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Facile Green Synthesis and Characterization of Titanium Dioxide Nanoparticles Using *Kigelia africana* (Lam) Benth., Aqueous Leaf Extract and its Antioxidant and Antibacterial Activity

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Titanium nanoparticles are toxic to bacteria and have a widespread applications in different fields of research. Hence the present study aimed to synthesize the titanium dioxide nanoparticles by adopting green synthesis methodology using *Kigelia africana* leave extract as a biological reducing agent. The UV absorption spectra show characteristic absorption maxima corresponding to TiO_2 nanoparticles at a wavelength of 512 nm confirms the formation of nanosized tin particles. The FT-IR spectrum of TiO_2 nanoparticles show absorption bands at 3609 cm^{-1} and 3227 cm^{-1} corresponding to O-H stretching in alcoholic and carboxylic compounds, respectively. Absorption peaks at 1607, 2834, 1654 and 1324 cm^{-1} correspond to aromatic C=C vibrations, C-H stretching in aldehydes, C-H bending vibrations and aromatic C-N stretching vibrations, respectively. This confirms the involvement of bioactive compounds from the plant extract. The SEM and EDX studies confirmed that the nanoparticles are spherical to oval shape with an average particle size of 46 nm. The metal content in the nanoparticles was found to be 58.71%. The synthesized nanoparticles have potential growth inhibition activity against Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The DPPH radical scavenging activity of the nanoparticles synthesized was compared with that of aqueous leaf extract and standard ascorbic acid and proved that the nanoparticles have enhanced activity than aqueous leaf extract. The IC_{50} of the leaf extract, nanoparticles and the standard was found to be 31.55, 75.82 and 84.95 µg/mL, respectively. *Kigelia africana* leaf is shown in this work to be a valuable bioagent in the biosynthesis of TiO_2 nanoparticles with increased biological activity.

Keywords: ***Kigelia africana*, Green synthesis, TiO_2 nanoparticles, SEM-EDX, Biological activity.**

INTRODUCTION

Nanobiotechnology is an engaging innovation that executes with nanometer-sized materials in various fields of science, for example, biotechnology, materials science, physical science, nanotechnology, as well as science. Other than a few substances and physical strategies, which have been made do with getting ready metallic nanoparticles, nanobiotechnology likewise helps as a significant novel process for the safe, clean and environmentally friendly fusion and collection of metallic nanoparticles [1]. In most of the recent couple of years, biosynthesis of nanoparticles has gotten huge thought attributable to the rising need in nontoxic synthetic compounds, antibacterial, antiviral, anticancer, directed medication conveyance ecological amiable solvents and sustainable materials [2].

Different nanoparticle sizes, distributions and consistent morphological properties have recently attracted considerable interest. There are several real-world applications for nanoscience and nanotechnology, including biomedical applications, manufacturing, food preservation as well as environmental protection [3]. Several toxic solvents were included in the formation of nanoparticles along with optimization of different critical parameters like temperature, reflux time, pressure, *etc.* [4]. To minimize the inclusion of toxic agents during the synthesis of nanoparticles, the present trend is to utilize green synthesis methods [5]. The minimization of the utilization of unsafe chemicals and maximizing the effectiveness of the synthesis with less time is the main principle involved in green chemistry [6].

Numerous researchers have been drawn to TiO_2 because of its comprehensive physical and chemical properties, environmental friendliness, clean photocatalytic nature, outstanding optical property, chemical and thermal durability and non-toxic property. TiO_2 nanoparticles have a tunable and low bandgap that may be utilized as an efficient photocatalytic ingredient to degrade different organic substances. It can effectively degrade organic molecules when it is illuminated with the source of UV/visible light [7].

The green synthesis of TiO_2 nanoparticles has many benefits due to the exclusion of the usage of different harmful chemical compounds, physical procedures. It also removes the need for a particular response condition like reagents, solvents, catalysts and maintenance of particular environmental conditions. Hence, the present work aimed to synthesize TiO_2 nanoparticles using green methodology and study of its biological applications.

