



Evaluation of *In vitro* Anti-Diabetic Properties of Biosynthesized Magnesium Oxide Nanoparticles from *Vernonia amygdalina* (Bitter Leaf) Aqueous Leaf Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Vernonia amygdalina* is a common shrub that is widely used and extracts from them have been traditionally used as remedies for treating diabetes mellitus in various parts of the world. The use of *V. amygdalina* to synthesize MgO nanoparticles has been used for various biomedical applications and it is compatible with anti-diabetic studies. This research investigates the in vitro anti-diabetic potentials of biosynthesized from aqueous *V. amygdalina* leaf extract.

Methodology: Aqueous extract of *V. amygdalina*-MgO nanoparticles were characterized using Fourier Transform Infrared, X-ray Diffraction and Scanning Electron Microscopy techniques. FTIR validated the presence of functional groups, the crystallization and size (66nm) of the nanoparticles was validated by XRD while SEM confirmed the shape of the nanoparticles synthesized.

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Result: Qualitative screening confirmed the presence of saponin, flavonoids, phenols, alkaloids, tannins, terpenoids, glycosides while their concentrations were evaluated quantitatively. *V. Amygdalina*-MgONPs extract and acarbose showed significant inhibition of α -amylase and α -glucosidase, with IC₅₀ values of 55.05% and 20.0% respectively.

Conclusion: The study found that biosynthesized MgONPs-*V. amygdalina* aqueous extract has strong anti-diabetic properties, indicating its potential for diabetes treatment and management.

Keywords: *Vernonia amygdalina*; antidiabetic activity; MgO nanoparticles.

1. INTRODUCTION

Diabetes mellitus is a widespread, chronic metabolic disorder symbolized by high glucose level in the blood, posing significant global health risks. The therapy of diabetes aims to maintain normal blood glucose levels after meals for diabetic patients, particularly Type 2. [1]. Postprandial hyperglycemia plays a crucial part in the occurrence of type 2 diabetes and its complications. A major therapeutic approach is to decrease the level of blood glucose right after a meal by inhibiting carbohydrate hydrolyzing enzymes such as alpha amylase and alpha glucosidase which in turns retard the absorption of glucose in the body [2]. Type 2 diabetes mellitus can also be called adult-onset diabetes. Type 2 diabetic patients are always resistant to the action of insulin [3]. About 5-7% of the world's population suffers this type of diabetes and it is usually managed via dietary therapy, exercise and hypoglycemic agents [3].

Traditional healers in Africa use various plants to treat diabetes mellitus [4]. Plants with bitter taste have been linked to improved symptoms of diabetes mellitus [5]. *V. amygdalina*, a medium-sized shrub with abundant bitter principles, is extensively harnessed in Nigeria for therapeutic and nutritive goals due to its therapeutic potentials. Extracts of this plant have been traditionally harnessed as traditional remedies for treating diabetes mellitus globally [6]. Farombi and Owoeye [7] reported *V. amygdalina* is widely used in Africa for conventional therapy of ailments like gastrointestinal issues, malaria, sexually transmitted diseases and infertility. Conventional utility of this plant extends beyond humans, as they are also used in the production of horse feed which provides chusandokin (a strengthening or fattening tonic) in the Northern part of Nigeria [8].

Nanoparticles (NPs) are rapidly known for their relevance in ecological remedy, medicine, consumer goods, and other fields [9]. NPs' unique properties have been harnessed in

various areas such as healthcare, beauty products, renewable energy, ecological remedy and biomedical applications [10]. Pathania et al [11] reported that MgNPs are highly popular due to their broad spectrum of bactericidal and fungicidal activity across various scientific applications. Green-based MgONPs synthesis involving bacteria, algae, fungi, and plants has gained popularity in medicine due to its eco-friendliness, low cost, toxicity, and long-lasting stability [12]. Generally, plants' phytochemicals are highly exploitable and harmless, with various metabolites acting as reducing agents in the synthesis of nanoparticles, benefiting various biological science fields [13]. Thus, this research work explores the antidiabetic potentials of biosynthesized MgO nanoparticles derived from *V. amygdalina* aqueous leaf extract.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Splant

V. amygdalina leaves were locally obtained from Sayedaro market at Ilaro, Ogun state, Nigeria. Identification and authentication were done at the University of Lagos by a botanist with voucher number No; 8767. The leaf, renowned for its medicinal content and abundance, was chosen to test its potential as a reducing and capping agent for MgNPs, potentially benefiting humanity.

