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Research Article

# Antioxidant and Antibacterial Activities of Magnesium Oxide Nanoparticles Prepared using Aqueous Extract of *Moringa Oleifera* Bark as Green Agents

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**Abstract**
In this research, magnesium oxide nanoparticles (MgONPs) was prepared from $MgCl_2$ solution using aqueous extract of *Moringa oleifera* (*M. oleifera*) bark as green agent. Preparation procedure involved mixing of $MgCl_2.6H_2O$ solution and the aqueous extract of *M. oleifera* bark, followed by drop wise addition of NaOH solution. The formation of MgONPs in this study was confirmed using UV-Vis absorption. The spherical crystal structure of MgONPs was confirmed by XRD analysis. The average particle size of the synthesized MgONPs was found between 60—100 nm using SEM and TEM images and PSA results. The MgONPs synthesized showed good antioxidant activity, as well as antibacterial activity against *S. aureus*, *E. faecalis*, *E. coli*, and *S. dysenteriae* bacteria.

**Keywords:** antibacterial, antioxidant, bark extract, green synthesis, magnesium oxide nanoparticles

## 1. INTRODUCTION

Green method is an experimental procedure that uses extracts from plants as agents to form nanoparticles. This method provides a variety of resources in the process of nanoparticle synthesis [1]–[3]. In addition, there are many other advantages in the use of natural materials in the synthesis of nanoparticles. The use of biodegradable raw materials for the synthesis of nanoparticles has no harmful impact on the environment, leads to a green technology policy, and is first and foremost environmentally friendly. Second, the efficiency of synthesis, which promotes further production through the availability of abundant natural raw materials. Third, the nanoparticles produced are biocompatible in comparison with conventionally seized nanoparticles [4].

Magnesium oxide nanoparticles (MgONPs) are multipurpose metal oxide nanoparticles which have many applications in various fields. MgONPs have been widely used as catalysts [2][5][6] and catalyst supports for various organic reactions [7], adsorbents [8]–[10] and electrochemical biosensors [11]. In biomedical applications, there are evidence showing MgONPs have antioxidant [12], antibacterial [13]–[15], antifungal [12][16], and anticancer [16] activities.

*Moringa Oleifera* (*M. oleifera*) is a multipurpose tropical plant from the *Moriceae* family which is spread throughout India, Asia, and sub-Saharan Africa [17]. The use of *M. oleifera* extract has been widely used in synthesizing metal nanoparticles and metal oxides. Moringa flower extract can be used to prepare palladium [18], silver [19], and hydroxyapatite [20] nanoparticles. On other hand, Moringa leaf extract is useful to prepare silver [21], zinc oxide [22], titanium oxide [23] and nickel oxide [24] nanoparticles. Phytochemical analysis of water extracts of *M. oleifera* bark shows that Moringa wood bark contains compounds such as alkaloids, phenolic acids, terpenoids, and flavonoids [25]. These compounds play a role in the chelation process and they are able to reduce metal ions to nanoparticles [26].

The effect of *M. oleifera* bark water extract for preparation of MgONPs was examined in this research. This research has been confirmed since the special use of these plant components for nanoparticle preparation is still limited. Characterization of the MgONPs was performed through UV-Vis absorption spectroscopy, X-Ray diffraction (XRD), Electron scanning microscopy (SEM), electron transmission microscopy (TEM), and particle size analyzer (PSA). The bioactivity of MgONPs was subsequently examined as antioxidant and antibacterial agents.

**Table 1.** Phytochemicals analysis of *M. oleifera* bark aqueous extract.

| Chemical constituents | Testing Methods | *M. oleifera* bark aqueous extract |
|---|---|---|
| Alkaloids | Dragendroff's test | + |
| Flavonoids | Shinoda test | + |
| Saponins | Foam test | + |
| Carbohydrate | Anthrone test | + |
| Polyphenols | Puncal-D | - |
| Proteins | Ninhydrin test | - |
| Amino acids | Millon's test | - |
| Phenolics | Ferric chloride test | + |
| Triterpens | Salkowski test | - |
| Anthraquinones | Borntragges test | - |

+ = present, - = absence

## 2. MATERIALS AND METHODS

### *2.1. Materials*

Fresh *M. oleifera* bark were collected from the plants that grow naturally around the City of Metro, Lampung, Indonesia during September 2019. Laboratory grade magnesium chloride hexahydrate ($MgCl_2.6H_2O$), Folin-Ciocalteu reagents, sodium carbonate ($Na_2CO_3$), gallic acid, catechin, aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) were purchased from Merck Sigma-Aldrich Reagent Pte, Singapore.