Kigelia africana (Lam) Benth., often known as the sausage tree, is a spreading tree with long, stalked gourd-like fruit and pendulous racemes of dull liver-coloured flowers. The plant contains several potentially valuable phytochemical constituents like naphthoquinones, dihydro isocoumarins, flavonoids and aldehydic iridoids [8]. It has analgesic, anti-inflammatory activities [9], antimicrobial [10-12], antioxidant [10], wound healing [10], antidiabetic and antioxidant activities [13]. The plant extract has potential inhibition activity against MCF-7 breast cancer cells [14]. The chemical constituents in different plant parts are proved as potential reducing substances for the green synthesis of different metal nanoparticles [15-17]. Hence, the present investigation is described the green TiO_2 nanoparticles synthesis and the study of biological activities such as antioxidant activity by DPPH free radical scavenging assay and antibacterial activity by agar plate well diffusion method of the synthesized TiO_2 nanoparticles.

EXPERIMENTAL

The fresh plant leaves of *Kigelia africana* were collected in Seshachalam Hills located in Tirupati, India and authenticated by the bonatist from P.B. Sidddharth College of Arts & Science, Vijaywada, India. The collected fresh leaves were cleaned, shade dried and preserved in Amber-coloured bottle. Titanium(IV) isopropoxide and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) were bought from Sigma-Aldrich, India. Additional chemicals/reagents used have been purchased from Merck Chemicals, India.

***Kigelia africana* aqueous leaf extract preparation:** *Kigelia africana* aqueous leaf extract was made using dried leaves. The leaves were powdered using a mortar and pestle to a uniform size and an accurately weighed 20 g of leaves powder was boiled for 20 min in 250 mL of double distilled water at 60 ºC. Extracts were then filtered through nylon mesh and a hydrophilic 0.2 µ millipore filter to remove impurities. The *Kigelia africana* aqueous leaf extract mediated TiO_2 nanoparticles were synthesized using the obtained extract.

Synthesis of TiO_2 nanoparticles: The TiO_2 nanoparticles synthesis was done as per the procedure given by Shanavas *et al.* [18]. The aqueous extract of *Kigelia africana* leaves mediated TiO_2 nanoparticles were synthesized by mixing 100 mL of 0.1 M of titanium(IV) isopropoxide as well as 25 mL of leave extract under continuous agitating for 4 h at 50 ºC. After obtaining a brownish-green gel-type blend, it was incubated for 24 h. Then, it was heated at 80 ºC for 8 h in a hot air oven. The resulting powder was rinsed multiple times with ethanol as well as with distilled water. The resultant powder was dried and calcinated for 3 h at 350 ºC in a muffle furnace. The resulting powder was preserved as TiO_2 nanoparticles and used for the charac-terization and activity studies.

Characterization of TiO_2 nanoparticles: The optical characterization of the synthesized TiO_2 nanoparticles was done using a UV-visible spectrophotometer (JASCO, Japan). The nanoparticles were dispersed in aqueous solution and the solution was scanned at a wavelength of 800-400 nm against distilled water as blank. The wavelength maximum of the UV-visible absorption spectra was evaluated and compared with the existing literature. The functional groups of the plant biomolecules included in the biosynthesis of TiO_2 nanoparticles were evaluated using an FT-IR spectrophotometer (Bruker, USA). The synthesized nanoparticles pellet was prepared with KBr and the prepared sample was scanned using FT-IR in the scan range of 4000-500 cm^{-1}. The absorption bands observed in the FT-IR spectrum was compared with the standard FT-IR table and functional groups that involved in the formation of TiO_2 nanoparticles. A field-emission SEM (NOVA NANOSEM 450, FEI, USA) was employed to assess the morphology as well as size of the formed TiO_2 nanoparticles. An X-ray diffractometer (Rigaku Corporation) has been used to evaluate the crystalline nature and lattice composition of the synthesized TiO_2 nanoparticles at a scanning rate of 2 º/min in the diffraction angles (2θ) from 20º-80º. Energy-dispersive X-ray spectroscopy ("RONTEC's EDX equipment, Model QuanTax 200, Germany") has been utilized to access the elemental structure of the synthesized TiO_2 nanoparticles.

