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Short communication

Titanium dioxide nanoparticles fabrication from *Parmotrema austrosinense* (Zahlbr.) Hale extracts and its antimicrobial efficacy against plant pathogens

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ABSTRACT

Green chemistry adoption in biosynthesis of nanoparticles has revolutionized the realm of phyto-medicine. Lichens that comprise the symbiotic association of fungi and either algae or cyanobacteria render eco-friendly and biocompatible applications. A plethora of secondary metabolites with redox potentials from lichens have wide applications as bioactive incumbents and bio-indicators of pollution. The present study reports, the synthesis of titanium dioxide nanoparticles (TiO_2-NPs) using an ultra-sonicated aqueous extract of the lichen Parmotrema austrosinense (Zahlbr.) Hale. The physico-chemical properties of TiO_2-NPs were characterized using UV–Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopic (SEM) techniques. Bio-synthesized TiO_2-NPs exhibited an average particle size of 10–80 nm indicating structural variability. Phytochemical properties were estimated prior to assessment of antibacterial efficacies elicited by the synthesized by testing it against selected bacterial and fungal plant pathogens such as *Bacillus subtilis, Erwinia chrysanthemi, Xanthomonas phaseoli*, *Fusarium oxysporum, Rhizoctonia solani,* and *Sclerotium rolfsii* respectively using agar well diffusion method. The results revealed that as prepared TiO_2 nanoparticles from lichens owned significant antimicrobial activity against *X. phaseoli* and *F. oxysporum.*

1. Introduction

Titanium dioxide (TiO_2) represents the dielectric component responsible for energy production using photocatalytic activity and electrochemical properties [1–3]. TiO_2 nanoparticles accounts for key component production in various fields posing significant applications in bio-catalysis, pharmaceutical applications along with cosmetics production [4]. Nevertheless, potential utility of nanoparticles in both key realms of agriculture and medicine show escalated research and product development for commercialization aspects too [5]. Response Surface Methodology (RSM) has been reported for biosynthesis of silver nanoparticles ($AgNO_3$) employing green chemistry in the lichen *Cetraria islandica* (L) Ach, showed biocompatible nanoparticles under increased temperature conditions and decreased nanoparticles size [6]. Methodologies for characterization and optimization for escalated bio-medical activities pose as a significant arena of research [7–9]. Titanium dioxide synthesized from *Phaseolus vulgaris* have been proved effective in phyto-toxicity, seed yield and morphometric augmentation that reveals plant growth promotion [10].

Titanium dioxide has been assessed for affirmed plant growth promotion activities in *Vicia faba* [11]. Moreover, titanium dioxide nanoparticles have been proved for versatile mechanisms in plant growth properties, chromosomal variations and cytotoxic activities in combating DNA damage and environmental safety [12], compared with silver nanoparticles (AgNPs) and also much more explored. Titanium dioxide nanoparticles synthesized from the lichen *Protoparmeliopsis muralis* showed that metal and metal oxide nanoparticles effectively combated multidrug resistant *Staphylococcus aureus* efficiently through inhibition of quorum sensing and antioxidant properties [13]. The titanium dioxide nanoparticles owing to its potent bactericidal and other

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similar bioactivities have been addressed to coat several non-living components, waste water treatment coatings and food package materials [14]. Thus, the utility of titanium dioxide nanoparticles in multiple optimal benefits was advocated to corroborate the nano medicinal properties in phytopathology fields. As the phyto-nanomedicine advancements are significantly representing potential upheaval, the present work was undertaken to synthesize and characterize TiO_2 nanoparticles using lichen *Parmotrema austrosinense* and evaluating the antifungal efficacy of TiO_2 nanoparticles against selected plant pathogens.

2. Experimental

2.1. Lichen collections

Lichens were intensively collected at various altitudes across the Western Ghats located in South India. The thallus margin damage was taken care and the specimens were cleaned and dried under sunlight. To cross verify, the samples were deposited in National Botanical Research Institute, Lucknow.

2.2. Morphological and taxonomical identification

Morphological features were studied using powerful lenses and trinocular zoom dissection microscope (Meiji optics, Japan). Spot test according to Santesson, [15], employing K (potassium hydroxide), C (sodium hypochlorite), KC and P tests (*para*-phenylenediamine), were done for the identification of the lichen. Thin layer chromatography (TLC) analysis was carried out according to a modified protocol of Culberson [16,17], method for metabolite profiling in the lichen. Identification protocol of the pioneering lichenologist Mason Hale's procedure was adopted as per the classical taxonomic outline [18].

