5 and HeLa, the impact of TiO_2 /DHQ treatment on ROS levels was significant (Figures 3C and 3D, respectively). The incubation of MRC-5 cells with TiO_2 /DHQ induced a significant reduction in the ROS level when cells were exposed to a TiO_2 /DHQ concentration equal to or higher than 5 mg/mL, while in the case of HeLa cells, a concentration-dependent reduction in the ROS level started from the lowest TiO_2 /DHQ concentration (1 mg/mL).

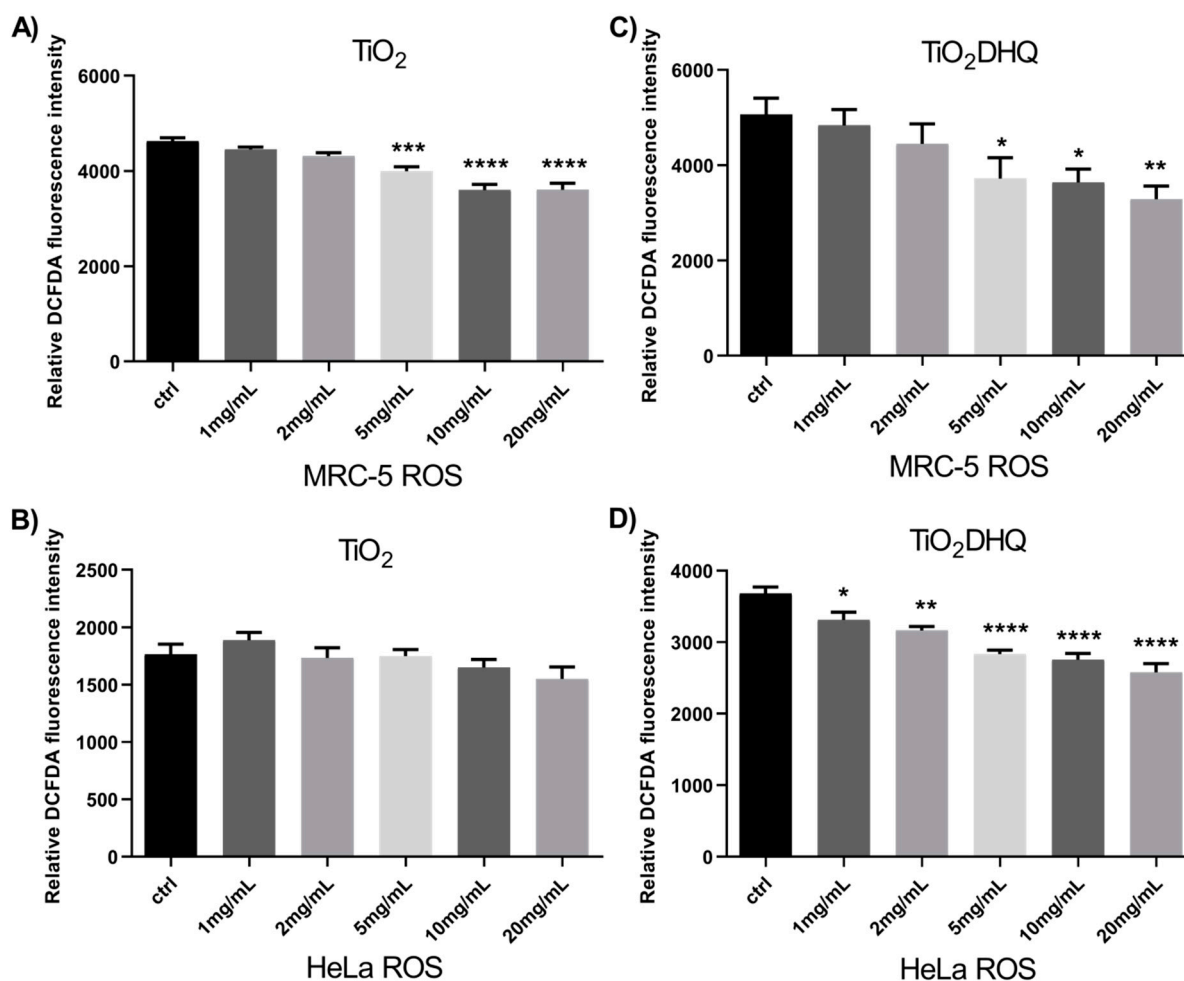


Figure 3. (A,B): The effect of TiO_2 at a range of concentrations (1, 2, 5, 10, and 20 mg/mL) on the production of reactive oxygen species (ROS) in MRC-5 cells (A) and HeLa cells (B), determined by the H2DCFDA assay, expressed as relative fluorescence intensity. (C,D): The effect of TiO_2 /DHQ on the production of reactive oxygen species (ROS) at a range of concentrations (1, 2, 5, 10, and 20 mg/mL) in MRC-5 cells (C) and HeLa cells (D), determined by the H2DCFDA assay. The data are expressed as mean + SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ versus control (by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post hoc test).

2.3. Genotoxicity and Antigenotoxicity

Besides their cytotoxic activity, we investigated the genotoxic potential of TiO_2 and TiO_2 /DHQ and the antigenotoxic effect of TiO_2 /DHQ. The results presented in Figure 4A,B, assessed by the comet assay, indicate the genotoxic effect of TiO_2 and TiO_2 /DHQ in MRC-5 cells. The pristine TiO_2 caused significant DNA damage compared to the control in the investigated concentration range (1–20 mg/mL).

