



RESEARCH ARTICLE - PHYSICS

Determine the anticancer activity of green synthesized MgO nanoparticles by *Spirogyra varians*

Sarah Yahya Haider ^{1*}, Ahmed Sahi Dweish ², Osama Abdul Aziz Dakhil ³

¹ Biology dept. / College of Science/ Mustansiriyah University, Baghdad, Iraq

² College of Pharmacy / Mustansiriyah University, Baghdad, Iraq

³ Physics Dep./ College of Science/ Mustansiriyah University, Baghdad, Iraq

* Corresponding author E-mail: sarahyahya10@yahoo.com

Article Info.	Abstract
<i>Article history:</i> Received 20 July 2024 Accepted 25 August 2024 Publishing 30 September 2025	<i>Spirogyra varians</i> were taken from 2nd February to 21st April 2023 from the water channel in Diyala Bridge and the lake at the University of Baghdad, Various parameters including temperature, acidity, turbidity, electricity, and the presence of nutrients nitrogen (NO₃) and phosphorus (PO₃) were recorded. Following the identification of <i>Spirogyra varians</i>, an algal extraction was conducted using methanol as the solvent. The algal extract was utilized to synthesize MgO nanoparticles (MgONPs), 0.05 M concentrations of Mg (NO₃)₂H₂O with reaction degree equal to 80° at 6 pH were used as conditions of the green synthesized. The green generated MgONPs were characterized using Ultraviolet – Visible spectrophotometry, Field Emission Scanning Electron Microscope (FESEM), energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD) techniques. The anticancer efficacy against MCF-7 breast cancer cells and its correlation with Reactive Oxygen Species (ROS) were assessed. The findings demonstrated significant efficacy and impact of the produced magnesium oxide nanoparticles (MgONPs) against MCF-7 breast cancer cells. Additionally, a clear correlation was seen between MgONPs and reactive oxygen species (ROS) in the cancer cell line.

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1. Introduction

Nanotechnology is a significant field of science that focuses on natural and manufactured materials with a size range in the nanometer scale (1-100 nm), which has garnered the interest of several scientists in the contemporary day [1]. Nanotechnology encompasses the study of the fundamental physics, chemistry, biology, as well as technology of materials at the nanoscale scale. Nanotechnology has seen rapid progress in recent years, creating several designed nanoparticles with exceptional optical, magnetic, catalytic, along with electrical capabilities [2]. Because of their superior physicochemical properties, such as optical property, magnetic property, catalytic property, and antimicrobial activity at the nano-size, nano-sized particles are distinguished from bulk materials that have a comparable chemical structure. This is due to the fact that nano-sized parts have an increased surface area/volume proportional and surface molecule fraction [3], [4]. The biomolecules included in *Spirogyra* spp. extract are used to synthesize magnesium nanoparticles and serve as a reducing agent and protein caps [5]. Anticancer, antifungal, antioxidant, and insecticidal effects are all due, in part, to MgONPs' inherent cytotoxic efficacy. Biological synthesis of nanomaterial is a safe, relatively simple, and environmentally friendly approach that uses fundamental

reducing rather than of hazardous materials and can be done using microorganisms (such as bacteria, fungi, and yeast), algae, or plant extracts [6], [7]. Nanostructured magnesium oxide stands out among them due to its impressive antibacterial action, excellent thermal stability, and low manufacturing costs [8]. On the other hand, microalgae (like *Spirogyra spp.*) are major sources of secondary metabolites with a variety of biological functions. These secondary metabolites can be used to alter and stabilize metal salts, which can be used to synthesize metal, metallic oxide, or bimetallic NPs [6]. It also can absorb high levels of metals from their environment and convert them into nanoparticles. This makes them one of the most suitable organisms for the green production of nanoparticles. Algae have a high ability to absorb metal and are abundant, making them an economical raw resource for the synthesis of NPs [9]. The current study is mostly about green synthesizing of magnesium oxide nanoparticles made by *Spirogyra varians* and determine their anticancer effects against breast cancer cells.

