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RESEARCH ARTICLE

Bioactivity of mycosynthesized titanium oxide nanoparticles using *Epicoccum sherrardiae*, an endophyte of *Plectranthus vettiveroides*

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Abstract

Fungal endophytes are known to confer innumerable beneficial properties to the host plants they reside in, by way of their metabolites being utilised in various plant pathways involved in growth, reproduction, pest resistance and so on. Metal oxide nanoparticles have been in the limelight for their myriad physicochemical and bioactive properties. Mycosynthesized nanoparticles have more significance owing to their sustainable, nontoxic and eco-friendly nature. In the present study, Titanium oxide NPs were successfully synthesised using *Epicoccum sherrardiae,* a leaf fungal endophyte of the medicinal plant *Plectranthus vettiveroides,* with SEM analysis revealing an average particle diameter of 70 nm and a UV-Vis absorption peak at 284 nm. The NPs demonstrated significantly better performance than the fungal extract across all applications with 95.03 % Radical Scavenging Activity (RSA), enhanced antimicrobial activity with 21 mm zone of inhibition and high photocatalytic degradation of Congo Red dye (77.53 %). The results emphasise their potential in biomedical and bioremediation applications, aligning with the United Nations SDGs 6 and 12.

Keywords: congo red dye; DPPH; *Epicoccum sherrardiae*; FTIR; nanoparticles; *Plectranthus vettiveroides*; photocatalytic

Introduction

Endophytes are organisms with broad ecological presence that are present in the tissues of the plants, during a part of their life without technically infecting the host. Endophytic fungi are crucial for their secondary metabolism, which plays an imperative role in various industries such as pharmacy, food and environmental protection (1). Recently, endophytic fungi have been acknowledged as potent bioreactors for the synthesis of nanoparticles (NPs). These fungi colonise plant tissues without causing disease and are known for producing a diverse range of bioactive metabolites, enzymes and compounds that can reduce metals (2, 3). Plants constitute one of the most essential sources of pharmaceuticals. A significant proportion of the medications available today are derived from plants. Medicinal plants are the main source of secondary metabolites that serve as medicines and healing essential oils. Utilising medicinal plants to address various health concerns offers several advantages, including their safety, cost-effectiveness, efficacy and easy availability. The benefits of these practices have caused traditional healers to integrate them into their daily routines in a comprehensive manner. Due to the metabolic versatility and capacity to grow in controlled environments, the endophytic fungi are considered promising candidates for nanomaterial biosynthesis. Furthermore, studies of endophytes from medicinal plants have demonstrated that these relationships frequently produce distinctive fungal species with significant biotechnological promise (4).

Titanium dioxide (TiO_2) has gained considerable focus in the field of science and chemistry due to its diverse characteristics and unique individuality (5). TiO_2consists of two forms, amorphous and crystalline and it's mainly present in three crystalline polymorphs. mostly used to conduct experimental and theoretical studies and is widely used in photocatalysis and the degradation of dyes (6). In comparison, fungi are most preferred by researchers for the biosynthesis of TiO_2NPs, as most of the fungi are extracellular, allowing easy recovery of the NPs. In addition, the fungi-mediated synthesis of TiO_2 is economically feasible for large-scale production (7).

This study describes the synthesis of nanoparticles using *Eppicoccum sherrardiae* endophytic fungus isolated from the medicinal plant *Plectranthes vettiveroides,* a member of the Lamiaceae family, which is a native herb from south India and is used in ayurvedic medicine for its aromatic, bitter, cooling effect is well recognised in traditional medicine for its antimicrobial, anti-inflammatory and hepatoprotective properties (8). The study is distinct in itself as compared to the conventional research of NP mediated by fungi, in which well-known or highly documented types of fungi are commonly used; the current study features the use of a fungal species that has not been reported commonly in relation to nanobiotechnology. The significance lies in the mutually advantageous relationship between the still-to-be-investigated *E. sherrardiae* and its host *P. vettiveroides*. It is hoped that this exclusive association will deliver a unique secretome of biological reducing and stabilising substances to help convert the titanium tetraisopropoxide (TTIP) precursor into NPs and to this end, this work is a valuable contribution to green nanotechnology (9). Despite the well-established medicinal properties of *P. vettiveroides*, its potential in nanotechnology applications remains unexplored. To the best of the authors' knowledge, this represents the first report utilizing *E. sherrardiae* as a sustainable, cost-effective, resource for the synthesis of TiO_2 nanoparticles particularly as a bio-reducing and stabilizing agent in the green synthesis of metal or metal oxide nanoparticles, offering a novel contribution to green nanotechnology. This study, therefore, seeks to bridge this gap by employing the fungal extract of *E. sherrardiae* in the eco-friendly synthesis of TiO_2 nanoparticles.

Materials and Methods

Cultivation of endophytic fungi from plant samples

The isolation of endophytic fungi from the *P. vettiveroides* was carried out using surface sterilisation methods using 70 % ethanol. After sterilisation, the plant leaves were cut and placed on Sabouraud dextrose agar (SDA) medium supplemented with 100 mg/L ampicillin and 50 mg/L streptomycin. Fungal colonies were allowed to grow on the plates by incubating them at 25 °C for 9 days (10).