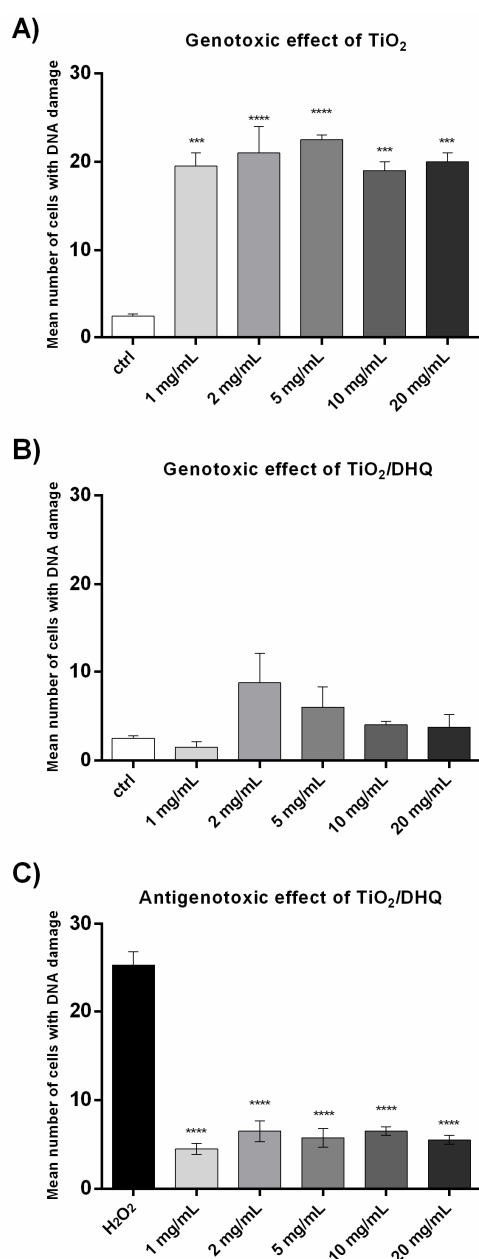


Figure 4. Genotoxic effect of (A) TiO₂ and (B) TiO₂/DHQ in MRC-5 cells and (C) antigenotoxic effect of TiO₂/DHQ against DNA damage induced by H₂O₂ in MRC-5 cells at a range of concentrations (1, 2, 5, 10, and 20 mg/mL), determined by the comet assay. The data are expressed as the mean number of cells with DNA damage + SEM. *** $p < 0.001$, **** $p < 0.0001$ versus control (by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparison post hoc test).

Since TiO₂/DHQ does not display genotoxicity, we investigated its antigenotoxic potential against DNA damage induced by oxidative stress. Figure 4C shows the antigenotoxic effect of TiO₂/DHQ studied in MRC-5 cells treated with H₂O₂. The pretreatment of cells with TiO₂/DHQ for 24 h resulted in a significant reduction in H₂O₂-induced DNA damage compared to the extent of DNA damage observed in cells exposed only to H₂O₂. TiO₂/DHQ displays an antigenotoxic effect over the entire investigated concentration range (1–20 mg/L), as opposed to the ICT complex between TiO₂ and caffeic acid, showing antigenotoxic properties at a lower concentration (0.5–1.6 mg/mL) [39]. This discrepancy is a consequence of either the different morphologies of TiO₂ samples or, more likely, the pronounced antioxidant properties of DHQ compared to caffeic acid. The change from genotoxic to antigenotoxic behavior in TiO₂ upon the formation of the ICT complex

with DHQ suggests that DHQ retains its antioxidant properties despite the consumption of two out of its five hydroxyl groups in the condensation reaction with TiO_2 surface hydroxyl groups.

2.4. Preliminary Antimicrobial Tests

Figure 5 shows the time- and concentration-dependent antimicrobial activity of TiO_2 /DHQ against the Gram-negative bacteria *E. coli*, including data for pristine TiO_2 (20 mg/mL) and the control (the absence of a photocatalyst). Results for two additional controls, the antibacterial activity of TiO_2 and TiO_2 /DHQ in the dark, are presented in the Supporting Information (Table S1). Despite limited experimental data, we can discern the following conclusions. First, pristine TiO_2 does not display antimicrobial activity against *E. coli* upon Vis light excitation; its time-kill curve practically overlaps with the control. Of course, neither of the photocatalysts (TiO_2 and TiO_2 /DHQ) display a biocidal effect in the dark. Second, for low concentrations (1 mg/mL), TiO_2 /DHQ does not show any antibacterial properties. Third, at higher concentrations (5, 10, and 20 mg/mL), TiO_2 /DHQ demonstrates concentration-dependent antimicrobial activity. For example, after five hours of illumination, the number of surviving bacteria was reduced by two and three orders of magnitude when the TiO_2 /DHQ concentration was 5 and 20 mg/mL, respectively. Fourth, unfortunately, the kinetic curves of antimicrobial activity are incomplete since the home-made light source, designed to cover the desired spectral range, has an overheating issue, preventing continuous operation for extended periods. However, based on their trends, complete bacterial destruction can be expected under prolonged irradiation.

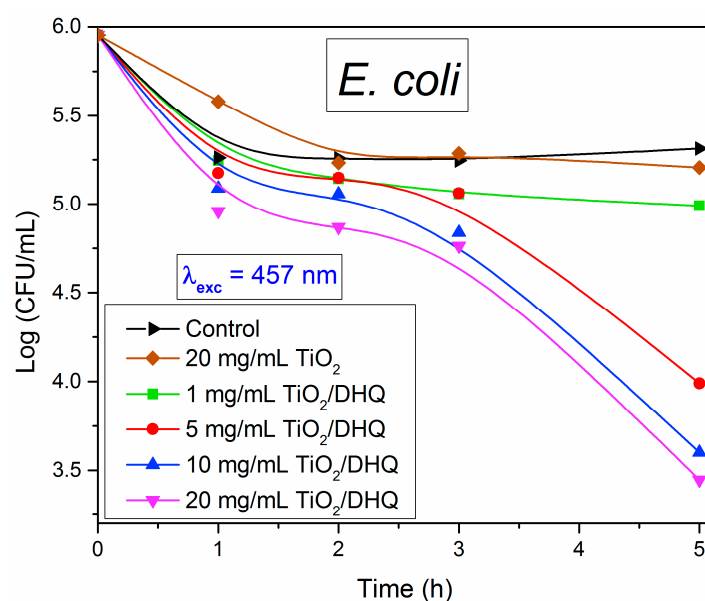


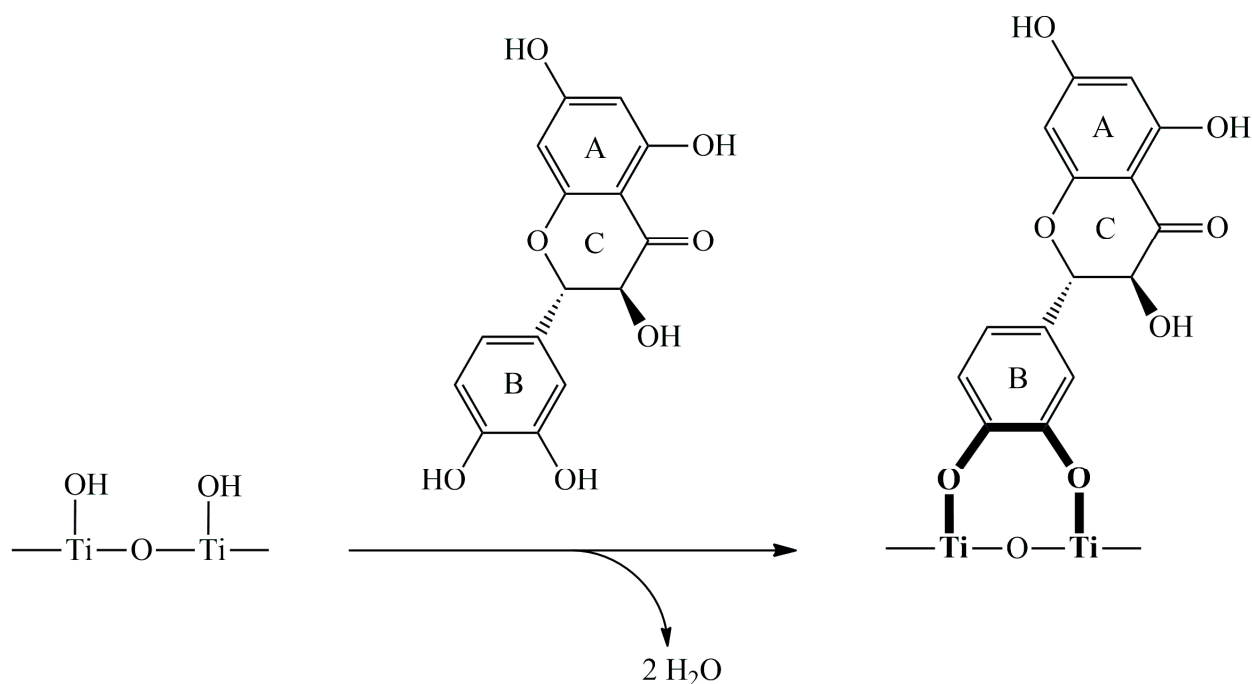
Figure 5. The time-dependent antimicrobial ability of pristine TiO_2 (20 mg/mL) and TiO_2 /DHQ (1–20 mg/mL) against *E. coli* under visible-light excitation (457 nm); control: experiments without photocatalyst.

