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Extracellular Synthesis of Magnesium Oxide at Nano and Bulk Scale: Antifungal Effect Against *Candida albicans, Aspergillus niger*

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ABSTRACT

The antifungal activity of magnesium oxide nanoparticles (MgO-NPs) prepared by the cactus plant (Opuntia ficus indica) at the nano and macro scale was evaluated against important human pathogens: Candida albicans and Aspergillus niger. The nanoparticles were characterized using UV–Vis, FTIR, DLS, EDX, and FESEM. UV–Vis analysis revealed a peak at 300 nm, and FT-IR analysis showed that the biomolecules played an important role in ions reduction, leading to the growth of MgO-NPs. A peak close to 400 cm−1 was observed, indicating Mg-O-Mg bonding. EDX analysis confirmed the presence of MgO-NPs. MgO-NPs were identified as nanospheres with diameters between 15.5 and 78.01 nm (average 42.28 nm), while MgO-Bulk was identified as macrospheres with lengths between 105.2 and 1313.9 nm (average 356.09 nm) using FESEM. Z-average sizes by DLS analysis were 46.04 nm and 377 nm. In vitro antifungal assays were evaluated using two methods: well diffusion and the microdilution method, and MgO-NPs showed the highest effect in both. The Minimum Inhibitory Concentration (MIC) for MgO-NPs was equal to 1.5 mg/mL and 6.25 mg/mL for C. albicans and A. niger, respectively.

Keywords: MgO nanoparticles; Biosynthesis; *ficus indica*; Antifungal activity; *Candida albicans; Aspergillus niger*.

1. INTRODUCTION:

From ancient times, fungal infections have significantly contributed to the ever-increasing morbidity and mortality rate[1]. The increasing clinical and microbiologic resistance of Candida spp. and Aspergillus spp. isolates to several antifungal agents is becoming a serious problem[2,3]. Death rates due to invasive fungal infections are often 50% or higher[4]. The risk of failure of surgical operations and medical treatment will increase in the absence of effective drugs[5]. Nanotechnology is a science that uses various techniques to synthesize nanoparticles[6]. Nanoparticles possess several properties compared to larger particles of the same material, mainly due to their large surface area in relation to volume. The influence of this factor is significant, as materials larger than 100 nm are expected to have physical and chemical properties regardless of size, but this is different for nanoparticles. Foremost among them is their antifungal properties[7,8]. Metal oxides were attractive because of their easy modifiability and a variety of shapes[9]. Among metallic nanoparticles, extensive research has been carried out using MgO-NPs and their applications in the biomedical field as drug delivery system[10], anti-microbial[11], and anti-cancer agents[12], water purification and also in pharmaceutical products[6].

MgO has gained keen interest due to its ecofriendly nature, the availability of greater specific area, biocompatibility, low cost, and the easy convenience of its sources[6]. In addition, The Food and Drug Administration (FDA) recommended MgO-NPs as safe materials[13]. Studies in this regard are few compared to other minerals. Presently, green synthesis of metal nanoparticles using plant extract gets a lot of attention since it is eco-friendly

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and cost-effective [9]. It has been known since the early 20th century[9]. Cactus is one of the plants used as bioactivators for the fabrication of nanoparticles, which has many types. In recent years, the cacti genus has received a lot of attention for its therapeutic benefits, in the foreground, its antimicrobial effect[14]. MgO-NPs synthesis via green route is limited in the literature and few studies on MgO-NPs green synthesis using *Nephelium lappaceum L.*[15], *Azadirachta indica*[16], *Aloe vera* [17], *Betel leaf* [18], *Sargassum wighti* [19], *Rhizophora lamarckii*, were reported previously. In this study, *Opunita ficus indica* extract mediated green synthesis of MgO-NPs, which was investigated for the first time. *O.F.I* is a well-known elaborate plant that belongs to *Cactaceae*[20]. It has been used in traditional folk medicine for its antifungal and antibacterial effects [21].

2. MATERIALS AND METHODS

1.2 Materials:

The Opuntia ficus indica (O.F.I) cladodes were freshly collected from wild plants growing in Homs, Syria (34°21′N 38°19′E). Double-distilled water (DDW) was used in the experiments. Sodium hydroxide 98% (Medex, UK), Ethanol GR (Eurolab, UK). All the chemical materials were obtained from Aldrich. Fungal species: Aspergillus niger was obtained from the Microbiology lab at Pharmacy College, Al-Baath University, Syria, Candida albicans ATCC 10231 was obtained from the Atomic Energy Commission, Damascus, Syria.