2.2 Preparation of Leaf Extract and Biosynthesis of MgONPs

The preparation of *V. amygdalina* leaves extract and synthesis of MgONPs were done according to [14]. The freshly plucked leaves of *V. Amygdalina* were rinsed to remove impurities like sand under running water, then washed with distilled water thrice. The washed leaves were air dried for two (2) weeks at room temperature and then ground using electrical grinding machine into powdery form. Fifty (50g) of the powdery form of the leaf was measured with a weighing balance into 500ml beaker and 500ml distilled

water was added. The substance was stirred continuously at 60°C for an hour, cooled to room temperature, and filtered using Whatman filter paper. A pale green color of filtrate was observed. A magnesium nitrate solution was prepared by adding 5g of magnesium nitrate in 100ml distilled water [15]. Thirty (30ml) *V. amygdalina* aqueous extract and 100ml of $Mg(NO_3)_2$ (freshly prepared) were put in a 500ml beaker and stirred for 2 hours at 80°C with a magnetic stirrer. The addition of Magnesium nitrate solution led to a significant change in color from pale green to brown, confirming the formation of MgO nanoparticles. The solution underwent centrifugation at 4000rpm/min for 4 minutes, washed with ethanol multiple times to remove impurities, and then air dried overnight.

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screenings of the aqueous leaf extract and MgONPs biosynthesized extract of *Vernonia amygdalina* were done with standard procedures that identify as well as quantify the phytochemical constituents.

2.4 Qualitative Analysis

The qualitative phytochemical screenings were done with standard procedures by Sofowara [16].

2.5 Test for Saponins

2g powdered sample boiled in 20cm³ of distilled water in a water bath was filtered and 10cm³ of the filtrate was taken and mixed with 5cm³ distilled water with vigorous shakes to form stable, persistent froth. 3 drops of olive oil was blended with the froth and vigorously shook to observed an emulsion formation.

2.6 Test for Phenol

1cm³ extracts was put in a test tube and approximately 2 drops of 5% $FeCl_3$ were added. A greenish precipitate signifies the presence of phenolic.

2.7 Test for Flavonoid

A portion of the plant extract was put in a beaker and 5cm³ diluted ammonia solution (10%) was added, then concentrated H_2SO_4 was also added to observe yellow color of the extract that confirms flavonoids present.

2.8 Test for Tannins

0.5g powdered sample boiled in 20cm³ water was filtered and ferric chloride (0.1%) was added dropwise to observe brown-green or blue-black color.

2.9 Test for Steroid

0.5g sample extract with 2cm³ H_2SO_4 was put in a beaker and 2cm³ acetic anhydride added. The changed color in which violet turns green shows that steroid is present.

2.10 Test for Terpenoids

2cm³ chloroform with 3cm³ concentrated H_2SO_4 was blended with 5cm³ sample extract to create a sheet. The interface's red-brown color indicates positive result.

2.11 Test for Phlobatanin

Plant extract and 1% HCl were boiled together to form red precipitate that indicates presence of phlobatanins.

2.12 Test for Alkaloid

5mg extract sample was melted in 3 ml acidified ethanol and filtered. The addition of Mayer's reagent and 1ml Dragendroff's reagent to 1ml filtrate resulted in the observation of turbidity.

2.13 Quantitative Analysis

Estimation of tannins: 500 mg sample extract was melted in 50ml distilled water with 1 hr shaking. 5ml filtrate aliquot was mixed with 2ml $FeCl_3$ (0.1 M) in 0.1M HCl and $8 \times 10^{-3}M$ $C_6FeK_4N_6$. Absorbance was read in 10 mins at 720.

Estimation of total phenolic compound: The study utilized Makkar and Becker's [17] procedures. 0.5g sample extract was melted in 50ml H_2O and 0.5ml of dissolved extract was mixed with 0.1ml Folin- Ciocalteu reagent (0.5 N), then, kept warm for 15mins at 37°C. 2.5ml Na_2CO_3 was added and incubated for another 30 mins at 37°C. Absorbance was computed at 760 nm and total phenol content was expressed as gallic acid equivalent (GAE).

Estimation of total flavonoid content: 1ml sample solution blended with 3ml methanol,

0.2ml of AlCl_3 (10%), 0.2ml of 1M $\text{CH}_3\text{CO}_2\text{K}$, and 5.6ml distilled water were incubated for 30 mins at 37°C and absorbance read at 415 nm. A curve of calibration was plotted by quercetin solutions.

Determination of Alkaloids: The mixture was filtered while hot, re-digested for 30 mins more, and evaporated with 50ml alcohol, then, distilled water, and 3 drops of 10% HCl were added. A homogeneous mixture was created with the mixture of the whole solution, 5ml zinc accurate and 5ml potassium ferricyanide solution and permit to stand, filtered. The alkaloids were extracted, and the residue was melted in 10ml hot distilled H_2O . The obtained residue was melted in 10ml hot distilled H_2O and 0.2g selenium was added, then the resulting solution was poured into a kjeldahl tube for digestion to obtain colorless solution. %Nitrogen was determined using kjeldahl distillation apparatus. Back titration was performed with 0.01N HCl and the value gotten was used to estimate the %Nitrogen using the formulae:

$\%N = \text{Titer value} \times \text{Atomic mass of Nitrogen} \times \text{Normality of HCl} \times 100 \div \text{weight of sample (mg)}$
 $\% \text{Alkaloid} = \% \text{Nitrogen} \times 3.26$