2.3. Aqueous extract preparation and extraction

The conventionally used, cold percolation extraction method was adopted. Briefly the lichen thalli were thoroughly washed and dried at room temperature, pulverized in an electronic grinder and 10.00 g of lichen powder was extracted with 100 ml of sterile distilled water at 70 °C in water bath and ultra-sonicated. The extract was filtered by No. 1 Whatman filter paper and the filtrate containing the water was evaporated by drying and the extract obtained was stored in a refrigerator.

2.4. Phytochemical analysis

Phytochemical analyses were carried out according to the methods of Alavi et al. [13]. Below enlisted are the protocols adopted for photochemical analysis.

2.4.1. Test for alkaloids, saponins, amino acid, anthraquinones, flavonoids, tannins, steroids, cardenolides, terpenoids, cardiac glycosides, phlobatannis, xanthoprotein, proteins and sugars

The 0.5 ml of extract from lichen sample was added with 1 % aqueous hydrochloric acid (HCl) and placed in water bath for 5 min and few drops of Wagner's reagent was added. Orange brown color formation indicates the presence of alkaloids. 1.00 ml of extract from lichen sample was added with 2 ml of distilled water and agitated for 15 min. The foam formation indicates the presence of saponins. Three drops of 5 % ninhydrin added to 1 ml of extract from lichen sample and boiled for 10 min. The bluish or purple color indicates the presence of amino acid. 0.5 ml of extract from lichen sample was added with 5.00 ml of chloroform and kept for 5 min shaking. It was filtered and equal volume of ammonia was added again and then kept in shaker for 5 min. Red /pink /violet color formation indicates the presence of anthraquinones. A few drops of 1 % ammonia were added to 1.00 ml of extract from lichen sample. Yellow color formation indicates the presence of flavonoid. A few drops of 1 % lead acetate were added to 2–3 ml extract from lichen sample. The yellow precipitate formation indicates the presence of tannins. 1 ml of extract from lichen sample was added to 10 ml of chloroform and equal volume of concentrated sulfuric acid was added. Yellow with green fluorescence and red color indicates the presence of steroids. 10 ml of extract from lichen sample was added to 1 ml of chloroform and acetic anhydride, 2 ml of concentrated sulfuric acid was added. Reddish violet color formation indicates the presence of terpenoids. 2 ml of glacial acetic acid was added with 1drop of ferric chloride and 5 ml of extract from lichen sample, 1 ml of concentrated sulfuric acid was added. Brown ring formation at interface, violet ring below and green ring below violet ring indicates the presence of cardiac glycosides. 5 ml of extract from lichen sample was added with 5 ml of 1 % aqueous HCl. The red precipitate formation indicates the presence of phlobatannis. 5 ml of extract from lichen sample was added with 1 ml of concentrated nitric acid, it was boiled and cooled and 40 % sodium hydro oxide was added. The deep orange color formation indicates the presence of xanthoprotein. 1 ml of extract from lichen sample was added with 4 % sodium hydro oxide along with few drops of 1 % copper sulphate. The violet red color formation indicates the presence of protein. 1 ml of extract from lichen sample was added with few drops of iodine. Deep blue color formation indicates presence of sugars. 2 ml of glacial acetic acid was added with 1 drop of ferric chloride and 0.5 ml of extract from lichen sample. Then, 1 ml of concentrated sulfuric acid was added. The brown ring at interface indicates the presence of cardenolides [19].

2.5. Bio-synthesis of TiO_2-NPs

Briefly, for synthesis of TiO_2-NPs, the Erlenmeyer flask containing 100 ml of titanium oxide (TiO_2) with 0.1 and 0.01 concentrations were prepared and stirred for 2 h. The lichen *P. austrosinense* extract was filtered over Whatman No. 1 filter paper and centrifuged at 6000 rpm for 30 min. Ten mL of resulted lichen extract was added to 90 ml of TiO_2 at room temperature under agitated condition for 24 h. Later, synthesized NPs solutions were centrifuged at 5000 rpm for 20 min. Reduction of TiO_2 with *P. austrosinense* extract resulted in light to dark brown colours [13,20].

2.6. Characterization of TiO_2-NPs

Characterization of synthesized nanoparticles for assessing the redox potential nature was performed various techniques such as UV–VIS spectroscopy, Fourier transform infrared radiation (FTIR), X-ray diffractometry (XRD) and Scanning electron microscopy (SEM).

2.6.1. UV/Vis spectroscopy

Biosynthesized TiO_2 nanoparticle was characterized by UV/Vis spectrophotometer (UV-1700, Shimadzu). TiO_2 nanoparticles were observed by the spectrum with the wavelength ranges between 200 and 700 nm.

