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GREEN SYNTHESIS AND CHARACTERISATION OF MAGNESIUM OXIDE NANOPARTICLES USING *Annona Muricata* LEAVES EXTRACT

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Abstract: The synthesis of magnesium oxide nanoparticles (MgO-NPs) using green synthesis has increased. In this study, green synthesis of MgO-NPs has been carried out by using *Annona muricata. Annona muricata*, popularly known as "graviola" or soursop, is a typical plant in countries with a tropical climate. The MgO-NPs were synthesised by mixing 30 mL of *Annona muricata* extract with 5 mM magnesium nitrate solution. The synthesised MgO-NPs were characterised by using Thermogravimetric Analysis (TGA) to identify the calcination temperature to form MgO-NPs, followed by a Fourier Transform Infrared (FTIR) analysis, X-ray Diffraction (XRD) analysis and Scanning Electron Microscopy (SEM). From TGA, temperatures at 700 and 900 have been chosen. For FTIR characterisation, the 450-600 cm^{-1} peaks were assigned to MgO stretching vibrations. Under SEM, the morphology of synthesised MgO-NPs seems to be in irregular shape and the aggregation of particles were observed.

Keywords: Magnesium oxide nanoparticle, MgO-NPs, green synthesis, metal oxide, *Annona muricata.*

Introduction

Nanotechnology is one of the most effective areas of study in new material science with wide applications in biotechnology and the biomedical field. A nanoparticle can be synthesised in specific characteristics such as shape, size and distribution. Using biological materials like plant extracts (from the leaves, flower, stem bark, fruit peels and seed), fungi, bacteria and algae for nanoparticles are eco-friendly and compatible with pharmaceuticals and other biomedical applications. No toxic chemicals are needed in the synthesis procedure (Dhillon *et al.,* 2012).

The green synthesis method is one of the best methods practised in nanoparticle research since it can reduce the use of toxic and hazardous waste materials. Besides, it also creates a renewable process for the environment by using nontoxic chemicals, renewable materials or biologically benign solvents. Several types of biomolecules could be used in order to synthesise metallic nanoparticles. They are vitamins, fungi, enzymes, biodegradable polymers and bacteria. The previous study of green synthesis of magnesium oxide nanoparticles (MgO-NPs) from *Neem* leaves extract has also agreed with the eco-friendly advancement of novel technology (Wani and Shah, 2012).

MgO-NPs is an essential material applied in many fields like catalysis, toxic waste remediation, paint, superconducting products and anti-bacterial activities against the foodborne pathogen (Tony Yiping, 2011; Mageshwari *et al.,* 2013; Mohd *et al.,* 2012). Common synthesis methods such as the solution combustion (Jiahai *et al.,* 2011), co-precipitation (Banele *et al.,* 2013), sol-gel, hydrothermal (Hiromichi and Yukiya, 2010), solvothermal (Hou and Song, 2003) and microwave-assisted synthesis (Hakimeh and Abolghasem, 2012) can be further modified and improved by using green

synthesis. One of the best methods to synthesise nanoparticles without agglomeration in the yield and size can be easily controlled is the co-precipitation method (Yusoff *et al*., 2020).

Green synthesis methods have made use of moderately pollutant-free chemicals to synthesise nanoparticles. It consists of water as a solvent and the natural extract (Masuo *et al.,* 2007). Usually, the green synthesis nanoparticle was carried out using parts from plants or an extract from fungi, bacteria and algae (Sastry *et al.,* 2003; Iravani, 2014; Hulkuti and Taranath, 2014; Ovais *et al.,* 2016). Major compounds found from plant extracts are flavonoids followed by alkaloids and phenols. Two main plant organs that are widely studied are the leaves and seeds. From the organic extracts, alkaloids have been identified as the common phytochemical. Alkaloids are naturally occurring compounds having a basic nitrogen atom. In this study, Annona muricata (also known as soursop) was chosen to synthesise MgO-NPs. Alkaloids reported in *Annona muricata* are mainly composed of isoquinoline, aporphine and protoberberine (Mohanty *et al.,* 2008). In a phenolic compound, there are 37 compounds which have been reported in *Annona muricata*. The important phenolic compounds in *Annona muricata* leaves are quercetin (Nawwar *et al.,* 2012) and gallic acid (Correa-Gordillo *et al.,* 2012). From a qualitative test, it recognises the presence of bioactive photo constituents in the leaf extracts. The organic molecule in the leaf extract acts as a reducing and stabilising agent.

