Assessment of Antioxidant, Anticancer, and Antibacterial Properties of Plant-Mediated Green Synthesized Magnesium Oxide Nanoparticles Derived from *Calamus palustris* Fruit Peel Extract

Javith J[1], Vinothini Gunasekaran[2], Chitra Shivalingam[3], Saheb Ali[2*]

[1]Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai.
[2]Department of Biomaterials (Prosthodontics), Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai.
[3]Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai.
Corresponding Author:
Dr. Saheb Ali
Department of Periodontics
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Chennai- 600 077
Corresponding Author Mail ID: sahebali.sdc@saveetha.com
Corresponding Author ORCID ID: 0000-0003-3427-9081

Abstract

Nanoparticles often exhibit distinct and enhanced physical, chemical, and biological properties compared to their bulk counterparts. These unique attributes enable the engineering of nanoparticles from diverse materials such as metals, semiconductors, polymers, and ceramics, with broad applications in medical fields. In this study, magnesium oxide (MgO) nanoparticles were synthesized using a green synthesis approach mediated by *Calamus palustris* fruit peel extract. The synthesized MgO nanoparticles were characterized through X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and UV-visible spectroscopy. The biological activities of the synthesized MgO nanoparticles were evaluated through antioxidant, cytotoxicity, and antibacterial studies. Antioxidant activity demonstrated significant free radical scavenging potential, with 19.66% activity at low concentrations and 63.86% at higher concentrations. Cytotoxicity was assessed using XTT and Neutral Red Uptake (NRU) assays, confirming dose-dependent cytotoxic effects. The MTT and NRU assays further demonstrated that MgO nanoparticles effectively induced apoptosis in head and neck squamous cell carcinoma (HNSCC) cells. The antibacterial activity of MgO nanoparticles, assessed using the well-diffusion method, revealed potent efficacy against bacterial pathogens, while genotoxicity studies confirmed their non-toxic nature. The findings indicate that plant-mediated MgO nanoparticles are promising as effective therapeutic agents for treating HNSCC by inducing apoptosis via physiological pathways. This study also highlights

the potential of *Calamus palustris* fruit peel extract as a novel and sustainable approach for the synthesis of tunable MgO nanoparticles, paving the way for further research in nanomedicine.

Key words: *Calamus palustris* , Antioxidant activity, Cytotoxicity, Genotoxicity, Antibacterial activity.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is sixth most prevalent cancer in the world. Majority of carcinomas developed in lower lip, tiny cancer on the ventral region of the tongue, and melanoma cramped to the grating to perform a better prediction, but persistence of patients with cancers in additional area impoverished. In 2023, 630,000 peoples were affected in this type of cancer and the expected amount of death ratio around 350,000 [1]. HNSCC in India was the general type of melanoma in males that might be developed due to people's way of life threats, as well as cigarettes, tobacco, alcohol and areca nuts. For cancer treatment, surgery, chemotherapy and radiotherapy are generally used. These all treatment has various side effects like loss of saliva, oral cavity necrosis, hypothyroidism, headache and dizziness. In order to overcome this side effects, nanoparticles mediated target delivery has showed promising results against cancerous cells [2].

Now a days, researchers have find several properties behind nanotechnology and science which plays a predominant part in this period. Nanotechnology clasp pledge for several advancements and play as a fundamental blocks for attracting researchers to study several fields. Nanomaterials are structural constituents with the scale level of 1-100nm nanometer. Nanoparticles are find in different shape and morphology like as spiral, flat, conical, hallow etc [3]. MgO NPs was effectively used in magnesium batteries, catalysts, biosensors, and toxic waste water removal [4]. Likely, metal oxides like as CuO, ZnO, MnO_2, TiO_2 and CoO_2 might be find. The diverse functional properties of MgO has properties like as nontoxicity, high-unique exterior position, and eco-friendly nature these kind of features attracted researcher's interest globally [5]. MgO nanoparticles have strongly used in a wide scope of bio-implementations such as gene delivery and drug, cell and tissue engineering and therapeutic uses etc. Along with these implementations, determination of the antibacterial and anticancer properties which leads to a less economic and a simple medicine with less size effects [6].

Till now, various techniques were used for the preparation of nanoparticles like as surface mediated method, solvent free method, precipitation method, electrochemical method, liqid-phase chemical precipitation method, ultrasonic method, and membrane-mediated precipitation method. Hence above mentioned methods has increase outcome capacity, but also they includes demerits likes as low energy ratios, needs of intricate, monotonous, moderate and harmful reaction [7]. However, needs to induce the dependable and well organized synthetic methods to restrict or reduce the above mentioned conditions *via* green nanotechnology. In fact, plant extract mediated synthesis nanoparticles has interesting functions like as non-toxic, quick synthesis protocol and less economic. The plant extract mediated nanoparticles develops an efficient way for synthesis with the help of herbal stabilizing and reducing substances [8].

Calamus palustris belongs to the family of Palmae, *C. palustris* rhizomes has been used as single tranquil or composites of definite tranquil diposition in the Indian traditional medication for psychoneurosis like sleeping disorder, and loss of memory. Additionally, this plant parts are used for fever, headache, sadness, hemorrhoids, stomachic, cancer, skin maladies, and emetic [9]. Therefore, the current research is focused through using *Calamus palustris* leaf extract as reducing and stabilizing agents for synthesis of MgO nanoparticles for the antibacterial and anticancer (Head and neck squamous cell carcinoma) implementations.

2. Materials and methodology

2.1 Materials

Fresh and healthy fruits of *C. palustris* were collected from rainforest of Andaman and Nicobar Islands, India along with latitude 11.9761◦N and longitude 92.9876◦E [10].

