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Making MnO_2 NPs from the Jatropha Maheshwarii Leaf and Analyzing their Physicochemical and Pharmaceutical Properties

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ABSTRACT: In this study, we demonstrated the green synthesis of manganese oxide nanoparticles (MnO_2 NPs) using Jatropha Maheshwarii leaf extract as both a reducing and capping agent. The synthesized materials were characterized through ultraviolet-visible spectroscopy and scanning electron microscopy. The obtained MnO_2 NPs appeared as pinkish-green crystals with a yield of 96 %. An absorption band at 334 nm was indicative of manganese oxide nanoparticles. Morphological analysis via SEM revealed that the nanoparticles possess a spherical morphology, with sizes averaging around 32 nm and showing no aggregation. Additionally, we investigated the antibacterial activity of the synthesized materials against both Gram-positive and Gram-negative bacteria. The MnO_2 NPs exhibited noteworthy antibacterial activity against various bacterial strains, including Bacillus subtilis, Bacillus cereus, Staphylococcus albus, and Pseudomonas aeruginosa. Specifically, a concentration of 7 μg/ml of the green MnO_2 NPs demonstrated significant efficacy against these strains, likely related to the presence of heterocyclic compounds derived from the plant. In summary, the use of Jatropha Maheshwarii for the biosynthesis of manganese oxide nanoparticles not only offers a sustainable and environmentally friendly production method but also yields nanoparticles with considerable antibacterial potential. This research opens up promising possibilities for future applications in both medical and environmental fields, providing a novel pathway for the potential use of MnO_2 NPs in clinical and drug development contexts.

KEYWORDS: Manganese oxide nanoparticles, Jatropha Maheshwarii leaf, Green synthesis, Gram-positive bacteria, Gram-negative bacteria

I. INTRODUCTION

People are becoming more aware of the benefits of medicinal plants, using them to stay healthy and prevent illnesses in an environmentally friendly way. One exciting area of scientific research is nanotechnology, which involves creating extremely small particles that are more effective than larger ones [1-5]. Recently, there has been a rise in the use of these tiny particles in various fields, including water treatment, healthcare, and manufacturing [6,7]. Researchers are exploring ways to produce these tiny particles more cheaply and effectively. Different methods of making them can affect both the cost and the amount produced [8-11]. Over the last few years, scientists have increasingly turned to "green" methods for creating these materials [12-14]. These environmentally safe techniques are economical, and efficient, and do not harm living organisms. One of the key advantages of green synthesis is that it typically uses safe materials like water and natural plant substances, which are non-toxic and safe for the environment. Scientists are particularly interested in increasing the production of these nanoparticles while also keeping costs down and ensuring they remain stable in different conditions [15,16].

Nanotechnology is defined as the precise manipulation of materials at the atomic level, employing a combination of engineering, chemistry, and biological approaches. This field primarily focuses on nanomaterials and structures that range from 1 to 100 nanometers, establishing it as a significant and rapidly advancing area within nanoscience and

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nanotechnology [17]. Particularly noteworthy are metal nanoparticles, which possess a high specific surface area and a considerable fraction of surface atoms. These attributes lead to unique physicochemical characteristics that have been extensively studied, including catalytic, optical, electronic, and magnetic properties, as well as notable antimicrobial activities [18-22]. As an emerging field, nanotechnology is poised for transformative developments in the coming decades, particularly through its integration with green chemistry principles, which emphasizes sustainability and environmental responsibility [23,24]. This convergence holds the potential to foster innovative applications that can significantly benefit various sectors.