Fungal filtrate preparation

The fungus was grown in Sabouraud Dextrose Broth (SDB) medium by dissolving 16 g of dextrose and 4 g of Peptone in 400 mL of distilled H_2O and autoclaving for 20 min at 121 °C. After autoclaving, the endophytic fungi were inoculated into the SD broth by transferring a small piece of fungal mycelium from the master plate into the broth using a sterile loop. The flasks with the culture were then incubated at 25 °C for 9 days with constant shaking using a shaker incubator (Remi RS-24 plus) at 150 rpm.

Mycosynthesis of TiO_2 NPs

The biosynthesis of TiO_2 NPs experiment was carried out by combining 20 mL of fungal extract, which was prepared by chopping the fungal biomass and spinning it at 400 rpm in distilled water for 3 hr in a 1:2 ratio (mycelium:water), with 20 mL of 0.34 M Titanium isopropoxide (TTIP) solution prepared in a 1:9 ratio using ethanol (11). The pH of the reaction mixture was adjusted and maintained at pH 6.5 ± 0.2 to ensure controlled hydrolysis of the precursor. The mixture was then placed in a shaker incubator at 200 rpm to ensure continuous agitation in a dark environment at 37 °C for 24 hr. A control was established under the same conditions. After ageing, the solution was centrifuged to obtain a pellet, which was washed several times using ethanol and deionised water (thrice) and centrifuged at 10000 rpm to remove unreacted substances and impurities (9). The obtained white-colored pellet was subjected to calcination for 2 hr at 300 °C (11).

Molecular identification of fungal endophyte

Molecular phylogenetic analysis was performed using DNA sequencing of the ITS (Internal Transcribed Spacer) regions as a basis. Following the purification of these PCR amplicons, DNA sequencing was performed using forward and reverse ITS-1 and 4 primers. The BDT v3.1 Cycle sequencing kit was utilised on the ABI 3730xl Genetic Analyzer Tool. The results obtained were analysed via BLAST analysis and compared to the GenBank database using nucleotide homology. The phylogenetic tree was constructed using the MEGA 10 tool. Consensus sequences were created from forward and reverse primers with the help of the aligner software. Amplified fragments of ITS regions were analysed for species identification using the multiple alignment software Clustal W, based on the identity score matrix derived from BLAST (Basic Local Alignment Search Tool) analysis in the GenBank database. A phylogenetic tree was then constructed using MEGA 10.

Characterisation of TiO_2 NPs

UV-spectroscopy

The UV spectral analysis was carried out between 200 and 800 nm by a UV spectrophotometer. (Shimadzu UV-1800ENG240V)) for UV–visible spectral analysis. The nanoparticle suspension was appropriately diluted using distilled water before analysis to avoid scattering effects. A blank solution containing only water without a precursor was used as the control.

Fourier transform infrared (FTIR)

The various functional groups engaged in the synthesis of nanoparticles were identified using the FTIR (Shimadzu IRSpirit) for TiO_2 NPs made in a green environment.

XRD Analysis

The crystalline structure and phase composition of the mycosynthesized titanium oxide nanoparticle were analysed using X -ray diffraction (XRD) (12). X-ray diffraction (XRD) analysis was conducted using a RigakuMiniFlex diffractometer operating at 30 kV and 15 mA with Cu Kα radiation (λ = 1.5406 Å).

SEM analysis

The surface morphology and elemental composition of the TiO_2 were examined using Field Emission Scanning Electron Microscopy (FE-SEM) coupled with Energy-Dispersive X-ray Spectroscopy (EDS), performed on a ZEISS Sigma HV microscope. The EDS spectra confirmed the presence and distribution of titanium (Ti) and other elements.

Antimicrobial assay

Antimicrobial activities for the synthesized metal oxide (TiO_2)NPs and Fungal extract were performed against Gram positive bacteria *Staphylococcus aureus* (ATCC 25923), Gram Negative bacteria *E. coli* (ATCC MG1655) using well diffusion method was used for the antimicrobial assay, cultures of the test organisms were made on nutrient agar plates under aseptic conditions. Plates were allowed to stand for 10 min to let the culture get absorbed. Then, wells were made using a sterile cork borer into the nutrient agar plates for testing the antimicrobial activity of TiO_2 nanoparticles and Fungal extract samples against the positive control Ampicillin (1 mg/mL) (13). A micropipette was used to add 30 µl of each sample of nanoparticle suspension and the extract was poured into each wells The plates were incubated overnight at 37 °C. After incubation, the zone of microbial growth suppression was observed (14, 15). The selection of Gram-negative *E. coli* and gram positive *S. aureus* enables an important comparative examination, as they represent the two leading pathogens linked to antimicrobial resistance and responsible for most community and hospital-acquired infections. Thus, they serve as vital reference points for evaluating novel antimicrobial approaches.