### *2.2. Methods*

#### *2.2.1. Plant extract*

A fresh, *M. oleifera* sample washed by floating water, dried and then poured into powder and stored at room temperature under direct sunlight. In 100 mL of distilled water, four grams of *M. oleifera* bark powder was soaked and heated for 20 minutes at 60 ° C. The mixture was then filtered 1 hour with Whatman Filter Paper 1 to isolate the extract from the residue. The mixture was left overnight.

#### *2.2.2 Phytochemicals analysis*

The aqueous extract was subjected to phytochemical analysis to detect the presence of carbohydrates, amino acids, glycosides, polyphenols, saponins, steroids, flavonoids, tannins, and alkaloids. Total phenolic was estimated using the Follin-Ciocalteu test [27], and the result was expressed in µg/mg gallic acid equivalent (GAE) unit. Total flavonoid content was determined by the colorimetric $AlCl_3$ method employing catechin as standard and expressed as µg/mg equivalent of catechin (CE) [28].

#### *2.2.3. Preparation of MgO nanoparticles (MgONPs)*

To synthesize MgONPs, 50 mL of an aqueous extract of *M. oleifera* barks was mixed with 50 mL $MgCl_2.6H_2O$ solution 1 mM in a beaker at 90 °C and stirred at 600 rpm. The 1 M NaOH solution was added drop wise until the color of the mixture faded out and precipitate was formed. The mixture was left for 3 hours to maximize the synthesis process. The MgONPs synthesized was centrifuged at 7,500 rpm at room temperature and re-dispersed in deionized water and methanol (99%) to remove biological residues. The process was repeated twice,

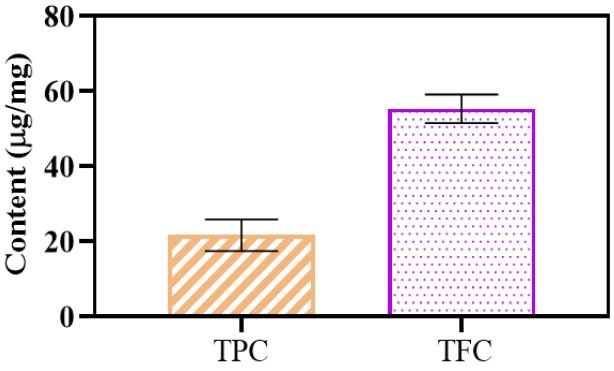


**Figure 1**. Total phenolic and flavonoid content in *M. oleifera* bark aqueous extract, Notes: value is mean ± SD.

and the solid was dried at 100 °C. The solid was subjected to calcination at 600 °C for 5 hours was used to optimize the formation of oxides.

### *2.2.4. Characterizations of MgO nanoparticles (MgONPs)*

Several techniques were used to characterize MgONPs. The confirmation of MgONPs synthesis was based on the change in the color of the mixture during the reaction and recorded with UV-Visible spectroscopy (Analytic Jena Specord 200 Plus) by scanning the spectrum in the range of 200 - 800 nm. The morphology of MgONPs was studied by SEM FEI Inspect-S50. The size and morphology of the MgONPs were investigated by TEM JEOL Jem 1400) and the average particle size by PSA Horiba SZ 100z). The crystal structure of the synthesized MgONPs nanoparticles was confirmed by XRD PANAnalytical Expert Pro.

### *2.2.5. Antioxidant activity*

Antioxidant activity of *M. oleifera* bark aqueous extract (BEM) and MgONPs was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay in accordance with the procedure described, using ascorbic acid as reference [29]. A DPPH 0.1 mM solution was prepared by dissolving DPPH in ethanol. As much as 1 mg of ascorbic acid was dissolved in 1 mL of methanol. Dilution was carried out to make a standard solution of ascorbic acid with different concentrations (50-500 µg/µL). For each tube containing a standard solution of ascorbic acid (200 µL), 1 mL of 0.1 mM DPPH solution was added and followed by the addition of 800 µL 50 mM Tris-HCl buffer (pH7.4). The final volume was adjusted to 4 mL using ethanol. Stock solutions for BEM and MgONPs were prepared by dissolving 1 mg of each sample in 1 mL of methanol and dimethylsulfoxide, respectively.