The Jatropha genus is part of the Euphorbiaceae family and includes 13 species in India. Its name comes from Greek words meaning "doctor" and "nutrition." Jatropha Maheshwarii I Subr. and Nayar is an endemic species found in the coastal areas and hills of Kanyakumari, Thoothukudi, and Tirunelveli in Tamil Nadu, extending to Thiruvananthapuram in Kerala [25]. Locally, it is called ‘Athalai,’ ‘Vel-thali,’ and ‘Kattamannaku.’ This evergreen shrub can grow up to 2 meters tall, with a thick stem and dark green, smooth oval leaves. It is drought-resistant and has traditional medicinal uses for rheumatism, eczema, and ringworms, and it serves as an insecticide. The latex can stop bleeding from eczema and treat mouth ulcers, while the leaves reduce inflammation. Local communities use fresh stems as toothbrushes. Extracts from the stems can combat Staphylococcus aureus and other bacteria and fungi. This study aims to test if Jatropha Maheshwarii (Figure 1) can inhibit the growth of selected human bacteria using agar well diffusion.

Figure 1. Image showing the picture of the Jatropha Maheshwarii plant

Researchers have been using nanotechnology and related fields to help in the early detection and prevention of diseases. A significant focus has been on developing small devices that utilize these particles, especially for tasks like gene sequencing. Metal nanoparticles, in particular, have unique properties that make them effective in various applications. Manganese oxide nanoparticles, which has shown promise for diagnosing and treating a range of health issues, including infections and even harder-to-treat conditions like cancer. Overall, the combination of plants and nanotechnology holds great potential for improving health while being gentle on the planet.

II. MATERIALS

The starting material, manganese acetate tetrahydrate [$Mn(OAc)_2 \cdot 4H_2O$], and sodium hydroxide (NaOH) for nanoparticle synthesis were procured from Sigma Aldrich. Deionized water was utilized throughout the reaction process.

III. METHODS

3.1 Analytical Instrumentation Analysis

The electronic absorption spectra were obtained using a Deep Vision, single beem 900 UV-visible spectrometer. X-ray photoelectron spectroscopy was recorded using a Bruker D 8 Advance Eco-Powder X-Ray Diffraction (XRD).

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Calcination was carried out on a Muffle furnace instrument at a heating rate of 10°C/min between 400°C and 500°C. SEM images were recorded on a Field Emission Scanning Electron Microscope (FESEM), SIGMA 300 Carl Zeiss microscope. The antibacterial activity based on the Zone inhibition method was performed in an Inverted microscope [26].

3.2 Plant collection and Preparation of leaf extract

Jatropha Maheshwarii plants were collected from near Terku Kallikulam 627113 in the Tirunelveli district of Tamil Nadu, India. The leaves were then dried in the shade for three days before being ground into a powder. A quantity of 5 g of the dried powdered leaves was extracted at 80 °C for 1 hour using 50 ml of double distilled water. The mixture was subsequently centrifuged at 7000 rpm. The resulting extract was filtered through Whatman No.1 filter paper, and the filtrate was stored in a refrigerator at 4 °C for further use (Figure 2).

Figure 2. Image showing the dry leaf, dry powder, and its water extract of the Jatropha Maheshwarii plant

3.3 Synthesis of manganese nanoparticles

Approximately 50 mL of a 1 g manganese acetate tetrahydrate solution in water was prepared in a 250 mL beaker. To this, 10 mL of a previously prepared Jatropha leaf extract was introduced. The mixture was subjected to heating at 80°C while being continuously stirred using a magnetic stirrer for two hours. Following this, the pH of the solution was adjusted to 14 by incrementally adding a dilute NaOH solution (0.01 M). Subsequently, the solution was concentrated using a rotary evaporator, and the resultant concentrated solution was allowed to stand undisturbed for 24 hours to facilitate the crystallization of manganese oxide nanoparticles. The resulting brownish crystals were then isolated and subjected to calcination at 400°C for three hours in a muffle furnace. This process yielded a dark brown crystalline powder with a yield of 96%.

IV. RESULT AND DISCUSSION

4.1 Macroscopic and microscopic studies

Macroscopic studies were conducted through basic determination techniques, examining features such as shape, size, color, odor, margin, and apex. The stem and leaf specimens were preserved in FAA, and microtome slides were prepared and stained. Photomicrographs at various magnifications were captured using a Nikon Labphot 2 microscope (Figure 3). Spectrophotometric analysis of the water extract revealed the presence of phenolics, flavonoids, and saponins, with concentrations of 3.9, 0.4, and 19.0 mg/g DM, respectively.

