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Full Length Article

Assessment of nutritional, antioxidant, and antidiabetic properties of the BF 1 herbal formulation via *in vitro* and *ex vivo* approaches

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ABSTRACT

The study investigated the dietary composition of the BF1 herbal formulation and its effect on carbohydrate hydrolyzing enzymes linked with type 2 diabetes (α-amylase and α-glucosidase enzymes) through *in vitro* and *ex vivo* parameters. *In vitro* antioxidant parameters, such as 1,1-diphenylhydraxyl (DPPH), Fe^{2+} chelating abilities, ferric reducing antioxidant property (FRAP), hydroxyl (OH) scavenging, and nitric oxide (NO), as well as *ex vivo* parameters, such as glutathione (GSH), malondialdehyde (MDA) level, and catalase, ENTPDase, and ATPase enzyme activities in iron-induced pancreatic injury were evaluated. The inhibitory assay for α-amylase and α-glucosidase enzymes was conducted and the results of these parameters revealed that the BF1 formulation could scavenge DPPH radicals compared to the control. At all concentrations, the BF1 formulation reduced ferric compounds, compared to the standard. The BF1 formulation chelated iron better at its lower concentration, similar to the control. Additionally, the BF1 formulation showcased a significant NO radical scavenging ability in comparison to the standard. The BF1 formulation reduced lipid peroxidation in the rat pancreas at a lower concentration than the control. Furthermore, the BF1 formulation demonstrated significant inhibition of α-amylase and α-glucosidase in comparison to the control (metformin). This suggests that BF1 formulation can be considered a potential antidiabetic treatment and can be used for supplement formulation.

1. Introduction

Diabetes mellitus is an increasing global health issue, with it currently reaching a worldwide epidemic level of 424.9 million affected adults (aged 20–79), representing 8.8 % of the worldwide adult population (Amdie et al., 2022; Mikhail et al., 2024; Otegenova et al., 2024). According to current estimates, by 2045, the figure is predicted to rise to 628.6 million people, impacting nearly 10 % of the worldwide adult population (Howarth et al., 2019; Wake, 2020; Reza et al., 2024). As of 2021, the worldwide prevalence was about 8.1–8.8 million individuals globally diagnosed with diabetes. Out of this number, 1.5 million (18 %) were below the age of 20 years (Zerihun et al., 2024). Diabetes mellitus is a metabolic condition that results in an increased amount of sugar in the bloodstream (hyperglycemia) caused by either the body producing little to no insulin or the body cells not being receptive to the produced insulin (Rachdaoui, 2020; Olaniyi et al., 2022). Insulin is a polypeptide

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hormone that is primarily secreted by β cells present in the pancreatic islets of Langerhans (Al-Suhaimi et al., 2022). Its function is to control blood glucose levels, specifically to lower or reduce blood glucose levels in the body. Insulin acts via an anabolic pathway. It converts sugar, starches, and other foods into energy (ATP) (Wolosowicz et al., 2022).

Current synthetic treatments often address individual pathways but are associated with side effects and limited accessibility, particularly in resource-constrained regions (Pei and Li, 2025). Medicinal plants, rich in bioactive compounds such as flavonoids, alkaloids, and phenolic acids, offer a promising alternative due to their multitargeted mechanisms of action (Amanat et al., 2025). These compounds regulate glucose metabolism, enhance insulin sensitivity, and mitigate oxidative stress, a key factor in diabetes complications (Gong et al., 2025). This makes medicinal plants an attractive area of research, bridging traditional knowledge and modern science to develop holistic therapies that address the complexities of diabetes. According to recent research, botanicals with antidiabetic properties are increasingly being used in large-scale commercial formulations due to their accessibility, affordability, and lack of adverse effects as compared to synthetic antidiabetic medications (Ogunlakin et al., 2023a; Blahova et al., 2021).

Herbal formulations, which can consist of different plant parts, including seeds, leaves, stem barks, roots, tubers, and aerial parts, are being considered as possible antidiabetic treatments because they contain phytochemical constituents that possess antidiabetic activity. These include flavonoids, alkaloids, quercetin, kaempferol, apigenin, and triterpenoids (Marella and Tollamadugu, 2018; Ojo et al., 2024). The investigation of BF 1 herbal formulation is a rational step in this context, as it combines medicinal plants known for their nutritional, antioxidant, and antidiabetic properties into a synergistic blend. BF 1 is designed to not only regulate blood glucose levels but also combat oxidative stress and provide essential nutrients, addressing the disease's multifaceted challenges. Its role fits seamlessly into broader research exploring plant-based solutions for diabetes, particularly in aligning with studies that emphasize the importance of nutrition and antioxidants in mitigating diabetes complications. By leveraging the multitargeted potential of medicinal plants, BF 1 exemplifies a sustainable, holistic approach to diabetes management, reflecting the growing demand for safe and accessible plant-based therapies in modern healthcare. The herbal formulation, BF1, claimed to have antidiabetic activity, has not been validated. Therefore, this study aimed to investigate the nutritional composition and antioxidant potential of the BF1 herbal formulation along with its effects on the activity of carbohydrate hydrolyzing enzymes related to type-2 diabetes (α-amylase and α-glucosidase enzymes).

2. Materials and methods

2.1. Chemicals

All the analytical-grade reagents and chemicals used were purchased from Sigma-Aldrich, Germany.

2.2. BF1 herbal formulation

The BF 1 powdered formulation was obtained on February 13, 2024 from Next Era Health, 11, Julius Kadir Street, Ifako Gbagada, Lagos, Nigeria. *Sesamum indicum*, *Gossypium barbadensis*, *Ficus exasperata*, and *Curcuma longa* were used to formulate BF 1. The formulation was prepared under a standard operating procedure by regulatory personnel and the pharmacognosist, Oyindamola Awosola.

2.3. Extraction of BF 1 formulation

A measured volume of 100 % methanol (1000 mL) was used to extract a measured mass of (350 g) of the BF 1 formulation powder in a large conical flask. This setup was covered and left to stand at room temperature for 3 days. The mixture was stirred at intervals within the days it was allowed to stand. The filtrate was then obtained using filter paper. The methanolic extract of the BF 1 formulation was obtained through the use of a rotary evaporator to concentrate the filtrate.

2.4. Proximate analysis

The powdered BF 1 formulation sample was subjected to a proximate analysis according to the Association of Analytical Chemists standard. The weight variation approach was utilized to determine the contents of ash and moisture. The fiber content was computed using the crucible and its contents' weight loss during ignition. The percentages of crude protein, ash, fat, and moisture were added up and deducted from 100 to obtain the nitrogen-free extract (Ogunlakin et al., 2023b).

2.5. Atomic absorption spectroscopy assay (AAS)

This was conducted using the accepted procedures (Ogunlakin et al., 2023b). In a ventilated area, 6.5 mL of nitric solution (HNO_3), sulfuric (H_2SO_4), and perchloric ($HClO_4$) acids were added to the 0.25 g powdered formulation sample. The mixture was boiled in the acidic solution with the use of a hot plate (model VWR VELP Scientifica, Germany), and white vapors were emitted from the flask, signifying that the digestion process was complete. Following a quick stir and the addition of a few drops of distilled water, the solution was left to cool. In 50 mL volumetric flasks containing the sample, distilled water was added to make these digested samples 50 mL in volume. A filter paper was used to collect the filtrate in plastic bottles. An atomic absorption spectrophotometer and a suitable hollow cathode lamp were used to confirm that the solutions contained the necessary elements (Shimadzu AA-670). Using equivalent standard calibration curves based on standard AR-grade solutions of the elements, the concentration of each element in the seed was measured (Ogunlakin et al., 2023b). The elements are Zn, Mg, Na, Pb, Cd, Co, Cu, and Fe.