Antioxidant Activity (DPPH Assay)

2,2-Diphenyl-1-picryl hydrazyl (DPPH), a commonly used assay, was employed to evaluate the fungal extracts and Titanium dioxide nanoparticles' antioxidant capacity. To make up the volume to 2 mL, a range of extract volumes (50, 100, 150 and 200 µL) was added to 1 mL of 0.2 mM DPPH solution and the volume was made up with pure methanol solution (16). The tubes were incubated in the dark conditions for 60 min and the absorbance was measured with a UV-visible spectrophotometer using ascorbic acid as the reference and methanol as the blank solution at 517 nm. The percentage antioxidant activity was calculated using the formula

$$\textbf{\% RSA} = \left(\frac{Ac - As}{Ac}\right) \times 100 \qquad \text{(Eqn. 1)}$$

where Ac is the absorbance of the control at 517 nm and As is the absorbance of the sample at 517 nm (Control was 0.2 mM DPPH) (17).

Photocatalytic activity

Titanium dioxide nanoparticles and Fungal extract were subjected to the degradation of Congo red dye under visible light irradiation to determine their photocatalytic activity. About 10 mg of the synthesised nanomaterials was dispersed in 1000 mL (1 ppm) of the Congo red dye suspension at a concentration of 10 ppm. To reach the adsorption equilibrium, the synthesised sample with Congo red dye was stored in the dark, maintained at room temperature for about 5 hr (18). The sample solution was withdrawn at varied intervals (10, 15, 20, 30, 45, 60 mins), this initial & final concentration (C_0 to C) and was determined by filtering using a filter syringe to determine the photocatalytic degradation of the nanoparticles. Results were checked using a UV-visible spectrometer at 495 nm (19). Titanium dioxide NPs suspended in water and filtered using a syringe filter served as the blank.

The percentage of dye degradation was calculated by using the following equation:

$$\text{\% degradation} = \left(\frac{co - c}{co}\right) \times 100 \qquad \text{(Eqn. 2)}$$

Where $C_0$is the absorbance at 0th min and $C_0$is the absorbance of the sample at 495nm

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 21. A One-way Analysis of Variance (ANOVA) was employed to determine if there were statistically significant differences in the means of the antioxidant activity, antimicrobial activity and photocatalysis activity across the different groups of TiO_2NPs and the control group. All statistical comparisons were evaluated at a significance level of $p < 0.05$.

Results and Discussion

Culturing and isolation of fungal endophytes

Plectranthus vettiveroides was isolated and subjected to washing and surface sterilisation using 70 % (v/v) ethanol and 5 % (v/v) sodium hypochlorite. The explants were inoculated in SDA media, at 25 °C (under dark conditions) to allow fungal growth. One of the endophytic fungi from the leaves of *P. vettiveroides* (*P. vettiveroides* leaf fungus PVLF1) was used for bioactive studies and the synthesis of Titanium oxide NP**.** Regular observations were made in order to check for myceli**a**l growth and contaminations if any and LCB staining was performed (Fig. S1). After four weeks of incubation, optimal mycelial growth was obtained and these cultures were then further sub-cultured in SDA and incubated for about 15 days at 25 °C under dark conditions for optimal mycelial growth (Fig. 1). The culture was further inoculated in SDB incubated for about 7 days at 25 °C (under dark conditions) (20).

Fig. 1. Fungal endophyte *Epicoccum sherrardiae* isolated from *Plectranthes vettiveroides* grown on sabouraud dextrose agar media.

Identification of the fungal endophyte by rDNA sequence analysis

The fungus PVLF1, isolated from *P. vettiveroides*, was identified by 28S rRNA sequencing by PCR amplification of ITS-1 and 4 regions. Based on an identity score matrix using the Basic Local Alignment Search Tool (BLAST) analysis in the GenBank database, amplified fragments of the ITS regions were analysed for species identification through the multiple alignment software program Clustal W Distance matrix. The strain was identified and deposited in GenBank and the accession number obtained was PV891893. The fungal endophyte was identified as *E. sherrardiae*, which demonstrated a high degree of similarity based on nucleotide phylogenetic analysis and homology (Fig. S2). Using MEGA 10, a Phylogenetic tree was constructed and is depicted in Fig. S3 (21).

Biogenic synthesis of TiO_2 NPs

The fungal culture was allowed to reach the required growth phase. The mycelium was obtained through a sterilised filter paper. The mycelium was chopped and spun in distilled water for three hr (Fig. 2). This filtrate comprised fungal metabolites and enzymes that could convert titanium ions into TiO_2NPs through the process of reduction. To accomplish this, the obtained filtrate was combined with a solution containing titanium ions (titanium isopropoxide). Then, the resulting mixture was allowed to age at 37 °C for 24 hr (22). The biosynthesis of TiO_2 NPs was controlled by the biomolecules and the enzymes in the fungal filtrate, which acted as reducing agents that catalysed the reduction and conversion of titanium ions into TiO_2 NPs (9, 11).

