AN EVALUATION OF BIO-SYNTHESIZED MAGNESIUM OXIDE NANOPARTICLES FOR ENHANCED ANTIOXIDANT AND ANTICANCER ACTIVITY

A dissertation

submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCES

IN

BIOTECHNOLOGY

By

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UNDER THE SUPERVISION OF

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CANDIDATE'S DECLARATION

I, Yogita Tomer, 2K22/MSCBIO/56 student of M.Sc. Biotechnology, hereby certify that the thesis entitled **"An Evaluation of Bio-Synthesized Magnesium Oxide Nanoparticles for Enhanced Antioxidant And Anticancer Activity"** in partial fulfilment of the requirement for the award of the Degree of Master of Sciences submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from Jan. 2024 to May 2024 under the supervision of Prof. Jai Gopal Sharma.

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LIST OF ABBREVIATION

MgO	Magnesium Oxide
MgOE	MgO Extract
MgOC	MgO Chemical
XRD	X-ray diffraction
SEM	Scanning Electron Microscopy
EDX	Energy Dispersive X-Ray Analysis
TEM	Transmission Electron Microscopy
DPG	*Dimorphocalyx glabellus*
DPGE	*Dimorphocalyx glabellus* extract
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
ROS	Reactive Oxygen Species
GC-MS	Gas Chromatography- Mass spectrometry
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid

ABSTRACT

The environmental benefits and potential medical uses of biologically synthesized metal oxide nanoparticles have attracted a lot of attention in recent years. This work is centered on the biosynthesis of Magnesium Oxide (MgO) Nanoparticles and assesses how well they function as antioxidants and anticancer agents. *Dimorphocalyx glabellus*, found in the evergreen forests of peninsula India and Shri Lanka, is a therapeutic agricultural plant but no thorough research on antioxidants and anticancer agents has been done yet. Thus, in this work, *Dimorphocalyx glabellus* plant extracts were used as stabilizing and reducing agents to create MgO nanoparticles. In order to ascertain the structural, morphological, and chemical characteristics of the produced nanoparticles, a number of methods were employed, such as X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis and transmission electron microscopy (TEM). Presence of magnesium and oxygen were further confirmed by EDX profile.

Standard assays like DPPH and ABTS, were used to evaluate the antioxidant activity of the MgO nanoparticles, and the results showed a substantial potential for scavenging free radicals. The differential distribution of anticancer phytochemicals in *Dimorphocalyx glabellus* were found by GC-MS analysis. Additionally, the anticancer efficacy was assessed utilizing MTT, ROS generation and wound healing assays against a human cancer cell line, HepG2. The potential of bio-synthesized magnesium oxide nanoparticles as potent anticancer agents was highlighted by their noteworthy cytotoxicity and ability to heal wounds in cancer cells.

CHAPTER 1

INTRODUCTION

As nanoparticles find increasing applications in various fields such as medicine, electronics, and environmental remediation, several upcoming issues need to be addressed. One major concern is the lack of comprehensive understanding of the long-term effects of nanoparticles on human health and the environment. Studies have shown that certain nanoparticles can accumulate in living organisms and have toxic effects. (Yuliarto et al., 2019a) One significant concern is the environmental impact of the chemicals and solvents used in their production. These substances can contribute to pollution and hazardous waste, posing risks to ecosystems and human health. (Heiligtag & Niederberger, 2013)Additionally, the energy-intensive nature of chemical synthesis processes contributes to greenhouse gas emissions and environmental degradation. Another issue is the potential toxicity of chemically synthesized nanoparticles, both in their production process and in their applications.(BHATTACHARYA & MUKHERJEE, 2008)

Biogenic synthesis is an environmentally sustainable method of producing nanoparticles that makes use of natural materials and safe processing techniques to minimize negative effects on the environment.(Yuliarto et al., 2019b) It is more secure and beneficial to the environment than traditional synthesis techniques since it uses less hazardous chemicals and solvents, which also minimizes waste production and pollution.(Franco et al., 2021) Along with being in line with green chemistry principles, this approach has additional benefits like biocompatibility, energy efficiency, affordability, adaptability, and scalability.(Nasrollahzadeh et al., 2020)

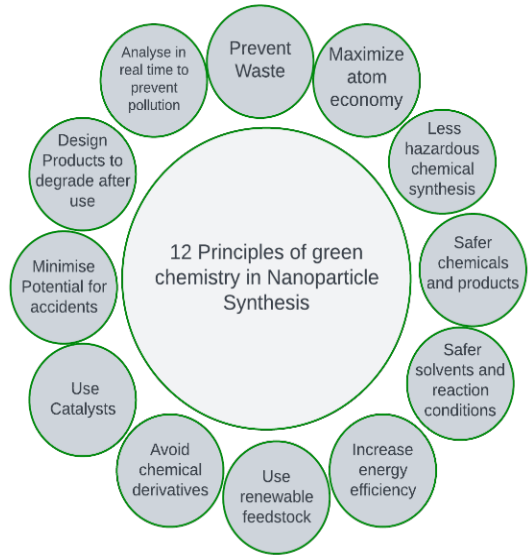

Figure 1: 12 principles of green chemistry in Nanoparticle synthesis. (Redrawn and adapted from [(Anastas & Eghbali, 2010)])

The application of the twelve principles of green chemistry in nanoparticle synthesis is a relatively new emerging field concerning its sustainability. This field has received great attention in recent years due to its capability to design alternative, safer, energy efficient, and less toxic routes towards synthesis.(PATIL et al., 2021)

A variety of nanoparticle materials can be synthesized using green synthesis techniques, such as enzyme, plant, microbial, and biomolecule assisted synthesis. These techniques provide special benefits. Green synthesis has many applications in the medical, electronics, and environmental science fields.(Gatou et al., 2024)Further study and development in this area will make green synthesis even more useful and contribute to a more sustainable future.

Nanoparticle synthesis involves the creation of nanoparticles, which are particles ranging from 1 to 100 nanometers in size, with unique properties compared to their bulk counterparts.(Baig et al., 2021) The synthesis methods can be broadly categorized into bottom-up and top-down approaches. Bottom-up methods build nanoparticles from smaller components, such as atoms or molecules, and include techniques like chemical reduction, sol-gel processes, microemulsion, and green synthesis. These methods offer control over size, shape, and composition and are often used for their simplicity and scalability. Top-down methods, on the other hand, break down larger materials to create nanoparticles and include techniques like ball milling, lithography, and electrospinning.(Dutta & Das, 2021) These methods are useful for creating nanoparticles with precise shapes and patterns. Overall, nanoparticle synthesis methods vary in complexity and applicability, providing a range of options for producing nanoparticles tailored to specific applications in medicine, electronics and environmental science.

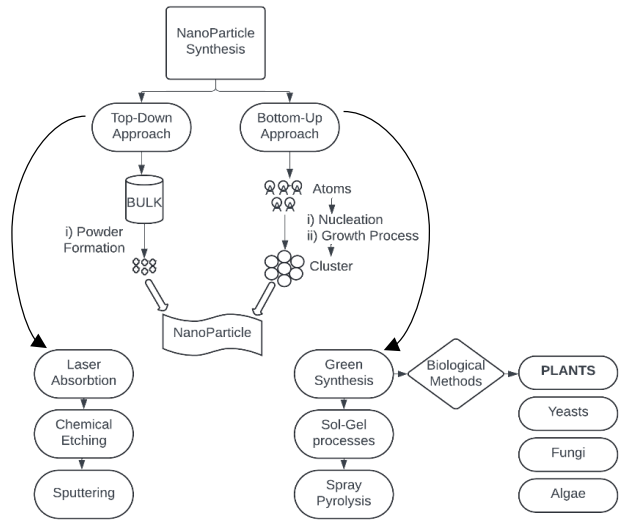

Figure 2: Process of nanoparticle synthesis. (Redrawn and adapted from [(Duan et al., 2015)])

CHAPTER 2

LITERATURE REVIEW

2.1.Magnesium Oxide Properties and Applications

2.1.1. Magnesium (Mg)

Magnesium is classified as an alkaline Earth metal (Group 2), with Mg (II) being its most stable oxidation state. With a weight percentage of about 2.1 weight percent, it is the eighth most prevalent element in Earth's crust and is also widely distributed in brine and ocean(S. V. S. Prasad et al., 2022). Magnesite MgCO3, dolomite $CaMg(CO_3)_2$, brucite $Mg(OH)_2$, and silicates, hydroxides, sulfates, and carbonates are the most frequent minerals that include it (González et al., 2021). After potassium, magnesium is the most prevalent metal ion present in living cells and is a crucial component of human physiology. It is a necessary nutrient for the upkeep of cells and organs and is involved in a number of physiological processes and enzymatic reactions. Magnesium can be found in chlorophyll, the green pigment that powers photosynthesis in cyanobacteria, algae, and plants. Consequently, Mg is associated, either directly or indirectly to multiple essential procedures (Peng et al., 2019).