2.6. Phytochemical screening

Following standard procedures, alkaloids, flavonoids, steroids, saponins, tannins, terpenoids, glycosides, and phenolics were screened both qualitatively and quantitatively. (Banu and Cathrine, 2015; Sonibare et al., 2022; Sobuj et al., 2024).

2.7. In vitro antioxidant activity

2.7.1. Nitric oxide radical scavenging

Nitric oxide scavenging assays were performed using the Griess reagent method described by Haenen and Bast (1999). First, 0.3 mL of sodium nitroprusside (5 mM) was added to 1 mL of each of the various concentrations of the extract. The test tubes were then incubated at 25 °C for 150 min. After 150 min, 0.5 mL of Griess reagent (equal volume of 1 % sulphanilamide on 5 % ortho-phosphoric acid and 0.01 % naphtylethylenediamine in distilled water, used after 12 h of preparation) was added. The absorbance was measured at 570 nm using.

2.7.2. DPPH (2,2- diphenyl-1-picrylhydrazyl) radical scavenging ability

The free radical scavenging ability of the extract against DPPH free radicals was evaluated as described by Fadogba et al. (2024) with modifications. One milliliter of various extract concentrations in methanol was mixed with 1 mL of a methanolic solution of 0.4 mM DPPH radicals. Blank control was also implemented by adding 2 mL of DPPH solution without the test sample. The mixtures were left in the dark for 30 min, after which the absorbance at 516 nm was measured spectrophotometrically. DPPH scavenging activity was expressed as the inhibition percentage of the extracted sample on free radicals by the following equation:

$$\% \ inhibition = \left[\frac{Ac - As}{Ac}\right] x\ 100$$

Where Ac and As are the absorbances of the blank control and the sample, respectively. The assay was repeated in triplicate for each extract concentration (2 mL, 3 mL, and 4 mL).

2.7.3. Iron chelating ability assay

The iron chelating ability of both extracts was determined using a modified method of Ogunlakin et al. (2024). Freshly prepared 500 μM $FeSO_4$ (150 μL) was added to a reaction mixture containing 168 μL of 0.1 M Tris–HCl (pH 7.4), 218 μL of saline, and the extracts (0–25 μL). The reaction mixture was incubated for 5 min before the addition of 13 μL of 0.25 % 1,10 phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated.

2.8. Experimental animals

For this study, ten healthy male Wister rats weighing 130–180 g, were procured. The rats had access to clean water and rat feed in pellet form during their ten-day acclimation period in decontaminated cages under specific conditions. An ethical approval number (BUAC/BCH/2024/0004) was obtained following the standards and directives stated in the National Institutes of Health (NIH) guidelines for the handling and use of experimental animals. 1.2 mL of ketamine was used to put the animal under anesthesia to perform animal euthanasia.

2.9. Ex vivo antioxidant assay

After homogenization with ice-cold phosphate buffer in an ice-cold mortar, the pancreases of ten carefully selected rats were weighed. The homogenates were centrifuged for 10 min at 5000 r.p.m. to separate the supernatant, which was then refrigerated for further analysis.

2.9.1. Pancreatic injury induction

The procedure outlined by Ogunlakin et al. (2024a) was carried out to treat damaged pancreas *ex vivo* with minimal modification. 200 μL of the organ residue, 100 μL of 0.1 mM $FeSO_4$, and different concentrations (30–240 mg/mL) of BF 1 formulation methanol extract were combined in the basic procedure. The samples were allowed to incubate at 37 °C for 30 min before being tested for biochemical analysis. These reaction compositions that contain the tissue supernatant and $FeSO_4$ functioned as the negative control, while the reaction composition containing only the organ supernatant served as the normal control.

2.9.2. Glutathione level

By Ogunlakin et al. (2024a) methodology, 600 μL of 10 % trichloroacetic acid was added to 600 μL of the tissue lysates to deproteinize them. The mixture underwent centrifugation for 10 min at 3500 rpm. A clean test tube was filled with 500 μL of the methanol extract, and 100 μL of Ellman reagent was mixed with it. At 415 nm, the absorbance was calculated following 5 min of incubation at 25 °C. GSH served as a standard.

2.9.3. Catalase activity

To evaluate the catalase activity assay for the methanol extract of BF 1, a few minor adjustments were made to the Ogunlakin et al. (2024a) approach. Tissue samples containing different concentrations of beet extract were filled in a volume of 20 μL, and 780 μL of 50 mM phosphate buffer was added. After adding 300 μL of 2 M H_2O_2, the absorbance was measured every 3 min for 240 min, with 1-min intervals.

2.9.4. Malondialdehyde (MDA) level

After adding 600 μL of thiobarbituric acid to a test tube, 200 μL of the sample is incubated for an hour at 95 °C and then immersed in water for 15 min. Spectrophotometer readings are made at 532 nm to determine the absorbance (Ogunlakin et al., 2024a).

2.9.5. Na/K^+ ATPase enzyme activity

The method described in Ogunlakin et al. (2024a) was modified slightly to determine the Na/K^+ ATPase activity. A mixture of 40 μL of 50 mM ATP, 1.3 mL of 0.1 M Tris-HCl buffer, 200 μL of 5 mM KCl, and 200 μL of the organ lysate containing varying amounts of G. barbadense leaf methanol extract was added. The reaction mixture was incubated using a mechanical shaker at 37 °C for 30 min before 1 mL of 1.25 percent ammonium molybdate and 1 mL of distilled water were added. Subsequently, the solution was mixed with 1 mL of 9 % ascorbic acid and allowed to stand for 30 min. The absorbance was taken at 660 nm.

2.9.6. E-NTPDase enzyme activity

By standard procedure (Ogunlakin et al., 2024a), 400 μL of a reaction mixture comprising 1.5 mM $CaCl_2$, 5 mM KCl, 0.1 mM EDTA, and 10 mM glucose was mixed with 40 μL of tissue lysates containing various concentrations of the methanol extract. 45 mM Tris-HCl and 225 mM sucrose). After that, the mixes were incubated for 10 min at 37 °C. The mixture was then incubated at 37 °C in a mechanical shaker after 40 μL of 50 mM ATP was added. A mixture containing 400 μL of 10 % TCA was added to stop the reaction. After 10 min of ice-cold incubation, the mixture's absorbance at 600 nm was determined.

2.10. Antidiabetic activity

2.10.1. α-amylase inhibition assay

The assay was performed by using the method reported in the studies of Rana et al. (2013) and Faboro et al. (2024) with modifications. Fifty microliters of the extract or acarbose (a well-known α-amylase inhibitor) solution in 0.02 M sodium phosphate buffer (pH = 6.9 with 0.006 M NaCl) was mixed with 50 μL of the enzyme solution porcine pancreatic α-amylase (EC 3.2.1.1.) (0.5 mg/mL) and incubated for 10 min at 25 °C. Subsequently, 500 μL of 1 % starch solution (0.02 M) was added to the mixture and incubated for 10 min at 25 °C. Then, the reaction was stopped by adding 1.0 mL of dinitrosalicyclic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and the absorbance at 540 nm was measured spectrophotometrically. The assay was repeated four times for each extract concentration. The α-amylase inhibitory activity was calculated using the following formula:

$$\% \ inhibition = \left[\frac{\text{ABS control} - \text{ABS sample}}{\text{ABS sample}}\right] x\ 100$$

Where ABS sample and ABS control represent the absorbance of the control (without the extracts or acarbose) and the acarbose or plant extract, respectively.