XRD analysis

The XRD pattern of TiO_2 NPs showed a dominant peak at the 2θ value of 25.25° (Fig. 3). This matches the (101) crystallographic plane of the TiO_2 anatase structure, indicating that the crystal composition is predominantly anatase. The XRD pattern exhibited distinct diffraction peaks at 2θ = 9.8°, 13.2°, 14.9°, 16.7°, 18.6°, 21.1° and 25.8°. The sharp and intense peaks confirmed the crystalline nature of the TiO_2 nanoparticles (23). The most prominent diffraction peak observed at 2θ = 25.8° corresponds to the (101) plane of the anatase phase of TiO_2 according to JCPDS cardno. 21-1272. These results validated the successful synthesis of anatase-phase TiO_2 nanoparticles with high crystallinity, which is favourable for photocatalytic and antimicrobial applications (23). The particle size was estimated using the Scherrer equation and was found to be ~22.8 nm

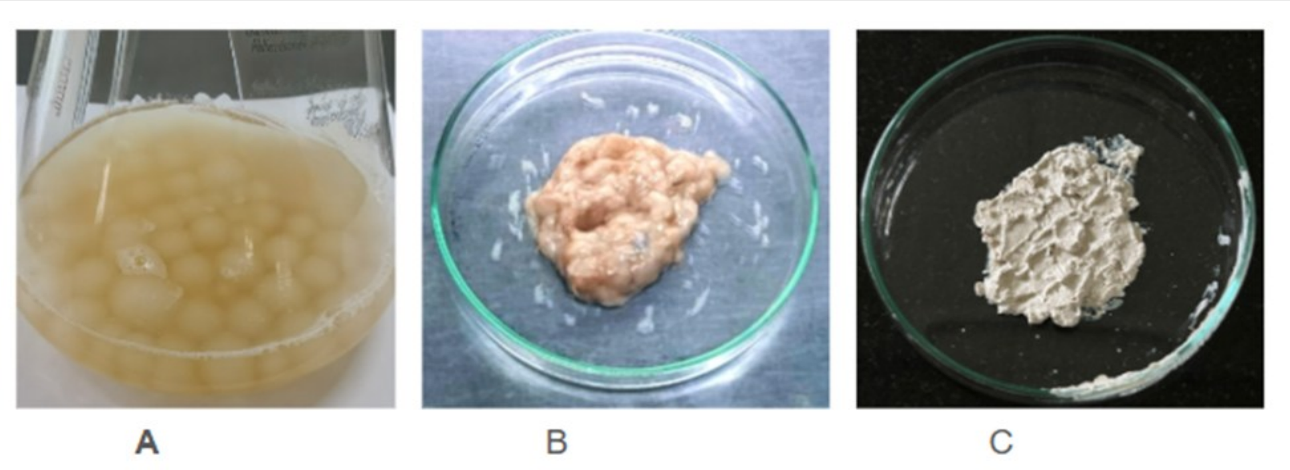


Fig 2. Preparation of fungal biomass and biosynthesised titanium oxide nanoparticles: **A.** Submerged fungal culture in Sabouraud Dextrose broth, **B.** Chopped fungal mycelium, **C.** Titanium oxide nanoparticles.

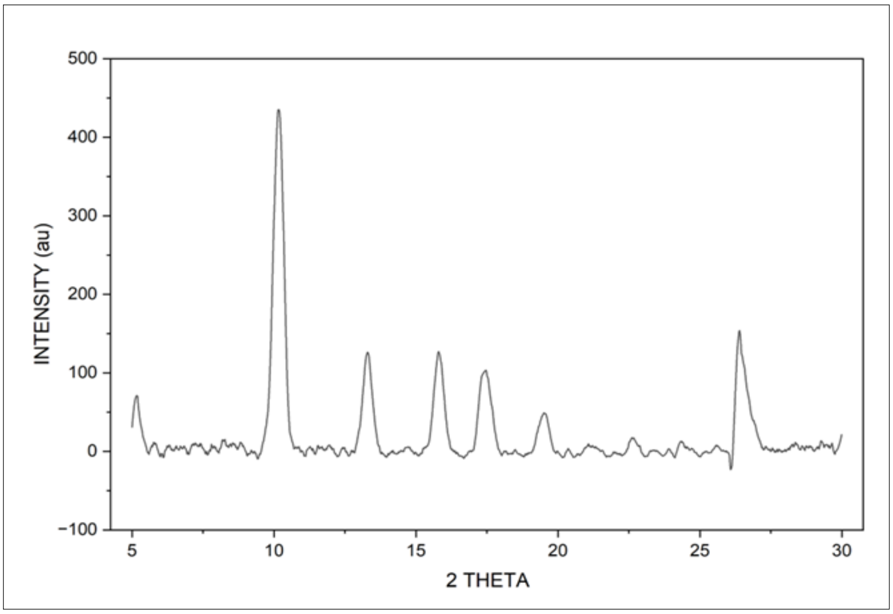


Fig 3. XRD analysis of synthesised TiO_2 nanoparticles using mycosynthesized *Epicoccum sherrardiae* extract.

FTIR analysis

A typical FTIR spectrum of the green synthesised TiO_2 NPs recorded in the series of absorption bands 500 cm^{-1} to 4000 cm^{-1} is shown in (Fig. 4). The band intensities in the different regions of the spectrum recorded for the powdered TiO_2 NPs were analysed. A broad peak at ~3525.35 cm^{-1} possibly attributed to the presence of the hydroxyl group and surface adsorbed water, strong peak at 1516 cm^{-1} is very commonly attributed to the bending vibration of adsorbed water (H-O-H bending) on the TiO_2 surface respectively absorption peak at 1339 cm^{-1} may correspond to Ti–O stretching and Ti–O–Ti bridging stretching modes (24–26).