2.2 *C. palustris* fruit extract preparation

Collected fruits were chapped into tiny pieces after eliminating its peel, then it was rinsed completely using DD water and shade dried for 2 weeks. After it was made into fine powder was collected mechanically through using electrical blender. Then the extract for synthesis of MgO NPs is obtained via easy green chemistry approach, 10g of fresh fruit power was mixed with 200ml of DD water and kept in boiling water bath for about 60 °C for 10mins. Then the extract was cooled after it was filtered by using Whatmann No.1 filter paper and then mixture was stored in 4 °C for following analysis [10].

2.3. Green synthesis of MgO NPs

0.5 M of Mg $(CH_3COO)_2$-$4H_2O$ was completely mixed with 100ml of *C. palustris* extract. Then the solution was stirred at 303K for 2 h till the clear solution was procured. The plant extract was act as a liquid, during the acetate in the precursor act as the oxidizer. The clear suspension was kept under microwave oven for 20 mins on the conduction step. After completion of the process, pure form of MgO nanoparticles powder was calcinated for 2h at 673 K [10,6].

2.4 Characterization of MgO NPs

The phase pureness, crystalline structure and lattice parameters of the obtained MgO NPs were determined and analyzed by using XRD technique via Defray-401 (JSC Scientific Instruments, Russia), at various 2θ ratios utilize chromium (Cr) as inception. The XRD pattern was carried out by 0.02 scan size at I s/mode analyzing ratio [11]. FTIR (Nicolet 380 Thermo Scientific, USA) techniques were utilized to analyze the formation of various functional components in the MgO NPs sample [12]. For FTIR analyses the sample were prepared through using KBr pellet approach. The FTIR spectrum were determined in the scale of 400-4000 cm^{-1} [13]. The MgO NPs sample were determine through SEM (Vega 3; TESCAN, Czech Republic) coupled with the EDX instrument (SDD-XMAS, Japan) to confirm the morphological shapes and elemental filling of the sample. For SEM analysis, 0.01g of MgO NPs was dispersed in 10ml of ethanol and the suspension was regulated by using sonication process for 30 mins. Then the sample was placed in carbon tube which is attached with SEM holder. After, a drop of the sample is completely dried then the sample was determined through regulating the powder of 10Kv [14]. The morphology of synthesized MgO NPs sample were confirmed through transmission electron microscope JEOL (TEM, JEM-2010, JEOL, Japan). For analyzing, 0.03g of obtained sample was dispersed in distilled water then the solution were sonicated for 10mins. Then, a single drop of NPs sample was coated on carbon-coated grid then it was dried at room temperature [15]. Synthesized MgO NPs absorption frequency were calculated by using UV–Vis spectrophotometer (Cary 8454; Agilent Technologies, Singapore). Absorption frequency were measured via visible and infrared wavelength scale of 180-800nm [16].

2.5 *In vitro* toxicity study

For *in vitro* toxicity study, OECD-203 protocol was followed. For analyzing sample 25 numbers of healthy undamaged zebrafish embryos were exposed to different ratios of (20, 40,

60, 80,100 and 120µg/ml) synthesized MgO NPs. Then the sample treated embryos were moved to 100ml of Hank's solution. After, the solution was moved to individual wells for the development of the organelles like head, tail, heart, eyes, and invertebrate columns were seemed by using 40x microscope at every 8h duration. During this assay, the water was regulated at particular temperature (24 °C). Total amount of mortality and dead eggs were regulated for every 8h time period and the death egg were eliminated in the test solution in order to restrict contamination. Death and hatching ratios of embryos were calculated for all the mentioned concentrations [17].

2.6. Antioxidant activity

Antioxidant activity is determined by using the di (phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) approach for MgO NPs [18]. Synthesized MgO NPs different ratios of (20, 40, 60, 80, 100 and 120µg/ml) were applied to determine the dose-mediated activity. Powder sample were placed in a vial along with various ratios of MgO NPs. 1.7ml of DPPH solution was poured drop wise through vortex for 3 mins. 1.7ml DPPH alone without MgO NPs used as control (C) and the vials including with MgO NPs plays as test sample (T). After, the solution was allowed to incubation at room temperature at 30mins. The supernatant solution is obtained at 11,963g for 2mins and then the optical density (OD) is evaluated at 517nm by using UV–Vis spectrophotometer (U-2900/2910; Hitachi, Japan) [19]. The total amount of free radical scavenging is evaluated using the control via following equation:

$$\% \text{ of DPPH scavenging} = \frac{CA\text{-}TA}{CA} \times 100$$

Since, CA and TA are mentioned as control absorbance and test absorbance.

2.7 Cytotoxic activity

2.7.1 MTT assay

Head and neck squamous cell carcinoma (HNSCC) cell line were applied in this assay. Cytotoxic activity of MgO NPs is determined through using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test [20]. For this assay, 150ml of cells were moved to 96-well plate (Becton Dickinson (BD), USA) at the solidity of 1×10^4 live cells/well and incubated for 24h for the cell attachment. During the attachment, the culture was changed with absolute medium (150ml/well) including MgO NPs (20, 40, 60, 80,100 and 120µg/ml) for 72h. Then the

cells were rinsed by using PBS and incubated along 100ml/well prepared media including 0.5mg/mL MTT. The MTT including culture is eliminated after 3h incubation on dark chamber. After, MTT formzan was diluted on 100ml/well DMSO and optical solidity is evaluated using ELISA plate reader (Bio-Tek, USA) [21]. The cell viability is evaluated through following formula:

$$\text{Cell viability (\%)} = (A_S/A_{Control}) \times 100$$

Since, A_S was the absorbance of the cell treated with MgO NPs and $A_{Control}$ was the absorbance of the cell incubated with the cell media alone.

