Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 3, 2024, 109
https://doi.org/10.33263/LIANBS133.109

In vitro free radical scavenging effects of Titanium dioxide Nanoparticles (TiO_2 NPs) biosynthesized using stem extract of *Cissus quadrangularis*

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Received: 23.05.2023; Accepted: 30.12.2023; Published: 25.07.2024

Abstract: Plant-mediated fabrication of nanoparticles has gained much attention in recent times owing to its inherent features such as simplicity, cost-effectiveness, eco-friendly, and biocompatibility. Therapeutic applications of nanoparticles are rapidly increasing for their utility in medicine and clinical research. The present study demonstrates the synthesis of Titanium dioxide nanoparticles (TiO_2 NPs) using the stem extract of *Cissus quadrangularis* (*C. quadrangularis*). The synthesized nanoparticles were characterized using different analytical techniques such as UV, FTIR, FE-SEM, EDX, and XRD. The sharp peak at 269 nm by UV spectra confirmed the synthesis of TiO_2 NPs. FTIR studies identified different functional groups involved in the effective capping of AgNPs. The shape and the morphology of the TiO_2 nanoparticles by the SEM analysis showed a spherical appearance. FE-SEM has shown that the average particle size is between 5 and 11 nm. The TiO_2 NPs exhibited good free radical scavenging activities and were analyzed using DPPH and ABTS. Both TiO_2 NPs and *C. quadrangularis* have bone healing properties and, after further detailed studies, could be used as a successful biomaterial in the future.

Keywords: TiO_2 nanoparticles; *Cissus qudrangularis*; free radical scavenging.

1. Introduction

Nanotechnologies have attracted great interest in recent years, providing immense results in many fields. Titanium dioxide (TiO_2) has become one of the most commonly used nanoparticles in recent years. TiO_2 nanoparticles (TiO2 NPs) have numerous benefits, such as large surface area, suitable band structure, high quantum performance, chemical inertness, and stability [1]. The TiO_2 NPs are exploited in various areas of life, such as the protection of the environment [2], the biomedical fields [3], food packaging [4], the cosmetics industry [5], agriculture [6], and also photocatalytic behavior [7]. The fabrication of TiO_2 NPs has been used for biomedical applications, especially in bone repair, due to its biocompatibility, comparatively inexpensive, better stability, and less toxicity [8, 9].

Several approaches exist for the chemical synthesis of TiO_2 NPs, such as sol-gel, solvothermal, hydrothermal, electrochemical, precipitation, etc. All these methods have their demerits of synthesis due to their chemical approach, longer duration, more expensive, and the particles will be larger. Thus, biological methods that are cost-effective and more stable for the larger-scale synthesis of TiO_2 NPs are becoming increasingly interesting due to less toxicity towards the environment [10]. In recent years, TiO_2 NPs have been synthesized biologically and were investigated for different applications, including environmental and biomedical fields [11].

Cissus quadrangularis L. (*C. quadrangularis*), commonly found in the tropical region of India, is fleshy and similar to the appearance of the cactus plant and has numerous medicinal properties. The primary property has been reported that roots and stems are widely used to cure bone fractures. Other pharmacological properties of the plants include antibacterial, antioxidant, antiulcer, antidiabetic, and analgesic [12]. The plant extracts can act as both a reducer and a capsuling agent in the biosynthesis of nanoparticles using titanium. Microorganisms are also used to biologically synthesize TiO_2 NPs. Compared with microorganisms, plants are more reasonable because of the lower aseptic conditions for TiO_2 NPs [13]. It is known that nanoparticles interact with oxygen and hydroxyl adsorbed on their surface to attain free oxygen radicals and free hydroxyl radicals capable of affecting both cell walls and cell membranes [14].

This work reports TiO_2 NPs synthesis using *C. quadrangularis* for the first time by the easy aqueous reducing method, and the free radical scavenging assays were evaluated. Since both TiO_2 and *C. quadrangularis* have bone-repairing properties, the TiO_2 NPs synthesized could be used as a promising material for osseointegration.

