Research Article

Green Synthesis of Manganese Dioxide (MnO_2) Nanoparticles with *Piper nigrum*, its Characterization, and Phytochemical Analysis

Raksha B [2], Dr. M Durga* [1], Vaishnavi M [2], Deepikaa R [2]

1. Assistant Professor, Department of Biochemistry and Bioinformatics, Dr. MGR Janaki College of Arts and Science for Women, University of Madras, Chennai-600028, Tamil Nadu, India.
2. Department of Biochemistry and Bioinformatics, Dr. MGR Janaki College of Arts and Science for Women, University of Madras, Chennai-600028, Tamil Nadu, India.

***Corresponding author's E-mail:** durgam2k7@gmail.com

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ABSTRACT

Manganese oxide (MnO_2) nanoparticles were synthesized utilizing Piper nigrum as a reducing agent through the green synthesis method. This technique was chosen due to its eco-friendly nature, which makes it preferable to other chemical methods. The plant was selected because of its easy availability, rich phytochemical composition, and high medicinal value. In recent times, MnO_2 nanoparticles have emerged as a fascinating subject for material science researchers due to their diverse applications in energy storage devices such as lithium-ion batteries and capacitors, as well as catalysts, adsorbents, sensors, imaging, and therapeutic activity. The distinct potential of these nanoparticles for various purposes, depending on the mode of action and application, necessitates a robust protocol for cheap, stable, biocompatible, and eco-friendly MnO_2 nanoparticles. This article entails the green synthesis method of MnO_2 nanoparticles, their characterization using Scanning Electron Microscopy (SEM), Ultraviolet (UV) spectroscopy, Raman spectroscope, and particle size analyzer, as well as their phytochemical analysis.

Keywords: Manganese Dioxide, *Piper nigrum*, Green Synthesis, Nanotechnology, Metal nanoparticles

INTRODUCTION

The manipulation of materials ranging from 1 to 100 nm is referred to as nanotechnology. Nanoparticles are defined as particles with sizes falling within the range mentioned above.[1] In the fields of optics, electronics, biomedical science, mechanics, drug delivery, chemical industry, optoelectronic devices, nonlinear optical devices, catalysis, space industries, energy science, and photoelectrochemical applications, nanotechnology is playing a crucial role through nanoscale structures (nanoparticles). Nanoparticles are highly sought after because of their high surface-to-volume ratio and small size (in nm), resulting in physical and chemical properties that differ from those of similar chemical compositions.[2]

MnO_2 NPs have been used in a variety of fields because they are simple to make and exhibit good stability. In both aqueous and organic electrolytes, the tunnel-like a-MnO_2 has been employed as an electrocatalyst for oxygen evolution and reduction. On the other hand, stacked birnessite can be used as a capacitor. Similarly, additional forms have also been employed as adsorbents and catalysts. They are also used in the field of biomedicine, and by doping the nanomaterials with other metals like Ni, Ag, etc., the activity of MnO_2 has been increased.[3]

Piper nigrum L., or black pepper, is a member of the Piperaceae family. Black pepper is a great source of vitamins, minerals, and nutrients. The chemical composition of 100 grams of black pepper seeds contains 10.2 g of fat, 10 g of protein, and 66.5 g of carbohydrates. They also have a comparatively high content of minerals such as potassium (1200 mg), phosphorus (160 mg), calcium (400 mg), magnesium (235.8–249.8 mg), and the reduced concentrations of sodium, iron, and zinc. These minerals are necessary components for human daily activities. Additionally, there is a notable concentration of vitamins, including C, B1, B2, and B3, in black pepper.[4]

The goal of this field of nanoscience should be to create environmentally safe and widely accepted nanoparticles. Both "top-down" and "bottom-up" are distinct approaches for combining NPs. A top-to-bottom strategy uses a variety of size reduction techniques, such as grinding, milling, sputtering, thermal/laser ablation, etc., to break down acceptable bulk material into smaller fine particles. While "bottom-up" approaches include chemical reduction, electrochemical procedures, and sono-decomposition, "bottom-to-top" methods generate NPs utilizing chemical and biological mechanisms by atoms self-assembling into new nuclei that grow into nanosized particles.[5]

Scientists are concentrating on avoiding hazardous, non-eco-friendly substances and shifting toward greener technology due to the latest environmental concerns. The recent rise in environmental awareness has compelled scientists to become more interested in biological methods for producing nanoparticles devoid of hazardous compounds as a byproduct.[6]