Where 3.26 is a constant

Determination of glycosides: 50ml chloroform was added to 10ml sample extract in a 250ml conical flask and shook for one hour using vortex mixer and filtered. 2ml sodium nitroprusside (20%) and 10ml Pyridine were added to the filtrate and shook for 10 mins, then, 3ml NaOH was added to obtain brown-yellow color. Glycoside standards were prepared using 100mg/ml standard glycoside ranging from 0 to 5mg/ml. Absorbance of both sample and standard were determined by spectronic 21D Digital spectrophotometer at a wave length of 510nm. %Glycoside was estimated by the formula:

$\% \text{Glycoside} = \text{Absorbance of sample} \times \text{Average speed} \times \text{Dilution factor} \div \text{weight of sample} \times 10000$

Determination of teroids: 0.05g plant extract was dissolved in a chloroform-methanol mixture, then alcoholic KOH added to the mixture which was heated in a water bath for 90mins, then cooled, petroleum ether added, and distilled water evaporated. The residue was then reacted with Liebermann Buchard reagent and analyzed using a spectrophotometer. 0.4mg/ml standard

steroid concentrations were made from 100mg/ml stock steroid solution and they were treated like the sample above. % steroid was estimated via this formula:

$\% \text{steroid} = \text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor} \div \text{Weight of sample} \times 10000$

2.14 Characterization of Nanoparticles

FTIR: FT-IR technique was used to examine surface functional groups in MgONPs synthesized from plant extract, confirming their presence using ATOM METHOD.a2m and Bruker IFS Affinity1 spectrometer.

X-RAY diffraction: X-ray diffraction studies were conducted on synthesized MgO nanoparticles to determine their crystalline or amorphous nature and determine their particle size using an X-ray powder diffractometer at a low angle range ($10^\circ - 70^\circ$).

SEM: SEM-EDX characterization studies on biosynthesized MgO nanoparticles were conducted using FEI Quanta 200 F, focusing on surface morphology and elemental studies. Details on applied voltage, magnification, and image size were incorporated.

2.15 In vitro Anti-Diabetic Assays

Determination of α -glucosidase enzyme inhibition: The study involved the preparation and separation of α -glucosidase from male wistar rats' small intestines. The mucosal tissue was excised, homogenized in phosphate buffer saline and then dialyzed overnight. Concentrated α -glucosidase from the animals was used to investigate inhibition by *V. amygdalina* leaves. The concentrated enzyme's protein content was estimated by Lowry procedures [18]. This study examined the impact of MgONPs-*V. amygdalina* extract on rats' intestinal α -glucosidase using Nagmoti and Juvekar's method [19]. Different concentrations of the extract were incubated with the enzyme and its activity was determined by glucose oxidase method.

Determination of α -amylase enzyme inhibition: The determination of α -amylase activity was carried out using a chromogenic method, involving mixing *V. amygdalina* extract with distilled water and soluble potato starch in phosphate buffer pH 6.9. Six hundred (600 μL) solution of enzyme was poured into test tubes

containing 300 µL 3,5-dinitrosalicylic acid color reagent. Then, the test tubes were put in a hot water bath for 15 minutes. The reaction mixture was made watery with distilled water, and absorbance estimated. Test incubations were made for different MgONPs-*V. amygdalina* concentrations, with blank and control incubations representing 100% enzyme activity. The tests were conducted in triplicate, and the net absorbance (A) as result of generated maltose was estimated using:

$$A_{540nm} \text{ MgONPs-} V. \text{ amygdalina} = A_{540nm} \text{ Test} - A_{540nm} \text{ Blank.}$$

The percentage of maltose derived was estimated using the maltose standard calibration curve, and the level of inhibition was calculated via this formula: % inhibition = 100 - % reaction.

3. STATISTICAL ANALYSIS

The data is presented as means ± standard deviations (SD) where n= 3.

4. RESULTS

4.1 Preliminary Phytochemical Screening

The result of qualitative phytochemical screening of *Vernonia amygdalina* aqueous leaf extract in Table 1 showed that the plant leaves gives positive results for tannins, flavonoids, terpenoids, alkaloids, tannins, reducing sugar, saponins, phenol, steroids and glycosides but phlobatanin was absent.

Table 1. The Qualitative phytochemical analysis of *V. amygdalina* aqueous leaf extract

| Phytochemicals | Result |
|----------------|--------|
| Saponins | + |
| Tannins | + |
| Phenolics | + |
| Flavonoids | + |
| Steroids | + |
| Terpernoids | + |
| Phlobatanin | - |
| Glycosides | + |
| Alkaloids | + |

Keys: + means test substance present, - means test substance absent

The result in qualitative phytochemical screening in Table 2 of biosynthesized MgO nanoparticle *Verenoniaamygdalina* aqueous leaf extract

shows the presence of Tanins, Saponin, Flavonoids, Steriods, Terpenoids, Cardiac Glycosides, Reducing Sugar, Phenols but Phlobatanin is absent.