2.7.2 NRU assay

The neutral red test (NRU) was determined by the identification of viable cells via uptake of dye. Viable cells has the capacity to intake of neutral red by vigorous move, comprise and attach to the dye towards its lysosomes whereas mortality or non-live cells are not possible to uptake the chromophore dye. After the cells are washed using PBS solution and the live cells are discolored the integrated dye during acidification filtered process. The amount of dye was applied to determine the total ratio of live cells. In this assay, the cytotoxic properties of synthesized MgO MPs at various concentrations (20, 40, 60, 80, 100 and 120µg/ml) was determined. The HNSCC cells are seeded in 96 well plates along with various ratios of MgO nanoparticles. Then the culture were incubated at room temperature for 2 h on a CO_2 humidified chamber. The NRU was analyzed through dissolving the stock 4mg/ml ratio through adding PBS solution in the ratio of 1:100 and solution kept under incubation at room temperature under the condition of 5 % CO_2. 100µl of NRU dye is added to the culture plate was incubated for extra 4 h at room temperature. After incubation, extra NRU dye was vanished and then 150µl solvent was added to plate. Then the solvent extract and NRU included with the cancer cells. Homogenously mixed using shaker to get extract of NRU dye and the absorbance was monitored at 540nm using ELISA plate reader (22, 23].

2.8 Genotoxicity assessment

2.8.1 Comet assay

Genotoxicity of MgO NPs is evaluated through using comet assessment, depends in the estimation of DNA movement under electrophoresis followed with few modification stated by

[24]. Then the cells were treated to MgO NPs (20, 40, 60, 80, 100 and 120µg/ml) for 24 h. the MgO NPs exposed cancer cells are then trypsinized to develop a single cell culture and 180ml of 1% normal melting point agarose (NMP) is gelled completely frozen (25nm) and 100ml 0.5% low melting agarose (LMP) including cell solution (20ml) was covered on the surface of the NMP agarose. Then the developed cell solution including surface, adding with 100ml of LMP agarose is transferred to the extra hole to develop an extra surface layer to enhance the space to the cells and gel layer. After, agar get solidification, the cells were kept on a lysis suspension [2.5 M NaCl, 100mM EDTA, 10mM Tris HCl (pH 10), 1% Triton X-100 and 10% DMSO are moved few minutes prior use) for 24h at 4 °C incubated using alkaline electrophoresis for 35 mins and kept in electrophoresis buffer (300mM NaOH/1mM EDTA, pH >13) and electrophoresed for 30 mins. Then, the comet were developed, then the alkaline gel was neutralized through washing the slides using buffer (0.4M Tris HCl, pH 7.5) 3 times. Then the slides were colored using SYBR Green I (SG) fluorescent dye then the amount of DNA breakage is calculated for each concentration [25].

2.9. Antibacterial activity

Various infection causing microbes were used to analyze the antibacterial properties testing by using gram-negative bacteria, namely *Escherichia coli* (MTCC 1692), and gram-positive bacteria, namely *Streptococcus pneumoniae* (MTCC 1935). Newly prepared nutrient media is incubated and allowed for incubation for 8 h at room temperature by using the above bacteria media to obtain an inoculum for antibacterial activity [26]. For this study Kirby-Bauer disk diffusion approach is utilized to determine the antibacterial features. Newly obtained inoculums were spread over the exterior of sterile Muller Hinton agar plates. Additionally, 5mm diameter disk were filled with various ratios of synthesized MgO NPs then the plates are placed on incubation for 24 h at room temperature. After, incubation time, the plates are seemed for antibacterial properties depends on the development of inhibition zone [27]. The plates are marked as disk A (Control), disk B (MgO NPs in various concentration 20, 40, 60, 80, 100 and 120µg/mL), disk C (leaf extract), and disk D (standard antibiotics).

3. Results and Discussion

3.1 Synthesis and chemical characterization of MgO NPs

The phytochemical components appears in the *C. palustris* fruit was seemed to be essential for the obtaining of MgO NPs. These phytochemical components plays a major role in

conversion of Mg acetate to MgO NPs. The obtaining process of MgO nanoparticles concluded through color alterations on the prepared solution from brownish red precipitation was seen in Fig.1. Then the obtained precipitate is further heated (80 °C) for 2h to attain MgO nanoparticle.

The phase, purity determination and shape of the synthesized MgO NPs was analyzed by XRD analysis. The formation of XRD spectrum speaks was shown in Fig. 2a and the obtained results depicts the shape intensity ~18.49° (101), 37.71° (200), 51.05° (102), 58.71° (220), 62.26° (311), and 72.22° (222). For particles confirmation the XRD results was matched with the JCPDS file no.076-0704 and the results depicted synthesized nanoparticles are hexagonal tightly packed structure (*hcp*) concluded for the development of crystalline nature of MgO NPs [28].

Fig. 2b reveals that the *C. palustris* fruit mediated synthesized MgO NPs, the FTIR results shows various IR spectrum frequencies like as 3673, 3270, 3010, 2353, 1988, 1614, 1405, 1061, 641, and 560 $cm^{-1.}$ Wavenumber at 3673 shows the formation of OH band due to presence of carbohydrates, proteins and polypeptides. IR spectrum at 3270 (NH_2), 3010 (CH stretching), 2353 (C-N group), 1988 (C=O), and 1624 (C=C group) shows the presence of amino acids, alkane, amide, ester groups and unsaturated compounds. In addition some of peaks seen at 1405 (C-O-C), 1061 (C-O), 641 (CH), and 560 (CH) and the peaks shows the presence of polysaccharides, carbohydrates, aromatic bands, and alhyl halides. The obtained results are matched with published literature of confirmation of synthesized MgO NPs functional compounds [29].

SEM analysis confirms that the *C. palustris* fruit synthesized MgO nanoparticles are irregular shape with the size scale of 50 to 748nm and the average nanoparticles size is ~247nm and the results was shown in Fig. 2 c-d. The absorption wavelength of the fruits extract medicated synthesized was seen at 219nm and 312nm (Fig. 2e) and the obtained results are matched with the published date for confirmation of MgO NPs [30,31].

