In vitro evaluation of bioinorganic magnesium-based nanomaterials using aqueous extract of *Bridelia ferruginea* in *hyperglycemia*

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Abstract

Diabetes mellitus is a chronic metabolic condition that results from absence of insulin secretion or activity (Kumar *et al*., 2011). The number of instances and prevalence of diabetes have gradually increased over the last decenary, and it is recognized as a quiet killer illness, impacting millions of people worldwide (Onyenibe and Udogadi, 2019). The vast majority of medicinal plants are used by inhibiting the enzymes involved in carbohydrates metabolism to treat diabetes and they mostly act, example of such is *Bridelia ferruginea*, but there is the need for improved activities of these plants in the body for better effects. Nanoparticle has recently been used to increase the effectiveness of bioactive compounds in plants. MgONPs were prepared using 150 ml of the freshly prepared 5mM Magnesium nitrate solution and 30ml of *Bridelia ferruginea* aqueous extract. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were used to characterize the nanoparticles. MgO nanoparticles was obtained after several hours from brown to pale yellow. The SEM images of biosynthesized MgO nanopartcles of *Bridelia ferruginea* (MgONPs-*Bf*) aqueous extract leave in this study are agglomerated and FTIR showed that carbonyl groups contributed to MgONPs-*Bf* synthesis. At increasing concentration of 20,40,60,80 and 100 µg/ml of *Bridelia ferruginea* the inhibitory capacity against α-amylase and α-glucosidase increased (51.82±0.52, 64.45±0.58, 74.15±0.49, 81.08±0.17, 88.08±0.12 and 46.48±0.078, 51.72±0.028, 58.99±0.170, 67.75±0.247, 83.08±0.170) as compared to acarbose. *Bridelia ferruginea* might aid in the development of MgONPs with considerably better pharmacokinetic characteristics and therapeutic performance in hyperglycemia.

Keywords: Nanomaterials; *Bridelia ferruginea*; Diabetes mellitus; hyperglycemia; α-amylase; α-glucosidase.

Introduction

Diabetes mellitus is a chronic metabolic condition that results from the absence of insulin secretion or activity. Chronic hyperglycemia results from insulin insufficiency, which disrupts carbohydrate, lipid, and protein metabolism (Kumar *et al*., 2011). Type 1 diabetes mellitus (DM-1) and Type 2 diabetes mellitus (DM-2) are the common types of diabetes. DM-2 is the most prevalent pathology among the diabetes kinds. This is because there is a clear link between the cause of this disease and people's dietary habits. According to well-established studies, the onset of DM-2 is caused primarily by increased resistance of insulin receptors (IRS-1), which are found in all tissues of the body (Galicia-Garcia *et al*., 2020). As a result, even if insulin is released in appropriate quantities, its physiologic response is less effective. Internalization of receptors in target tissues causes activity decrease in some circumstances (Ozougwu *et al*., 2013). Despite breakthroughs in understanding of the hazards associated with DM-2, most new compounds issued on the pharmaceutical market are considered to be advances in the pharmacokinetic characteristics of existing medicines (Gohil and Enhoffer, 2014). Current medications act on targets that are inefficient for treatment, these facts motivate the growing quest for therapeutic development for DM-2 (de-Lima *et al*., 2020). The antidiabetic qualities of plants have been explored due to the negative cause of medicines and the proximity of plants to inhabitants in underdeveloped areas. (Kumar *et al*., 2010). Traditional plants of different species of over 400 have been identified (Ramachandran *et al*., 2011). *Bridelia ferruginea* is one of these plants that has been extensively examined for it's antihyperglycemic effects, particularly the root and leaf extracts; nevertheless, leaves do not show substantial antihyperglycemic activity, despite being traditionally used to treat diabetes (Cimanga *et al*.,2011). As a result, more study, scientific observations, and continuous justification are needed to emphasize its value added advantage in diabetes therapy and associated ailments, hence the synthesis of magnesium oxide nanoparticle from *Bridelia ferruginea* in this study. Nanotechnology has several intriguing applications, including bio-sensing, biomedical, food, feed, and medication delivery, cosmetics, and cancer therapy, among others (Ahmed *et al*., 2016). Magnesium oxides are of special importance since these compounds are not only fixed through a rigorous process, but they are also largely recognized as safe materials for humans and animals (Huang *et al*., 2000). Magnesium oxide