Table 2. The Qualitative phytochemical analysis of MgONPs-*V. amygdalina* aqueous leaf extract

| Phytochemicals | Results |
|----------------|---------|
| Saponins | + |
| Tannins | + |
| Phenolics | + |
| Flavonoids | + |
| Steroids | + |
| Terpernoids | + |
| Phlobatanin | - |
| Glycosides | + |
| Alkaloids | + |

Keys: + means test substance present, - means test substance absent

The result in Table 3 reveals the quantitative phytochemical analysis of *Vernonia amygalina* aqueous leaf extract showing the mean ± standard deviation value. The quantitative test shows that Reducing Sugar, Phenol, Flavonoids, Steriods, Alkaloids, and Tanins were present with values 47.26±0.09,52.91±1.48,48.96±0.09,46.64±3.54,38.99±1.69 and 47.14±3.42 respectively.

Table 3. Quantitative Phytochemical Analysis of *V. amygdalina* aqueous leaf extract

| Phytochemicals | Concentration (mg/100g) |
|----------------|-------------------------|
| Phenols | 48.96±0.09 |
| Flavonoids | 46.64±3.54 |
| Steroids | 38.99± 1.69 |
| Tannins | 47.26±0.09 |
| Alkaloids | 47.14±3.42 |

Keys: values are expressed in mean ± standard error.

The result in Table 4 reveals the quantitative analysis of biosynthesized MgO nanoparticles of Alkaloids, tannins, reducing sugar, phenol, flavonoids and steroid were present with values of 44.60± 0.48, 47.67 ± 0.48, 44.60 ±0.48,26.88 ± 2.63, 24.03± 0.25 and 26.36±0.48 respectively.

4.2 Characterization

4.2.1 FTIR spectroscopy

The FTIR spectra revealed the O-H stretching of intermolecular bonded alcohol at 3436cm⁻¹, the

presence of C=O at 2928cm⁻¹ and 2861cm⁻¹, and the stretching of ester at 1743cm⁻¹. The FTIR spectrum of *V. amygdalina* leaf extract reveals C=C stretching at 1644cm⁻¹, methylene C-H bending, -H alcohol bending, and C-O stretching at 1462 cm⁻¹, 1376 cm⁻¹ and 1212 cm⁻¹, with prominent peaks in the wave number range of 3436.69 cm⁻¹ to 2861.80 cm⁻¹.

Table 4. Quantitative Phytochemical Analysis of MgONPs-*V. amygdalina* aqueous leaf extract

| Phytochemicals | Concentration (mg/100g) |
|----------------|-------------------------|
| Phenols | 44.60±0.48 |
| Flavonoids | 26.88±2.63 |
| Steroids | 24.03±0.25 |
| Tannins | 44.60± 0.48 |
| Alkaloids | 26.36±0.48 |

Key: values are expressed in mean ± standard error.

4.2.2 X-ray diffraction analysis

The XRD pattern of synthesized MgONPs, confirms the crystalline cubic structure of MgONPs through the sharp peaks observed. The peak at 2θ values of 18.3°, 19.34°, 37.77°, 58.88°, and 59.97° indicated the hexagonal shape of MgO-NPs (JCPDS)01- 073-2966). The average crystallite size of synthesized MgONPs was determined using the Scherrer formula $D = k\lambda/\beta\cos\theta$ [20]. D was calculated with values = 93°, 28°, 14°, 106°, 84° and 72°nm at 2θ peaks, therefore, D = 66nm.

4.2.3 Scanning electron microscopy

Particles appear clustered at 500x magnification and some of the individual crystals are clearly seen but at higher magnification of 4000x, the

hexagonal shapes of the nanoparticles are evident and separated.

4.2.4 Antidiabetic activity Of Mgonps-*V. amygdalina* aqueous leaf extract

Table 5 shows the inhibition of α-Amylase by MgONPs-*V. amygdalina* aqueous leaf extract and acarbose. The alpha amylase enzyme was observed to be inhibited at different concentrations of 20, 40, 60, 80, 100µg/ml of MgO nanoparticles *V. amygdalina* and the % inhibition were found to be 34.54%, 38.45%, 41.40%, 59.75%, and 72.56% respectively with IC₅₀ of 55.05.

Table 6 revealed that alpha glucosidase enzyme was found to be blocked at different concentrations of 20, 40, 60, 80 and 100 µg/ml of MgO nanoparticles *V. amygdalina* and the percentage inhibition were found to be 21.82%, 29.65%, 43.25%, 56.10% and 67.68% respectively with IC₅₀ of 66.43.

5. DISCUSSION

Diabetes mellitus, chronic endocrine ailment affecting carbohydrate metabolism, indicated by hyperglycemia due to insufficient insulin production or cell response [21]. The therapeutic approach to diabetes involves reducing postprandial hyperglycemia [22]. This can be attained by blocking enzyme activities of α-amylase and α-glucosidase that hydrolyze carbohydrates [23].