India is the most active region conducting research in the subject of green synthesis, with over 1400 published papers published between 1996 and 2018. We can conclude that materials science is a promising field, ranking second in terms of publications, behind the fields

of biochemistry, genetics, and molecular biology. Researchers have experimented with several techniques, including chemical and physical ones, to synthesize nanoparticles. These techniques, however, were expensive, tedious, and required complex electrical or electronic equipment. These techniques weren't environmentally friendly and used hazardous chemicals that pose numerous health risks. Thus, green synthesis, which included significant medicinal plants, was showing promise and being beneficial in this area.[7]

Green synthesis is a sustainable, cost-effective, eco-friendly, and clean way to create nanomaterials. Certain conventional synthesis procedures require the use of toxic or hazardous chemicals during the preparation process, which might result in the development of toxic or deadly substances that could be damaging to people and the environment. On the contrary, green synthesis produces ecologically safe nanoparticles by substituting dangerous chemical products and creating new processes to minimize or even completely remove materials that are bad for the environment and human health.[6]

The need for environmentally friendly technologies prompted nanoscience to investigate the biology and offer a fresh platform for the aseptic synthesis of nanomaterials using bacterial, fungal, and plant extracts. The process of creating nanomaterials using plant extracts, microorganisms, fungi, proteins, and biological components including sugar, enzymes, and entire cells is known as "green synthesis".[6]

1. MATERIALS AND METHODS:

1.1. Preparation of Plant Extract

Fruits of *Piper nigrum* were dried and ground into a fine powder with a mortar and pestle. 10 g of dried powder was added to 200 ml of distilled water and boiled for 1 hr with continuous stirring. The extract was cooled down and then filtered with Whatman No.1 filter paper. The filtrate was stored at 4˚C for further use.

1.2. Green Synthesis of MnO_2 NPs

50 ml of 0.1 M manganese acetate tetrahydrate was taken in a beaker. 100 ml plant extract was added to the prepared manganese acetate tetrahydrate solution. It was heated at 80˚C for 2 hours with constant stirring. The solution's colour was changed from light brown to dark brown colour indicating the reduction of manganese acetate tetrahydrate to manganese oxide (MnO_2) NPs.

The mixture was filtered using Whatman No.1 filter paper. The filtrate was dried in an oven until all the water evaporated. The powdered nanoparticle is stored for further studies.

The whole process of green synthesis of MnO_2 NPs by using the dried fruit extract of *Piper nigrum* was found to be successful. The process is summarized in a flow chart (Figure 1).

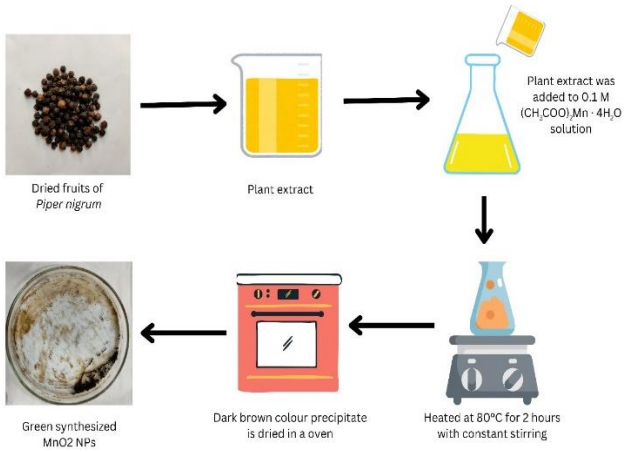


Figure 1: Summary of Green Synthesis of MnO_2 NPs with *Piper nigrum*

3.3. Phytochemical Analysis of *Piper nigrum*

Table 1: Procedure for phytochemical analysis of *Piper nigrum:*

S.NO	Name of the test	Reagents required	Procedure	Observation	Inference	Reference
			TEST FOR ALKALOIDS			
1	**Hager's test**	Hager's reagent (saturated picric acid solution)	Few drops of Hager's reagent (saturated picric acid solution) added to 2 ml of the respective plant extract.	Bright yellow colour is observed	Presence of Alkaloids	[8]
			TEST FOR CARDIAC GLYCOSIDES			
2	**Baljet's Test**	Baljet's reagent (picric acid, ethanol and sodium hydroxide)	A few drops of the Baljet's reagent (picric acid, ethanol and sodium hydroxide) were added to 2-3 mg of sample	Reddish orange colour is observed	Presence of Cardiac glycosides	[9]
			TEST FOR TANNINS			
3	**Alkaline reagent test**	1N Sodium hydroxide	A volume of 2 ml of 1N of NaOH was added to 2 ml of extract samples.	Appearance of yellow to red color is observed	Presence of tannins	[8]