2.6.2. FTIR analysis

FT-IR of the sample was obtained using a spectrometer (Shimadzu) in the spectral range of 400–4000 cm^{-1}. The samples were formed into pellets with KBr and the spectra were recorded on a Shimadzu FTIR spectrometer for the infrared absorption spectrum. The information about the way in which the absorbed molecules are bonded to the surfaces as well as the structural information of the solids were elucidated by IR spectroscopy.

2.6.3. XRD analysis

The powdered samples of TiO_2 nanoparticles were investigated with X-Ray diffraction method. The spectral details were derived in XPERT-PRO diffractometer using specifications as per the instrument manufacturer at optimal conditions. Scherrer formula for calculating the spectral details to estimate crystalline deposits were performed.

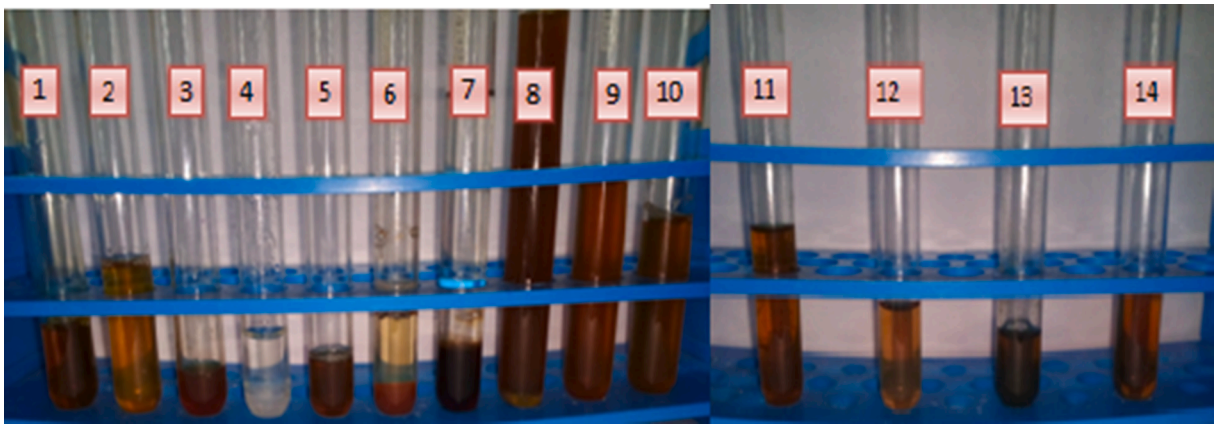

Fig. 1. *P. austrosinense* lichen collected from Western Ghats area and its phytochemical analysis for *P. austrosinense* extracts.

Debye – scherrer's formula; $d = 0.9\lambda/\beta\cos\theta$

Where d is the mean diameter of nanoparticles, λ is the wavelength of X-ray diffraction source which is 1.54056; β is the angular FWHM of the XRD peak at the diffraction angle θ [21]. Fig. 1a shows the XRD pattern of the sample shows the peaks (2θ = 31.6527°, 45.4106°, 75.4866°) which determines the biological synthesis of TiO_2 nanoparticles. The corresponding 2θ and FWHM are 31.6527° (0.1476°), 45.4106° (0.2460°) and 75.4866° (1.1808°). The particle size was estimated by Debye-Scherrer's formula also confirms the titanium dioxide nanoparticles. The obtained patterns were equivalently identified in the biosynthesis using *Lecanora muralis* lichen which was proved to possess antibacterial and antifungal activities in biomedical applications [21]. The phytopathogenic activity testing can be redressed to the above results for prominent inference for agglomerating the plant protection phenomenon.

2.6.4. SEM analysis

SEM analysis was done to determine the size and characteristics of the sample. The size of the synthesized TiO_2 nanoparticles was determined by the focused electron beams. The SEM micrographs to assess the sample size distribution patterns were analyzed [22].

2.7. Antimicrobial activity

Antimicrobial activity of the TiO_2 nanoparticles was determined using the agar well diffusion assay method [23]. Two ml of molten and cooled nutrient MHA agar media was poured into sterilized Petri dishes. The selected bacterial pathogens of plant, *Bacillus subtilis, Erwinia chrysanthemi, Xanthomonas phaseoli* were grown in nutrient broth and the fungal pathogens of plant, *Fusarium oxysporum, Rhizoctonia solani,* and *Sclerotium rolfsii* were grown in potato dextrose broth. These plant pathogens were inoculated by spread plate technique with cotton swab. The bacterial pathogens were incubated at 37 °C for 24 h and the fungal pathogens at 30 °C for 72 h. Using sterilized stainless-steel cork borer, agar wells were prepared and loaded with different concentrations of (75 and 100 μl) TiO_2 nanoparticles. Antibiotic streptomycin and nystatin were used as positive control for bacterial and fungal pathogens respectively to compare the growth inhibition. After incubation, the antimicrobial activity was evaluated by measuring the diameter of inhibition zone in millimeter (mm).