This work focuses on the green synthesis of MgO-NPs using *Annona muricata* leaves extract. The synthesised MgO-NPs were further characterised by various methods, which include Fourier Transforms Infrared (FTIR), X-Ray Diffraction (XRD), Thermogravimetric Analysis (TGA) and Scanning Electron Microscopy (SEM).

Materials and Methods

All the chemicals and materials that were used in green synthesis and characterisation of magnesium oxide nanoparticles using soursop leaves extract were distilled water, magnesium nitrate solution, deionised water, magnesium nitrate, 1 M NaOH, ethanol, concentrated sulfuric acid, diluted ferric chloride solution, Fehling's solution, 1% aqueous hydrochloric acid, acetic anhydride, iodine solution, 10% iron (III) chloride solution, potassium iodide and soursop leaves.

Characterisations of the obtained samples were carried out using Thermogravimetric Analysis (TGA) to identify the calcination temperature to form MgO-NPs, followed by a Fourier Transform Infrared (FTIR) analysis, X-ray Diffraction (XRD) analysis and Scanning Electron Microscopy (SEM).

Preparation of *Annona muricata* (Soursop) Leaves Extracts

Leaves were rinsed with distilled water thoroughly to eliminate dirt and other attached particles. 5 g of soursop leaves were cut into smaller pieces and placed in a 100 mL Erlenmeyer flask and filled with 50 mL of distilled water. Then, the mixture was boiled for 5 minutes. The solution was removed from the heat source and left at room temperature. Then, it was filtered through a normal filter paper followed by a Whatman filter paper No.1. The extract was kept in a refrigerator at 4 for further experiments.

Phytochemicals Test

Alkaloid Test

0.5 mL of crude extract was treated with a few drops of Wagner's reagent (iodine in potassium iodide). The colour change was observed, and a brown precipitate formed, showing the presence of an alkaloid.

Flavonoid Test (test)

0.5 mL of crude extract was treated with a few drops of concentrated . The colour change was observed and the formation of orange colour was specified as the existence of flavonoid.

Phenolic Content Test

0.5 mL of crude extract was put into the test tube. About 2 mL of distilled water was added, followed by 2 or 3 drops of 10% iron (III) chloride solution. The existence of green or blue colour was indicated as the presence of the phenolic compound.

Tannin Test

0.5 mL of crude extract was added to distilled water; later 1 to 2 drops of dilute ferric chloride solution were added. A dark green or blue-green colouration was indicated as the existence of tannin.

Saponin Test (Foam Test)

1 mL of aqueous was added to a few volumes of distilled water in a test tube. The solution was shaken vigorously, and a stable, persistent froth for 10 minutes was observed.

Steroid Test (Libermann-Burchard Test)

0.5 mL of one crude extract was treated with a few drops of acetic anhydride. It was boiled and set cold. A few drops of concentrated sulfuric acid were added to the test tube and the formation of a brown ring in the middle of two layers was observed. A positive steroid test was indicated when green colouration of the upper layer occurred.

Phlobatannin Test

0.5 mL of crude extract was boiled with 1% aqueous hydrochloric acid. The presence of red precipitate was indicated as the presence of phlobatannin.

Carbohydrate Test (Iodine Test)

0.5 mL of one crude extract was preserved with a few drops of iodine solution. The presence of carbohydrates was proved as purple or dark blue colour is formed.

Synthesis of MgO-NPs

30 mL of the plant extract was taken and placed in a 500 mL beaker. 150 mL of the freshly prepared 5 mM magnesium nitrate solution was added drop by drop using a burette and 1 M NaOH was added dropwise with continuous stirring for 2 hours at the temperature of 80. With the addition of the magnesium nitrate solution, a sharp change in colour from pale green to brown was detected. This observation has confirmed the formation of a precipitate. Then, the solution was centrifuged, washed with ethanol several times to remove other impurities, and dried in an oven for 8 hours.

