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Green Synthesized Iron Nanoparticles Using *Gongronema latifolium* Leaf Extract: Characterization, Antioxidant, and Antidiabetic Activities

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ABSTRACT
Green nanoparticles are derived from natural sources possessing unique properties for biomedical applications. Inhibition of key enzymes (-amylase and -glucosidase) involved in the digestion of starch and protection against free radicals and lipid peroxidation could be part of the therapeutic approach towards the management of hyperglycemia. The study was carried out to synthesize and characterize Iron Oxide nanoparticles coated with ethanolic leaf extracts of *Gongronema latifolium* (Gl-FeONPs) using highly advanced techniques for its use as antioxidant and antidiabetic. The study compared the in vitro antioxidant capacities of ethanolic leaf extracts of *Gongronema latifolium* (Et-Gl), iron oxide nanoparticles (FeONps) and Gl-FeONPs by evaluating the Metal Chelating Activity (MCA), Nitric Oxide Scavenging Activity (NOSA), Ferric Reducing Ability Power (FRAP) and Lipid Peroxidation (LPO). The in-vitro anti-diabetic capacities were determined against -amylase and -glucosidase. Results showed that, Et-Gl and Gl-FeONPs displayed MCA (IC50= 29.48 - 14.77 g/mL), NOSA (IC50= 2.157 - 1.480 g/mL), FRAP (IC50= 10.658 - 4.352 g/mL), LPO (14.17 – 94.38%), -amylase (IC50= 2.81 - 4.83 mg/mL), and -glucosidase (IC50= 2.02 – 3.52 mg/mL). Overall, Gl-FeONPs indicated the potential to prevent postprandial hyperglycemia by slowing down carbohydrate hydrolysis and oxidative stress.

Keywords: *Nanotechnology, Green nanoparticle, Antioxidant, Anti-diabetic, Gongronema latifolium*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition typified by a lack of insulin action, production, or both, which results in hyperglycemia (Cole Florez, 2020). Chronic hyperglycemia (high blood sugar) with problems with major macromolecule metabolism is the outcome of its lack. Diabetes mellitus can cause long-term harm, malfunction, and organ failure, particularly to the liver, kidneys, eyes, nerves, heart, pancreas, eyes (retinopathy), kidneys (nephropathy), and blood vessels (Deshpande et al., 2008). According to recent estimates, there were 455 million diabetics globally in 2017, and by 2045, that number is predicted to rise to 693 million. It was also shown that around 50% of diabetics go undiagnosed. The cost of treating diabetic patients was predicted to be USD 850 billion worldwide in the same year (Ganasegeran et al., 2020). It is estimated that 11.2 million Nigerians, or 5.77% of the population, have diabetes (Uloko at al., 2018). Because the digestible polysaccharides do not absorb glucose quickly, causing a hyperglycemic peak, the nutrition approach successfully lowers postprandial glycemia (Srisongkram et al., 2022). α-amylase is essential for the digestion of polysaccharides. The absorption of monosaccharides (such as glucose or maltose) and abrupt high glycemic peaks, which lower insulin sensitivity, are prevented by inhibition of these two enzymes (Alqahtani et al., 2019). A family of oral hypoglycemic medications known as alpha-glucosidase inhibitors alters how carbohydrates are absorbed in the intestines, lowering blood glucose levels. Type 2 diabetes mellitus is treated with them (Alqahtani et al., 2019). Patients who are overweight

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or obese may benefit from lowering their blood glucose absorption through food modification (Kaur et al., 2021). Synthetic substances such as acarbose, miglitol, and voglibose are utilized in clinical settings. However, some of these substances' side effects might include gastrointestinal (GI) abnormalities, such as bloating, abdominal discomfort, GI cramps, or diarrhea (Dirir et al., 2022). More promising than synthetic medicines (such as acarbose or voglibose) for decreasing postprandial glycemia with fewer side effects such as bloating, stomach discomfort, and diarrhea are natural items that may block α-amylase and α-glucosidase activity (Alqahtani et al., 2019; Kaur et al., 2021; Dirir et al., 2022).