3. Discussions

3.1. Basic Properties of ICT Complex Between TiO_2 and DHQ

The coordination of catechol to the TiO_2 surface is almost intuitive since the formation of the ICT complex occurs through the condensation reaction between hydroxyl groups from the inorganic and organic components of the ICT complex. The remaining question after the infrared spectroscopy analysis of the TiO_2 –catechol complex and its components—does catechol coordinate to the TiO_2 surface by bridging or chelating coordination—was

solved by applying Job's method of continuous variation [10], and the formation of bridging coordination was, in addition, supported by density functional calculations (DFT) [18]. Of course, due to the presence of multiple hydroxyl groups in DHQ, it is mandatory to check how DHQ coordinates to the TiO_2 surface; i.e., is it over neighboring hydroxyl groups at positions 3' and 4' (ring B), with hydroxyl groups at positions 5 and 7 (ring A) and 3 (ring C) remaining free? The infrared analysis of free and bound DHQ to TiO_2 , supported by a DFT calculation using the $[\text{Ti}_{18}\text{O}_{31}(\text{OH})_8]/\text{DHQ}$ cluster as a model system, undoubtedly proved the above-mentioned coordination of DHQ to the TiO_2 surface [29]. For clarity, the formation mechanism of TiO_2/DHQ , based on the condensation reaction, and the surface structure of the ICT complex are presented in Scheme 1.



Scheme 1. Schematic presentation of the formation mechanism of the TiO_2 -based ICT complex with DHQ.

The way in which DHQ binds to the TiO_2 surface significantly affects the bioactivity of the ICT complex since hydroxyl groups on the phenolic ring display antioxidant activity. The presence of multiple hydroxyl groups increases the ability of the phenolic compound to donate hydrogen atoms to reactive free radicals, stabilizing them. In addition, the aromatic ring of phenolic compounds increases their antioxidant activity. So, on the one hand, although two hydroxyl groups at positions 3' and 4' (ring B) are consumed in the formation of the $\text{Ti}-\text{O}-\text{C}$ linkage between TiO_2 and DHQ, the remaining three free hydroxyl groups can significantly reduce the toxicity of TiO_2 . On the other hand, although the TiO_2 -based ICT complex with DHQ should have increased photoinduced antimicrobial performance due to the visible-light response, the ability of free hydroxyl groups to scavenge ROS can somewhat diminish its antimicrobial ability.

3.2. Cytotoxicity

The functionalization of TiO_2 with DHQ increased the cytotoxic potential of cervical cancer cells, consistent with previous findings of DHQ cytotoxicity in HeLa cells [40]. It should be mentioned that the inhibitory effect on cell viability was more pronounced in HeLa cancer cells than in normal MRC-5 cells, which is most likely a consequence of different DHQ effects in cancer versus normal cells. In a recent study, Mohammed et al. [41]

indicated that DHQ and its metabolites have better inhibitory activity on the colon HCT-116 cancer cell line than on HEK-293 normal cells in the tested concentration range. Further, a study by Chahardoli et al. [42] showed that biosynthesized TiO₂ nanoparticles using quercetin had dual effects in normal and cancer cells, with IC₅₀ values below 100 and 50 µg/mL for human breast cancer cells of the MCF-7 cell line and melanoma cancer cells of the A375 cell line, respectively. However, biosynthesized TiO₂ nanoparticles did not significantly affect normal skin fibroblast cells at up to 50 µg/mL.

In conclusion, the TiO₂-based ICT complex with DHQ has increased antioxidative potential compared to pristine TiO₂ in both types of cells, and this observation is in accordance with related studies. Previous results indicate that DHQ inhibits intracellular ROS generation in human cells in a dose-dependent manner, showing the pronounced inhibition of ROS generation with an increase in DHQ concentration from 10 to 100 µg/mL [43]. Also, DHQ displays a reduction in the activity of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX)) in pretreated cells, along with decreased intracellular ROS production [44]. Li et al. [45] observed that DHQ can efficiently scavenge •OH, DPPH•, and ABTS•⁺ (hydroxyl, 2,2-diphenyl-1-picrylhydrazyl, and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radicals, respectively) and protect bone marrow-derived mesenchymal stem cells from •OH-induced cytotoxicity. Also, DHQ prevented oxidative damage, such as ROS generation, glutathione depletion, and single-strand break formation, in human primary dermal fibroblasts (NHDFs) and epidermal keratinocytes (NHEKs) [46]. In addition, in a previous study, we showed that free hydroxyl groups at positions 3, 5, and 7 of the DHQ moiety in TiO₂/DHQ enable a reduction in the ABTS•⁺ radical cation, while exposure to UV light further enhances this reduction. In the presence of TiO₂, photogenerated electrons reduce ABTS•⁺, whereas in the presence of TiO₂/DHQ, both photogenerated electrons and hydroxyl groups contribute to the decrease in ABTS•⁺, enabling the ICT complex to exhibit both photocatalytic and radical-scavenging properties. Also, TiO₂ did not affect the DPPH radical concentration, while TiO₂/DHQ induced a 35% decrease in the relative DPPH concentration due to the radical-scavenging potential of the hydroxyl groups on the DHQ moiety attached to the TiO₂ surface [29].

