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In-Vitro Evaluation of Antimicrobial Efficacy of Spherical Green Ag-Tio2 NPs Using Sajne Leaves Extricate of Moringa Oleifera in Aqueous Media

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ABSTRACT

Environmentally friendly and biocompatible methods were employed for the green synthesis of Ag-TiO2 nanoparticles (NPs) using the leaf extract of Moringa oleifera as a capping and reducing agent. The successful synthesis of Ag-TiO2 NPs was observed through a wavelength shift within one hour of reaction. The formation of nanoparticles was confirmed through multiple analytical techniques, including UV–VIS spectroscopy, FTIR, XRD, EDX, and SEM analysis. X-ray diffraction and electron microscopy confirmed the spherical structure and size of the nanoparticles. EDX analysis verified the presence of silver ions in the doped TiO2 lattice, while SEM revealed the spherical morphology of Ag-TiO2 NPs. Bacterial concentrations of 1.8×10^8 CFU/ml were tested using 20 ml of three samples: Moringa oleifera leaf extract, TiO2 NPs, and Ag-TiO2 NPs at concentrations of 12.5 mg/L, 25 mg/L, and 50 mg/L, in three separate trials. The nanoparticles demonstrated significant antimicrobial activity against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli), as well as antifungal activity against Aspergillus niger (A. niger). These findings suggest that Ag-TiO2 NPs hold potential for applications in biomedical fields.

Introduction

Recently, nano-biotechnology represents a significant neighborhood of research for fusion and designing of NPs due to diversity of unique specifications i.e., small dimension, large surface area, allocation, highly reactive nature and bio morphology of NPs [2-3] with special ratio of chemical constituents, shapes and size.In running decade, the plant based biogenesis of doped and single metal NPs [4,5] has acknowledged for considerable interest due to its relieve, eco-friendliness and extensive photo catalytic action [6-9] and economical that can minimally increase the entire assembly of NPs [10]. The rate of eco-compatible quick biosynthesis of NPs brings into being more constant tools [11-13]. The plants processed bioactive synthesis of NPs suggested as precious and advantageous slant [14-16] safe, grime free, dependable, bio-eco-compatible, speedy and lead to ecological byproducts. The nature, makeup and dimension of NPs depend on the type of the plant extricate with temperature of oxide formation.

The common name of *Moringa oleifera* is *sajne* belong to the family Meliaceae. *Moringa oleifera* is a species of India, Burma, Thailand, Bangladesh, Cambodia, Indonesia, Iran, Malaysia, Nepal, Pakistan, Sri Lanka and Vietnam [17-19]. *Moringa oleifera*has showing its biological behaviors such as antibacterial [20], antifungal [21], antiviral [22], anticandidal [23] and anticarcinogenic activities [24, 25]. For the treatment of eye disorders, bloody nose, leprosy, stomach upset, intestinal worms, skin ulcers, loss of appetite, heart diseases and, diabetes, fever and in liver problems Sajne leaf is used. In light of its cardio active phytochemic property, it is a famous cardenolides drug and a wide range of these drugs can be isolated from plant *Moringa oleifera.* Proteolytic enzymes, sterol, triterpenoids, tannins, cardenolides, flavonoids, and terpenes like biologically active compounds present in it help in the diminution of metal.

Ti- oxide (TiO_2) is a potential photo catalyst, due to its band energy gap, the TiO_2 has broadsubmissions, counting self-cleaning, photo catalysis, ecological refinement and elevated quantum efficiency. The development of TiO_2 NPs in which metal Ti forms complex with plant extricate, the method is eco-friendly and biocompatible. In the proposed work, TiO_2 NPs were fused through green method, and Ag alters its band gap values. The consequence of Ag doping on the crystal frame of TiO_2 NPs were studied using spectroscopic and microscopic techniques. In the continuation of our research work on NPs and their doped derivatives and applications [25-29], here author describes the effect of NPs by using aqueous extricate of *Moringa oleifera* is. In this synthesis, the TiO_2 NPs were synthesized by using titaniumisopropoxide as primary reactant and *Moringa oleifera* is used as reducing, capping and alleviating agent. The capping agents present in *Moringa oleifera* directs the size of Nps with high surface energy which prevent its accumulation resulting novel application as reducing cum capping as well as surface stabilizing agent to report spherical-shaped Ag-TiO_2-NPs. The UV-visible (UV-VIS) spectroscopyreveals the optical properties where as other characterization had been done by XRD, FTIR, EDX.

Synthesis / Material and Methods –

The chemicals were used for the Ag-TiO_2-NPs are of analytical grade, purified solvents and double distilled water. Titaniumisopropoxide (Merck), Silver nitrate (Merck), concentrated hydrochloric acid (Merck) were purchased from Hi-media.