Free radicals cause oxidative stress, which builds up in the body and causes reactive oxygen and nitrogen species to accumulate (Teleanu et al., 2022). This disrupts normal aging processes and causes a variety of metabolic abnormalities. These free radicals are produced either naturally during human metabolism or as a result of ultraviolet radiation and chemical pollutants in the environment. Excessive free radicals, DNA damage, and cell malignancy are all directly linked to the development of cancer, according to scientific investigations (Yang et al., 2022). A major contributing component in the development of type II diabetes mellitus has also been identified as oxidative stress. Cost-effective alternatives are urgently needed to lessen the burdens on people and society because the medical therapy for these diseases is extremely expensive (Teleanu et al., 2022). In other words, antioxidants are molecules that diminish or stop the effects of free radicals. Antioxidants are substances that shield cells from the harm produced by free radicals. Free radicals receive electrons from them, which lessens their reactivity. Many plants and animals create anti-oxidants to prevent oxidation from damaging healthy cells or making damaged cells worse (Achan et al., 2011). Plant-derived substances have been of great interest due to their versatile applications (Krishnamurti Rao, 2016).

Gongronema latifolium is a native of Esanland, Edo State, Nigeria. It is often referred to as "Utezi" in Esanland and "arokeke" in Yoruba communities in Nigeria (Okon et al., 2022). In addition to being consumed raw or as a vegetable in soup, *G. latifolium* has been used extensively in folk medicine to help maintain normal blood glucose levels (Ojo et al., 2020; Omonhinmin et al., 2022). The residents of Edo State, Nigeria's Esanland are well-known for consuming a lot of vegetables, some of which are included in meals taken under particular circumstances, such as illness or during recovery periods (Omonhinmin et al., 2022).

Modern technology development frequently focuses on the provision of healthcare as a fundamental human right (Anjum et al., 2021). Nanotechnology stands out among all cutting-edge technologies for its success in guaranteeing high-quality medical care. Although nanotechnology has many applications across many industries, it is particularly capable of using nanoparticles to encapsulate medications (Adewuyi et al., 2018). Utilizing plant extracts to synthesize metallic nanoparticles is a promising approach since it has shown to be less expensive, safer, faster, simpler, and easier than traditional approaches (Adeyemi et al., 2022). The green synthesis of iron nanoparticles and their biomedical applications have been extensively reported (Kalpana et al., 2018; Vijayakumar et al., 2022). However, there are synthesis methods and important properties and characteristics that still need to be investigated. Therefore, in this study we synthesized iron nanoparticles using *G. latifolium* extract as a reducing agent in the redox synthesis of the nanomaterials. We characterized the nanostructures by spectroscopic and microscopic analyses and studied their in-vitro free radical scavenging activities. We also demonstrated the in vitro screening of α-amylase inhibitory activities. Moreover, the results here described give insight towards the development of novel and more effective antioxidant and antidiabetic therapies for different applications.

MATERIALS AND METHODS

Plant Materials

In the current research, the leaves of *Gongronema latifolium* were selected based on the ethno-medicinal claims of the Esan tribe of the Edo people, Nigeria. The leaves of *G. latifolium* were retrieved from the forest located in Ogwa village. The plant material was identified and authenticated by Dr. E.S. Osagie, Technical Officer, Plant Genetic Resources, University of Benin. Herbariums were deposited at JNTBGRI (TBGT 86816/dated November 25, 2022).

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Preparation of Extracts

Gongronema latifolium was freshly collected, washed thoroughly with distilled water, and left to dry for two weeks. The leaves were homogenized by a mechanical blender, model TT-I777. The resulting powdered leaves were macerated with ethanol and distilled water solvents. This solution was left for 72 hours to ensure proper concentration. The solution was appropriately filtered using a mesh-like structure. The plant solutes were properly disposed of while the subsequent filtered solvent underwent extraction and concentration to extract the initially present ethanol and to concentrate the plant extract using a rotary evaporator, vacuum pump, and water bath simultaneously.

Synthesis of Iron Nanoparticle Coated with Ethanolic Leaf Extracts of *Gongronema latifolium* (Gl-FeONPs)

According to Ali et al. (2016), Gl-FeONPs were prepared as follows: 3.33 g of Iron(III) Chloride hexahydrate and 1.5 g of Iron(II) Chloride were dissolved in 100 mL of distilled water. The solution was mixed under dry gas in an oven at 80°C for 10 minutes. Subsequently, 15 mL of plant extract was added drop-wise into the mixture under continuous stirring for 10 minutes. After 5 minutes, 60 mL of 1 M NaOH was added to the solution. The reaction further proceeded until the color of the solution, which was initially yellow, transformed to dark brown.

Characterization of Iron Nanoparticle Coated with Ethanolic Leaf Extracts of *Gongronema latifolium* (Gl-FeONPs)

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was used to identify the functional group of FeONPs (Perkin Elmer, 147 spectrum RSI 83303). The components were mixed with KBr, shaped into pellets, and then inspected at a wavelength of 400–4,500 cm^{-1}.