is recognized as a highly effective antibacterial agent when compared to organic antibacterial agents (Stoimenov *et al*., 2002). Magnesium oxide is a basic oxide that has a variety of applications including catalysis, adsorption, and the production of refractory ceramics (Choudhary *et al*., 2009). Nano Magnesium oxide (MGONPS) has the advantage of being made from commonly accessible and inexpensive precursors and solvents, making it a significant bactericidal material under basic conditions (Roselli *et al*., 2002). Apart from that, MGONPS are quite essential since they have distinct features when compared to bulk materials. The exceptional features of MGONPS include great chemical stability, strong photocatalytic activity, high electrical permittivity, and non-toxicity. The majority of medicinal plants are used to treat diabetes, and they mostly operate by blocking enzymes involved in glucose metabolism. However, there is a need to boost these plants' activity in the body for better results. In this study, the anti-diabetic properties of produced MgO nanoparticles synthesized *Bridelia ferruginea*. were investigated in hyperglycemia. Two key enzymes that are therapeutic targets in diabetes management are α-amylase and α-glucosidase (Krentz and Bailey, 2005). These two enzymes are involved in the breakdown of starch to glucose, which increases the quantity of glucose in the circulation; however, inhibiting α-amylase and α-glucosidase activity slows glucose absorption and thereby moderates postprandial blood glucose rise" (Bischoff, 1994). α-amylase is a key enzyme that breaks down large, soluble starch molecules found in pancreatic juice and saliva into smaller and digestible units (Afifi *et al*., 2008). α-amylase and α-glucosidase inhibitors have been shown to be helpful in decreasing level of postprandial hyperglycemia (Matsui *et al*., 2000).

Methodology

Chemicals and reagents required

Analytical Grade chemicals and reagents were employed in this investigation. Magnesium nitrate was bought from a medical supply store in Lagos island, Lagos, Nigeria, along with distilled water and ethanol.

Preparation of samples

Bridelia ferruginea was gotten from a farmland along Igbese road, Ilaro, Ogun State, Nigeria. The plant extract was prepared in the manner described by (Vergheese and Vishal, 2018) with minor modifications. For two weeks, *Bridelia ferruginea* leaves were left to dry in the shade. Using a blender, the dried leaves were finely processed and powdered. The leaf powder was then used to prepare the leaf extract. In a clean 500ml beaker, distilled water (500ml) was mixed with 50g of the powder. It was continually shaked at 60^{o}C for an hour, cooled at 25^{o}C, and the entire mixture was passed through double layered muslin cloth followed by separation filtration with Whatman filter paper, yielding a pale green color.

Preparation of Magnessium Nitrate Solution

Distilled water (100ml) was used to dissolved 5g of magnesium nitrate to make a solution. Munjal, *et al*., (2017) described how to make a magnesium nitrate solution. These was carried out according to their methods

Synthesis of Magnessium Nitrate Nanoparticles

The magnesium oxide nanoparticles were produced from *Bridelia ferruginea* leaf extract in the same manner as Vergheese and Vishal, 2018, with a few changes. In a beaker of 500ml capacity,30ml of the plant extract was transferred, and magnesium oxide was added in drops for 2 hours at room temperature. The addition of magnesium nitrate solution caused a significant change in color from pale green to brown, demonstrating the synthesis of $Mg(OH)_2$ nanoparticles. After centrifuging the solution for 4 minutes, the precipitate was washed multiple times with ethanol to eliminate contaminants before air drying overnight.

Characterization of nanoparticles synthesized from *Bridelia ferruginea* leaf

Coates (2000) described the use of fourier-transform infrared spectroscopy (FTIR) to evaluate biosynthesized MgO nanoparticles and scanning electron microscopy (SEM) as described by Olajire *et al*. (2017).

Pytochemical Screening

The aqueous extract and powdered material were subjected to phytochemical screening using established protocols to detect bioactive compound as described by Sofowara (1993).