FE-SEM and EDX Analysis

The distinct surface morphology of biosynthesised TiO_2 was characterised using FE-SEM. The micrographs revealed clustered NPs exhibiting predominantly cubic and spherical morphologies (Fig. 5a). The particle size was analysed using ImageJ software and the average size was found to be approximately 70 nm. The EDS spectra show the sharp signals corresponding to the Ti peak at approximately 0.5 and 4.5 keV and the O peak at 0.5 keV (Fig. 5b), confirming the successful formation of TiO_2 NPs. EDS profiles of biosynthesised Ti NPs also show signals corresponding to carbon; the presence of carbon is commonly associated with organic compounds originating from the *E. sherrardiae* fungal extract used in the green synthesis process. These organic signals suggest the surface adsorption of phytochemicals such as polyphenols, flavonoids or other bioactive constituents onto the surface of the TiO_2 NPs. The detection of these elements supports the phytochemical-assisted synthesis route and further explains the enhanced biocompatibility and functional properties of the resulting TiO_2 NPs.

Absorption spectroscopy analysis

The absorption measurement of TiO_2 nanoparticles was recorded using distilled water at room temperature using a UV-Vis spectrophotometer and the absorption spectrum is shown in (Fig. 6). The strong peak at 284 nm in the range of 200 to 800 nm confirmed the biosynthesis of TiO_2 NPs (27). The absorption spectrum of metal NPs is sensitive to several factors, including particle size, shape and particle–particle interaction (agglomeration) with the medium (28). Similar studies were conducted with peaks ranging around 280 nm in UV–Vis spectroscopy, confirming the presence of titanium oxide NP (29, 30).

Antioxidant assay

The antioxidant activity of the fungal extract and nanoparticles was evaluated using the DPPH radical scavenging assay and compared with a standard antioxidant, ascorbic acid. The absorbance values at 517 nm for different concentrations (0, 0.25, 0.5, 0.75 and 1 mg/mL)

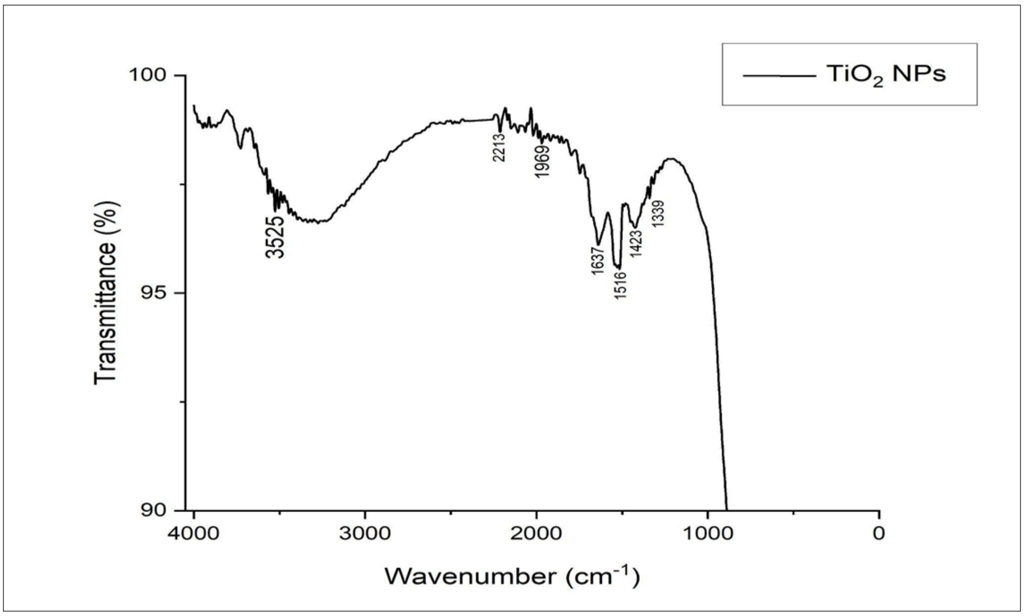


Fig. 4. FTIR spectrum of synthesised TiO_2 NPs mycosynthesized using *Epicoccum sherrardiae* extract.

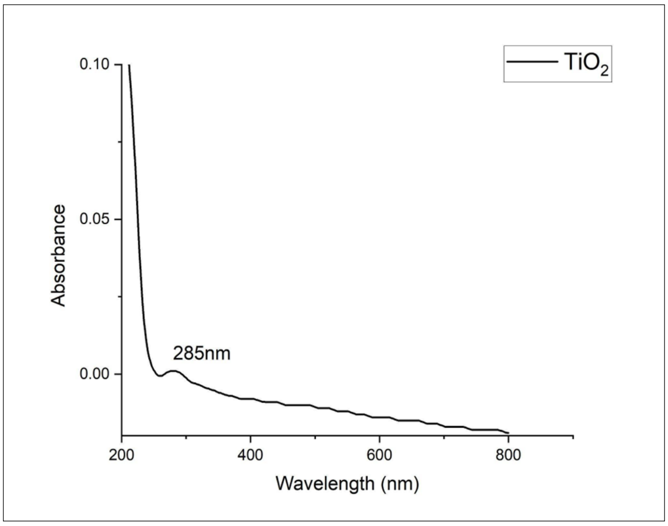


Fig. 5. UV-Vis absorption spectrum of synthesised Titanium oxide NPs mycosynthesized using *Epicoccum sherrardiae* extract.

