EVALUATION OF INVITRO ANTI-DIABETIC POTENTIALS OF BIOSYNTHESIZED MgO NANOPATICLES FROM *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 in vitro anti-diabetic potentials of MgONPs biosynthesized from aqueous *V. amygdalina* leaf extract.

Methodology: Aqueous extract of *V. amygdalina*-MgO nanoparticles were~~as~~ characterized using FTIR, XRD and SEM 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.

Result: ~~The phytochemical screenings of the crude and MgONPs *V. amygdalina* extract were also carried out and~~ Qualitative screening confirmed the presence of saponin, flavonoids, phenols, alkaloids, tannins, terpenoids, glycosides while their concentrations were evaluated quantitively. MgONPs-*V. amygdalina* extract's anti-diabetic potentials were evaluated via α-amylase and α-glucosidase inhibition analysis with acarbose as the standard drug in the laboratory. *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).

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Traditional healers in Africa use various plants to treat diabetes mellitus (2). Plant-based bitter principles have been linked to improved symptoms of diabetes mellitus(3).*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(4). (5)reported *V. amygdalina* is ~~also~~ widely used ~~widely~~ 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(6).

Nanoparticles (NPs) are rapidly known for their relevance in ecological remedy, medicine, consumer goods, and other fields(7). NPs' unique properties have been harnessed in various areas such as healthcare, beauty products, renewable energy, ecological remedy and biomedical applications(8). (9) 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(10). 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(11). Thus, this research work explores the antidiabetic potentials of biosynthesized MgO nanoparticles derived from *V. amygdalina* aqueous leaf extract.

MATERIALS AND METHODS

Collection and authentication of plant ~~leaves of *V. amygdalina*~~

V. amygdalina leaves were locally obtained~~gotten~~ from Sayedaro market at Ilaro, Ogun state, Nigeria. Identification and a~~A~~uthentication ~~and identification~~ 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 extract and biosynthesis of MgONPs

The preparation of *V. amygdalina* leaves extract and synthesis of MgONPs were done according to(12). 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. 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 Whitman 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(13). 30 ml *V. a~~A~~mygdalina* 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

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underwent centrifugation at 4000rpm/min for 4 minutes, washed with ethanol multiple times to remove impurities, and then air dried overnight.

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 PBS, 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. This study examined the impact of MgONPs-*V. amygdalina* extract on rats' intestinal α-glucosidase using Nagmoti and Juvekar's method. Different concentrations of the extract were incubated with the enzyme and its activity was determined by glucose oxidase method. The IC_{50} value was used to express the enzyme inhibition data.

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Determination of α-amylase enzyme inhibition

The ~~investigation~~ 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 ~~a pH 6.9~~ phosphate buffer pH 6.9. 600µL solution of enzyme was poured into test tubes containing 300 µL DNSA 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:

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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.

The methods used for the determination of Phytochemicals, FTIR, XRD and SEM were not included. Please provide the methodology.

RESULT& DISCUSSION

PRELIMINARY PHYTOCHEMICAL SCREENING

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

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

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.

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.

