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Green synthesis of MgO Nanoparticles Using *Anona Muricata* Leaf Aqueous Extract and its Antidiabetic Activity

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Abstract

Green synthesis of metal oxide nanoparticles leads to the formation of non-harmful nanoparticles as a result of numerous phytochemicals present that have pharmaceutical applications. Synthesis of MgO nanoparticles using A. muricata is a simple technique that is easily used for various biomedical applications and this method makes biosynthesized MgO nanoparticles compatible with anti-diabetic studies. The MgO nanoparticles synthesized from A. muricata were characterized using the FTIR technique that confirmed the presence of functional groups; XRD confirmed the crystallinity and *size (41nm) of the nanoparticle while SEM confirmed the surface morphology of the synthesized nanoparticle. Phytochemical screening of the aqueous extract of MgO nanoparticles synthesized in A. muricata leaves confirmed the presence of alkaloids, tannin, flavonoids, steroids, phenol, glycoside and terpenoids. The total phenol and flavonoid content were evaluated to be at values 48.54±0.58 mg and 46.45±0.642 mg. In vitro, anti-diabetic potentials of the MgO nanoparticles synthesized extract were evaluated using α-amylase and α-glucosidase inhibition with acarbose as a reference drug. The extract of MgO nanoparticles synthesized A. Muricata and acarbose (standard) showed IC_{50}values of 66.00µg/ml and 20.03µg/ml in inhibition of α-amylase while values of 73.42µg/ml and 29.5µg/ml in inhibition of α-glucosidase. The results showed that the aqueous extract of MgO nanoparticles synthesized by Anona muricata exhibits powerful anti-diabetic effects and this supports its use in treating and managing diabetes.*

Keywords*: Anona muricata, Antidiabetic activity, MgO nanoparticles, α-amylase, α-glucosidase.*

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Introduction

Metal oxide nanoparticle synthesis by green methods has become the center of attraction in medicine recently because it is more eco-friendly, it has low cost and toxicity and it exhibits lasting stability (Metz*et al*,2015). Green synthesis nanoparticles are generally obtained from bacteria, algae, fungi and green plants (Sastry *et al*, 2003; Iravani, 2014, Hulkoti & Taranath, 2014; Ovais*et al*, 2016). A large amount of phytochemicals in plants are greatly assessable, and safe and have numerous metabolites that act as reducing agents in nanoparticle synthesis which are beneficial in many biological science fields (Jeevanandam et al., 2017). MgO (Magnesium oxide) is a metal oxide material that is usually harnessed as a catalyst, electrochemical biosensor, pharmaceutical industry and paints (Choudary*et al*, 2003; Lu *et al*, 2010; Shen *et al*, 2007; Huang *et al*, 2005). The strong crystal-like MgO nanoparticles have low electrical conductivity but high heat stability. The structural morphology of crystalline magnesium oxide nanoparticles exhibits surface area and reactivity that is very specific due to the huge amount of structural defects and corner sites on their surfaces. Unlike TiO_2, Ag, Cu and other types of solid bactericides, MgO nanoparticles can be prepared from easily assessable

and low-cost precursors and solvents (Roselli*et al*, 2003; Sawai, 2003; Sawai *et al.*, 2000). Normally, MgO nanoparticles are synthesized through diverse techniques like hydrothermal, sol-gel and wet precipitation, and chemical gas phase deposition methods which are dangerous and not useful in medical applications. Hence, nanoparticle synthesis from green methods is very efficient and nontoxic (Karthikeyan *et al*, 2012).

Annona muricata is a fruit tree from the family of Annona ceae and has since been in use since the days of old. *A. muricata* also called soursop is an ageless plant mostly seen in the equatorial regions of the globe (Patel & Patel, 2016). The bark, leaves, roots and seeds of the soursop tree have medicinal properties. Soursop (*Anonna muricata*) leaves have antihypertensive, antispasmodic, sedative, hypoglycemic, anticancer, emetic and vermifuge properties (Hardoko et al., 2015). Leaf juice is used to prevent unconsciousness and leaf extracts are used to treat parasites both external and internal, as well as cystitis, diabetes, migraines and insomnia (Solanki et al., 2020). Most parts of the plant have since been in use for treating various ailments which include malaria, diabetes and parasitosis (Chan *et al*, 2020). Hence, research was aimed at investigating the antidiabetic potentials of biosynthesized MgO oxide nanoparticles using *Anona muricata* aqueous leaf extract.