I. Preparation of *Moringa oleifera* plant extricate
II. Green synthesis of Ag-TiO_2-NPs.

Preparation of Moringa oleifera plant extricate –

Selected fresh leaves of *Moringa oleifera* plant was collected from Dr Shakuntala Misra National Rehabilitation University, Lucknow, Campus. The leaves gathered from the campus were washed under running water 2-3 times to remove the dust of NPs, then dried in oven at 60°C for 2-3 hours and then crushed into powdered form. 5 gm of crushed powder along with 250 ml of deionized water was taken into a 500 ml beaker and boil the solution for 2-3 hrs and filter the resultant. Hence, that extricate was used further for the synthesis of NPs.

Green synthesis of Ag-TiO_2NPs –

The TiO_2 NPs were manufactured by adding 50ml of *Moringa oleifera* aqueous plant extricate and 5gm of Titanium isopropoxide together with 15 ml 0.5 M $AgNO_3$ solution in a 250 ml round bottom flask. Concentrated HCl was added to adjust the pH value. Mixture of Titanium isopropoxide and sajne leaf extricate of *Moringa oleifera* plant extricate were heated at 60˚C for 2 hrs. under high-stirring. After 2 hours of the solution was allowed to settle down over night and then centrifuge at 15000 rpm for 10 min. The collected NPs were washed again and again for 2-3 times with a mix of ethanol and water. Separated NPs were dried in hot air oven at 80˚C and put in the Muffle Furnace for 2 hrs. for calcination at 600˚C and for examine spectral behaviour, grind the NPs using mortal pistol.

Results and Discussion:-

Plausible mechanism for formation of TiO_2 NPs:-

Chemical conformation of *Moringa oleifera* favors the establishment of nano TiO_2 it encompasses considerable amount of *Cis* and *Trans* -sabinene hydrate, terpinen-4-ol, α-terpineol and linalyl acetate. The hydroxyl group contemporaneous as a functional group in the bioorganic phytochemical ingredients under plant extricates to dry out titanium isopropoxide to Tio_2 NPs by refluxing it by way of plant extricate solution for 30-40 min at 60-70 ^{0}C. Terpinen-4-ol, α-terpineol and sabinene hydrate servs as a catalyst in thermal decomposition reaction due to the breakdown of titanium isopropoxide $[Ti(OCH(CH_3)_2)]_4$ into titanium hydroxide $Ti(OH)_4$ and cyclopropanol $(CH_2)_2CHOH$ promoted condensation reaction removes water molecule and form titanium oxide NPs which on progressively acclimated and studied (fig 1).

