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GREEN SYNTHESIS OF MgO NANOPARTICLES USING *MORINGA OLEIFERA* LEAF AQUEOUS EXTRACT FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT. Nanoparticle fabrication using plant extracts is an important alternative method because it is non-toxic, biocompatible, and environmentally friendly. In this study, green synthesis of MgO nanoparticles using *Moringa oleifera* leaf water extracts was conducted by mixing the extract and a solution of magnesium chloride. The product was characterized using different techniques, *i.e.* UV-Visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The UV-Vis spectrum of MgO nanoparticles shows an absorption at 280 nm. The size of the synthesized MgO nanoparticles ranges from 20-50 nm. The antibacterial activity of MgO nanoparticles was seen from the zone of inhibition against *Staphylococcus aureus* (6.3 mm) and against *Escherichia coli* (6 mm). MgO nanoparticles have been successfully fabricated using *Moringa oleifera* leaf aqueous extracts, providing an alternative method for synthesizing MgO nanoparticles.

KEY WORDS: Antibacterial activity, *Escherchia coli*, MgO nanoparticles, *Moringa oleifera*, *Staphylococcus aureus*

INTRODUCTION

In recent years, a lot of interest has arisen to synthesize nanoparticles of Fe, Ni, Cu, Ti, and Mg because they have superior properties and can be applied in variousfieldssuch as sensors [1], catalyst [2], and biomedical applications [3-5]. Of all-metal oxide nanoparticles, magnesium oxide (MgO) nanoparticles have good activity for use as an antibacterial. Leung *et al*. [6] explained that the antibacterial activity of MgO nanoparticles can be observed based on the absence of reactive oxygen species (ROS). The mechanism of antibacterial activity that occurs is possible by destruction of cell membranes.

Several researchers have synthesized MgO nanoparticles using several methods, including sol-gel [7], sonochemical [8], co-precipitation [9, 10], and chemical reduction [11]. Green synthesis techniques use reagents that are free of chemicals that are harmful to the environment such as using water solvents or plant extracts [12, 13]. The use of *Moringa* leaf extract in the process of synthesis of metal oxide nanoparticles has been widely carried out by previous researchers. Ezhilarasi *et al*. [3] successfully synthesized nickel oxide nanoparticles (NiO) using *Moringa oleifera* leaf extract with water and ethanol as asolvent. The synthesized NiO crystal is a face-centered cubic with a crystalsize of 9.69 nm.

The activitities of MgO against bacteria have been studied by several researchers previously. Mirhosseini and Afzali [14] studied the antibacterial activity of MgO suspension against *Escherichia coli* (*E. coli*) in milk. Scanning using an electron microscope was performed to characterize the morphological changes of the *E. coli* after antibacterial treatment. The results

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obtained indicate that the presence of MgO combined with pressure can damage cell membranes, resulting in a leakage of the cell contents, and eventually, the bacterial cells die. Das *et al.* [4] synthesized MgO nanoparticles using *Bauhinia purpurea* extract as an antibacterial against *Staphylococcus aureus* (*S. aureus*). The antibacterial activity of MgO was tested through colony forming unit analysis with fluorescence microscope and FE-SEM. The results showed that the MgO produced had high activity as an antibacterial agent against *S. aureus* with a small dose (250 µg/mL). Amrulloh *et al.* [15] tested the bioactivity of MgO nanoparticles prepared using aqueous extract of *M. oleifera* bark. The average particle size of the synthesized MgO nanoparticles lied between 60-100 nm using SEM and TEM images and PSA results. MgO NPs synthesized showed good antioxidant activity and antibacterial activity against *S. aureus*, *E. faecalis*, *E. coli* and *S. dysenteriae* bacteria.

In this research, the synthesis of MgO nanoparticles was carried out using *M. oleifera* leaf aqueous extracts and studied the antibacterial activity against *E. coli* and *S. aureus*.