Equipment: Ultrasonic bath (POWERSONIC 405, Hwashin Technology Co., Korea), Sensitive balance (Sartorius TE214, Germany), UV-1800 spectrophotometer (Shimadzu, Japan), Rotary evaporator (Heidolph Instruments, Germany), Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan), Zetasizer instrument (DLS; Zeta-size Nano-ZS; Malvern Instruments, UK), FTIR spectrometer (SHIMADZU), mixer (Germany DI 18 basic), FESEM (European).

2.2 Methods: Experiments were performed triples.

1.2.2 Preparation of the extract: The healthy Opuntia ficus indica was collected, washed under running tap water, dried for 5 minutes at room temperature, and finely chopped. Briefly, 30 g of the plant was suspended in a 500ml beaker (100:50 mL) (DDW: Ethanol) and treated with ultrasound for 30 min at 50°C. The obtained extract was filtered through Whatman number 1 filter paper, and it was concentrated by a rotary evaporator until it removed all the ethanol. A freshly prepared extract was used for the synthesis of MgO-NPs.

2.2.2 Biosynthesis of MgO-NPs: In the experiment, MgO-NPs were prepared by slowly mixing 10 ml of plant extract with 90 ml of 10^(-3) M aqueous solution of magnesium nitrate dropwise under vigorous agitation by a mixer and adding NaOH (0.1 N) until pH reached 11. Then, the mixture was left overnight by magnetic stirring and at 35°C. The magnesium nitrate ions were reduced to magnesium oxide nanoparticles using the plant extract. The formation of MgO-NPs has been observed by a color change. Thereafter, the solvent of nanoparticles was evaporated by a rotary evaporator. Finally, the obtained precipitate was dried at 60°C and calcinated at 500°C for 3 hours to produce MgO-NPs.

3.2.2 Characterization of MgO-NPs:

the formation of MgO-NPS was confirmed by:

- **Ultraviolet-Visible (UV–Vis) Spectroscopy**

The optical features of MgO-NPs were determined using UV–Vis spectroscopy. The highest absorbance (λmax) of a stock solution of the MgO-NPs, diluted with distilled water (1:1 ratio), was measured in the wavelength range from 200 to 600 nm to confirm the presence of the specific Surface Plasmon Resonance (SPR) peak of MgO-NPs.

- **Fourier Transform Infrared Spectroscopy (FTIR) Analysis**

The MgO-NPs, which were calcinated at 500°C, and the dried form of the extract at 80°C were pelletized with KBr and analyzed using an FTIR spectrometer[22]. The absorption was recorded in the field extending from 400 to 4000cm^{-1}.

- **Dynamic Light Scattering (DLS)**

DLS was used to measure the mean diameter and polydispersity index (PDI). It was performed by the Zetasizer instrument at 25°C. The particle diameter determined in this way is known as the hydrodynamic diameter. The value of PDI ranges between 0 and 1, with a value close to 0 being mono-dispersed and a value close to 1 representing a poly-dispersed sample. It is important to synthesize nanoparticles with a lower size and higher absorbance[23].

- **Field Emission Scanning Electron Microscope (FE-SEM) Analysis**

Samples were prepared by placing a drop of colloidal on a slide and drying it at 25°C. FE-SEM images were taken, and Image J and Origin 2017 were utilized to prepare histograms for dimensions.

- **Energy-dispersive X-ray spectroscopy (EDX)**:

EDX analysis confirmed the presence of MgO -NPs. EDX relies on the characteristic X-ray emission of MgO.

***4.2.2 In Vitro* Antifungal Activity of MgO-NPS**

The antifungal activity of the MgO-NPs was evaluated using agar well diffusion against Aspergillus niger and Candida albicans ATCC 10231. The tested fungal strains were grown on Sabouraud Dextrose agar plates and incubated for 1, 5 days at 37°C and 25°C for C. albicans and A. niger, respectively[2] [24]. The turbidity of the suspension: for *Candida Albicans*, the turbidity was adjusted by spectrophotometry to an optical density of 0.08-0.1[2], and for *Aspergillus niger* was an optical density of 0.09 to 0.13[24]. One milliliter was uniformly distributed on Czapek dox agar plates. Using a sterile cork-borer, wells (8 mm) were cut; The wells were closed with a drop of agar so that the nanoparticles would not settle to the bottom of the well, 50,100 µL of MgO-NPs and 50,100 µL of MgO-Bulk were transferred to each well individually and left for 2 h at 25 °C, and then the plates were incubated for 1, 5 days at 25, 35°C for *C. Albicans, A. niger* respectively. After incubation, the inhibition zones are determined and recorded. Moreover, different concentrations of MgO-NPs were evaluated as antifungal to detect the minimum inhibitory concentration (MIC).