Based on the results of this study and the literature data, the TiO₂-based ICT complex with DHQ has increased cytotoxicity and a pronounced antioxidant effect compared with pristine TiO₂ due to DHQ's antioxidant properties. This conclusion agrees with a report showing that titanium dioxide nanotubes loaded with quercetin are more effective anticancer and ROS-reducing agents than titanium dioxide nanotubes or quercetin themselves [47].

3.3. Genotoxicity and Antigenotoxicity

As already shown, TiO₂ nanoparticles can cause adverse reactions, leading to cell damage, inflammation, and immune responses [48]. The comet assay, used in this study, is commonly used in the genotoxicity testing of DNA damage and repair in various experimental models [49]. Several research groups have already indicated the potential risks of genotoxicity associated with exposure to TiO₂ nanoparticles, showing that they can cause DNA and chromosome damage and gene mutations either in in vitro or in vivo experiments [50–52]. The form and degree of damage highly depend on the physical and chemical properties of TiO₂, which govern its reactivity and bioavailability [48]. Its genotoxic effects do not only depend on the morphology of TiO₂ particulates (size and shape) but also on the test subjects (cells/animals) and the type, concentration, and duration of exposure [48,50–52]. We showed that pristine TiO₂ can cause significant DNA damage in MRC-5 cells. Although three mechanisms are recognized for the genotoxic effects of TiO₂

particulates, ranging from nano- to micro-sizes, the available data suggest that oxidative stress, e.g., nanoparticle-induced ROS production, is a signal of other physiological effects, including cytotoxicity and genotoxicity [50,53].

However, the TiO₂-based ICT complex with DHQ did not induce DNA damage in MRC-5 cells under the same experimental conditions (Figure 4B). These results are consistent with the literature data obtained for ICT complexes between nanometer-sized TiO₂ particles with ascorbic acid [31] and caffeic acid [39], showing no DNA damage in leukocytes from whole blood cells (0.4–8.0 mg/mL). Also, the ICT complex between TiO₂ powder (Degussa P25), the same one used in this study, and caffeic acid did not induce DNA damage when orally administered to mice (2000 mg/kg) [33].

The change from genotoxic to antigenotoxic behavior in TiO₂ upon the formation of the ICT complex with DHQ suggests that DHQ retains its antioxidant properties despite the consumption of two out of its five hydroxyl groups in the condensation reaction with TiO₂ surface hydroxyl groups.

3.4. Preliminary Antimicrobial Tests

Particulate matter's growing applications in various fields have raised concerns about its toxicity, and therefore, the use of functionalized TiO₂ particles with DHQ is advantageous from an ecological perspective. An additional advantage of TiO₂/DHQ is its enhanced harvesting ability for solar radiation. While many studies have demonstrated the antimicrobial activity of TiO₂ under UV light [2–5,7,8], even though ICT complexes are eco-friendly and Vis light sources can be used instead of harsh UV light sources, the literature on the biocidal action of ICT complexes under Vis light is almost non-existent [30]. So, in this study, we performed a preliminary antimicrobial test of the TiO₂-based ICT complex with DHQ under Vis light excitation (457 nm is the maximum emission wavelength of the light source; see Figure 1) against *E. coli* to estimate if this system is worth further investigation in this direction.

The presented results are a step forward in the design of a non-toxic antimicrobial agent. Owing to free hydroxyl groups, the coordination of DHQ to the TiO₂ surface confers antioxidant behavior to the entire ICT architecture, alongside enhanced optical properties. However, since free hydroxyl groups diminish the antimicrobial potency of the ICT complex, further research in this area should focus on increasing the efficiency of antimicrobial activity while keeping the ICT complex non-toxic.

4. Material and Methods

4.1. Synthesis and Optical Characterization of ICT Complex Between TiO₂ and DHQ

Dihydroquarcetin (DHQ; commercial product of SIBPRIBOR OOO, Irkutsk, Russia, 97% purity) was obtained thanks to Professor Lada Živković (Faculty of Pharmacy, University of Belgrade). TiO₂ (Aeroxide[®] P25) was purchased from ACRÖS ORGANICS (Geel, Belgium). All other used chemicals were high-grade (Sigma-Aldrich, St. Louis, MO, USA). Milli-Q deionized water with a resistivity of 18.2 MΩ cm^{−1} was used as a solvent.

The TiO₂-based ICT complex with DHQ was prepared following the procedure described in our recent publication [29]. Briefly, 0.5 g of TiO₂ powder was combined with 317 mg of DHQ dissolved in 250 mL of deionized water. The dispersion was vigorously stirred for 3 h at 40 °C and left without stirring at room temperature for another 48 h. The powder was almost immediately colored, indicating the formation of the ICT complex. Then, the solid was separated by centrifugation, thoroughly washed five times with 20 mL of water, and dried in the oven at 40 °C.

Reflection spectroscopy measurements of pristine TiO₂ and TiO₂/DHQ were carried out using a Shimadzu UV–Visible UV-2600 spectrophotometer (Kyoto, Japan) equipped

with an integrated sphere (ISR-2600 Plus, Kyoto, Japan). The content of DHQ in the TiO₂/DHQ sample was determined by thermogravimetric analysis (TGA) using a Setaram Setsys Evolution-1750 instrument, Austin, TX, USA. The measurements were performed from room temperature to 700 °C at a heating rate of 10 °C/min in a dynamic air atmosphere with a flow rate of 20 cm³/min. Detailed information concerning composition, based on thermogravimetric analysis, surface structure, i.e., the coordination of DHQ to the surface of Ti atoms, based on infrared measurements, and the energy alignment of TiO₂/DHQ, based on DFT calculations, can be found in reference [29].

4.2. Biological Effects of ICT Complex Between TiO₂ and DHQ

4.2.1. Treatment Preparation

TiO₂ and TiO₂/DHQ stock solutions (40 mg/mL) were prepared by suspending powders in a complete RPMI medium (RPMI 1640 medium (Biowest, Nuaille, France), 10% fetal calf serum (FCS, Gibco, Waltham, MA, USA), and 1% antibiotic–antimycotic solution (Capricorn Scientific GmbH, Ebsdorfergrund, Germany)). The suspensions were left for 24 h in a humidified incubator with 5% CO₂ at 37 °C to allow for the release of any material attached to the TiO₂ surface. After 24 h, the supernatant was separated from the solid by centrifugation at 3000× *g* for 10 min. Then, the supernatant was diluted with fresh RPMI medium in serial dilutions to reach final concentrations (1, 2, 5, 10, and 20 mg/mL) to be used in further experiments.

4.2.2. Cell Culture

Human fetal lung fibroblasts (MRC-5) and human cervical carcinoma cells (HeLa) were propagated in 25 cm² tissue culture flasks in a humidified incubator with 5% CO₂ at 37 °C. MRC-5 and HeLa cells were grown in a complete medium. After reaching 70% confluence, the cells were trypsinized (0.25% trypsin–EDTA solution, Institute for Virology, Vaccines, and Sera “Torlak”, Belgrade, Serbia), seeded in 96-well plates (1.5 × 10⁴ cells/well), and left to attach to wells for 24 h at 37 °C (5% CO₂).

