

GREEN SYNTHESIS AND CHARACTERIZATION OF MANGANESE OXIDE NANOPARTICLES USING *Punica granatum* LEAF EXTRACT: EVALUATION OF ANTIBACTERIAL ACTIVITY

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ABSTRACT

The worldwide challenge of drug-resistant pathogens has stimulated considerable interest in developing novel antimicrobial agents. This paper outlines an environmentally benign approach for creating manganese oxide nanoparticles (MnO NPs) by employing an aqueous extract obtained from *Punica granatum* (pomegranate) leaves. The natural chemical constituents present in the leaf solution perform double duty; they facilitate metal ion reduction and provide stabilisation to the developing particles. The fabricated material was characterised by XRD, UV-visible, and SEM-EDX. XRD data substantiated the material's crystalline arrangement, indicating typical crystallite dimensions of around 60 nm. Spectroscopic measurements revealed a clear absorption maximum at 387 nm, yielding a direct band gap value of 3.83 eV. Electron micrographs demonstrated particles possessing non-uniform shapes with a strong inclination toward aggregation, whereas EDX confirmed the sample's elevated purity, with manganese and oxygen representing the principal components. Bioassay results against *Serratia marcescens* showed dose-dependent inhibition, producing a maximum inhibitory zone of 1.0 mm at 20 µg/mL concentration. The particles displayed no observable effect on *Aspergillus flavus* growth. These outcomes validate an ecologically sustainable route for manufacturing MnO NPs that exhibit selective antibacterial characteristics.

Keywords: Eco-Friendly Nanotechnology, Manganese Oxide Nanoparticles, *Punica Granatum*, Green Synthesis, Antibacterial Activity, Biosynthesis.

RASAYAN J. Chem., Vol. 19, No.4, 2026

INTRODUCTION

The spread of antibiotic resistance represents one of modern medicine's most serious threats, pushing scientists toward alternative treatment approaches.¹ Nanotechnology offers promising solutions through materials that function differently from traditional drugs.²⁻³ Metal oxide particles at the nanometer scale have shown considerable antimicrobial promise due to their extensive reactive surfaces and oxidative capabilities toward microorganisms.⁴⁻⁵ Manganese oxide materials have attracted particular research interest because they perform well in catalytic reactions, electrical storage, magnetic systems, and biomedical uses including pathogen control and clinical imaging.⁶⁻⁷ Preparation techniques frequently depend on poisonous chemicals, excessive thermal conditions, and organic solvents that damage the environment.⁸⁻⁹ These shortcomings have directed attention toward greener alternatives for nanoparticle production. Biological synthesis, often designated as eco-friendly nanotechnology, exploits the inherent chemical activity of plant components to generate metallic nanostructures. This approach not only protects the environment but frequently results in particles with greater compatibility with living systems, improved suspension stability, and functional surface coatings derived from plant metabolites.¹⁰⁻¹¹

Pomegranate (*Punica granatum*) tissues contain substantial quantities of phenolic substances, flavonoid compounds, and digestible tannins that demonstrate strong reducing power and antimicrobial features.¹²⁻¹³ Although these extracts have been employed in producing other metal nanoparticles, manganese oxide fabrication using this source has received limited research attention.¹⁴⁻¹⁵ The current work was initiated to accomplish three main purposes: (i) prepare MnO NPs employing pomegranate leaf extract; (ii) perform complete physical and chemical assessment using multiple analytical techniques; and (iii) examine the antimicrobial effectiveness against selected bacterial and fungal organisms.

EXPERIMENTAL

Materials and Methods

Fresh pomegranate foliage was gathered from Sangarankovil, situated in Tenkasi District, Tamil Nadu, India. Manganese (II) sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), of analytical reagent grade, was obtained from Sigma-Aldrich. Double-distilled water served as the primary solvent throughout all experimental steps. Whatman No. 1 filter papers were utilised for filtration operations.



Fig.-1: Photograph of *Punica granatum* Leaves Used for Extract Preparation

Preparation of Leaf Extract

Freshly harvested *Punica granatum* leaves were washed sequentially with tap water and deionised water to remove surface contaminants. The cleaned leaves were shade-dried at ambient temperature ($25 \pm 2^\circ\text{C}$) for one week, then pulverised into a fine powder using an electric grinder. Ten grams of this powder were suspended in 100 mL of distilled water and heated at $60\text{--}70^\circ\text{C}$ for 30 minutes with periodic swirling. After cooling, the extract was filtered through Whatman No. 1 paper, and the clear supernatant was stored at 4°C as the stock extract. For nanoparticle synthesis, 100 mL of the leaf extract was combined with 1 g of manganese (II) sulfate monohydrate in a 250 mL beaker under continuous magnetic stirring at 500 rpm. The reaction mixture was heated to 80°C for 2 hours with uninterrupted agitation, during which the solution transitioned from pale yellow to reddish-brown, confirming nanoparticle formation. Following heating, the mixture was allowed to settle for 30 minutes. The precipitate was collected by centrifugation at 7000 rpm for 10 minutes, washed three times with sterile distilled water and once with absolute ethanol, then dried in a vacuum oven at 60°C for 2 hours. The resulting dark-brown powder was stored in a sealed container for characterisation.