5.2.2 NCCLS Microdilution Method

This method was used as described in the National Committee for Clinical Laboratory Standards. The MIC of MgO-NPs was defined as the lowest concentration which resulted in a prominent decrease in turbidity compared to that of growth-control wells[25].

3. RESULTS AND DISCUSSION

1.3 UV–visible spectroscopy:

MgO ions were bioreduced to MgO-NPs when Opuntia ficus-indica extracts were added. The formation of MgO-NPs was confirmed by UV-Vis spectrophotometer studies within a range of 200-600 nm. The specific SPR peak of MgO-NPs after 24 hours was found to be centered at 300 nm in the spectrum, indicating the presence of MgO-NPs. This finding aligns with studies by Dobrucka R[26], and Prasanth R *et al* [27]**.** The obtained SPR peak confirms the reduction of Mg (NO3)2 to MgO-NPs, and it is evident that the phytochemicals present in O.F.I may function as a capping and stabilizing agent toward the MgO-NPs. MgO-Bulk showed a modest peak at 300 nm, lower than the nanoparticle peak. As shown in Figure 1, the frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticle[28].

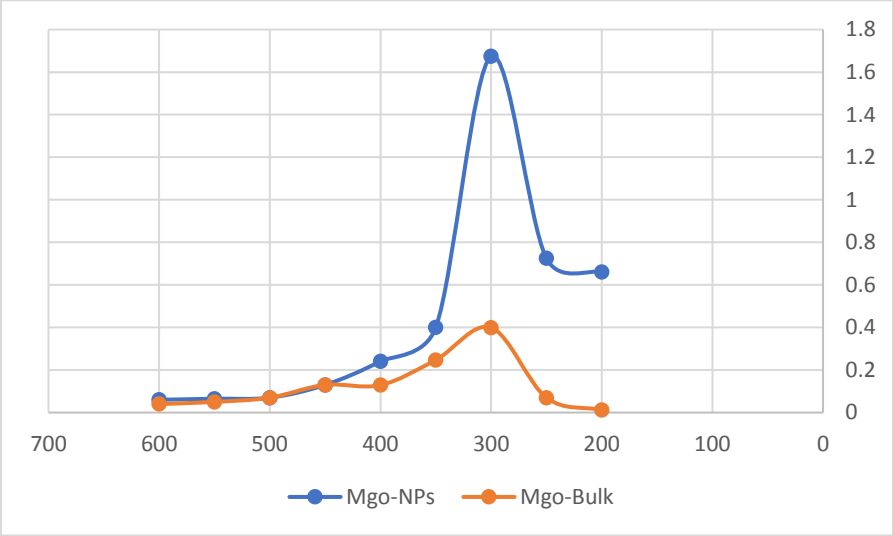
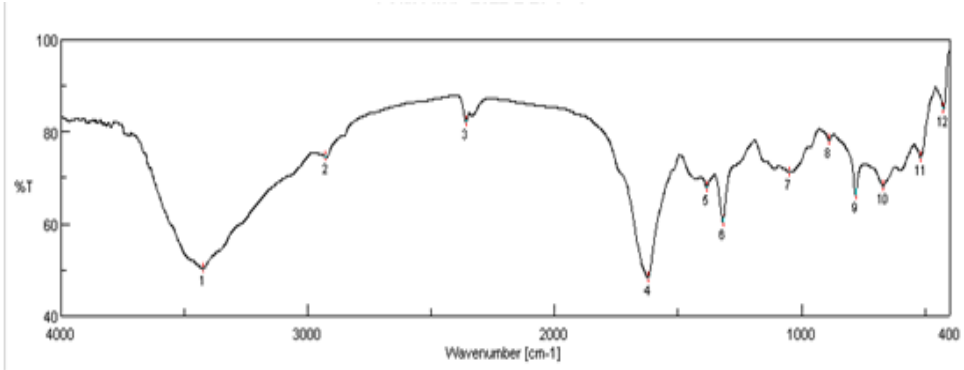