3. Results and discussion

3.1. Phytochemical analysis

The collected lichen was identified as *Parmotrema austrosinense* (Fig. S1). The phyto-chemical analysis was carried out for the aqueous lichen extract and the results are depicted in Table 1. Among the fourteen phytochemical studies, lichen extract has eleven components except amino acids, protein and anthraquinones. The absence of these components might have possibly occurred due to poor extraction ability of the solvent used during aqueous extraction process. Therefore, advanced solvent extraction protocols and optimization studies are necessitated. The phytochemical color reaction of the lichen extracts and reagents are shown in Figs. 1 and 2. $AgNO_3$/lichen ratio has been addressed to biocompatibility of the nanoparticles earlier [6]. Hence, further studies regarding the bioactivity, biocompatibility and choice of nanoparticles for lichen influence in plant growth promotion and crop protection needs further investigation. Titanium nanoparticles in phytomedicine are increasingly studied for augmenting plant growth and the requirement of cellular recognition assessment and genetic regulation has been stressed by many phytologists [10]. However, lichenologists apart from taxonomic revisiting, pose alternative strategies for effectuating lichen bioactivity and amelioration of plant diseases. Biosynthesis of nanoparticles in lichens have been extensively reviewed for indicating cost-effective applications, biocompatible nature for human welfare and environmentally friendly benefits [24]. Thus, the novelty and uniqueness in corroborating the present study relies on phyto-nanomedicine rather than the usually adopted biomedical scenario as agricultural benefits mediated by lichens in sustainable production through phyto pathological analysis is envisaged.

Table 1
Phytochemical constituents of lichen extract.

S. No	Phytochemicals	Results
1	Alkaloids	+
2	Saponins	+
3	Amino acids	–
4	Anthraquinone	–
5	Flavonoids	+
6	Tannins	+
7	Steroids	+
8	Terpenoids	+
9	Cardiac glycosides	+
10	Phlobatannins	+
11	Xanthoprotein	+
12	Protein	–
13	Sugar	+
14	Cardenolides	+

3.2. UV/Visible spectrum

The biogenic synthesized TiO_2-NPs were primarily evident by colour development. The comparative characterization of TiO_2 nanoparticle formation by UV–Visible, FTIR and X-ray diffraction method are show in

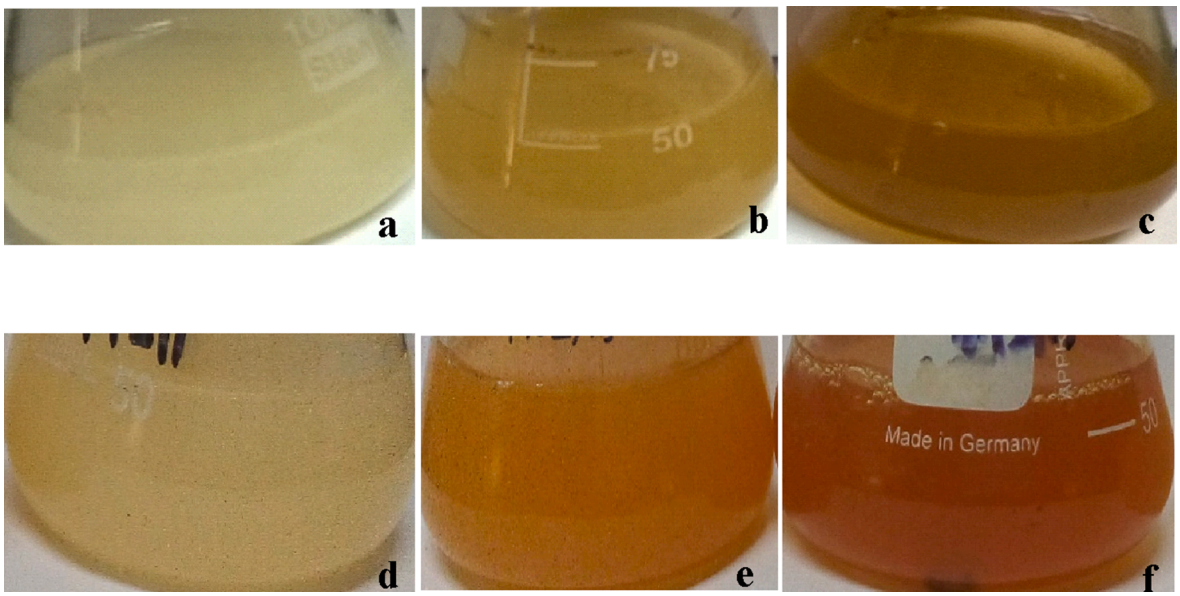

Fig. 2. Biosynthesis of *P. austrosinense* extract mediated TiO_2 nanoparticles before **(a, b, c)** and after **(d, e, f)** incubation; **a)** 0.1 mM of TiO_2 with 0.5 ml of lichen extract, **b)** 0.1 mM of TiO2 with 2.5 ml of lichen extract, **c)** 0.1 mM of TiO_2 with 5 ml of lichen extract.