2. Materials and Methods

2.1. Preparation of aqueous extract of C. quadrangularis.

Fresh stem samples of *C. quadrangularis* were collected from Kadapakkam village, Tamil Nadu, India. Samples were cleaned with running tap water to remove dirt and shade dried. About 5 g of the sample was finely ground into a paste with pestle mortar and blended with 100 mL of deionized water in a glass beaker. The aqueous blend was boiled at 60°C in a heating mantle for 10 min. The decoction was filtered with Whatman no. 1 filter paper, and the aqueous extract was stored for experimental purposes.

2.2. Biosynthesis of TiO_2 NPs.

Around 20 mL of the aqueous extract was added to 80 mL of 0.5 M $TiCl_4$ in an Erlenmeyer flask. The reaction mixture was stirred well using a magnetic stirrer at 50°C for 4 to 5 h [15]. The development of a light green solution indicated the formation of titanium dioxide nanoparticles (TiO_2 NPs).

2.3. Characterization.

The biosynthesised TiO_2 NPs were characterized using UV-visible spectroscopy (UV–vis Shimadzu 1800); Fourier Transformed Infrared Spectroscopy (FTIR Perkin Elmer system); X-ray Diffraction (XRD Rigaku smart lab) and Field Emission - Scanning electron microscopy (FE-SEM) with Energy Dispersive X-ray Spectroscopy (EDX) (SUPRA 55-CARL ZEISS) and Transmission Electron Microscopy (TEM) (Titan Themis 300kV FEI).

2.4. Free radical scavenging activity.

2.4.1. DPPH assay.

The scavenging effect of free radicals of TiO_2 NPs has been demonstrated by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay [16]. Various concentrations of TiO2 (5-320 µg/mL) were mixed with 500 µL of DPPH solution (0.01 mM) and comprised up to 3 mL of methanol solution. The mixture was thoroughly mixed and held in a dark state for 30 minutes at 28°C. Solution uptake was measured at 517 nm with a UV-vis spectrophotometer. Ascorbic acid has been used for standards. The radical scavenging activity (RSA) was calculated as a percentage using the given formula,

$$\% \text{ RSA} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (1)$$

2.4.2. ABTS assay.

The 2, 2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), the potential for TiO2 NP radical scavenging was determined using standard procedure. Different levels of TiO2 NPs (5-320 µg/ml) and 100 µL of ABTS solution were added and incubated at 28°C for 30 minutes. Here, ascorbic acid is utilized as standard. After 30 minutes, the absorbance of the reaction solution was measured at 750 nm using a UV-vis spectrophotometer.

$$\% \text{ RSA} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (2)$$

3. Results and Discussion

3.1. UV-vis spectroscopy.

UV-vis analysis of the biosynthesized TiO2 NPs showed maximum absorbance at 269 nm due to surface plasmon resonance (SPR), demonstrating the effective bioreduction of the metallic nanoparticle (Figure 1). This technique is most frequently used to characterize the optical properties of nanoparticles and confirm the formation of nanoparticles [17]. The obtained result was in agreement with TiO_2 NPs biosynthesized with leaf extract of *Cola nitida* leaf extract [18], *Echinacea purpurea* [19], *Cola nitida* [18], *Aloe vera* [20], and respectively.

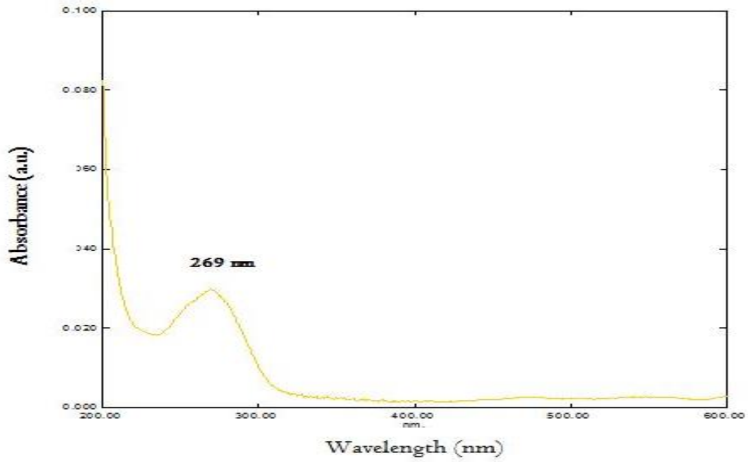

Figure 1. UV-vis absorption spectra of TiO_2 NPs biosynthesized stem extract of *C. quadrangularis.*