Antibacterial activity of TiO_2 nanoparticles: The antimicrobial activity of TiO_2 nanoparticles has been performed using the agar plate well diffusion technique [19]. The antibacterial activity was carried against two Gram-positive bacteria such as *S. aureus* (MTCC–1430) and *B. subtilis* (MTCC-1427), two Gram-negative bacteria such as *P. aeruginosa* (MTCC–1748) and *E. coli* (MTCC-294). A sterilized and aseptically chilled agar medium was used to make a 25 mL solution in an aseptic petri dish and left overnight to check the possible contamination to appear. Simultaneously in the nutrient agar broth, the selected microorganism was grown separately for 24 h. In the non-contaminated plates, 100 µL of the broth was spread like bacterial lawns and a sterilized stainless-steel cork borer was utilized to form agar plate wells. Each well included 100 µL of sterile micropipette loaded sterile TiO_2 nanoparticles in selected and varying concentrations. The antibacterial drug gentamicin was employed as a positive control, while distilled water was utilized as a negative control, with all plates incubated for 24 h at 37 ºC. The antibacterial activity of synthesized TiO_2 nanoparticles was determined using a zone of inhibition is expressed in millimeters (mm).

DPPH radical scavenging assay of TiO_2 nanoparticles: The approach explained by Thirunavukkarasu *et al.* [20] was used to conduct the DPPH free radical scavenging experiment on synthesized TiO_2 nanoparticles. It took 30 min to incubate the reaction mixture, which included 1 mL each of TiO_2 nanoparticles and an extract from *Kigelia africana* aqueous leaf, in 1 mL of 0.135 mM methanolic DPPH solutions. A spectro-