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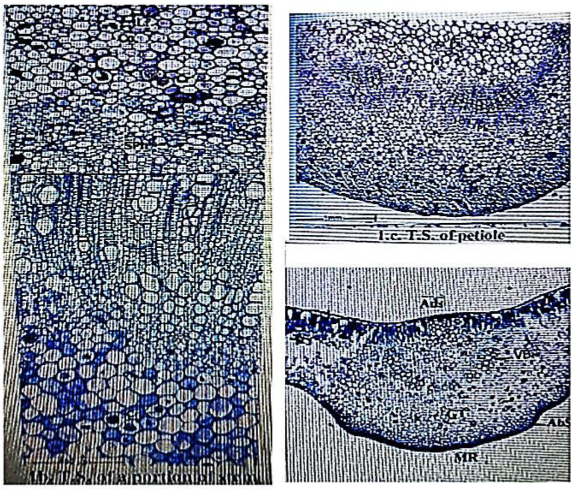

Figure 3. Microscopic image of the Jatropha Maheshwarii plant cell structure

4.2 Photophysical analyse

The 0.05 mM concentration of MnO_2 nanoparticles (NPs) was thoroughly analyzed under UV-visible light in the range of 200 to 900 nm, clearly demonstrating the presence of nanoparticles through the reduction of manganese ions in the solution (refer to Figure 4).

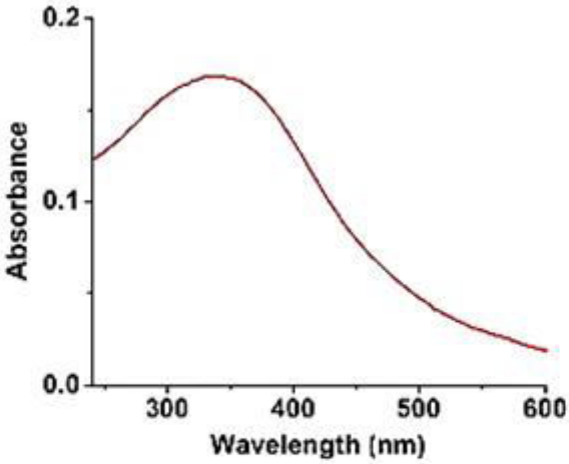

Figure 4. The absorption spectrum of MnO_2 NPs in water

The MnO_2 NPs were placed in a quartz cuvette for precise wavelength scanning, utilizing distilled water as a reference. Notably, an absorption peak was observed at 334 nm, a definitive characteristic of MnO_2 nanoparticles.

4.3 Surface morphological study

The surface morphology of the synthesized MnO_2 nanoparticles was characterized using scanning electron microscopy (SEM). The analysis reveals that the MnO_2 nanoparticles predominantly exhibit spherical and oval geometries with sizes ranging from 30 to 50 nm. Notably, the high colloidal stability of these nanoparticles, along with the observed coating layer in the SEM images, suggests that the phytochemicals extracted from Jatropha Maheshwarii leaf juice function as effective capping agents. This stabilization process is further corroborated by the consistent water dispersibility of the MnO_2 nanoparticles, which have an average hydrodynamic diameter (Dh) of 32 nm (Figure 5).

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Figure 5. **SEM images of MnO_2 nanoparticle**

4.4 Antibacterial assay

The agar well diffusion method was systematically utilized with Petri plates containing 20 ml of Mueller Hinton medium [27-30], which were inoculated with a 3-hour culture of various bacterial strains: Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), Pseudomonas aeruginosa (PA), Escherichia coli, and Klebsiella pneumoniae (KP). Using a well cutter, approximately 10 mm wells were created, into which 50 µl samples were carefully added. The antimicrobial properties of the leaf extract effectively diffuse into the medium, resulting in interaction with the freshly seeded test organisms. Following this, the plates were incubated at 37 °C for 24 hours. Antibacterial activity was rigorously assessed by measuring the diameter of the inhibition zone formed around each well, recorded in millimeters. This meticulous study was performed in duplicate with the aid of an inverted microscope. Notably, the results indicated that these nanoparticles possess remarkable antibacterial activity, particularly at a concentration of 7 µg/mL. As such, this nanoparticle demonstrates a strong potential as an effective agent against nearly all tested bacteria (refer to Figure 6 and Table 1).