Thermogravimetric Analysis (TGA) to identify the calcination temperature to form MgO-NPs, followed by a Fourier Transform Infrared (FTIR) analysis. For FTIR, its response was approved through the wavenumber range from 400-4000 by using the KBr pellet technique at room temperature. X-ray Diffraction (XRD) analysis was done to determine the crystallinity and purity of samples and finally, Scanning Electron Microscopy (SEM) to observe the morphology and shape of the samples.

Results and Discussion

Synthesis of MgO-NPs

Phytochemical tests were carried out on *Annona muricata* leaves extract to investigate the chemicals acting as reducing agents for reducing metal ions to nanoparticles. Figure 1 shows the *Annona muricata* leaves extract preparation. The extract obtained was further used to synthesise MgO-NPs. Figure 2 shows precipitate formation after mixing the extract solution with magnesium nitrate and a few drops of sodium hydroxide (NaOH) solution.

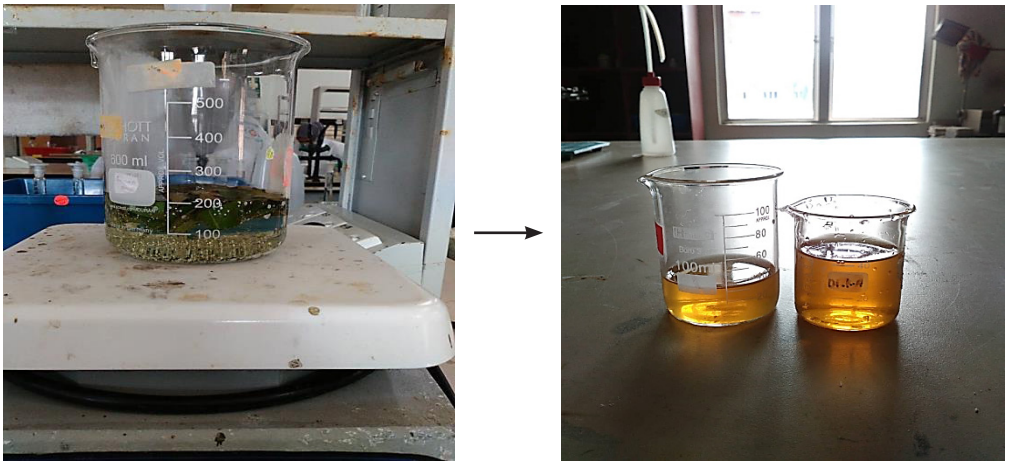


Figure 1: *Annona muricata* leaves extract

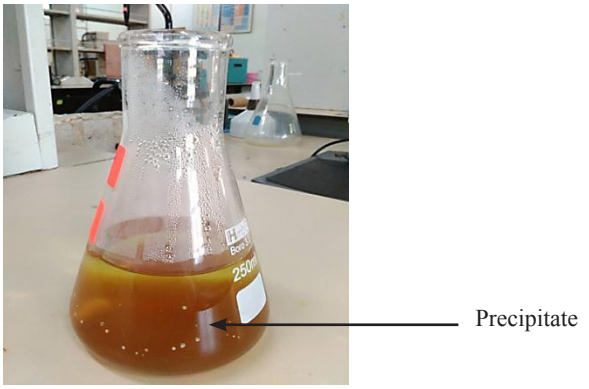
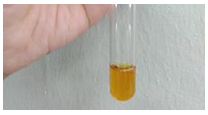
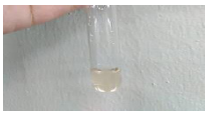
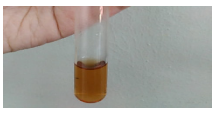


Figure 2: Formation of $Mg(OH)_2$ precipitate

Phytochemicals Test

A total of eight phytochemical tests were conducted. Phytochemical analysis was carried out to determine the presence of carbohydrate, tannin, alkaloid, flavonoid, saponin, phlobatannin, phenolic content test and steroid. Table 1 shows the results of the phytochemical test for *Annona muricata* extract. Three replicates were carried out for each test, and only tests that were positive in two or more tests were considered to confirm the presence of the investigated compounds in *Annona muricata* leaves extract.