2. Methods:

2.1. Fieldwork measurement: During the fieldwork measurement from 2nd February to 21st April 2023, both the chemical and physical parameters were assessed and calibrated before being used. A portable multi-meter was used in order to monitor the following parameters: the pH level, temperature, electricity (EC), salinity, and the total amount of dissolved solids. In order to categorize and identify the algae, light microscopy has been employed as the method of identification for the algal sample. For the purpose of imaging, cameras (Canon-Japan) and categorization sources were used.

2.2 The production of algae powder: The process of preparing algae powder involves the careful collection of samples, followed by rinsing them with tap water and removing any sand, mud, and other debris that may be present. It is common practice to let them dry fully at room temperature over a few days before grinding into powder using an electrical grinder and storing them in dry jars at a temperature of 4 degrees Celsius until they are used [10].

2.3. Production of algal extract: The process of preparing algae powder involves the careful collection of samples, followed by rinsing them with tap water and removing any sand, mud, and other debris that may be present. It is common practice to let them dry fully at room temperature over a few days before grinding into powder using an electrical grinder and storing them in dry jars at a temperature of 4 degrees Celsius until they are used [10].

2.4. Green synthesis of magnesium oxide nanoparticles: Over the course of twenty-seven treatments, three different concentrations of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 0.025 M were combined with algal extraction (9:1 w/w) at temperatures of eighty Celsius, with pH values of six. Through the process of changing the fluid from greenish to brown, the formation of MgO nanoparticles was visually assessed. After being left at room temperature for a full 24 hours, the solution went through a centrifuge for ten minutes at a speed of 10,000 rpm, and the magnesium oxide nanoparticles were washed with sterile water that had been distilled in a gentle manner. Nanoparticle purity was guaranteed by the use of double centrifugation and washing. The pellets were dried in a hot air oven at 300 Celsius for three hours. Generally, the green manufacturing of MgO nanoparticles was proven by a number of experiments [11], [12].

2.5. Characterization of magnesium oxide nanoparticle properties:

2.5.1. UV-Vis. Spectroscopy: The UV-Vis spectroscopic analysis of nanoparticle fabrication was performed using a nanoparticle suspension in pure water. The absorption spectra exhibited a uniform augmentation in the distinctive peak as a function of both reaction duration and the concentration of biological samples containing salt ions [13].

2.5.2. XRD: X-Ray Diffraction is a flexible and non-destructive method used to identify and evaluate the crystalline structure of samples in substance and biological studies. X-ray fluorescence is a non-invasive

analytical technique used to determine the elemental composition and crystalline structure of a material. The X-ray diffraction (XRD) technique was employed to investigate the crystal structure of magnesium oxide nanoparticles (MgONPs) using the Ultima IV instrument [14].

2.5.3. FESEM and EDS: SEM (FEI QUANTA 450) images at 20 kV HV were used to investigate MgONP morphology. Elemental magnesium in solid specimens was measured by EDX. The German BRUKER Q200 was utilized for this procedure. X-ray spectrometer and FESEM showed electron emission mapping and analysis of points simultaneously [15].

2.6. Cell cultures and antitumor test: The MCF-7 breast cancer cell line was collected from high educational labs of University of Baghdad/Iraq, and they were used to evaluate the anticancer effects of MgONPs. The MCF-7 cell line was cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented via 10% fetal bovine serum (FBS) and 100 µg/ml of both streptomycin and penicillin. The living cell growth was grown in a sealed incubator at 37°C with a CO₂ level of 5%. The cells were treated with MgONPs for 24 hours at doses ranging from 1 to 100 µg/ml. The MTT test was used to evaluate the effectiveness of the anticancer treatment [16]. The intracellular levels of reactive oxygen species (ROS) were quantified by using 2'-7'-dichlorodihydrofluorescein diacetate (H2DCFDA) [17].

2.7. Statistical analysis: The data was examined via a one-way analysis of variance (ANOVA) using Dennett's multiple comparison analyses. The significance level was set at $p < 0.05$. The experiment produced quantifiable data in the form of mean $\pm$ SD.

