Green Synthesis, characterization and biological evaluation of MgO Nanoparticle from Foxnut extracts

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Abstract

Nanoparticles, with the potential to revolutionize various fields, were synthesized using plant extracts in biogenic magnesium oxide nanoparticles (MgONPs). Magnesium oxide (MgO), a white, hygroscopic solid with a lattice structure of Mg2+ and O2- ions, is nontoxic and environmentally friendly, making it suitable for medical and biotechnological applications. Through green synthesis with foxnut extract and magnesium sulfate heptahydrate, MgONPs of 14 nm size and high crystallinity were produced, yielding 0.45g. X-ray diffraction (XRD) confirmed their trigonal shape. MgONPs showed significant anticancer activity against HT29, HCT116, and Vero cells, with an IC50 value of 164.1 µg/mL for HT29 cells, consistently reducing cell viability with increasing concentrations.

Keywords: MgO, nanoparticles, green synthesis, foxnut, anticancer

1. INTRODUCTION

Nanotechnology is a technology developed on shape and size matter, with its particle size ranging from 1 to 100 nm [1]. This means that we can synthesize substances and devices from an individual atom using this process. Particles have unique properties such as physical, chemical and biological characteristics based on their size and shape [2]. The unique properties of nanoparticles, such as their small particle size [3], high surface area-to-volume ratio, controllable morphology, and reactivity relative to bulk materials of the same compositions[4], it has been widely applied in different areas including optics, electronics [5], drug delivery [6] and catalysis [7]. To synthesize green chemistry nanoparticles, plants are considered as a potential candidate source because they are novel, safe to use and more suitable for phytochemical fabrication [8]which work as reducing and stabilizing agents in nanoparticle synthesis processes [9].

The principles of sustainable and green chemistry involve reducing inherent hazards and risks associated with chemical processes [10]. Sustainable chemicals are designed to prevent pollution of the environment and depletion of resources. Green chemistry focuses on the development of new, clean, non-toxic technological alternatives for synthesis processes [11]. Conventional chemical methods used for synthesis of nanoparticles are often associated with production expenses and environmental toxicity, thus necessitating the need to develop an eco-friendly approach employing plants and microorganisms in nanoparticle synthesis [12]. Plant extracts, fungi, algae, and bacteria have been successfully used in this context to synthesize various nanoparticles in a safe manner [13].

Foxnut *(Euryale ferox)* has a short, thick root and large, round leaves that are dark green on top and purple underneath, with a spiky texture. The plant's stems and flowers are also spiky, with small flowers featuring green outer petals and purple inner petals. Its cone-shaped fruit, up to 11 cm long, retains the flower's covering [14]. Foxnuts are nutrient-dense, and rich in essential minerals such as calcium, iron, and potassium. They are an excellent source of protein, especially for vegetarians. The roasting of foxnuts increases their protein and mineral content as well as the total phenolics providing several health benefits such as antioxidant property, low glycemic index which is suitable for management of obesity and diabetes. Foxnuts are a low-calorie, high-protein snack options with hypocholesterolemic property and anticancer potential which makes a versatile health-food choice[15].

2. METHODOLOGY

2.1 Material and Methods

In current literature, MgO nanoparticles were synthesized using a green synthesis method. This process utilized Magnesium sulfate heptahydrate as the initial precursor and Foxnut *(Euryale ferox)* seed extract, which may function as both an oxidizing and reducing agent.

2.2 Preparation of Foxnut seed Extract

Foxnut Seeds were purchased from a local market, washed several times with water to remove dust and other impurities, and dried in shade. The dried seeds were then ground into powder form using a grinding mill. Extract was prepared by mixing the Foxnut powder with different solvents at various ratios (table 1), filtered through Whatman filter paper to eliminate any residues and used as the substrate for nanoparticle synthesis.

Table 1: *Preparation of foxnut extract of different solvent*

Entry	Solvent (10 mL)	Foxnut powder (g)	Stirring Time (hours)	Temp. (C°)	Yield %
1	Ethanol	0.1	1	60	10
2	Ethanol	0.5	3	60	20
3	Methanol	0.15	2	50	10
4	Methanol	0.4	2	50	25
5	Dimethyl sulfoxide	0.1	3	60-70	5
6	Dimethyl sulfoxide	0.5	3	60-70	20
7	ethyl acetate	0.1	2	70	8
8	ethyl acetate	0.5	3	70	15
9	10% Methanol	0.1	2	50-55	5
10	30% Methanol	0.3	2	50-55	15
11	50% Methanol	0.5	3	50	20

2.3 General Procedure for Foxnut Extract Preparation

To prepare foxnut extract, take 10mL of solvent in conical flask and add small concentration of foxnut powder in it. The solution was mixed thoroughly with magnetic stirrer on hot plate at specific temperature for 1 hour, after an hour, take the container from the heat source and let it cool to room temperature. After cooling, strain the liquid to separate the foxnut extract from the solid particles.