4.2.3. Cytotoxicity Evaluation

The MRC-5 and HeLa cells were treated with TiO₂ and TiO₂/DHQ in the concentration range described in Section 4.2.1. (total volume of 100 µL per well). Following incubation with the treatments or medium alone (control) at 37 °C for 24 h, we performed an MTT assay. MTT reagent at a working concentration of 0.5 mg/mL (thiazolyl blue tetrazolium bromide, Sigma Aldrich, St. Louis, MO, USA) was added (10 µL per well), and the cells were left for 2 h in the dark at 37 °C for the reaction to occur. Then, purple formazan crystals were dissolved with sodium dodecyl sulfate (10% SDS in 0.01 M HCl, Sigma Aldrich, St. Louis, MO, USA), and the absorbance was measured at 570 nm on a microplate reader (BioTek ELx800, Winooski, VT, USA) after the complete solubilization of the crystals. Three independent experiments in triplicate were performed (*n* = 9).

4.2.4. H2DCFDA Assay (2', 7'-Dichlorofluorescein Diacetate)

To evaluate ROS production in MRC-5 and HeLa cells, the medium in the cell cultures, prepared according to the procedure outlined in Section 4.2.2., was exchanged with 100 µL/well treatments with various concentrations of TiO₂ and TiO₂/DHQ prepared as described in Section 4.2.1. After 24 h, treatments were removed, and cells were rinsed with PBS. Further, we followed the manufacturer's instructions for the DCFDA assay. So, 5 µM of the cell-permeable oxidation-sensitive probe, H2DCFDA (Merck Millipore, Burlington, MA, USA, 2', 7'-Dichlorofluorescein Diacetate-CAS 4091-99-0-Calbiochem), using PBS as the diluent, was added to the cells and left for 45 min in the dark. Next, the cells were washed with PBS and exposed to PBS alone (control) or 200 µM of H₂O₂ (positive control).

After 1 h of incubation and the conversion of non-fluorescent H2DCFDA to the highly fluorescent 2',7'-dichlorofluorescein (DCF), the intracellular ROS generation level in cells was determined by measuring the fluorescence at 535 nm upon excitation at 485 nm using a fluorescent plate reader (Wallac 1420 multilabel counter Victor 3V, Perkin Elmer, Shelton, CT, USA). Data were expressed as relative fluorescence intensity. Three independent experiments in triplicate were performed ($n = 9$).

4.3. Comet Assay

Before processing for the alkaline comet assay, MRC-5 cells were incubated for 24 h with TiO₂ or TiO₂/DHQ at a range of concentrations in a complete RPMI medium in 96-well plates for genotoxicity testing. For the antigenotoxic effect, after rinsing with PBS, 50 μ M of H₂O₂ was added to a serum-free medium to induce DNA damage in the 24 h-incubated cells with TiO₂/DHQ. After the incubation period, the treatments were rinsed with PBS. Then, the cells were trypsinized with a 0.25% trypsin–EDTA solution and centrifuged at $300\times g$ for 5 min. The cell pellets were resuspended in a complete medium to achieve a density of approximately 1×10^4 cells.

The comet assay was performed according to Tice et al. [54]. Cells were suspended in 0.5% low-melting-point agarose (Sigma Aldrich, St. Louis, MO, USA) and pipetted onto slides pre-coated with 1% normal-melting agarose (Sigma Aldrich, St. Louis, MO, USA). The slides were covered with coverslips and stored at 4 °C for 5 min to solidify the agarose. After removing the coverslips, the slides were immersed in a pre-cooled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% dimethyl sulfoxide; pH 10, adjusted by NaOH) and stored overnight at 4 °C.

The next day, the slides were placed in electrophoresis buffer in a horizontal tank, and electrophoresis was conducted at 4 °C for 30 min at a constant voltage (0.8 V/cm). After electrophoresis, the slides were neutralized and stained with ethidium bromide (20 μ g/mL). The comets were examined under an Olympus BX 50 microscope (Olympus Optical Co., GmbH, Hamburg, Germany), equipped with a mercury lamp HBO (50 W, 516–560 nm, Zeiss, Oberkochen, Germany). Comets were visually scored and classified into five categories (A, B, C, D, and E) based on the extent of DNA damage, as described by Anderson et al. [55]. The degree of DNA damage was expressed as the mean number of cells in comet classes B + C + D + E. Two replicate slides were prepared for each treatment, and 100 randomly selected cells per slide were analyzed. The entire experiment was repeated three times.

4.4. Antimicrobial Activity

The photoinduced bactericidal activity of TiO₂/DHQ was evaluated against the Gram-negative bacteria *E. coli* (ATCC 25922) under nearly monochromatic Vis light illumination (457 nm, see Figure 1). The microbial inoculum was prepared by transferring the microorganisms into 3 mL of tryptone soy broth (TSB) supplemented with 0.6% yeast extract (TSB1Y) and incubating them overnight at 37 °C (~18 h). The incubated test cultures were diluted to a working concentration of approximately 10^5 CFU/mL.

Various amounts of the TiO₂/DHQ samples (25, 50, and 100 mg) were placed in glass Petri dishes containing 5 mL of the microbial suspension in saline solution (8.5% NaCl). The Petri dishes were incubated under the light for 5 h. After a series of dilutions, a 0.1 mL aliquot of the solution was transferred into a sterile Petri dish and overlaid with tryptone soy agar (1.5% agar in TSB1Y). The inoculated plates were incubated at 37 °C for 24 h, and the surviving cells were counted (CFU/mL; CFUs—colony-forming units).

To obtain unambiguous information about the photocatalytic antibacterial activity of TiO₂/DHQ under exclusive Vis light excitations, we performed the following control

experiments: the time-dependent reduction of *E. coli* under Vis light illumination in the absence of any photocatalyst, the time-dependent reduction of *E. coli* in the presence of TiO₂/DHQ in the dark, and the time-dependent reduction of *E. coli* in the presence of pristine TiO₂ in the dark, as well as under Vis light illumination.

5. Conclusions

In conclusion, we prepared surface-modified TiO₂ with a potent plant-derived antioxidant dihydroquercetin (DHQ) and assessed its bioactivity. Besides inducing red-shifted absorption due to ICT complex formation, the coordination of DHQ to the surface of Ti atoms reduced the genotoxicity of TiO₂ generated by reactive oxygen species (ROS) formed upon the excitation of the hybrid architecture. We demonstrated that the TiO₂-based ICT complex with DHQ is non-toxic to MRC-5 cells up to a concentration of 10 mg/mL and exhibits significant antigenotoxic properties compared with pristine TiO₂ nanopowder. Furthermore, preliminary antibacterial tests indicated that the TiO₂-based ICT complex with DHQ efficiently inactivates the Gram-negative bacteria *E. coli* when exposed solely to Vis light. We are confident that further research in this direction is worthwhile, considering that, on the one hand, expensive and harmful UV light sources are replaceable by cost-effective, harmless Vis light sources, while, on the other hand, the release of toxic materials, for example, silver, into the environment can be avoided.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms26041475/s1>.