Determination Of α-glucosidase Enzyme Inhibition

Rat intestinal α-glucosidase preparation and separation of rat small intestine α-glucosidase:

Under anesthesia a male wistar rat was killed, the small intestine of the animal (180g) was taken. The mucosal tissue of the epithelial layer was obtained by completely washing the gut with saline and then removing the luminal surface with a spatula. For 15 minutes, phosphate buffered saline (PBS) pH 7.4 with 1% triton X 100 was used to homogenize the mucosal scrapings before centrifugation at 12000 rpm. Rat small intestine α-glucosidase was found in the supernatant fraction. Butanol was added in a 1:1 ratio to the supernatant fraction and centrifuged at 15000 rpm for 15 minutes. Overnight, the aqueous layer was purified against the same buffer. Following dialysis, the concentrated enzyme was utilized as crude α-glucosidase enzyme to study the inhibition by *B. ferruginea* leaf extract. All of the preparations were done at 4 °C. The enzyme prepared by protein content was calculated by The Lowry technique (Lowry *et al*. 1951)

Determination Of α -amylase Enzyme Inhibition

The chromogenic technique reported by Ali *et al* (2019) was used to measure α-amylase activity. 120µL of P. dulce methanolic extract (20 mg/mL) in DMSO was mixed with 480µL of distilled water and in a test tube, 0.5% w/v soluble potato starch (1.2 mL) was dissolved in 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride. The process started (0 min) using 600µL of enzyme solution (4 units/mL in distilled water). A 600µL portion of the mixture was removed and immersed in hot water. After 3 minutes, add 30µL DNSA color reagent (1 g of 3, 5-dinitrosalicylic acid (96 mM), 30g of sodium potassium tartarate, and 20 mL of 2 N sodium hydroxide to a final volume of 100 mL in distilled water). A Shimadzu UV-160 spectrophotometer (Kyoto, Japan) was used to measure absorbance at 540 nm. P. dulce concentrations of 5, 7.5, 10, 15, and 20 mg/mL were also used to test for concentration-dependent inhibition. At the start of the process, the enzyme mixture was replaced with 600µL of distilled water to establish blank incubations for each concentration. 100% control incubations.

Enzyme activity were carried out in the same way, but using 120µL DMSO instead of P. dulce. All tests were performed in duplicate. A540nmP. dulce = A540nmTest - A540nmBlank net absorbance (A) owing to the maltose produced. The percentage (w/v) of maltose derived from the obtained value was determined using the maltose standard calibration curve equation (0-0.1% w/v maltose). The inhibition percentage (%) was calculated as follows: At t=3min, % inhibition equals 100% response. Where % response = sample mean maltose/control mean maltose.

Statistical analysis

The data was evaluated using one-way ANOVA, which was followed by Tukey's test for post-hoc analysis and graphical representation of results using the GraphPad Prism 8.5 Program (GraphPad Software, San Diego, CA, USA). All values were presented as mean SEM. At p 0.05, statistical differences were considered.

Results

Phytochemical Screening of Aqueous Extract *B. ferruginea* Leaves
Table 1: Qualitative Phytochemical Screening on aqueous Extract of *Bridelia ferruginea*.

S/N	TEST	*Bridelia ferruginea* Extract
1	Tannins	+
2	Phlobatanins	-
3	Saponin	-
4	Flavonoids	+
5	Steroids	+
6	Terpenoids	+
7	Glycosides	+
8	Alkaloids	+
9	Phenols	+

Table 2: Quantitative Phytochemical Screening on Aqueous Extract of *Bridelia ferruginea*.

S/N	TEST	*Bridelia ferruginea* EXTRACT
1	Tannins	61.56±0.054
2	Phenols	63.76±0.055
3	Flavonoids	76.09±0.38
4	Steroid	58.79±1.79
5	Alkaloids	50.32±0.96
6	Reducing sugar	50.59±0.23

Table 3: Qualitative Phytochemical Screening on Aqueous Extract Biosynthesized MgO Nanoparticle *Bridelia ferruginea*.