2.4 Preparation of Magnesium Sulfate and Sodium Hydroxide

1M solution of $MgSo_4.7H_2O$ was prepared in 100ml by weighing 24.647g $MgSO_4.7H_2O$ using balance and transfer into flask, Add small amount of water about 50ml and stirrer well then further add water to reach the flask to 100ml mark.

4M NaOH solution was prepared by dissolving 8g of NaOH in 50ml and the mixture was stirrer well to ensure the solution is homogeneous. The main purpose of NaOH is to adjust the reaction mixture pH and to enhance the precipitation of MgO nanoparticle.

2.5 Synthesis of MgO Nanoparticles

Different concentration of foxnut extract (concentrated), magnesium sulfate heptahydrate and sodium hydroxide was taken in flask (table 2). After that the solution was stirrer vigorously using magnetic stirrer on hot plate for 2 hours at 60-70°C. The reaction mixture was centrifuge at high speed around 10,000 rpm for 10-15 minute to separate the precipitated nanoparticle. The reaction mixture was filtered using Whatmann filter paper. After filtration the precipitate was washed with distilled water and was dried at room temperature for 1 day to obtained nanoparticle.

Table 2: *Different concentration use for preparation of MgO Nanoparticle*

Entry	Concentration			Stirring Time (h)	Temp. ºC	Yield (g)
	Foxnut extract of different solvent (conc)	$MgSO_4.7H_2O$ 1M in 100mL	NaOH 4M in 50mL			
1	2mL Foxnut extract of Dimethyl Sulfoxide	11mL	6mL	2	60-70	0.499
2	0.8mL Foxnut extract of Ethanol	5mL	3mL	1	60	0.195
3	3mL Foxnut extract of 50% Methanol	16.5mL	9mL	3	50-55	0.931
4	1mL Foxnut extract of 10% Methanol	5.5mL	3mL	1	50-55	0.316
5	2mL Foxnut extract of ethyl acetate	11mL	6mL	2	70	0.305

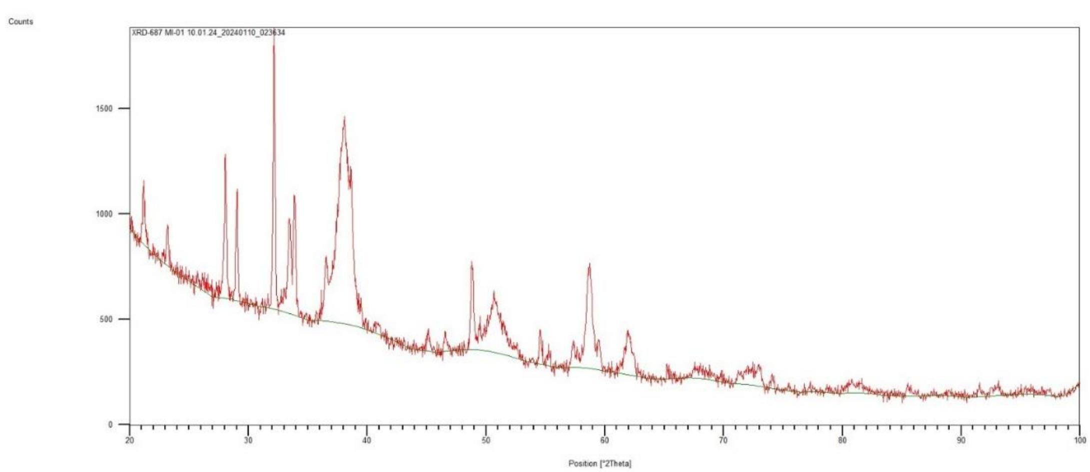
3. Result and Discussion

Magnesium oxide (MgO) was synthesized via a green synthesis method using magnesium sulfate heptahydrate ($MgSO4\cdot7H2O$) and NaOH, with Foxnut (Euryale ferox) seed extract as a reducing agent. The synthesis conditions were optimized by examining the influence of various parameters, including stirring temperature, concentration of foxnut extract, and calcination temperature, on the formation of magnesium oxide nanoparticles (MgONPs).