Test	Result	Set 1	Set 2	Set 3
Carbohydrate		-	-	-
Tannin		+	+	+
Alkaloid		+	+	+
Flavonoid		+	+	+
Saponin		+	+	+
Phlobatannin		-	-	-
Steroid		-	-	-
Phenolic		+	+	+

(+) = presence of active compounds

(-) = no active compounds

Five tests showed positive results from these tests: tannin, alkaloid, flavonoid, saponin, and phenolic. This finding agrees with a study by Gavamukulya *et al.,* 2014 and Hasmila *et al.*, 2019 and contains all the prominent second metabolites. The other three phytochemical tests showed negative results: carbohydrate, phlobatannin and steroid. Our study uses water extract for the green synthesis route, while other studies use ethanol and methanol solvents; hence more selected compounds can be separated (Agu and Okolie, 2017 and Hasmila *et al.*, 2019).

Characterisation of MgO-NPs in Annona muricata leaves

Thermogravimetric Analysis (TGA)

TGA helps select the calcination temperature to form pure MgO nanoparticles. The temperature chosen was 700 and 900. The calcination temperatures were checked for the synthesis of MgO-NPs, and the conditions were optimised (Moorthy *et al.,* 2014). The obtained sample from the experiment was characterised using TGA to observe the weight loss curve to determine the calcination temperature. Two sets of samples of MgO before calcination were used to perform TGA analysis. This is to confirm the consistency of the method used. The analysis of MgO before calcination was performed twice at a heating rate of 1 / min. Figure 3 shows the TGA graph of two sets of MgO samples before calcination. The weight loss of the sample observed at room temperature to 100 ºC was due to the evaporation of water molecules, whereas 100 ºC to 600 ºC was due to the evaporation of organic and inorganic materials (Guadix-Montero *et al.,*2017).

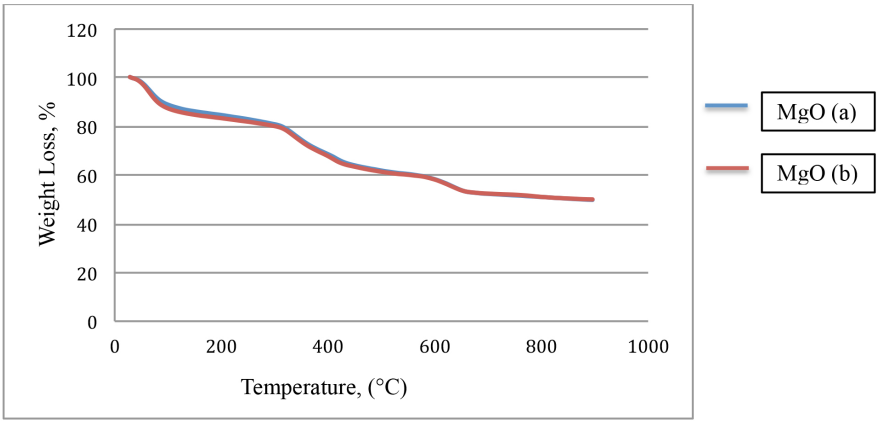


Figure 3: TGA graph of two sets of MgO samples before calcination

The calcination temperatures used to obtain MgO-NPs were 700ºC and 900ºC. The result showed that the weight loss percentage continuously decreased until 700 ºC and started flat after that. TGA analysis was observed at room temperature to 900 ºC.

Fourier Transform Infrared (FTIR)

FTIR spectroscopy is used to define the vibrational frequency of stretching and indirect modes of the molecules as well as potential biomolecules, which are liable for the reduction and capping of MgO-NPs (Abinaya *et al*., 2021). Figure 4 shows the FTIR of commercial MgO, synthesised powder before calcining, and MgO-NPs calcined at 700 ºC and 900 ºC having a different bands with various functional groups.