2.10.2. α-glucosidase inhibitory activity

The assay was carried out using the procedure reported in the study of Dej-Adisai et al. (2022) with modifications. One hundred microliters of α-glucosidase (EC 3.2.1.20) solution in 0.1 M phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. Here, p-nitro phenyl-glucopyranoside (pNPG) was used as a substrate. The reaction mixture, containing 100 L of phosphate buffer (100 mM, pH 6.8), 20 μL of α-glucosidase (1 U/mL), and 40 μL of plant extract or acarbose solution, was left for 15 min at 37 °C. Subsequently, 40 μL of pNPG (5 mM) solution was added, and the mixture was left for 20 min at 37 °C. The reaction was terminated by adding a solution of 1 % Na_2CO_3 (0.1 M, 2 mL). The mixture was spectrophotometrically measured at 400 nm. The assay was repeated three times. The inhibitory effect of the extract on α-glucosidase was calculated as follows:

$$\% \ inhibition = \left[\frac{\text{Acontrol} - \text{Asample}}{\text{Asample}}\right] x\ 100$$

where Acontrol and Asample represent the absorbance of the control (without the extracts or acarbose) and the acarbose or plant extract, respectively.

2.11. Statistical analysis

Each test was conducted in triplicate, and the findings are shown as mean ± SD, percentages, and numbers. The data was evaluated using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The graphs were plotted using GraphPad Prism 9 edition. A significant threshold of $p < 0.05$ is considered.

3. Results

3.1. Proximate, AAS, and phytochemical analysis of BF 1 formulation

Table 1 presents an analysis of the BF1 formulation and illustrates the formulation's protein, ash content, moisture content, crude fiber, crude fat, and nitrogen-free extract. The investigation reveals that the protein and nitrogen-free extract have the highest composition. The percentages of their concentrations were given. Table 2 indicates a few of the BF 1 formulation's mineral components. Parts per million (ppm) are used to denote the concentrations of the constituent minerals. The components of the BF 1 formulation are listed in the table: zinc, magnesium, sodium, lead, cadmium, cobalt, and iron. The highest quantities of magnesium were discovered, whereas no copper was found at all. Table 3 summarizes the results of the BF 1 formulation's phytochemical screening. The formulation is exceptionally high in alkaloids and low in glycosides and tannins, according to the data. Table 4 presents findings of the quantitative phytochemical screening of the BF 1 formulation, along with the quantities of anthraquinone, flavonoids, alkaloids, tannins, saponins, and phenols. Saponin concentrations were found to be abundant. Every concentration was reported as milligrams per milliliter (mg/mL) except for the flavonoid concentration, which was reported as grams (g).

3.2. Antioxidant assay

Figs. 1–3 and Table 5 show the antioxidant activity of the BF 1 formulation methanolic extract in comparison to standards for each assay. In Fig. 1, BF 1 formulation was seen to have a lower ability to scavenge DPPH radicals when compared to the standard used, which is quercetin. It was also seen that an increase in concentration caused a corresponding increase in the DPPH scavenging ability. There is a very close similarity between the BF 1 formulation methanolic extract and the standard used in the NO radical scavenging assay (Fig. 2). The NO radical ability is shown to be lower in comparison to quercetin, which was the standard used. The potential of the methanolic extract of the BF 1 formulation to chelate iron (Fe^{2+}) according to Fig. 3, has the same trend as the control, quercetin, that was used. As the concentration of BF 1 formulation increases, its iron chelating ability also increases.

Table 1
Proximate analysis of the BF1 formulation.

S/N	Proximate content	BF 1 (%)
1	Ash content	3.00 ± 0.01
2	Moisture contents	5.1 ± 0.02
3	Crude fat	2.1 ± 0.12
4	Crude fibre	5.95 ± 0.15
5	Protein	19.69 ± 0.2
7	Nitrogen free extract	64.16 ± 0.12

Table 2
AAS analysis of the BF1 formulation.

S/N	Elements	BF 1 (ppm)
1	Zn	0.1 ± 0.0044
2	Mg	19.479 ± 0.0086
3	Na	5.676 ± 0.0038
4	Pb	0.436 ± 0.0010
5	Cd	0.322 ± 0.0010
7	Co	1.048 ± 0.0003
8	Cu	ND
9	Fe	6.728 ± 0.0015

Table 3
Qualitative phytochemical screening of the BF1 formulation.

S/N	Test	BF 1
1	Phenols	+
2	Glycosides	–
3	Terpenoids	++
4	Alkaloids	+++
5	Flavonoids	+
7	Saponins	+
8	Tannins	–
9	Anthraquinone	+

+ = indicates presence of phytochemicals; - = indicates absence of phytochemicals; ++ = shows moderate concentration; +++ = shows high concentration.

Table 4
Quantitative phytochemical screening result of the BF1.

S/N	Test	BF 1
1	Phenols (mg/mL)	0.55 ± 0.12
2	Tannin (mg/mL)	0.14 ± 0.09
3	Saponin (mg/mL)	3.04 ± 0.09
4	Alkaloids (mg/mL)	1.88 ± 0.12
5	Anthraquinone (mg/mL)	1.031 ± 0.20
7	Flavonoids (g)	1.55 ± 0.12

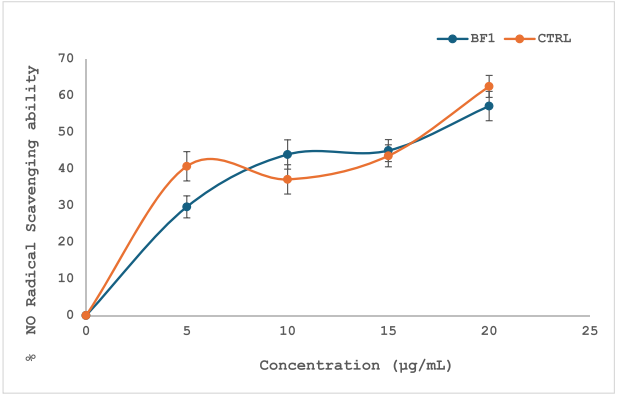

Fig. 1. NO radical scavenging potential of the BF1 formulation
CTRL: control (quercetin); A mean ± SD (n = 3) is used to represent data.

3.3. Ex vivo study

Also, as shown in Fig. 4, the BF 1 formulation and the untreated show similar GSH activity. For catalase enzyme activity in Fig. 5, BF1 shows a higher catalase activity compared to the untreated sample. The level of malondialdehyde (MDA) inhibition is higher in the untreated than in the BF 1 formulation, as seen in Fig. 6. The ENTPase activity of BF1 is more than that of the untreated in Fig. 7. In Fig. 8, the BF 1 formulation shows a higher Na/K ATPase activity than the untreated. Furthermore, the

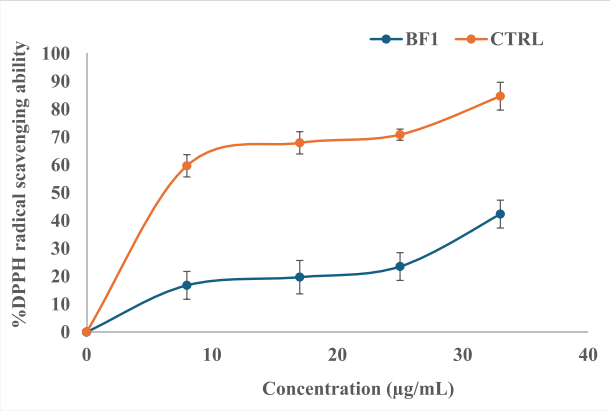

Fig. 2. Percentage of DPPH radical scavenging activity of the BF1 formulation. CTRL: control (quercetin); Data are represented as mean ± SD (n = 3).