2.1.2. Magnesium Oxide

Magnesium oxide, or MgO, is a useful metal oxide that is formed by calcining the mineral magnesite. It has a defined geometry, regular crystalline structure, and predominant ionic character. It also presents intriguing physicochemical features. Electrostatic forces primarily explain the chemical bonding in magnesium oxide (MgO). A symmetric charge distribution encircles the magnesium (II) ion, which is encircled by six oxide anions.(Ercan et al., 2018; Pilarska et al., 2017)

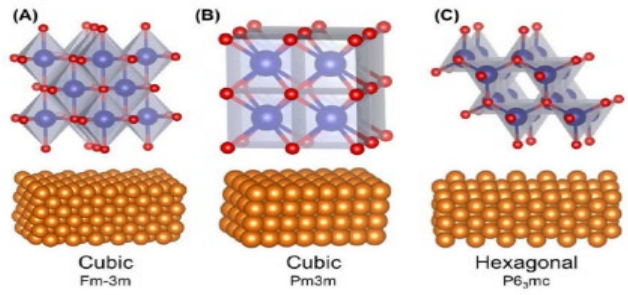

Figure 3: MgO crystallizes in three main structures [Redrawn and adapted from (Silva et al., 2022)]

The valence shell of the Mg (II) ion ($2s^2$ $2p^6$ $3s^0$) is closed, which contributes to MgO's simple crystalline structure and its physical and chemical characteristics. Additionally, unlike transition metal oxides, MgO lacks electrons in its d orbitals(J. P. Singh et al., 2020a). MgO is a versatile material that finds use in a wide range of industries and may be generated in both micro and nanostructure(Hazra Chowdhury et al., 2015). Different MgO nanostructures result in various physical phenomena and applications, such as optical and dielectric characteristics, which make nano MgO behave differently than bulk MgO.(Patil & Bhanage, 2013) The MgONPs exhibit greater porosity, a larger surface area, a higher volume, and a wider band gap than bulk MgO and as a result, they're frequently used in industrial catalysis as well as the elimination of different hazardous pollutants including dyes, soluble toxic ions, and heavy metals from wastewater. (Khalaj et al., 2020)

2.2. Green Synthesis

The utilization of natural chemicals in the chelation, reduction, and/or precipitation of a metal ion precursor has attracted attention in comparison to traditional chemical and physical synthetic approaches. This process is known as the "green synthesis of metal and metal oxides." (Jeevanandam et al., 2016) Green synthesis is regarded as an eco-friendly method because of the following reasons:

- **i.** employs fewer dangerous chemicals and solvents;
- **ii.** doesn't generate any hazardous wastes;
- **iii.** conducted under benign experimental circumstances, such as ambient pressure, low energy input, and temperature;
- **iv.** has a comparatively easy to use interface;
- **v.** low cost;
- **vi.** is expandable.

Microorganisms and plant extracts are the most commonly used renewable resources in the formation of new products. The green production of metal nanoparticles (MeNPs) like Cu (Pakzad et al., 2019), Ag, Au(Rani et al., 2022), and Pd (Nasrollahzadeh et al., 2020)as well as metal oxide nanoparticles (MeONPs) like FeO_x, CuO, MnO_2, MgO, $TiO2_2$(Verma et al., 2022), and ZnO has made considerable use of plants. Plants are abundant in nature and contain a variety of phytochemicals with different structural variations. The polyphenols stand out among them due to their strong chelating and

reducing properties, which, depending on the metal ion, might encourage the production and stabilization of nanoparticles.(Rana et al., 2020)

In comparison to the bulk materials, the nanoparticles have distinct physical, chemical, electrical, and magnetic properties, which typically results in better performance for a variety of applications.(Asha & Narain, 2020) The large surface area to volume ratio affects several characteristics, including mechanical, electrical, and thermal conductivity, characteristics, optical qualities, magnetism, and catalytic activity capacity.(J. P. Singh et al., 2020b) Nevertheless, aggregation is hampered by the high surface tension and a downward spiral that can be stopped by stabilizing or chelating agents, and the polyphenols found in plants are also crucial in hindering the mechanisms that deactivate NPs.

2.3. Dimorphocalyx glabellus

Dimorphocalyx glabellas is a plant used in Sri Lankan traditional medicine. However, knowledge of its therapeutic properties is limited to a few traditional physician-families in Sri Lanka. *D. glabellus* (Welliwenna) is classified as a controversial medicinal plant. This plant is widely distributed in Southern India and Sri Lanka(Yu & Van Welzen, 2020).The majority of traditional physician families employ this plant as a purgative in the treatment of Sarpavisha Vedakama (therapy for snake bites), Kadum Bindum Vedakama (traditional orthopaedic treatment), either as a standalone medication or in conjunction with other components. In addition, it is used in tiny doses to treat neurological system diseases.(Chakrabarty & Krishna, 2020) The portions used in medicine are leaves, roots and stem bark. As a seldom used medicinal plant, unknown to many, action is produced to disseminate the knowledge about the medicinal herb, *D. glabellus* (Welliwenna).

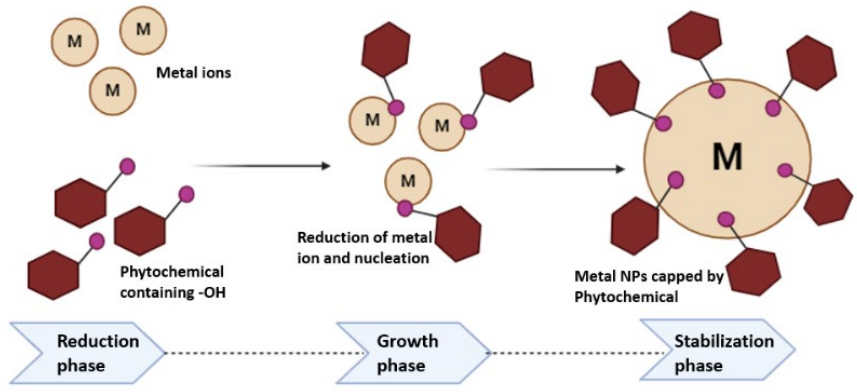

Figure 4: Schematic illustration of the method and mechanism involved in green synthesis of nanoparticles using plants as reducing agents. [Redrawn and adapted from (H. Singh et al.,

2.4. Hepatoprotective activity

The hepatoprotective properties of plant medications against chronic liver illnesses have been demonstrated, and 600 commercial herbal formulations are marketed globally. In India, forty patented polyherbal compositions contain 93 different medicinal herbs. Because of the presence of polyphenols, flavonoids, alkaloids, coumarins, carotenoids, lignans, and other chemicals, herbs have an antioxidant property that underpins the mechanism (Girish & Pradhan, 2012). Combining *Cassia occidentalis, Tamarix gallica, Capparisspinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna,* and *Achillea millefolium* in a clinical trial demonstrated liver-protective effects against hepatitis B virus infection and alcohol-induced hepatic damage, both of which are significant risk factors for liver cancer (Gallo et al., 2021; Girish & Pradhan, 2012). In addition to preventing chronic liver disease, a number of medicinal plants and herbal formulations have been thoroughly investigated in relation to HCC. Certain traditional extracts and formulations, like Jiedu Granule, Fuzheng Jiedu Xiaoji formulation, *Trametes robiniophila,* and *Coriolus versicolor*, have been shown in clinical trials to improve overall survival and progression-free survival rates in addition to standard medical treatment. Traditional medicinal plant extracts have been shown to induce apoptosis and suppress tumor growth in a mouse model. These extracts include *Azadirachta indica, Schizocapsa plantaginea, Paris polyphylla var. yunnanensis*, and a combination of green tea, curcumin, quercetin, and resveratrol (Kim et al., 2022) According to a recent study, HepG2 cells were exposed to polyphenol-rich leaf extracts from *Camellia sinensis* and *Withania somnifera*, as well as seed extracts from *Vitis vinifera*, which caused cell death (Venkatachalapathy et al., 2021). Numerous research has indicated that the well-known Indian saffron, *Curcuma longa*, containing curcumin, has anti-hepatocarcinoma action (S. Darvesh et al., 2012).