Scanning Electron Microscopy (SEM)

SEM (JEOL.JSM-6360LV.149, Japan) was used to examine surface morphology. Powdered FeONPs were encapsulated in gold by fully utilizing the sputtering method. The SEM study was employed to improve electrical conductivity and the quality of the green nanoparticle. This was conducted at 151 micrographs.

Zeta Potential Analysis

Zeta potential is a measure of the electric charge or the electric potential difference between the surfaces of a particle suspended in a liquid medium. It provides information about the stability and behavior of the particle in a dispersion.

In Vitro Antioxidant Activity

In vitro antioxidant activity of *G. latifolium*, the green nanoparticle (Gl-FeONPs), and Iron oxide nanoparticles (FeONPs) was evaluated using standard procedures as mentioned below.

Metal Chelating Activity (MCA)

The MCA was determined using the spectrophotometric method as depicted by Dinis et al. (1994). The reaction mixture, which contains 0.5 mL of Gl-FeONPs (50–500 μg/mL), 1.6 mL of distilled water, 0.05 mL of $FeCl_3$ (2 mM), and 0.1 mL of 1,10-phenanthroline (5 mM), was incubated at 40°C for 10 minutes, and the absorbance was measured at 562 nm. The metal chelating activity of the extracts was also measured as described above. Furthermore, a similar protocol was carried out for FeONPs (0.5 mL, 50–500 μg/mL), and ascorbic acid was used as the standard. The percentage metal chelating activity was determined using the formula:

$$\text{MCA (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Nitric Oxide Radical Scavenging Activity (NOSA)

The scavenging effect on nitric oxide (NO•) radical was measured according to the method of Marcocci et al. (1994). 0.5 mL of the leaf extract was added in the test tubes to 1 mL of sodium nitroprusside solution (25 mM) and the tubes incubated at 37°C for 2 hours. An aliquot (0.5 mL) of the incubation solution was removed and diluted with 0.3 mL of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against deionized water as blank with catechin (50 μg) used as standard. Results were expressed as percentage radical scavenging activity (RSA):

$$\text{NOSA (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

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Ferric Reducing Antioxidant Power (FRAP)

This was estimated using the method of Bensie Strain (1996). 2.5 mL of 200 mM of phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ were added to various concentrations of the extract. The mixture was incubated for 2 minutes at 50°C and then centrifuged at 1000 g for 8 minutes. 5 mL of the supernatant was then mixed with 5 mL of distilled water and 1 mL of 0.1% $FeCl_3$. The absorbance of the mixture was measured at a wavelength of 700 nm. Catechin was used as the standard.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity of Et-Gl was performed according to procedures outlined by Halliwell (1990). 0.1 mL of sample were mixed with 1 mL of the reagents solution (4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid). The tubes were incubated for 90 minutes at 95°C. When the samples were cooled to room temperature, the absorbance of the aqueous solution was quantitatively determined at 695 nm.

Anti-Lipid Peroxidant Activity

The anti-lipid peroxidation effects of Et-Gl, FeONPs, and Gl-FeONPs were evaluated in vitro by the method of Suja et al. (2004). 0.5 g of rat liver tissue was sliced and homogenized with 10 mL of 150 mM KCl-Tris-HCl-buffer (pH 7.2). The reaction mixture contained 0.25 mL of liver homogenate, 0.05 mL of 0.1 mM ascorbic acid (AA), 0.1 mL of Tris-HCl-buffer (pH 7.2), and 0.05 mL of various concentrations of plant extract/serial fraction. The mixture was kept for incubation at 37°C for 1 hour. After that, 0.2 mL of 9.8% sodium dodecyl sulfate (SDS), 0.5 mL of 0.1 N HCl, 0.9 mL of distilled water, and 2 mL of 0.6% thiobarbituric acid (TBA) were added to each of the tubes and shaken vigorously. The tubes were placed in a boiling water bath at 100°C for half an hour. The flocculent precipitate formed after cooling was removed by addition of 5 mL of n-butanol and centrifuged at 3000 rpm for 20 minutes. The absorbance of the supernatant was measured at 523 nm. Malondialdehyde (MDA) produced was expressed as % Control.