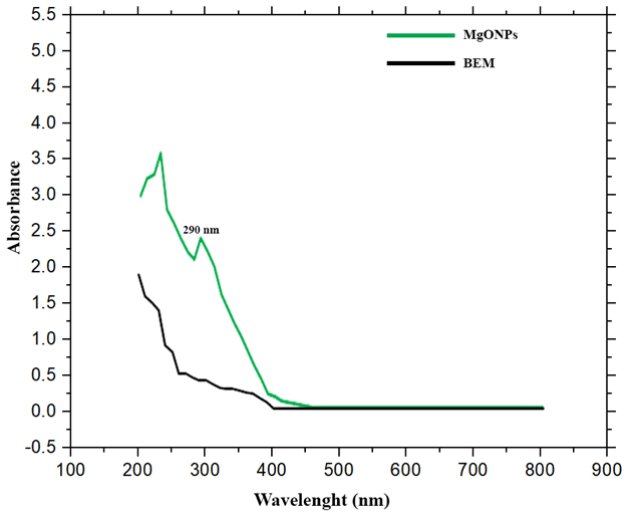


**Figure 2.** UV-Vis spectrum of *M. oleifera* aqueous extract and MgONPs.

Different aliquots of stock solution (50-500 µg) were added to separate tubes, and the final volume was adjusted to 2 mL using ethanol. A total of 1 mL of 0.1 mM DPPH solution and 800 µL 50 mM Tris-HCl buffer (pH7.4) was added to each tube. The control was made by mixing 1 mL DPPH 0.1 mM, 800 µL 50 mM Tris-HCl buffer (pH7.4), and 2 mL ethanol. Absorbance was recorded after incubation for 30 minutes at room temperature, measured by UV-Vis spectrophotometer at 517 nm. The percentage of antioxidant activity (% Inhibition) was calculated using the following equation:

$$(\%\ Inhibition) = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\ x\ 100\ \% \quad \textbf{(1)}$$

The mean and standard deviation (SD) were calculated based on triplicate measurements by repeating three times.

### *2.2.6. Antibacterial activity*

#### *2.2.6.1. Microorganism and inoculum preparation*

The antibacterial activity of BEM and MgONPs nanoparticles was evaluated against both gram-positive (*S. aureus* and *E. faecalis*) and gram-negative (*E. coli* and *S. dysenteriae*) obtained from the microbiology laboratory of Airlangga University. Bacterial cultures for testing were cultivated on nutrient agar (NA) tilted by selecting a colony from the Mueller-Hinton agar plate (MHA) after 24 hours.

A single bacterial or fungal colony is selected and transferred to the Mueller-Hinton (MHB) broth using a sterilized loop, followed by shaking at 100 rpm at 37 °C overnight for a normalized population. The optical density of bacterial or fungal suspense was maintained at the standard 0.5 for MacFarland by addition of sterilized MHB for the test of antibacterial and antifungal activities. The inoculum is therefore composed in about 106-107 CFU / mL of several fungi or bacteria.