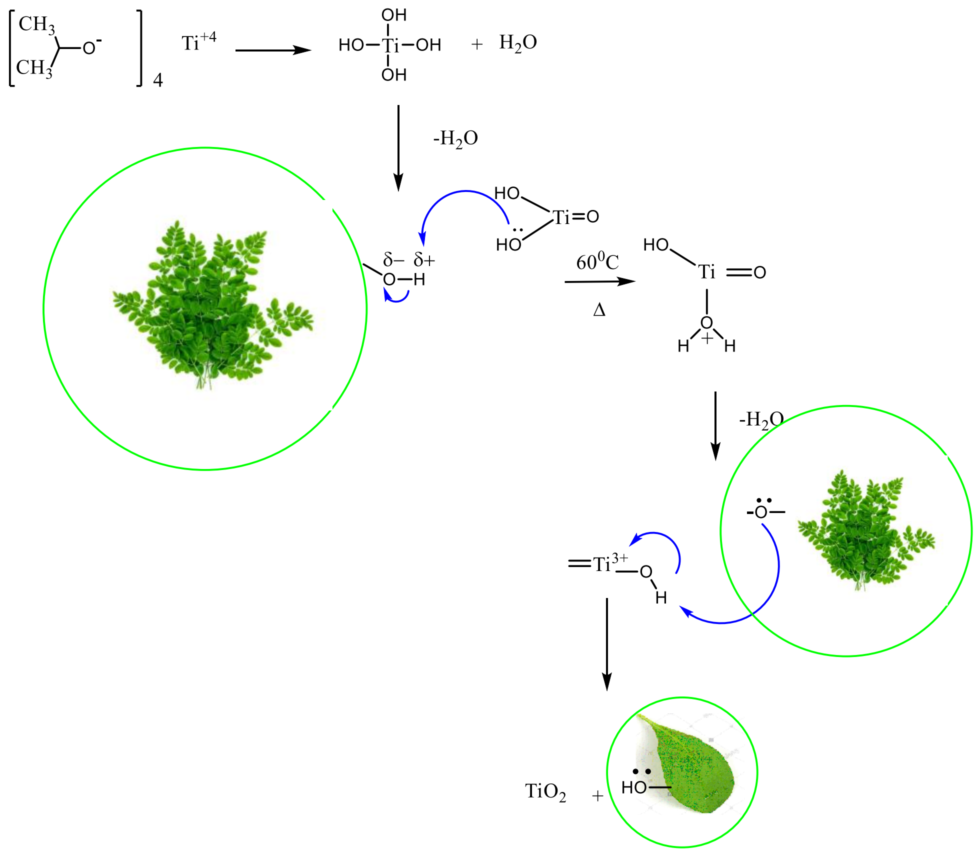

Fig 1- Plausible route for the dehydration of titanium isopropoxide to TiO_2 NPs

The poisonousness [34] and applicability [35] of TiO_2 and doped TiO_2 NPs has received substantial attention because of the captivating ability of TiO_2 NPs to yield combative oxygen based species in separately as well as fond of with other metals. As a part of our persistent effort for the enlargement of efficient green organization in the field of biosynthesis of NPs we have also report herein the fixing of silver with TiO_2 NPs and form titania silver NPs.

Reactions for construction of TiO_2NPs can be discussed as following: [36]

Hydrolysis:
$$Ti(OCH(CH_3)_2)_4 + 4H_2O \longrightarrow Ti(OH)_4 + 4(CH_2)_2CHOH \ldots (1)$$

Condensation:
$$Ti(OH)_4 \longrightarrow TiO_2.xH_2O + (2-x)H_2O \ldots\ldots(2)$$

Crystallisation via calcination:
$$TiO_2.xH_2O \longrightarrow TiO_2 \ldots\ldots\ldots(3)$$

where x = number of water molecules.

The OH group favors the hydrolysis of titanium isopropoxide monitored by condensation which designed an unstable intermediate $Ti(OH)_4$ and precipitation takings room. The white precipitate occurs due to establishment of $TiO_2.xH_2O$ as displayed in equation (2). This precipitate goes through calcinations in muffle furnace at temp range 200-700 ^{0}C and shows inestimable size reliant on changes.

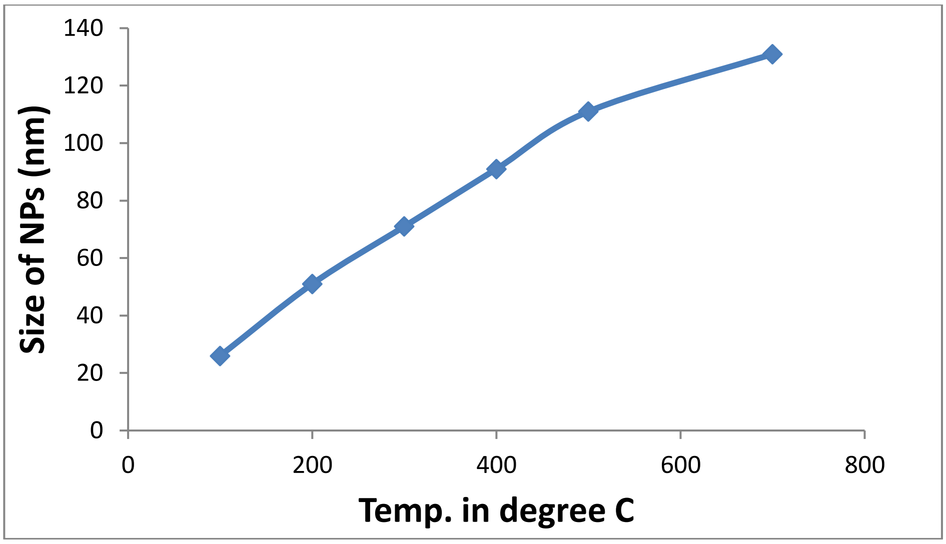

Fig 2- Temperature size graph

The crystal-like size of TiO_2 NPs be subject to the calcinating high temperature as the heat increases size of NPs also upturns. Thermally stimulated crystalline growth can observed when temperature ranges from 200-500 ^{0}C and also about 700 ^{0}C the particle size turn out to be very large overdid 140 nm (fig 2). Nucleation take place in growth of TiO_2 NPs at sophisticated temperature and shows accumulation arrangement in SEM analysis (Fig 6).