In Vitro Anti-Diabetic Activity

Determination of α-Amylase Inhibition

The inhibition of α-amylase activity was measured using the spectrophotometric method described by Anigboro et al. (2021). The Gl-FeONPs (0.5 mL, 50-500 μg/mL) respectively, 0.5 mL of α-amylase, were incubated for 10 minutes at 37°C, then 5 μL of 1% starch was added, incubated for 10 minutes at 50°C, thereafter 200 μL DNS, incubated in a boiling water bath for 5 minutes and cooled; 3 mL of distilled water was added and absorbance read at 540 nm. Additionally, the same procedure was followed while working with FeONPs (0.5 mL, 50-500 μg/mL), and acarbose was used as the standard. The percentage α-amylase inhibitory activity was calculated using the formula:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the absorbance of control (without Gl-FeONPs/standard) and A_1 is the absorbance in the presence of Gl-FeONPs. The IC_{50} value was calculated using Microsoft Excel.

Determination of α-Glucosidase Inhibition

The inhibition of α-glucosidase activity was measured using the spectrophotometric method as described by Anigboro et al. (2021). The Gl-FeONPs (0.5 mL, 50-500 μg/mL), 100 μL of α-glucosidase in 0.1 M phosphate buffer (pH 6.9) was incubated at 25°C for 10 minutes. 50 μL of 5 mM p-nitrophenol and 1 mL of 1% starch solution in 0.1 M phosphate buffer was added, the mixture was incubated at 25°C for 5 minutes and read at 405 nm. Additionally, the same procedure was followed while working with Gl-FeONPs (0.5 mL, 50-500 μg/mL), and acarbose was used as the standard. The percentage α-glucosidase inhibitory activity was calculated using the formula:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the absorbance of control (without Gl-FeONPs/standard) and A_1 is the absorbance in the presence of Gl-FeONPs. The IC_{50} value was calculated using Microsoft Excel.

Statistical Analysis

All assays were performed in triplicates and results expressed as Mean ± SEM. Experimental data were

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analyzed by one-way analysis of variance (ANOVA) to evaluate significant difference at $p < 0.05$.

1 Results and Discussions

Nanoparticles are very effective for drug transportation and delivery, particularly because they can precisely locate and carry encapsulated medicines to diseased cells (Nasimi & Haidari, 2013). The absorption and bio-distribution capacity is determined by the analysis of the chemical and physical properties of the nanoparticles, such as size, shape, and surface charge (Umrani & Paknikar, 2014; Manne et al., 2020).

FTIR analysis was carried out to determine the different functional groups present in Et-Gl and Gl-FeONPs. The conspicuous wavelengths present were 3454 cm^{-1}, 2913 cm^{-1}, 2847 cm^{-1}, 2480 cm^{-1}, 1775 cm^{-1}, 1636 cm^{-1}, 1452 cm^{-1}, and 871 cm^{-1}, corresponding to functional groups O-H Stretching indicating an alcohol group, O-H and N-H Stretching indicating an alcohol and primary amine group respectively, S-H Stretching indicating a thiol group, C-H Bending indicating an alkane functional group, C=C Stretching and N-H bending indicating α, β-unsaturated ketone, C-H Bending indicating an alkane. For Gl-FeONPs, the wavelengths at 3454 cm^{-1}, 2906 cm^{-1}, 2847 cm^{-1}, 1749 cm^{-1}, 1550 cm^{-1}, and 1379 cm^{-1} correspond to O-H Stretching indicating an alcohol functional group, O-H and N-H Stretching indicating an alcohol and primary amine group respectively, C-H Bending indicating an alkane group, N-O Stretching indicating the presence of nitro compounds, and O-H Bending indicating an alcohol functional group respectively. Similar observations were reported by Bensie & Strain (1996). FTIR studies of Et-Gl and Gl-FeONPs were performed to characterize the chemical nature of the nanoparticles.

Scanning electron microscopy (SEM) is a technique employed to determine the surface morphology of the nanoparticle by providing information about its size, shape, and morphology. The surface morphology of the green nanoparticle (Gl-FeONPs) was examined by SEM due to its capability of detecting scattered electrons from the particle's surface. The SEM images show that the particle size, calculated to be 5.0 m (5000 nm), was dispersed at 5% by mass. The SEM pictures confirm the formation of Gl-FeONPs, showing the spherical shape of the nanoparticles. Similar observations were reported by Mirza et al. (2019) for zinc oxide nanoparticles coated with plant extracts.