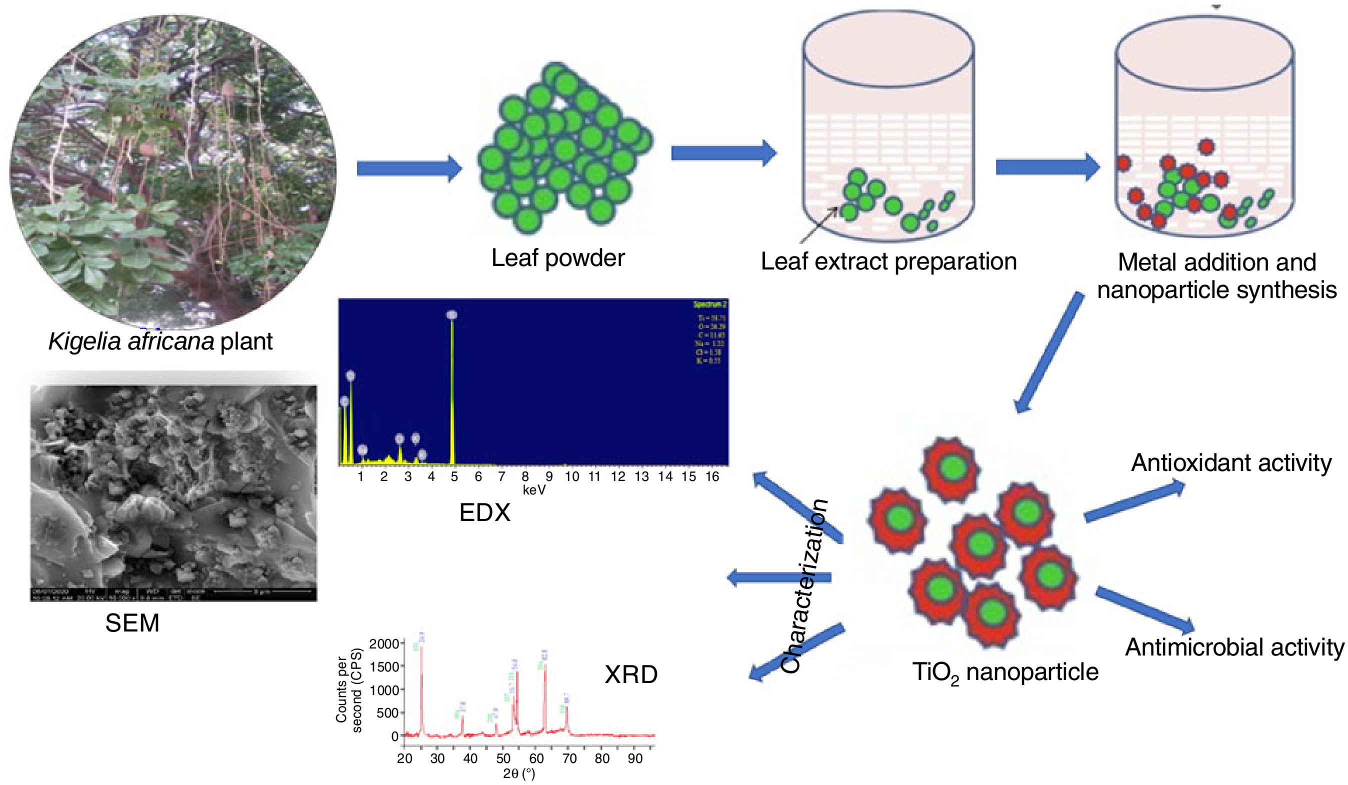

Fig. 1. Schematic representation of nanoparticle synthesis process

photometer was used to measure the absorbance of the final solution at 517 nm. A comparison of the DPPH inhibitory activity of the formed nanoparticles to the control values was made using absorbance values.

RESULTS AND DISCUSSION

The beneficial aspect of the nanotechnology field was shifted rapidly from laboratory to large scale industrial production and present proposed method provide a green synthetic approach for the synthesis of TiO_2 nanoparticles using aqueous extract of *Kigelia africana* leaves as a biological reduction agent. In the synthesis of TiO_2 nanoparticles, the formation of nanoparticles was initiated by the addition of aqueous plant extract and metal solution. The initiation of reaction was confirmed by observing the colour change from light greenish red to dark brown in the reaction mixture. It is also observed that the intensity of the colour was increased by passing the incubation time confirmed the nanoparticles formation was increased with increase in time. The schematic representation of TiO_2 nanoparticles synthesis is given in Fig. 1.

FTIR analysis: The synthesized TiO_2 nanoparticles were analyzed by using FT-IR spectroscopy for the evaluation of the possible functional groups of bioactive molecules from the aqueous extract of the *Kigelia africana* leaves that are actively involved in the formation of nanoparticles. The FT-IR transmittance bands were recorded at 3609 cm^{-1} as well as 3227 cm^{-1} corresponding to O-H stretching in alcoholic and carboxylic compounds, respectively. In aldehydes, the transmission peak at 2834 cm^{-1} corresponds to C-H stretching. To identify the aromatic C=C vibrations, the peak at 1607 cm^{-1} is accountable. The C-H bending vibrations in the nanoparticles were confirmed by the presence of a band at 1654 cm^{-1}. The aromatic C-N stretching vibrations in the nanoparticles were confirmed by the band at 1324 cm^{-1} [21]. The existence of Ti-O-Ti structure in the nanoparticles was verified by the detection of FT-IR transmission peak about 800-500 cm^{-1} in the spectra that are attributable for metal-oxygen stretching vibrations (Fig. 2) [22,23].