2.5.Antioxidant Activities

The antioxidant properties of natural substances in food or biological systems are assessed using a variety of techniques, with differing degrees of success. Two free radicals, 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), are frequently employed to measure antioxidant activity in vitro. But neither of these radicals is native to a living system. A potent oxidizing agent, such as potassium permanganate or potassium persulfate, is reacted with the ABTS salt to produce the ABTS. The suppression of the characteristic long wave (734 nm) absorption spectrum of the blue-green ABTS radical is used to quantify the reduction of the solution colored by the radical by hydrogen-donating antioxidant. The absorption band of DPPH, a stable free radical, is located at 517 nm. When decreased by a free radical species or an antioxidant, it loses this absorption. The DPPH technique is frequently used to assess the antioxidant and antiradical properties of natural plant extracts and purified phenolic compounds. According to (Schaich et al., 2015), the majority of phenolic antioxidants react with DPPH slowly, taking at least 1-6 hours to achieve a steady state. This implies that measurements of antioxidant activity using DPPH must to be made periodically. The procedure is widely used and exhibits good repeatability. It has little to no relevance to biological systems, nevertheless, just as ABTS.

AIM & OBJECTIVE

The aim of the dissertation project was to synthesis and characterize Magnesium Oxide Nanoparticles biogenically and study their anti-oxidant and anti-cancer activity.

The project had the following objective:

1. To extract phytochemicals from the plant *Dimorphocalyx glabellus* and characterize the phytochemicals present in it.
2. To biogenically synthesize and characterize Magnesium oxide nanoparticle and compare the functionality with chemically synthesized Magnesium oxide.
3. To perform a comparative study of the anti-oxidant property and anti-cancer property of the synthesized nanoparticles.

CHAPTER 3

METHODOLOGY

3.1. Extract preparation

The leaves of Dimorphocalyx glabellus plant were brought to the lab, where they were cleaned with distilled water to get rid of any debris. They were then wiped, dried for eight days in the shade, and pulverized. A total of 10 g of the leaf powder was diluted with 100 mL of distilled water, the mixture was then heated on a hot plate (80°C) for 60 mins. After completion of the heating process, the mixture was cooled and filtered using Whatman No. 1 filter paper, the resulting filtrate was stored at 4° C and was analyzed for its phytochemical properties through GC-MS analysis.(Abinaya et al., 2021)

3.2. Green Synthesis of MgO nanoparticles

MgO nanoparticles were synthesized using the aqueous extract of Dimorphocalyx glabellus. In order to create MgO NPs, the hydroxyl and carbonyl groups present in it, served as coprecipitation agents, they acted as stabilizing and reducing substances for the MgO nanoparticle synthesis.40 mL of aqueous extract of Dimorphocalyx glabellus were combined with 10 mL of 0.1 M $Mg(NO_3)_2$, and the mixture was constantly stirred for 45 minutes on a hot plate with the help of a magnetic stirrer. To the mixed suspension 6.0 ml of 0.2 M NaOH was added drop by drop, until a noticeable precipitate was formed. The color change from yellow to dark brown was used to monitor the creation of nanoparticles. Moreover, the solution was then maintained at 25 ± 3 °C. the suspension was centrifuged at 7,000 rpm for 10 mins followed by washing of the precipitates with Milli Q. The precipitate was first dried in an oven at 70”C for 2 hours and then calcined for four hours at 873 K to produce a pure, fine powder. (Moawad et al., 2010)

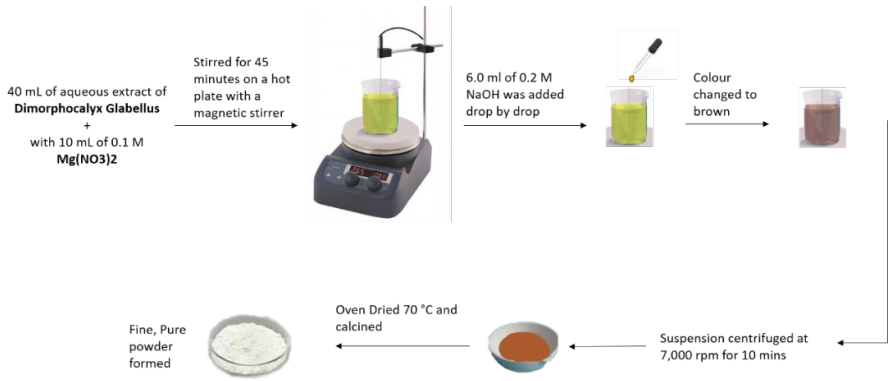

Figure 5: Protocol for nanoparticle synthesis

3.3. Chemical Synthesis of MgO Nanoparticles

For the chemical synthesis of MgO Nanoparticles, 0.1 mM of Magnesium Nitrate Hexahydrate solution was prepared by adding 1.8g of the salt to 100 ml of Milli Q in order to obtain the precursor solution. To this, equal volume of 0.1 M of NaOH solution was added slowly and stirred for 2 hours on a magnetic stirrer. The white precipitate so formed indicate the formation of Magnesium Hydroxide Nanoparticles. The solution was stirred for another 2 hours and centrifuged at 4000 rpm for 10 minutes. The precipitate was washed with water and ethanol until the pH reached 7. The washed solution was then calcined at 400°C for 4 hours.

3.4. Characterization

3.4.1. Transmission Electron Microscopy (TEM)

TEM analysis was performed to examine the size and form of the synthesized NPs. The MgO nanoparticles were dispersed in Milli Q and a thin droplet of the dispersion was placed on the staining mat. With the coated side facing up, the copper grid coated with carbon was placed into the drop and dried to be screened in a Transmission Electron Microscope.

3.4.2. Scanning Electron Microscopy (TEM)- EDX

The comprehensive analyses were conducted using an energy dispersive analysis of X-rays (EDX)-equipped scanning electron microscope (SEM) operating at 15 kV, which was made possible by the SEM apparatus. Surface morphology, elemental composition, and elemental distribution mapping were all examined

3.4.3. X-Ray Diffraction (XRD)

XRD analysis was performed in the range of 20–80° (2θ) using CuKα radiation.

3.5. Antioxidant Assays

3.5.1. DPPH radical scavenging assay

The assay was performed as per (Brand-Williams et al., 1995), with slight modifications. 0.1mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was freshly prepared and adjusted to a concentration by diluting with methanol (if needed) so as to achieve an absorbance of 0.980 at 517nm (λmax) using UV spectrophotometer. A series of dilutions were prepared for the aqueous plant extract (0-30µg/ml) and magnesium oxide nanoparticles in the range 0-1200µg/ml (both chemically and green synthesized), to which DPPH solution was added to make up the volume up to 1 ml. These serial dilutions were then incubated at room temperature in the dark for 30 min. The absorbance for each dilution was recorded after 30 minutes of incubation time at 517nm using 1ml of the DPPH stock solution as the control and methanol as blank. The readings for each nanoparticle and the plant extract were taken in triplicate and the free radical scavenging activity for each was calculated using the following formula:

$$\text{Free radical scavenging activity \%} = \frac{(\text{Absorbance of control} - \text{Absorbance of Sample}) * 100}{\text{Absorbance of control}}$$

3.5.2. ABTS radical scavenging assay

ABTS assay was performed as described by (Unuofin et al., 2018). For ABTS radical scavenging assay, 2.45mM of Potassium Persulphate ($K_2S_2O_8$) was prepared by dissolving 6.6mg of potassium persulphate in 10 ml of distilled water. Similarly, 7mM of ABTS solution was prepared by adding 38.4 mg salt of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid to 10 ml of distilled water. Equal volumes of both the solutions were mixed and incubated in dark at room temperature for about 12-16 hours to allow the formation of ABTS radical. After incubation a visible

color change confirmed the formation of ABTS radical solution. This solution was then diluted with methanol (if needed) in order to attain an absorbance value of 0.950 at wavelength of 734nm (λmax). Similar to DPPH Assay, serial dilutions were prepared for the plant extract (0-30µg/ml) and the MgO nanoparticles (0-1200µg/ml).

These dilutions were made up to 1 ml by adding ABTS radical solution and kept for incubation for 5-6 minutes in dark at room temperature. The absorbance for each dilution was recorded after 5 minutes of incubation time at 734nm using 1ml of the ABTS stock solution as the control and methanol as blank. The readings for each nanoparticle and the plant extract were taken in triplicate and the free radical scavenging activity for each was calculated using the following formula:

$$\text{Free radical scavenging activity \%} = \frac{(\text{Absorbance of control} - \text{Absorbance of Sample}) * 100}{\text{Absorbance of control}}$$

3.6. Cell Culture and treatment

The standard protocol was followed for cultivating cells in a humidified incubator with a CO_2 (5%) environment at 37 ◦C (A. Prasad et al., 2022) .