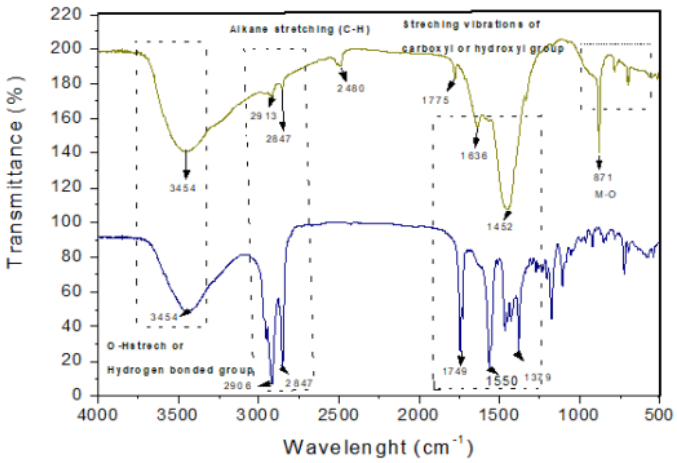

Figure 1: FTIR spectra of Et-Gl and Gl-FeONPs. The curve shows the emission spectra of Et-Gl (blue line) and Gl-FeONPs (green line).

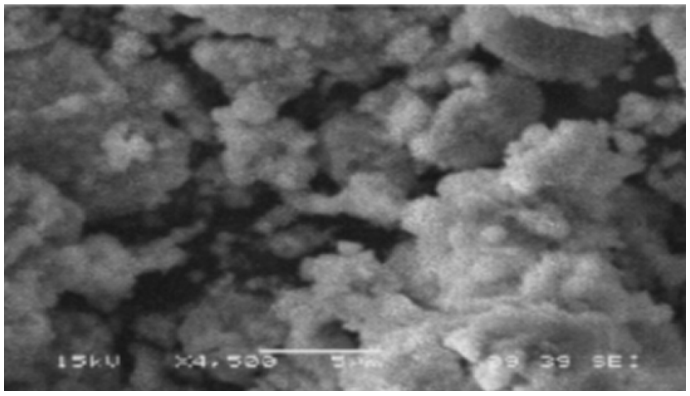

Figure 2: The SEM image for Gl-FeONPs with 4500 magnification.

Zeta potential is a measure of the electric charge or the electric potential difference between the surfaces of the particle suspended in a liquid medium. It provides information about the stability and behavior of the particle in a dispersion. A Zeta-potential of -50 millivolts (mV) indicates that the negative charge arises from the presence of functional groups or ions on the surface of the particles, which can be either naturally occurring or intentionally introduced through surface modification or functionalization. The magnitude of the zeta potential provides information about the stability and behavior of the particle in dispersion. Generally, larger absolute values of zeta-potential indicate greater stability, as the repulsion between particles with similar charges

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prevents them from aggregating or flocculating. Gl-FeONPs showed a zeta-potential of -50 mV, indicating a relatively high degree of stability for the dispersion of FeONPs. Similar observations were made by Hu et al. (2021). The Zeta potential can be used to optimize the formulations of suspensions, emulsions, protein solutions, as well as predict interactions with surfaces.

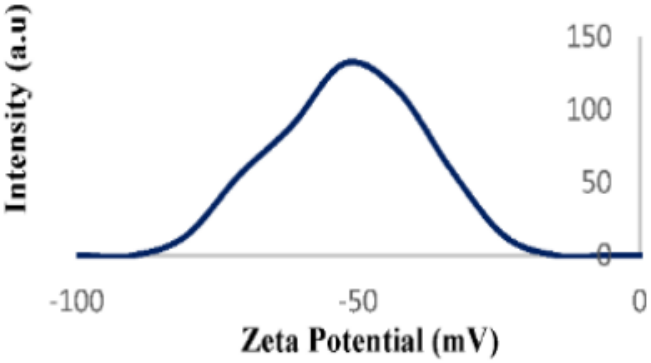

Figure 3: The ZETA Potential plot for Gl-FeONPs at pH 2-5.

In this study, the antioxidant activity of Et-Gl, FeONPs, and Gl-FeONPs were investigated in vitro. As excess free heavy metals have been implicated in the induction and formation of free radicals in vivo, Et-Gl, FeONPs, and Gl-FeONPs were tested in a metal chelating assay in a concentration range of 50-500 mg/mL respectively. Although Et-Gl at 500 μg/mL indicated no inhibition of the chelating of heavy metals, FeONPs and Gl-FeONPs showed dose-dependent inhibitions of metal chelators utilizing ascorbic acid as a standard control. The in vitro metal chelating activity of Et-Gl, FeONPs, and Gl-FeONPs are shown in Figure 4, with all nanoparticles compared to that of ascorbic acid. The IC_{50} value of Et-Gl, FeONPs, and Gl-FeONPs were 29.48 g/mL, 871.67 g/mL, and 14.77 g/mL respectively, compared to the standard ascorbic acid whose IC_{50} was 24.445 g/mL. The metal chelating activity assay works on the principle of reduction/chelation of metals participating in redox reactions. Hence, Et-Gl and Gl-FeONPs were observed to be effective chelators of ferrous ion radicals compared to ascorbic acid. Similar observations were made by Faisal et al. (2021).