TEST FOR FLAVONOIDS						
4	**Flavonoid test**	20% Sodium hydroxide	The powdered sample (1 g) was boiled with 10 ml of distilled water for 5 min and filtered while it was hot. A few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate.	No colour change is observed	Absence of flavonoids	[9]
TEST FOR SAPONINS						
5	**Saponin test**	Sodium bicarbonate	2mg of sodium bicarbonate was added to aqueous extract and shaken vigorously	Froth formation is observed	Presence of saponins	[8]
TEST FOR STEROLS						
6	**Salwoski's test**	Concentrated sulphuric acid	2 to 3 drops of concentrated sulphuric acid were added to form a lower layer	Reddish-brown color at the interphase	Presence of steroidal ring	[10]
TEST FOR ANTHRAQUINONES						
7	**Anthraquinone test**	2% Hydrochloric acid	A few drops of 2% Hydrochloric acid were added to the tested plant	No colour change is observed	Absence of Anthraquinones	[8]
TEST FOR ANTHOCYANIN						
8	**Anthocyanin test**	Concentrated sulphuric acid	To the plant extract, a few ml of concentrated sulphuric acid was added.	No colour change is observed	Absence of anthocyanin	[8]
TEST FOR COUMARINS						
9	**Coumarin test**	10% Sodium hydroxide	1 ml of 10% sodium hydroxide was added to 1 ml of plant extract	Yellow color is observed	Presence of coumarins	[8]
TEST FOR QUINONE						
10	**Quinone detection test**	Concentrated sulphuric acid	1 ml of concentrated sulphuric acid was added to 1 ml plant extract sample	No colour change is observed	Absence of quinone	[8]
TEST FOR ACIDS						
11	**Acid detection test**	Sodium bicarbonate	1ml plant extract sample was treated with a solution of sodium bicarbonate	No Effervescence is observed	Absence of acid	[8]
TEST FOR CARBOHYDRATES						
12	**Molisch test**	Molisch reagent, Concentrated sulphuric acid	Few ml of plant extract was reacted with molisch reagent and Concentrated sulphuric acid was added along the sides of the test tubes	Violet color ring was observed at the junction	Presence of carbohydrates	[11]
13	**Benedict test**	Benedict's reagent	To 2 ml of plant extract, few ml of Benedict's reagent was added and heated	No characteristic change is observed	Absence of reducing sugar	[11]
14	**Fehling's test**	Fehling's A and Fehling's B solution	Fehling's A and B solutions were added to 2 ml of plant extract	Reddish brown precipitate was observed	Presence of aldoses	[11]
TEST FOR PROTEIN						
15	**Ninhydrin test**	Ninhydrin reagent	To a few ml of plant extract, ninhydrin reagent was added and heated.	No colour change is observed	Absence of protein	[11]
16	**Xanthopr-oetic test**	Concentrated nitric acid	Concentrated nitric acid was added to 2 ml of plant extract and heated	No color change is observed	Absence of protein	[11]

RESULTS AND DISCUSSION

Characterization and Phytochemical Analysis

The produced MnO_2 nanoparticles were put through several spectrum investigations, including SEM examinations, UV, Raman spectroscope, and particle size analyser (MAL-1049897).

Particle Size Analyser

Using a particle size analyser (MAL-1049897), the diameter of MnO_2 nanoparticles in the 0.1–10,000 nm range was detected. Two peaks were observed at 899.5 nm with an intensity of 90.1% and at 38.54 nm with an intensity of 9.9% (Figure 2). The peak with the highest intensity of 90.1% is observed at 899.5 nm because of aggregation and agglomeration on the nanoparticles.