Fig 1. UV-vis absorption spectrum of MgO-NPs, MgO-Bulk

2.3 Fourier transform infrared spectroscopy (FTIR) analysis: FT-IR measurements were conducted to identify the possible biomolecules responsible for the reduction of Mg^(+2) and capping of the MgO-NPs synthesized using O.F.I extract. From the two spectra (Figure 2a, b), we observed a decrease in the quantities of plant components (alcoholic, amino compounds, alkanes, alkenes, carboxylic acids, etc.)[29]. This decrease is indicative of their participation in the fabrication of nanoparticles, as evident in the peak that expresses phenols and alcohols, which decreases in the spectrum of nanoparticles. There is also the appearance of a clear peak (7) in the nanoscale sample at 466 cm^(-1) (Fig. 2b), which corresponds to Mg-O [30,17]. This result is similar to an Indian study that showed a peak at 450-560 cm $^{-1}$[31 31], and in contrast to the result in Poland in 2016, they used the *Artemisia abrotanum* plant, which gave a peak at 407-419 cm $^{-1}$[26].

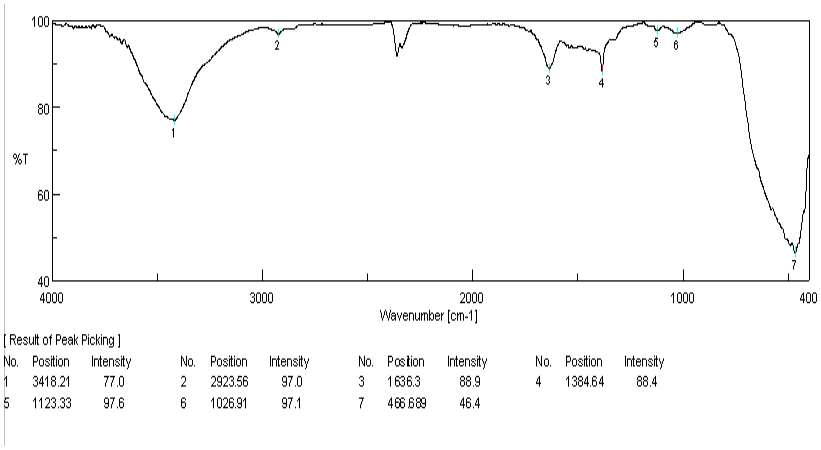
(a)



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3424.96	50.3	2	2926.45	74.6	3	2358.52	82.2	4	1621.84	48.2
5	1383.68	68.0	6	1318.11	60.5	7	1049.09	71.2	8	888.059	78.2
9	780.065	66.4	10	670.142	68.2	11	517.793	74.4	12	424.263	85.1

(b)



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3418.21	77.0	2	2923.56	97.0	3	1636.3	88.9	4	1384.64	88.4
5	1123.33	97.6	6	1026.91	97.1	7	466.689	46.4			

Fig 2. FTIR spectrum of (a) *Opunita ficus indica*, (b) MgO-NPs synthesis by *Opunita ficus indica*

3.3 Dynamic Light Scattering (DLS): DLS analysis of MgO-NPs synthesized using *O.F.I* and MgO-Bulk is shown in Fig. 3, with a Z-average size of 46.90nm. This differs from a study conducted in 2020 in which the dimensions of nickel oxide fabricated by *O.F.I* was 20-35nm[32], and this is due to the difference in the metal's ability to ionize[33], and it showed Z-average size at 377.3 nm for MgO-Bulk.