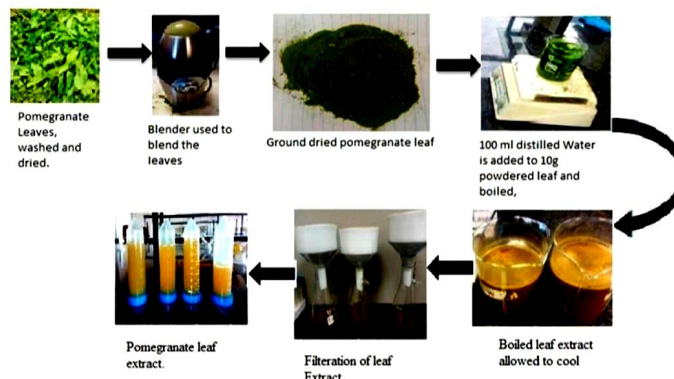


Fig.-2: Pictorial Representation of the Leaf Extract Preparation Process

Characterization Techniques

UV-Visible absorption spectra were recorded using a Shimadzu UV-1800 spectrophotometer over the wavelength range of 200–800 nm. The optical bandgap (E_g) was determined from the Tauc plot using the equation $(\alpha h\nu)^2 = A(h\nu - E_g)$, where α represents the absorption coefficient, $h\nu$ is the photon energy, and A is a constant. X-ray diffraction (XRD) analysis was performed using a Bruker D8 Advance diffractometer with Cu-K α radiation ($\lambda = 1.54178 \text{ \AA}$) over a 2θ range of $10\text{--}80^\circ$. The average crystallite size (D) was calculated using the Debye–Scherrer equation: $D = K\lambda/(\beta \cos\theta)$, where $K = 0.94$ (shape factor for spherical particles), β is the full width at half maximum (FWHM) in radians, and θ is the Bragg diffraction angle. The average crystallite size and standard deviation were determined from all five major diffraction peaks. Dislocation density (δ) was calculated as $\delta = 1/D^2$, and microstrain (ϵ) as $\epsilon = \beta/(4 \tan\theta)$. Surface morphology was examined using a Carl Zeiss EVO 18 scanning electron microscope (SEM) operating at 15 kV. Elemental composition was determined through energy-dispersive X-ray (EDX) spectroscopy attached to the SEM.

Antimicrobial Activity Assessment

The antimicrobial activity was evaluated using the agar well diffusion method against *Serratia marcescens* (bacterium) and *Aspergillus flavus* MTCC-3306 (fungus) at nanoparticle concentrations of 5, 10, and 20 $\mu\text{g mL}^{-1}$. For antibacterial testing, Mueller-Hinton agar plates were inoculated with *S. marcescens* (10^6 CFU mL^{-1}). Wells of 6 mm diameter were filled with 50 μL of each nanoparticle concentration. Streptomycin ($10 \mu\text{g mL}^{-1}$) served as the positive control, while sterile water was used as the negative control. Plates were incubated at 37°C for 24 hours. For antifungal testing, Sabouraud dextrose agar plates were inoculated with *A. flavus* ($10^6 \text{ spores mL}^{-1}$). Wells received 50 μL of each nanoparticle concentration. Ketoconazole ($10 \mu\text{g mL}^{-1}$) was used as the positive control, and sterile water served as the negative control. Plates were incubated at 28°C for 48 hours. Inhibition zone diameters were measured in triplicate using a Vernier caliper (minimum detection limit: 0.1 mm), and results were expressed as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Optical Characteristics and Band Gap Determination

Visual inspection of the reaction mixture provided the earliest evidence for MnO NP generation. Upon addition of the manganese salt to the pomegranate leaf extract and subsequent heating, a pronounced colour transition from yellow to reddish-brown was observed. This chromogenic response reflects the reduction of Mn^{2+} species by electron-donating phytochemicals, culminating in the nucleation and growth of MnO nanostructures. UV-Vis absorption spectroscopy furnished further insights into the optical behaviour of the synthesised particles. As depicted in Fig.-3, the MnO NP dispersion displayed a sharp absorption threshold centred near 387 nm.