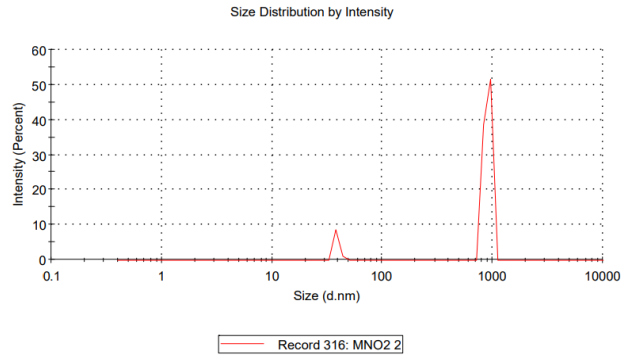


Figure 2: Particle size analysis of MnO_2 nanoparticles

UV Spectroscope Analysis

The sample was subjected to UV Spectroscope analysis. The synthesized MnO_2 nanoparticles showed maximal absorbance at 339.41nm, 350.45 nm and, 364.70 nm in the UV region (Figure 3).

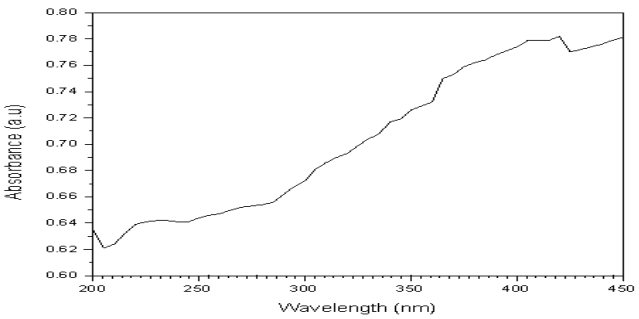


Figure 3: UV Spectroscope Analysis of MnO_2 nanoparticles

UV Spectroscopy analysis of MnO_2 NPs should theoretically have an absorption peak between 350 and 410 nm. The production of MnO_2 NPs is clearly indicated by the emersion of the absorption boundary at 339.41nm, 350.45 nm, and 364.70 nm which lies in the range of 350 nm to 410 nm.[12] This is explained by the electrons being excited by light from the valence band into the conduction band. The optical band gap (E_g) was determined as 3.45 eV.[13]

Raman Spectroscopy

The obtained MnO_2 NPs were subjected to Raman spectroscopy analysis. The peaks were observed at 331 cm^{-1} and 644 cm^{-1} (Figure 4) which is according to the literature.[14] These results confirm the presence of MnO_2 NPs.

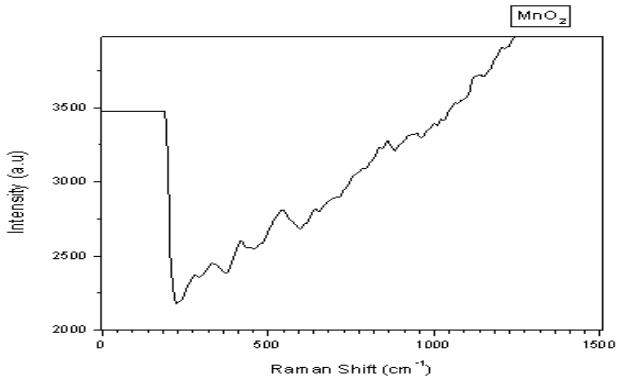


Figure 4: Raman Spectroscope Analysis of MnO_2 nanoparticles

Morphological Analysis using SEM

After sonication, the MnO_2 NPs were subjected to SEM analysis. The nanoparticles were found to be in the sizes 68.5 nm, 71.1 nm, 80.8 nm, 86.9 nm, and 120.6 nm (Figure 5). In previous studies, the mean particle size obtained from the SEM analysis was 50–100 nm.[15] Our results coincide with the above-mentioned range. Previously agglomerated nanoparticles as per PSA have been reduced in size after sonication. The accumulation might be due to the high surface area and surface energy[16] and the high surface-to-volume ratio of the formed NPs. That might have increased the attractive forces among the NPs which is evident from our PSA results. The results we have obtained from the SEM analysis are under the nanoparticle size range. So, we can confirm that these are MnO_2 NPs.