3.2. *In vitro* toxicity studies

Various concentration of MgO NPs are diluted in Hank's solution for *in vitro* toxicity assay. The embryonic mortality of live zebrafish embryo was analyzed after the treatment of synthesized MgO NPs. Fig. 3a reveals that the MgO NPs treated embryos and hatched eggs at 24 and 72 hpf are seemed by using 40x magnification (light field microscope). The outcome of this study was confirmed that the higher ratio of MgO NPs treated eggs have delayed hatching time and less death rate in the prior stage of exposure. Fig. 3 a,b and Table 1 reveals that the obtained

MgO NPs exposed (20µg/ml) embryonic sample showed 1.5% mortality whereas at higher ratio of (60µg/ ml) showed 4.8% mortality rate. 72 h treated MgO NPs exposed eggs are seemed by using microscope for clear observation of tail, head, eye, internal vertebrates formation and deformations, whereas sample treated eggs at 92 and 120h reveals that the maximum of embryos are hatched. Fig.3a depicts that the toxicological analysis of MgO nanoparticles in the developmental period eggs shows the death ratios are effectively low. Fig. 3a, b and Table 1 depicts that the MgO nanoparticles have no toxicological properties in the zebrafish study [32].

Table 1. *In vitro* toxicity studies of zebrafish death percentage from *Calamus palustris* fruit extract synthesized MgO NPs towards different time and dosage.

	Concentration (µg/ml)	Mortality (%)				
		24 h	48 h	72 h	96 h	120 h
MgO NPs	Control	0.1 ± 0.4	0.7 ± 0.2	0.5 ± 0.3	0.3 ± 0.4	0.1± 0.3
	20	0.3 ± 0.2	1.3 ± 0.7	1.0 ± 0.4	0.4 ± 0.7	0.0 ± 0.3
	40	0.8 ± 0.3	1.8 ± 0.5	1.5 ± 0.3	1.0 ± 0.3	0.0 ± 0.7
	60	1.1 ± 0.6	2.0 ± 0.4	2.4 ± 0.7	1.3 ± 0.4	0.0 ± 0.2
	80	1.5 ± 0.9	1.3 ± 0.8	4.8 ± 0.3	1.7 ± 0.7	0.0 ± 0.1
	100	0.3 ± 0.3	0.7 ± 0.6	1.9 ± 0.2	1.3 ± 0.8	0.0 ± 0.2
	120	0.2 ± 0.2	0.2 ± 0.5	1.4 ± 0.1	0.7 ± 0.3	0.0 ± 0.1

The *in vitro* toxicological result showed low toxicological results was confirmed by the similar published data [33,34] The obtained results concluded that the synthesized MgO NPs effectively used for biomedicine and microbial disease treatment. .

3.3 Antioxidant activity

. The free radical scavenging percentage of synthesized MgO NPs via the DPPH assessment. The assay is depends on time and concentration reaction. The synthesized MgO NPs

free radical scavenging was depicted in fig.4 and table 1. This assay is depend on dose and time mediated activity. Figure depicted that the fruit extract exposed sample shows 16.80% in (20µg/ml)

Concentration (µg/ml)	Ascorbic Acid	MgO NPs
20	16.80	19.66
40	23.33	29.90
60	30.16	37.33
80	39.20	44.16
100	48.06	58.90
120	53.93	63.86

scavenging activity and 53.93% in (120µg/ml) scavenging percentage in standard ascorbic acid solution whereas synthesized magnesium oxide nanoparticles showed at (20µg/ml), 19.66% and in higher concentration (120µg/ml), shows 63.86% of free radical scavenging activity. For confirmation, the obtained results are matched with published data and the data concluded that the procured results have higher percentage of activity [25, 19].

Table 2. Antioxidant activity of synthesized MgO NPs synthesized from *Calamus palustris* fruit extract.

3.4. Cytotoxic assessment

3.4.1. MTT assay

The MTT test was common test for the determination of cytotoxicity of synthesized MgO NPs against cancer cells. This study was analyzed in the human head and neck squamous cell

carcinoma cells using various concentrations of (20–150µg/ml) synthesized MgO NPs. After the exposure with the various ratios of nanoparticles, the exposed cells are subjected to MTT study to picturing the activities of MgO NPs in the cells depends on the optical density calculations. This assay used to evaluate the cell viability in the cancer cells line by using cellular activities. Fig. 5 depicts that the ratio of MTT was directly proportional to the reduction of cell viability. 20µg/ml concentration of MgO NPs shows 93.90% of cancer cell mortality whereas at 120 µg/ml shows 30.89% at 6h. In 24 h treated cancer cells shows at 20µg/ml up to 81.90% of cell reduction whereas at 120µg/ml shows 16.89% of cell reduction and the results concluded that the activity was depends on time and concentration reaction. For supporting the data, the obtained results are well matched with published literature and the data concluded that the synthesized MgO NPs might effectively destroy the cancer cells [35, 36]

3.4.2. NRU assay

NRU assessment was applied to evaluate the cytotoxic features of synthesized MgO NPs on the HNSCC cancer cells through determine the level of live cells. When the viable cells are exposed to HNSCC cells to neutral red dye, the dye was started to destroy in the process of lysosome due to pH difference on lysosome and intracellular cytoplasm. The cell viability was determined by using absorbance of dye by the cell. Fig. 6 depicts that the NRU was also time and concentration mediated property. In low concentration 20µg/ml treated sample shows 95.79% and 120µg/ml shows up to 33.81% of dye absorption at 6h, whereas in 24h MgO NPs treated sample shows at 82.80% in 20µg/ml concentration and higher concentration at 120µg/ml sample shows 18.98% of NRU dye absorption which reveals that the higher ratio of cell mortality. The obtained NRU results revealed closer similarity to MTT assay, which showing the mitochondrial and lysosomal damage happens in both assay because of the similar cellular death pathway [37, 38]. The HNSCC cell mortality can be mediated to the MgO NPs formation resulting to the oxidative stress induced cellular death.