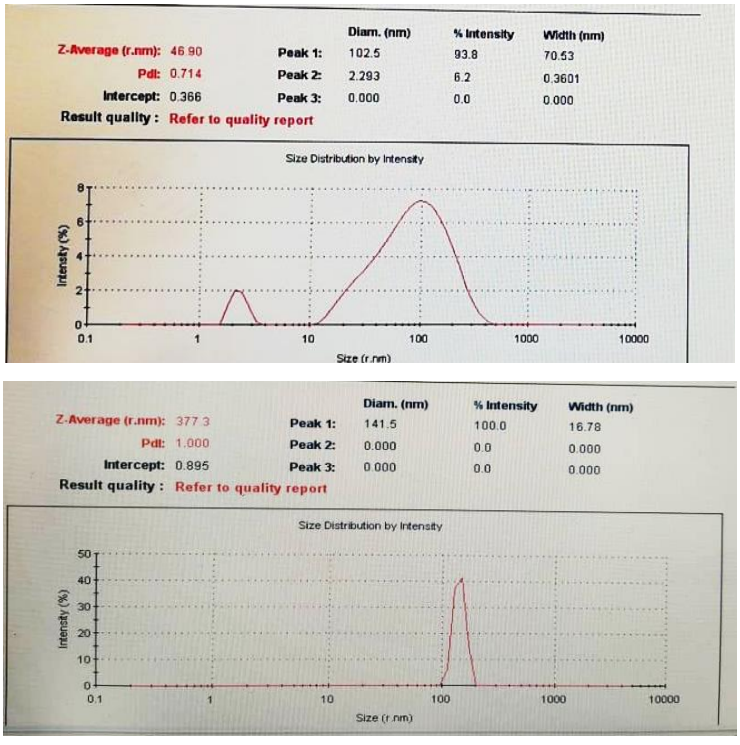
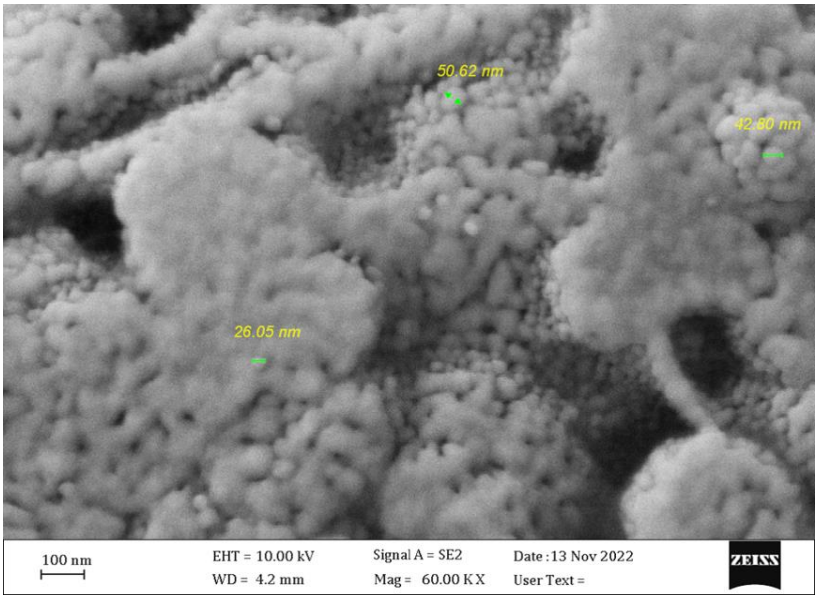


Fig 3. DLS of MgO-NPS, MgO-Bulk

4.3 Field Emission Scanning Electron Microscopy (FESEM):

FE-SEM analysis of biosynthesized MgO nanoparticles indicated a spherical shape with agglomeration and crystalline solid at both size nano and bulk particles. The size of particles which were synthesized by the green method was (42.28 as average) (Fig. 4), and MgO-Bulk was (356.04nm as average) (Fig. 5), due to histogram, and this is similar to the previously described. With DLS, this confirms the ability of plant metabolites to return salt ions.

(a)



(b)