Materials and Methods

The reagents and chemicals used for the research were of scientific grade. Magnesium nitrate $Mg(NO_3)^2$ was bought from Lagos, Nigeria.

Collection and authentication of plant leaves of *A. muricata*

Anonna muricata fresh leaves were obtained from Ilaro town in Ogun state, Nigeria and were taken to the Botany Department, University of Lagos, Nigeria for recognition and authentication with voucher number 8768.

Preparation of leave extract

The preparation of plant extract was carried out according to Vergheese & Vishal (2018) with slight modification. The fresh plant leaves obtained was washed under running tap water and then washed again twice or three times with distilled water. The preparation of solutions was done using distilled water.

The leaves were properly cleaned and allowed to air dry for two weeks. Using a grinder, the dried leaves were thoroughly ground into powder form. 50g of the powder was placed in a 500ml beaker with 500ml of distilled water and was agitated continuously for an hour at 60°C. It cooled to room temperature before being filtered twice with muslin cloth and once with Whatman filter paper. The filtrate was seen to be a light green colour.

Preparation of biosynthesized Magnesium oxide Nanoparticle

The preparation of MgO nanoparticles from *Annona muricata* leaf extract was carried out according to Vergheese and Vishal (2018) with a slight modification. 30ml plant extract was put into a 500ml beaker and 100ml freshly prepared Magnesium nitrate solution was added in drops with a burette and was stirred continuously for 2hrs using a magnetic stirrer at 80° C. The addition of $Mg(NO_3)^2$ solution caused a prompt colour change from light green to brown which confirmed the MgO nanoparticles' formation. The synthesized MgO nanoparticles solution was centrifuged for 4 minutes at 4000rpm/mins and the precipitate was washed severally with ethanol so that contaminants are removed, afterwards, put under a shade for air-drying overnight.

Characterization of Biosynthesized Magnesium Oxide Nanoparticle

Characterization of the synthesized MgO nanoparticles was performed using the following techniques. FTIR analysis was done using the wave number range from 650-4000cm-1 by atom method to confirm the functional groups in the synthesized MgO nanoparticle. The air-dried MgO nanoparticle was mixed with 150g of dry potassium bromide powder in

a mortar and the mixture into a mould by pressing. The Fourier-transformed infrared spectrophotometer was recorded on an FTIR (BrukerIFS66v/s, Germany) equipped with OPUS version 3.1. Software for windows in the region of 4000–40 cm^{-1} and at a resolution of 4 cm−1. XRD technique was done to determine the crystallinity nature of the nanoparticles. The X-ray powder diffractometer was used to analyze the powdered sample of manufactured MgO nanoparticles in the low angle range (5^o to 70^o at 2**θ**). EI Quanta 200F was used for SEM characterization experiments to examine the nanoparticle's surface shape. For this research, gold was sputtered after a small portion of biosynthesized MgONP was put and spread on the top of the sample holder. For elemental analyses, the nanolayer-coated MgO nanoparticles were analyzed.

In vitro anti-diabetic studies of biosynthesized Magnesium oxide Nanoparticle Inhibition of alpha-amylase enzyme

500µl of test samples and the reference drug (100–1000 g/ml) were added to 500µl of a solution that contained 0.5 mg/ml of -amylase in a 0.20 mM phosphate buffer (pH 6.9). Each test tube received 500µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) after the mixture had been incubated at 25°C for 10 mins. Following this, the individual test tubes were incubated again at 25°C for 10 min. The test tubes were placed in a water bath for 5 mins to stop the reaction and they were allowed to cool to room temperature. The reactions in the test tubes were then diluted with 10 ml of distilled water, and absorbance at 540 nm was taken. The control represents 100% enzyme activity and the procedure was carried out similarly but the extract was replaced with a vehicle (Thalapaneni*et al*., 2008; Heidari et al., 2005).

Inhibition of alpha glucosidases enzyme

1ml starch substrate solution (2 % w/v maltose or sucrose) was incubated with 0.2 M Tris buffer (pH 8.0) and different concentrations of the test sample at 37°Cfor 5 min. The reaction mixture was initiated by adding 1ml α-glucosidase enzyme (1U/ml), then incubating at 37°Cfor 10 mins. The reaction mixture was topped by being heated in a boiling water bath for 2 mins. The glucose oxidase peroxidase method was used to measure the amount of glucose released (Andrade-Cetto, Becerra-Jimenez and Cardenas-Vazquez, 2008); Matsuura *et al.*, 2002; Tietz, 1999).