Result and Discussion-

UV-Visible Absorption Spectroscopy-

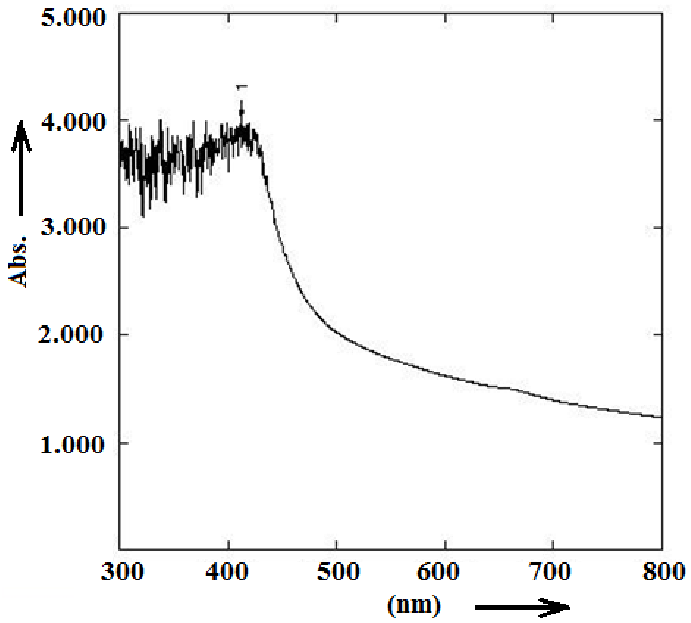

Fig. 3: UV visible Study

The production of NPs by reduction reaction of metalic ion under aqueous condition by *Moringa oleifera* plant extricates can easily be scrutinized by using double beam UV-Visible spectrophotometer (Shimadzu Model Number. 1800). The absorption peak found at wavelength range of 390 nm characterized the presence of Ag NPs peak and absorption peak at 430.0 nm accounts for this linkage with TiO_2 NPs in UV-Visible absorption spectra. [30]

FTIR Analysis

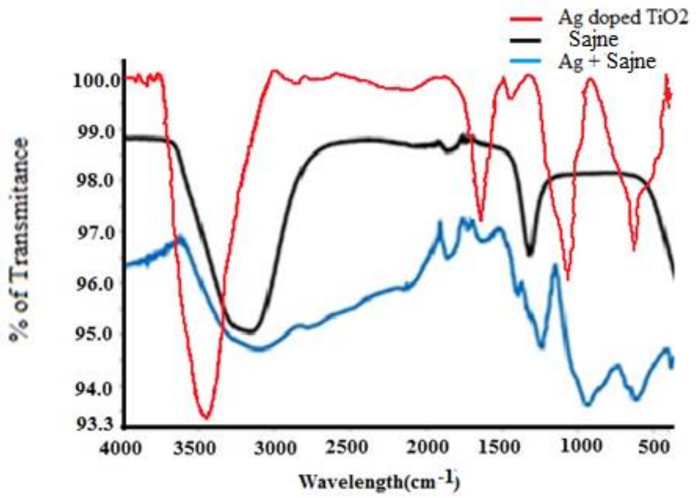

Fig. 4: FTIR spectra of Ag doped TiO2 NPs, Sajne, and Ag-Sajne

The triplet role of the plant extricate as reducing, capping as well as stabilizing cause was deep-rooted by FTIR investigation (Fig. 4) of the organized Ag doped TiO2NPs. The results of the of leaf extricates of Moringa oleifera plant and Ag doped TiO2 NPs were synthesized after the bio-reduction noted in the range of 400 to 4000 cm-1. The KBr method has been used to record the FTIR spectra of NPs. The band near 705 cm-1 resembles to the Ti-O stretching mode, 3200 to 3600 cm-1 match to the stretching vibration of the N-H for NH2 group and OH group for water present on the surface of test-sample and sajne leaf extricate molecules [31]. The Ag-O stretching vibration band confirmed at 1630 cm-1 and it is absent in case of TiO2.

X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis is a reliable technique for determining the crystalline phases and structural properties of nanoparticles (NPs). The TiO_2 NPs were confirmed to be nanocrystalline, with diffraction peaks observed at $2\theta = 33.21°$ and $58.97°$, corresponding to the (111) and (220) crystal planes, respectively (Fig. 5). Based on the Joint Committee on Powder Diffraction Standards (JCPDS) card number 21-1272, the particles were identified as spherical in shape. The strong and sharp diffraction peaks indicate a high degree of crystallinity in the TiO_2 NPs. The XRD pattern showed only the TiO_2 phase, with no detectable impurities. The crystalline size of both pure and silver-doped TiO_2 nanoparticles was estimated using the Debye-Scherrer equation:

$$D = K\lambda/\beta \cos\theta$$

Here, D is the crystallite size, λ represents the X-ray wavelength (0.1541 nm), β is the full width at half maximum (FWHM) of the diffraction peak, K is the Scherrer constant (ranging between 0.9 and 1), and θ is the Bragg angle.