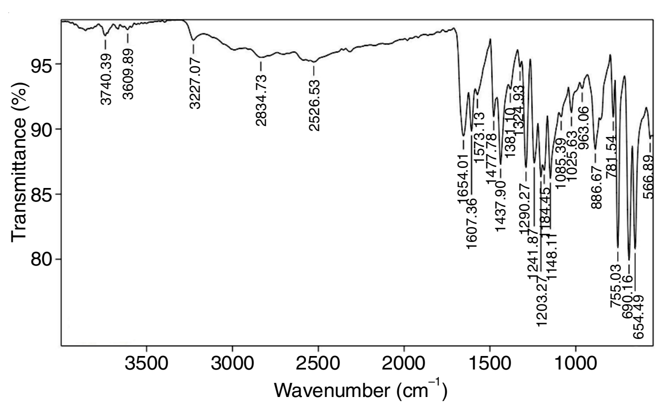

Fig. 2. FTIR spectrum (cm^{-1}) of TiO_2 nanoparticles

Optical absorption study: The absorption spectrum of metal nanoparticles is sensitive to several factors, including particle size, shape and particle-particle interaction (agglomeration) with the medium. The optical absorption ability of synthesized TiO_2 nanoparticles was determined using a UV visible spectrophotometer and the scanning spectra is shown in Fig. 3. The TiO_2 nanoparticles show high absorption in the

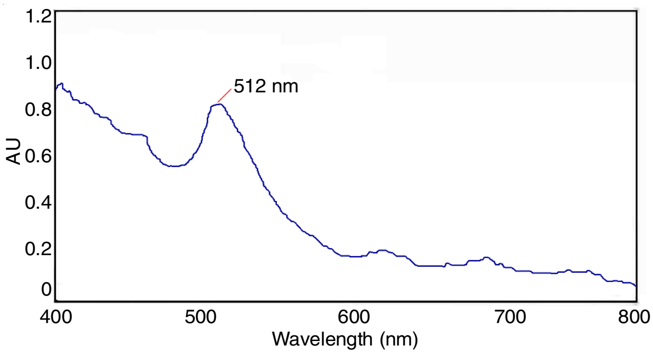

Fig. 3. UV absorption spectra of TiO_2 nanoparticles

visible region. Due to the spontaneous recombination of free excitons after collisions between oxygen defects and Ti metal ions, TiO_2 nanoparticles have a distinctive emission peak at 512 nm. This finding proved that the particle are the nano-sized particles which exhibited the characteristic absorption maxima. A similar UV-visible absorption pattern was observed for the TiO_2 nanoparticles reported by Shanavas *et al.* [18].

Structural characterization: The use of SEM made it possible to provide information on the size and shape of the TiO_2 nanoparticles and hence HR-SEM analysis of the synthesized TiO_2 nanoparticles was performed to evaluate the size and shape of the nanoparticles. The SEM data demonstrate that the nanoparticles were equally spread on the surface and that aggregate nanoparticles formed (Fig. 4). Nanoparticles' size ranged between 38-63 nm have been seen in the micrograph, which shows the synthesis of TiO_2 nanoparticles aggregates.