Calculation of 50% Inhibitory Concentration (IC_{50})

IC_{50} was calculated using percentage scavenging activities of 5 different concentrations of the extract.

I% (Percentage inhibition) was calculated with this formula:

$$I\% = \frac{Ac - As}{Ac} x100,$$

where Ac is the absorbance of the control and As is the absorbance of the sample (Shai, *et al*, 2010).

Results and Discussion

Synthesis of MgO nanoparticles

It was observed during MgO nanoparticle formation that the addition of colourless 5mM Magnesium Nitrate [$Mg(NO_3)_2$] solution (dropwise) to the extract of *Anona muricata* caused a colour change from light green to brown which confirmed the synthesis of MgO nanoparticles (Vergheese & Vishal, 2018).

Fourier Transform Infrared Spectroscopy

FTIR is an interference and absorption-based technique that involves vibrations in the molecules after absorption of precise infrared radiation (Singh, Joshi and Ramola, 2019)). It is used to identify the type of bonds present on the nano-materials over a frequency range of 4000 to 650cm^{-1}. FTIR identified the biomolecules that caused the reduction and capping of MgONPs (Prasanth *et al*, 2019). Figure 1 shows the FTIR responses for the biosynthesized MgONPs *Anona muricata* aqueous extract. The FTIR analysis was performed by wave number range from 650-4000cm-1 using the Atom method at room temperature. The bands at 3261, 1632 and 1349 cm^{-1} can be ascribed to some functional groups matching -OH vibration of alcohols, carboxylic acids and phenols, C-H of methyl groups and CH_2 group. The

FITR peaks obtained in this research match the observed peaks from publications that used *A. muricata* leaves and fruit extracts (Badmus *et al*, 2020). Badmus *et al.*, (2020) also reported that polyphenols like phenols and flavonoids may contribute to the reduction process leading to the biosynthesis of nanoparticles capped with proteins or amines.

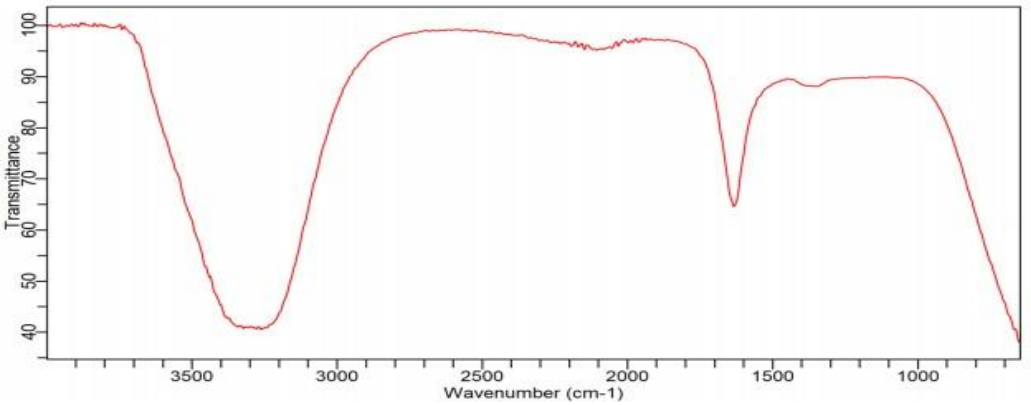

Fig I: FTIR response of Magnesium oxide nanoparticle synthesized from *A. Muricata*.

X-ray Diffraction Analysis

X-ray diffractometer identified and confirmed the crystallographic structure of the biosynthesized MgO nanoparticles. Fig.2 exhibits the XRD pattern of the synthesized MgONPs. The crystalline cubic structure of MgONPs was confirmed by the sharp peaks observed in Fig. 2. The peak was observed at 2θ values ranging from 21.5°, 25.80°, 32.14°, 34.6°, 59.8° which indicated hexagonal shape of MgO-NPs (JCPDS)01-073-2966). The calculation of the average crystallite size of the synthesized MgONPs was carried out using the Scherrer formula $D = k\lambda/\beta\cos\theta A$ (Athithanetal.,2020), where D = average crystalline size in Å, k = shape factor, λ = wavelength of X-ray(0.1540Å) Cu-Kα radiation, B= full width at half maximum (FWHM), and θ = angle of diffraction, therefore, 15°, 37°, 78°, 25° and 50°nm were the values of D at the peaks that appeared at 2θ, hence, average crystalline size of synthesized MgONPs was 41nm.