S/N	TEST	*Bridelia ferruginea* Extract
1	Tannins	+
2	Phlobatanins	-
3	Saponin	+
4	Flavonoids	+
5	Steroids	+
6	Terpenoids	+
7	Cardiac glycosides	+
8	Reducing Sugar	+
9	Phenols	+

Keys: + = Test Substance Present, - = Test Substance Absent

Table 4: Quantitative Phytochemical Screening on Aqueous Extract Biosynthesized MgO Nanoparticle *Bridelia ferruginea*.

S/N	TEST	*Bridelia ferruginea* EXTRACT
1	Tannin	51.91±2.470
2	Reducing sugar	43.38±0.331
3	Phenol	53.58±0.659
4	Flavonoids	39.66±0.525
5	Steroids	39.89±0.525
6	Alkaloids	30.153±0.250

FTIR SPECTRUM

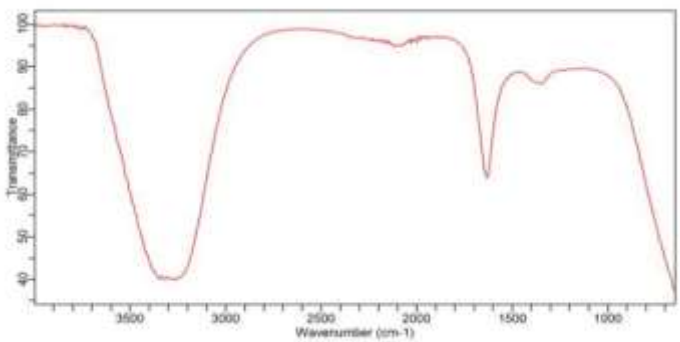

Figure 1 : FTIR Spectrum Data of MgONPs *Bridelia ferruginea*

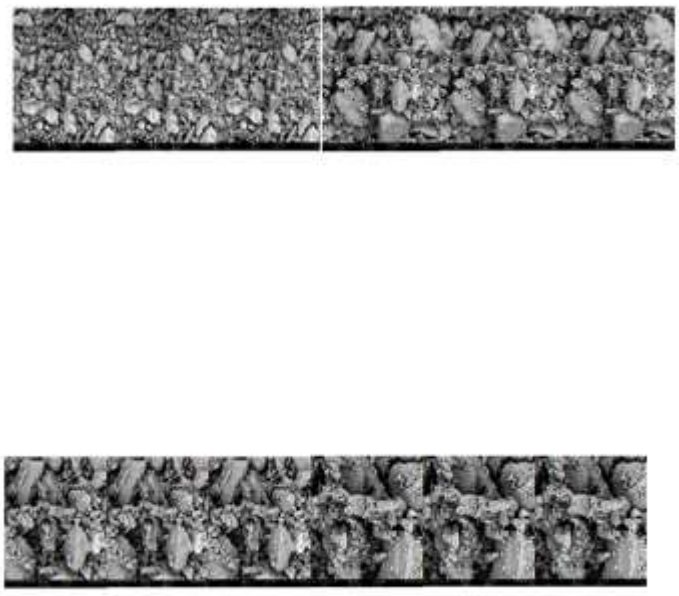

Figure 2: SEM MICROGRAPH OF BIOSYNTHESIZED MGONPS

Results of Invitro Anti-diabetic Activity of Biosynthesized MgO Nanoparticle *B. ferruginea.*

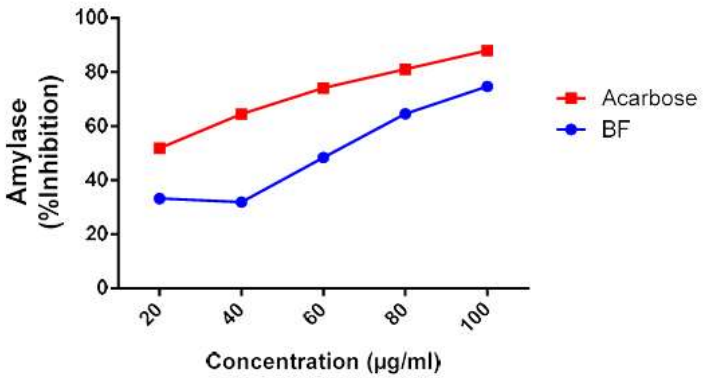

Figure 3: α-amylase activity of the Aqueous extract *B. ferruginea* leaves extract and Acarbose(control). The data represent the percentage inhibition of α-amylase. Each point represent the values obtained from two experiments, perfoermed in duplicate (mean±S.D).