3.2. Fourier Transformed Infrared Spectroscopy..

Fourier transform infrared spectroscopic analysis was performed to recognize the key biomolecules engaged [21] in the nanoparticle capping (Figure 2). An intense peak located at 3414 cm^{-1} corresponds to the intense stretching vibration frequency of the hydroxyl (-OH) group [22-23]. A small peak spotted at 1621 cm^{-1} denotes O-H bending of the water molecular adsorbed to the surface of the synthesized material. The peak at 1364 cm^{-1} represents the C-C bond stretching at the aromatic ring, respectively [24]. The presence of a peak at 1257 cm^{-1} corresponds to the C-O stretching vibration of the alcohol or carboxylic acid group [25-26]. The absorption of a band at 1036 cm^{-1} represents the C=O vibrations of carboxylic acid [27]. A minor peak at 442 cm^{-1} was attributed to the stretching vibration of O-Ti-O, which confirmed the presence of TiO_2 NPs [28].

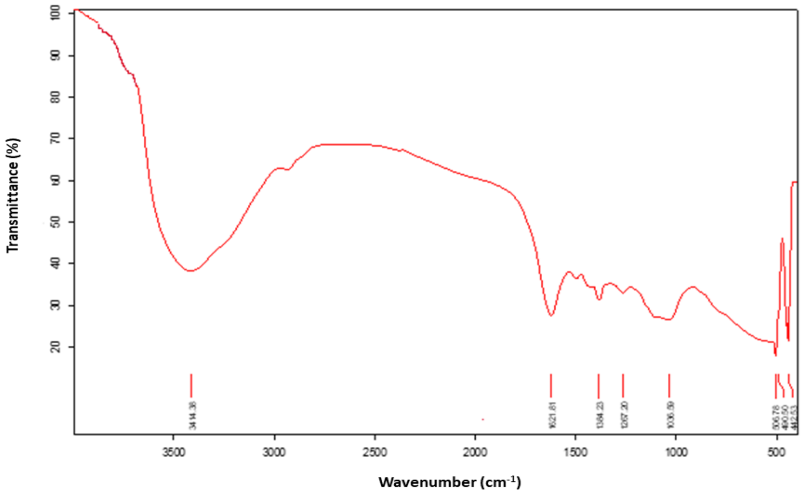

Figure 2. FTIR spectra of TiO_2 NPs biosynthesized with stem extract of *Cissus quadrangularis.*

3.3. X-ray Diffraction.

The crystalline property of the biosynthesized TiO_2 NPs has been confirmed through X-ray diffraction (Figure 3). The anatase phase was confirmed by the presence of 2θ values corresponding to 27.12, 35.33°, 41.14°, 43.69°, 54.170°, 62.92°, 69.04° indexed to lattice planes (101), (004), (111), (210), (105), (204), (116) which was in agreement with TiO_2 biosynthesized with *Cinnamomum tamala (C. tamala) leaf* extract [29]. XRD results were in concordance with JCPDS (File no. 84-1285). A sharp peak was noted at 25.3° (101) reflection, and the diffractogram pattern validated the face-centered tetrahedral in nature [30]. The particle size reduces as the peak width increases, suggesting that the current biosynthesized TiO_2 material is in the nanometer range [31]. Most importantly, the presence of the prominent diffraction peaks: 27.12° (rutile form), 35.33° (anatase form), 41.14° (rutile form), and 54.17° (anatase form) was considered as an attributive significance of the biologically synthesized TiO_2 crystallites [32-33].