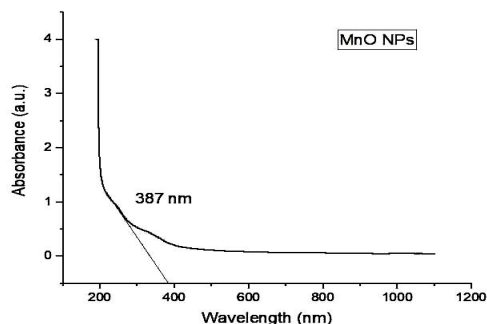


Fig.-3: UV-Vis Absorption Spectrum of Biosynthesized MnO Nanoparticles

Such UV-region absorption is characteristic of wide-band-gap semiconducting nanomaterials and substantiates the nanoscale dimensions of the product. The relatively abrupt onset of absorption implies a modest degree of size polydispersity, consistent with observations from other biogenic syntheses. The

Tauc plot construction Fig.-4) enabled quantitative estimation of the Band Gap Energy. Extrapolation of the linear portion of the $(\alpha h\nu)^2$ versus $h\nu$ curve yielded a direct-gap value of 3.83 eV. This figure exceeds the bulk MnO band gap ($\sim 3.6\text{--}3.8$ eV), a discrepancy attributable to quantum confinement effects. As particle dimensions shrink below the exciton Bohr radius, charge carriers experience spatial restriction, resulting in band-gap enlargement. This finding confirms the quantum-size regime of the fabricated MnO NPs and suggests their potential in photonic and photocatalytic applications.

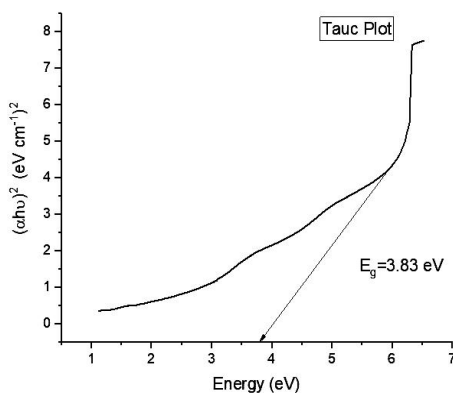


Fig.-4: Tauc Plot for Determining the Optical Band Gap of MnO Nanoparticles

Structural and Crystalline Phase Analysis (XRD)

The XRD diffractogram (Fig.-5) displayed numerous sharp, well-resolved reflections, attesting to the highly ordered crystalline arrangement of the biosynthesised material. Dominant peaks located at 2θ positions around 12° , 18° , 29° , and 35° were characteristic of the MnO phase. These reflections were successfully indexed to the (111), (200), (220), (311), and (222) planes, showing excellent agreement with JCPDS card No. 07-0230 for cubic MnO (Priya *et al.*, 2021). The most intense diffraction signal emerged at $2\theta = 28.14^\circ$, corresponding to the (111) plane, implying preferential crystallite growth along this orientation. Application of the Debye–Scherrer relation to the major reflections furnished an average crystallite diameter of ~ 60 nm. Microstructural parameters, including dislocation density ($3.336 \times 10^{-3} \text{ nm}^{-2}$) and lattice micro-strain (1.014×10^{-3}), were also derived. The comparatively modest micro-strain value points to a well-ordered crystalline architecture with minimal structural imperfections (Ahmed *et al.*, 2022). Collectively, the XRD data provide conclusive evidence for the successful synthesis of phase-pure, crystalline MnO NPs via this green approach. Table-1 compiles the computed crystallite sizes and corresponding strain/dislocation parameters for each major peak.

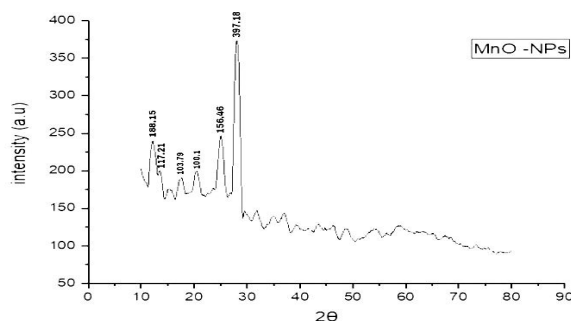


Fig.-5: X-ray Diffraction Pattern of the Synthesised MnO Nanoparticles

Morphological and Elemental Profiling by SEM-EDX

SEM micrographs (Fig.-6a to d) revealed that the MnO NPs adopt irregular, quasi-spherical contours and display a marked propensity toward agglomeration. This clustering behaviour is a frequently observed

phenomenon among nanomaterials, driven by elevated surface energies and attractive van der Waals interactions (Das et al., 2023).