Table 1: Zone inhibition of MnO_2 NPs with Bacteria

S. No.	Bacteria	Manganese Oxide nanoparticles @ 7 µg/mL	Control (STREPTOMYCIN)
1	Bacillus subtilis	18	22
2	Bacillus cereus	21	20
3	Pseudomonas aeruginosa	23	12
4	Staphylococcus albus	16	12
5	Klebsiella pneumoniae	18	13
6	E.coli	22	11

Figure 6. Antibacterial activity of Manganese Oxide nanoparticles

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V. DISCUSSION AND FUTURE SCOPE

The diversity of methodologies for the green synthesis of nanoparticles is extensive, yet the approaches for synthesizing manganese nanoparticles remain limited [31-33]. Given the range of manganese oxides, including MnO, Mn_5O_8, Mn_2O_3, MnO_2, and Mn_3O_4, coupled with the variety of biological entities—such as plants, fungi, bacteria, yeasts, algae, and actinomycetes—that can facilitate this synthesis, there is significant potential for further exploration in this domain. Manganese oxide nanoparticles exhibit a myriad of properties and applications, spanning antibacterial and antifungal activities to roles in gene therapy and neuroprotection [34-36]. Recent studies have indicated that manganese nanoparticles can mitigate neural inflammation, influence apoptotic pathways, and enhance antioxidant defenses in neural tissues. Furthermore, these nanoparticles may promote immune cell infiltration, suggesting their therapeutic potential against neurotoxins. Employing physical techniques like high gravity has emerged as a viable strategy to augment the rate of electron transfer during synthesis, thereby improving collision efficacy among reactants.

In this investigation, we propose a novel approach to the green synthesis of manganese oxide nanoparticles utilizing Jatropha Maheshwarii, alongside an examination of their potential biomedical applications, particularly antibacterial efficacy. These natural components are cost-effective and largely devoid of side effects, making them suitable for medicinal use. The components involved in this synthesis include various green agents such as sugars, ascorbic acid, and starches. By manipulating macroscopic parameters during the experimental procedure, we can effectively control the particle size of the synthesized nanoparticles. Future research could focus on plants exhibiting high antioxidant properties for the synthesis of metallic nanocomposites. The green synthesized MnO2 nanoparticles have already found applications across multiple sectors, as previously detailed, but the characterization of these nanoparticles could yield insights into their utility in medical, biological, and environmental contexts [37-39]. Moreover, the integration of magnetic nanoparticles with existing and novel synthesis techniques may open new avenues for application. Super-paramagnetic materials have emerged as particularly relevant for analytical chemistry and molecular diagnostics, owing to their ability to interact with diverse biomolecules and their distinctive magnetic characteristics [40,41]. In upcoming studies, the exploration of perovskite manganite nanoparticles could be beneficial for DNA purification and extraction processes.

VI. CONCLUSION

This study presents a comprehensive review of green synthesis methods, including the use of leaf extracts and low-temperature synthesis techniques. The structural and dimensional properties of the green synthesized manganese dioxide nanoparticles (MnO_2 NPs) were evaluated, revealing an average size of 32 nm as determined by scanning electron microscopy (SEM) analysis. Additionally, various applications of these green synthesized MnO_2 NPs have been examined. Notably, the distilled water extract exhibited significant antibacterial activity against all bacterial strains assessed. The antibacterial effects of the leaf extracts from J. Maheshwarii were favorably compared to those of the standard antibiotic streptomycin, indicating that the MnO_2 NPs demonstrated the highest levels of activity. Consequently, it is concluded that the leaves of J. Maheshwarii could serve as an effective source of medicinal compounds for addressing a range of bacterial infections in humans. Therefore, this study affirms the recognition of these plant parts as promising antimicrobial agents, while also highlighting the necessity for further sophisticated research to ensure their effective application in the field of healthcare.