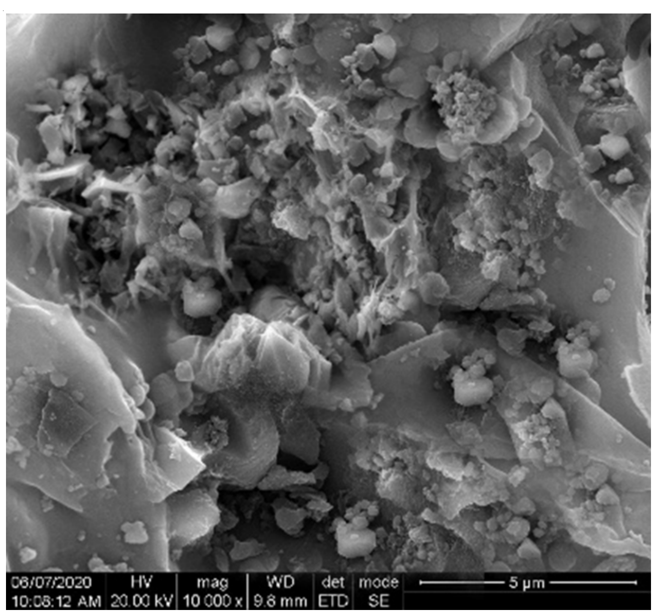

Fig. 4. SEM image of TiO_2 nanoparticles

The homogeneity of the synthesized TiO_2 nanoparticles and its elemental distribution of elements was investigated using EDX spectroscopy and Fig. 5 shows the EDX spectrum of TiO_2 nanoparticles synthesized using aqueous leaf extract of *K. africana*. The EDX spectrum of TiO_2 nanoparticles shows high intense peak at Kα of 4.8 keV which was attributed to

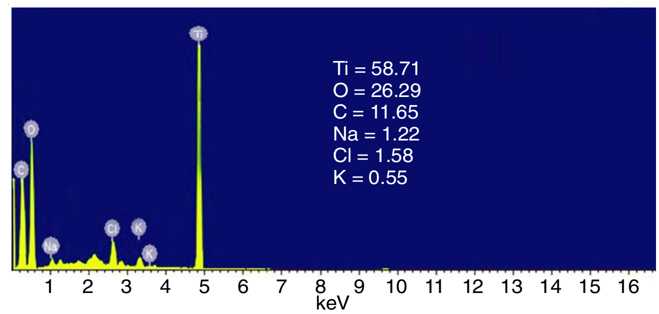

Fig. 5. EDX spectra of TiO_2 nanoparticles

metallic Ti confirms the existence of Ti in the green synthesized nanoparticles. The peaks corresponding to oxygen and carbon were detected in the EDX spectra, which correspond to the plant biomolecules. The % composition of Ti in TiO_2 nanoparticles was found to be 58.71%. Thus, it can be confirmed that the synthesized nanoparticles having high percentage of elemental Tin along with oxygen and carbon.

XRD studies: The crystallinity and phase purity of the synthesized TiO_2 nanoparticles was confirmed by XRD studies and the XRD spectra observed in the study is shown in Fig. 6. The XRD patterns of TiO_2 nanoparticles shows diffraction peaks at 24.9º, 37.6º, 47.9º, 53.7º, 54.6º, 62.8º and 69.7º corresponds to 101, 004, 200, 105, 211, 204 and 116 planes, which confirm the formation of well crystalline Ti and it is well evident with the JCPDS (File no. # 21-1272). Scherrer's equation was used to compute the nanoparticles' size of the particles and determined to be 46 nm.