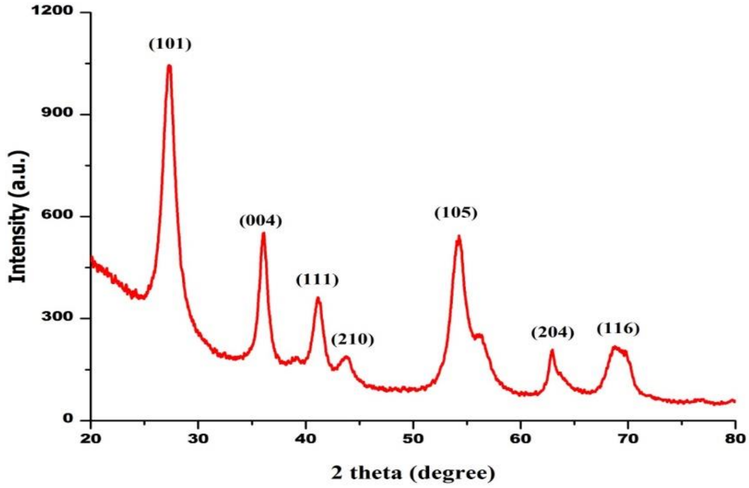

Figure 3. X-ray diffraction spectra of TiO_2 NPs using stem extract of *Cissus quadrangularis.*

3.4. Scanning Electron Microscopy.

SEM microscopic analysis of the biosynthesized TiO_2 NPs showed the presence of spherical morphology. The individual nanoparticles appeared fine, spherical, and oval-shaped along with aggregate structures [32]. The micrograph showed that the nano-scaled TiO_2 NPs exist in spherical agglomerate structures (Figure 4a), similar to that of rutin-mediated synthesis of titanium dioxide nanoparticles [34-36]. The nanoparticles were measured to be in a size ranging between 5 to 11 nm. Agglomeration of the particle depends on the nature of the extract and its compounds and the intra-molecular bond of hydrogen between them [37]. Further, EDX spectra confirmed the presence of elemental Ti and O (Figure 4b).

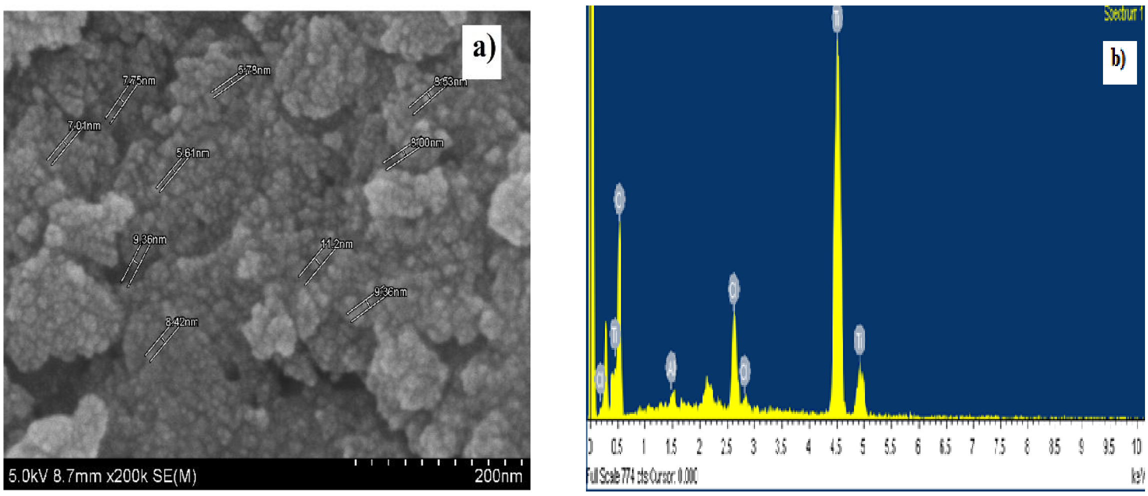

Figure. 4 a) SEM; **b)** EDX analysis of TiO_2 NPs biosynthesized with stem extract of *Cissus quadrangularis.*

High-intense signals of titanium (Ti) were observed from the EDX signal. However, the spectra displayed a less intense signal of oxygen (O) because of the dissociation of the precursor substance (Titanium tetrachloride, $TiCl_4$) used during the biosynthesis process [24, 38]. Hence, SEM-EDX analysis confirmed that the green synthesis process assisted by *Cissus quadrangularis* resulted in the bioreduction of the titanium ions into metallic nanoparticles.

3.5. Transmission Electron Microscopy.

The particle size of the biosynthesized TiO_2 was also examined using TEM analysis (Figure 5), which revealed a range of sizes between 20 and 25 nm, and the particle is spherical in shape [39]. Additionally, TEM investigation shows that TiO_2 closely matches the level predicted based on the XRD pattern [27].