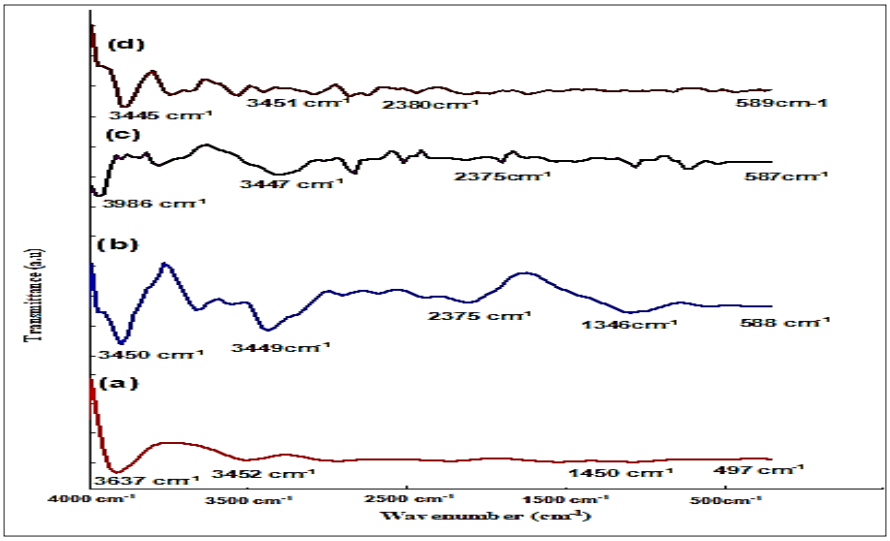


Figure 4: FTIR spectru am of (a) commercial MgO, (b) synthesised sample before calcine, (c) calcined MgO-NPs at 700 and (d) calcined MgO-NPs at 900

The peaks in the 450-600 cm^{-1} are allocated to Mg-O stretching vibrations for all samples. The broad peaks at the higher region 3440-3450 cm^{-1} are due to stretching –OH groups (Ogunyemi *et al.,* 2019 and Yusoff *et al.,* 2020). A reduction in peak intensity was noticed when compared with each other. This suggests that the organic molecules have been involved in forming MgO-NPs. Peaks in the range between 2100 -2500 cm^{-1} indicate the stretching of alkynes, while peaks at 2375-2385 cm^{-1}, 1633 cm^{-1} and 1346 cm^{-1} correspond to C=O stretching (amide linkages), C=C stretching (alkenes) and –CH stretching (alkanes), respectively. It is also observed that with nanoparticle formation, the intensity of peak 3500 cm-1 assigned to N-H stretching was found to decrease. The peaks in the range between 588-694 cm^{-1} corresponded to Mg-O stretching vibrations (Yusoff *et al*., 2020). A study from Yusoff *et al.* (2020) claims that the presence of these functional groups is due to the compounds present in the plant extract. They highlight the biological properties of metal oxide derived from the plant extract. Table 2 shows the results of FTIR analysis for commercial MgO, synthesised samples before calcine, and samples calcined at 700 and 900.

Table: 2 FTIR peak assignments

Samples	Functional group	Wavenumber (cm^{-1})
Commercial MgO	O-H	3637
	CH Stretching (alkanes)	1450
	Mg-O	497
	O-H	3452
Synthesised sample before calcine	C=O	2375
	CH Stretching (alkane)	1346
	O-H	3450
	Mg-O	588

Calcined at 700	C-O	2375
	Mg-O	587
Calcined at 900	C-O	2380
	Mg-O	589

X-Ray Diffraction (XRD)

Samples were analysed by a Cu Kα – X-Ray Diffractometer to confirm the presence of MgO nanoparticles. Figure 5 shows the XRD pattern obtained from MgO-NPs synthesised using *Annona muricata* extract.

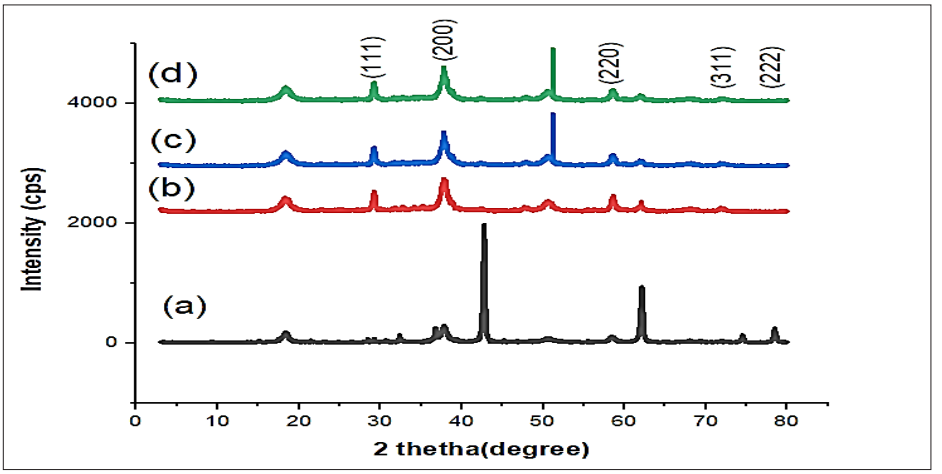


Figure 5: The XRD pattern obtained of MgO-NPs synthesised (a) commercial MgO, (b) synthesised sample before calcine, MgO-NPs calcined at (c) 700 and (d) 900

