EVALUATION OF INVITRO ANTI-DIABETICPROPERTIES OF BIOSYNTHESIZED MAGNESIUM OXIDE NANOPARTICLESFROM*Vernonia amygdalina*(BITTER LEAF) AQUEOUS LEAF EXTRACT

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 invitro anti-diabetic potentials ofbiosynthesized fromaqueous*V. amygdalina*leaf extract.

Methodology: Aqueous extract of *V. amygdalina*-MgO nanoparticleswere characterized usingFourier Transform Infrared, X-ray Diffraction and Scanning Electron Microscopytechniques. FTIRvalidated 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.

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 IC_{50} 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.

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 nutritivegoals 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 widelyused 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 provideschusandokin (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).Pathaniaet al (11)reported thatMgNPs 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 plantshas 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, thisresearch work explores the antidiabetic potentials of biosynthesized MgO nanoparticles derived from *V. amygdalina*aqueous leaf extract.

MATERIALS AND METHODS

Collection and authentication of ssplant

*V. amygdalina*leaveswere locally obtained from Sayedaro market atIlaro, 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.

Preparation of leaf extractand biosynthesis of MgONPs

The preparation of *V. amygdalina*leaves extract and synthesis of MgONPswere 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^{o}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℃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.

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.

Qualitative analysis

The qualitative phytochemical screenings were done with standard procedures by Sofowara (1993)(16).

Test for saponins

2g powdered sample boiled in 20cm3 of distilled water in a water bath was filtered and $10cm^3$ of the filtrate was taken and mixed with $5cm^3$ 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.

Test for phenol

$1cm^3$ 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.

Test for flavonoid

A portion of the plant extract was put in a beaker and 5cm^3 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.

Test for tannins

0.5g powdered sample boiled in 20cm^3 water was filtered and ferric chloride (0.1%) was added dropwise to observe brown-green or blue-black color.

Test for steroid

0.5g sample extract with 2cm^3 H_2SO_4 was put in a beaker and 2cm^3 acetic anhydride added. The changed color in which violet turns green shows that steroid is present.

Test for terpenoids

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

Test for phlobatanin

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

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.

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 $8x10^{-3}$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 (1997) 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^{o}C. 2.5ml Na_2CO_3 was added and incubated for another 30 mins at 37^{o}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 CH_3CO_2K, and 5.6ml distilled water were incubated for 30 mins at 37^{o}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% HCI 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 HCI and the value gotten was used to estimate the %Nitrogen using the formulae:

%N= Titer value x Atomic mass of Nitrogen x Normality of HCI x 100weight of sample (mg) %

Alkaloid = % Nitrogen x 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:

% Glycoside = Absorbance of sample x Average speed x Dilution factor weight of sample x 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: % steroid = Absorbance of sample x Average gradient x Dilution factor Weight of sample x 10000.

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 Affinity1spectrometer.

X-RAY difraction

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 (10θ -70 θ).

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.

Invitro 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 salineand 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(17).This study examined the impact of MgONPs-*V. amygdalina*extract on

rats' intestinal α-glucosidase using Nagmoti and Juvekar's method(18). 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 determinationof α-amylase activity was carried out using a chromogenic method, involving mixing *V. amygdalina*extract with distilled water and soluble potato starch in phosphate bufferpH 6.9. Six hundred(600 µL) solution of enzyme was poured into test tubes containing 300 µL 3,5-sdinitrosalicylic 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:

A540nmMgONPs-*V.amygdalina*= A540nmTest – A540nmBlank.

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.

Statistical analysis

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

RESULT

PRELIMINARY PHYTOCHEMICAL SCREENING

TABLE 1: The Qualitative phytochemical analysis of *V. amygdalina* aqueousleaf extract

PHYTOCHEMICALS	RESULT
Saponins	+
Tannins	+
Phenolics	+
Flavonoids	+
Steroids	+
Terpernoids	+
Phlobatanin	-
Glycosides	+
Alkaloids	+

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

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 2: The Qualitative phytochemical analysis of MgONPs-*V.amygdalina*aqueousleaf extract

PHYTOCHEMICALS	RESULTS
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 3: Quantitative Phytochemical Analysis of *V. amygdalina*aqueousleaf 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 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 4: Quantitative Phytochemical Analysis of MgONPs-*V. amygdalina*aqueousleaf 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.