EXPERIMENTAL

Materials

Fresh *M. oleifera* leaf was 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 ($NaNO_2$), sodium hydroxide (NaOH), and Mueller-Hinton agar were purchased from Merck Sigma-Aldrich Reagent Pte, Singapore. Bacterial cultures (*E. coli* and *S. aureus*) were obtained from the microbiology laboratory of Airlangga University.

Moringa oleifera leaf extract preparation

Fresh *Moringa oleifera* leaf was washed using flowing water, and then dried under direct sunlight, and finally ground into powder and stored at room temperature. *M. oleifera* leaf extraction was conducted with reference to the method reported in Elumalai, *et al.* [16] and Das *et al*. [4]. *Moringa* leaf powder sample of 4 g was mixed with 100 mL of distilled water. The mixture was then heated to 60 °C for 20 min with stirring until all the *Moringa* sample powder was evenly mixed. After heating, the solution was allowed to cool and filtered using filter paper (Whatman filter paper), and the filtrate was collected. The resulting filtrate was used as a stock solution for the synthesis of MgO nanoparticles (fresh extract was used for each synthesis and testing process).

Free radical scavenging activity and antioxidant potential of M. Oleifera leaf aqueous extract

DPPH scavenging ability

The test method using DPPH (1,1-diphenyl-2-picrylhydrazyl) refers to the research by Das *et al.* [4]. 200 µL of *Moringa* extract was mixed with 0.1 mM DPPH dissolved in ethanol and 800 µL 50 mM Tris-HCl buffer (pH 7.4). The solution was incubated at room temperature (27-30 °C) for 30 min, then the DPPH free radical reduction activity was determinedby measuring the absorbance at 517 nm wavelength using a UV-Vis spectrophotometer. Ascorbic acid (1 mg/mL) was used as a comparison of free radical activity, and the solutions without *Moringa*/ascorbic acid were used as controls. The activity of anti-free radicals against DPPH (%) was calculated using the formula 1 [17]:

$$DPPH\ scavening\ activity\ (\%) = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\ x\ 100\ \% \quad (1)$$

Total phenolic content

Total phenolic content of the *M. oleifera* leaf aqueous extract was determined by spectrophotometer using the Folin-Ciocalteu reagent, according to the study by Das *et al*. [4]. Two mL of *M. oleifera* extract was mixed with 10 mL of Folin-Ciocalteu reagent solution with a concentration of 1/10 in deionized water as a solvent, and left for 2 min. After 2 min incubation, 8 mL of 1 M sodium carbonate solution was added to the mixture of extract and Folin-Ciocalteu reagents. The mixture was incubated in a dark room for 2 hours at room temperature. To determine the phenolic levels, the absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Standard curve was constructed using gallic acid solutions with a concentration range from 50 to 500 µg/mL as standards. The results obtained showed equivalent levels of gallic acid in µg/mL unit contained in the samples [18].

Total flavonoid content

The total flavonoid content of the extract was measured using the colorimetric method with aluminum chloride. One ml of *M. oleifera* leaf extract was mixed with 4 mL of distilled water, and the $NaNO_2$ solution with a concentration of 0.3% (w/v) was added and left for 5 min. After 5 min incubation, the mixture was added with a solution of $AlCl_3$ 0.3% (w/v) and then left for 6 min. Then 2 mL of 1 M NaOH solution was added, and the final mixture was added with distilled water until the volume of the mixture became 10 mL. The mixture was left for 15 min, and the absorbance value was measured at a wavelength of 570 nm [14]. Total flavonoid levels of Moringa extracts were determined using a standard curve of catechin solution with a concentration range from 100 to 1000 mg/L.

Green synthesis MgO nanoparticles

The synthesis of MgO nanoparticles was commenced by adding 10 mL of the extractin to 10 mL of 1 mM $MgCl_2$ solution and stirred at 600 rpm at 90 °C. After the temperature of 90 °C has been reached, 2 M NaOH solution was added dropwise to the mixture, and the mixture was left for 3 h aging process in order to optimize the formation of the $Mg(OH)_2$ precipitate. The precipitate formed was separated from the mixture by centrifugation with a rotation of 7500 rpm at room temperature for 20 min and the precipitate was washed twice using ethanol (99%). The precipitate was dried using an oven to remove any residual water and ethanol. After drying, the precipitate was calcined at 600 °C for 5 hours.