CHARACTERIZATION

FTIR SPECTROSCOPY

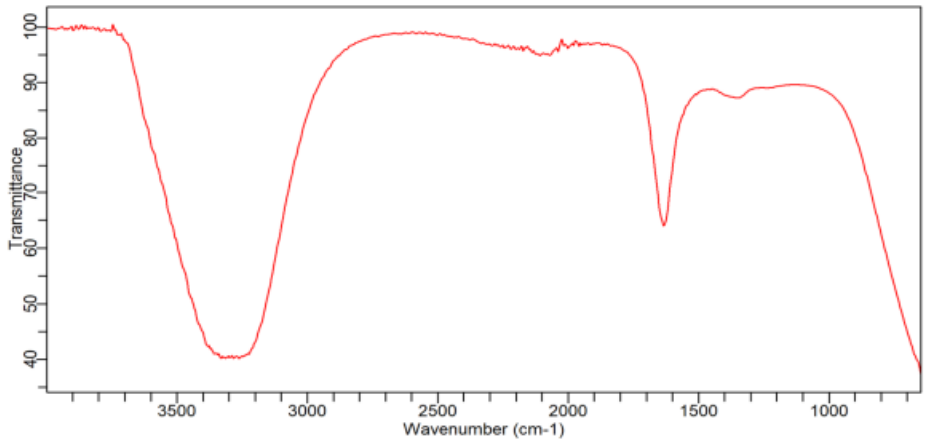

Fig: 1: FTIR Spectrum data of MgONPs-*Vernonia amygdalina* aqueous leaf extract

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X-RAY DIFFRACTION ANALYSIS

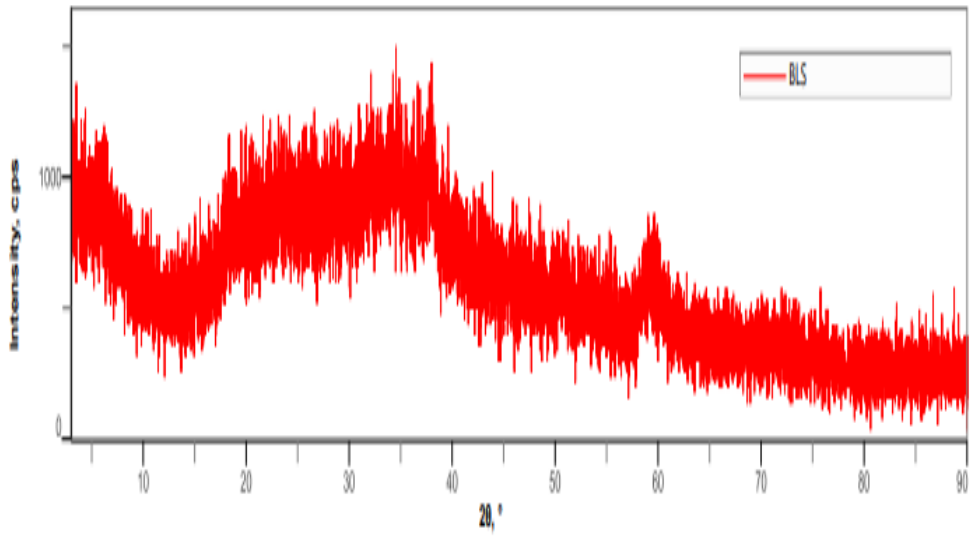

Fig 2: XRD Spectrum Pattern of MgONPs-*Vernonia amygdalina* leaf aqueous extract

SCANNING ELECTRON MICROSCOPY

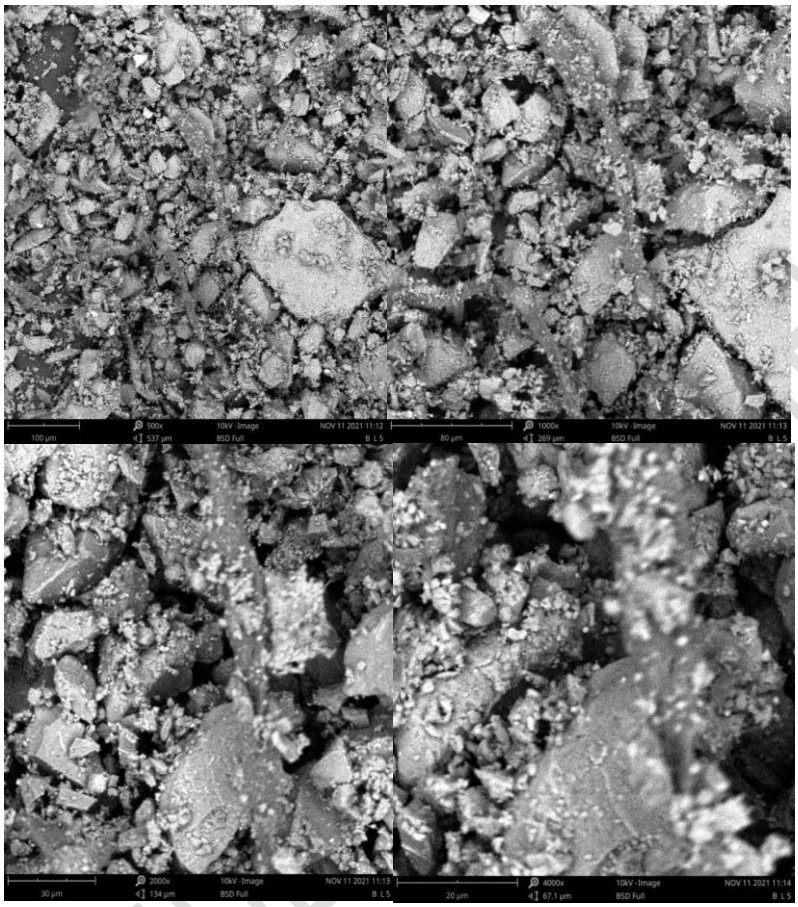

Fig 3: SEM Micrographs of MgONPs-*V. amygdalina*aqueous leaf extract

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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). P< 0.05 considered as IC50 significant when compared to the standard drug (Acarbose).

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). P< 0.05 considered as IC50 significant when compared to the standard drug (Acarbose).

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Diabetes mellitus, chronic endocrine ailment affecting carbohydrate metabolism, indicated by hyperglycemia due to insufficient insulin production or cell response(14). The therapeutic approach to diabetes involves reducing postprandial hyperglycemia~~.~~(15). This can be attained by blocking enzyme activities of α-amylase and α-glucosidase that hydrolyze carbohydrates~~.~~(16).
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 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, ~~It was also revealed from the result that~~mean values of ~~the quantitative~~ phytochemical constituents of the biosynthesized MgO nanoparticles extracts ere~~are~~ reduced compared to ~~the values of~~ the crude extract ~~especially~~ particularly flavonoids and alkaloids.~~,~~ ~~this~~ This ~~can~~ confirmed that part of the phytochemical constituents contributed to the biosynthesis of MgONPs from *V. amygdalina* which are in accordance with(17).

FTIR is a technique that uses interference and absorption to study molecules' vibrations after absorption of precise infrared radiation(18). FTIR analysis identified biomolecules that can be utilized for the reduction and capping of MgONPs(19). 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}.

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

SEM was used to analyze the shape of the biosynthesized MgONPs. (21)reported that the structure of biosynthesized MgO nanoparticles leave aqueous extract is~~are~~ 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.

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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(15).Thus, ~~hinderance~~hindrance of α-amylase and α-glucosidase activities can decrease postprandial hyperglycemia and decrease the likelihood of developing diabetes(22).

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 the inhibition of α-Amylase by MgONPs-*V. amygdalina* aqueous leaf extract and acarbose. The alpha amylase enzyme was observed to be significantly 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 5 shows that the biosynthesized MgONPs- *V. amygdalina* aqueous leaf extract showed that at 100µg/ml high percentage inhibition of 72.56±0.640. Table 6 revealed that alpha glucosidase enzyme was found to be significantly 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 IC_{50} of 66.43. Table 6 showed that the biosynthesized MgONPs-*V. amygdalina* aqueous leaf extract of showed that at a concentration of 100µg/ml, a high % inhibition of 61.33±0.742% was observed. 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,(23).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.

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CONCLUSION

This study supports the application of MgONPs-*V. amygdalina* aqueous leaf extract in the treatment of diabetes mellitus. This ~~project~~ 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.

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