The nitric oxide scavenging assay (NOSA) is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which reacts with oxygen to

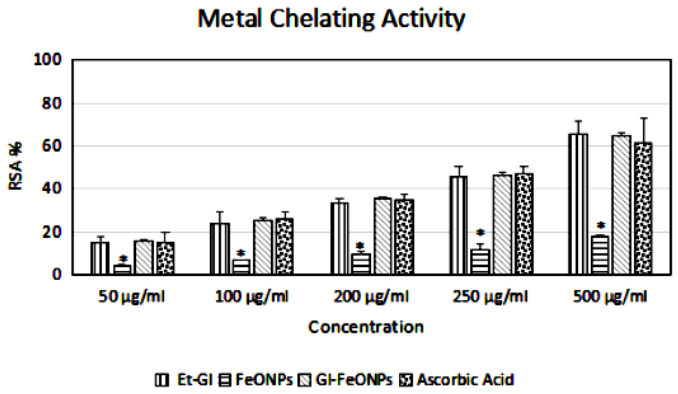

Figure 4: A plot for metal chelating activity of Et-Gl, FeONPs, Gl-FeONPs, and Ascorbic acid. *Values are expressed as means ± SD. Compared with control (Ascorbic Acid): P i 0.05.

yield nitrite ions that can be estimated using Griess reagent. In the present study, Et-Gl, FeONPs, and Gl-FeONPs demonstrated a dose-dependent increase in nitric oxide radical scavenging activity, fully utilizing ascorbic acid as a standard control. The in vitro nitric oxide scavenging activity of Et-Gl, FeONPs, and Gl-FeONPs are shown in Figure 5, with all nanoparticles compared to that of ascorbic acid. The graphical trend of % inhibition against concentration was similar to that of standard ascorbic acid, indicating metal chelating activity of the nanoparticles. The IC_{50} value of Et-Gl, FeONPs, and Gl-FeONPs were 2.157 g/mL, 22.961 g/mL, and 1.480 g/mL respectively, compared to the standard ascorbic acid whose IC_{50} was 1.838 g/mL. The half-maximal activity (IC_{50}) of Et-Gl and Gl-FeONPs was lower than that of the standard respectively. However, FeONPs displayed significantly (Pi0.05) higher inhibition in comparison with that of ascorbic acid, which served as a standard for the assay. Similar observations were reported by Abu-Serie & Abdelfattah (2020) for iron nanoparticles, which served as good scavengers of nitric oxide radicals.

Ferric reducing ability power of biologically active compounds are electron donors that can reduce the oxidized intermediates, such as those in the lipid peroxidation processes. It is centered on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form at low pH. The in vitro ferric reducing antioxidant power of Et-Gl, FeONPs, and Gl-FeONPs are shown in Figure 6, with all nanoparticles

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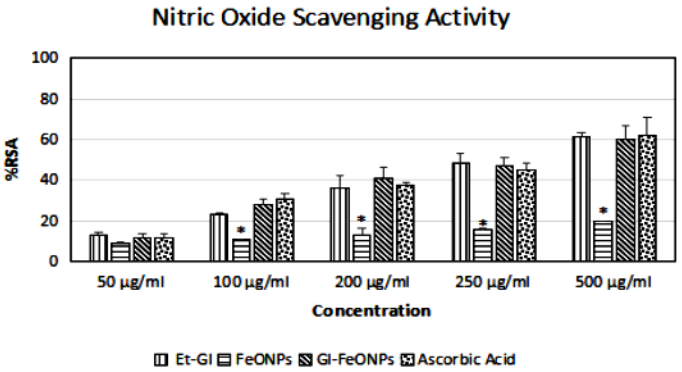

Figure 5: A plot for Nitric Oxide Scavenging Activity (NOSA) of Et-Gl, FeONPs, Gl-FeONPs, and Ascorbic acid. *Values are expressed as means ± SD. Compared with control (Ascorbic Acid): P ¡ 0.05.

compared to that of ascorbic acid. The graphical trend of % inhibition against concentration was similar to that of standard ascorbic acid, indicating metal chelating activity of the nanoparticles. The IC_{50} value of Et-Gl, FeONPs, and Gl-FeONPs were 115.91 g/mL, 332.71 g/mL, and 64.43 g/mL respectively, compared to the standard ascorbic acid whose IC_{50} was 67.30 g/mL. Et-Gl and Gl-FeONPs displayed a significantly higher inhibition in comparison with that of ascorbic acid, which served as a standard for the assay. Similar observations were made by Rajput et al. (2013) for zinc oxide nanoparticles synthesized from plant extracts, which served as good ferric-reducing agents.