Human hepatocarcinoma HepG2 cells were obtained from the National Centre for Cell Science's (NCCS, Pune, India) cell repository. A high-glucose DMEM medium containing 10% (v/v) fetal bovine serum (FBS) and antibiotics (10 µg/ml streptomycin and 100 U/ml penicillin) was used to grow HepG2 cells at seedling densities of 10×10^3 (96-well plate) and 2×10^5 (6-well plate) cells/well for 24 hours, before the cells were treated. The stock solution (made in DMSO) for further treatments. Treatment at 50–60% cell confluency was used in all experiments, with the exception of the wound healing assay, which required

70–80% confluency. Phosphate buffer saline (PBS), pH 7.2, was used to wash the cells in all studies both before and after the addition of dye and treatment.

3.7. MTT Assay

HepG2 cells were used to test the anticancer potential of the green and chemically synthesized MgO Nanoparticles using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (Chen et al., 2022). 5–400 µg/ml of DGPE, MgOC, MgOE were added to cells cultured in a 96-well plate at a density of 10×10^3 cells per well until 60% confluency in SFM for 6, and 12 hours. As the vehicle control, a maximum DMSO level of 0.05% was used. Following treatment, cells were incubated for two hours in 100 µl of new media containing 0.5 mg/ml MTT. MTT is transformed by living cells into formazan crystals, which are then dissolved in 100 µl DMSO. Each well's absorbance was measured at 575 nm using an ELISA microplate reader.

3.8. Wound Healing Assay

The cell scratch assay was used to test the Magnesium Oxide nanoparticles' ability to repair wounds. Utilizing BJ-5Ta normal fibroblast cell lines, the wound healing activity was tested. In this experiment, 2×10^5 cells/mL of cells were seeded in DMEM, the standard cell culture medium, which was enhanced with 10% phosphate buffer saline (PBS) and M199 medium. The cells were seeded and then cultured in a CO2 incubator for 24 hours. The cells in the middle of the culture well were scratched by sharp tips when they were in a monolayer conformation at roughly 70–80% confluence. After that, twice PBS washes were used to remove the ruptured cells. The DGPE, MgOC and MgOE, were then put into the wells that had been scrapped. Once more, the culture plates were incubated for 24 hours. (Roy et al., 2023)

3.9. Investigation of reactive oxygen species (ROS)

The amount of ROS production was determined as described earlier by (Shi et al., 2020) with minimal alterations. It was predicated on dichloro-dihydro-fluorescein diacetate's (DCFHDA) fluorescence intensity. The Hepg-2 cell line were treated with 100 µg/ml of DGPE, MgOC and MgOE that contained 2×10^5 cells/mL cells in a 6 well plate. The cells were treated for 6 h, were washed and incubated with dyes in SFM for 30 min. After that cells were again washed, collected in cold PBS, and transferred to a quartz cuvette for spectrofluorimetric measurement with a spectrofluorophotometer. Fluorometric parameters for the fluorophores H2DCFDA was λ_{ex} = 488 nm and λ_{em} = 500–550. Experimentally for

fluorescence microscopy, cells were cultured on coverslips in 6-well plates (2 × 105 cells/well), and after 24 h, treated with extracts for 6 h. Then cells were washed with PBS and incubated with staining solution (fluorescent dyes mixed in SFM) for 45 min in a CO2 incubator in the dark. Then cells were washed three times with PBS and analyzed fluorescence microscope at 10X magnification and in green channel.

CHAPTER 4

RESULTS

4.1. Characterization of the Extract and MgO Nanoparticles.

4.1.1. GC-MS Analysis

The biomolecules identified in high concentration from the GC–MS analysis are listed in Table 1.

Table 2: Gc-Ms Spectrum Showing Characteristic Peaks of Phytochemicals Present in The Extract.

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	12.907	152463	1.03	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
2	13.498	58935	0.40	HEXADECANOIC ACID, METHYL ESTER
3	14.019	3573129	24.07	n-Hexadecanoic acid
4	14.964	144026	0.97	Octadecanoic acid, 2-propenyl ester
5	15.190	123784	0.83	6-Octadecenoic acid, methyl ester, (Z)-
6	15.686	6998666	47.15	Oleic Acid
7	15.891	773975	5.21	9-OCTADECENOIC ACID (Z)-
8	16.540	136732	0.92	2-HYDROXY-3-[(9E)-9-OCTADECENOYLOXY]PROPY
9	16.764	55883	0.38	Octadecanoic acid, 2-propenyl ester
10	18.901	479898	3.23	1,2-BENZENEDICARBOXYLIC ACID
11	20.027	156642	1.06	9-OCTADECENOIC ACID (Z)-, 2,3-BIS[(TRIMETHYLS
12	21.034	193991	1.31	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23
13	22.080	975578	6.57	CHOLEST-5-EN-3-YL NONANOATE
14	24.431	1019773	6.87	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5,6-Dimethylhe
		14843475	100.00	

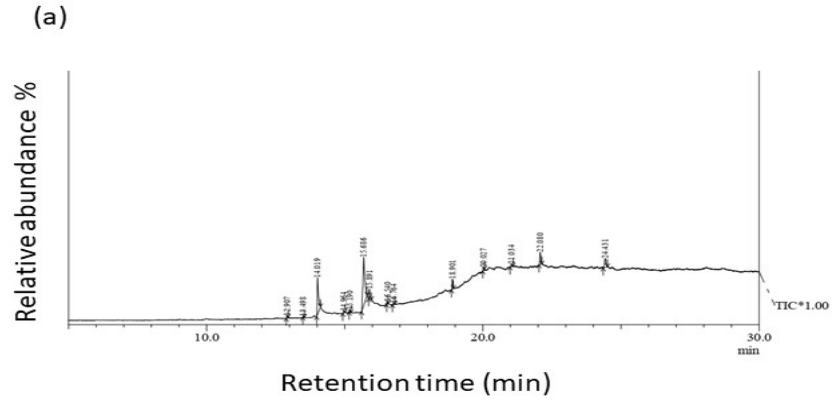

Figure 6(a): GC-MS-based molecular profiling of methanolic extracts of *Dimorphocalyx glabellus*

4.1.2. SEM- EDX Analysis

Figure 7. shows the morphology of MgO NPS. The microstructure exhibits distinct ensembles of hexagonal particles with an average size of 37.3 nm. The particles were evenly dispersed over the material's surface, exhibiting a high surface area-to-volume ratio, and they exhibited very little agglomeration.

A sample's qualitative and quantitative element composition can be ascertained by gathering and processing the EDX (or EDS) spectrum, which displays the energy versus relative counts of detected X-rays. Figure 7 (c) shows EDX result that magnesium (12%) and oxygen (87%) were the elemental compounds.

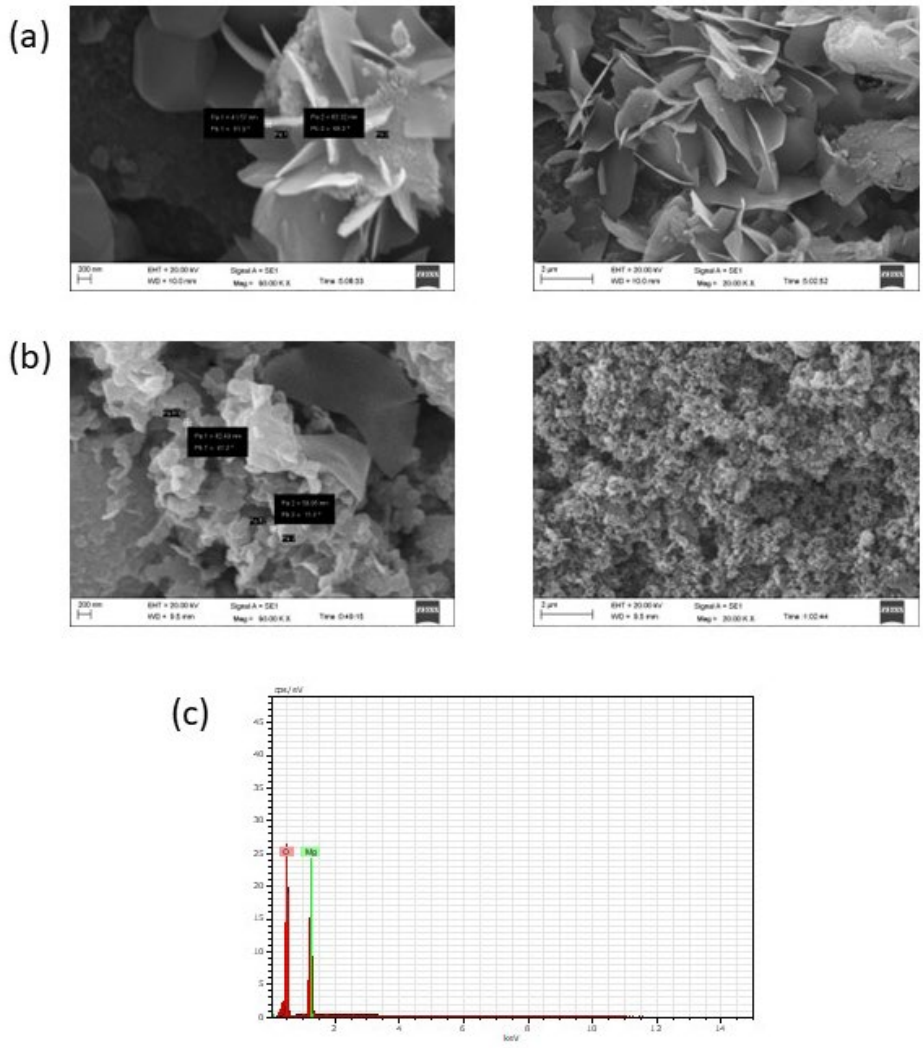

Figure 7: SEM image of Magnesium Oxide Nanoparticle (a)MgOC (b) MgOE (c) Energy dispersive x-ray microanalysis of MgO NPs