3. Results

3.1. Samples collection: Algae samples were taken from 2nd February to 21st June 2023. During this period, many parameters including temperature, acidity, turbidity, electricity, and the presence of nutrients nitrogen (NO₃) and phosphorus (PO₃) were measured and recorded. Generally, temperature was recorded 9, 13, and 18 °C and pH values were 8.2, 8.0, and 7.6 in February, March, and April respectively. The turbidity was ranging from 408 to 452 mg/l with average equal to 430 mg/l, while electricity mean was 930 and ranging from 910 to 1024 Ms/cm. on the other hand, nitrite and phosphate means were 4.8 and 0.25 mg/l respectively. Temperature and pH are negatively correlated. The higher temperature was meted with the lower pH, while turbidity increases when electricity was increased. The pH was higher in the colder months and lower in the summer months. Temperature affects water hydrogen bonding. The acidity degree in the current study had consistently measured by multiple researches [17]. Temperature affects surface water salt concentration. Salts, nutrients, and compounds in water rise with temperature, raising electrical conductivity. NO₃ and PO₄ levels correlated with aqua system species, particularly phytoplankton [18]. Nitrate is the main factor affecting phytoplankton levels in aquatic systems, according to several studies. Additionally, nitrate is known to restrict algae development [18], [19], [20]. According to [17], climate fluctuations during the research period may have caused the observed outcomes.

3.2. Algae isolation: The filamentous, branched-out green algae that develop as free-floating mats were found to be *Sporangia spp.* algae, which were separated from shallow waters. The majority of the time, it was found growing in the shady littoral zones of water bodies, meandering streams, and sluggish waterways such as ponds and canals. As is well knowledge, they belong to the Chlorophyta class and the Zygnematales order. They take their name from the peculiar helical or spiral shape of the chloroplast that is characteristic of the genus. There were *Spirogyra* species that seemed to be encircled with a slimy, jelly-like material when seen under a microscope. This substance is the exterior wall of the organism that dissolves in water.

Mucilage also surrounds the filaments, which helps to keep them together so that their bodies can form clumps in water. Furthermore, the highest amount of mucilage that was collected was recorded in the

month of June, which was in agreement with the findings of many other studies that were conducted in the past [4]. These studies pointed out that the growth of algae begins at the beginning of spring and achieves its peak in the months of autumn, fig.1 shows the collected samples of *Spirogyra varians* in the current study.



Fig.1. A specimen of the collected *Spirogyra varians* under the microscope (10X and 40X).

3.3. MgONPs green synthesis by *Spirogyra varians* : The progressive transition in the solution's color from a greenish hue to a yellowish-brown tint confirmed the green synthesis of magnesium oxide nanoparticles (MgONPs). This color change served as a marker for MgONPs production. Fig.2 shows the algal extract combined with a solution containing various concentrations of Mg (NO₃)₂H₂O at room temperature for 24 hours. Several studies have demonstrated that the transition from a greenish hue to a yellowish-brown can serve as a reliable signal of the synthesis of MgONPs [2], [12], [21]. The hue of the solution changes with bio-reduction activation, which indicates the reduction of metal ions and the formation of nucleation. Algal cells release substances and enzymes that initiate these processes. Nucleated metal elements join together during development to form thermodynamically stable nanoparticles (NPs) of various shapes and sizes. Upon reaching the final termination stage, NPs have achieved their ultimate form. Factors such as temperature, pH, duration, static conditions, substrate concentration, and mixture agitation might influence the physical properties of nanoparticles (NPs) [2], [22]. Algae can produce nanoparticles (NPs) either inside their cells (intracellular) or outside their cells (extracellular). Because bioactive components reduce bioavailability, it is probable that nanoparticles (NPs) are more concentrated on the cell membrane than in the cytoplasm. Algal cells produce nanoparticles (NPs) in a manner that depends on the dosage occurring inside the cells. Conversely, metabolites, primarily proteins, attach to the outer surface of the cells and reduce the quantity of metal ions outside the cells. It is easy to distinguish between NPs, and they are easier to manufacture outside of cells [21]