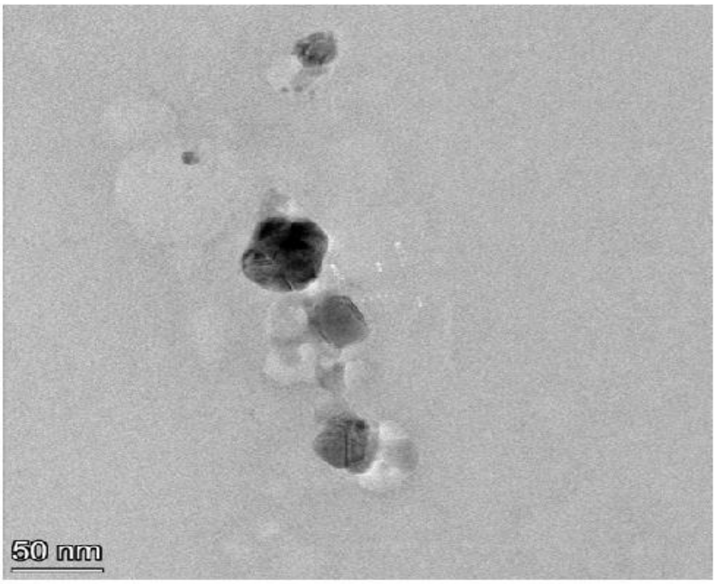

Figure 5. TEM analysis of TiO_2 NPs biosynthesized with stem extract of *Cissus quadrangularis.*

3.6. Free radical scavenging assay.

3.6.1. DPPH assay.

The free radical scavenging effect of the biosynthesized TiO_2 NPs was observed in concentration-dependent manner i.e., enhanced radical scavenging effect with increased levels of the nanoparticle [40-41]. At a minimum dose of TiO_2 (5 µg/mL), the radical inhibition percentage was found to be less, and it was increased at a maximum dose of TiO_2 (320 µg/mL). The IC_{50} value of DPPH was found to be 108.76 µg/mL (Figure 6). The radical scavenging effect was attributed to the presence of phytoconstituents such as alkaloids, flavonoids, lignin, tannins, phenols, terpenoids, glycosides, saponins present in the aqueous extract of stem parts of *C. quadrangularis* [42]. The stem portion of C. quadrangularis was especially abundant in flavonoids, which would have assisted in scavenging the free radicals [43]. Vijayalakshmi et al., 2013 have also documented that the flavonoid fraction of the aerial part of *C. quadrangularis* demonstrated enhanced antioxidant activity against DPPH radicals [44]. A previous study has reported the maximum radical scavenging effect against DPPH radical: 58.2 % and 65.5 % of TiO_2 nanoparticles that were biosynthesized with leaf extract of *Lagenaria* *siceraria* and *Pithecellobium dulce* [45]. A recent study has documented that the TiO2 biosynthesized with leaf extract of *Cola nitida* showed a maximum of ~60% inhibition effect against DPPH free radical [18].

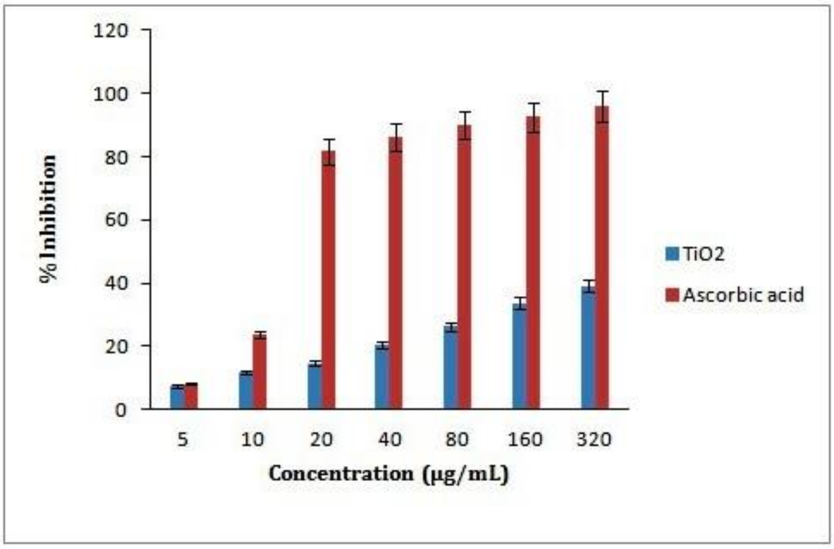

Figure 6. DPPH radical scavenging effect of biosynthesized TiO_2 NPs using stem extract of *Cissus quadrangularis.*