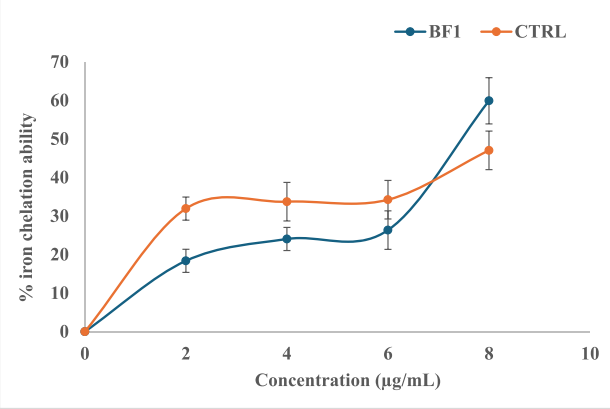

Fig. 3. Percentage of iron chelation ability of the BF1 formulation
CTRL: control (quercetin); A mean ± SD (n = 3) is used to represent data.

Table 5
IC_{50} values of the antioxidant potentials of BF 1 formulation methanol extract.

Samples	IC_{50} (µg/mL)		
	Iron chelation ability	DPPH radical scavenging activity	NO scavenging ability
BF 1	7.80 ± 0.28	43.54 ± 0.33	15.73 ± 0.20
Standard (quercetin)	8.28 ± 0.82	13.54 ± 0.26	15.17 ± 1.10

Data = mean ± SD; n = 3.

activity of each parameter increases with an increase in the concentration of BF 1 formulation except for MDA, where increased concentrations of BF 1 formulation led to increased MDA inhibition activity.

3.4. Antidiabetic activity of BF1 formulation

According to Fig. 9, the α-amylase activity of the BF 1 formulation is slightly lower than that of the control metformin. The figure also shows an increased α-amylase activity with increased concentrations in the BF 1 formulation. In Fig. 10, the two samples show strong similarity, as seen because the graph plots follow each other very closely. The BF 1 formulation showed a lower α-glucosidase inhibiting ability compared to the control metformin. Although at the highest and lowest concentrations of the sample and the control, BF 1 formulation extract showed higher inhibitory activity at those concentrations.

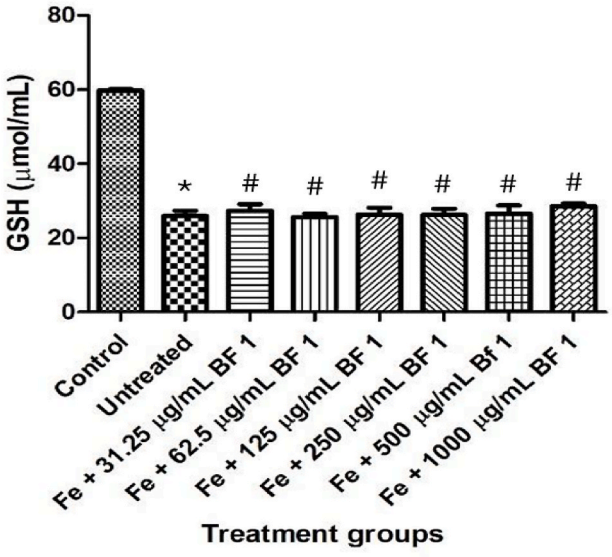

Fig. 4. BF1 formulation effect on GSH levels in iron-mediated oxidative pancreatic injury.
A mean ± SD (n = 3) is used to represent data. *Statistically significant compared to control, while # statistically significant compared to the untreated group at $P < 0.05$.

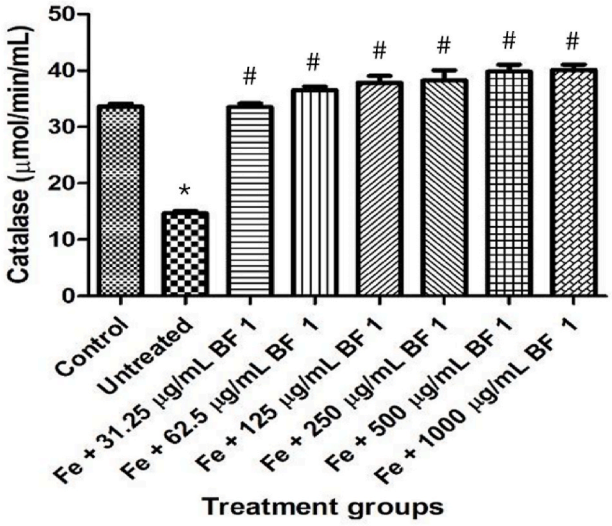

Fig. 5. The effect of BF1 formulation on catalase enzyme activity in iron-mediated oxidative pancreatic damage.
Data = mean ± SD; (n = 3). *Statistically significant compared to control, while # statistically significant compared to the untreated group at $P < 0.05$.

4. Discussion

4.1. Proximate analysis, atomic absorption spectroscopy (AAS), phytochemical screening

The proximate analysis of the BF 1 formulation showed the highest proportions of protein, crude fiber, and moisture content. This indicates its potential to serve as a dietary supplement. The atomic absorption spectroscopy assay of the BF 1 formulation showed the strong presence of magnesium, sodium, and iron, with magnesium being the highest quantity and iron found in average amounts. One major macronutrient that is necessary for human health is magnesium (Mg), which is involved in several physiological functions (Rukat et al., 2024). Studies show that

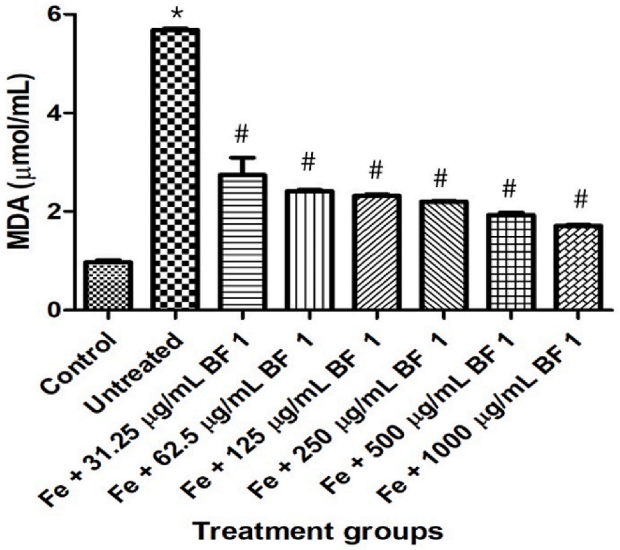

Fig. 6. Effect of BF1 on MDA levels in oxidative pancreatic injury mediated by iron.
A mean ± SD (n = 3) is used to represent data. *Statistically significant compared to control, while # statistically significant compared to the untreated group at P < 0.05.

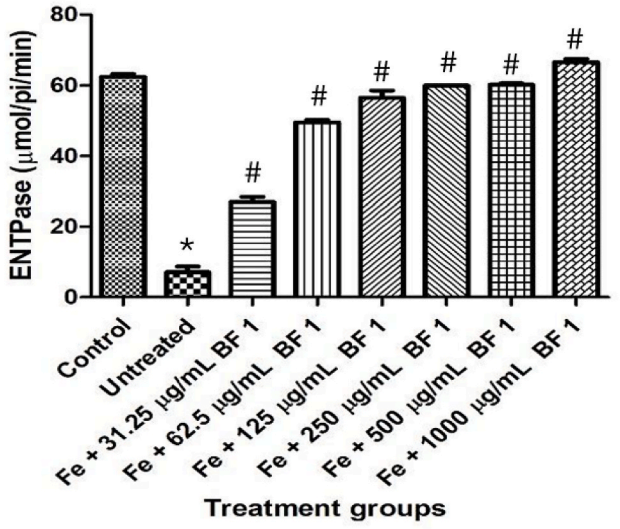

Fig. 7. Effect of BF1 in non-mediated oxidative pancreatic injury on ENTPase activity.
A mean ± SD (n = 3) is used to represent data. *Statistically significant compared to control, while # statistically significant compared to the untreated group at P < 0.05.

people with diabetes, particularly those with type 2 diabetes mellitus, frequently have magnesium deficiencies (Fatima et al., 2024). That is, lower levels of magnesium correspond to increased insulin resistance (Humphries et al., 1999). Magnesium increases glucose uptake by cells by increasing GLUT-4 levels; this, in turn aids blood glucose regulation (Fapohunda and Balogun, 2019). Magnesium interacts with insulin receptors to function as a cofactor for multiple enzymes that regulate insulin synthesis and activity in target tissues (such as insulin receptor tyrosine kinase) (Pelczyńska et al., 2022; Nik et al., 2023).