3.5. Genotoxicity assessment

3.5.1. Comet assay

Comet assay was standard method and specific test for evaluating genotoxicity. This assay calculate the entire tail length and also damaged and non-arranged morphology are depicted by using genomic components of the damage cells while the migration on the agarose gel [39]. For this assessment, the Head and neck squamous cell carcinoma cell lines treated with

synthesized magnesium oxide nanoparticles are applied to determine the raises in tail length (DNA movement) on the agarose gel. Fig.7a-b depicts that the comet assay results obtained through using cancer cell line treated to different concentrations of (20, 40, 60, 80, 100 and 120µg/ml). Fig. 7a reveals 88.90% damage of genomic DNA at low concentration (20µg/ml) and 15.98% of damage in increase concentration (120µg/ml). Fig. 7b depicts 10.89% olive tail movement at low concentration (20µg/ml) and 78.98% movement at higher concentration (120µg/ml). For confirmation, the obtained results are matched with published literature [40,41] and the data showed 22% and 5.37% of olive tail movement at 20 µg/ml and 8 µg/ml, based on time and concentration based activity. Hence, the current study reveals closer range of DNA damage and olive tail movement [42]. The cell damage raises when the concentration was significantly increases which shows that the increase ratio of synthesized MgO nanoparticles was applied for the cancer cell line resulting to cellular death. The DNA strand breakage after exposure of MgO NPs reveals the cancer cells are undergone apoptosis. The MgO NPs concentration raises the reactive oxygen species at the exterior area, which resulting to the development of free radical formation inside the cell it leads to cancer cell death [43,44].

3.6. Antibacterial activity

Antibacterial activity of the synthesized MgO nanoparticles was analyzed towards different human infection generating bacteria and the procured results are depicted in Fig. 8, 9, and Table. 3 The antibacterial properties of MgO nanoparticles towards gram positive bacteria like as *S. pneumonia* MTCC 1935 and *Staphylococcus aureus* MTCC 7443, and Gram-negative bacteria like *P. aeruginosa* MTCC 2582 and *E. coli* MTCC 1692 were tested in this activity. The procured zone of inhibition was observed to be *Staphylococcus aureus* MTCC 7443 (18 ± 0.56 mm) and for *S. pneumonia* MTCC 1935 (22 ± 0.24mm). Closely, the zone of inhibition for Gram-negative bacteria was seemed to be *P. aeruginosa* MTCC 2582 (25 ± 0.60mm), and for *Proteus vulgaris* MTCC 744 (23 ± 0.80mm) in a 120µg/ml concentration. For comparison, all the test bacterial samples were differentiate and the maximum zone of inhibition was seemed for *S. pneumonia* MTCC 1935 (22 ± 0.24mm) and *P. aeruginosa* MTCC 2582 (25 ± 0.60mm). The variations in the zone of inhibition shows a stimulated percentage of property.

Table 3. Antibacterial activity of MgO NPs studied towards various pathogens

Microorganisms	Zone of Inhibition (mean ± SD (mm))			
	DMSO	Leaf extract	MgO NPs	Standard Antibiotics
S. epidermidis MTCC 2639	--	8 ± 0.82	18 ± 0.56	15 ± 0.78
B. subtilis MTCC 1133	--	12 ± 0.18	22 ± 0.24	18 ± 0.93
P. aeruginosa MTCC 2582	--	15 ± 0.10	25 ± 0.60	22 ± 0.58
E. coli MTCC 1692	--	18 ± 0.32	23 ± 0.80	19 ± 0.49

Prior studies concluded that the metal oxide nanoparticles startlingly rupture the cell membrane and then enter inside the cell [45]. Form that prior study stated that the low ratio of magnesium oxide nanoparticles shows insufficient to generate H_2O_2. The MgO NPs at low concentration does not generate toxic features in humans. Daily consumption of Mg through food was a needed component for metabolic properties [46]. MgO has a properties towards intestinal tract breakage from *E. coli*. The pH on the stomach level from 2 to 5. When MgO connect to acid, Mg ions are developed. This ions initiate the enzymes like as carbonic anhydrase carboxyl peptidase, and alcohol dehydrogenase which are needed factors for alcohol and carbohydrate digestion [47]. Fig. reveals that the MgO nanoparticles has two main properties: 1. Formation of ROS, and 2. Stimulation of cell mortality. The MgO nanoparticles induces ROS genesis inside the cell and stimulate oxidative stress, which leads to rupture of cellular constituents like lipids, proteins, and DNA. Moreover, MgO nanoparticles induces toxic effects inside the bacterial cells which leads to cell death was represented in fig. 10 [48].

4. Conclusion

Present research was intended to analyze the significant implementation of MgO nanoparticles as an anticancer drug. The MgO nanoparticles are synthesized through green chemistry method by using the extract of *Calamus palustris* . The synthesized MgO NPs are polycrystalline structure that is concluded via XRD analysis. FTIR analysis confirmed that the

various functional compounds present in the obtained MgO NPs sample. The SEM analysis results concluded irregular shape. The obtained antioxidant activity showed better free radical scavenging activity. The MgO NPs cannot cause zebrafish embryo mortality which concluded that the synthesized particles are safe to use human healthcare applications. The cytotoxicity of MgO nanoparticles was analyzed to found cell-mortality cycle exposed through head and neck squamous cell carcinoma cell line. MTT and NRU depends on mitochondrial and lysosomic cytotoxic assessment conclude the cytotoxicity of MgO NPs was time and dose mediated activity. The comet assessment depicts that the synthesized MgO NPs stimulate ROS formation that might cause DNA breakage which resulting in apoptosis mediated cancer cell death in head and neck squamous cell carcinoma cell line. In conclusion, the experimental results evidently concluded that the fruit extract mediated MgO NPs might be an effective anticancer drug. This research not only synthesis MgO NPs by using phytochemicals of *Calamus palustris* but also found to be substitute for generating a non-toxic, eco-friendly potential anticancer drug.