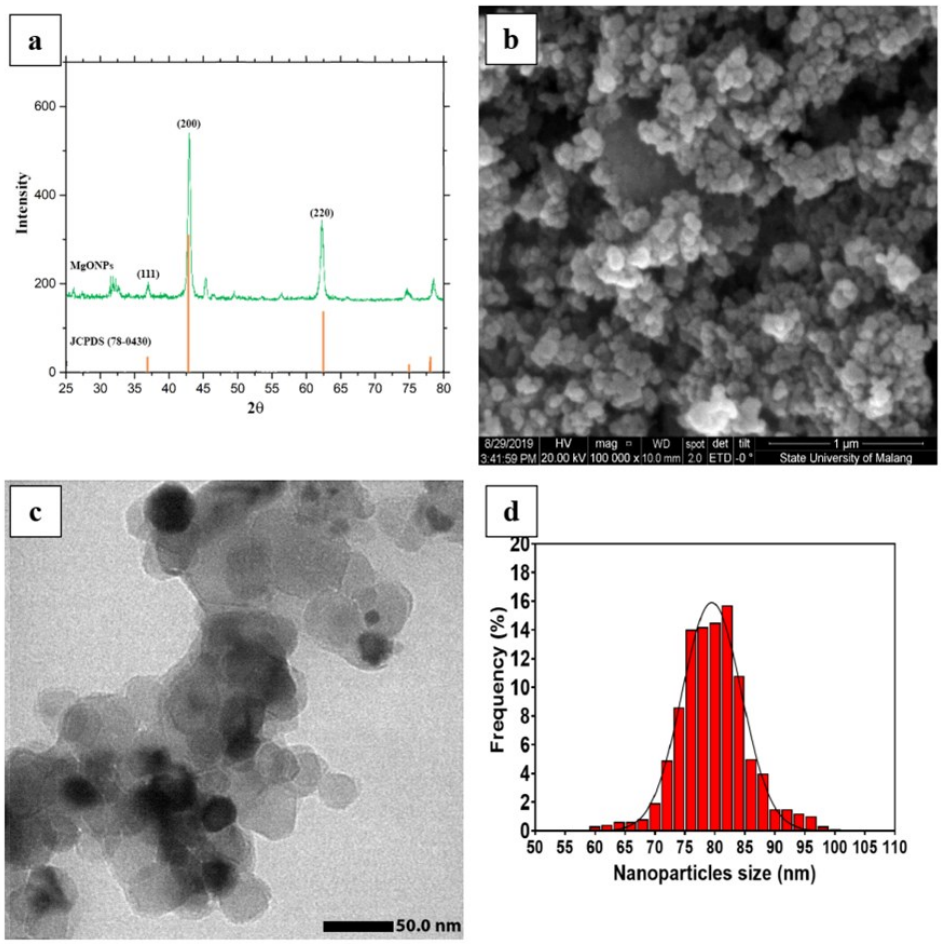


**Figure 3**. (a) diffraction pattern, (b) SEM image, (c) TEM image and (d) particles size distribution of synthesized MgONPs.

*2.2.6.2. Minimum inhibition concentration (MIC) determination*

The resazurin microtiter test was used to assess the minimum concentration of inhibition. This approach has been chosen as the easiest and most economical way to simultaneously scan for multiple microorganism isolates and produces satisfactory performance. A 270 mg tablet of resazurin in 40 mL of sterile distilled water was prepared to dissolve the resazurin solution. The test was conducted in aseptic conditions in 96-well plates. The samples were transferred into the plate well with a volume of 100 µL containing 600 mg/mL. Subsequently, the checked sample was applied to all other wells by 50 µL of bacterial suspense and diluted severely. Each well was subsequently supplemented with 10 µL resazurin solution. The plate was covered with film to avoid dehydration and incubated for 24 hours at 37 °C. The change in color was noticed visually. A change in blue to rose color signaling growth of the cells was considered. In case of a color change, the MIC was registered at the lowest concentration. The mixtures between the sterile distilled water and the dimethylsulfoxide solvents and the nutrient broth were used as adverse controls by Streptomycin (antibacterial) at 0.02 µg/µL concentration.

## 3. RESULT AND DISCUSSIONS

*3.1. Phytochemical screening of M. oleifera bark aqueous extract*

Different methods are used to qualitatively test phytochemical compounds contained in *M. oleifera* (BEM) extracts. The test method used refers to the report Das et al. [29] and the qualitative evaluations of several chemical contents are shown in Table 1.

Table 1 shows BEM extract contains phytochemical compounds such as alkaloids, flavonoids, saponins, and phenolics. These compounds play a role in the chelation process and are able to reduce metal ions to nanoparticles [26].

Total phenolic and flavonoid levels in BEM extract 21.65 ± 4.25 µg/mg GAE and 55.31 ± 3.82

µg/mg CE respectively, as shown in Figure 1. Extracts of some parts of the plant contain different constituents with distinct functional groups, which can act as reducing or chelating agents in the formation of nanoparticles. Total phenolic and flavonoid levels in bark water extracts are lower than total phenolic and flavonoid levels in water extracts of other *M. oleifera* plants that have been previously reported. Siddhuraju and Becker [30] reported total phenolic content in leaves is 74.30 ± 9.00 µg/mg GAE, while Mohammed and Manan [31] reported total phenolic content in ore is 101.79 ± 2.89 µg/mg GAE. The total flavonoid content in leaf water extract was reported by Okumu et al. [32] amounted to 79.13 ± 13.04 (µg / mg CE).