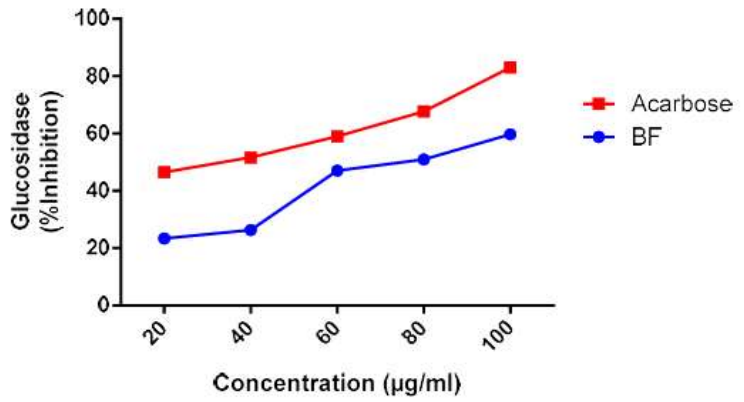

Figure 4 α-glucosidase activity of the Aqueous extract *B. ferruginea* leaves extract and Acarbose(control). The data represent the percentage inhibition of α-glucosidase.Each point represent the values obtained from two experiments, perfoermed in duplicate (mean±S.D).

Discussion

Phytochemical Analysis

Phytochemicals are naturally occurring compounds in plants that act as medication to protect humans from sickness; they are non-nutritive plant chemicals with protection or disease prevention qualities (Cheng *et al*., 2002). *Bridelia ferruginea* aqueous leaf extract was screen for phytochemicals, (Table 1) shows the presence of Steroids, terpenoids, flavonoids, alkaloids, reducing sugar, phenols, and tannin while saponin and phlobatanin were absent but this is in accordance with Sofowara, (1993).The result (Table 2) reveals the quantitative phytochemical analysis of *B. ferruginea* leaves aqueous extract showing the mean ± standard deviation value. The quantitative test shows that Tannins, Reducing sugar, Phenol, Flavonoids, Steroids, Alkaloids were present with 61.56±0.054, 63.76±0.055, 50.59±0.23, 50.32±0.96, 58.79±1.79, 76.09±0.38 respectively.

The result (Table 3) showed active substances (phytochemicals) present in biosynthesized MgO nanoparticle *B. ferruginea* which includes; Tannins, Saponin, Flavonoids, Steroids, Terpenoids, Cardiac glycosides, Reducing Sugar, Phenols and also indicated that phlobatanin was absent. The result (Table 4) reveals the quantitative phytochemical analysis of *B. ferruginea* showing the mean ± standard deviation value. The quantitative test shows that Tannins, Reducing sugar, Phenol, Flavonoids, Steroids, Alkaloids were present with 51.91±2.470, 43.38±0.331, 53.58±0.659, 39.66±0.525, 39.89±0.525, 30.153±0.250. These types of biochemicals, particularly alkaloids, flavonoids, and tannins, are known to be physiologically active and so have anti-diabetic properties (Kolawale *et al*., 2006).

FTIR Spectrum Analysis

FTIR is a technology that combines vibrations in molecules after precise infrared light absorption based on interference and absorption (Ajay *et al*., 2019). At frequencies ranging from 4000 to 650 cm-1, the FTIR technique is used to determine the kind of chemical interactions present on nanomaterials. Prasanth and Subramanian (2013) used FTIR to identify probable biomolecules involved in the reduction and capping of MgONPs. The FTIR response was done by utilizing the Atom technique throughout a wave number range of 650-4000 cm^{-1}. The FTIR spectrum of MgONPs biosynthesized from aqueous *Bridelia ferruginea* leaf extract (MgONPs-Bf) shows that the absorption bands are less than 5, indicating that *Bridelia ferruginea* is either a simple compound with a low molecular weight or an inorganic substance. The peak contained the single bond area (2400-4000 cm^{-1}) which shows that there is a broad absorption band which reveals the presence of hydrogen bond. This band confirms the existence of hydrate (H_2O), hydroxyl (OH), ammonium or amino (Nandiyanto, 2019). The presence of an active carbonyl group, such as anhydrides, halide acids, or halogenated carbonyl, or ring carbonyl carbons, such as lactone or organic carbonate, is indicated by the wavelength range 1750 to 1700. It also describes simple carbonyl compounds such as ketones, aldehydes, esters or carboxyl (Nandiyanto, 2019). These compounds have a carbonyl component, no triple bond material, and since the peaks are only two, they are small.