Certain disorders can be prevented and treated with the aid of phytochemicals with antioxidant activity, such as flavonoids, carotenoids, and phenolic compounds. Plants' ability to act as antioxidants or antidiabetic agents is directly influenced by the concentration and type of

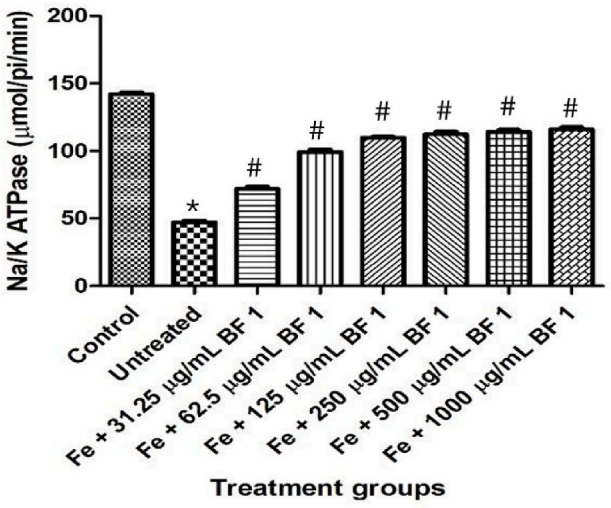

Fig. 8. Effect of BF1 in iron-mediated oxidative pancreatic injury on Na/K ATPase activity
A mean ± SD (n = 3) is used to represent data. *Statistically significant compared to control, while # statistically significant compared to the untreated group at P < 0.05.

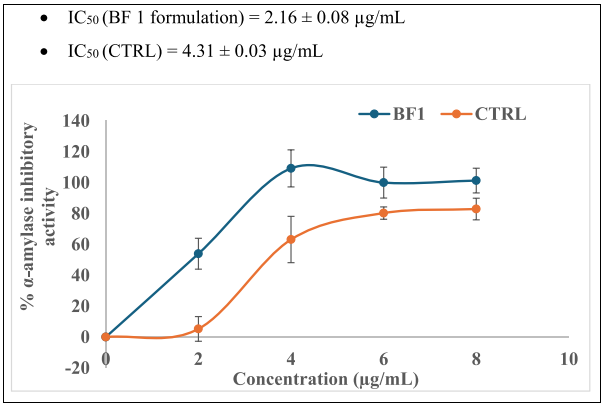

Fig. 9. BF1 formulation's percentage of α-amylase inhibitory activity
CTRL: Control (Metformin); Mean ± SD (n = 3) is used to represent data.

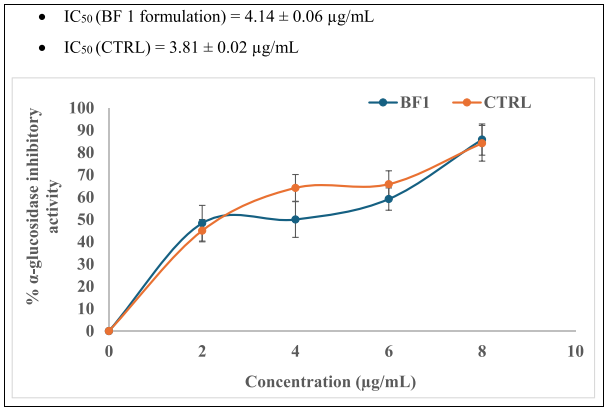

Fig. 10. Percentage of BF1 formulation's α-glucosidase inhibitory activity.
CTRL: control (metformin); Mean ± SD (n = 3) is used to describe the data.

phytochemicals they contain (Ogunlakin et al., 2023a; Wang et al., 2023; Ogunlakin et al., 2024b). The qualitative phytochemical screening results of BF1 formulation show a strong presence of alkaloids and

terpenoids. The quantitative phytochemical screening showed a high quantity of saponins. Alkaloids and flavonoids effectively contribute to hyperglycemia by promoting the synthesis of glycogen and the use of glucose. Their intricate process involves modifying the actions of various enzymes that are either directly or indirectly involved in the metabolism of carbohydrates to regulate sugar levels in the blood (Behl et al., 2022; Feng et al., 2024; Maache et al., 2024).

4.2. Antioxidant activity

Oxidative stress is another element that contributes to diabetes complications. Oxidative stress results from an imbalance between the development and buildup of reactive oxygen species (ROS) and the biological system's potential to conduct detoxification of these reactive products in cells and tissues (Afzal et al., 2023; Singh et al., 2019). Antioxidants usually help with this balance by scavenging the amount of ROS and free radicals in the body, in turn aiding the immune system (Muscolo et al., 2024). Antioxidants are sometimes unable to regulate the excessive buildup of reactive oxygen species (ROS) and free scavenging radicals (Morshed et al., 2023). Numerous health problems, such as diabetes, cancer, Alzheimer's disease, and problems with the metabolism and cardiovascular system have all been connected to this. Membrane lipids, DNA, proteins, cells, organs, and tissues can be severely damaged because of oxidative stress. Therefore, a few diseases, which include diabetes, cancer, and other metabolic disorders can be treated by reducing oxidative stress (Hajam et al., 2022; Akbari et al., 2022; Gatou et al., 2023). To measure how well antioxidants scavenge free radicals by collecting electrons and hydrogen radicals from antioxidant molecules, DPPH, a stable free radical, is used (Adedayo et al., 2015; Ogunlakin et al., 2023b). The highest percentage of DPPH scavenging activity of the BF 1 formulation methanol extract demonstrated a dose-dependent antioxidant potential when compared to the standard (quercetin). Iron's chelating qualities lower the concentration of the transition metal that catalyzes lipid peroxidation, which results in its contribution to lipid peroxidation through the Fenton reaction (Ademiluyi et al., 2013; Barbouti et al., 2021; Ojo et al., 2023). The concentration-dependent iron chelating activity observed in the methanolic extract of BF1 formulation suggests that the extract's capacity to chelate Fe^{2+} is dose-dependent. The flavonoid, saponin, terpenoids, phenol, alkaloids, and anthraquinone, composition of the extract might be responsible for its capacity to chelate ions and scavenge radicals (Sahoo et al., 2024). Therefore, BF 1 formulation is an effective free radical scavenger.

Nitric oxide (NO) synthesis and diabetes mellitus are linked (Suresh and Reddy, 2021). The methanolic extract of BF 1 formulation had higher nitric oxide inhibitory efficiency in comparison to quercetin, the standard. This suggests that BF 1 formulation is an excellent nitric oxide scavenger. Iron exposure in its ferrous form (Fe^{2+}) has been associated with a sustained drop in GSH and CAT levels and thus poses a risk to the pancreas (Ferreyra et al., 2024; Barbouti et al., 2021). The enzyme catalase (CAT) hydrolyses the toxic ROS hydrogen peroxide (H_2O_2) converting it into oxygen and water. It is essential for shielding the cells from oxidative damage (Rigoletto et al., 2024). In addition to scavenging reactive oxygen species, glutathione (GSH) aids in the regeneration of other antioxidants (Aquilano et al., 2014). Since the negative control had lower CAT and GSH levels, the investigation's findings indicate that OS had increased in the pancreas. The higher MDA levels in the negative control group relative to the positive control may be an indication of lipid peroxidation in the pancreatic tissue (Ogunlakin et al., 2024c). As the BF 1 formulation methanol extract concentration increases, the MDA level in the treated groups decreases, strongly indicating the formulation's beneficial effects on pancreatic tissue, since OS has a negative influence on glucose metabolism.