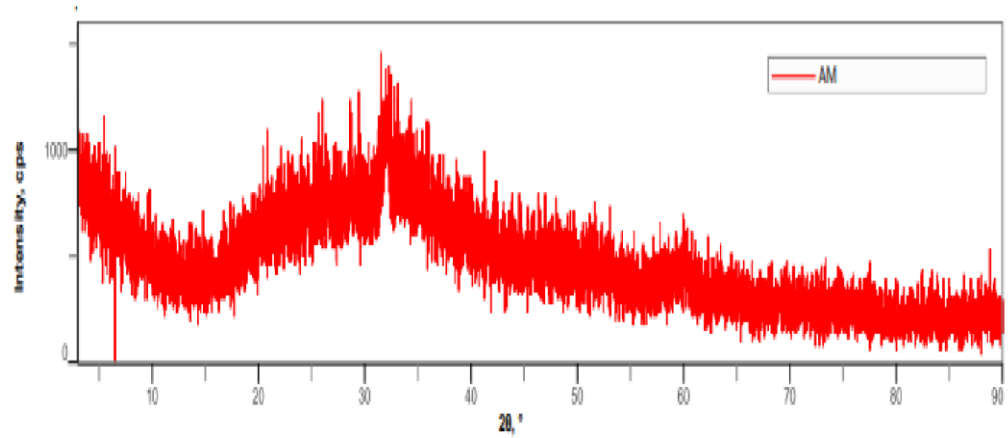

Fig II: XRD Spectrum Pattern of MgONPs *A. muricata*

Scanning Electron Microscopy

The shape of the biosynthesized MgONPs was analyzed by SEM. In previous studies, Athithan *et al*, (2020) found that the morphology of biosynthesized MgO nanoparticles using Aloe vera extracts shows a rock-like structure. In this study, the SEM images of biosynthesized MgO nanoparticles of *Annona muricata* leave aqueous extracts showed intermittent breakdown of the fibrillar structure into individualized fibrils. Fig.3 shows that the particles have rock-like structures and are hexagonal. The range of dimensions of the biosynthesized MgONPs was between 36.7 and 69.6nm.