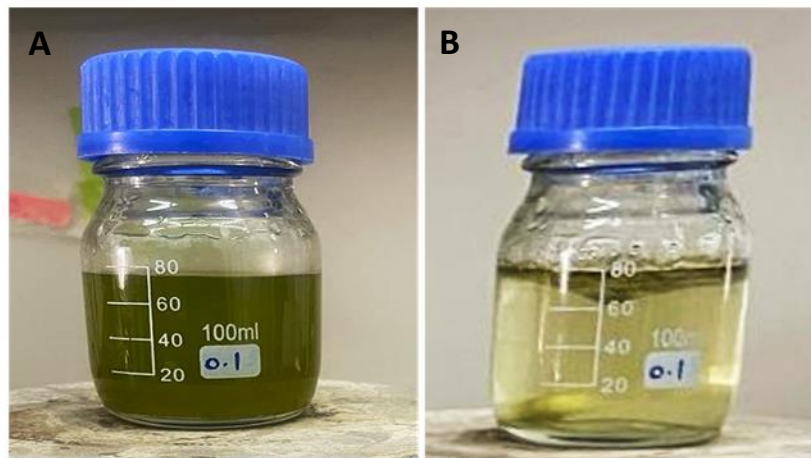


Fig.2 Visual indicator of MgO-NPs green synthesis.

A: solution before MgONPs synthesis B: Solution after MgONPs synthesis

3.4. Characterization of green synthesized MgONPs.

3.4.1. Ultraviolet visible spectrophotometer: The optical properties of MgONPs from all seven trials were examined using UV-Vis spectroscopy over a wavelength range of 200-800 nm. A prominent absorbance peak was seen in the wavelength region of 3237 nm during UV-visible spectroscopy, indicating the existence of minute MgO particles. The green synthesized magnesium oxide nanoparticles (MgONPs) frequently display surface plasmon resonance, leading to absorption in the ultraviolet-visible (UV-Vis) region with distinct optoelectronic properties. The observation of a well-defined absorbance peak at approximately 330 nm in UV-visible spectroscopy indicates the formation of MgO particles with small diameters [2] as seen in fig.3.

3.4.2. Results of FESEM & EDS: Scanning electron microscopy (SEM) is employed to examine the surface of magnesium oxide nanoparticles (MgONPs). The SEM was used to investigate all six treatments. Fig.4. displays the outcomes of all treatments [23]

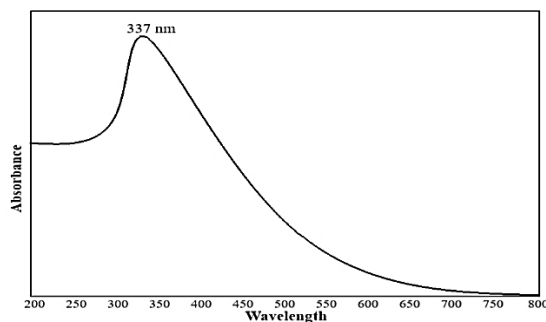


Fig.3. UV-Vis analysis of synthesized MgONPs (0.05M, 6 pH, and 80 °C).