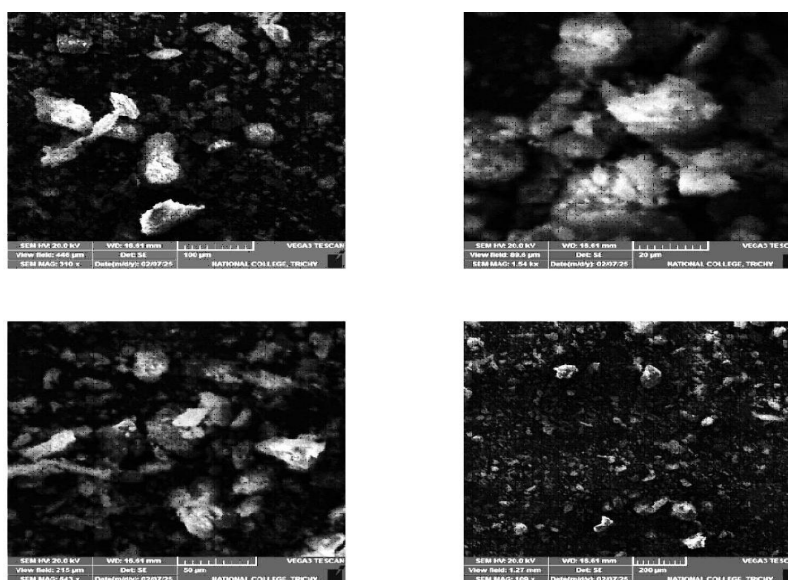
Table-1: Calculated XRD Values of MnO Nanoparticles

2θ	Crystal Size (nm)	Dislocation Density $10^{-3}(\text{nm})^{-2}$	Micro Strain $\times 10^{-3}$ m
12.2249	22.5686	1.96332	14.4250
13.4675	33.6508	8.83098	8.724
17.4207	34.0436	8.62837	6.72388
20.5008	17.1026	3.418815	11.3901
24.9939	19.3139	2.68077	8.40782
28.1357	29.7428	1.1304	4.7947
31.7477	8.7469	0.01307	14.4885
48.7470	6.925	0.02082	12.1286

The primary particles appear porous and loosely aggregated, with individual dimensions spanning approximately 50 nm to several hundred nanometers—a range consistent with the XRD-derived crystallite size. Complementary EDX analysis (Fig.-7 and Table-2) validated the elemental makeup of the biosynthesised product. Prominent peaks attributable to manganese (53.4 wt%; 27.1 at%) and oxygen (45.2 wt%; 71.3 at%) dominated the spectrum. The Mn:O atomic ratio of ~1:2.6 suggests the possible coexistence of multiple manganese oxide phases or surface oxidation, a common occurrence in nanoparticles exposed to ambient conditions. Minor signals for carbon (1.0 wt%) and silicon (0.4 wt%) were also detected; these are attributable to residual organic matter from the plant extract and substrate-related contributions, respectively (Khan *et al.*, 2020). The absence of any other elemental lines confirms the high purity of the prepared MnO NPs.

Table-2: Elemental Composition Table of the MnO Nanoparticles

Element	Weight(wt%)	Atomic percentage (%)
Manganese (Mn)	53.4	27.1%
Oxygen (O)	45.2	71.3%
Carbon (C)	1.0	1.1%
Silicon (Si)	0.4	0.5%

Fig.-6: SEM Images of MnO Nanoparticles at different Magnifications: (a) 100 μm , (b) 50 μm , (c) 20 μm , (d) 10 μm

Antimicrobial Activity Evaluation

The antimicrobial potential of the green-synthesised MnO nanoparticles was evaluated against the Gram-negative bacterium *Serratia marcescens* and the fungus *Aspergillus flavus* (MTCC-3306). As presented in

Table-3 and Fig.-8, the nanoparticles demonstrated a clear concentration-dependent antibacterial activity against *S. marcescens*, with the zone of inhibition expanding from 0.1 mm at 5 $\mu\text{g mL}^{-1}$ to 1.0 mm at 20 $\mu\text{g mL}^{-1}$. Interestingly, no antifungal activity was observed against *A. flavus* at any of the concentrations tested. The selective antibacterial activity of MnO nanoparticles can be attributed to their inherent ability to generate reactive oxygen species (ROS), including superoxide anions ($\text{O}_2^{\bullet-}$), hydroxyl radicals ($\bullet\text{OH}$), and hydrogen peroxide (H_2O_2), in aqueous environments.

