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Green Synthesis of Manganese Oxide Nanoparticles Mediated by Butea Monosperma Pod Extract: Characterization and Antibacterial Screening

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Abstract: ***This study reports a completely green, eco-friendly, and cost-effective method for synthesizing manganese oxide (MnO) nanoparticles using the aqueous pod extract of Butea monosperma as a natural reducing and stabilizing agent. The phytoconstituents within the pod extract successfully mediated the reduction of manganese precursors, eliminating the need for toxic chemical reagents. The synthesized MnO nanoparticles were comprehensively characterized using UV-Visible, FTIR and XRD, confirming the formation of stable nanoparticles. Furthermore, MnO nanoparticles were screen for antibacterial activity via the agar well diffusion method against both Gram-positive and Gram-negative bacterial pathogens. These findings highlight the potential of Butea monosperma as an excellent bio-resource for nanotechnology, positioning the resulting (MnO) nanoparticles as promising candidates for biomedical applications.***

Keywords: Green synthesis, *Butea Monosperma*, Manganese oxide nanoparticles, Characterization, Antibacterial activity

1. Introduction

In recent years, the rapid advancement of nanotechnology has revolutionized various scientific and industrial fields, particularly biomedicine, pharmacology, and environmental remediation. Nanoparticles, typically defined as materials with at least one dimension spanning 1 to 100 nanometers, possess unique physicochemical properties- including an exceptionally high surface area to volume ratio, distinct magnetic traits, and high surface reactivity, that dramatically set them apart from their bulk counterparts [1]. Among various transition metal oxides, manganese oxide (Mn O) nanoparticles have emerged as highly attractive candidates for diverse applications due to their multivalent oxidation states, low cost, abundant natural availability, and environmental compatibility [2]-[3]. Specifically, MnO nanoparticles exhibit significant potential as catalysts, energy storage materials, water purification agents, and targeted therapeutic or antimicrobial systems [4]. Peroxidase, glutathione peroxidase, oxidase, catalase, dismutase, and superoxide are among the enzyme-like activities exhibited by MnO nanoparticles [5].

Nanoparticles can be produced using a number of tried-and-true techniques, such as chemical, physical, and green synthesis methods. Physical synthesis, which includes methods like plasma chemistry, vapour deposition, microwave radiation, pulsed laser, sonochemical reduction, and gamma radiation, is preferred due to its high purity, controlled shape, and high yield [6]. Another common method is chemical synthesis, which makes use of reducing agents in polyol, microemulsions, heat breakdown, and electrochemical synthesis. However, both physical and chemical synthesis methods have obstacles, including the requirement for high-grade resources, meticulous techniques, considerable budgets, and potential biological toxicity due to hazardous residues. These drawbacks make these techniques less suitable for creating NPs meant for in vivo medicinal applications [7]-[8]. Consequently, there is an urgent and growing paradigm shift toward green chemistry principles [9]. This eco-friendly alternative prioritizes the use of benign, renewable biological resources such as plant extracts [10]-[12], fungi [13]-[15], and bacteria [16]-[17] to fabricate functional nanomaterials without environmental toxicity.

Plant-mediated green synthesis, or phytosynthesis, has garnered immense preference over microbial routes because it eliminates the tedious, time-consuming steps of cell culture maintenance, isolation, and sterile laboratory scaling [18]. Plant extracts act simultaneously as natural reducing agents and capping/stabilizing matrices that prevent the agglomeration of the generated nanoparticles [19]. *Butea monosperma*, popularly known as "Flame of the Forest" or *Palash*, is a prominent medicinal plant native to the Indian subcontinent belonging to the family Fabaceae. Various parts of this plant have been historically utilized in traditional medicine due to their rich phytochemistry, which includes a dense concentration of polyphenols, flavonoids, glucosides, tannins, and organic acids that exhibit notable hepatoprotective, antitumoral, and wound-healing properties [20]-[21].