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.

CHARACTERIZATION

FTIR SPECTROSCOPY

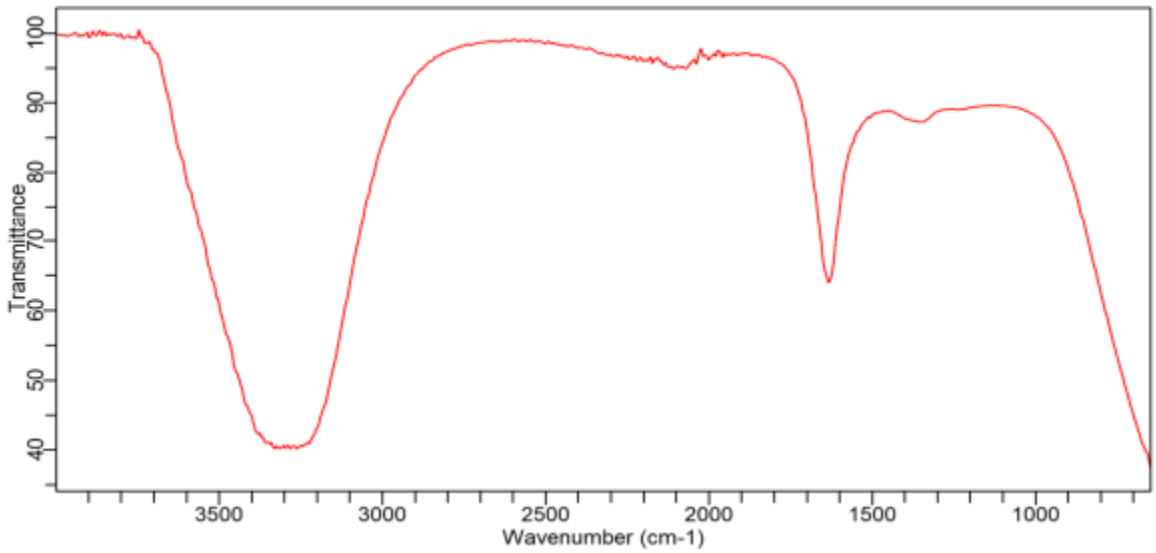

Fig: 1: FTIR Spectrum data of MgONPs-*Vernonia amygdalina* aqueous leaf extract

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}.