The Commercial MgO sample showed peaks which are corresponding to [111], [200], [220], [311] and [222] planes (Yusoff *et al.*, 2015). While for a sample before calcine and MgO-NPs calcined at 700 °C and 900 °C showed MgO peaks corresponding to [111], [101] and [200]. Table 3 shows the peak assignment of commercial MgO, synthesised sample before calcine, and MgO-NPs calcined at 700 and 900.

Samples	**2**	**d (angles)**	**d references (angles)**	**hkl**	**Peak assignment**
Commercial MgO	18.63	4.7629	4.7400	001	$Mg(OH)_2$
	36.84	2.4397	2.4347	111	$Mg(OH)_2$
	38.11	2.3012	2.3615	101	MgO
	42.81	2.1123	2.1085	200	MgO
	62.17	1.4931	1.4909	200	MgO
	74.55	1.2729	1.2715	311	MgO
	78.50	1.2184	1.2173	222	MgO

Synthesised sample before calcine	18.29	4.8502	4.8502	102	$Mg(OH)_2$
	29.42	3.036	3.034	111	$Mg(NO_3)_2.6H_2O$
	36.84	2.4397	2.4347	111	MgO
	38.12	2.3608	2.3605	101	MgO
	50.48	1.808	1.801	231	$Mg(NO_3)_2.6H_2O$
	62.17	1.4931	1.4909	200	MgO
	72.52	1.3035	1.3023	152	$Mg(NO_3)_2.6H_2O$
MgO-NPs calcined at 700	18.29	4.8502	4.8502	102	$Mg(OH)_2$
	29.42	3.036	3.034	111	$Mg(NO_3)_2.6H_2O$
	36.84	2.4397	2.4347	111	MgO
	38.12	2.3608	2.3605	101	MgO
	50.48	1.808	1.801	231	$Mg(NO_3)_2.6H_2O$
	62.17	1.4931	1.4909	200	MgO
	72.52	1.3035	1.3023	152	$Mg(NO_3)_2.6H_2O$
MgO-NPs calcined at 900	18.29	4.8502	4.8502	102	$Mg(OH)_2$
	29.42	3.036	3.034	111	$Mg(NO_3)_2.6H_2O$
	36.89	2.4319	2.4421	111	MgO
	38.12	2.3608	2.3605	101	MgO
	50.48	1.808	1.801	231	$Mg(NO_3)_2.6H_2O$
	62.17	1.4931	1.4909	200	MgO
	72.52	1.3035	1.3023	152	$Mg(NO_3)_2.6H_2O$

Scanning Electron Microscopy (SEM)

SEM was used to view the morphology of the obtained samples. To better view the shape and size of synthesised MgO-NPs, two magnifications were used (5000X and 10,000X).