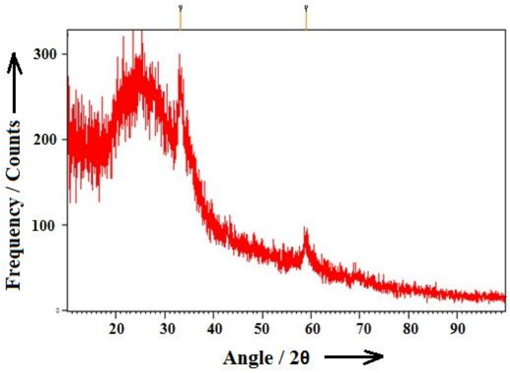

Fig. 5: X-ray Diffraction Analysis (XRD)

SEM and Energy-Dispersive X-ray (EDX) Analysis

Energy-Dispersive X-ray (EDX) spectroscopy, also known as EDAX or EDS, is utilized to determine the elemental composition of materials. EDX systems are typically paired with electron microscopy techniques such as Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM), allowing for precise imaging and compositional analysis of the sample. SEM analysis of the synthesized nanoparticles (Fig. 6) indicated spherical morphology with diameters ranging from 27.11 nm to 60.70 nm.

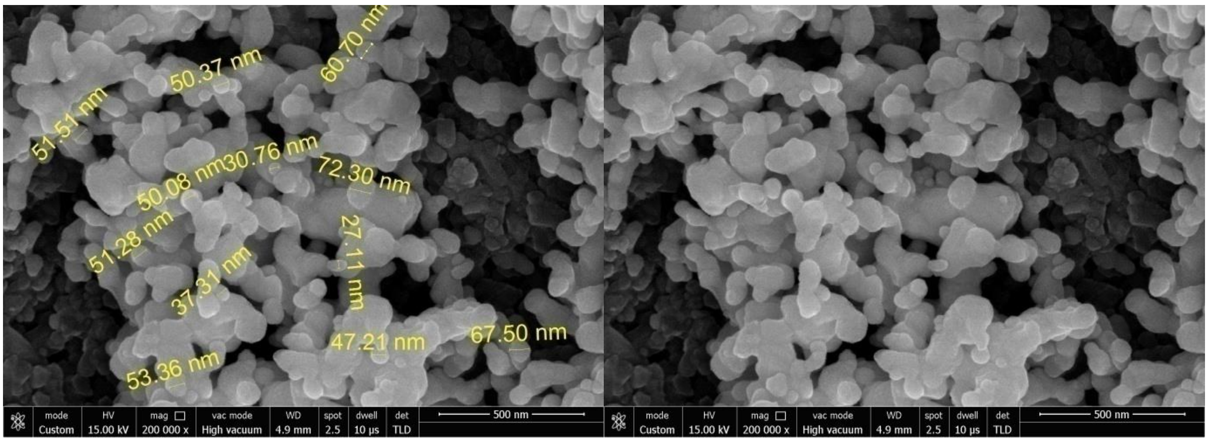

Fig. 6: SEM of Ag-TiO_2 nano particle

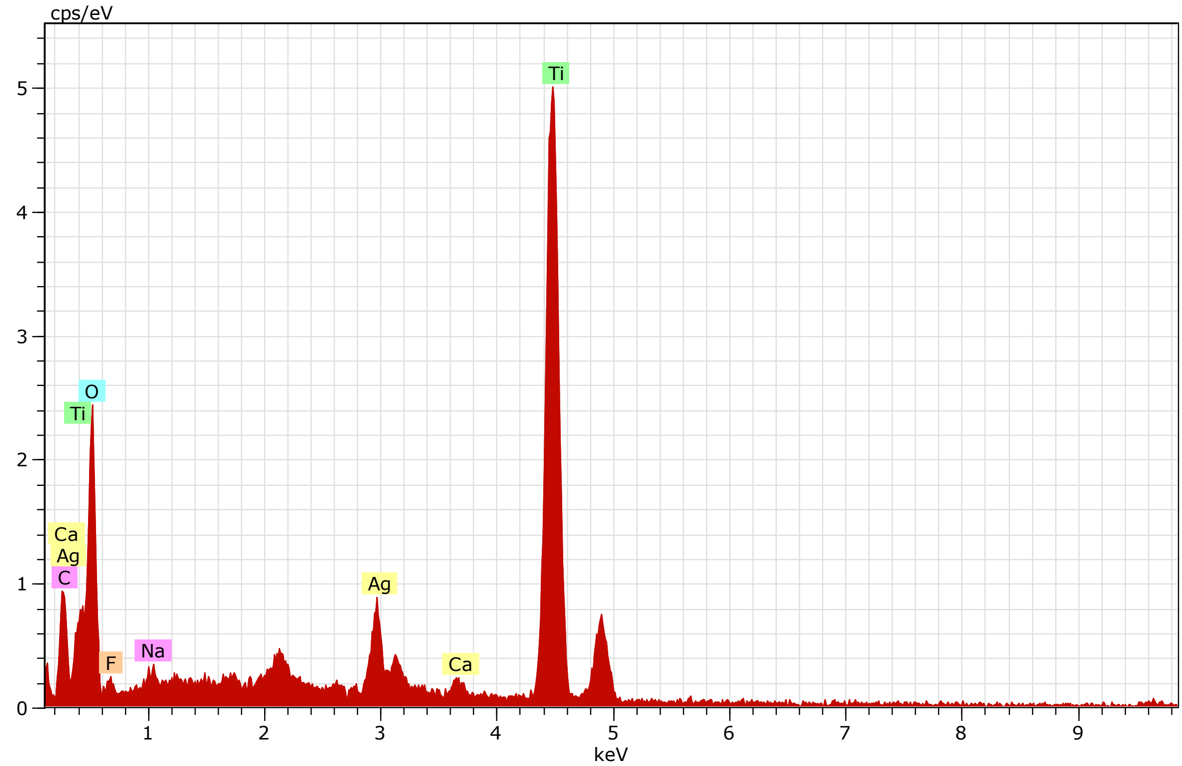

Fig. 7: Energy Dispersive X-Ray Analysis (EDX) of Ag-TiO_2nano particle

The EDX data (Fig. 7) revealed spectra with peaks corresponding to the elemental composition of the sample, confirming the presence of titanium, silver, oxygen, chlorine, sulfur, and trace amounts of carbon. The trace carbon content suggests the involvement of plant-derived phytochemicals in the reduction and stabilization of the TiO_2 NPs. Additionally, the analysis confirmed the successful incorporation of silver into the TiO_2 matrix, verifying the composition and quality of the biosynthesized nanoparticles.

Biological Activity:

Antibacterial Activity

The antibacterial potential of the synthesized nanoparticles (NPs) was assessed using the well-diffusion method against the gram-negative bacterium *E. coli* (MTCC 1721) and the gram-positive bacterium *S. aureus* (MTCC 3160). All media were sterilized under conditions of 125°C, 15 PSI, and a duration of 35 minutes. A bacterial suspension with a concentration of 1.8×10^8 CFU/ml was tested with 20 ml of three samples: *Moringa oleifera* leaf extract, TiO_2 NPs, and Ag-TiO_2 NPs, at concentrations of 12.5 mg/L, 25 mg/L, and 50 mg/L, conducted in three separate trials. The plates were incubated for 24 hours at a temperature range of 35–37°C.