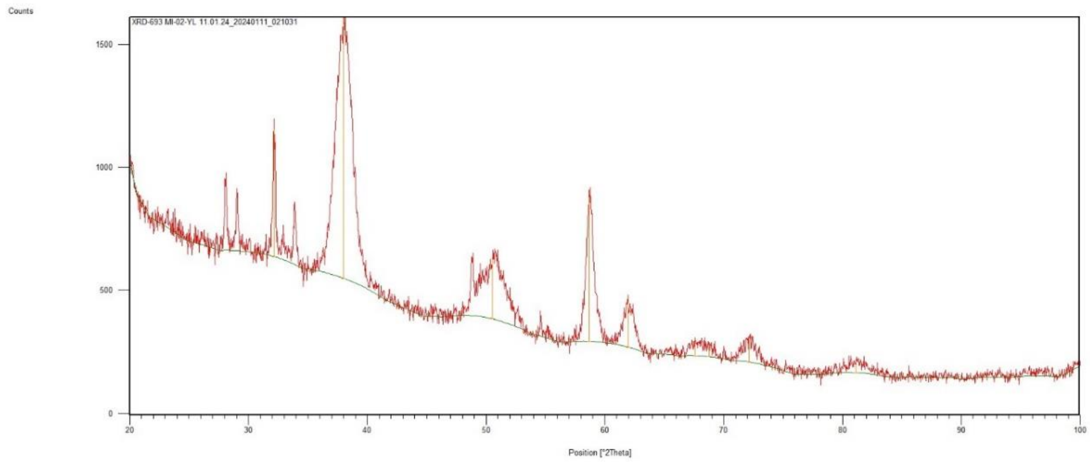
3.1 X-Ray Diffraction (XRD)

X-ray diffraction (XRD) involves directing X-rays onto a substance to observe how they scatter off its atoms. This scattering produces a diffraction pattern, where atoms disperse in various directions due to interactions with the surrounding electron clouds. Analyzing the angles and intensities of these scattered X-rays enables the determination of the material's crystal structure. XRD thereby provides valuable insights into the atomic arrangement within the substance, facilitating the identification and quantification of different phases present. X-ray diffraction (XRD) is particularly valuable in the analysis of materials like minerals because it offers insights into phase ratios, crystal structures, and phase identification. These aspects are crucial for diverse scientific and industrial applications [16].

A white powder of magnesium oxide nanoparticles (MgONPs) was obtained. Distinct and significant peaks observed in the XRD analysis of the MgONP sample, as shown in Figure 1(a&b), confirm the formation of magnesium oxide. The XRD study indicates that the synthesized MgONPs possess a high degree of crystallinity and exhibit a trigonal structure. The average size of the MgONPs synthesized from Foxnut (Euryale ferox) seed extract was determined to be approximately 14 nm, as confirmed by the XRD analysis.



(a)



(b)Figure 1(a&b) : *XRD spectra of magnesium oxide nanoparticle*

4. Anticancer activity evaluation

The cytotoxic potential of magnesium oxide (MgO) nanoparticles derived from foxnut seed extract was evaluated using the AlamarBlue assay on Vero and HCT116 cancer cells. The experiment demonstrated minor cytotoxic effects on both cell lines, as no IC50 threshold was reached after 24 and 48 hours of treatment. Sequential seeding was employed, wherein cells were initially seeded and allowed to grow for a day before treatment with various doses of MgO nanoparticles. This method showed lower toxicity compared to the co-seeding method, affecting the observed cytotoxicity outcomes. Minimal effects were observed on normal cells, indicating selective cytotoxicity on these cells after 24 and 48 hours, with notable toxicity only at concentrations exceeding 100 µg/mL.

Additionally, the cytotoxicity of biogenic MgO nanoparticles, synthesized via eco-friendly methods, was assessed using the Neutral Red Uptake (NRU) colorimetric assay. This assay measures cell viability and structural integrity by quantifying the ability of live cells to incorporate neutral red dye into their lysosomes. Higher concentrations of MgO nanoparticles correlated with decreased neutral red absorption by HT29 cells, indicating concentration-dependent cytotoxicity. The IC50 value for HT29 cells was determined to be 164.1 µg/mL, signifying the concentration needed to inhibit 50% of cell growth. As MgO nanoparticle concentrations increased, the viability of HT29 cells declined steadily, with all concentrations demonstrating suppressed cell growth compared to the control group.

5. Conculsion

Green synthesis, an eco-friendly method, uses natural sources like foxnut seeds as reducing agents to produce magnesium oxide (MgO) nanoparticles, avoiding harsh chemicals and reducing pollution. The process begins with extracting bioactive compounds from pulverized foxnut seeds using solvents, then combining the extract with a magnesium salt precursor, facilitated by NaOH. X-ray diffraction (XRD) analysis confirms the crystalline structure and 14 nm size of the MgO nanoparticles. These nanoparticles, with enhanced properties due to their small size, have significant potential in biomedicine, optoelectronics, environmental remediation, and catalysis. This sustainable approach offers an environmentally friendly alternative to traditional nanoparticle synthesis methods.

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