MgO nanoparticles characterization

The synthesis of MgO nanoparticles was followed by UV-Visible spectrophotometer analysis (Analytic Jena Specord 200 Plus). An aliquot of 1 mL of reaction mixture was placed in a glass cuvette and the absorbance of the sample was scanned from 200 to 800 nm wavelength. The MgO nanoparticles produced were characterized using different instrumental techniques. The surface morphology was studied using scanning electron microscopy (SEM, FEI Inspect-S50). The crystal structure was studied using X-ray diffraction (XRD, PAN Analytical Expert Pro) techniques. To detect surface functional groups, the samples were characterized using a Fourier Transform Infrared spectrophotometer (FTIR, Shimadzu IR Prestige 21).

Antibacterial activity

The antibacterial activities of *M. oleifera* leaf aqueous extract, MgO nanoparticles and different antibiotics (Ampicillin, Chloramphenicol, and Erythromycin) were evaluated against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* obtained from the microbiology laboratory of Airlangga University using the modified paper disc method of Katata-Seru *et al.* [19]. Briefly, isolates were grown on nutrient agar at 37 °C for 18 to 24 h. The bacterial suspensions were then swabbed on the Mueller-Hinton agar (MHA) plates using sterile cotton swabs. Sterile Whatman No 1 paper discs at 6 mm dimension were impregnated with MgO nanoparticles. The discs with different antibiotics (Ampicillin, Chloramphenicol, and Erythromycin) located on the plates were maintained as references. The discs were gently pressed in MHA plates and incubated in inverted position for 24 h at 37 °C to determine the zone of inhibition.

RESULTS AND DISCUSSION

Free radical scavenging activity and antioxidant potential of M. oleifera leaf aqueous extract

DPPH scavenging ability

Antioxidant activity of the *M. Oleifera* leaf aqueous extract measured by DPPH method is shown in Table 1. A testing anti-free radical activity using DPPH is a must in every test of antioxidant and phytochemical ability of a plant extract [20–22]. The antioxidant activity of *M. oleifera* leaf aqueous extract displays a good DPPH inhibition with 64.60±0.69% RSA (radical scavenging activity) as compared ascorbic acid showed around 71.72±0.56% inhibition of DPPH.

Total phenolic content

Gallic acid used as a standard to determine total phenolic content spectrophotometrically (765 nm) [15]. Amount of phenolic compound present in the leaf extract calculated using gallic acid standard curve. Total phenolic content of the *M. oleifera* leaf aqueous extract of 683.95±0.74 GAE per mL was found. It can be infered from Table 1 that the phenolic content of the leaf extract sample may be responsible for the reduction of Mg^{2+} ion to form MgO nanoparticles [23].

Total flavonoid total

Total flavonoid content was measured spectrophotometrically used aluminium chloride method at 510 nm [4]. Total flavonoid content of the *M. oleifera* leaf aqueous extract was found to be 514.08±0.12 µg CE (catechin equivalent) per mL (Table 1). This finding is in agreement with report by others which show that flavonoids have antioxidant activity in vitro and also act as antioxidant in vivo [24].

Tabel 1. Free radical scavening activity and antioxidant potential of *M. oleifera*leaf aqueous extract.