X-RAY DIFFRACTION ANALYSIS

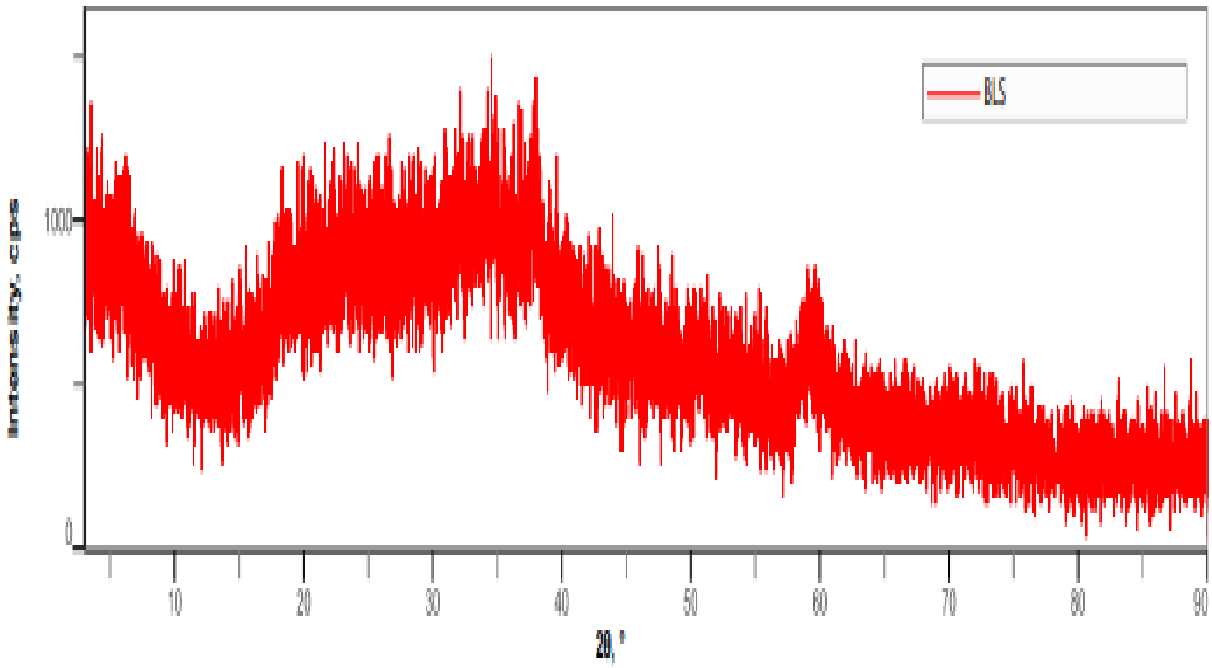

Fig 2: XRD Spectrum Pattern of MgONPs-*Vernonia amygdalina* leaf aqueous extract

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 = kλ/βcosθA(19). D was calculated with values = 93°, 28°, 14°, 106°, 84° and 72°nm at 2θ peaks, therefore, D = 66nm.