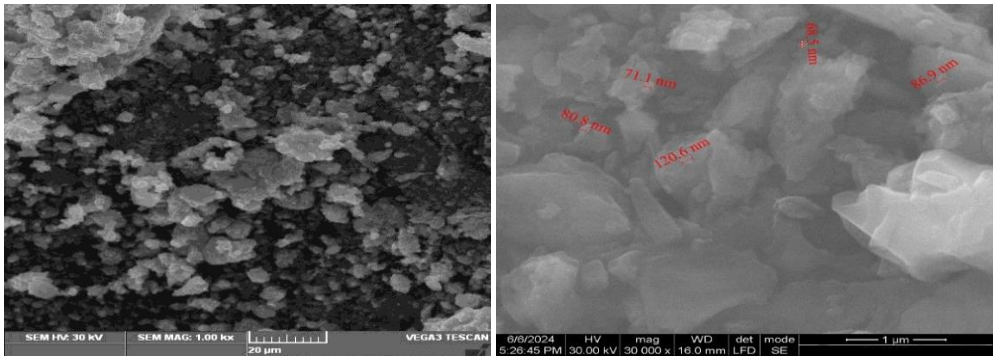
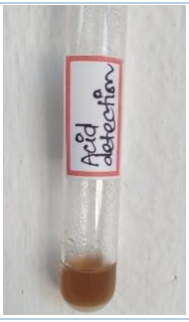


Figure 5: SEM Analysis of MnO_2 nanoparticles

1.3. Phytochemical Analysis

Table 2: Results of phytochemical analysis of *Piper nigrum*

S.NO	Name of the Test	Observation		Inference
TEST FOR ALKALOIDS				
1	**Hager's test**	A bright yellow colour is observed		Presence of Alkaloids
TEST FOR CARDIAC GLYCOSIDES				
2	**Baljet's Test**	Reddish-orange colour is observed		Presence of Cardiac glycosides
TEST FOR TANNINS				
3	**Alkaline reagent test**	Appearance of yellow to red colour is observed		Presence of tannins
TEST FOR FLAVONOIDS				
4	**Flavonoid test**	No colour change is observed		Absence of flavonoids
TEST FOR SAPONINS				
5	**Saponin test**	Froth formation is observed		Presence of saponins

TEST FOR STEROLS				
6	**Salwoski's test**	A reddish-brown colour formed at the interphase		Presence of steroidal ring
TEST FOR ANTHRAQUINONES				
7	**Anthraquinone test**	No colour change is observed		Absence of Anthraquinones
TEST FOR ANTHOCYANIN				
8	**Anthocyanin test**	No colour change is observed		Absence of anthocyanin
TEST FOR COUMARINS				
9	**Coumarin test**	Yellow color is observed		Presence of coumarins
TEST FOR QUINONE				
10	**Quinone detection test**	No colour change is observed		Absence of quinone
TEST FOR ACIDS				
11	**Acid detection test**	No Effervescence is observed		Absence of acid

TEST FOR CARBOHYDRATES				
12	**Molisch test**	Violet colour ring was observed at the junction		Presence of carbohydrates
13	**Benedict test**	No characteristic change is observed		Absence of reducing sugar
14	**Fehling's test**	A reddish-brown precipitate was observed		Presence of aldoses
TEST FOR PROTEIN				
15	**Ninhydrin test**	No colour change is observed		Absence of protein
16	**Xanthoproetic test**	No colour change is observed		Absence of protein

Table 3: Summary of phytochemical investigation of *Piper nigrum* in aqueous extract

Constituents	Observation
Alkaloids	+
Cardiac glycosides	+
Tannins	+
Flavonoids	-
Saponins	+
Sterols	+
Anthraquinones	-
Anthocyanin	-
Coumarins	+
Quinone	-
Acids	-
Carbohydrates	+
Protein	-

CONCLUSION

The use of an extract derived from Piper nigrum, commonly known as black pepper, has been proven to be a highly effective method for reducing the synthesis of MnO_2 nanoparticles. These nanoparticles were examined using various advanced techniques such as scanning electron microscopy (SEM), ultraviolet-visible spectroscopy (UV), Raman spectroscopy, and particle size analyzer. The SEM analysis confirmed that the synthesized MnO_2 nanoparticles were within the size range of 50-100 nm. The green synthesis process using plant extract is not only simple and cost-effective but also doesn't require the use of any catalysts or expensive materials, making it a sustainable option. The degrading ability of the Piper nigrum was proved by the synthesis of MnO_2 NPs, proving that the synthesis utilizing plant extract is viable by a simple reaction

at standard pressure and temperature, without the need for catalysts, cast, or costly material. Additionally, the phytochemical analysis of Piper nigrum extract provides a comprehensive understanding of all the components present in it, allowing for further exploration of potential applications. However, further research is required to fully unveil the potential of MnO_2 nanoparticles.

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