4.1.3. TEM Analysis

According to the TEM evaluation, the particles had a hexagonal shape (Figure 4). Furthermore, they had good size uniformity and were monodispersed, having an average diameter of 36.7 nm

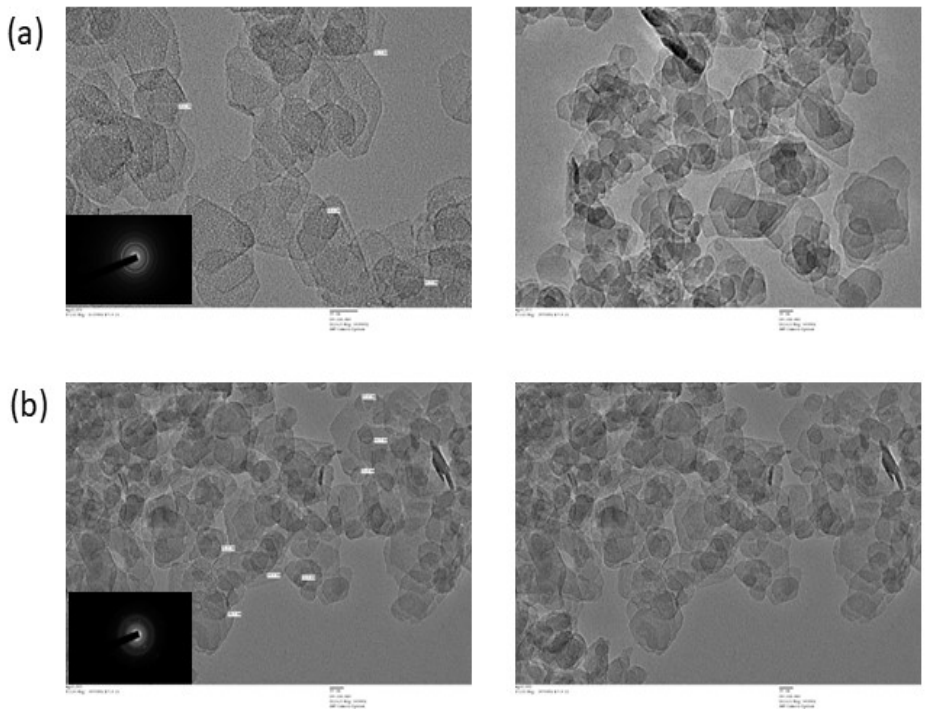

Figure 8: TEM images of MgO Nanoparticles (a) MgO-Chemical (b) MgO-Extract

4.1.4. XRD Analysis

The MgONPs were evaluated using XRD to look at the sample's phase purity and crystalline structure. The diffraction pattern has multiple peaks, as can be seen in the diffractogram displayed in Figure 5. As previously shown in the SEM and TEM data, the peaks match those in the standard reference file in terms of both angular location and intensity, confirming the creation of MgO's hexagonal phase. Moreover, the spectrum Figure 5b shows additional peaks, indicating the presence of few phytocompounds that have become a part of the crystalline system without hindering the actual lattice of the metallic nanoparticle.

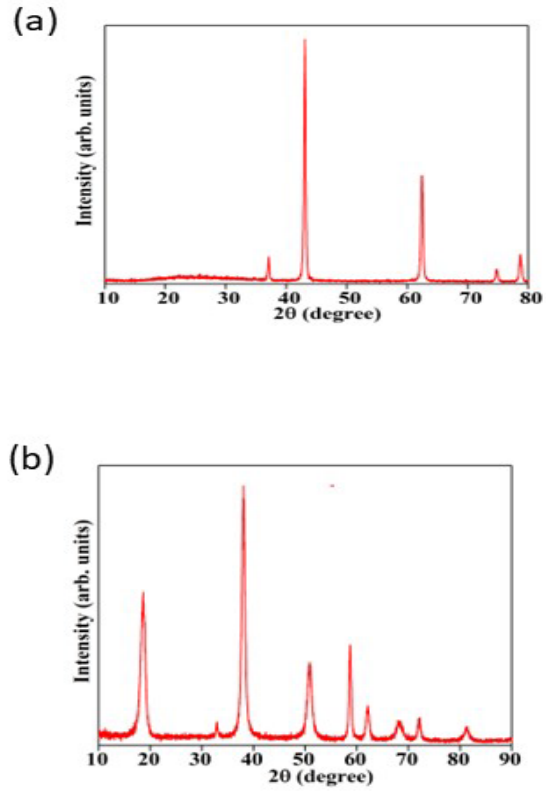

Figure 9: XRD pattern of MgO NPs showing monophasic MgO (a) MgO-Chemical (b) MgO-Extract

4.2. DPPH and ABTS Assay

The nanoparticle with the DPG extract exhibited DPPH radical scavenging activity in the order DPGE > MgOE > MgOC in a concentration-dependent manner (Figure 6a, 7a–b). DGPE demonstrated a 11.87%, 20.60%, 32.79%, 45.68%, 51.97%, 61.42%, 69.23% and 79.28% at 5,10, 15, 20, 25, 30, 35 and 40 µg/ml concentrations. While MgOC showed 15.27%, 22.37%, 30.63%, 40.21%, 51.44 and 64.57% DPPH free radical scavenging scavenged at 200, 400, 800, 1000, and 2000 µg/ml. MgOE also showed DPPH scavenging activity, with 18.61%, 28.73%, 34.88%, 46.14%, 58.86%, 68.91% at 200, 400, 800, 1000, and 2000 µg/ ml concentrations, respectively (Figure 7a). The IC50 values and the associated regression equations for DGPE, MgOC, and MgOE were 16.29 µg /ml ($Y = 2.346x– 11.7$, $R^2 = 0.973$), 988.6 µg /ml ($Y = 0.05125x – 0.6679$, $R^2 = 0.937$), and 832.87 µg /ml ($Y = 0.05043x+7.998$, $R^2 = 0.968$) respectively. These values indicated the concentration at which each extract could scavenge 50% of the DPPH radicals.

Additionally, DGPE, MgOC and MgOE demonstrated the ability to scavenge ABTS free radicals in the same order as that of DPPH radical. The ABTS radical scavenging capacity of DGPE was 22.9%, 29.66%, 44.79%, 55.92%, 82.49%, 88.05%, 96.91% and 99.69%. concentrations of 5,10, 15, 20, 25, 30, 35 and 40 µg/ml, respectively (Figure 6b), with an IC50 value of 17 µg /ml ($Y = 1.865x+2.486$, $R^2 = 0.984$). MgOC exhibited ABTS radical scavenging percentages of 27.55%, 43.52%, 54.90%, 62% and 78.07% at concentrations of 200, 400, 800, 1000, and 2000 µg/ml respectively (Figure 7d), with an IC50 value of 420 µg/ml ($Y = 0.2325x+ 1.771$, $R^2 = 0.954$). For MgOE, the scavenging percentages were 24.28%, 38.65%, 64.56%, 72.26% and 82.58% at concentrations of 200, 400, 800, 1000, and 2000 µg/ml (Figure 7f), and its IC50 value was determined to be 500 µg /ml using the regression equation $Y = 0.3038x+ 10.90$ ($R^2 = 0.9711$)