are shown in Fig. 6. The radical scavenging property for all the dilutions of the standard and extracts was estimated by substituting the values in the prescribed formula (17). The synthesised TiO_2 nanoparticles and fungal broth displayed notable antioxidant properties, comparable to the standard ascorbic acid (Fig. 7). The radical scavenging activity (RSA) increased with the rising concentrations (20). The highest concentrated sample showed the best RSA for mycosynthesized TiO_2 NP (95.03 %) and fungal extract (90.5 %).

Antibiotic susceptibility assessment of TiO_2 nanoparticles and Fungal extract

The nanoparticle and the fungal extract showed a broader spectrum of antibacterial activity against both classes of bacteria. The mycosynthesized NPs showed good antimicrobial activity against the positive control ampicillin and the zone of inhibition was measured to be 20 mm and 21 mm for the test organisms *S. aureus and E. coli,* respectively and the fungal extract showed ZOI of 14 mm and 15 mm for the test organism respectively. The increased biocidal potential exhibited by the fungal-mediated nanoparticles and the fungal extract was shown toward Gram-negative bacteria *E.coli* (Fig. 8). These variations could be attributed to their differences in the structure of the cell wall, virulence factors or inherent resistance mechanisms of Gram-negative bacteria *Staphylococcus aureus* (31). This could be significant in clinical settings as many members of Gram-negative bacteria like *Klebsiella pneumoniae, Acinetobacter*

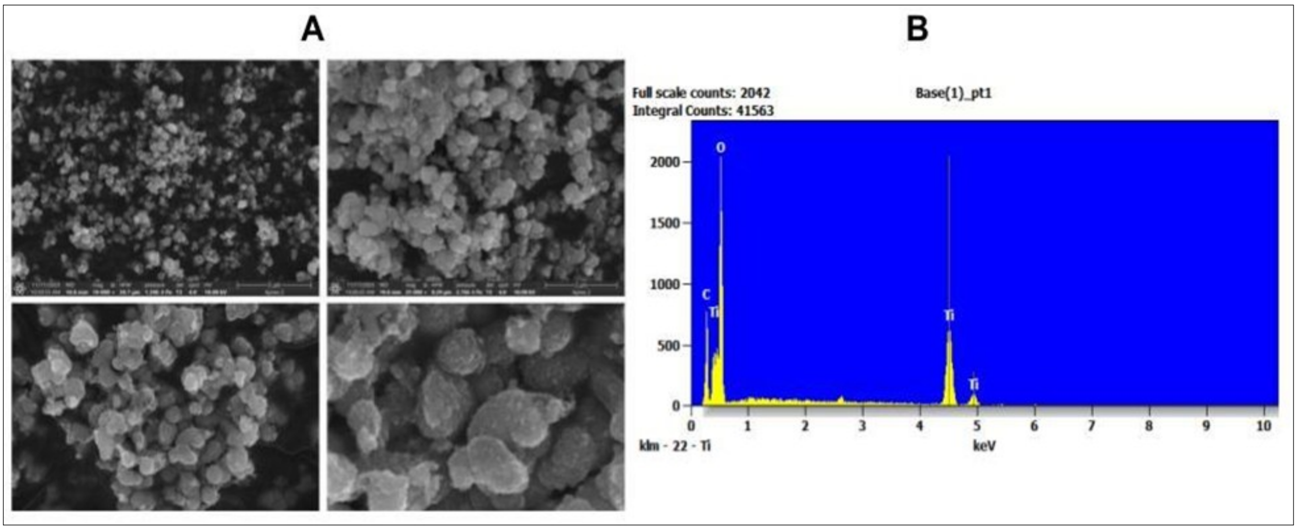


Fig. 6. Surface morphology of mycosynthesized Titanium oxide nanoparticles using *Epicoccum sherrardiae* extract: **A.** SEM images, **B.** EDS spectrum with the peak corresponding to titanium.