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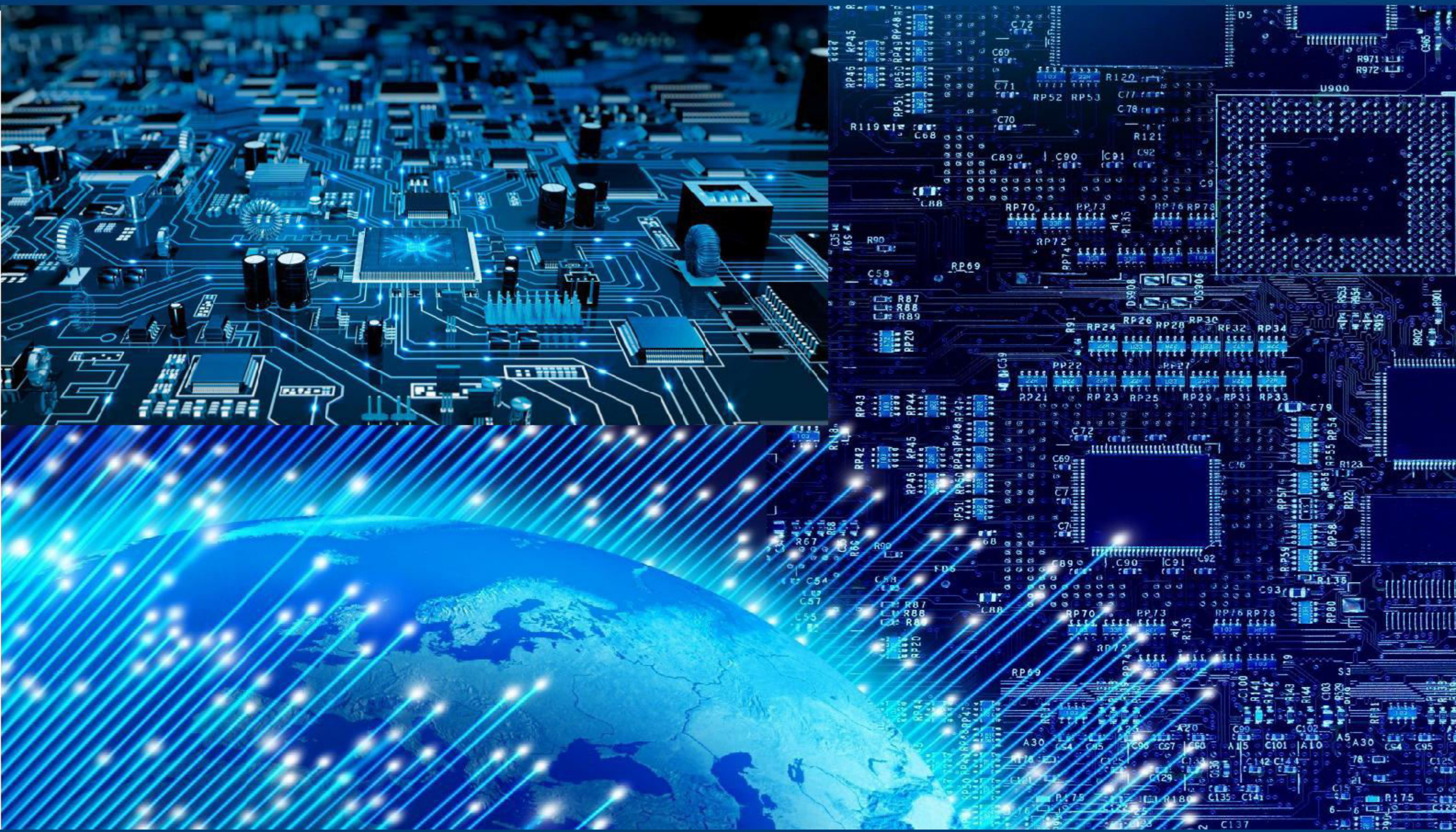

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