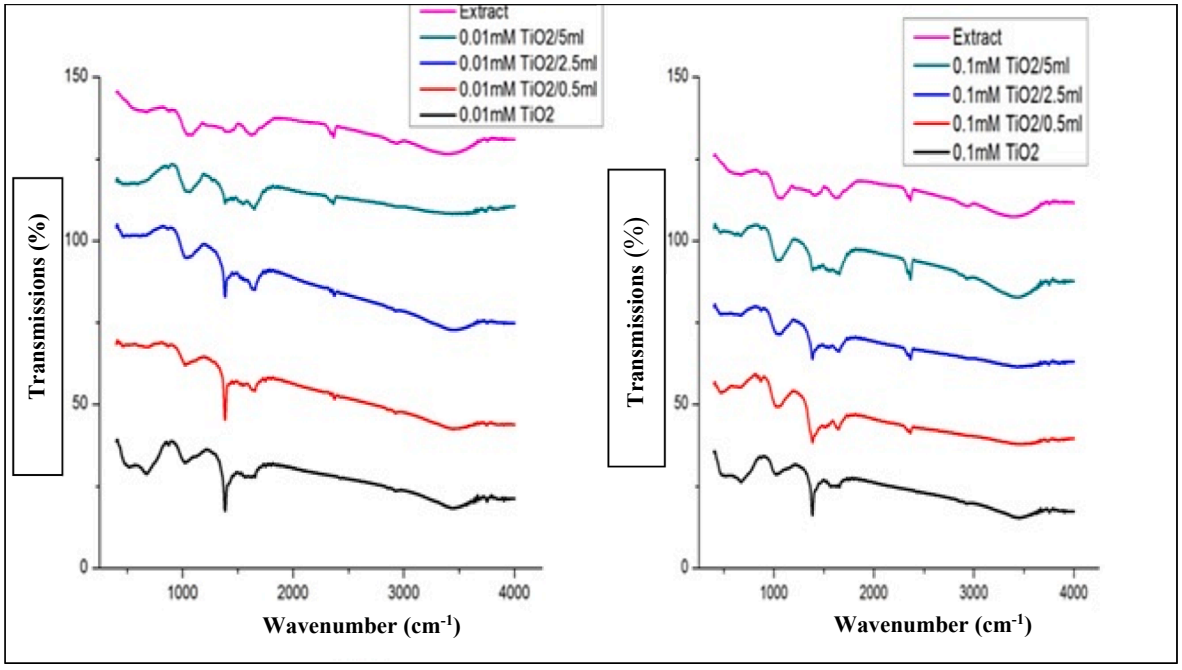

Fig. 3. FTIR for *P. austrosinense* extract mediated TiO_2 nanoparticles.

Figs. 3 and 4, they displayed with light brown to dark brown colour with TiO_2-NPs according to the concentrations of titanium dioxide. The optimal absorption of 0.1 mM of TiO_2 nanoparticles per 0.5 ml extract was observed in the range between 420 and 450 nm indicating the biosynthesis of nanoparticles (Fig. 4a). The results are in correspondence to the plasmon resonance and scattering of light due to optical properties and radiation in the visible region as reported earlier [25]. Similar results were also obtained for biogenic titanium dioxide nanoparticles synthesis and bioactivity using *Ananas comosus* plant leaf extracts [26]. Thus, the authenticity of the results in the present study is affirmative.

3.3. FTIR and XRD analysis

The FTIR spectra for the synthesized TiO_2 nanoparticle exhibited prominent peaks at 1741, 1647, 1627, 1033.83, 547 cm^{-1}. The peak at 1741 cm^{-1} shows C═O stretch and peak at 1647 cm^{-1} indicates the presence of —C═C— stretching. 1627 cm^{-1} indicates the presence of N—H stretching bands. The peak range at 547 cm^{-1} explains the presence of the TiO_2 nanoparticles and the peak range at 642 cm^{-1} shows the ═C— bending due to the action of amines. The results in Fig. 3, shows the existence of reactive hydroxyl groups at the surface of both the hydrophilic and hydrophobic TiO_2 particles. The FTIR results are evident to the similarity that the Titanium dioxide nanoparticles coating with polyethylene glycol for systemic drug delivery systems in cancer