The aqueous extract of the leaves of MgONPs-*V. amygdalina* can be the basis for herbal medicine to efficiently treat various diseases that affect humans such as diabetics. The phytochemical screenings revealed that saponins, glycosides, terpenoids, phenols, flavonoids, steroids, tannins and alkaloids were present except phlobatanin that was absent in both crude and MgONPs

Table 5. Effect of MgONPs-*V. amygdalina* aqueous leaf extract on α-amylase

| Concentration (µg/ml) | MgONPs- <i>V. amygdalina</i> leaf extract (% Inhibition) | Acarbose (% Inhibition) |
|-----------------------|--|-------------------------|
| 20µg/ml | 34.54±0.095 | 51.82±0.365 |
| 40µg/ml | 38.45±0.465 | 64.45±0.410 |
| 60µg/ml | 41.40±0.210 | 74.15±0.350 |
| 80µg/ml | 59.75±0.100 | 81.08±0.120 |
| 100µg/ml | 72.56±0.640 | 88.07±0.175 |
| IC ₅₀ | 55.05 | 20.03 |

Values are represented as mean ± S.D (in duplicate).

Table 6. Effect of MgONPs- *V. amygdalina* aqueous leaf extract on α -glucosidase

| Concentration ($\mu\text{g/ml}$) | MgONPs- <i>V. amygdalina</i> leaf extract(% Inhibition) | Acarbose (% Inhibition) |
|------------------------------------|---|-------------------------|
| 20 $\mu\text{g/ml}$ | 21.82 \pm 0.29 47.42 \pm 0.037 | |
| 40 $\mu\text{g/ml}$ | 29.65 \pm 0.30 52.72 \pm 0.018 | |
| 60 $\mu\text{g/ml}$ | 43.25 \pm 1.05 59.99 \pm 0.320 | |
| 80 $\mu\text{g/ml}$ | 56.10 \pm 0.65 68.65 \pm 0.430 | |
| 100 $\mu\text{g/ml}$ | 67.68 \pm 0.75 84.09 \pm 0.530 | |
| IC50 | 66.43 | 29.57 |

Values are represented as mean \pm S.D (in duplicate).

synthesized extracts, however, both extracts are abundantly rich in phenols, flavonoids, steroids, tannins and alkaloids. Therefore, the phytochemicals present in this plant might be responsible for the reported anti-diabetic activities. In this study, mean values of phytochemical constituents of the biosynthesized MgO nanoparticles extracts are reduced compared to the crude extract particularly flavonoids and alkaloids. This confirmed that part of the phytochemical constituents such as Tanins, Flavonoids, Steroids, Alkaloids and Phenols contributed to the biosynthesis of MgONPs from *V. amygdalina* which are in accordance with [24].

FTIR is a technique that uses interference and absorption to study molecules' vibrations after absorption of precise infrared radiation [25]. FTIR

analysis identified biomolecules that can be utilized for the reduction and capping of MgONPs [26]. The FTIR responses for the biosynthesized MgONPs-*V. amygdalina* aqueous extract are depicted in fig. 1. The FTIR spectra revealed the O-H stretching of intermolecular bonded alcohol at 3436 cm^{-1} , the presence of C=O at 2928 cm^{-1} and 2861 cm^{-1} , and the stretching of ester at 1743 cm^{-1} . The FTIR spectrum of *V. amygdalina* leaf extract reveals C=C stretching at 1644 cm^{-1} , methylene C-H bending, -H alcohol bending, and C-O stretching at 1462 cm^{-1} , 1376 cm^{-1} and 1212 cm^{-1} , with prominent peaks in the wave number range of 3436.69 cm^{-1} to 2861.80 cm^{-1} . The FTIR results confirmed the presence of alkanes, alkenes, carboxylic acid and alcohol in the plant extract of *V. amygdalina* is also in agreement with previous studies conducted by Bashir et al [27].