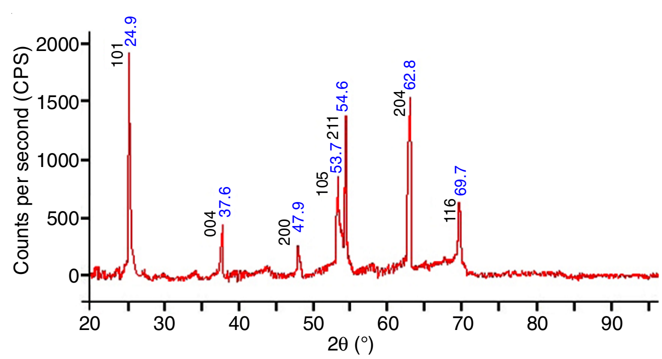

Fig. 6. XRD spectra of TiO_2 nanoparticles

Based on the findings in the synthesis and characterization, it was confirmed that the TiO_2 nanoparticles were synthesized using aqueous extract of *Kigelia africana* leaves as green reducing agent. The formation of nanoparticles was initiated by the addition of aqueous leaf extract to the titanium metal solution. While addition of the extract to the metal solution, the reducing agents present in the plant extract will acts as bio-capping and reducing agent in the formation of nanoparticles [24]. Although the exact mechanism of biosynthesis of titanium metal nanoparticles using aqueous leaf extract of *Kigelia africana* is not clear, the role of the secondary metabolites in biosynthesis process is undeniable. The presence of alkaloids, phenols, flavonoids, *etc.* in the leaf extract that contains alcoholic, carboxylic acid, aldehydes and aromatic nitrogen compounds that are detected in the FTIR study were actively participated in the formation of nanoparticles. The nanoparticles were irregular shaped with an average particle size of 46 nm and having a metal composition of 58.71%. Further the synthesized nanoparticles were evaluated for its antibacterial activity and antioxidant activity by DPPH free radical scavenging assay.

Antibacterial activity study: The antibacterial activity of the green synthesized TiO_2 nanoparticles was evaluated using agar plate well diffusion method and the results were compared with standard gentamycin. The results confirmed that the TiO_2 nanoparticles having significant activity on the growth inhibition of bacteria in the study. The high inhibition activity at a low dose was observed for *E. coli*. At 10 µg/mL concentration, the growth inhibition was reported to be 3.9 mm for *E. coli* and 3.2 mm for *P. aeruginosa*. At this very low dose, the inhibition of *B. subtilis* was found to be 1.1 mm whereas very less inhibition of 0.8 mm was observed for *S. aureus*. However, at a high dose of 100 µg/mL, the inhibition zone was found to be elevated for all the bacterial strains in the study. The antibacterial activity of TiO_2 nanoparticles confirms that they are particularly efficient towards Gram-negative bacteria whereas less potent towards Gram-positive bacteria. This confirmed that the green synthesized TiO_2 nanoparticles was identified to be having potential antimicrobial activities. It has been confirmed that the TiO_2 nanoparticles have the capacity to hold fast to the bacterial cell membrane and penetrate the cytoplasm. The results of the zone inhibition study of TiO_2 nanoparticles are given in Table-1.

TABLE-1
GROWTH INHIBITION STUDY RESULTS

Bacteria name	Inhibition zone (mm)		
Conc. →	10 µg/mL	50 µg/mL	100 µg/mL
B. subtilis	1.1	3.1	6.1
S. aureus	0.8	2.6	5.7
E. coli	3.9	8.1	11.6
P. aeruginosa	3.2	7.3	12.5

DPPH radical scavenging assay: The DPPH scavenging activities are recognized to be due to the hydrogen donating abilities of antioxidants. The DPPH scavenging activity of the synthesized TiO_2 nanoparticles, aqueous leaf extract of *Kigelia africana* was studied and the results were compared with the standard ascorbic acid. The synthesized TiO_2 nanoparticles as well as plant aqueous extract had DPPH radical scavenging activity when comparing with normal ascorbic acid, according to the results of this study. It was calculated that the IC_{50} of standard ascorbic acid was 25.79 µg/mL, whereas the green synthesized TiO_2 nanoparticles was reported to be 31.29 µg/mL and the aqueous plant leaf extract was recorded to be 61.52 µg/mL. The findings show that green produced TiO_2 nanoparticles have better radical inhibition activity as compared to aqueous leaf extract and are comparable to the standard. Table-2 summarized the findings of the DPPH inhibition investigation, whereas Fig. 7 depicted the comparison graph.