Scanning Electron Microscope

SEM was used to analyze the surface morphology of the generated MgO nanoparticles (Athithan *et al*., 2020). The development of highly crystalline MgONPs (Figure 2), which are spherical in shape and well agglomerated with variable sizes, was revealed by SEM examination of MgONPs. Previous research suggested that the structure of Magnesium Oxide nanoparticles was spherical, whereas Anantharanan *et al* (2016) revealed that the structure of MgO nanoparticles synthesized using aloe vera extract is rock-like. At 500x magnification, the particles look agglomerated, with some individual crystals plainly visible, but at 3000x magnification, the hexagonal forms of the nanoparticles are obvious, as are isolated nanoparticles. As stated by Suresh *et al*., (2014).

Antidiabetic Activity

The Comparison of α-amylase inhibition of acarbose and MgO nanoparticles

α -amylase breaks starch (polysaccharide) into disaccharides and oligosaccharides before it enters the colon. **α**-glucosidase catalyzes the degradation of disaccharides to produce glucose, which is then transported into the circulation from the small intestine. Inhibiting these enzymes might slow starch breakdown in the gastrointestinal tract, delaying carbohydrate digestion and absorption and altering the increase in post-meal hyperglycemia (Kwon *et al*., 2007). The MgO nanoparticles were tested in vitro utilizing α-amylase inhibition framework. The efficacy of synthesized MgO nanoparticles compared with the diabetes drug Acarbose was studied. Figure 3 depicts a comparison of α-amylase inhibition of acarbose and MgO nanoparticles *B. ferruginea*. At varying concentrations (20, 40, 60, 80, 100 µg/ml) MgO nanoparticles *B. ferruginea* increased in its inhibitory capacity against α-amylase enzyme, and the percentage inhibition was reported to be 33.19,31.90,48.34,64.56, and 74.69, with an IC50 of 66.00 µg/ml. Therefore, this result suggests that the biosynthesized MgO nanoparticles using *B. ferruginea* gives a high level of inhibitory activity under in vitro conditions and this is as reported by Athithan *et al*., (2020). The impact of *B. ferruginea* leaf extracts on the activities of α-amylase and α-glucosidase was investigated in this study.

The Comparison of α-glucosidase inhibition of acarbose and MgO nanoparticles

B. ferruginea were shown in figure 4. The activity of α-glucosidase enzyme was highly decreased by MgO nanoparticles *B. ferruginea* at varyingconcentrations (20, 40, 60, 80, and 100), with the percentage inhibition being 23.41,26.27,47.05, 52.96, and 59.74, respectively, with an IC50 of 74.51µg/ml. Figure 4 demonstrates that at a concentration of 100µg/ml, the MgONPs-Bf had a high percentage inhibition of 59.74±0.898. However, the α-amylase inhibitory activities of MgONPs-Bf are greater than their α-glucosidase inhibitory activities, which is consistent with Nagmoti *et al* (2012). In the case of α-glucosidase, the aqueous extract strongly inhibits enzyme activity. This is consistent with the findings of Kwon *et al* (2007).

Conclusion

In conclusion, Magnesium Oxide nanoparticles can act as antidiabetic agents because they inhibit α-amylase and α-glucosidase. Green or biological method is a good alternative over chemical methods due to its high efficiency. The synthesized MgONPs were well characterized by FTIR and SEM techniques.

Recommendation

This study suggest that further invivo investigation should be carried out on Biosynthesized magnesium oxide nanoparticle of *Bridelia ferruginea* leaf aqueous exract in other to validate the antidiabetic activity of *Bridelia ferruginea* in the management of diabetic mellitus.

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