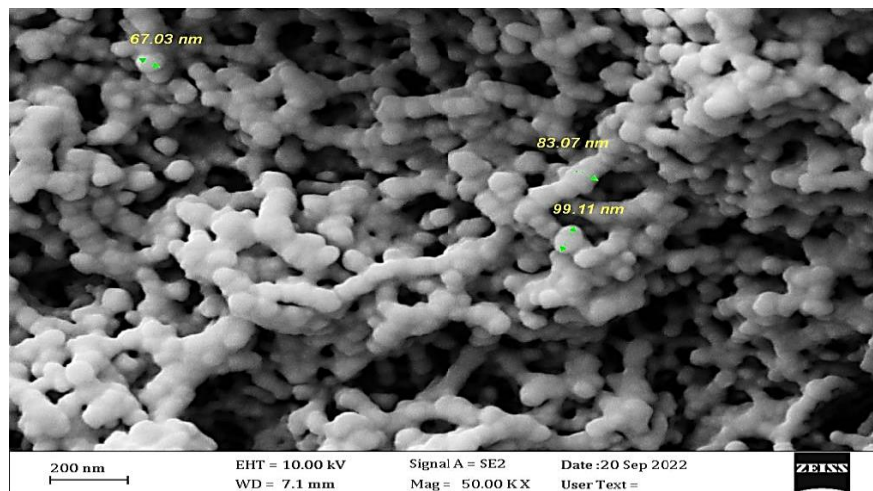


Fig.4. The FESM of MgONPs synthesized from *S. varians* treatment

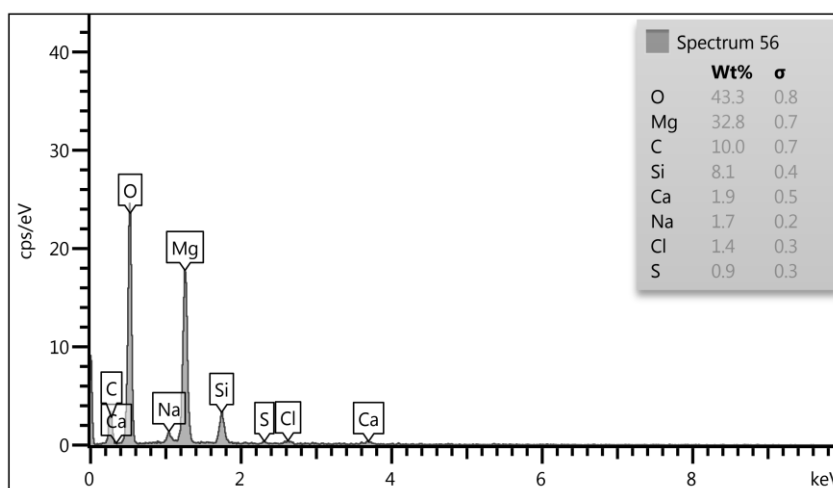


Fig.5. The EDS of MgONPs synthesized from *S. varians*.

3.4.3. Results of XRD: The phase and composition of the magnesium nanoparticle samples that were created as a result were determined using the X-ray diffraction analysis (XRD) technique. The XRD result is shown in Fig.6, Using FESEM, the shape and size of the biosynthesized MgONPs were evaluated. Furthermore, MgONPs production was enhanced by biomolecules derived from *Spirogyra varians* extract. Rod-shaped particles can be seen in FESEM images of magnesium oxide nanoparticles (MgONPs) from several investigations. Magnesium (Mg) and oxygen (O) showed high peaks in the EDS analysis as shown in fig 5, with no additional peaks that would have an impact on their functionality. The optimal conditions for the green production of magnesium oxide nanoparticles (MgONPs) were met by the current study in line with many other previous publications [24]

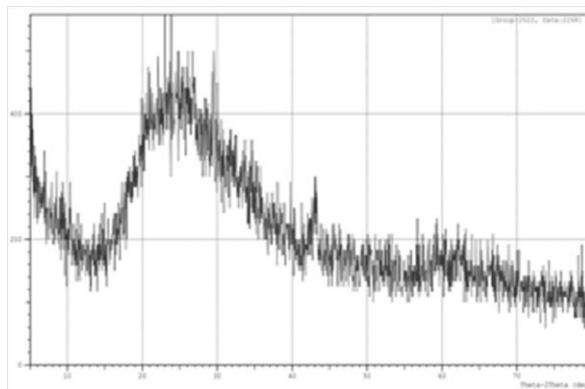


Fig.6. XRD pattern of synthesized MgONPs.

3.5. Results of anticancer activity of MgONPs: There is an increasing interest in researching the effectiveness of magnesium oxide nanoparticles (MgONPs) as a possible treatment for cancer [17]. MgO nanoparticles that were manufactured in an environmentally responsible manner were examined for their anticancer effects using human breast cancer cells (MCF-7). Following the exposure of neoplastic cells to MgONPs at doses ranging from 1 to 50 $\mu\text{g/ml}$ for a period of 24 hours, the MTT assay was utilized to evaluate the effectiveness of the MgONPs in inhibiting the growth of disease-causing cells. The dose-dependent cytotoxicity of magnesium oxide nanoparticles (MgONPs) on MCF-7 cancer cells is illustrated in Fig.7. MgO nanoparticles were found to have IC₅₀ values of 29.5 $\mu\text{g/ml}$, as determined by the measurements, and IC₈₀ were found in concentration equal to 100 $\mu\text{g/ml}$.