### *3.2. Characterization*

#### *3.2.1. UV-Vis spectroscopy of BEM and MgONPs synthesized*

Synthesis of MgONPs using Moringa aqueous extract followed by a change in color during the synthesis process. The color of the solution changes from clear ($MgCl_2$ solution) to dark brown when added with Moringa extract. After addition of NaOH, the color of the solution changes to brighter, indicating the formation of MgO and $Mg(OH)_2$ complexes in the solution. Adsorption spectrum of MgONPs measured in the range of 200—800 nm. Figure 2 shows the UV spectrum with a sharp peak at around 290 nm, which confirms the formation of MgO nanoparticles [28]. Besides, the precursor ion $Mg^{2+}$, $MgCl_2$ salt does not show a spectrum at the specified wavelength. The existence of a peak of about 280—290 nm can be attributed to the formation of metal oxide nanoparticles after the addition of plant extracts and NaOH solution [28].

#### *3.2.2. Size and morphology of MgONPs synthesized*

In order to correctly assess the atomic position in the lattice structure, a crystalline stage and structure of the synthesized MgONPs were studied using XRD technology. The XRD patterns of MgONPs are shown in Figure 3(a). The MgONPs showed a high intensity peak with two peaks at 42.915 and 62.304 and a low intensity of 31.636, 74.729 and 78.629. The obtained results have been verified using XRD data (No: 78-0430) from JCPDS. A high purity of synthesized MgO nanoparticles does not appear as major peaks from Mg or other impurities observed on the diffractogram. The average crystalline (D) diameter was calculated with the formula of Scherrer (equation 2) for (200) planes of 20–30 nm.

$$D = \frac{K\lambda}{\beta \cos\theta} \quad \textbf{(2)}$$

Where K is a constant dimension depending on the particular geometry of the target, λ is the wavelength of X-ray radiation, β is the full width at half maximum (FWHM) of the significant peaks in radians, and θ is the Bragg's angle. SEM has been used to carry out morphological tests of MgONPs synthesized with extract. Figure 3(b) shows a micrograph image of the MgONPs. The resulting MgONPs are in the form of spherical particle of 20 to 80 nm.

Figure 3 (c) and (d) shows TEM and PSA of MgONPs prepared. The results of PSA show that the MgO with particle size in the range of nanometer with relatively narrow distribution was produced. The samples obtained have the particle size in the range of 60—100 nm. The existence of spheres particles with a size ranging from 60—100 nm was supported by the results of TEM. Based on these results, MgO nanoparticles have been successfully produced using aqueous extracts of bark of *M. oleifera* plant, providing an alternative method for synthesizing MgO nanoparticles. Amrulloh et al. [33] reported the use aqueous extract of Moringa leaves as a green agent in synthesizing MgONPs. The water extract sample of Moringa leaves contains phytochemical compounds such as alkaloids, saponins, carbohydrates, polyphenols, proteins, and amino acids with