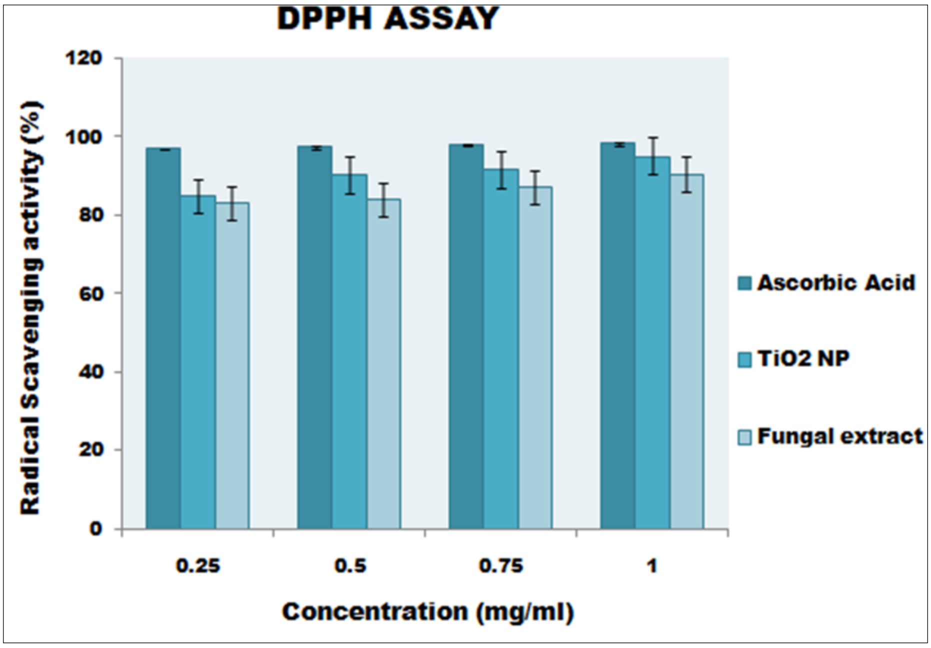
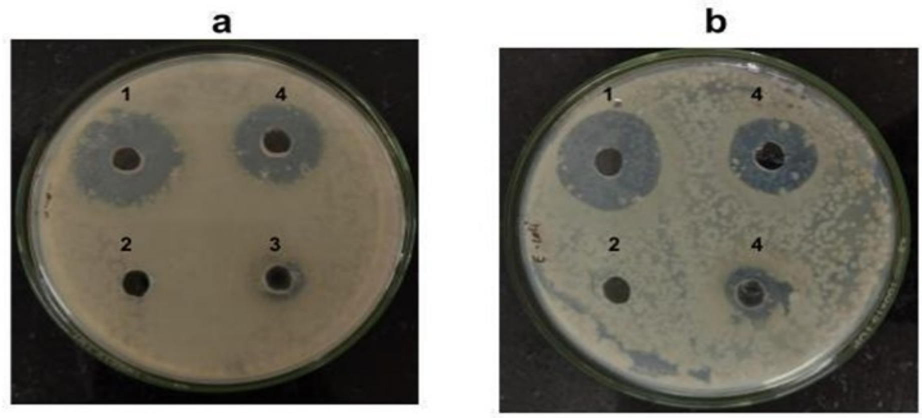


Fig. 7. Radical Scavenging activity by DPPH assay of *Epicoccum sherrardiae* endophytic fungal culture filtrate, mycosynthesized Titanium oxide nanoparticles and standard antioxidant (ascorbic acid) at different concentrations. The values are represented as means of three independent determinations (n = 3), indicating that the values are significantly different from the treated samples using one-way ANOVA, $p < 0.05$ using SPSS software.



c

	Zone of inhibition (mm)			
Bacterial species	Ampicillin (1)	Negative control (2)	Fungal extract (3)	Titanium oxide nanoparticle (4)
S. aureus	24	-	14	20
E. coli	25	-	15	21

Fig. 8. Antibacterial test of *Epicoccum sherrardiae* fungal extract and titanium oxide nanoparticles by well diffusion. **a.** *Staphylococcus aureus,* **b.** *Escherichia coli,* **c.** Zones of inhibition.

baumannii, Pseudomonas, Neisseria gonorrhoeae and *aeruginosa, Salmonella,* are dangerous human pathogens and are becoming increasingly resistant to antibiotics (32). At present, many studies are on pipiline to investigate how metal and metal oxide nanoparticles interface with bacteria. Studies have demonstrated that metal nanoparticles cause the bacterial surface's outer membrane to dissolve, releasing harmful substances (33, 34). Titanium dioxide nanoparticles (TiO_2 NPs) are recognised for their ability to generate oxidative stress by producing reactive oxygen species (ROS), which can damage the integrity of bacterial cell walls by initiating lipid peroxidation and altering the fluidity of the membrane (35).

Photo-catalytic activity

The photodegradative potential of biosynthesised TiO_2 NPs and fungal extract was analysed initially with a UV–vis spectrophotometer. The maximum degradation attained after 1 hr was 76.52 % for the mycosynthesized nanoparticle and 50 % for the fungal extract (36). Light is crucial for the photo-catalytic breakdown of dye, as the photo-chemical activity is linked to the fragmentation and conversion of Congo Red (CR) dye into low-molecular-weight by-products. The effect of irradiation time was studied for a duration of 1 hr at an optimised initial dye concentration of 20 ppm for fungal broth and mycosynthesized nanoparticles under room temperature and visible light irradiation. The decrease in degradation efficiency till 60th min is depicted in (Fig. 9) (37).