The current findings provide credence to a number of earlier research that have demonstrated the anticancer capabilities of magnesium oxide nanoparticles (MgONPs) on a variety of cancer cell types. In the investigations, the process of synthesizing nanocomposite with anticancer characteristics in human malignancies of the colon HC116 and carcinoma of the breast MCF7 cells was presented. This process was documented in the current study work [17], [25] in the past. Previous research has demonstrated that magnesium oxide nanoparticles (MgONPs) are effective in combating cancer. In 2019, Verma and colleagues conducted a study in which they found that human breast cancer cells (MCF-7) demonstrated sensitivity when they were exposed to magnesium oxide nanoparticles (MgONPs). Recent studies [26], [27] have studied the anticancer characteristics of magnesium oxide nanoparticles (MgONPs) that are generated from plant and algae extracts. These nanoparticles were tested on human melanoma tumors [28].

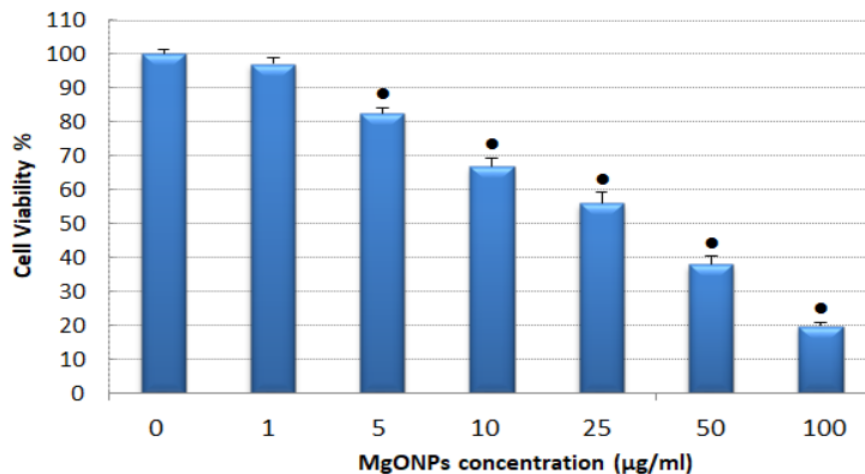


Fig.7. Anticancer effects of MgONPs in human breast cancer cells (MCF-7) • ($p < 0.05$).

According to the findings, there was a positive link between the levels of reactive oxygen species (ROS) in the MCF-7 cancer cells and the manufactured magnesium oxide nanoparticles (MgONPs), as could be shown in Fig.7. The rise in the oxidative stress response, namely the quantity of reactive oxygen species (ROS), was found to have a direct association with the concentration of magnesium oxide nanoparticles (MgONPs).

Reactive oxygen species (ROS) are implicated in a diverse range of disease processes and serve crucial roles in cellular functioning. Within the cell, mitochondria play a crucial role in producing reactive oxygen species (ROS). Pugazhendhi and colleagues conducted a recent study which revealed that the accumulation of intracellular oxygen species that are reactive (ROS) was the cause of apoptosis activation in cancer cells that had encountered magnesium oxide nanoparticles (MgONPs) [29]

.4. Conclusion:

The study revealed that the use of MgO nanoparticles shown notable anticancer properties when applied to breast cancer cells (MCF-7) during testing. Oxidative stress was found to be involved in the anticancer effect of MgO nanoparticles, as evidenced by the generation of reactive oxygen species (ROS) in cancer cells following exposure to these nanoparticles. The results of this study offer crucial knowledge on the potential application of eco-friendly synthesized magnesium oxide nanoparticles (MgONPs) as a novel medical treatment for cancer-related microbial infections.

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