



Preliminary evaluation of the antidiabetic and antioxidant potentials of a herbal formulation

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Abstract

Diabetes mellitus is a disease that characterised by elevated blood glucose. The adverse effects of currently available medications call for a search for viable alternatives especially from medicinal plants. This study investigated the antioxidant and anti-diabetic activity of a herbal formulation, MHF containing *Momordica charantia* and *Hunteria umbellata* dried leaves. Phytochemical screening of the extract was done to determine the phytochemicals in the product. Quantification of the total flavonoids (TF) and total phenolics (TP) were done using ultraviolet spectrometry. Assessment of antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) inhibition, total antioxidant capacity (TAC) and ferric reducing antioxidant capacity (FRAP) assays was done. Alpha-amylase and alpha-glucosidase inhibitory activity were investigated to determine the *in vitro* anti-diabetic potential. Reducing sugars, cardiac glycosides, triterpenoids, steroids, tannins, phenolic compounds and flavonoids were present in the product with total phenolics of 25.36 ± 0.19 mg/g gallic acid equivalent (GAE) and total flavonoids of 77.51 ± 0.51 mg/g rutin equivalent (RE). The product had peak DPPH inhibition of 91.91 ± 0.11 %, TAC of 35.52 ± 0.32 mg/g Ascorbic acid equivalent and FRAP of 15.93 ± 0.29 μ M Fe²⁺/ml. The product had significant alpha-amylase and alpha-glucosidase activity 90.60 ± 0.21 % and 91.72 ± 0.20 % respectively. The product had good antioxidant and anti-diabetic activities.

Keywords: Herbal formulation, Alpha-amylase, alpha-glucosidase, antioxidant, anti-diabetic

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Introduction

Diabetes mellitus is a disease marked by persistently high blood glucose levels caused due to insufficient insulin synthesised by the pancreas or due to insulin resistance marked by unresponsive cells to insulin causing underutilisation of glucose (Fradkin *et al.*, 2013). Diabetes is classified as type 1, type 2 and gestational diabetes (Fradkin *et al.*, 2013). In type 1 diabetes, the β -cells of the pancreas are selectively destroyed leading to decrease of insulin blood levels causing insufficiency. It is marked by age-related differences causing a different perspective of management to be fashioned for each patient (Fradkin *et al.*, 2013). Type 1 diabetes is primarily seen in children and young adults and affected patients have to be on insulin everyday (Fradkin *et al.*, 2013). In type 2 diabetes, it is either that the body cells do not utilise it effectively or the insulin produced is insufficient in quantity to achieve optimum blood glucose levels. It is the most widespread form of diabetes and elements such as obesity, being overweight and a family history of diabetes are predisposing factors (Fradkin *et al.*, 2013). Type 2 form of diabetes can occur at any age even childhood. The occurrence of this disease can be hampered if steps are taken to reduce the risk factors (Sharma *et al.*, 2025).

Gestational diabetes results due to pregnancy and may stop after birth. People who have had gestational diabetes have a higher risk of developing type 2 diabetes in their lifetime while most diabetes diagnosed in pregnancy is type 2 (Sharma *et al.*, 2025). Patients that are classified as prediabetes have blood sugar that is high but not sufficient enough to be classified as type 2. Monogenic diabetes occurs due to a single gene aberration. Diabetes can also come up due to a surgery to remove the pancreas or from some pancreatic injury caused by cystic fibrosis or pancreatitis. Patients diagnosed with diabetes may develop different health issues such as heart disease, nerve damage, sexual problems, kidney damage and sight problems. A successful management of diabetes involves incorporating lifestyle changes in conjunction with monitoring blood glucose frequently levels and medication or insulin therapy. With time, the patients may suffer time damage to the heart, kidneys, feet, and eyes. Symptoms of diabetes include polyuria, polydipsia, polyphagia, blurred vision, fatigue, chronic wounds, infections such as urinary tract infections, skin infections or yeast infections (Sharma *et al.*, 2025). Researchers have no little knowledge of what causes type 2 diabetes but an inevitability to likely to develop type 2 diabetes occurs in obesity or in patients age 35 or older. Children and teenagers develop type 2 diabetes with risk higher with age, individuals with family history of type 2 diabetes, Africans, sedentary lifestyle, smokers, history of gestational diabetes or in mothers who give birth to baby who weigh over 4 kg (Sharma *et al.*, 2025).

Alpha-amylase and alpha-glucosidase are important enzymes for digestion and metabolism of sugars but are multipurpose

(Shalini *et al.*, 2025). Alpha-amylase is an enzyme that is essentially charged with breaking down starch and glycogen into smaller units. It is present in plants, animals, and microorganisms (Shalini *et al.*, 2025). It is a chief enzyme in the alimentary system, found specifically in the mouth, liver and small intestine (Shalini *et al.*, 2025). In the mouth, salivary alpha-amylase starts the activity of starch breakdown while pancreatic alpha-amylase carries on with this in the small intestine (Shalini *et al.*, 2025). Alpha-amylase is abundant in living organisms. In the human digestive system, an alpha-amylase called ptyalin is manufactured by the salivary glands while pancreatic amylase is produced by the pancreas and secreted into the small intestine (Shalini *et al.*, 2025). The optimum pH range of alpha-amylase is neutral [6.7–7.0] (Shalini *et al.*, 2025). This enzyme activates the disintegration of α -1,4-glycosidic bonds in starch and glycogen, breaking them down into smaller polysaccharides, disaccharides, and oligosaccharides. Alpha-amylase is very important in human digestion and of therapeutic importance in treating certain gastrointestinal disorders which are involved in several industrial applications, such as the production of syrups, biofuels, and in the food industry (for example in the preparation of sweeteners and baking) (Shalini *et al.*, 2025). Over activity or underutilisation of alpha-amylase can cause many medical conditions such as pancreatic disorders or other problems (Shalini *et al.*, 2025).