5. Novelty

This study demonstrates a novel, eco-friendly approach for synthesizing magnesium oxide (MgO) nanoparticles using *Calamus palustris* fruit peel extract, revealing their multifunctional potential. The research highlights the dual role of phytochemicals in facilitating MgO nanoparticle synthesis and enhancing biological activities. Characterization results confirm the hexagonal crystalline structure and functional groups integral to the nanoparticles' activity. The study uniquely integrates diverse assays to evaluate antioxidant, cytotoxic, genotoxic, and antibacterial properties, showing significant free radical scavenging, effective apoptosis induction in head and neck squamous cell carcinoma cells, and potent antibacterial activity against both Gram-positive and Gram-negative pathogens. Importantly, the synthesized MgO nanoparticles demonstrate low toxicity, offering a promising therapeutic avenue for biomedical applications and microbial disease management while contributing to sustainable nanomaterial production. This research establishes a foundation for further exploration of plant-mediated MgO nanoparticles as innovative agents in nanomedicine and biotechnological applications.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors are grateful to the Saveetha Dental College and Hospital for providing the facility to complete this research successfully. Authors are thankful to (i) the Council of Scientific & dustrial Research - Human Resource Development Group (CSIR-HRDG: 324–4730–129/2k24/1) and (ii) the Anusandhan National Research Foundation (ANRF), Confederation of Indian Industry (CII) and Prim Drugs & Pharmaceuticals (India) Private Limited for Prime Minister Fellowship for Doctoral Research (PMFDR: 2023(43).

Declaration of generative AI in scientific writing

This article was formatted and edited for grammar and readability with the help of ChatGPT 4o. The authors conducted final edits and are responsible for the scientific accuracy of the content.

Data availability

No data was used for the research described in the article

References

1. Iype, E.M.; Pandey, M.; Mathew, A.; Thomas, G.; Sebastian, P.; Nair, M.K. Oral cancer among patients under the age of 35 years. *J Postgrad Med.* **2001**, 47, 171−176.
2. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet−Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA Cancer J Clin*, **2015**, 65, 87−108.
3. Jayakaran, P.; Nirmala, G.; Selvaraju, S.; Naveen Prasad B.P.S.; Nachiappan, S.; Gizachew A.K.; Preparation and Characterization of Magnesium Oxide Nanoparticles and Its Application for Photocatalytic Removal of Rhodamine B and Methylene Blue Dyes. *Journal of Nanomaterials*. **2022**, 64, 1-6.
4. Balakrishnan, B.; Velavan, R.; Batoo, K.M.; Raslan, E.H. Microstructure, optical and photocatalytic properties of MgO nanoparticles. *Results in Physics*, **2020**, 16, 103013.
5. Fouda, A.; Hassan, S.E.D.; Saied, E.; Hamza, M.F. Photocatalytic degradation of real textile and tannery effluent using biosynthesized magnesium oxide nanoparticles (MgO-NPs), heavy metal adsorption, phytotoxicity, and antimicrobial activity. *Journal of Environmental Chemical Engineering*. **2021**, 9, 105346.
6. Karthik, K.; Dhanuskodi, S.; Prabu Kumar, S.; Gobinath, C.; Sivaramakrishnan, S. Microwave assisted green synthesis of MgO nanorods and their antibacterial and anti-breast cancer activities. *Materials Letters*. **2017**, 206, 217–220.

7. Khan, M.I.; Akhtar, M.N., Ashraf, N. Green synthesis of magnesium oxide nanoparticles using *Dalbergia sissoo* extract for photocatalytic activity and antibacterial efficacy. *Applied Nanoscience*. **2020**, 10, 2351–2364.
8. Amina, M.; Al Musayeib, N.M.; Alarfaj, N.A. Biogenic green synthesis of MgO nanoparticles using *Saussurea costus* biomasses for a comprehensive detection of their antimicrobial, cytotoxicity against MCF-7 breast cancer cells and photocatalysis potentials. **2020**, 15, 0237567.
9. Alok, R.; Paras, J.; Binod, S.; Pallavi, S.; Sharma, H.P. *Acorus Calamus* L.: An Insight Review of Botany, Chemistry, Medicinal Uses and Cultural Practice. *JCBPS*. **2016**, 6, 1027-1045.
10. Sathishkumar, G.; Pradeep, K., Jha Vignesh, V., Rajkuberan, C.; Jeyaraj, M.; Selvakumar, M.; Rakhi, J.; Sivaramakrishnan, S. Cannonball fruit (*Couroupita guianensis*, Aubl.) extract mediated synthesis of gold nanoparticles and evaluation of its antioxidant activity. *Journal of Molecular Liquids*. **2016**, 215 229–236.
11. Sudha, K.G.; Ali, S.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Gorshenkove, M.V.; Rajeshkumar, M.P. *Cyrtrandroemia nicobarica*-Synthesized ZnO NRs: A New Tool in Cancer Treatment. *JOM*. **2020**, 1-23.
12. Sundarajan, M.; Suresh, J.; Gandhi, R. A comparative study on antibacterial properties of MgO nanoparticles prepared under different calcinations temperatures, digest. *Journal of Nanomaters and. Bioscience*. **2012**, 7, 983–989.
13. Sudha, K.G.; Ali, S.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Gorshenkove, M.V.; Rajeshkumar, M.P. An eco-friendly production of ZnO NRs using *Knema andamanica* (Warb) extracts for photocatalytic and anticancer applications. *Inorganic Chemistry Communications*, 134, **2021**, 109030.
14. Ali, S.; Sudha, K.G.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Rajeshkumar, M.P. Green synthesis of stable antioxidant, anticancer and photocatalytic activity of zinc oxide nanorods from *Leea asiatica* leaf. *Journal of Biotechnology*. **2021**, 329, 65-79.
15. Manoj, K.; Patel, M.D.; Zafaryab, M.; Rizvi, M.A.; Agrawal, V.V.; Ansari, Z.A.; Malhotra, B.D.; Ansari, S.J. Antibacterial and cytotoxic effect of magnesium oxide nanoparticles on bacterial and human cells. *Journal of nanoengineering and nanomanufacturing*. **2013**, 3, 162–166.
16. Ali, S.; Sudha, K.G.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Gorshenkove, M.V.; Rajeshkumar, M.P. Novel *Leea grandifolia* leaves mediated synthesis of ZnO nanorods for photocatalytic and anticancer applications. *Appl Organomet Chem*. **2021**, 6239.