While *Butea monosperma* bark has been utilized to derive carbon quantum dots [22], and its leaves or flowers have mediated silver, gold, and cobalt nanoparticles [23]-[24] [20], its heavy, fibrous pods remain largely underutilized as a bio-resource. The aqueous extract of *Butea monosperma* pods contains complex biomolecules rich in hydroxyl (OH) and carbonyl (C=O) functional groups, making them ideal candidate pools for the green reduction of manganese precursors into stable Mn nanoparticles without adding synthetic chemical stabilizers. Simultaneously, the emergence of multi-drug resistance (MDR) bacterial infections around the world has posed a serious threat to contemporary healthcare, making many traditional antibiotics useless. Alternative antibacterial techniques must be developed immediately due to microbial biofilms and adaptive mutations. The use of biogenic metal oxide nanoparticles as a weapon against these hardy infections is quite effective [25]. Excellent bactericidal properties against both Gram-positive and Gram-negative microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, have been shown in recent studies assessing green-synthesized manganese oxide nanoparticles against sporadic infections [26]-[27].

Motivated by these interconnected needs for eco-friendly material processing and potent alternative antimicrobials, this study investigates the completely green synthesis of manganese oxide (MnO) nanoparticles mediated by the aqueous pod extract of *Butea monosperma*. To the best of my knowledge, this represents a unique exploration utilizing this specific botanical byproduct for manganese reduction. The synthesized MnO nanoparticles are subjected to comprehensive characterization using UV-Visible spectroscopy, Fourier-Transform Infrared (FTIR) spectroscopy and X-ray Diffraction (XRD), to understand their optical properties, functional capping layers and crystalline nature. Finally, the antibacterial efficacy of these biogenic nanoparticles is evaluated against clinically relevant Gram-positive and Gram-negative bacterial strains to ascertain their potential as next-generation antimicrobial agents.

2. Experimental Details

2.1 Reagents and Chemicals

Manganese chloride ($MnCl_2$) was procured from SD Fine-Chem Limited (SDFCL), India, and used as received without further purification. All aqueous solutions and experimental dilutions were prepared using triple-distilled de-ionized water to ensure the highest purity and eliminate ionic interference.

2.2 Collection and Processing of Plant Material

Fully grown pods of *Butea monosperma* were collected from the field regions of Dhamangaon, located in the Amravati district of Maharashtra, India. To remove surface dust and particulate contaminants, the collected pods were initially washed thoroughly with running tap water, followed by two successive rinses with de-ionized distilled water. The cleaned pods were then allowed to air-dry completely at room temperature. Once thoroughly dried, the pods were pulverized into a fine powder using a domestic mixer. The resulting plant powder was stored in an airtight container for subsequent extraction and experimental use [28].

2.3 Preparation of *Butea monosperma* Pod Extract

The aqueous extract of *Butea monosperma* pods was prepared by dispersing 5 g of the processed pod powder into 100 mL of triple-distilled de-ionized water in a 250 mL beaker. The mixture was heated at a controlled temperature for 20 min to facilitate the extraction of bioactive phytochemicals. Following the thermal extraction, the mixture was allowed to cool naturally to room temperature. The crude extract was then filtered through Whatman No. 1 filter paper to separate the insoluble plant debris. The clear resultant filtrate was collected and stored at 4°C in a refrigerator for subsequent synthesis and experimental applications.

2.4 Biosynthesis of Manganese Oxide (MnO) Nanoparticles

For the green synthesis of manganese oxide (MnO) nanoparticles, a 5 mL aliquot of the freshly prepared aqueous *Butea monosperma* pod extract was added dropwise to 50 mL of a 0.1 M manganese chloride ($MnCl_2$) stock solution under continuous stirring. To optimize particle nucleation and achieve a highly reduced, tiny particle size, the pH of the reaction mixture was adjusted to 8 by the meticulous, dropwise addition of a dilute sodium hydroxide (NaOH) solution under gradual and constant agitation. The resulting mixture was then transferred to a magnetic stirrer and heated at a constant temperature of 65°C under continuous stirring for 6 hours. During the reaction, a distinct color transition of the solution to a dark brown hue was observed, visually confirming the successful biosynthesis and reduction of the precursor into MnO nanoparticles. To isolate the nanoparticles, the colloidal solution was subjected to centrifugation at 4000 rpm for 20 minutes. The supernatant was carefully discarded, and the obtained pellet was washed three times with triple-distilled de-ionized water to thoroughly eliminate any unreacted precursor ions or residual plant metabolites. Finally, the washed pellet was allowed to air-dry completely at room temperature to obtain the stable, fine MnO nanoparticle powder, which was stored for further characterization and application [29].