The antimicrobial effectiveness was evaluated by measuring the diameter of the zone of inhibition (in mm) and the minimum inhibitory concentration (MIC) in µg/ml for the tested samples: *Moringa oleifera* (MO) leaf extract, TiO_2 NPs, and Ag-TiO_2 NPs, as detailed in Table 1. Ag-TiO_2 NPs demonstrated the highest inhibition zones at all tested concentrations, with inhibition diameters of 13.53 mm, 18.00 mm, and 20.00 mm for *S. aureus* at concentrations of 12.5, 25, and 50 mg/disc, respectively. In comparison, smaller inhibition zones of 11.50 mm, 16.00 mm, and 18.00 mm were observed for *E. coli*.

The results highlighted a direct relationship between increasing concentration and larger inhibition zones, particularly for *S. aureus* (MTCC 3160). Among the tested samples, Ag-doped titanium oxide NPs exhibited superior antibacterial activity compared to both plant extract and TiO_2 NPs, demonstrating higher efficacy at 50 mg/L against *S. aureus*.

Table 1: Observation of antibacterial activity

S.N	Name of Bacteria	Test Sample	Concentration(mg/L)	Zone of inhibition (mm)
1	*E. Coli* (MTCC 1721)[a]	*Moringa oleifera* (MO) leaf extricate	12.5	4.0
			25.0	4.0
			50.0	6.0
		TiO_2 Nps	12.5	7.5
			25.0	6.0
			50.0	11.0
		Moringa oleifera (MO) leaf extricate + Ag-TiO_2Nps	12.5	11.5
			25.0	16.0
			50.0	18.0
2	*S. Aureus* (MTCC3160)[b]	*Moringa oleifera (MO)* leaf extricate	12.5	5.5
			25.0	6.0
			50.0	7.5
		TiO_2 Nps	12.5	9.5
			25.0	8.0
			50.0	12.5
		Moringa oleifera (MO) leaf extricate + Ag-TiO_2Nps	12.5	13.5
			25.0	18.0
			50.0	20.0

[a] Gram negative bacteria: EC, *Escherichia coli.*
[b] Gram positive bacteria: SA, *Staphylococcus aureus;*