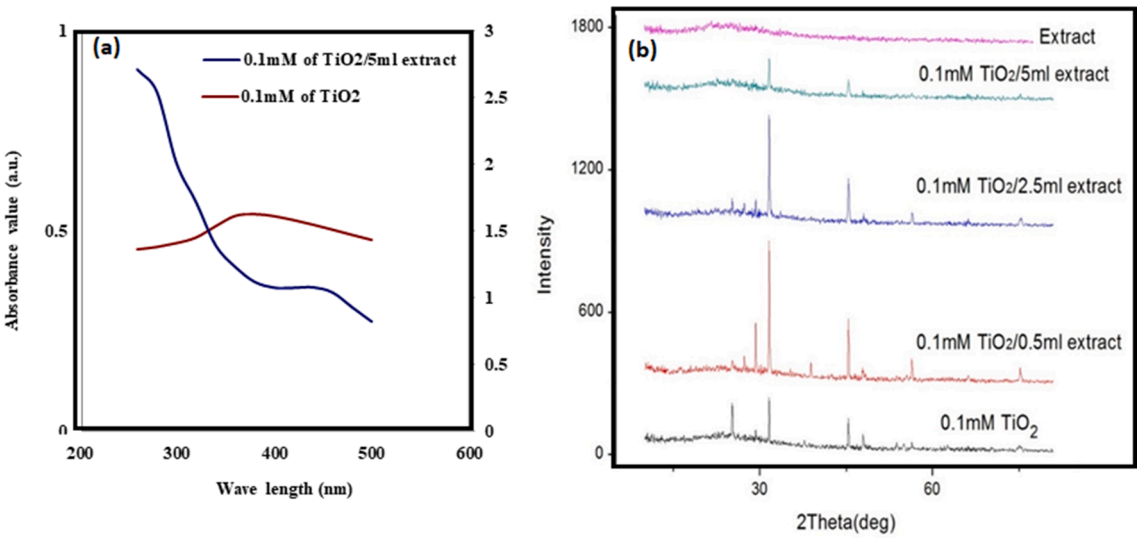

Fig. 4. (a) UV–Vis spectra of TiO_2 nanoparticle (b) X-ray diffraction pattern of TiO_2 nanoparticles compared with extract addition.