Author Contributions: V.N.: methodology, investigation. A.P.: visualization, methodology, investigation, writing—original draft. B.S.-P.: methodology, validation, data curation. L.Ž.: methodology, validation, data curation. D.T.: investigation, formal analysis. J.M.N.: writing—review and editing, conceptualization. V.L.: writing—review and editing, conceptualization, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract Numbers: 451-03-66/2024-03/200017, 451-03-65/2024-03/200161, and 451-03-66/2024-03/200019) and by the Science Fund of the Republic of Serbia, Program PRISMA, Grant No. 5354, Multifunctional visible-light-responsive inorganic–organic hybrids for efficient hydrogen production and disinfection—HYDIS.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Hoffmann, M.R.; Martin, S.T.; Choi, W.; Bahnemann, D.W. Environmental Applications of Semiconductor Photocatalysis. *Chem. Rev.* **1995**, *95*, 69–96. [[CrossRef](#)]
2. Carré, G.; Hamon, E.; Ennahar, S.; Estner, M.; Lett, M.-C.; Horvatovich, P.; Gies, J.-P.; Keller, V.; Keller, N.; Andre, P. TiO₂ Photocatalysis Damages Lipids and Proteins in Escherichia coli. *Appl. Environ. Microbiol.* **2014**, *80*, 2573–2581. [[CrossRef](#)] [[PubMed](#)]
3. Wei, Y.; Wu, Q.; Meng, H.; Zhang, Y.; Cao, C. Recent advances in photocatalytic self-cleaning performances of TiO₂-based building materials. *RSC Adv.* **2023**, *13*, 20584–20597. [[CrossRef](#)] [[PubMed](#)]
4. Ziental, D.; Czarczynska-Goslinska, B.; Mlynarczyk, D.T.; Glowacka-Sobotta, A.; Stanisz, B.; Goslinski, T.; Sobotta, L. Titanium Dioxide Nanoparticles: Prospects and Applications in Medicine. *Nanomaterials* **2020**, *10*, 387. [[CrossRef](#)]

5. Chouirfa, H.; Bouloussa, H.; Migonney, V.; Falentin-Daudré, C. Review of titanium surface modification techniques and coatings for antibacterial applications. *Acta Biomater.* **2019**, *83*, 37–54. [\[CrossRef\]](#)
6. Zhang, W.; Rhim, J.W. Titanium dioxide (TiO₂) for the manufacture of multifunctional active food packaging films. *Food Packag. Shelf.* **2022**, *31*, 100806. [\[CrossRef\]](#)
7. Haider, A.J.; Anbari, R.H.A.; Kadhim, G.R.; Salame, C.T. Exploring potential Environmental applications of TiO₂ Nanoparticles. *Energy Procedia* **2017**, *119*, 332–345. [\[CrossRef\]](#)
8. Nguyen, N.T.T.; Nguyen, L.M.; Nguyen, T.T.T.; Liew, R.K.; Nguyen, D.T.C.; Van Tran, T. Recent advances on botanical biosynthesis of nanoparticles for catalytic, water treatment and agricultural applications: A review. *Sci. Total. Environ.* **2022**, *827*, 154160. [\[CrossRef\]](#)
9. Rajh, T.; Nedeljkovic, J.M.; Chen, L.X.; Poluektov, O.; Thurnauer, M.C. Improving Optical and Charge Separation Properties of Nanocrystalline TiO₂ by Surface Modification with Vitamin C. *J. Phys. Chem. B* **1999**, *103*, 3515–3519. [\[CrossRef\]](#)
10. Shin, S.; Shafian, S.; Ryu, K.Y.; Jeon, Y.K.; Kim, W.S.; Kim, K. Solution-Processed TiO₂ Nanoparticles Functionalized with Catechol Derivatives as Electron Transporting Layer Materials for Organic Photovoltaics. *Adv. Mater. Interfaces* **2022**, *9*, 2200118. [\[CrossRef\]](#)
11. Lazić, V.; Nedeljković, J.M. Photocatalytic Reactions over TiO₂-Based Interfacial Charge Transfer Complexes. *Catalysts* **2024**, *14*, 810. [\[CrossRef\]](#)
12. Finkelstein-Shapiro, D.; Davidowski, S.K.; Lee, P.B.; Guo, C.; Holland, G.P.; Rajh, T.; Gray, K.A.; Yarger, J.L.; Calatayud, M. Direct Evidence of Chelated Geometry of Catechol on TiO₂ by a Combined Solid-State NMR and DFT Study. *J. Phys. Chem. A* **2016**, *120*, 23625–23630. [\[CrossRef\]](#)
13. Higashimoto, S.; Nishi, T.; Yasukawa, M.; Azuma, M.; Sakata, Y.; Kobayashi, H. Photocatalysis of titanium dioxide modified by catechol-type interfacial surface complexes (ISC) with different substituted groups. *J. Catal.* **2015**, *329*, 286–290. [\[CrossRef\]](#)
14. Nsib, M.F.; Maayoufi, A.; Moussa, N.; Tarhouni, N.; Massouri, A.; Houas, A.; Chevalier, Y. TiO₂ modified by salicylic acid as a photocatalyst for the degradation of monochlorobenzene via Pickering emulsion way. *J. Photochem. Photobiol. A* **2013**, *251*, 10–17. [\[CrossRef\]](#)
15. Božanić, D.K.; Garcia, G.A.; Nahon, L.; Sredojevic, D.N.; Lazić, V.; Vukoje, I.; Ahrenkiel, S.P.; Djoković, V.; Šljivančanin, Ž.; Nedeljković, J.M. Interfacial Charge Transfer Transitions in Colloidal TiO₂ Nanoparticles Functionalized with Salicylic acid and 5-Aminosalicylic acid: A Comparative Photoelectron Spectroscopy and DFT Study. *J. Phys. Chem. C* **2019**, *123*, 29057–29066. [\[CrossRef\]](#)
16. Fujisawa, J.-I.; Matsumura, S.; Hanaya, M. A single Ti–O–C linkage induces interfacial charge-transfer transitions between TiO₂ and a π -conjugated molecule. *Chem. Phys. Lett.* **2016**, *657*, 172–176. [\[CrossRef\]](#)
17. Fujisawa, J.-I.; Eda, T.; Giorgi, G.; Hanaya, M. Visible-to-Near-IR Wide-Range Light Harvesting by Interfacial Charge-Transfer Transitions between TiO₂ and *p*-Aminophenol and Evidence of Direct Electron Injection to the Conduction Band of TiO₂. *J. Phys. Chem. C* **2017**, *121*, 18710–18716. [\[CrossRef\]](#)
18. Barbieriková, Z.; Dvoranová, D.; Brezová, V.; Džunuzović, E.; Sredojević, D.N.; Lazić, V.; Nedeljković, J.M. Visible-light-responsive surface-modified TiO₂ powder with 4-chlorophenol: A combined experimental and DFT study. *Opt. Mater.* **2019**, *89*, 237–242. [\[CrossRef\]](#)
19. Fujisawa, J.-I.; Kato, S.; Hanaya, M. Substituent effects on interfacial charge-transfer transitions in a TiO₂-phenol complex. *Chem. Phys. Lett.* **2023**, *827*, 140688. [\[CrossRef\]](#)
20. Ikeda, S.; Abe, C.; Torimoto, T.; Ohtani, B. Photochemical hydrogen evolution from aqueous triethanolamine solutions sensitized by binaphthol-modified titanium(IV) oxide under visible-light irradiation. *J. Photochem. Photobiol. A* **2003**, *160*, 61–67. [\[CrossRef\]](#)
21. Yao, B.; Peng, C.; Lu, P.; He, Y.; Zhang, W.; Zhang, Q. Fabrication of Tiron-TiO₂ charge-transfer complex with excellent visible-light photocatalytic performance. *Mat. Chem. Phys.* **2016**, *184*, 298–305. [\[CrossRef\]](#)
22. Maldaye, E.M.; Subramaniam, S.K. Fabrication of Visible Light Sensitive Electrospun TiO₂ Nanofibers Using Squaric Acid for Photocatalytic Application. *J. Nanomater.* **2023**, *2023*, 4213684. [\[CrossRef\]](#)
23. Lazić, V.; Sredojević, D.; Ćirić, A.; Nedeljković, J.M.; Zelenková, G.; Férová, M.; Zelenka, T.; Chavhan, M.P.; Slovák, V. The photocatalytic ability of visible-light-responsive interfacial charge transfer complex between TiO₂ and Tiron. *J. Photochem. Photobiol. A* **2024**, *449*, 115394. [\[CrossRef\]](#)
24. Agrios, A.G.; Gray, K.A.; Weitz, E. Photocatalytic Transformation of 2,4,5-Trichlorophenol on TiO₂ under Sub-Band-Gap Illumination. *Langmuir* **2003**, *19*, 1402–1409. [\[CrossRef\]](#)
25. Agrios, A.G.; Gray, K.A.; Weitz, E. Narrow-Band Irradiation of a Homologous Series of Chlorophenols on TiO₂: Charge-Transfer Complex Formation and Reactivity. *Langmuir* **2004**, *20*, 5911–5917. [\[CrossRef\]](#)
26. Kose, O. In Vitro Toxicity of Titanium Dioxide Nanoparticles on Human Lung Cells—Impact of the Physicochemical Features. Ph.D. Thesis, Chemical and Process Wngineering, Université de Lyon, Lyon, France, 2020. (In English).
27. Afşar, O.; Oltulu, Ç. Evaluation of the cytotoxic effect of titanium dioxide nanoparticles in human embryonic lung cells. *Turk. J. Med. Sci.* **2023**, *53*, 1648–1657. [\[CrossRef\]](#)