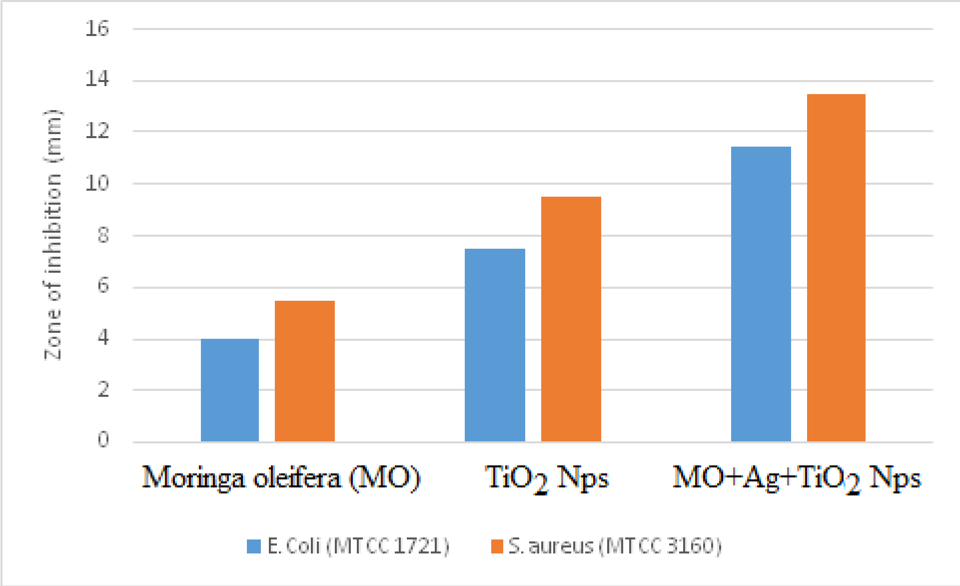

Fig 8a: Graph at concentration 12.5 mg/L

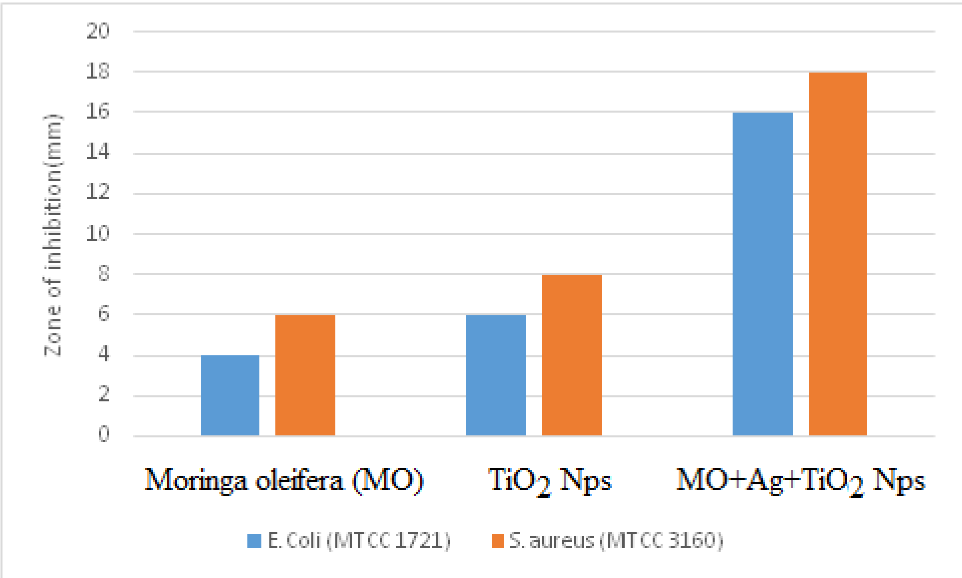

Fig 8b: Graph at concentration 25 mg/L

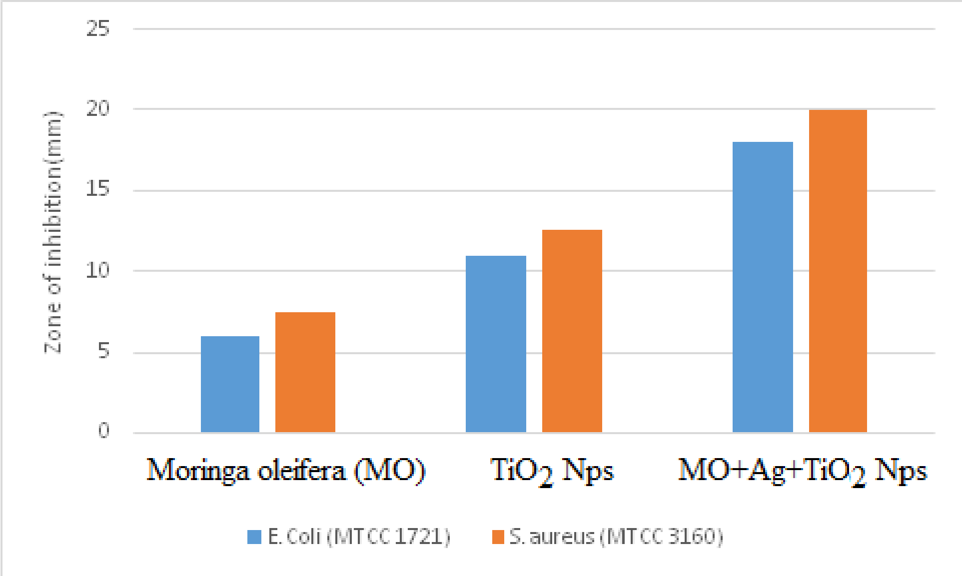

Fig 8c: Graph at concentration 50 mg/L

Antifungal Activity:
The antifungal efficacy of *Moringa oleifera* (MO) plant extract, TiO_2 nanoparticles (NPs), and Ag-doped TiO_2 NPs was evaluated against *Aspergillus niger* using the well diffusion method. The experiment was conducted on Potato Dextrose Agar (PDA) medium inoculated with a spore suspension of *Aspergillus niger* at a concentration of 1×10^5 CFU/ml.