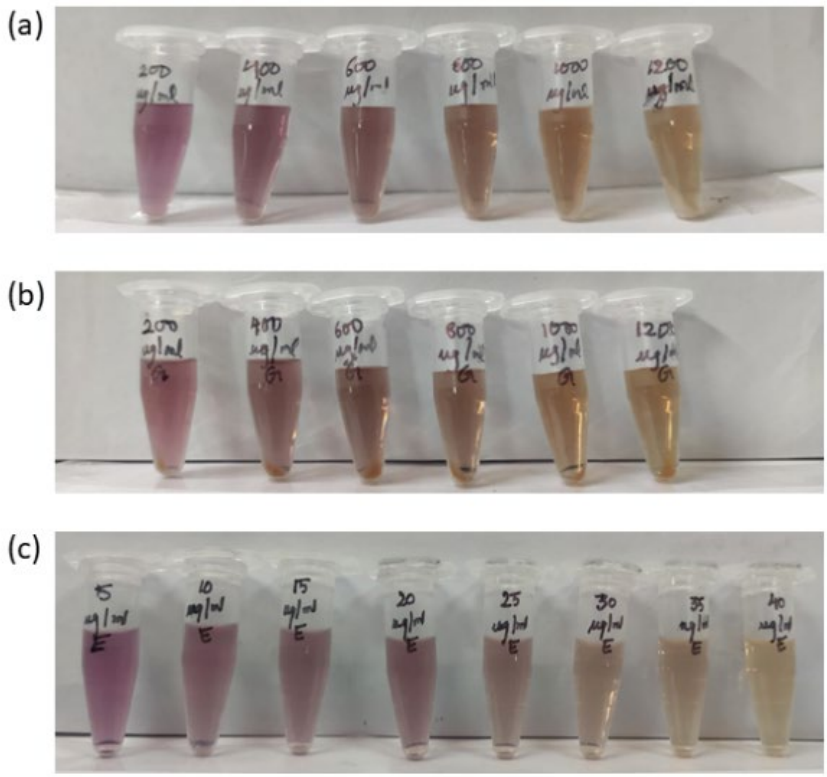

Figure 10: The observed colour changes in DPPH Assay in the serial dilutions of (a) aqueous plant extract (b) MgO-Green synthesized (c) MgO-Chemically synthesized

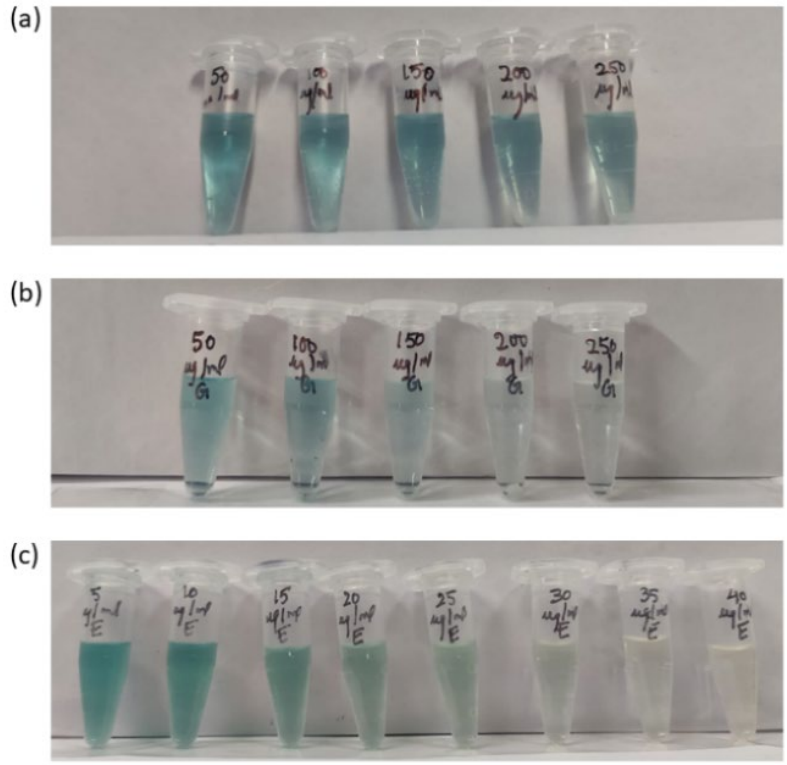

Figure 11: The observed colour changes in ABTS Assay in the serial dilutions of (a) aqueous plant extract (b) MgO-Green synthesized (c) MgO-Chemically synthesized

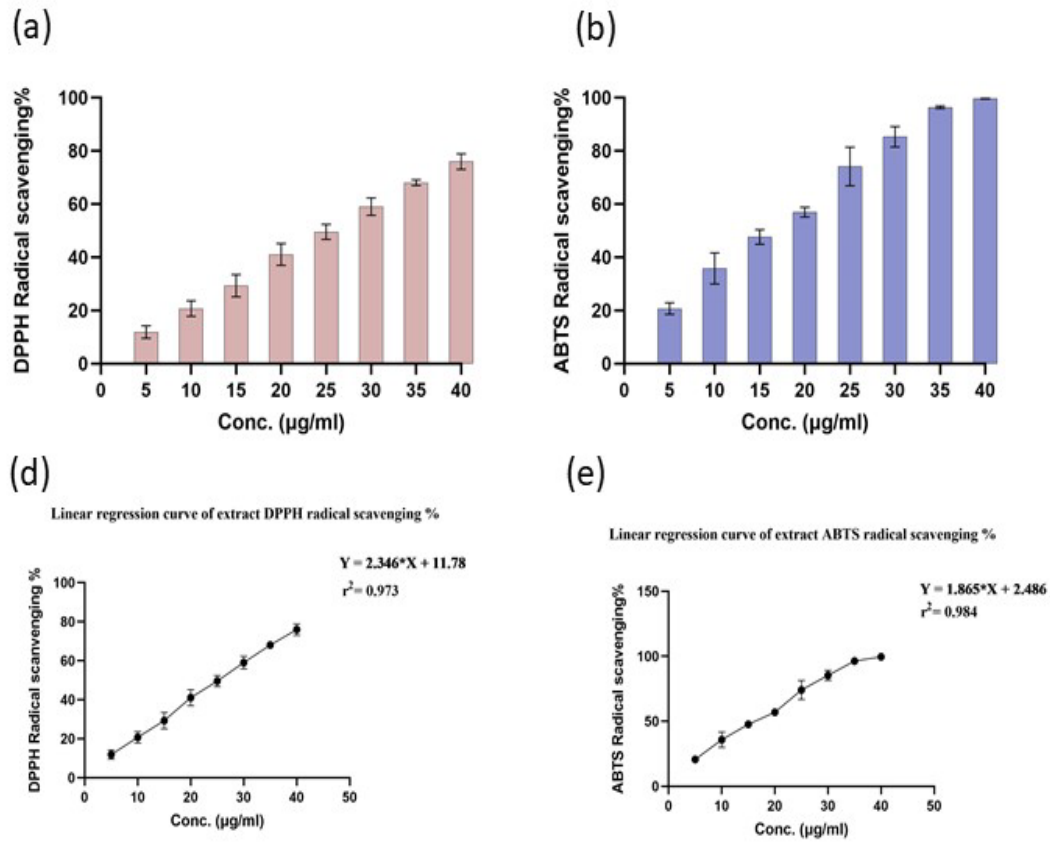

FIGURE 12: (A) DPPH RADICAL SCAVENGING ACTIVITY OF DGPE (B) ABTS RADICAL SCAVENGING ACTIVITY OF DGPE (C) LINEAR REGRESSION CURVE OF DGPE DPPH RADICAL SCAVENGING % (D) LINEAR REGRESSION CURVE OF DGPE ABTS RADICAL SCAVENGING