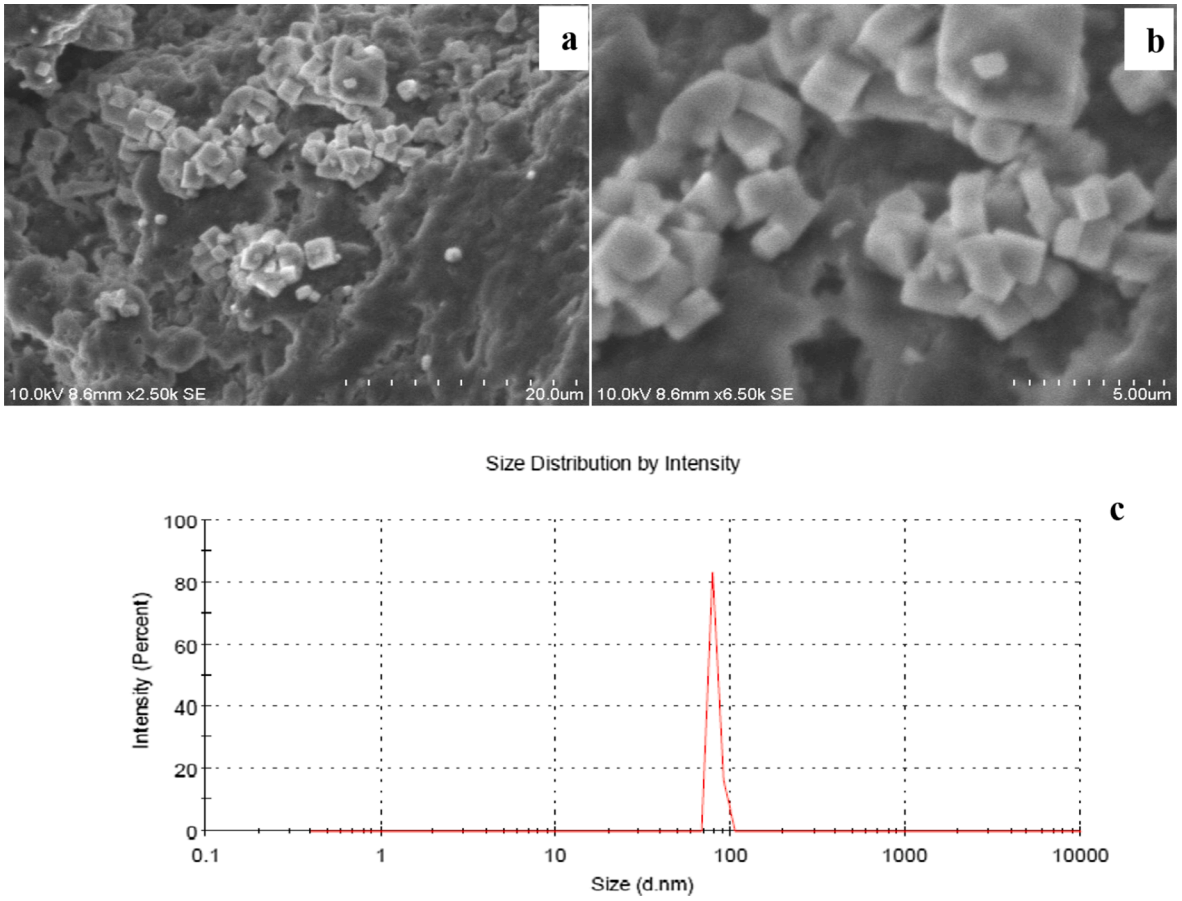

Fig. 5. (a-b) SEM images of the TiO_2 nanoparticles, (c) Nanoparticle size analysis.

treatment for biomedical applications [27], and the present assessment could have potential implications in environmental and agricultural specificity and sensitivity for phyto protection and plant growth. Moreover, the results also prove the environmental indicator applications according to a study indicating the catalytic properties of the titanium dioxide nanoparticles [28].

Fig. 4b shows the XRD pattern of the sample prepared by using various amount extract and the peaks (2θ = 31.6527°, 45.4106°, 75.4866°) which determines the biological synthesis of TiO_2 nanoparticles. The corresponding 2θ and FWHM are 31.6527° (0.1476°), 45.4106° (0.2460°) and 75.4866° (1.1808°). The particle size was estimated by Debye-Scherrer's formula also confirms the titanium dioxide nanoparticles. Fig. 5 depict the diffraction pattern of the synthesized titanium dioxide nanoparticles. The obtained patterns were equivalently identified in the biosynthesis using *Lecanora muralis* lichen which was proved to possess antibacterial and antifungal activities in biomedical

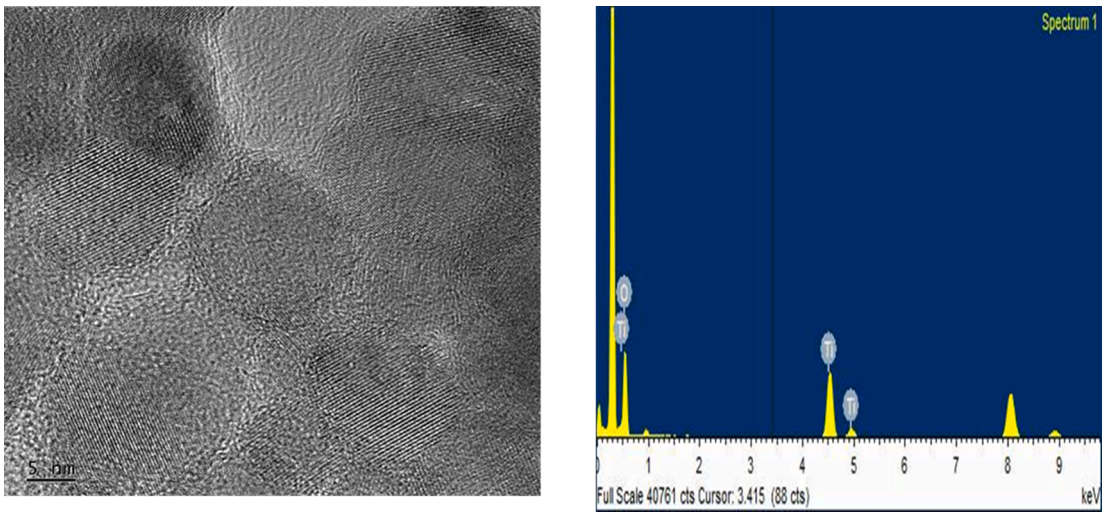

Fig. 6. TEM images of as prepared TiO_2 nanoparticle and its confirmation by EDX analysis.

Table 2
Antimicrobial activity of TiO_2-NPs against tested plant pathogens.