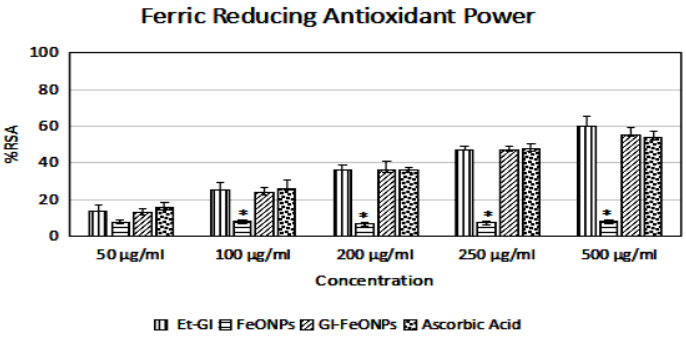

Figure 6: A plot for Ferric Reducing Antioxidant Power (FRAP) of Et-Gl, FeONPs, Gl-FeONPs, and Ascorbic acid. *Values are expressed as means ± SD. Compared with control (Ascorbic Acid): P ¡ 0.05.

Total antioxidant capacity (TAC) is a measure of the overall antioxidant capacity of a substance or biological sample. The TAC assay measures the ability of a substance to neutralize free radicals or inhibit oxidative reactions. It provides an overall assessment of the cumulative antioxidant activity of the sample. The working principle of measuring the total antioxidant capacity (TAC) is based on the ability of antioxidants to neutralize or inhibit the harmful effects of free radicals by subjecting the ethanolic plant extract (Et-Gl) to a specific assay that assesses its ability to counteract or scavenge free radicals. Using the garlic acid equivalent which is a standard for determining the total anti-oxidant capacity, the TAC was estimated to be 1.8 mg, indicating the plant quantitatively contained 1.8 mg of antioxidant properties. This correlates with findings by Gulcin (2020).

Anti-lipid peroxidation mechanisms aim to prevent or interrupt this chain reaction by neutralizing or scavenging the free radicals or ROS that initiate lipid peroxidation. Overall, the working principle of anti-lipid peroxidation involves the use of antioxidants and other mechanisms to neutralize free radicals, scavenge ROS, and interrupt the chain reaction of lipid peroxidation. By protecting cell membranes and lipid components, anti-lipid peroxidation strategies aim to prevent oxidative damage and maintain cellular integrity, and assess the impact of antioxidants on health and disease. The in vitro anti-lipid peroxidant activity of Et-Gl, FeONPs and Gl-FeONPs are shown in Figure 7. The IC50 value of Et-Gl, FeONPs and Gl-FeONPs were 37.298 g/mL, 96.310 g/mL and 32.829 g/mL respectively. In the present study, Et-Gl, FeONPs and Gl-FeONPs demonstrated a dose-dependent increase in the anti-lipid peroxidation activity corresponding with Ali et al. (2020) who demonstrated that iron nanoparticles coated with Aframomum angustifolium were capable of inhibiting the formation of Malondialdehyde (MDA) products.

Consistent management of blood glucose is one of the effective strategies for combating type II diabetes mellitus. Postprandial blood glucose level rises due to the activity of carbohydrate hydrolyzing and degrading enzymes: pancreatic α-amylase and intestinal α-glucosidase. Inhibitors of these enzymes are currently used as oral hypoglycemic drugs for the control of hyperglycemia, especially in patients with type II diabetes mellitus. The inhibitors delay the release of monosaccharide from polysaccharides present in food (Oboh et al., 2012; Avwioroko et

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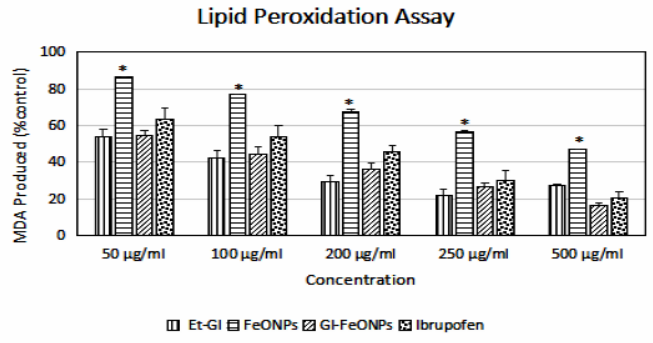