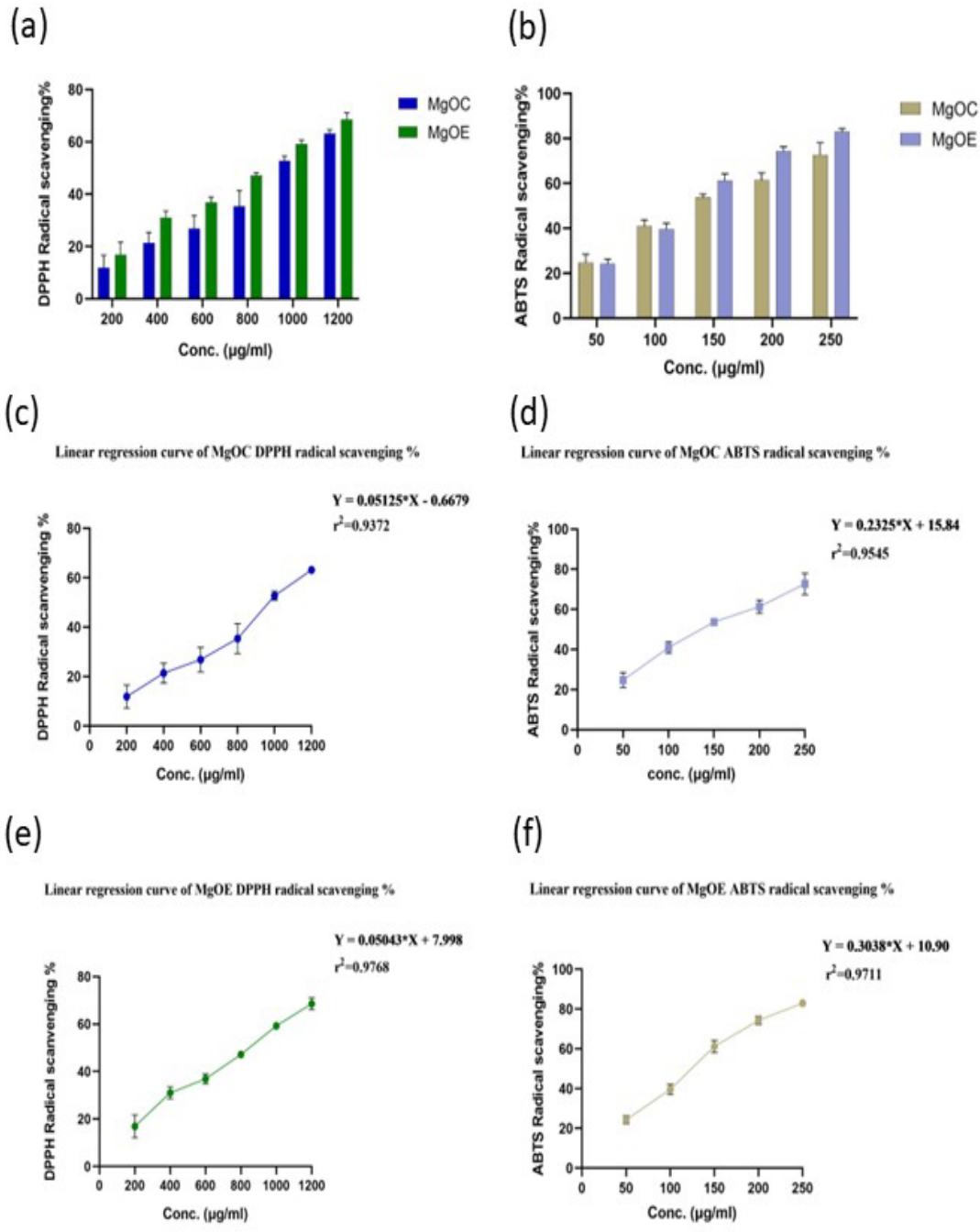

FIGURE13: (A) DPPH RADICAL SCAVENGING ACTIVITY OF MGOC AND MGOE NANOPARTICLES (B) ABTS RADICAL SCAVENGING ACTIVITY OF MGOC AND MGOE NANOPARTICLES (C) LINEAR REGRESSION CURVE OF MGOC DPPH RADICAL SCAVENGING % (D) LINEAR REGRESSION CURVE OF MGOC ABTS RADICAL SCAVENGING % (E) LINEAR REGRESSION CURVE OF MGOE DPPH RADICAL SCAVENGING % (F) LINEAR REGRESSION CURVE OF MGOE ABTS RADICAL SCAVENGING %.

4.3. MTT Assay

The results demonstrate that MgO NPs significantly and dose-dependently decreased HepG-2 viability. In HepG-2 cells, cell viability was significantly decreased to 90% at a dose of 200 µg/mL. Up to an ideal dose of 80µg/mL, which was used in this study. For the HepG-2 cell line, the IC50 value of DGPE, MgOC, MgOE were found to be 60, 75, 82 µg/mL. Consequently, it may be said that MgOE have a greater harmful effect on cancer cells than MgOC. These findings demonstrate the MgO NPs' specific cytotoxicity to tumor cells.

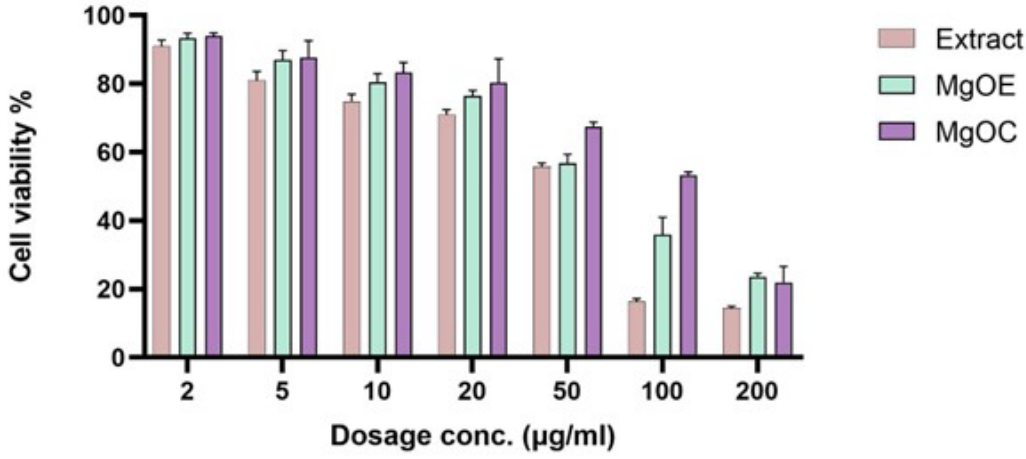

Figure 12: MTT Assay: Graph depicting the cell viability percentage as a function of varying extract and nanoparticle concentration.

4.4. Wound Healing Assay

The wound healing assay confirmed the inhibitory effects of the nanoparticles on cell migration, as suggested by a 45.33, 18.63, 6.34 and 4.9% of wound closure in cell treated with DGPE, MgOC and MgOE at 24h, which is significantly lower compared to the 78.9% of the control group. The treatment caused severe cell death, leading to the detachment of cells from the monolayer and enlargement of the scratch. The results indicate that DGPE has the ability to inhibits the migration of HepG2 cells, thereby substantiating its tumor-suppressive role in tumor.