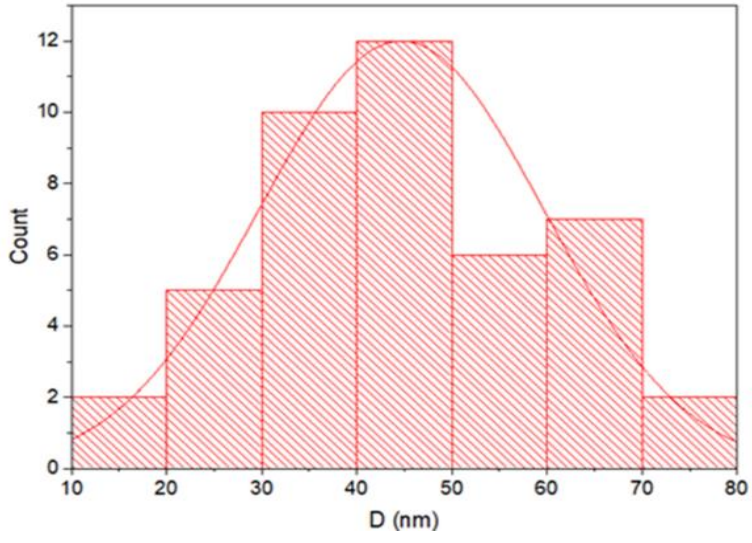
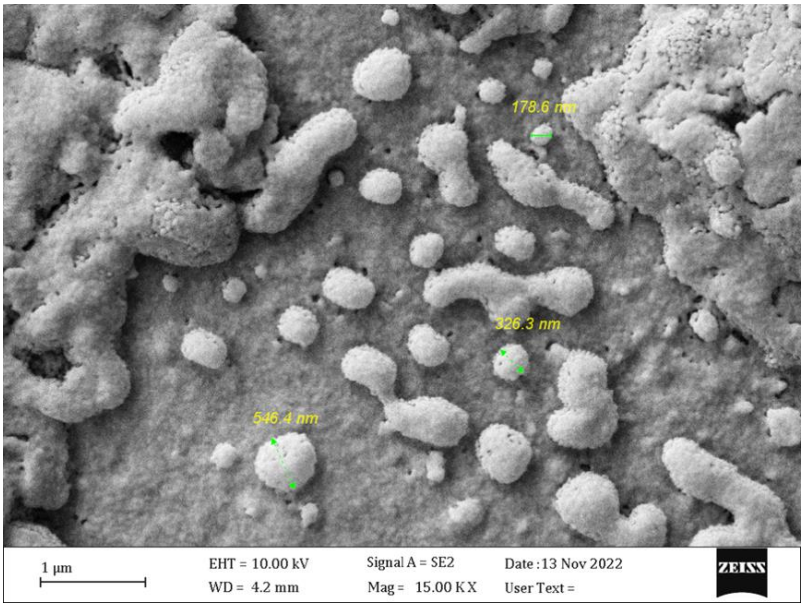


Fig 4. a: FESEM for MgO-NPs, b: Histogram of diameters

(a)



(b)

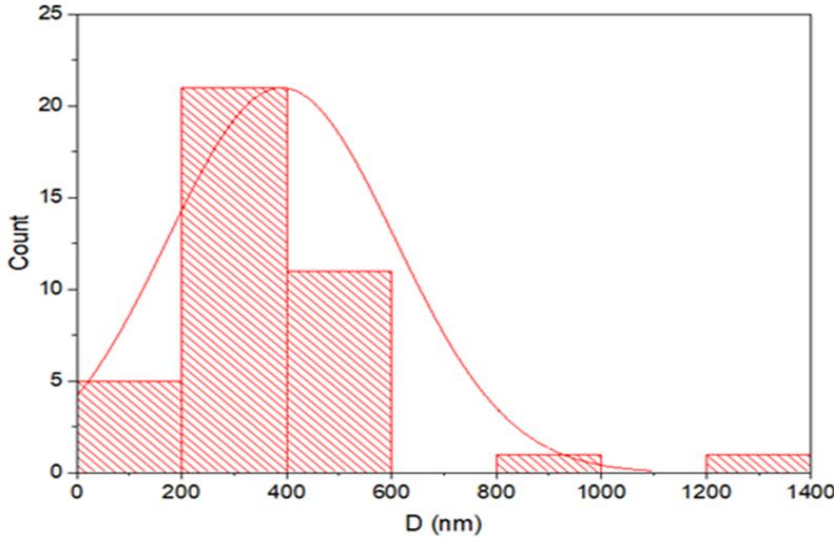


Fig 5. a: FESEM for MgO-Bulk, b: Histogram of diameters

5.3 Energy-dispersive X-ray spectroscopy (EDX):

The EDX analysis showed good signals for Mg,O together with remarkably stronger peaks, confirming that the pellets were MgO-NPs. The other elements are thought to have originated from the plant extract which is depicted in Fig. 6, thus confirming the formation of MgO-NPs.