Potato Dextrose Broth (PDB) was prepared and incubated in a shaking incubator for 48 hours, after which the fungal spores were introduced. Once the PDA solidified, it was evenly seeded with fungal spores. Wells of 6 mm diameter were created in the medium and filled with varying concentrations (50, 100, 500, and 1000 ppm) of *Moringa oleifera* plant extract, TiO_2 NPs, and Ag-doped TiO_2 NPs. The plates were then incubated at a temperature of 25–28°C for 48 hours.

After incubation, the antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm). The results revealed a direct relationship between the concentration of the test substances and their antifungal activity against *Aspergillus niger*. Among the tested samples, Ag-doped TiO_2 NPs derived from *Moringa oleifera* exhibited the highest antifungal activity at 1000 ppm concentration.

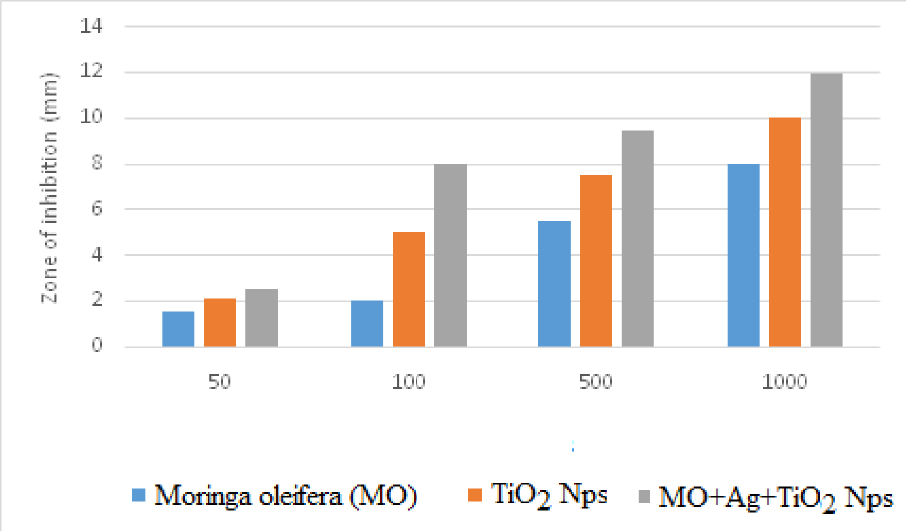

Fig 9: Comparison of Antifungal activity of Aspergillus *niger* at conc. (50 ppm, 100 ppm, 500 ppm, 1000 ppm).

Table 2: Observation of antifungal activity.

S.N	Name of Fungi	Concentration	Test Sample	Zone of inhibition (mm)
1	*Aspergillus niger*	50 ppm	*Moringa oleifera(MO)* leaf extricate	1.5
			TiO_2Nps	2.1
			(MO) + Ag-TiO_2Nps	2.5
		100 ppm	*Moringa oleifera(MO)* leaf extricate	2.0
			TiO_2Nps	5.0
			(MO) + Ag-TiO_2Nps	8.0
		500 ppm	*Moringa oleifera(MO)* leaf extricate	5.5
			TiO_2Nps	7.5
			(MO) + Ag-TiO_2Nps	9.5
		1000 ppm	*Moringa oleifera(MO)* leaf extricate	8.0
			TiO_2Nps	10.0
			(MO) + Ag-TiO_2Nps	12.0

Conclusions

We have developed convenient and green procedure for the synthesis of spherical Ag doped TiO_2 NPs from tropical based novel plant *Moringa oleifera*. The average sizes of the NPs are 50 nm, confirmed by SEM characterization techniques. The employed plant, *Moringa oleifera* plays important role as capping agent on the surface of metal NPs. The NPs showed good antimicrobial activity against *S. aureus* and *E. coli* bacteria. However, NPs were comparatively more effective inhibit the growth of *S. aureus* than *E. coli* bacteria. The antifungal activity of sphere-shaped Ag doped TiO_2 NPs from *Moringa oleifera* in contrast to *Aspergillus niger* fungus, increases with the rise of concentration of NPs. These results recommend that NPs can be of interest reflected for use in biomedical applications.

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