Sample	Free radical scavenging activity and antioxidant potential of *M. oleifera* leaf extract				
	DPPH scavenging ability (%)	Total phenolic content (µg GAE mL^{-1})	Total flavonoid content (µg CE mL^{-1})	IC_{50} (µg/mL)	Antioxidant activity index (AAI)
Ascorbic acid	71.72 ± 0.56	-	-	1.17 ± 0.43	33.65 ± 0.72
M. oleifera aqueous leaf extract	64.60 ± 0.69	683.95 ± 0.74	514.08 ± 0.12	27.52 ± 0.12	1.43 ± 0.31

Synthesis and characterization of nanoparticles MgO

UV-Visible spectroscopy

Synthesis of MgO nanoparticles using *Moringa oleifera* leaf aqueous extract was followed by a change in color during the synthesis process. The color of the solution changed from clear ($MgCl_2$ solution) to dark brown after the Moringa extract was added. After adding NaOH, the color of the solution changes to brighter, indicating the formation of MgO and $Mg(OH)_2$ complexes in the solution. The colloidal mixture was scanned over the wavelength in the range of 200-800 nm, and the UV spectrum produced is shown in Figure 1. As can be seen, the spectrum is characterized by the appearance of the peak at around 280 nm, which confirms the formation of MgO nanoparticles [12, 15].

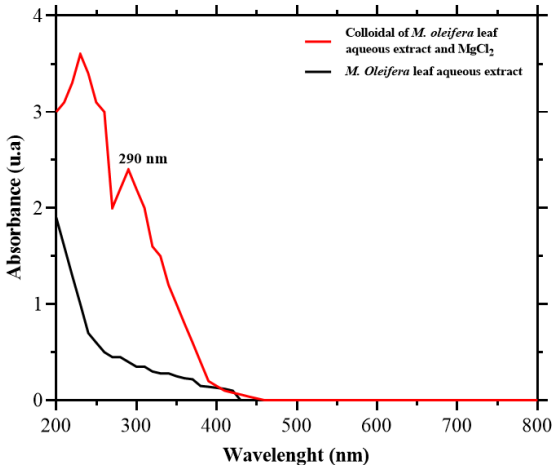

Figure 1.UV-Visible absorption peaks of*M. oleifera* leaf aqueous extract and colloidal of *M. oleifera* leaf aqueous extract and $MgCl_2$.

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 [4]. This phenomenon can be related to the formation of MgO nanoparticles from their saline solution precursor [25].

XRD

The crystalline phase and structure of the synthesized MgO nanoparticles were investigated using X-ray diffraction techniques to properly study the position of the atoms in the lattice structure. Figure 2 shows the XRD pattern of MgO nanoparticles. The two highest peaks at the XRD diffractogram are specific for MgO nanoparticles (at 2θ = 42.915, 62.304) and supported by three small peaks (at 2θ = 31.636, 74.729, 78.629). Confirmation of the results obtained is verified using the JCPDS standard XRD data (No: 78-0430). No significant characteristic peaks appear from Mg or other impurities detected on the diffractogram indicating the high purity of the synthesized MgO nanoparticles. The average diameter of crystalline (D) was measured using the Scherrer's formula (Equation 2) for the (200) plane obtained at 21.07 nm [20, 21].

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

where K is a constant dimension depending on the specific geometry of the object, λ is the wavelength of X-ray radiation, β is the full width at halfmaximum (FWHM) of the significant peaks in radians, and θ is the Bragg's angle [28].

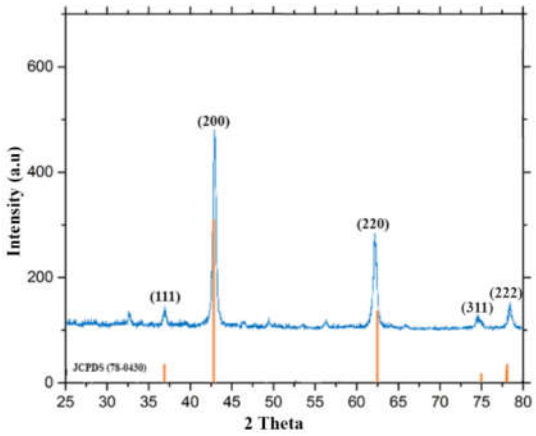

Figure 2. XRD pattern of MgO nanoparticles.