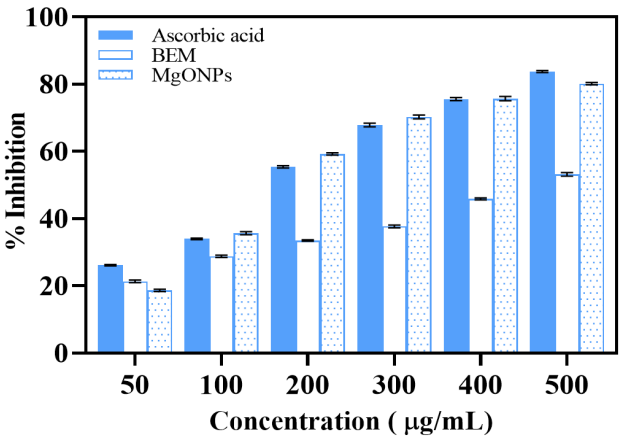


**Figure 4.** Antioxidant activity of BEM and MgONPs.

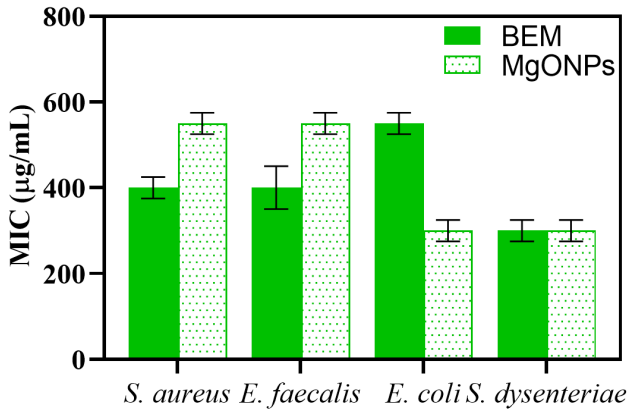


**Figure 5.** Antibacterial activity of BEM and MgONPs.

phenolics and flavonoids contents of 34.75 ± 4.03 µg/mg GAE and 74.28 ± 4.82 µg/mg CE, respectively. The structure of the synthesized MgONPs was confirmed by the spherical structure. The average particle size of the synthesized MgONPs measured between 40—70 nm. The MgONPs synthesized from leaf aqueous extract have narrower particle size distribution than that of MgO produced using bark aqueous extract, which was attributable to the presence of antioxidants-rich compounds (e.g. flavonoids, phenolic acids, reducing sugars, etc.) [34][35]. These biomolecules play a vital role as outstanding bio-reducing and/or bio-capping agents towards generation of the nanoparticles [36].

### *3.3. Antioxidant*

The antioxidant activity of BEM and MgONPs was assessed by DPPH test using ascorbic acid as a positive control. The free radical capture activity of DPPH BEM and MgONPs is directly related to their concentration. DPPH is a stable compound and accepts hydrogen or electrons from BEM or MgO nanoparticles. This test is often used for the antioxidant activity of compounds present in medicinal plant extracts [37]. The antioxidant activity of MgONPs tends to be higher than that of BEM (Figure 5). Researchers have reported similar observations of antioxidant activity by MgO [28], ZnO [38], and CuO [39] nanoparticles.

### *3.4. Antibacterial*

The potential antibacterial activity of BEM and MgONPs was evaluated against gram-positive (*S. aureus* and *E. faecalis*) and gram-negative (*E. coli* and *S. dysenteriae*) bacteria clinically isolated in vitro. MIC values of the presence of BEM against *S. aureus*, *E. faecalis*, *E. coli*, and *S. dysenteriae* are in the range 300—550 g/mL (Figure 5). The antibacterial activity of MgONPs nanoparticles was observed with MIC values (300—550 µg/mL). If the susceptibility between the bacteria tested is compared, *S. dysenteriae* is very susceptible to BEM and MgONPs compared to other test bacteria. Medicinal plants, including *M. oleifera*, are traditionally used to treat various diseases because of their antibacterial activity [40].

The antibacterial activity of MgONPs against gram-positive and gram-negative test bacteria showed different results. The difference between gram-positive and gram-negative bacteria is mainly in the structure of their cell walls. Gram-positive bacteria have a thick layer of peptidoglycan without an outer membrane and contain teichoic acid. In contrast, gram-negative bacteria have a thin layer of peptidoglycan with an outer membrane that contains lipopolysaccharides. Because of this difference, each type of bacteria shows a different sensitivity [41].

## 4. CONCLUSIONS

Green synthesis of MgONPs that were prepared using *M. oleifera* bark aqueous extract was successfully carried out. The formation of MgONPs in this synthesis was confirmed using UV-Vis absorption. The spherical crystal structure of MgONPs was confirmed by XRD analysis. The average particle size of the synthesized MgO nanoparticles measured between 60—100 nm using SEM and TEM images and PSA results. Our study shows that MgO nanoparticles synthesized exhibit a good antioxidant activity, which is higher than ascorbic acid as a positive standard. We also demonstrated that water extracts of *M. oleifera* bark and MgONPs have medium antibacterial activity against *S. aureus*, *E. faecalis*, *E. coli*, and *S. dysenteriae* with MIC values in range of 300—550 g/mL.

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