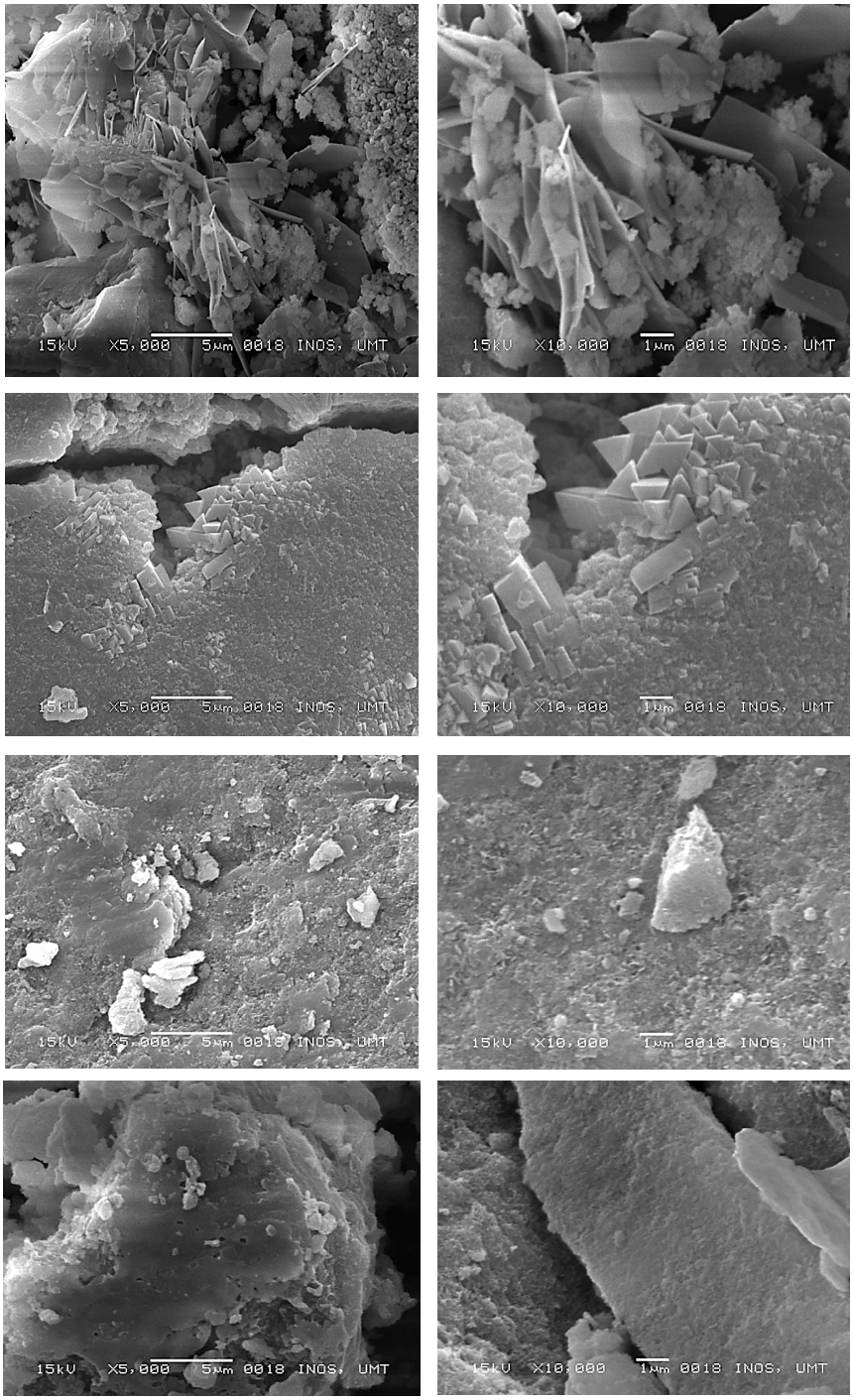


Figure 6 : SEM images of (a) 5000X and (b) 10,000X magnification for commercial MgO, at magnification of (c) 5000X and (d) 10,000X for powder before calcination, MgO-NPs calcined at 700 °C at magnification of (e) 5000X and (f) 10,000X, and lastly MgO-NPs calcined at 900 °C at magnification of (g) 5000X and (h) 10,000X

Figure 6a and 6b show the commercial MgO, which appeared as flakes under the SEM. Meanwhile, samples before (Figure 6c & 6d) and after calcined at 700 (Figure 6e & 6f) are irregular. However, SEM images for calcined samples at 900 show larger structures than expected, irregular shapes, and agglomerated. This might be due to the high surface energy of the nanoparticles (Yusoff *et al.*, 2020 and Hassan *et al.,* 2015).

Conclusion

In this study, we reported on the green synthesis of magnesium oxide nanoparticles from *Annona muricata* leaf extract. The organic molecules present in the leaf extract functioned as a reducing and stabilising agent. Samples were characterised using TGA, FTIR, XRD and SEM. The peaks within 450-600 cm^{-1} in FTIR were assigned to Mg-O stretching vibrations. From XRD, the calcination temperature at 700 and 900 ^{o}C still show traces of precursors from $Mg(NO_3)_2.6H_2O$. The shape of MgO-NPs seems to be larger than expected and in irregular shape when viewed under SEM. To further improved this study, calcination should be carried out at a higher temperature of more than 1000 ^{o}C.

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