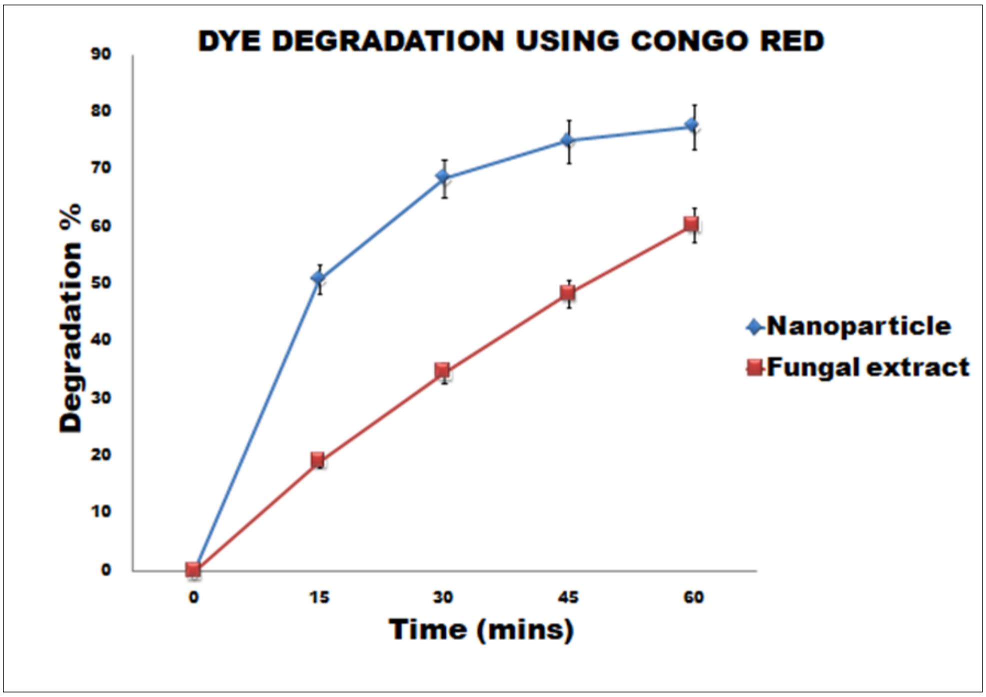


Fig. 9. Dye degradation efficiency of the extract of the endophytic fungus, *Epicoccum sherrardiae* and mycosynthesized Titanium oxide nanoparticles on Congo Red. The values are represented as means of three independent determinations (n = 3), indicating that the values are significantly different from the treated samples using one-way ANOVA, $p < 0.05$ using SPSS software.

The concentration of the solution affects the absorbance measurement at 0 min (A_0); a higher concentration leads to a greater absorbance at A_0, while a lower concentration results in lower absorbance. This phenomenon is associated with an increasing number of dye molecules, in which the amount of light (photon quantum) that penetrates the dye solution reaches the catalyst surface is reduced due to obstacles in the light path, which causes the absorbance value to increase. Moreover, the percentage of degradation value at a given time (t) increases along with the concentration of Congo red solution (5). This can be explained by the fact that UV irradiation promotes the valence band to the conduction band (CB), which leads to the formation of a photoelectron. These highly energetic photoelectrons, in turn converts the hydroxyl ion (OH–) into hydroxyl radical (•OH), which is responsible for the photochemical degradation of Congo red (38). The fungal endophyte *E. sherrardiae* was found to enhance TiO_2 performance by acting as stabilisers to increase the surface area and as surface modifiers to enable efficient visible light activation, leading to superior photocatalytic dye degradation.

Conclusion

The present work depicts a sustainable mycosynthetic method of the preparation of titanium dioxide nanoparticles using the endophytic fungus *E. sherrardiae* which was isolated through the medicinal plant *P. vettiveroides.* In the XRD and SEM analyses, the crystallite size of the TiO_2 nanoparticles was found with an average diameter of the particles at approximately 70 nm. In the UV-Vis absorption, it was established that the nanomaterial was formed successfully, as the nanoparticles showed a peak at 284 nm. The nanoparticles were tested functionally as they showed much improved antioxidant activity (95.03 % RSA) and strong antibacterial effect, giving an inhibition zone of 21 mm on the growth of *E.coli* and 20 mm on *S. aureus* (better than the fungal extract). Their photocatalytic activity was also higher at 77.53 % degradation of Congo Red on the surface, as opposed to 60.04 with the fungal extract and demonstrated greater reactivity of the surface and catalytic ability. The example of using *P. vettiveroides* and *E. sherrardiae* as a source of a green, inexpensive and environmentally unbiased synthetic pathway to minimise the number of chemical inputs and maximise the stability of nanoparticles via biologically mediated functionalization. The findings also align strongly with global sustainability priorities by contributing to SDG 3 through improved antimicrobial and antioxidant solutions for health applications, SDG 6 through the development of efficient and eco-friendly dye degradation strategies for cleaner water and SDG 12 through the adoption of a responsible, non-toxic, biologically driven production method. In general, the analysis demonstrates that mycosynthesized TiO_2 nanoparticles based on *P. vettiveroides*-associated *E. sherrardiae* are a promising platform for developing sustainable biomedical and environmental technologies.

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Authors' contributions

SS contributed to conceptualisation, supervision, reviewing and editing of the manuscript. MK contributed to methodology, Software, Investigation and writing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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