4.3. Purinergic enzyme activity and antidiabetic activity

The untreated group's ATPase activity was higher than the control and other treatment groups. The attack that reduced ATP levels was partially due to pancreatic tissue's acid/base composition (Zhou et al., 2023). The optimal pH environment, which is required for pancreatic functions, is produced by the ATPases present on the membrane surface (Petersen et al., 2021). Elevated ATPase levels have been shown to enhance invasion and motility in diabetics (Son et al., 2019; Al-Bataineh et al., 2016). The findings obtained demonstrate that the pancreas generated more ATP following treatment with the BF 1 formulation, which may have resulted from enhanced glucose metabolism in the pancreatic tissues (ShamsEldeen et al., 2022; Li et al., 2022). The BF 1 formulation's ability to prevent diabetes is based on its ability to inhibit two enzymes that break down carbohydrate bonds. They are α-amylase, which breaks down carbohydrates into simpler sugars by acting on the α-1,4 glycosidic link, and α-glucosidase, which breaks down simpler sugars into glucose (Liu et al., 2021). When these enzymes are inhibited, the conversion of starch to glucose (α-amylase) is slowed down, which prevents blood sugar increasing after meals (postprandial hyperglycemia) (Abdulkareem et al., 2024). BF 1 formulation's methanolic extract demonstrated the potential to inhibit both α-amylase and α-glucosidase compared to the standard, metformin. Therefore, advanced methods, such as NIRS for rapid screening, inductively coupled plasma mass spectrometry (ICP-MS) for minerals, and isothermal titration calorimetry (ITC), which provide a more detailed understanding of the binding kinetics and thermodynamics between the BF 1 herbal formulation's bioactive compounds and the target enzymes, are hereby recommended.

5. Conclusion

The methanol extract of BF 1 showed notable antioxidant activity. The results also showed that the BF 1 formulation contains a range of phytochemicals in moderate to high concentrations that have been proven to have antidiabetic qualities. The nutritional assessment of the BF 1 formulation shows that it contains some essential macro and micro minerals especially magnesium, which has the highest quantity and has been proven to have an antidiabetic effect. The formulation BF1 exhibited a good α-glucosidase and α-amylase inhibitory capacity and antioxidant properties. This suggests that BF1 can be considered as a potential antidiabetic treatment and can also be used as a food supplement.

CRediT authorship contribution statement

Akingbolabo Daniel Ogunlakin: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ruth Omokhafe Elagauma:** Visualization, Validation, Software, Methodology, Investigation. **Ayobami Tosin Adegbenro:** Methodology, Investigation, Funding acquisition. **Favour Inijesunimi Olagookun:** Methodology, Investigation, Funding acquisition. **Sophie Adedamola Adeyeye:** Methodology, Investigation, Funding acquisition. **Oluwafemi Adeleke Ojo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation. **Oyindamola Esther Awosola:** Methodology, Investigation, Funding acquisition. **Oluwaseun Abigael Ogunlakin:** Methodology, Investigation. **Blessing Obianuju Ezea:** Methodology, Investigation, Funding acquisition. **Victoria Seseyon Paul-Adio:** Methodology, Investigation, Funding acquisition. **Mubo Adeola Sonibare:** Validation, Project administration, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdulkareem, M.A., Owolabi, B.A., Saheed, E.S., Aromolaran, R.F., Bashiru, R.M., Jumah, T.A., Chijioke, D.U., Amaechi, O.J., Adeleke, F.C., Charles, O.O., Oluokun, T. S., 2024. Genetic factors and the role of pancreatic amylase in the pathogenesis of type 2 diabetes. Egyptian Journal of Medical Human Genetics 25 (1), 33. Mar 13.

Adedayo, B.C., Oyeleye, S.I., Ejakpovi, I.I., et al., 2015. Effects of hot water treatment on the radicals scavenging, lipid peroxidation, and α-amylase and α-glucosidase inhibitory abilities of Crassocephalum crepidioides leaves. Nutrafoods 14, 217–225.

Ademiluyi, A.O., Oboh, G., 2013. Aqueous extracts of Roselle (Hibiscus sabdariffa Linn.) varieties inhibit α-amylase and α-glucosidase activities *in vitro*. J. Med. Food 16 (1), 88–93.

Afzal, S., Abdul Manap, A.S., Attiq, A., Albokhadaim, I., Kandeel, M., Alhojaily, S.M., 2023. From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Front. Pharmacol. 14, 1269581. Oct 18.

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., Mahdavi Abhari, F., 2022. The role of plant-derived natural antioxidants in reduction of oxidative stress. Biofactors 48 (3), 611–633. May.

Al-Bataineh, M.M., Alzamora, R., Ohmi, K., Ho, P.Y., Marciszyn, A.L., Gong, F., Li, H., Hallows, K.R., Pastor-Soler, N.M., 2016. Aurora kinase A activates the vacuolar H+-ATPase (V-ATPase) in kidney carcinoma cells. Am. J. Physiol. Ren. Physiol. 310 (11), F1216–F1228. Jun 1.

Al-Suhaimi, E.A., Aljfary, M.A., Khan, F.A., 2022. Endocrine Pancreas and Glucose Metabolism. In: InEmerging Concepts in Endocrine Structure and Functions. Springer Nature Singapore, Singapore, pp. 247–285. Apr 21.

Amanat, M., Lal, K., Singh, T.G., Singh, R., 2025. Molecular insights of diabetic nephropathy and chemical constituents-based treatment approach. Phytochem. Rev. 1–44. Jan 27.

Amdie, F.Z., Luctkar-Flude, M., Snelgrove-Clarke, E., Sawhney, M., Alemu, S., Woo, K., 2022. Feasibility of virtual simulation-based diabetes foot care education in patients with diabetes in Ethiopia: protocol for a randomized controlled trial. Diabetes, Metab. Syndrome Obes. Targets Ther. 995–1009. Jan 1.

Aquilano, K., Baldelli, S., Ciriolo, M.R., 2014. Glutathione: new roles in redox signaling for an old antioxidant. Front. Pharmacol. 5, 196. Aug 26.

Banu, K.S., Cathrine, L., 2015. General techniques involved in phytochemical analysis. Int. J. Adv. Res. Comput. Sci. 2 (4), 25–32. Apr.

Barbouti, A., Lagopati, N., Veroutis, D., Goulas, V., Evangelou, K., Kanavaros, P., Gorgoulis, V.G., Galaris, D., 2021. Implication of dietary iron-chelating bioactive compounds in molecular mechanisms of oxidative stress-induced cell ageing. Antioxidants 10 (3), 491. Mar 21.

Behl, T., Gupta, A., Albratty, M., Najmi, A., Meraya, A.M., Alhazmi, H.A., Anwer, M.K., Bhatia, S., Bungau, S.G., 2022. Alkaloidal phytoconstituents for diabetes management: exploring the unrevealed potential. Molecules (Basel) 27 (18), 5851. Sep. 9.

Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., Omelka, R., 2021. Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. Pharmaceuticals 14 (8), 806. Aug 17.

Dej-Adisai, S., Sakulkeo, O., Wattanapiromsakul, C., Pitakbut, T., 2022. Flavonoid constituents and alpha-glucosidase inhibition of Solanum stramonifolium Jacq. inflorescence with *in vitro* and in silico studies. Molecules (Basel) 27 (23), 8189. Nov 24.