2.5 Characterization of Biosynthesized MnO Nanoparticles

The structural, optical, and surface properties of the biosynthesized MnO nanoparticles were systematically evaluated using standard spectroscopic and diffractometric techniques. The optical absorption profile and successful reduction of the precursor metal ions were monitored using a Systronics UV-Visible Spectrophotometer-119 within a scanning range of 200–800 nm. To identify the key functional groups and bioactive plant metabolites responsible for the reduction, capping, and stabilization of the nanoparticles, Fourier-transform infrared spectroscopy was conducted on a Shimadzu IR Spectrophotometer by recording the transmittance spectra in the mid-infrared region of 4000–400 cm^{-1}. Furthermore, the crystalline nature, phase purity, and average crystallite size of the air-dried nano-powder were evaluated by X-ray diffraction (XRD) analysis on a Rigaku Miniflex 600 diffractometer operating with Cu-Kα radiation ($\lambda = 0.15406$ nm) over a 2θ scanning range of 10° to 80°.

2.6 Evaluation of Antibacterial Efficacy

The antibacterial efficacy of the synthesized MnO nanoparticles was evaluated against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacterial strains using the standard agar well diffusion technique. Freshly cultured bacterial strains were used to prepare the inocula, and a 100 μL aliquot of each inoculum was uniformly spread over nutrient agar plates (Hi Media Laboratories, India) containing approximately 15 mL of sterile medium per plate. The inoculated plates were allowed to dry completely under strict aseptic conditions inside a laminar airflow hood. Subsequently, wells of 6 mm diameter were aseptically punched into the agar using a sterile borer. The test compound was dissolved in triple-distilled de-ionized water to achieve a stock concentration of 1 mg/mL, and a 30 µL volume of this colloidal solution was introduced into the designated wells. Streptomycin (25 µg/well) was utilized as the positive reference control. The plates were then incubated at 37°C for 24 hours under aerobic conditions. The antibacterial activity was quantitatively evaluated by measuring the diameter of the zones of inhibition formed around each well in millimeters.

3. Results and Discussion

3.1 UV-Visible Spectroscopic Analysis

The primary confirmation of the formation and optical properties of the biosynthesized manganese oxide (MnO) nanoparticles was obtained via UV-visible absorption spectroscopy. The electronic absorption spectrum of the colloidal solution, synthesized using *Butea monosperma* pod extract, is illustrated in Figure 1. The spectrum displays a distinct and prominent absorption peak centered at approximately 325 nm. This well-defined absorption maximum (λ_{max}) in the near-UV region is highly characteristic of manganese oxide nanoparticles, arising from the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions as well as surface plasmon resonance (SPR) effects typical of nanostructured manganese oxides [30]-[31]. The appearance of this specific absorption band confirms the successful reduction of manganese chloride ($MnCl_2$) precursor ions into stable MnO nanoparticles mediated by the bioactive phytochemicals- such as polyphenols, flavonoids, and organic acids- present in the *Butea monosperma* pod extract [32]. The sharp intensity and clear resolution of the peak indicate a narrow size distribution and the formation of tiny, uniform particles, which was successfully achieved through the controlled optimization of the reaction mixture at pH 8. Furthermore, the absence of any other prominent absorption bands in the visible region (400–700 nm) highlights the optical purity and baseline stability of the biosynthesized nano-powder.