SEM

Morphological analysis of MgO nanoparticles synthesized was carried out using SEM. Figure 3 shows a scanning electron microscopy (SEM) image of MgO nanoparticles, which shows that the resulting MgO nanoparticles are in the form of spherical with particle sizes between 20 to 50 nm. Essien *et al.* [23] synthesized MgO nanoparticles using the aqueous extract of *Chromolaena odorata* leaf. UV–visible (UV–Vis) spectrophotometric assessment indicated the formation of MgO nanoparticles by the presence of a peak at 270 nm, SEM showed good surface properties, energy-dispersive X-ray analysis (EDX) confirmed the presence of MgO in the sample, transmission electron microscopy (TEM) revealed cubic-shaped MgO nanoparticles with average size of 12.3 nm, X-ray diffractometry (XRD) confirmed the formation of MgO phase. Amrulloh *et al.* [15] synthesize MgO nanoparticles using *M. oleifera* bark aqueous extract as green agent. The spherical crystal structure of MgO nanoparticle was confirmed using XRD and SEM analysis. The average particle size of the synthesized MgO nanoparticles between 60-100 nm was revealed by SEM and TEM images and PSA results.

FTIR

FTIR analysis was carried out to investigate absorbed molecules or functional groups on the surface of MgO nanoparticles synthesized using *M. oleifera* leaf aqueous extract. Also, it can be used in investigating the mechanism of synthesis of MgO nanoparticles using biological compounds. Figure 4 shows the FTIR spectra of MgO nanoparticle samples synthesized, displaying sharp absorption bands at wavenumbersof 1190 cm^{-1} and 1460 cm^{-1}, which show the presence of Mg-O interactions [29]. Also, smallbands in the area of 2000-2400 cm^{-1} indicate a stretch of C-H of the remaining organic compounds and a band in the region of 3500 to 3800 cm^{-1}, which is related to the stretching of the N-H group [23]. From the spectrum, it can be concluded that the MgO nanoparticles produced on the surface do not contain other molecules or functional groups.

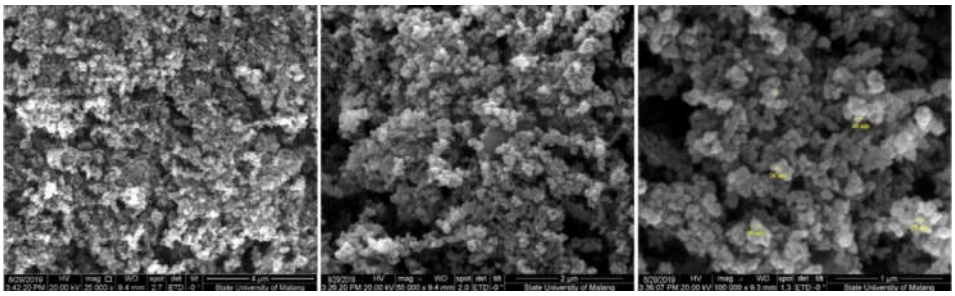

Figure 3. SEM image of MgO nanoparticles.