17. Suriyaprabha, R.; Rajendran, V. In vitro and in vivo characteristics of biogenic high surface silica nanoparticles in A549 lung cancer cell lines and Danioreriomodel systems for inorganic biomaterials development. *Artif. Cells Nanomed. Biotechnol*. **2018**, 46, 1415– 1424.
18. Serpen, A.; Capuano, E.; Fogliano, V.; Gokmen, V. A new procedure to measure the antioxidant activity of in soluble food components. *J. Agric. Food Chem*. **2007**, 55, 7676–7681.
19. Karunakaran, G.; Suriyaprabha, R.; Manivasakan, P.; Yuvakkumar, R.; Rajendran, V.; Kannan, N. Screening of in vitro cytotoxicity, antioxidant potential and bioactivity of nano-andmicro-ZrO2 and -TiO2 particles. *Ecotoxicology and Environmental Safety*, **2013**, 93, 191–197.
20. Moloudi, K.; Samadian, H.; Jaymand M. Iron oxide/Gold nanoparticles- decorated reduced graphene oxide nanohybrid as the thermo-radiotherapy agent. *IET Nanobiotechnol*. **2020**, 14, 428–432.
21. Mortazavi-Derazkola, S.; Ebrahimzadeh, M.A.; Amiri, O. Facile green synthesis and characterization of Crataegus microphylla extract-capped silver nanoparticles (CME@ Ag-NPs) and its potential antibacterial and anticancer activities against AGS and MCF-7 human cancer cells. *J Alloys Compd*. **2020**, 820, 153186.
22. Uzar, N.K.; Abudayyak, M.; Akcay, N.; Algun, G.; Özhan, G. Zinc oxide nanoparticles induced cyto- and genotoxicity in kidney epithelial cells. *Toxicol. Mech. Methods.* **2015**, 25, 334-339.
23. Nidhi, C.; Manish, P.S.; Preeti, S.; Shadma, A.; Nand, K.S. Biosynthesis Zinc Oxide Nanoparticles Using Senna Occidentalis Leaf Extract and Evaluation of their Cytotoxic Effect on SW480 Colon Cancer Cell Line. *Research square*, **2022**, 11, 1-22.
24. Patlolla, A.K.; Berry, A.; May, L.B. Genotoxicity of silver nanoparticles in Vicia faba: a pilot study on the environmental monitoring of nanoparticles. *IJERPH*. **2012**, 5, 1649–1662.
25. Hemali, P.; Sumitra, C. Synthesis of silver nanoparticles using *Ziziphus nummularia* leaf extract and evaluation of their antimicrobial, antioxidant, cytotoxic and genotoxic potential (4-in-1 system), Artificial Cells. *Nanomedicine, and Biotechnology*, **2021**, 49, 1, 354-366.
26. Karunakarana, G.; Cho, E.B.; Suresh Kumar, G.; Kolesnikovc, E.; Karpenkovd, D.Y.; Gopinathane, J.; Pillai, M.M.; Selvakumar, R.; Boobalan, S.; Gorshenkov, M.V. Sodium dodecyl sulfate mediated microwave synthesis of biocompatible superparamagnetic mesoporous hydroxyapatite nanoparticles using black Chlamys varia seashell as a calcium source for biomedical applications. *Ceramics International*. **2019**, 45, 15143–15155.
27. Karunakarana, G.; Cho, E.B.; Suresh Kumar, G.; Kolesnikovc, E.; Karpenkovd, Y.; Gopinathane, J.; Pillai, M.M.; Selvakumar, R.; Boobalan, S.; Gorshenkov, M.V.; Kuznetsov, D. Ascorbic Acid-

Assisted Microwave Synthesis of Mesoporous Ag Doped Hydroxyapatite Nanorods from Biowaste Seashells for Implant Applications. *ACS Appl. Bio Mater*. **2019**, 2, 2280−2293.