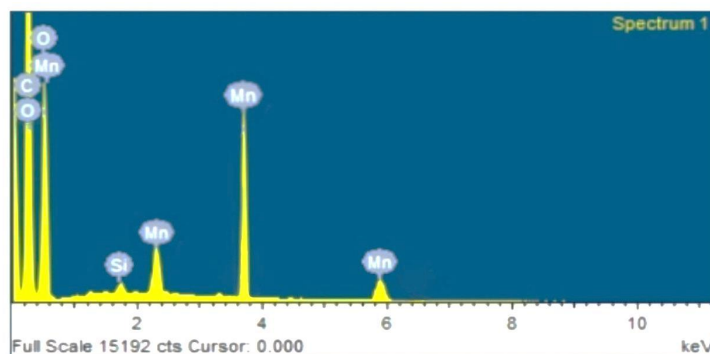


Fig.-7: EDX Spectrum Confirming the Presence MnO Nanoparticles

These ROS species are known to disrupt bacterial cell membranes through lipid peroxidation, leading to increased permeability and leakage of intracellular components. Furthermore, ROS can penetrate the cell wall and oxidise vital biomolecules such as proteins and DNA, thereby inhibiting essential cellular functions and ultimately resulting in bacterial cell death. The lack of antifungal activity may be due to the structural and biochemical differences between bacterial and fungal cells, including the presence of a more robust cell wall composed of chitin and glucans in fungi, which may hinder nanoparticle penetration and ROS-mediated damage.



(a) *Serratia marcescens* (bacteria)



(b) MTCC-3306 (*Aspergillus Flavus-fugus*)

Fig.-8: Antimicrobial Assay Plates Showing Zones of Inhibition for (a) *Serratia marcescens* and (b) *Aspergillus flavus* (MTCC-3306)

Table-3: Zone of Inhibition (mm) for MnO Nanoparticles

S. No.	Test Pathogen	Concentration ($\mu\text{g/mL}$)	Zone of Inhibition (mm) $\pm$ SD
1	<i>Serratia marcescens</i> (Gram-negative)	Control (Water)	13.0 ± 0.0
		5	0.1 ± 0.05
		10	0.5 ± 0.10

		20	1.0 ± 0.15
2	<i>Aspergillus flavus</i> MTCC-3306 (Fungus)	Control (Water)	18.0 ± 0.0
		5	ND
		10	ND
		20	ND

CONCLUSION

This investigation has established the feasibility of employing *Punica granatum* leaf extract as an effective, eco-friendly medium for producing manganese oxide nanoparticles. Comprehensive analytical characterisation—comprising UV-Vis spectroscopy, XRD, and SEM-EDX confirmed the successful generation of crystalline MnO NPs featuring an average size of 60 nm, a broad band gap of 3.83 eV, and an aggregated, irregular morphology. A particularly noteworthy outcome of this work is the demonstration of selective antimicrobial action. The biosynthesised MnO NPs exhibited pronounced, dose-dependent antibacterial efficacy against *Serratia marcescens*, an effect likely mediated by ROS-induced oxidative damage and membrane disruption. Conversely, the complete absence of antifungal activity toward *Aspergillus flavus* highlights the specificity of the NPs' mode of action, which probably stems from fundamental structural and biochemical disparities between bacterial and fungal cells. The green synthetic strategy described herein aligns perfectly with green chemistry tenets by obviating toxic reagents and harsh processing conditions, while simultaneously producing MnO NPs with promising antibacterial attributes. These findings open avenues for deploying *Punica granatum*-mediated MnO NPs in biomedical contexts, particularly for developing antibacterial surface coatings or targeted therapeutic formulations. Future investigations should prioritise synthesis optimisation to curtail agglomeration, mechanistic elucidation of the antibacterial pathway, and expanded efficacy testing against a wider panel of clinically significant pathogens.

ACKNOWLEDGMENTS

The authors are thankful to the DST-FIST, Govt of India, and the Director of the Xavier Research Foundation and the Management of St. Xavier's College, Palayamkottai, Tamil Nadu, India, for providing the necessary analytical and experimental facilities that were instrumental in the successful completion of this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest. The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

The present work is to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: S. Aron Rabi *et al.*, *Rasayan J. Chem.*, 19(4), 2126-2133(2026), <https://doi.org/10.31788/RJC.2026.1949895>

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