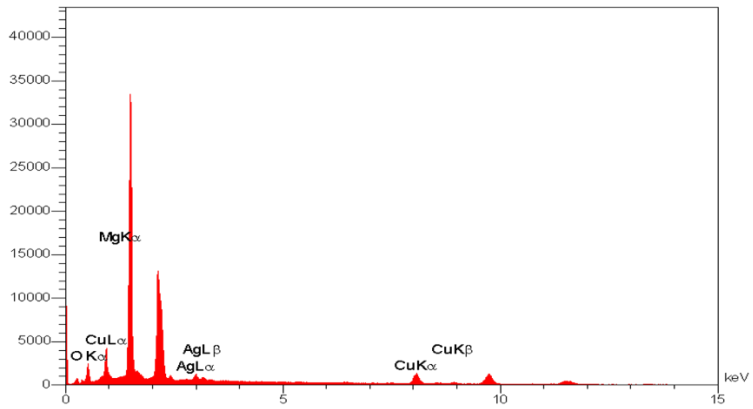


Fig 6. EDX analysis of MgO-NPs fabricated by green synthesis method

2.3 Antifungal effect:

1.2.3 well diffusion methods:

After 1 and 5 days of incubating the dishes in the microbiological incubator, the antifungal activity of the 100 and 50 mg/mL MgO-NPs solution was evaluated. The antifungal activity of the MgO-NPs solution was compared with MgO-Bulk against C. albicans and A. niger. The nanoparticles showed clear inhibition zones with diameters of 30 ± 0.5 and 25 ± 0.5 mm, respectively, whereas the bulk particles had no effect at both concentrations against C. albicans. The antifungal mechanism of MgO-NPs is not clear, unlike bacteria whose mechanism of action mainly depends on oxygen free radicals (ROS). For C. albicans, the mechanism may be due to the destruction of the integrity of the fungal cell membrane 34. It can be concluded that the effectiveness is directly related to the concentration used and increases with its increase, similar to the study of J. Chen et al [35]. In our results, the diameters of the inhibition zones were similar to the study of S. Vijayakumar et al [36] but in contrast to the study of E. Vidhya et al, which reported diameters of 21 ± 1.70 mm[37]. This difference may be due to the variation in the number of particles used. The study by Vidhya et al used 20 µL of nanoparticle suspension, while in our study, 100 µL was used, which directly affects the effectiveness[27].

Regarding *A.niger,* MgO-Bulk gave a small zone inhibition 17±0.5, 15±0.5 mm compared with MgO-NPs which was 25±0.5, 20±0.5 nm. This is similar to the study Vijayakumar S *et al* [36] where the inhibition zone was 24 ± 0.72 mm, and in contrast to the study by Vidhya E *et al* [38] which reported an inhibition zone of 16 mm. This may explain the difference in the pattern of the studied fungi. The different antifungal effect between the nano and bulk particles may be explained by the higher concentration of reactive oxygen species (ROS) in nanoparticles compared to bulk particles, causing oxidative stress on fungal cells, disrupting membrane structure, and altering fungal permeability, which may damage the cell and exhibit antifungal activity[39].

We infer that, due to the different sizes of the nanoparticles, it influences the antifungal effect[5]. Many reports indicate that size is the most important factor in the effectiveness of antibiotics, as size is a critical factor in the destruction of bacterial and fungal systems for many reasons. Small sizes allow accumulation and penetration of bacterial cells, causing damage and, thus, death of bacteria5. Metal oxide nanoparticles larger than 10 nm in size enhance permeability upon contact with the organism, and this is due to the surface area-to-volume effect that affects the specific surface. For this reason, they can also have an effect on direct toxicity mechanisms against bacteria and, consequently, subsequent death[5].

2.2.3 Microdilution methods:

Using broth microdilution, all species demonstrated sensitivity to MgO-NPs, as shown in Table 1. The MIC for MgO-NPs was 1.5 and 6.25 mg/mL against C. albicans and A. niger, respectively. This is an effective value as observed in in-vitro studies, and higher MIC levels were noted for MgO-Bulk against A. niger at 25 mg/mL. This finding aligns with the study by Nguyen NY[40]. The different antifungal effects between nano and bulk particles may be explained by the higher concentration of reactive oxygen species (ROS) in nanoparticles compared to bulk particles. This higher concentration causes oxidative stress on fungal cells, disrupts membrane structure, and alters fungal permeability, potentially damaging the cells and exhibiting antifungal activity[39].