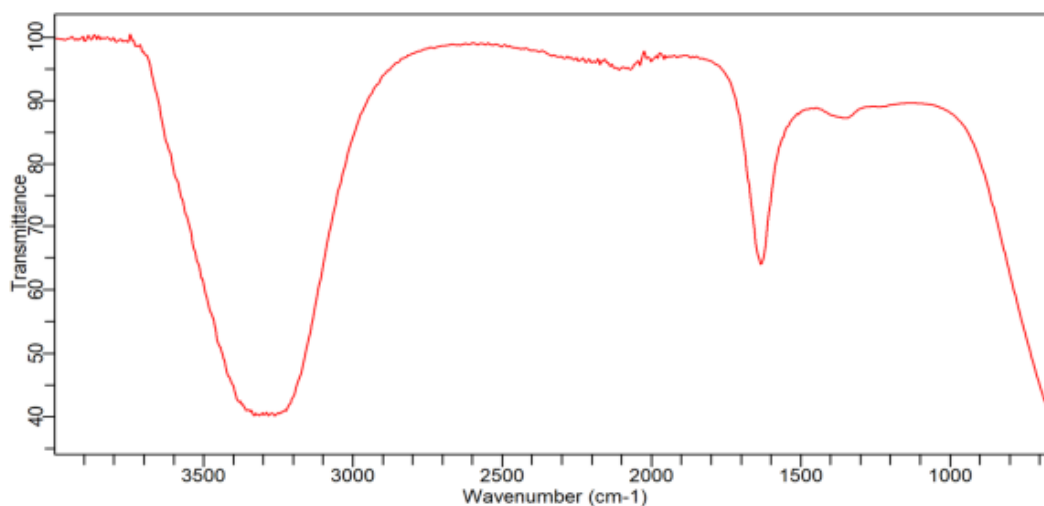


Fig. 1. FTIR Spectrum data of MgONPs-*Vernonia amygdalina* aqueous leaf extract

X-ray diffractometer (XRD) was used to identify the crystallographic structure of the biosynthesized MgONPs material. The XRD pattern of synthesized MgONPs, as shown in fig. 2, confirms the crystalline cubic structure of MgONPs through the sharp peaks observed at 2θ values of 18.3° , 19.34° , 37.77° , 58.88° , and 59.97° and the location of the peaks in the graph are in accordance with the report by Vergheese et al [14]. MgONPs through the sharp peaks observed at 2θ values of 18.3° , 19.34° , 37.77° , 58.88° , and 59.97° and the location of the peaks in the graph are in accordance with the report by Vergheese et al [14]. MgONPs through the sharp peaks observed at 2θ values of 18.3° , 19.34° , 37.77° , 58.88° , and 59.97° and the location of the peaks in the graph are in accordance with the report by Vergheese et al [14].

SEM was used to analyze the shape of the biosynthesized MgONPs. [28] reported that the structure of biosynthesized MgO nanoparticles leave aqueous extract is in the form of cluster. In fig.3, at 500x magnification, the particles appear clustered and some of the individual crystals are clearly seen but at higher magnification of 4000x, the hexagonal shapes of the nanoparticles are evident and separated and this is in accordance with a report by Suresh et al [28] which also confirmed the hexagonal shape of MgO nanoparticles.

α -glucosidase and α -amylase are crucial enzymes for the breakdown of carbohydrates, with amylase breaking down long-chain carbohydrates and α -glucosidase breaking down starch and disaccharides into glucose [22]. Thus, hindrance of α -amylase and α -glucosidase activities can decrease postprandial hyperglycemia and decrease the likelihood of developing diabetes [29]. The study investigated the inhibitory action of biosynthesized MgO nanoparticles from *V. amygdalina* leaves extract and Acarbose (standard drug) that reduces carbohydrates digestion by blocking the action of pancreatic amylase [20]. Table 5 shows that the biosynthesized MgONPs- *V. amygdalina* aqueous leaf extract has the highest value of percentage inhibition of 72.56 ± 0.640 at $100 \mu\text{g/ml}$. Table 6 showed that the biosynthesized MgONPs-*V. amygdalina* aqueous leaf extract has the highest value of % inhibition of $61.33 \pm 0.742\%$ at a concentration of $100 \mu\text{g/ml}$. However, the blockage activities of biosynthesized MgONPs-*V. amygdalina* aqueous extract on α -glucosidase is higher than the inhibitory activity on α -amylase and this is consistency with a report by, [30]. The result of this research indicates that MgONPs-*V. amygdalina* aqueous leaf extract exhibits good anti-diabetic activity and was able to decrease the activities of carbohydrate enzymes.

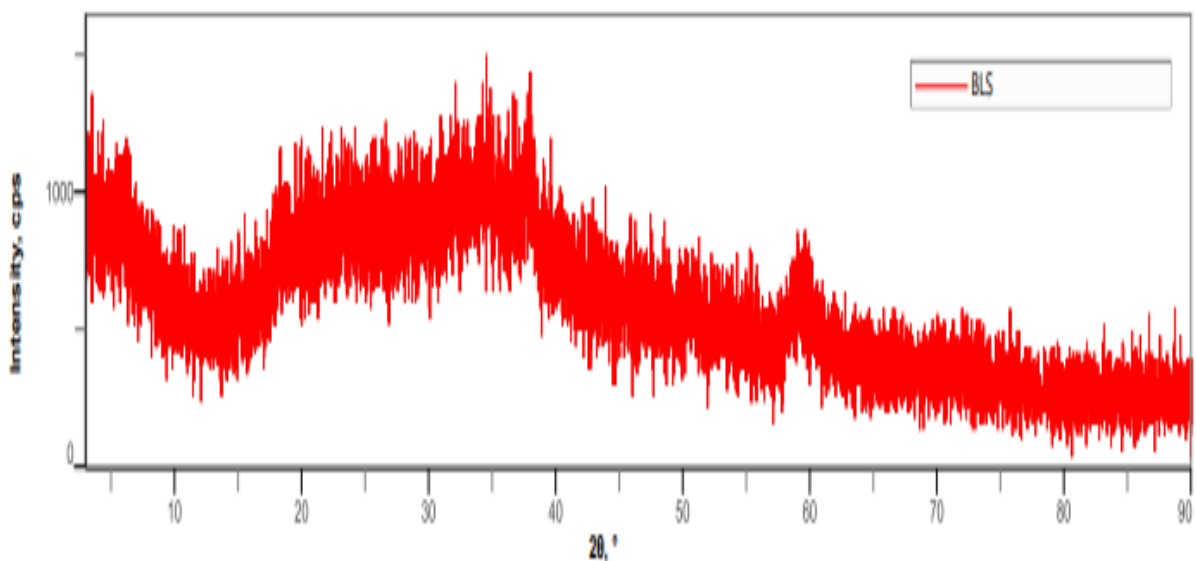


Fig. 2. XRD Spectrum Pattern of MgONPs-*Vernonia amygdalina* leaf aqueous extract