Figure 7: A plot for Anti-lipid peroxidant Activity (ALPO) of Et-Gl, FeONPs and Gl-FeONPs. *Values are expressed as means ± SD. Compared with control (Ibuprofen): P ¡ 0.05.

al., 2020). Alpha-amylase is a pancreatic enzyme involved in the degradation of starch. The inhibition of Alpha-amylase activity helps in managing diabetes mellitus and in reducing postprandial blood glucose (Manne et al., 2020). The addition of α-amylase to a reaction mixture of starch and water produces maltose which serves as the substrate for the reaction, dinitrosalicyclic acid (DNS) served as an indicator for the product formed. Et-Gl, FeONPs and Gl-FeONPs were analyzed for alpha-amylase activity in a concentration range of 1–3 μg/mL. The IC50 value of Et-Gl, FeONPs and Gl-FeONPs were 4.85 and 2.50 mg/mL respectively, compared to the standard acarbose whose IC50 was 3.37 mg/mL. The inhibition of α-amylase by Et-Gl, FeONPs and Gl-FeONPs showed little significant differences (¡0.05) from that observed for acarbose. Similar observations were reported by Anigboro et al. (2021).

Alpha-glucosidase is an intestinal enzyme that breaks down monosaccharides and starch into glucose (Peng et al., 2016). The inhibition of glucosidase activity helps in managing diabetes mellitus. α-glucosidase catalyzes the conversion of the substrate 4-nitrophenyl-α-D-glucopyranoside to α-D-glucopyranoside and p-nitrophenol. Et-Gl, FeONPs and Gl-FeONPs were analyzed for α-glucosidase activity in a concentration range of 1–3 mg/mL with no significant differences (P¡0.05) from that observed for acarbose. The IC50 value of Et-Gl, FeONPs, Gl-FeONPs were 65.42, 55.49 and 25.25 respectively, compared to the standard acarbose whose IC50 was 337.84 μg/mL. Similar findings were reported by Anigboro et al. (2021).

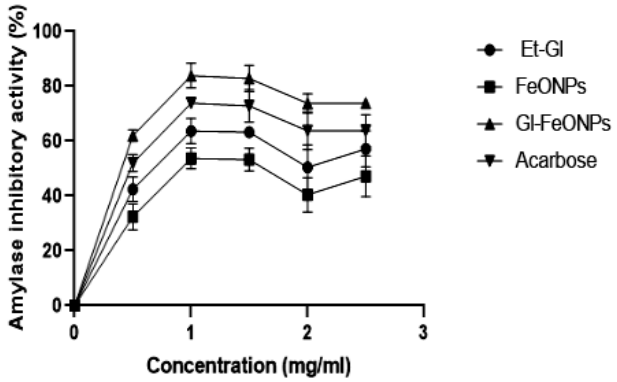

Figure 8: α-Amylase inhibitory activities of Et-Gl, FeONPs and Gl-FeONPs. *Values are expressed as means ± SD. Compared with control (Acarbose): (P ¡ 0.05).

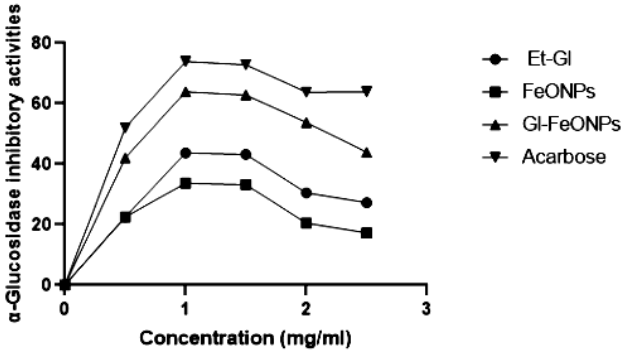

Figure 9: α-Glucosidase inhibitory activities of Et-Gl, FeONPs and Gl-FeONPs. *Values are expressed as means ± SD. Compared with control (Acarbose): P ¡ 0.05.

Conclusion

The results of this study indicated that the iron oxide nanoparticles coated with *Gongronema latifolium* (Gl-FeONPs) displayed potent *in vitro* antidiabetic potential. Additionally, their potent antioxidant activity helps combat oxidative stress, which plays a critical role in the progression of diabetes and associated complications. These findings are crucial as they provide insight into the use of green synthesized nanoparticles for the management of diabetes and its related complications. Further *in vivo* studies to ascertain the effect of metabolic reactions are suggested.

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