28. Sredojević, D.; Lazić, V.; Pirković, A.; Periša, J.; Murafa, N.; Spremo-Potparević, B.; Živković, L.; Topalović, D.; Zarubica, A.; Krivokuća, M.J.; et al. Toxicity of Silver Nanoparticles Supported by Surface-Modified Zirconium Dioxide with Dihydroquercetin. *Nanomaterials* **2022**, *12*, 3195. [\[CrossRef\]](#)
29. Nikšić, V.; Šimunková, M.M.; Dyrčíková, Z.; Dvoranová, D.; Brezová, V.; Sredojević, D.; Nedeljković, J.M.; Lazić, V. Photoinduced reactive species in interfacial charge transfer complex between TiO₂ and taxifolin: DFT and EPR study. *Opt. Mater.* **2024**, *152*, 115454. [\[CrossRef\]](#)
30. Shahriari-Khalaji, M.; Zabihi, F.; Bahi, A.; Sredojević, D.; Nedeljković, J.M.; Macharia, D.K.; Ciprian, M.; Yang, S.; Ko, F. Photon-driven bactericidal performance of surface-modified TiO₂ nanofibers. *J. Mater. Chem. C* **2023**, *11*, 5796–5805. [\[CrossRef\]](#)
31. Bajić, V.; Spremo-Potparević, B.; Živković, L.; Čabarkapa, A.; Kotur-Stevuljević, J.; Isenović, E.; Sredojević, D.; Vukoje, I.; Lazić, V.; Ahrenkiel, S.P.; et al. Surface-modified TiO₂ nanoparticles with ascorbic acid: Antioxidant properties and efficiency against DNA damage in vitro. *Colloids Surfaces B* **2017**, *155*, 323–331. [\[CrossRef\]](#)
32. Lazić, V.; Pirković, A.; Sredojević, D.; Marković, J.; Papan, J.; Ahrenkiel, S.P.; Janković-Častvan, I.; Dekanski, D.; Jovanović-Krivokuća, M.; Nedeljković, J.M. Surface-modified ZrO₂ nanoparticles with caffeic acid: Characterization and in vitro evaluation of biosafety for placental cells. *Chem.-Biol. Interactions* **2021**, *347*, 109618. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Dekanski, D.; Spremo-Potparević, B.; Bajić, V.; Živković, L.; Topalović, D.; Sredojević, D.N.; Lazić, V.; Nedeljković, J.M. Acute toxicity study in mice of orally administrated TiO₂ nanoparticles functionalized with caffeic acid. *Food Chem. Toxicol.* **2018**, *115*, 42–48. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Kirkland, D.; Aardema, M.J.; Battersby, R.V.; Beevers, C.; Burnett, K.; Burzlaff, A.; Czich, A.; Donner, E.M.; Fowler, P.; Johnston, H.J.; et al. A weight of evidence review of the genotoxicity of titanium dioxide (TiO₂). *Regul. Toxicol. Pharm.* **2022**, *136*, 105263. [\[CrossRef\]](#)
35. Živković, L.; Bajić, V.; Topalović, D.; Bruić, M.; Spremo-Potparević, B. Antigenotoxic Effects of Biochaga and Dihydroquercetin (Taxifolin) on H₂O₂-Induced DNA Damage in Human Whole Blood Cells. *Oxid. Med. Cell. Longev.* **2019**, *2019*, 5039372. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Abhijit, D.; Baidya, R.; Chakraborty, T.; Samanta, A.K.; Roy, S. Pharmacological basis and new insights of taxifolin: A comprehensive review. *Biomed. Pharmacother.* **2021**, *142*, 112004. [\[CrossRef\]](#)
37. Orlova, S.V.; Tatarinov, V.V.; Nikitina, E.A.; Sheremeta, A.V.; Ivlev, V.A.; Vasilev, V.G.; Paliy, K.V.; Goryainov, S.V. Molecularbiological problems of drug design and mechanism of drug action. *Pharm. Chem. J.* **2022**, *55*, 1133–1137. [\[CrossRef\]](#)
38. Mukhtar-Fayyad, D. Cytocompatibility of new bioceramic-based materials on human fibroblast cells (MRC-5). *Oral. Surg. Oral. Med. O.* **2011**, *112*, e137–e142. [\[CrossRef\]](#)
39. Lazić, V.; Vukoje, I.; Milicevic, B.; Spremo-Potparevic, B.; Zivkovic, L.; Topalovic, D.; Bajić, V.; Sredojevic, D.; Nedeljković, J.M. Efficiency of the interfacial charge transfer complex between TiO₂ nanoparticles and caffeic acid against DNA damage in vitro: A combinatorial analysis. *J. Serb. Chem. Soc.* **2019**, *84*, 539–553. [\[CrossRef\]](#)
40. Rokaitytė, A.; Zaborskienė, G.; Baliukonienė, V.; Jovaišienė, J.; Gustienė, S. Cytotoxicity of carnosine, taxifolin, linalool and their combinations with acids to the cells of human cervical carcinoma (HeLa). *Biologija* **2018**, *64*, 274–284. [\[CrossRef\]](#)
41. Mohammed, H.A.; Almahmoud, S.A.; El-Ghaly, E.-S.M.; Alam Khan, F.; Emwas, A.-H.; Jaremko, M.; Almulhim, F.; Khan, R.A.; Ragab, E.A. Comparative Anticancer Potentials of Taxifolin and Quercetin Methylated Derivatives against HCT-116 Cell Lines: Effects of O-Methylation on Taxifolin and Quercetin as Preliminary Natural Leads. *ACS Omega* **2022**, *7*, 46629–46639. [\[CrossRef\]](#)
42. Chahardoli, A.; Jalilian, F.; Shokoohinia, Y.; Fattahi, A. The role of quercetin in the formation of titanium dioxide nanoparticles for nanomedical applications. *Toxicol. In Vitro* **2023**, *87*, 105538. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Xie, X.; Feng, J.; Kang, Z.; Zhang, S.; Zhang, L.; Zhang, Y.; Li, X.; Tang, Y. Taxifolin protects RPE cells against oxidative stress-induced apoptosis. *Mol. Vis.* **2017**, *23*, 520–528. [\[PubMed\]](#)
44. Bruić, M.; Pirković, A.; Borožan, S.; Aleksić, M.N.; Jovanović Krivokuća, M.; Spremo-Potparević, B. Antioxidative and anti-inflammatory effects of taxifolin in H₂O₂-induced oxidative stress in HTR-8/SVneo trophoblast cell line. *Reprod. Toxicol.* **2024**, *126*, 108585. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Li, X.; Xie, H.; Jiang, Q.; Wei, G.; Lin, L.; Li, C.; Ou, X.; Yang, L.; Xie, Y.; Fu, Z.; et al. The mechanism of (+) taxifolin's protective antioxidant effect for •OH-treated bone marrow-derived mesenchymal stem cells. *Cell. Mol. Biol. Lett.* **2017**, *22*, 31. [\[CrossRef\]](#)
46. Svobodová, A.R.; Ryšavá, A.; Čížková, K.; Roubalová, L.; Ulrichová, J.; Vrba, J.; Zálešák, B.; Vostálová, J. Effect of the flavonoids quercetin and taxifolin on UVA-induced damage to human primary skin keratinocytes and fibroblasts. *Photochem. Photobiol. Sci.* **2022**, *21*, 59–75. [\[CrossRef\]](#)
47. Gulla, S.; Lomada, D.; Araveti, P.B.; Srivastava, A.; Murikinati, M.K.; Reddy, K.R.; Inamuddin; Reddy, M.C.; Altalhi, T. Titanium dioxide nanotubes conjugated with quercetin function as an effective anticancer agent by inducing apoptosis in melanoma cells. *J. Nanostruct. Chem.* **2021**, *11*, 721–734. [\[CrossRef\]](#)
48. Shabbir, S.; Kulyar, M.F.-E.; Bhutta, Z.A.; Boruah, P.; Asif, M. Toxicological Consequences of Titanium Dioxide Nanoparticles (TiO₂NPs) and Their Jeopardy to Human Population. *BioNanoScience* **2021**, *11*, 621–632. [\[CrossRef\]](#)
49. Langie, S.A.S.; Azqueta, A.; Collins, A.R. The comet assay: Past, present, and future. *Front. Genet.* **2015**, *6*, 266. [\[CrossRef\]](#)