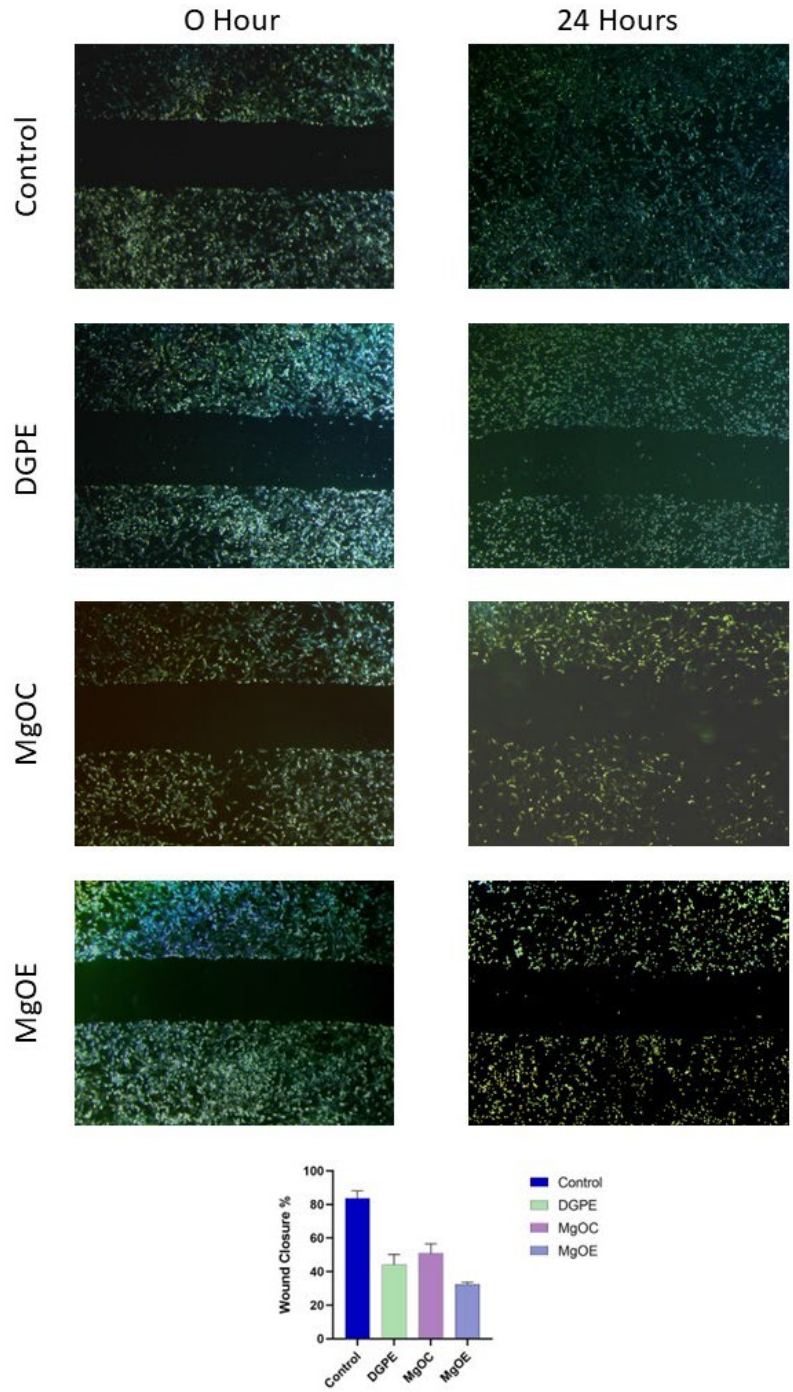

Figure 13: DGPE Extract, MgOC and MgOE inhibits cell migration in hepatocellular carcinoma cells. (a) Images of HepG2 cells exposed to 100 µg/ml of DGPE, MgOC and MgOE for 0 h and 24 h. The cell migration inhibition (wound healing) potential of each group at 24 was compared to the respective 0 h. (b) Statistical significance of changes in migration (wound closure) at 24 h.

4.5. ROS measurement

Spectrofluorometry based analysis revealed that treatment of DGPE, MgOC and MgOE in HepG2 cells, increased intracellular ROS by 2.3, 1.8, and 2.4 fold (Figure 8). The results were further confirmed by fluorescence microscopic analysis resulted in increase of intracellular ROS which were indicated by higher green fluorescence of DCF dye.

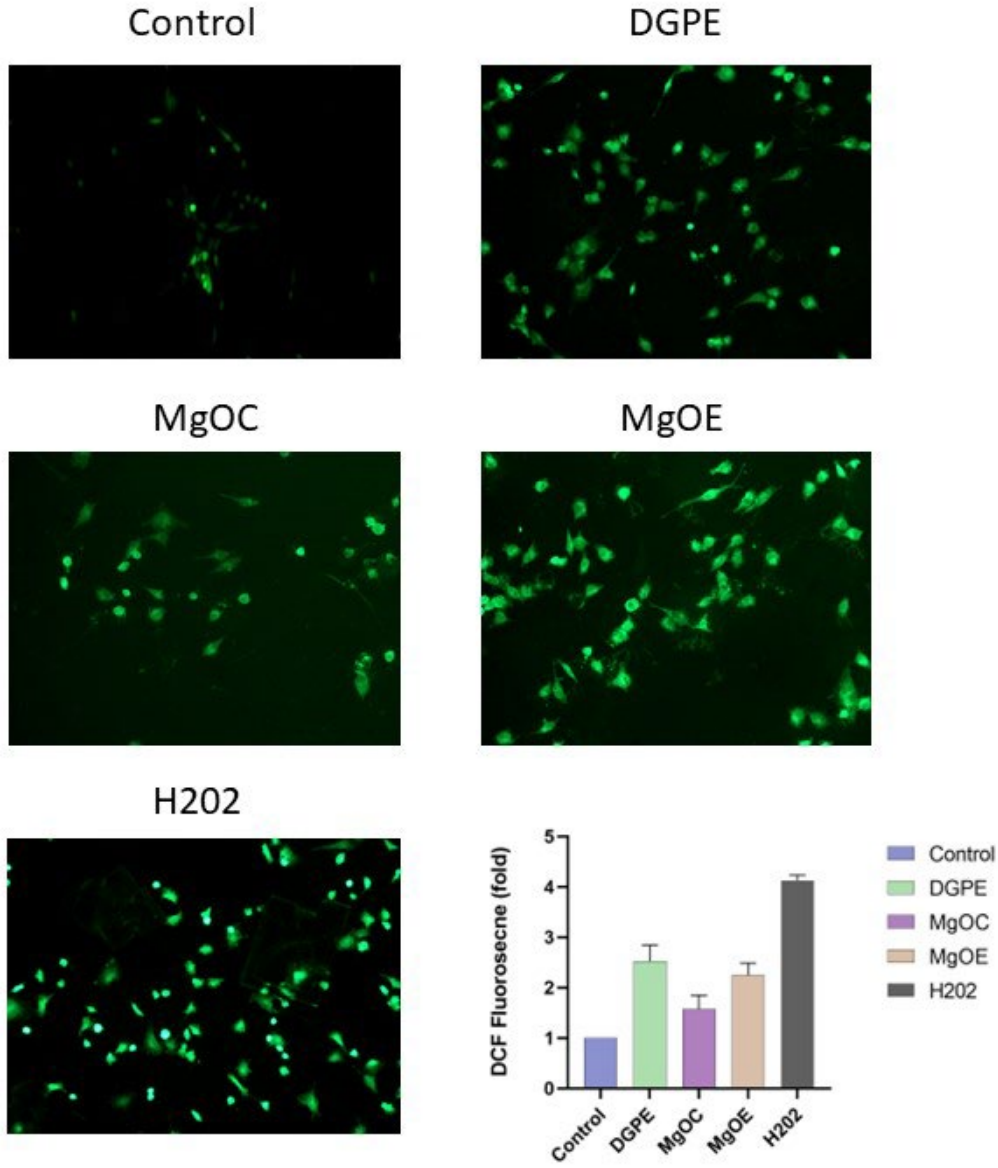

Figure 14: (f) Effect of extract, MgOC, MgOE on cytosolic ROS in HepG2 cells, treated with 100 µg/ml of for 3 h and stained with the fluorophores H2DCFDA were analyzed spectrofluorometrically for detecting variation. (a-e) Confirmation of spectrofluorometry data in (f) by fluorescence microscopy.

DISCUSSION

In Asia and Sub-Saharan Africa, hepatocellular carcinoma (HCC) is the second most common cause of cancer-related mortality, making up around 75% of cases of liver cancer (A. K. Singh et al., 2023). With a cumulative survival rate for all stages of liver cancer of only 20%, the situation is even more difficult and taxing on the healthcare system (Siegel et al., 2022).

Since, the potential anticancer activity of the aqueous extract of *Dimorphocalyx glabellus* has remained unexplored, our study aimed to investigate the potential in vitro anticancer effects in a mechanistic manner in liver cancer, HepG2 cells and antioxidant effects, using the MgO NPs synthesized biogenically with the help of aqueous extract of *Dimorphocalyx glabellus.*

In this study, we first investigated the phytochemical profiling which identified some bioactive phytochemicals in *Dimorphocalyx glabellus* listed in Table 1.

The aqueous extract of *Dimorphocalyx glabellus* outperformed the two nanoparticles synthesized chemically and biogenically in antioxidant radical scavenging assay. The nanoparticles and the extract were assessed in terms of their ability to scavenge DPPH and ABTS radicals as well as reduce Fe^{3+} and Mo^{6+}. This suggests that extract of the plant has the strongest in vitro antioxidant capability. The nanoparticle with the DPG extract exhibited DPPH radical scavenging activity in the order DPGE > MgOE > MgOC in a concentration-dependent manner and same was the case with ABTS radical scavenging activity.

CONCLUSION

The intriguing finding of GC-MS concludes that the Dimorphocalyx glabellus extract possesses antioxidant capacity in addition to bioactive substances. The information serves as a foundation for evaluating the beneficial impact of plant-based therapy in scavenging free radicals. MgO nanoparticles have been successfully characterized and synthesized using the aqueous extract of the leaves of Dimorphocalyx glabellus. The successful production of MgO nanoparticles was verified through investigations conducted using SEM, TEM, EDX and XRD. The biosynthesized nanoparticles' surface shape and crystallinity are supported by the XRD and SEM analyses. Strong anticancer properties were shown by the biosynthesized magnesium oxide nanoparticles. Therefore, this kind of synthesis process is straightforward, safe, and non-toxic, and it will find utility in pharmaceutical applications.

The study comes to the conclusion that biosynthesized magnesium oxide nanoparticles have improved anticancer and antioxidant qualities, indicating their potential for medicinal uses. According to our research, Dimorphocalyx glabellus, exhibits encouraging antioxidant and hepatocellular carcinoma-fighting properties in vitro. In addition to offering an ecologically acceptable substitute for traditional chemical procedures, this green synthesis strategy produces nanoparticles with enhanced biological activity, opening new avenues for future investigations and advancements in the field of nanomedicine.

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