Plant pathogens	Zone of Inhibition (mm)/Concentration (µl)			
	50.00	**75.00**	**100.00**	**Standard***
Bacterial pathogens				
Bacillus subtilus	11.00	19.02	20.08	32.03
Erwinia chrysanthemi	12.50	20.07	21.01	34.01
Xanthomonas phaseoli	14.10	25.00	27.04	30.04
Fungal pathogens				
Fusarium oxysporum	10.20	18.20	19.08	25.04
Rhizoctonia solani	07.05	10.21	11.01	12.09
Sclerotium rolfsii	NA$	08.03	10.00	15.08

* Streptomycin – antibacterial drug, Nystatin - antifungal drug.
$ NA – No activity.

applications [20]. The phytopathogenic activity testing can be redressed to the above results for prominent inference for agglomerating the plant protection phenomenon.

3.4. Electron microscopy analysis

The size of the synthesized TiO_2 nanoparticles was analyzed by scanning electron microscopy. The synthesized TiO_2 nanoparticle was rod, rectangular and spherical shaped as well as some aggregates. The size rages from 10 to 80 nm (Fig. 5 a-b). Similar nanofabrication results were obtained in 50–85 nm (Fig. 5c) particle size revealing the agglomeration range and bioactivity profiles [20]. Hence, future studies can also involve a holistic approach of utilizing either multiple nanoparticles synthesis from plants or multitude metal nanoparticles biosynthesis. A recent comprehensive review in compiling bionanofabrication applications synthesized by lichens enumerates large scale field applications in various fields except plant pathology or crop protection strategies [29]. Thus, the present assessment can be attributed as the few reports addressing fabricated titanium dioxide nanoparticles for applications against plant pathogens. Similar results were also obtained from the lichen *P. austrosinensei* showing the size of the nanoparticles as a key factor for redox potentials in eliciting bioactivity [22]. Fig. 6 shows the TEM images of as prepared TiO_2 nanoparticle and energy dispersive X-ray analysis could provide the element content of the prepared materials. The scale value of 5 nm mentioned in the micrograph image showing fine lattice of titania nanoparticles in spherical morphology.

3.5. Antimicrobial efficacy

MHA medium was used and by agar well diffusion method the antimicrobial activity against plant pathogens was determined. 75.00 and 100.00 µl of the sample was added and after incubation period, the diameter of inhibition zone was measured. 100.00 µl of the sample shows better result when compare to 75.00 µl of the sample. The synthesized TiO_2 nanoparticles have great activity on the tested strains. Table 2 shows the minimum inhibitory concentration as emancipated by the titanium dioxide nanoparticles against plant pathogens. The results show varied patterns and effectiveness precisely against the bacterial pathogen *X. phaseoli* and the fungal pathogen *F. oxysporum* (Table 2). The antimicrobial efficacy of the TiO_2 nanoparticles is attributed to its small size and large surface area which causes imposition of oxidative stress on the bacteria due to possible release of Ti + ions from TiO_2. In addition, the penetrative power of this nanoparticle proves to be bactericidal [30]. Biomedical pathogens were combated similarly using *P. praesorediosum* synthesized silver nanoparticles [31]. However physicochemical parameters including pH, temperature also play pivotal roles in cytotoxicity against colon cancer and microbial infections [32,33], indicating future gap to be addressed.

4. Conclusion

Green synthesis of TiO2 nanoparticles mediated ultra-sonicated aqueous extract of the lichen *P. austrosinense* under effective, eco-friendly and cost effective strategy. The characterization of TiO2 nanoparticles with UV–Vis, XRD, TEM and SEM results showed that the nanoparticles were globular and ranged in dimensions from 10 to 80 nm. FTIR reveals the existence of the =C— bending due to the action of amines. The antimicrobial features of TiO_2-NPs, revealed combat fungal phytopathogens causing high yield loss, such as *X. phaseoli* and *F. oxysporum*. Overall results displayed TiO_2-NPs owned significant antifungal against tested plant pathogens. However, it requires further studies to confirm the genetic crosstalk, cellular interaction, protein interaction and further field trials for wide outreach.

CRediT authorship contribution statement

Fatmah Ali Alasmary: Conceptualization, Methodology, Data curation, Writing – original draft, Project administration, Funding acquisition, Formal analysis. **Shyam Kumar Rajaram:** Conceptualization, Methodology, Data curation, Writing – original draft, Project administration, Writing – original draft, Writing – review & editing. **R.J.**

Ramalingam: Conceptualization, Methodology, Data curation, Writing – original draft, Project administration. **Ganesh Moorthy Innasimuthu:** Writing – original draft, Project administration, Writing – review & editing. **Karthikumar Sankar:** Resources, Writing – review & editing. **Shahila Stephen Muthaiah:** Resources, Writing – review & editing. **Abdullah M.A. AlKahtani:** Formal analysis, Writing – review & editing. **Amani salem almalki:** Formal analysis, Writing – review & editing. **Hassna Mohammed Alhajri:** Data curation, Funding acquisition, Formal analysis.

Declaration of Competing 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.

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