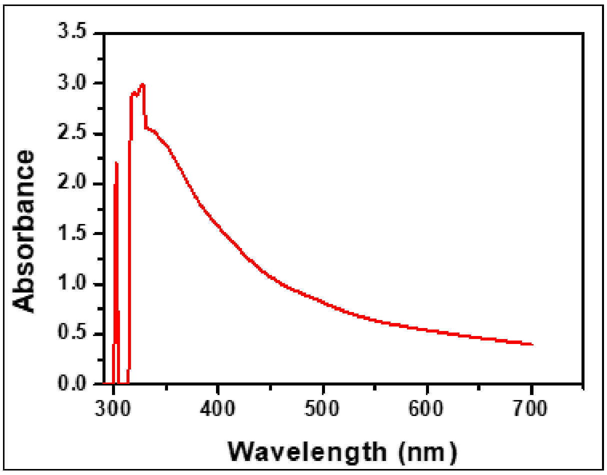

Figure 1: uv-visible spectra of MnO nanoparticles

3.2 Fourier-Transform Infrared (FTIR) Spectroscopic Analysis

Fourier-transform infrared spectroscopy was utilized to identify the functional groups involved in the green reduction and subsequent stabilization of the MnO nanoparticles. Figure-2 and Figure-3 illustrates the comparative FTIR spectra of the crude *Butea monosperma* pod extract and the biosynthesized MnO nanoparticles.

In the spectrum of the plant extract (Figure 2), a broad and intense absorption band is prominent at approximately 3400–3300 cm^{-1}, corresponding to the stretching vibrations of hydroxyl (-OH) groups belonging to phenolic compounds, flavonoids, and water molecules present in the botanical matrix. The peak observed at roughly 2925 cm^{-1} is attributed to the asymmetric stretching vibrations of aliphatic C-H groups. A sharp peak situated around 1630 cm^{-1} signifies the presence of conjugated carbonyl (C=O) or amide I stretching configurations, typical of plant proteins and organic acids. Further down, the bands appearing between 1400 cm^{-1} and 1000 cm^{-1} correspond to aromatic C=C skeletal stretching and C-O stretching vibrations of polyols and glycosidic bonds.

Upon the formation of the MnO nanoparticles (Figure 3), notable structural alterations and shift dynamics in the vibrational profiles are evident. The broad -OH stretching band shifts slightly and becomes distinct, indicating the active engagement of phenolic hydroxyl groups in coordinating with and reducing the Mn^{2+} ions. Concurrently, the aliphatic C-H peak around 2925 cm^{-1} decreases significantly in relative intensity. The shifting and attenuation of the carbonyl/amide peak near 1600 cm^{-1} alongside modifications within the fingerprint region (1400–1000 cm^{-1}) strongly signify that these biogenic molecules served as capping agents to prevent particle agglomeration. Crucially, the appearance of a new, sharp, high-intensity absorption band below 600 cm^{-1} (specifically localized in the 500–600 cm^{-1} region) explicitly confirms the successful formation of the metal-oxygen bond (Mn-O stretching vibration). This structural marker definitively validates the biogenic synthesis of stable, capped MnO nanoparticles using the *Butea monosperma* pod extract.

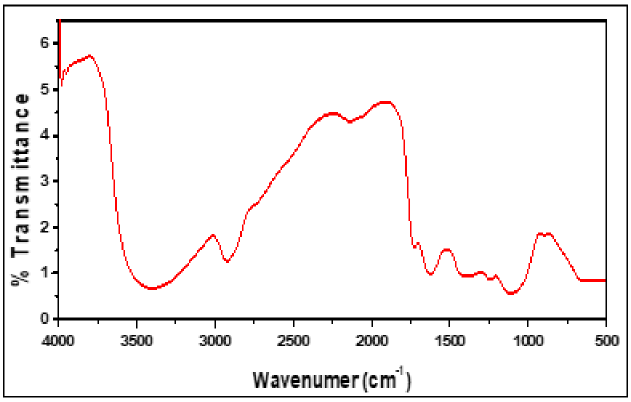

Figure 2: IR spectra of pod extract

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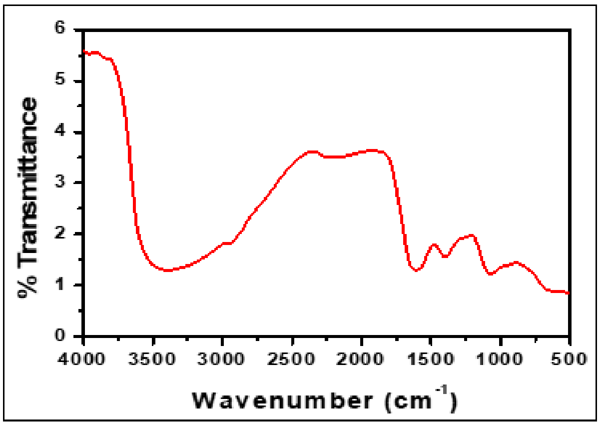