Table .1: Minimum inhibitory concentration of MgO-NPs, MgOBulk against *c. albicans, A. niger* (mg/mL)

DMSO	MgO-Bulk	MgO-NPS	MIC
-	25	6.25	***Aspergillus niger***
-	-	1.56	***Candida albicans***

* Values were expressed as the means of Triples replicates.

4. CONCLUSIONS

Nanomaterials represent an emerging field that enables the more efficient use of antimicrobial compounds. In this study, the antifungal activity of MgO-NPs fabricated using Opuntia ficus indica extract was evaluated. The synthesized MgO-NPs underwent characterization through UV–Vis spectroscopy, FTIR, DLS, EDX, and FESEM analyses. The SPR peak at 300 nm observed during nanoparticle characterization confirmed the formation of MgO-NPs. FTIR analysis identified the functional groups responsible for the bioreduction and stabilization of the synthesized MgO-NPs using the extract. The dimensions of nano and bulk particles were measured through DLS and FE-SEM analysis, revealing spherical shapes, and the EDX spectrum confirmed the presence of magnesium (Mg) and oxygen. Furthermore, MgO NPs exhibited significant antifungal activity against Aspergillus niger and Candida albicans ATCC 10231, assessed through both Well Diffusion and Microdilution Methods, and were compared with bulk particles.

We believe that MgO-NPs hold great potential for applications in the pharmaceutical industry, serving as an alternative to antifungal agents facing resistance. MgO nanoparticles, whether used alone or in combination with other antifungal compounds, could present a superior choice for various future applications, such as coating medical devices or preserving food. Additionally, their cost-effectiveness makes them a favorable option for patients.

Conflicts of Interest: The authors have declared that there are no competing interests.

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الاصْطنَاع الخارج خلوي لجسيمات أوكسيد المغنيزيوم في المستوى النّانويّ والمايكروني باستخدام نبات الصّبار كمعمل حيويّ: وفعاليّتها المضادة لفطور المبيضات البيض والرشاشية السّوداء

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ملخص

صُنعت جسيمات أوكسيد المغنيزيوم النّانويّة والمايكرونية بواسطة صبار التين الشوكي وتم تقييم فعاليتها المضادة للفطور المسببة للأمراض الهامة عند البشر كمبيضات البيض والرشاشيات السّوداء. وتم توصيف الجسيمات الناتجة بِـ UV–Vis، FTIR، DLS، EDX، FESEM. أظهر تحليل مطيافية الأشعة المرئية وفوق البنفسجية قمة عند 300 نانومتر، وبيّن اختبار الأشعة تحت الحمراء أن المستقلبات الحيوية في النبات تلعب دورًا مهمًا في إرجاع أيونات المعدن ثم نموها لتعطي في النهاية جسيماتٍ نانويّة، كما يبين التحليل قمة قريبة من 400 $^{-1}$سم تعود للرابطة Mg-O-Mg. يؤكد تحليل الـ EDX وجود جسيمات أوكسيد المغنيزيوم. تراوحت أبعاد جسيمات أوكسيد المغنيزيوم النّانويّة ذات الشكل الكروي بين 15.5 و 78.01 نانومتر (بمتوسط 42.28 نانومتر)، أما أبعاد الجسيمات المايكرونية الكروية تراوحت بين 105.2 و 1313.9 نانومتر (بمتوسط 356.09 نانومتر) وذلك باستخدام المجهر الإلكتروني الماسح عالي النفاذية FESEM. بينما كان متوسط الأبعاد المحسوب بطريقة التشتت الضوء الديناميكي DLS مساوياً لِـ 46.04 و 377 نانومتر. اختبار الفعالية المضادة للفطور في المختبر *In vitro* للجسيمات الناتجة تمت دراسته بواسطة طريقتين مايكروبيولوجيتين هما الانتشار في الآبار وتمديد المرق الدقيق في كلتا الطريقتين كانت للجسيمات النّانوية تأثير أكبر من الجسيمات بالحجم الأكبرضد الفطور، وكان التركيز المثبط الأدنى الـ MIC لجسيمات أوكسيد المغنيزيوم النّانويّة مساوياً لِـ 1.5 ، 6.25 ملغ / مل لكل من المبيضات البيض والراشاشيات السوداء على التوالي.

الكلمات الدالة: أوكسيد المغنيزيوم النّانويّ، صبار التين الشوكي، التأثير المضاد للفطور، فطور المبيضات البيض، الرشاشية السّوداء.

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