Faboro, E.O., Ogunlakin, A.D., Ayeni, P.O., Opaleye, O.D., Ojo, O.A., Ajayi-Odoko, O.A., Ayeleso, A.O., Ayokunle, D.I., Sonibare, M.A., Alanzi, A.R., 2024. Kigelia africana (Lam.) Benth. fruit inhibits iron-induced lipid peroxidation and?-amylase enzyme activity. Plant Science Today 11 (2), 496–502.

Fadogba, O.A., Ogunlakin, A.D., Ajayi, A.M., Sonibare, M.A., 2024. Antioxidant and anti-arthritic activity of Bombax buonopozense P. Beauv. leaves. InAnnales Pharmaceutiques Françaises 82 (4), 673–684. Jun 1; Elsevier Masson.

Fapohunda, O., Balogun, O., 2019. Oral magnesium supplementation modulates hepatic and intestinal expression of some carbohydrate metabolizing genes in type 2 diabetic rats. Int J Mol Biol Open Access 4 (6), 189–194.

Fatima, G., Dzupina, A., Alhmadi, H.B., Magomedova, A., Siddiqui, Z., Mehdi, A., Hadi, N., Mehdi, A., 2024. Magnesium matters: a comprehensive review of its vital role in health and diseases. Cureus 16 (10). Oct 13.

Feng, Y., Ren, Y., Zhang, X., Yang, S., Jiao, Q., Li, Q., Jiang, W., 2024. Metabolites of traditional Chinese medicine targeting PI3K/AKT signaling pathway for hypoglycemic effect in type 2 diabetes. Front. Pharmacol. 15, 1373711. May 10.

Ferreyra, M.R., Romero, V.L., Fernandez-Hubeid, L.E., Gonzales-Moreno, C., Aschner, M., Virgolini, M.B., 2024. Ferrostatin-1 mitigates cellular damage in a ferroptosis-like environment in Caenorhabditis elegans. Toxicol. Sci., kfae066. May 16.

Gatou, M.A., Vagena, I.A., Lagopati, N., Pippa, N., Gazouli, M., Pavlatou, E.A., 2023. Functional MOF-based materials for environmental and biomedical applications: a critical review. Nanomaterials (Basel) 13 (15), 2224. Jul 31.

Gong, T., Wang, D., Wang, J., Huang, Q., Zhang, H., Liu, C., Liu, X., Ye, H., 2025. Study on the mechanism of plant metabolites to intervene oxidative stress in diabetic retinopathy. Front. Pharmacol. 16, 1517964. Feb 5.

Haenen, G.R., Bast, A., 1999. [50] Nitric oxide radical scavenging of flavonoids. Methods Enzymol. 301, 490–503. Jan 1.

Hajam, Y.A., Rani, R., Ganie, S.Y., Sheikh, T.A., Javaid, D., Qadri, S.S., Pramodh, S., Alsulimani, A., Alkhanani, M.F., Harakeh, S., Hussain, A., 2022. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. Cells 11 (3), 552. Feb 5.

Howarth, F.C., Smail, M.M., Qureshi, M.A., Shmygol, A., Singh, J., Al, Kury L., 2019. Contraction and intracellular calcium transport in epicardial and endocardial ventricular myocytes from streptozotocin-induced diabetic rat. Hamdan Medical Journal 12 (3), 111–118. Jul 1.

Humphries, S., Kushner, H., Falkner, B., 1999. Low dietary magnesium is associated with insulin resistance in a sample of young, nondiabetic Black Americans. Am. J. Hypertens. 12 (8), 747–756. Aug 1.

Li, J., Yan, H., Xiang, R., Yang, W., Ye, J., Yin, R., Yang, J., Chi, Y., 2022. ATP Secretion and metabolism in regulating pancreatic beta cell functions and hepatic glycolipid metabolism. Front. Physiol. 13, 918042. Jun 21.

Liu, Y., Zhu, J., Yu, J., Chen, X., Zhang, S., Cai, Y., Li, L., 2021. A new functionality study of vanillin as the inhibitor for α-glucosidase and its inhibition kinetic mechanism. Food Chem. 353, 129448. Aug 15.

Maache, S., Laaroussi, H., Soulo, N., Nouioura, G., Boucetta, N., Bouslamti, M., Saghrouchni, H., A Bin Jardan, Y., Ibenmoussa, S., Bourhia, M., Lyoussi, B., Elarabi, I., 2024. The antioxidant, antidiabetic, and antihyperlipidemic effects of the polyphenolic extract from Salvia blancoana subsp. mesatlantica on induced diabetes in rats. Bioresour Bioprocess 11 (1), 62. Jun 26.

Marella, S., Tollamadugu, N.V., 2018. Nanotechnological approaches for the development of herbal drugs in treatment of diabetes mellitus–a critical review. IET Nanobiotechnol. 12 (5), 549–556. Aug.

Mikhail, N., Wali, S., 2024. Semaglutide for treatment of obesity-related heart failure with preserved ejection fraction in patients with and without diabetes. Journal of Diabetes and Clinical Research 6 (1), 18–23.

Morshed, M.N., Ahn, J.C., Mathiyalagan, R., Rupa, E.J., Akter, R., Karim, M.R., Jung, D. H., Yang, D.U., Yang, D.C., Jung, S.K., 2023. Antioxidant activity of Panax ginseng to regulate ROS in various chronic diseases. Appl. Sci. 13 (5), 2893. Feb 23.

Muscolo, A., Mariateresa, O., Giulio, T., Mariateresa, R., 2024. Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases. Int. J. Mol. Sci. 25 (6), 3264. Mar 13.

Nik, W.N., Zulkeflee, H.A., Ab Rahim, S.N., Ismail, T.S., 2023. Association of vitamin D and magnesium with insulin sensitivity and their influence on glycemic control. World J. Diabetes 14 (1), 26. Jan 1.

Ogunlakin, A.D., Onifade, T.R., Ojo, O.A., Adesanya, E.O., Berena, G.A., Ayeni, P.O., Omolekan, T.O., Ogunlakin, M.A., Iyinkristi, D.A., Sonibare, M.A., Fategbe, M.A., 2023a. Antidiabetic potential of Carica papaya L. and its constituents: from folkloric uses to products development. Bioactive Compounds in Health and Disease-Online 6 (6), 126–144. ISSN: 2574-0334; Print ISSN: 2769-2426.Jun 30.

Ogunlakin, A.D., Ojo, O.A., Gyebi, G.A., Adebodun, G.O., Elbasyouni, A., Adebodun, S. A., Ogunlakin, B., Olanrewaju, A.A., Sonibare, M.A., 2024b. Effect of Triclisia subcordata Oliv.(Menispermaceae) leaves on hormonal imbalance and genes expression in the ovaries of letrozole-induced polycystic rats via in vivo and computational approaches. J. Mol. Struct. 1318, 139275.

Ogunlakin, A.D., Ojo, O.A., Gyebi, G.A., Akinwumi, I.A., Adebodun, G.O., Ayokunle, D.I., Ambali, O.A., Ayeni, P.O., Awosola, O.E., Babatunde, D.E., Akintunde, E.A., 2023b. Elemental evaluation, nutritional analysis, GC-MS analysis and ameliorative effects of Artocarpus communis JR Forst. & G. Forst. seeds' phytoconstituents on metabolic syndrome via in silico approach. J. Biomol. Struct. Dyn. 1–21.

Ogunlakin, A.D., Adetunji, J.B., Iyobhebhe, M., Ajiboye, T.A., Gyebi, G.A., Ayeni, P.O., Ayokunle, D.I., Sonibare, M.A., Onoja, J.O., Adesanya, E.O., Ajayi-Odoko, O.A., 2024a. 3-(4-methoxyphenyl) acrylic acid halts redox imbalance and modulate purinergic enzyme activity in iron-induced testicular injury. Pure Appl. Chem. (0) Mar 25.