SCANNING ELECTRON MICROSCOPY

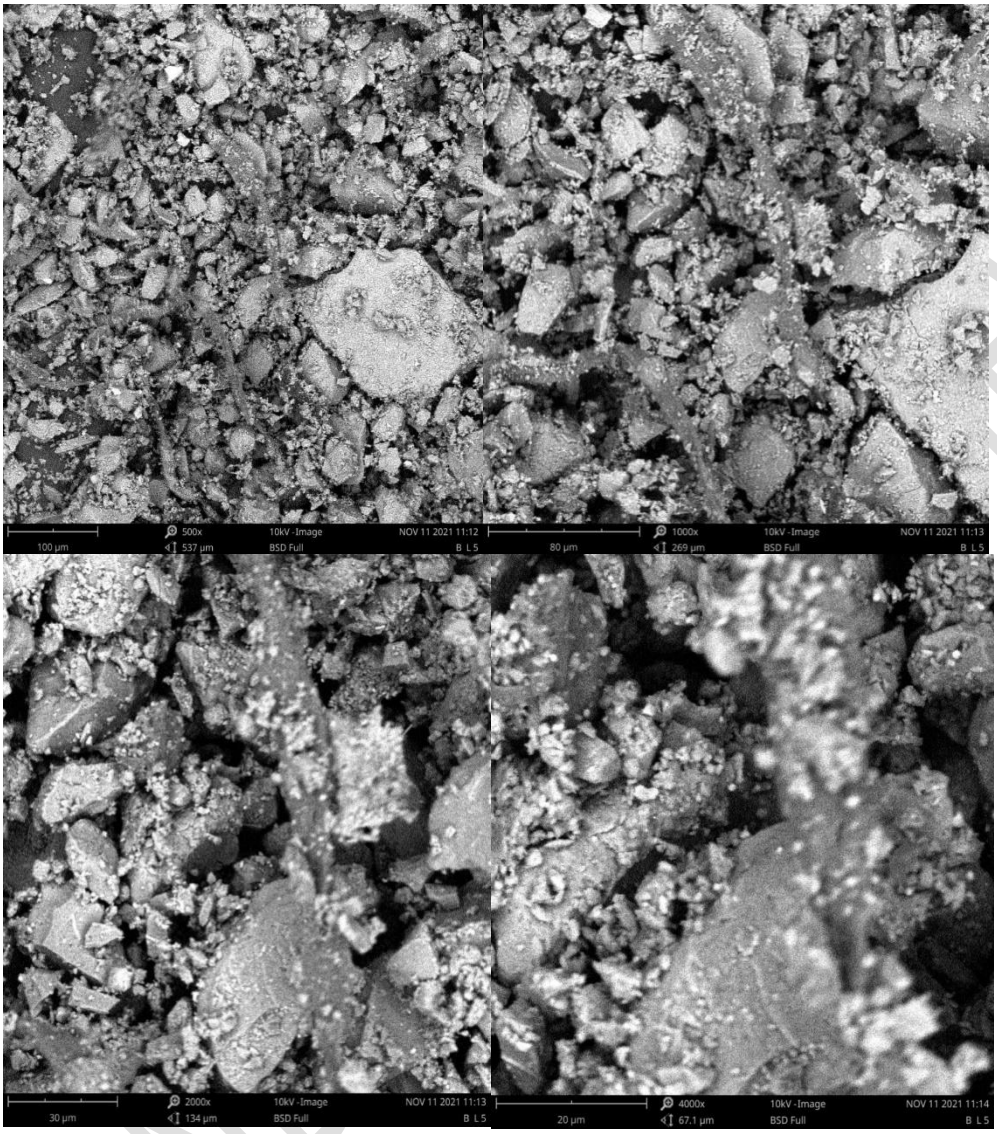

Fig 3: SEM Micrographs of MgONPs-*V. amygdalina*aqueous leaf extract

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.

ANTIDIABETIC ACTIVITY OF MgONPs-*V.amygdalina*AQUEOUS LEAF EXTRACT

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

Concentration (µg/ml)	**MgONPs- *V. amygdalina* 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 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 IC_{50} of 55.05.

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

Concentration (µg/ml)	MgONPs- *V. amygdalina* leaf extract(% Inhibition)	Acarbose (% Inhibition)
20µg/ml	21.82±0.29	47.42±0.037
40µg/ml	29.65±0.30	52.72±0.018
60µg/ml	43.25±1.05	59.99±0.320
80µg/ml	56.10±0.65	68.65±0.430
100µg/ml	67.68±0.75	84.09±0.530
IC_{50}	66.43	29.57

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

Table 6 revealed thatalpha glucosidase enzyme was found to be blocked at different concentrations of20, 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 IC_{50} of 66.43.

DISCUSSION

Diabetes mellitus, chronic endocrine ailment affecting carbohydrate metabolism, indicated by hyperglycemia due to insufficient insulin production or cell response(20). The therapeutic approach to diabetes involves reducing postprandial hyperglycemia(21).This can be attained by blocking enzyme activities of α-amylase and α-glucosidase that hydrolyze carbohydrates(22).
The aqueous extract of the leaves of MgONPs-*V.amygdalina*can be the basisfor 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 exceptphlobatanin that was absentinboth crude and MgONPs 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 extractsere reduced compared to the crude extractparticularlyflavonoids and alkaloids.This confirmed that part of the phytochemical constituentssuch as Tanins, Flavonoids, Steriods, Alkaloids and Phenolscontributed to the biosynthesis of MgONPsfrom*V. amygdalina*which are in accordance with(23).

FTIR is a technique that uses interference and absorption to study molecules' vibrations after absorption of precise infrared radiation(24). FTIR analysis identified biomolecules that can be utilized for the reduction and capping of MgONPs(25). The FTIR responses for the

biosynthesized MgONPs-*V.amygdalina*aqueous extract are depicted in Figure 1. 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}.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(2020)(26).

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 observedat 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 (2018)(14).

SEM was used to analyze the shape of the biosynthesized MgONPs.(27)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 (2018)(27) 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(21).Thus,hindrance of α-amylase and α-glucosidase activities can decrease postprandial hyperglycemia and decrease the likelihood of developing diabetes(28).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(19).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 100µg/ml.Table 6 showed that the biosynthesized MgONPs-*V. amygdalina*aqueous leaf extracthas the highest value of% inhibition of 61.33±0.742% at at a concentration of 100µg/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 consistence with a report by,(29).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.

CONCLUSION

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

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