Figure 3: IR spectra of MnO nanoparticles

3.3 X-Ray Diffraction (XRD) Analysis

Crystalline phase identification was carried out by X-ray diffractometer. Figure 4 shows the XRD pattern of the green-synthesized manganese oxide nanoparticles exhibited a broad diffuse profile without well-resolved Bragg reflections, indicating that the product is predominantly amorphous or possesses very low crystallinity. The absence of distinct diffraction peaks suggests that the nanoparticles are either extremely small or contain a significant amount of amorphous material, likely due to the influence of phytochemicals from the pod extract of *Butea monosperma* [33].

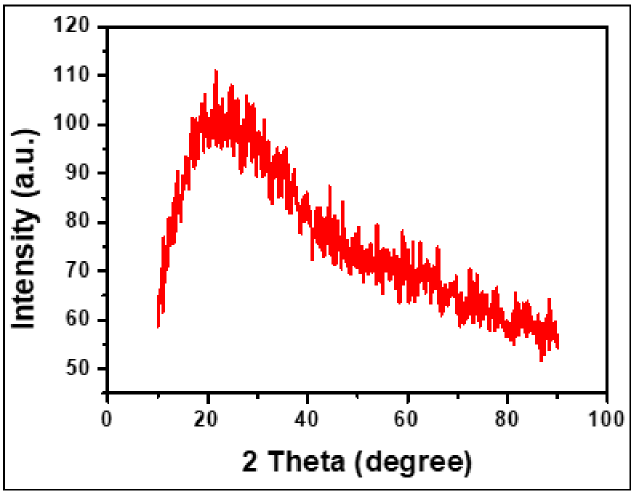

Figure 4: XRD pattern of MnO nanoparticles

3.4. Antibacterial Evaluation

The in vitro antibacterial efficacy of the green-synthesized MnO nanoparticles was assessed using the agar well diffusion method against representative Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. Streptomycin was utilized as the standard positive control antibiotic.

While the standard streptomycin control demonstrated prominent, well-defined zones of inhibition confirming the susceptibility of both test strains, the green-synthesized MnO nanoparticles did not display any significant zone of inhibition against either *E. coli* or *S. aureus* at the evaluated concentrations.

4. Conclusions

In this study, an eco-friendly and sustainable biogenic route was successfully developed for the synthesis of manganese oxide nanoparticles using the pod extract of *Butea monosperma*. X-ray diffraction (XRD) structural profiling revealed that the green-synthesized nanomaterial possesses an amorphous to poorly crystalline nature, characterized by broad, low-intensity features rather than sharp, definitive Bragg reflections. Consequently, a specific, highly ordered crystalline phase could not be definitively indexed or assigned based on the XRD data alone, indicating a highly disordered atomic framework or an ultra-small cluster regime governed by the dense organic capping matrix of the plant extract.

In vitro biological screening via the agar well diffusion assay against *Escherichia coli* and *Staphylococcus aureus* showed no significant zones of inhibition when compared to the standard streptomycin control. This biological inertness is likely due to the heavily wrapped organic shell from the phytoconstituents and the lack of a defined, reactive crystalline lattice capable of spontaneously leaching ions or generating reactive oxygen species (ROS) under standard dark testing conditions.

Despite the low crystallinity and lack of direct antibacterial activity, the structurally disordered and amorphous nature of these green-synthesized manganese oxide nanoparticles holds distinct advantages for environmental remediation. Amorphous nanomaterials frequently exhibit high surface areas and abundant coordinatively unsaturated surface-active sites. Thus, this synthesized material serves as a my be highly sustainable, benign, and promising candidate for heterogeneous catalysis, specifically for the degradation of toxic organic dyes in wastewater treatment.

Conflict of Interest

The author declares that there is no conflict of interests.

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