Ogunlakin, A.D., Ojo, O.A., Iyobhebhe, M., Ajisafe, T.L., Adeoye, E.O., Ayokunle, D.I., Sonibare, M.A., Ambali, O.A., Adebodun, G.O., Ajayi-Odoko, O.A., Adetunji, T.L., Oguntibeju, O.O., 2024c. Sodium 3-phenylpropanoate alleviate oxidative stress and iron-induced testicular toxicity in Wistar rats. J. Appl. Pharmaceut. Sci. 14 (3), 88–94.

Ojo, O.A., Ogunlakin, A.D., Maimako, R.F., Gyebi, G.A., Olowosoke, C.B., Taiwo, O.A., Elebiyo, T.C., Adeniyi, D., David, B., Iyobhebhe, M., Adetunji, J.B., Ayokunle, D.I., Ojo, A.B., Mothana, R.A., Alanzi, A.R., 2023. Therapeutic study of cinnamic acid derivative for oxidative stress ablation: the computational and experimental answers. Molecules (Basel) 28 (21), 7425. Nov 4.

Ojo, O.A., Gyebi, G.A., Ezenabor, E.H., Iyobhebhe, M., Emmanuel, D.A., Adelowo, O.A., Olujinmi, F.E., Ogunwale, T.E., Babatunde, D.E., Ogunlakin, A.D., Ojo, A.B., 2024. Exploring beetroot (Beta vulgaris L.) for diabetes mellitus and Alzheimer's disease dual therapy: *in vitro* and computational studies. RSC Adv. 14 (27), 19362–19380.

Olaniyi, K.S., Atuma, C.L., Sabinari, I.W., Hadiza, M., Saidi, A.O., Akintayo, C.O., Ajadi, I. O., Olatunji, L.A., 2022. Restoration of cardiac metabolic flexibility by acetate in high-fat diet-induced obesity is independent of ANP/BNP modulation. Can. J. Physiol. Pharmacol. 100 (6), 509–520. Jun 1.

Otegenova, A., Kazbekova, A., Kulzhanov, M., Akanov, Z., 2024. Navigating the global challenge of diabetes mellitus: insights from Kazakhstan's healthcare landscape and strategies for improved management. Interdisciplinary Approaches to Medicine 5 (1), 30–40. Jun 20.

Pei, X., Li, Z., 2025. Narrative review of comprehensive management strategies for diabetic retinopathy: interdisciplinary approaches and future perspectives. BMJ Public Health 3 (1). Jan 16.

Pelczyńska, M., Moszak, M., Bogdański, P., 2022. The role of magnesium in the pathogenesis of metabolic disorders. Nutrients 14 (9), 1714. Apr 20.

Petersen, O.H., Gerasimenko, J.V., Gerasimenko, O.V., Gryshchenko, O., Peng, S., 2021. The roles of calcium and ATP in the physiology and pathology of the exocrine pancreas. Physiol. Rev. 101 (4), 1691–1744.
Rachdaoui, N., 2020. Insulin: the friend and the foe in the development of type 2 diabetes mellitus. Int. J. Mol. Sci. 21 (5), 1770. Mar 5.
Rana, N., Walia, A., Gaur, A., 2013. α-Amylases from microbial sources and its potential applications in various industries. Natl. Acad. Sci. Lett. 36, 9–17. Feb.
Reza, M.S., Amin, R., Yasmin, R., Kulsum, W., Ruhi, S., 2024. Improving diabetes disease patients classification using stacking ensemble method with PIMA and local healthcare data. Heliyon 10 (2), e24536. Jan 30.
Rigoletto, M., Laurenti, E., Tummino, M.L., 2024. An overview of environmental catalysis mediated by hydrogen peroxide. Catalysts 14 (4), 267. Apr 17.
Rukat, M., Przyborowska, K., Kwiecień, J., Getka, B., Wiejak, K., Łata, M., 2024. The relationship between magnesium deficiency and anxiety, the therapeutic effects of magnesium supplementation–literature review. Journal of Education, Health and Sport 53, 91–101. Jan 17.
Sahoo, C.R., Bishoyi, A.K., Paidesetty, S.K., Dehury, B., Kaneria, M., Padhy, R.N., 2024. Chemical Constituents from a Selected Plant with Antioxidant Activity. In: InHerbal Formulations, Phytochemistry and Pharmacognosy. Elsevier, pp. 271–280. Jan 1.
ShamsEldeen, A.M., El-Aal, S.A.A., Aboulhoda, B.E., AbdAllah, H., Gamal, S.M., Hassan, F.E., Mehesen, M.N., Rashed, L.A., Mostafa, A., Sadek, N.B., 2022. Combined systemic intake of K-ATP opener (Nicorandil) and mesenchymal stem cells preconditioned with nicorandil alleviates pancreatic insufficiency in a model of bilateral renal ischemia/reperfusion injury. Front. Physiol. 13, 934597.
Singh, A., Kukreti, R., Saso, L., Kukreti, S., 2019. Oxidative stress: a key modulator in neurodegenerative diseases. Molecules (Basel) 24 (8), 1583. Apr 22.
Sobuj, M.K., Shemul, M.S., Islam, M.S., Islam, M.A., Mely, S.S., Ayon, M.H., Pranto, S.M., Alam, M.S., Bhuiyan, M.S., Rafiquzzaman, S.M., 2024. Qualitative and quantitative phytochemical analysis of brown seaweed Sargassum polycystum collected from Bangladesh with its antioxidant activity determination. Food Chemistry Advances 4, 100565. Jun 1.
Son, S.W., Chau, G.C., Kim, S.T., Um, S.H., 2019. Vacuolar H+-ATPase subunit V0C regulates aerobic glycolysis of esophageal cancer cells via PKM2 signaling. Cells 8 (10), 1137. Sep. 24.
Sonibare, M.A., Onifade, T.R., Ogunlakin, A.D., Akinmurele, O.J., Adebodun, S.A., 2022. Microscopic evaluation and antioxidant activity of glyphaea brevis (spreng.) monach.(family tiliaceae). Free Radic. Antioxid. 12 (1), 27–32. Aug 2.
Suresh, V., Reddy, A., 2021. Dysregulation of nitric oxide synthases during early and late pathophysiological conditions of diabetes mellitus leads to amassing of microvascular impedement. J. Diabetes Metab. Disord. 20, 989–1002. Jun.
Wake, A.D., 2020. Antidiabetic effects of physical activity: how it helps to control type 2 diabetes. Diabetes, Metabolic Syndrome and Obesity 2909–2923. Aug 19.
Wang, W., Li, H., Lv, J., Khan, G.J., Duan, H., Zhu, J., Bao, N., Zhai, K., Xue, Z., 2023. Determination of the anti-oxidative stress mechanism of isodon suzhouensis leaves by employing bioinformatic and novel research technology. ACS Omega 8 (3), 3520–3529. Jan 10.
Wolosowicz, M., Prokopiuk, S., Kaminski, T.W., 2022. Recent advances in the treatment of insulin resistance targeting molecular and metabolic pathways: fighting a losing battle? Medicina 58 (4), 472. Mar 25.
Zerihun, E., Abera, F., Kune, G., Girma, F., Tesgera, M., Robi, M., 2024. Undiagnosed status and associated factors of diabetes mellitus among adults living in eastern Ethiopia: unmasking a silent killer of prevalence of diabetes mellitus. Clinical Epidemiology and Global Health 25, 101483. Jan 1.
Zhou, Y., Chang, W., Lu, X., Wang, J., Zhang, C., Xu, Y., 2023. Acid–base homeostasis and implications to the phenotypic behaviors of cancer. Genom. Proteom. Bioinform. 21 (6), 1133–1148. Dec.