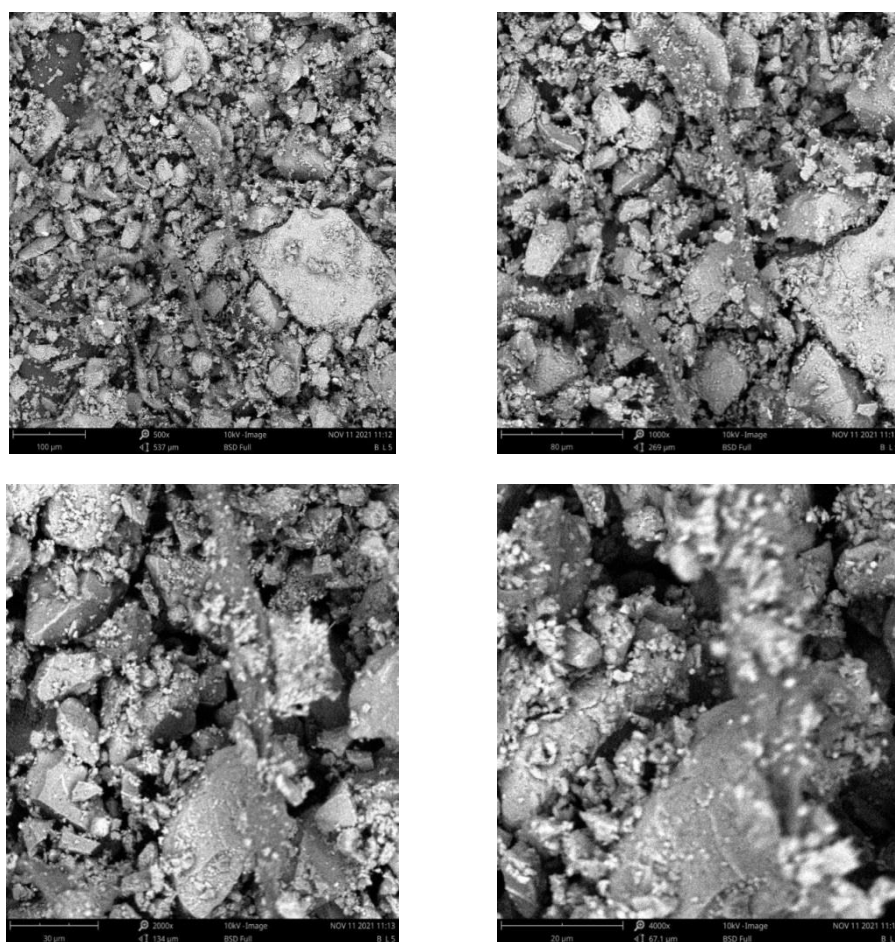


Fig. 3. SEM Micrographs of MgONPs-*V. amygdalina* aqueous leaf extract

6. CONCLUSION

This study supports the application of MgONPs-*V. amygdalina* aqueous leaf extract in the treatment of diabetes mellitus. This study recommends that MgO nanoparticles should be synthesized from other parts of the plant and their phytochemical constituents and antidiabetic activities be analyzed for further investigation.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462–70.

2. Bays H, Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab*. 2004;89(2):463–78.
3. Onyenibe N, Victoria D, Udogadi N. Ameliorative effect of fermented *Pentaclethra macrophylla* (African oil bean seed) on high fat diet and sucrose drink induced metabolic syndrome in male New Zealand rabbits. *J basic Appl Res Biomed*. 2019;5(2):42–8.
4. Keter LK, Mutiso PC. Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya. *J Ethnopharmacol*. 2012; 139(1):74–80.

5. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed.* 2012; 2(4):320–30.
6. Shukia R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. *Indian J Clin Biochem.* 2000;15(Suppl 1):169–77.
7. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and garcinia biflavonoid. *Int J Environ Res Public Health.* 2011;8(6): 2533–55.
8. Kadiri O, Olawoye B. *Vernonia amygdalina*: An underutilized vegetable with nutraceutical potentials – a review. *Turkish J Agric - Food Sci Technol* [Internet]. 2016;4(9):763–768. Available:<http://www.agrifoodscience.com/index.php/TURJAF/article/view/570>
9. Graf C, Vossen DLJ, Imhof A, van Blaaderen A. A General Method To Coat Colloidal Particles with Silica. *Langmuir* [Internet]. 2003;19(17):6693–700. Available:<https://doi.org/10.1021/la0347859>
10. Ghosh Chaudhuri R, Paria S. Core/shell nanoparticles: Classes, properties, synthesis mechanisms, characterization, and applications. *Chem Rev* [Internet]. 2012;112(4):2373–433. Available:<https://doi.org/10.1021/cr100449n>
11. Pathania D, Kumar S, Thakur P, Chaudhary V, Kaushik A, Varma RS, et al. Essential oil-mediated biocompatible magnesium nanoparticles with enhanced antibacterial, antifungal, and photocatalytic efficacies. *Sci Rep.* 2022;12(1):11431.
12. Afuye OO, Olasunkanmi AA. Green synthesis of MgO Nanoparticles Using Anona Muricata Leaf Aqueous Extract and its Antidiabetic Activity. 2022;3(2):59–67.
13. Jeevanandam J, Chan YS, Danquah MK. Biosynthesis and characterization of MgO nanoparticles from plant extracts via induced molecular nucleation. *New J Chem* [Internet]. 2017;41(7):2800–14. Available:<http://dx.doi.org/10.1039/C6NJ03176E>
14. Vergheese M, Vishal Sk, Mary Vergheese C. Green synthesis of magnesium oxide nanoparticles using *Trigonella foenum-graecum* leaf extract and its antibacterial activity. ~ 1193 ~ *J Pharmacogn Phytochem.* 2018;7(3):1193–200.
15. Munjal S, Singh A, Kumar V. Synthesis and Characterization of MgO Nanoparticles by Orange Fruit Waste through Green Method. *Int J Adv Res Chem Sci.* 2017;4(9):36–42.
16. Sofowora A. Research on medicinal plants and traditional medicine in Africa. *J Altern Complement Med.* 1996;2(3):365–72.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265–75.
18. Nagmoti DM, Juvekar AR. In vitro inhibitory effects of *Pithecellobium dulce* (Roxb.) Benth. seeds on intestinal α -glucosidase and pancreatic α -amylase. *J Biochem Technol.* 2013;4(3):616–21.
19. Athithan AS, Sundari JJ, renuga D. Annona muricata fruit mediated biosynthesis, physicochemical characterization of magnetite (Fe₃O₄) nanoparticles and assessment of its in vitro antidiabetic activity. *Rasayan J Chem.* 2020;13:1759–66.
20. Edem DO, Edagha I, Ette BB, Agwuigwo PE. Effects of *Tapinanthus Globiferus* Leaf Extract on Blood Glucose and Pancreatic Histology in alloxanized and normoglycemic rats. *Arch Diabetes Endocr Syst.* 2020;3:34–43.
21. Mohammed SA, Yaqub AG, Sanda KA, Nicholas AO, Arastus W, Muhammad M, et al. Review on diabetes, synthetic drugs and glycemic effects of medicinal plants. *Sect Title Pharmacol.* 2013;7(36):2628–37.
22. Dirir AM, Daou M, Yousef AF, Yousef LF. A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochem Rev.* 2022;21(4):1049–79.
23. Badmus JA, Oyemomi SA, Adedosu OT, Yekeen TA, Azeez MA, Adebayo EA, et al. Photo-assisted bio-fabrication of silver nanoparticles using *Annona muricata* leaf extract: exploring the antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities. *Heliyon.* 2020;6(11):e05413.
24. Ajay Singh, Naveen Chandra Joshi MR. Magnesium oxide nanoparticles (MgONPs): Green synthesis, characterizations and antimicrobial activity. *Res J Pharm Technol.* 2019;12(10): 4644–6.
25. Prasanth R, Dinesh Kumar S, Jayalakshmi A, Singaravelu G, Govindaraju K, Ganesh

- Kumar V. Green synthesis of magnesium oxide nanoparticles and their antibacterial activity. *Indian J Geo-Marine Sci.* 2019; 48(8):1210–5.
26. Bashir RA, Mukhtar Y, Chimbekujwo IB, Aisha DM, Fatima SU, Salamatu SU. Phytochemical screening and fourier transform infrared spectroscopy (FT-IR) analysis of *Vernonia amygdalina Del.(Bitter leaf)* methanol leaf extract. *FUTY J Environ.* 2020;14(2):35–41.
27. Suresh J, Pradheesh G, Alexramani V, Mahalingam S, Hong SI. Green synthesis and characterization of hexagonal shaped MgO nanoparticles using insulin plant (*Costus pictus D. Don*) leave extract and its antimicrobial as well as anticancer activity. *Adv Powder Technol.* 2018;29.
28. Heo SJ, Hwang JY, Choi JI, Han JS, Kim HJ, Jeon YJ. Diphlorethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent alpha-glucosidase and alpha-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Eur J Pharmacol.* 2009;615(1–3):252–6.
29. Nurdin S, Sukohar A, Ramadani OA. Antiglucosidase and antioxidant activities of ginger, cinnamon, turmeric and their combination. *Int J Pharm Pharm Res.* 2017;10(1):296–306.
30. Makkar HP, Becker K. Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. *The Journal of Agricultural Science.* 1997;128(3):311-22.

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