The findings achieved in the pharmacological activities such as antibacterial and DPPH radical scavenging assay confirmed that the green synthesized TiO_2 nanoparticles having

TABLE-2
DPPH RADICAL SCAVENGING ASSAY RESULTS

Conc. (µg/mL)	DPPH inhibition (%)		
	Ascorbic acid	Aqueous extract	TiO_2-NPs
5	7.29	0.90	2.76
10	11.32	2.44	4.63
15	17.13	4.44	10.83
20	31.31	9.51	15.65
25	42.24	13.50	32.29
30	65.72	20.04	46.57
35	75.78	27.45	59.27
40	84.95	31.55	75.82

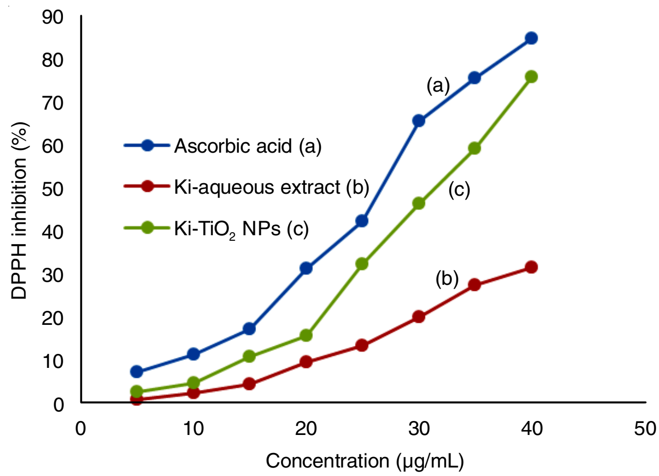

Fig. 7. Comparative graph of DPPH radical scavenging assay study outcomes

potential antibacterial as well as DPPH radical inhibition activity. The growth inhibition by the TiO_2 nanoparticles is primarily due to the formation of pores in the bacterial cell wall accompanied with changes in cell membrane permeability due to deposition of TiO_2 nanoparticles at these sites. Several underlying mechanisms involved in antibacterial action of TiO_2 nanoparticles have been proposed. Possibly, the disruption of the thiol group in the electron transport chain enzymes followed by the binding of the TiO_2 nanoparticles to the cell wall and membrane of bacterial cell accounts for the inhibitory effect of these TiO_2 nanoparticles. The TiO_2 nanoparticles interact with bacterial cell wall through attraction between the microbial cell wall's negative charge and nanoparticles' positive charge. Due to this interaction, the permeability function of the cell membrane changes and, hence, the bacterial integrity disrupts and causes cell death. Hence having potential antibacterial activity. The results achieved in the DPPH radical scavenging assay strongly recommend the application of TiO_2 nanoparticles as useful natural antioxidants for health preservation against different oxidative stress associated with degenerative diseases.

Conclusion

To synthesize titanium dioxide nanoparticles, we looked into the distinctive biological properties of a phytochemically less explored plant, *Kigelia africana*. It is possible to synthesize TiO_2 nanoparticles using bioactive chemicals found in the *Kigelia africana* leaves. The nanoparticles synthesized in the study were observed to be irregular in shape with an average particle size of 46 nm with metal composition of 58.71%. The

IC_{50} concentration in DPPH radical scavenging assay was calculated to be 25.79, 31.29 and 61.52 μg/mL respectively for standard ascorbic acid, green synthesized TiO_2 nanoparticles and aqueous plant leaf extract. The results proved that the DPPH radical scavenging activity of nanoparticles was enhanced than the leaf extract and the activity was similar to the standard ascorbic acid. The nanoparticles were observed to be potent on the growth of bacteria in study. At a very low concentration 10 μg/mL, the growth inhibition zone was observed to be 3.9, 3.2, 1.1 and 0.8 mm for *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*, respectively confirms that the TiO_2 nanoparticles were very effective even at low concentrations. The synthesized TiO_2 nanoparticles were characterized and are having antioxidant and antibacterial activities. The activities are due to the existence of bioactive functional groups on the surface of TiO_2 nanoparticles. Moreover, TiO_2 nanoparticles have a strong antibacterial activity which is due to their smaller size and having capping agents. Hence, it can be concluded that the synthesized TiO_2 nanoparticles might be used as antioxidant and antimicrobial agents in the future due to their cheap, eco-friendly and high efficiency.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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