28. Sushma, N.J.; Prathyusha, D.; Swathi, G.; Madhavi, T.; Deva Prasad Raju, B.; Mallikarjuna, K.; Kim, H.S. Facile approach to synthesize magnesium oxide nanoparticles by using *Clitoriaternatea*-characterization and *in vitro* antioxidant studies. *Appl. Nanosci.* **2016**, 6, 437-444.
29. Rao K.G.; Ashok C.H.; Rao K.V. Eco- friendly synthesis of MgO nanoparticles from orange fruit waste. *Int. J. Adv. Research Phys. Sci*. *IJARPS*. **2015**, 2, 1–6.
30. Kushwaha, A.; Bagchi, T. MgONPs synthesis, capping and enhanced free radical effect on the bacteria and its cell morphology. *AIP Conf. Proc*. **2018**, 1961, 030010-030011.
31. Hussein, H.Z.; Abdul-Karim, E.K.; Mutar, S.S. Possibility of using nanoparticles (ZnNPs &MgONPs) in keeping cucurbit fruit from infection by *Pythium aphanidermatum*. *Int. J. Sci. Res*. **2015**, 6, 2319–7064
32. Sudha, K.G.; Ali, S.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Gorshenkove, M.V.; Rajeshkumar, M.P. *Cyrtrandroemia nicobarica*-Synthesized ZnO NRs: A New Tool in Cancer Treatment. *JOM*. **2020**, 1-23.
33. De-Rui Di, M.E.; He, Z. 'A new nano-cryosurgical modality for tumor treatment using biodegradable MgO nanoparticles, *Nanomed. Nanobiotechnol*. **2012**, 8, 1233–1241.
34. Ali, S.; Sudha, K.G.; Karunakaran, G.; Kowsalya, M.; Kolesnikov, E.; Rajeshkumar, M.P. Anticancer and photocatalytic activities of zinc oxide nanorods synthesized from *Manilkara littoralis* leaf extract. *Materials Chemistry and Physics*, **2022**, 277, 125541.
35. Veerla, S.C.; Kim, J.; Sohn, H.; Yang, S.Y. Controlled nanoparticle synthesis of Ag/Fe co-doped hydroxyapatite system for cancer cell treatment. *Mat. Sci. Eng. C-Mater*. **2019**, 98, 311–323.
36. Ponnuvelu, D.V.; Selvaraj, A.; Suriyaraj, S.P. Ultrathin hexagonal MgOnanoflakes coated medical textile and their enhanced antibacterial activity. *Material Res. Express*. **2016**, 3, 105005.
37. Lee, K. X.; Shameli, K.; Mohamad, S. E.; Yew, Y. P.; Mohamed Isa, E. D.; Yap, H-.Y.; Lim, W. L.; Teow, S.-Y. Bio-mediated synthesis and characterisation of silver nanocarrier, and its potent anticancer action. *Nanomaterials*. **2019**, 9, 1423.
38. Izadiyan, Z.; Shameli, K.; Miyake, M.; Hara, H.; Mohamad, S.E.B.; Kalantari, K.; Taiba, S.H.M.; Rasoulic, E. Cytotoxicity assay of plant-mediated synthesized iron oxide nanoparticles using Juglans regia green husk extract. *Arab. J. Chem.* **2018**, 1–27.

39. Sriranjani, R.; Srinithya, B.; Vellingiri, V. Silver nanoparticle synthesis using Clerodendrum phlomidis leaf extract and preliminary investigation of its antioxidant and anticancer activities. *J Mol Liq*. **2016**, 220, 926–930.
40. Ke, Y.; Al Aboody, M.S.; Alturaiki, W.; Alsagaby, S.; Alfaiz, F.A.; Veeraraghavan, V.P.; Mickymaray, S. Photosynthesized gold nanoparticles from Catharanthus roseus induces caspase-mediated apoptosis in cervical cancer cells (HeLa). *Artif. Cells Nanomed.Biotechnol*. **2019**, 47, 1938–1946.
41. Qian, L.; Su, W.; Wang, Y.; Dang, M.; Zhang, W.; Wang, C. Synthesis and characterization of gold nanoparticles from aqueous leaf extract of Alternanthera sessilis and its anticancer activity on cervical cancer cells (HeLa). *Artif. Cells Nanomed. Biotechnol*. **2019**, 47, 1173–1180.
42. Xu, Z.; Feng, Q.; Wang, M.; Zhao, H.; Lin, Y.; Zhou, S. Green Biosynthesized Silver Nanoparticles with Aqueous Extracts of Ginkgo Biloba Induce Apoptosis via Mitochondrial Pathway in Cervical Cancer Cells. *Front. Oncol*. **2020**, 10, 575415.
43. Singh, A.K.; Tiwari, R.; Singh, V.K.; Singh, P.; Khadim, S.R.; Singh, U.; Laxmi; Srivastava, V.; Hasan, S.; Asthana, R. Green synthesis of gold nanoparticles from Dunaliella salina, its characterization and in vitro anticancer activity on breast cancer cell line. *J. Drug Deliv. Sci. Technol*. **2019**, 51, 164–176.
44. Karunakaran, G.; Jagathambal, M.; Gusev, A.; Minh, N. V.; Kolesnikov, E.; Mandal, A. R.; Kuznetsov, D. Nitrobacter sp. extract mediated biosynthesis of Ag2O NPs with excellent antioxidant and antibacterial potential for biomedical application. *IET Nanobiotechnol*. **2016**, 10, 425−430.
45. Karunakaran, G.; Jagathambal, M.; Gusev, A.; Kolesnikov, E.; Mandal, A. R.; Kuznetsov, D. Allamanda cathartica flower's aqueous extract-mediated green synthesis of silver nanoparticles with excellent antioxidant and antibacterial potential for biomedical application. *MRS Commun*. **2016**, 6, 41−46.
46. Mahendran, D.; Kavi Kishor, P.B.; Geetha, N.; Manish, T.; Sahi, S.V.; Venkatachalam, P. Efficient antibacterial/biofilm, anti-cancer and photocatalytic potential of titanium dioxide nanocatalysts green synthesised using *Gloriosa superba* rhizome extract. *Journal of Experimental Nanoscience*. **2021**, 16, 1, 11-30.

47. Cittrarasu, V.; Balasubramanian, B.; KaliannaN, D. Biological mediated Ag nanoparticles from Barleria longiflora for antimicrobial activity and photocatalytic degradation using methylene blue. *Artif Cells Nanomed Biotechnol*. **2019**, 47, 2424–2430.
48. Metryka, O.; Wasilkowski, D.; Mrozik, A. Insight into the Antibacterial Activity of Selected Metal Nanoparticles and Alterations within the Antioxidant Defence System in *Escherichia coli*, *Bacillus cereus* and *Staphylococcus epidermidis*. *Int. J. Mol. Sci*. **2021**, 22, 11811.