50. Cao, Y.; Chen, J.; Bian, Q.; Ning, J.; Yong, L.; Ou, T.; Song, Y.; Wei, S. Genotoxicity Evaluation of Titanium Dioxide Nanoparticles In Vivo and In Vitro: A Meta-Analysis. *Toxics* **2023**, *11*, 882. [[CrossRef](#)]
51. Ling, C.; An, H.; Li, L.; Wang, J.; Lu, T.; Wang, H.; Hu, Y.; Song, G.; Liu, S. Genotoxicity Evaluation of Titanium Dioxide Nanoparticles In Vitro: A Systematic Review of the Literature and Meta-analysis. *Biol. Trace Element Res.* **2021**, *199*, 2057–2076. [[CrossRef](#)]
52. Shi, J.; Han, S.; Zhang, J.; Liu, Y.; Chen, Z.; Jia, G. Advances in genotoxicity of titanium dioxide nanoparticles in vivo and in vitro. *NanoImpact* **2022**, *25*, 100377. [[CrossRef](#)]
53. Chen, T.; Yan, J.; Li, Y. Genotoxicity of titanium dioxide nanoparticles. *J. Food Drug Anal.* **2014**, *22*, 95–104. [[CrossRef](#)] [[PubMed](#)]
54. Tice, R.R.; Agurell, E.; Anderson, D.; Burlinson, B.; Hartmann, A.; Kobayashi, H.; Miyamae, Y.; Rojas, E.; Ryu, J.C.; Sasaki, Y.F. Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagen.* **2000**, *35*, 206–221. [[CrossRef](#)]
55. Anderson, D.; Yu, T.-W.; Phillips, B.; Schmezer, P. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutat. Res. Mol. Mech. Mutagen.* **1994**, *307*, 261–271. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.