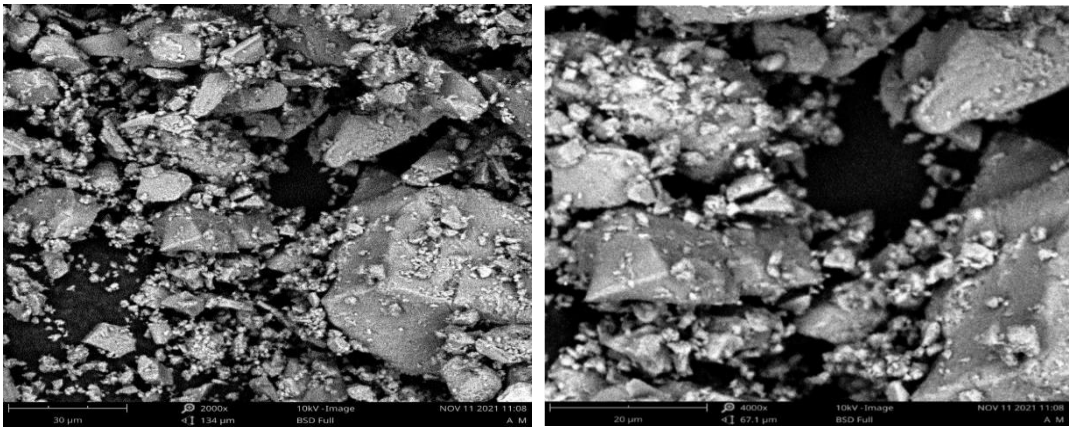

Fig III: SEM image of MgONPs *A. muricata*

Anti-diabetic Activity

Type II diabetes mellitus linked to postprandial hyperglycemia can be effectively controlled byα -amylase and α -glucosidase inhibitors (Badmus*et al*, 2020). The α-(1,4) D-glycosidic bond hydrolysis of starch and other glucose polymers is catalyzed by α-Amylase, therefore the inhibitors of this enzyme are of use in treating or managing diabetes mellitus (Agu*et al*, 2019). The inhibitory action of biosynthesized MgO nanoparticles of *Annona muricata* leaves aqueous extract and standard anti-diabetic drug, Acarbose was investigated. Acarbose slows down carbohydrates digestion and hinders the activity of pancreatic amylase breaking down oligosaccharides and disaccharides into monosaccharides for absorption (Athithan *et al.*, (2020). Table 1 exhibits the inhibition of α-Amylase by acarbose and MgO nanoparticles *A. muricata*. The MgO nanoparticles *A. muricata* significantly inhibited α-Amylase enzyme at different concentrations of 20, 40, 60, 80 and 100 while the percentage inhibition was found to be 32.59, 36.69, 39.16, 50.15 and 69.68 respectively with IC50 of 66.00µg/ml. The result in table 1 revealed that the biosynthesized MgO nanoparticle leave aqueous extract of *Annona muricata* exhibited a high percentage inhibition of 69.68±0.38 at 100µg/ml. The α-glucosidase anti-diabetic action of the extract of biosynthesized MgO nanoparticle A. muricata result is shown in Table 2. α-glucosidase acts by catalyzing the breakdown of glycosidic bonds by the retaining or inverting mechanisms of anomeric configuration (Mugiyanto *et al.*, 2019). α-glucosidase in the intestine hydrolyzes complex carbohydrates to glucose and other monosaccharides in the small intestine, therefore, the inhibition of this enzyme lowers the rate at which complex carbohydrates are digested, thus, reducing the amount of glucose absorbed (Nair, *et al*., 2013). Table 2 shows the inhibition of α-glucosidase by acarbose and MgO nanoparticles *A. muricata*. The α-glucosidase enzyme was significantly inhibited at various concentrations of 20, 40, 60, 80 and 100 of MgO nanoparticles *A. muricata* and the percentage

inhibition was found to be 20.98, 23.41, 45.57,52.96 and 61.33 respectively with IC50 of 73.42µg/ml. The result in Table 2 revealed that the biosynthesized MgO nanoparticle leaf aqueous extract of *Annona muricata* exhibited a high percentage inhibition of 61.33±0.742 at a concentration of 100µg/ml.

TABLE I: α-amylase anti-diabetic activity of biosynthesized MgO nanoparticle *A. muricata*

(CONCENTRATION)	**(PERCENTAGE INHIBITION)**	
(µg/ml)	**MgONPs *A. muricata* leaves**	**Acarbose**
	Aqueous extract	
20	32.59±0.26	51.83±0.26
40	36.69±0.13	64.45±0.29
60	39.16±0.23	74.15±0.25
80	50.15±0.39	81.08±0.13
100	69.68±0.38	88.08±0.12
IC50	66.00	20.03

All values were duplicates and expressed as mean ± SEM.

TABLE II: α-glucosidase anti-diabetic activity of biosynthesized MgO nanoparticle *A.muricata*

(CONCENTRATION)	**(PERCENTAGE INHIBITION)**	
(µg/ml)	**MgONPs *A. muricata* leaves**	**Acarbose**
	Aqueous extract	
20	20.98±0.127	46.48±0.078
40	23.41±0.445	51.72±0.028
60	45.57±0.134	58.99±0.170
80	52.96±0.389	67.75±0.247
100	61.33±0.742	83.08±0.170
IC50	73.42	29.57

All values were duplicates and expressed as mean ± SEM.

Conclusion

This research investigates the anti-diabetic activity of biosynthesis of Magnesium Oxide nanoparticles using aqueous leaf extract of *Anonna muricata*. The leaf extract contains organic molecules acting as reducing and stabilizing agents. The characterisation f the formed MgONPs was performed by techniques like FTIR, XRD and SEM. The FTIR confirmed the

functional groups present in the biosynthesized MgONPs. The average size of the biosynthesized MgONPs was calculated to be 41nm from the XRD analysis. SEM confirmed the hexagonal shape of MgONPs with nanometer-sized dimensions. The Antidiabetic activity of the biosynthesized MgO nanoparticle plant extract was studied by determining α -amylase andα -glucosidase inhibitory potentials and the values of their IC_{50}were 66.00µg/ml and 73.42µg/ml. Thus, this MgONPs *A. Muricata* exhibits good anti-diabetic activity. This research confirms the use of MgONPs *A. Muricata* in the management of diabetes mellitus.

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