3.6.2. ABTS assay.

The biosynthesized TiO2 NPs exerted concentration-dependent radical scavenging effects against the ABTS radicals. At minimum concentration (5 μg/mL) of TiO_2 NPs, the radical inhibition percentage was low and showed a good effect with an increase in concentration. The IC_{50} value of ABTS was found to be 112.78 μg/mL (Figure 7). A recent study has reported maximum inhibition of ~80 % at a high dose concentration-100 μg/mL of TiO_2 NPs, and the same study has identified that Ag-TiO_2 nanocomposite biosynthesized with leaf extract of *Origanum majorana* exhibited ~80-90% radical inhibition effect against ABTS free radical [46].

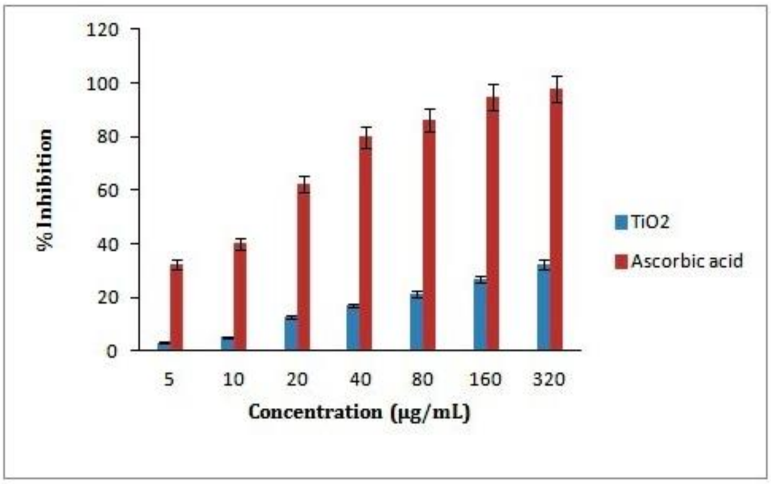

Figure 7. ABTS radical scavenging effect of biosynthesized TiO_2 NPs using stem extract of *Cissus quadrangularis.*

4. Conclusion

The study demonstrated the green synthesis of TiO_2 NPs using stem extract of *Cissus quadrangularis* as an efficient biological reducing agent. The biosynthesized TiO_2 NPs were spectroscopically characterized by UV-vis, XRD, FTIR, and EDX techniques. The reduction of TiO_2 NPs was primarily confirmed by UV-vis spectroscopy. XRD depicted the rutile (2 θ: 27.12°, 41.14°) and anatase (35.33°, 54.17°) form of the crystalline characteristic of the biosynthesized TiO_2 NPs. FTIR analysis of the biogenic TiO_2 NPs revealed the presence of hydroxyl, carboxylic acid, and other functional moieties as bio-capping agents for stabilizing the nanoparticles. SEM microscopy confirmed the presence of spherically shaped TiO_2 NPs with aggregate morphology. *In vitro* antioxidant tests such as DPPH and ABTS assays proved the free radical scavenging effect of the biosynthesized TiO_2 NPs against DPPH and ABTS radicals. Further studies should be carried out to evaluate the synthesized nanoparticles. Hence, it is concluded that the green synthesis of TiO_2 NPs using *Cissus quadrangularis* could have a better scope for developing novel nano-therapeutic materials with enhanced biomedical effects for their application in bone formation.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment

The authors are thankful to the Centre for Materials Engineering and Regenerative Medicine, Bharath Institute of Higher Education and Research, Chennai, and the Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology, Chennai, for providing instrumentation facilities.

Conflict of interest

The authors have no conflict of interest.

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