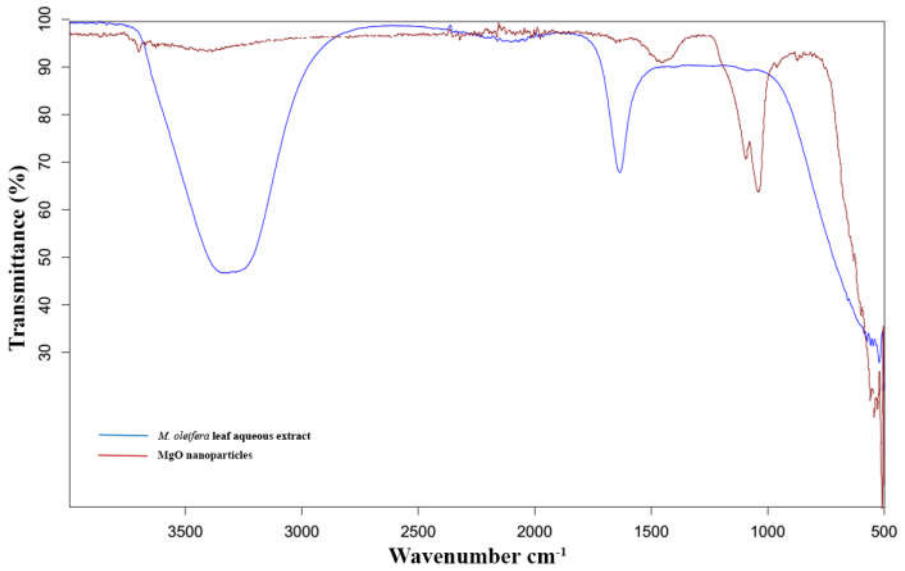

Figure 4. FTIR spectra of *M. oleifera* extract and MgO nanoparticles.

Antibacterial activity of MgO nanoparticles

The relative antibacterial activity of MgO nanoparticles was evaluated against pathogenic bacteria *S. aureus* (gram-positive) and *E. coli* (gram-negative) using the impregnation method. The zone of inhibition results (mm) from antibacterial testing of MgO nanoparticle samples and standard antibiotics (ampicillin, chloramphenicol, erythromycin) shown in Table 2. MgO nanoparticles have antibacterial activity against *S. aureus* and *E. coli* with a zone of inhibition against gram-positive bacteria is larger than that for gram-negative bacteria.

The green synthesized magnesium oxide nanoparticles demonstrated its antibacterial activity against both gram-positive and gram-negative bacteria, it was found that the antibacterial effect of MgO nanoparticles was higher against gram-positive (*S. aureus*) bacteria than that against gram-negative (*E. coli*), with the inhibition zones of 6.3 mm and 6 mm, respectively (Table 2). 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 [33]. The experimental results demonstrate that the synthesized MgO nanoparticles possess a moderate activity against the tested pathogens compared to the standard drug. The diameter of the zone inhibition reflects the magnitude of the susceptibility of microbes. Compared with other metal oxide nanoparticles, synthesized MgO nanoparticles have larger zone inhibition than those of CuO and ZnO nanoparticles, but smaller than that of Fe_2O_3 nanoparticles.

Table 2. Antibacterial activity.

Metal oxide nanoparticles	Bacteria	Zone inhibition (mm)	Reference
MgO	*S. aureus*	6.3	*
MgO	*E. coli*	6	*
Fe_2O_3	*S. aureus*	13	[30]
Fe_2O_3	*E. coli*	12	[31]
CuO	*S. aureus*	3.2	[32]
CuO	*E. coli*	4	[32]
ZnO	*S. aureus*	3	[32]
ZnO	*E. coli*	3.5	[32]
M. oleifera leaf	*S. aureus*	3.2	*
M. oleifera leaf	*E. coli*	2.8	*
Ampicillin	*S. aureus*	10	*
Ampicillin	*E. coli*	17	*
Chloramphenicol	*S. aureus*	7.7	*
Chloramphenicol	*E. coli*	11	*
Erythromycin	*S. aureus*	4.3	*
Erythromycin	*E. coli*	12	*

*Present work.

CONCLUSION

Green synthesis of MgO nanoparticles using plant extracts is a good alternative in the synthesis of a metal oxide nanomaterial. The results obtained in this study show that MgO nanoparticles can be produced using *Moringa oleifera* leaf aqueous extracts. The synthesized MgO nanoparticles were studied for their optical and structural properties by using UV-Visible spectroscopy, FTIR spectroscopy, X-ray diffraction technique and SEM analysis. In UV-Visible spectrum, the existence of MgO is indicated by the presence of a characteristic absorption at 290 nm. The FTIR analysis shows no functional groups other than those associated with MgO, suggesting a high purity of the sample. XRD and SEM studies confirmed the formation of nanoparticles of spherical shape with the particle size between 20 to 50 nm. The antibacterial experiments against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria demonstrated the potential of MgO nanoparticles as antibacterial agent for both *S. aureus* and *E. coli*.

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