Alpha-glucosidase inhibitors are abundant in many plants and herbs, and can be used to treat type 2 diabetes (Shalini *et al.*, 2025). They work by reducing the digestion of carbohydrates which control blood sugar levels. Alpha-glucosidase is found in the small intestine and specifically catalyses the hydrolysis of the terminal non-reducing α -D-glucosidic linkages in oligosaccharides and disaccharides, converting them into monosaccharides like glucose (Shalini *et al.*, 2025). This enzyme is important in the final stages of carbohydrate digestion because it converts oligosaccharides into glucose that are then absorbed into the bloodstream. Alpha-glucosidase has important implications in both human health and disease (Shalini *et al.*, 2025). In individuals with diabetes, alpha-glucosidase inhibitors such as acarbose, are frequently used to control blood glucose levels by retarding the absorption of glucose after meals (Shalini *et al.*, 2025). Inhibition of alpha-glucosidase activity can lead to dysfunctional glucose metabolism which is a important factor in diabetes management because it plays a role in some metabolic disorders and diseases related to malabsorption of carbohydrates. Studying these enzymes is important in comprehension of carbohydrate digestion and metabolism as well as their involvement in various diseases such as metabolic disorders, diabetes and obesity. Understanding the mechanisms by which these enzymes carry out their functions can lead to the development of new bioactive agents and biomolecules aimed at managing glucose metabolism which are important in managing type 2 diabetes and other

metabolic conditions. Additionally, these enzymes have practical applications in industries such as food processing, biofuel production, and pharmaceuticals (Febriyanti *et al.*, 2025). Summarily, the study of alpha-amylase and alpha-glucosidase focus on their biological roles in carbohydrate digestion, their significance in metabolic disorders as well as their applications in many industries. Researchers still explore these enzymes functions and mechanisms to develop effective drugs for diabetes and other related metabolic disorders (Shalini *et al.*, 2025).

Antioxidant activity of secondary metabolites activities refers to the processes by which antioxidants in plant neutralise toxic molecules known as free radicals. Free radicals are highly reactive, can damage cells, proteins, and DNA, leading to oxidative stress, which has been linked to various diseases, aging, and other health conditions such as cancer and other chronic disorders (Gulcin, 2025). Medicinal plants have antioxidant properties that may help protect against disease. Plants are a source of phytochemicals rich in antioxidants, important for normal plant functioning and adaptation to environmental cues and delivering helpful properties for human health. These properties are due to the presence of phytochemicals like polyphenols, tannins, and leucoanthocyanin and others such as vitamins and enzymes (Gulcin, 2025). Antioxidants neutralise free radicals by donating electrons, preventing them from causing cellular damage, regeneration of other antioxidants. Some antioxidants can regenerate other antioxidants that may have been used up in previous reactions prolonging their protective effects and chelation of metal ions. Antioxidants bind to metal ions (like iron or copper) that catalyse free radical formation thus reducing oxidative stress and inhibition of oxidative enzymes. Antioxidants also inhibit enzymes that produce free radicals like lipoxygenase and cyclooxygenase (Gulcin, 2025). Common antioxidants that are secondary metabolites include vitamin C, vitamin E, flavonoids, steroid, saponins and so on. These compounds are typically found in various fruits, vegetables, nuts, and seeds (Gulcin, 2025). Some herbal drugs can increase insulin sensitivity which can help with diabetes and stop the liver from producing glucose and can help with diabetes. Antioxidants protect cells and tissues from damage caused by oxidative stress, which occurs when free radicals (reactive oxygen species, ROS) accumulate in

the body also reducing oxidative damage and inflammation and improving insulin sensitivity. The mechanism of antioxidant activity is predominantly centered on their ability to neutralise or scavenge these free radicals.

Free Radical Scavenging: Free radicals are unstable molecules that have unpaired electrons, making them highly reactive. Antioxidants donate an electron to these free radicals, stabilizing them and preventing them from causing further cellular harm (Kaur *et al.*, 2022).

Some antioxidants act as enzymes to catalyse reactions that neutralise free radicals. Key examples include allium sulphur compounds from bulbs such as garlic and onions, anthocyanins from colour rich berries, beta-carotene from cucurbits, catechins from tea and copper from seafood, lean meat, and nuts. These enzymes inactivate free radicals to such as oxygen and water (Kaur *et al.*, 2022).

Certain antioxidants, such as vitamin C, help regenerate other antioxidants like vitamin E. After vitamin E neutralises a free radical, it can be regenerated by vitamin C, thereby prolonging the antioxidant defense (Kaur *et al.*, 2022).

Antioxidants reduce the production of pro-inflammatory cytokines, truncating the inflammatory process that can lead to oxidative damage (Kaur *et al.*, 2022).

This study aimed to investigate the antioxidant and anti-diabetic activity of a Nigerian herbal product with claims of hypoglycaemic activity in the management of type 2 Diabetes mellitus.

Materials and Methods

Plant Collection, Identification and Extraction

Plant was purchased from Mushin market, Lagos state, Nigeria and were identified and authenticated at the Lagos University Herbarium (LUH) domiciled at the Department of Botany, Faculty of Science, University of Lagos. They were assigned voucher numbers and herbarium specimens prepared and deposited (Table 1). The recipe is made of dried leaves *Momordica charantia* Linn. (Bitter melon) and *Hunteria umbellata* (K. Schum) Haillier f. combined in the ratio 1:2 (w/w) respectively.

Table 1: Plants used in the study

S/N	Botanical name	Family	Common name	Voucher number
1	<i>Momordica charantia</i> L.	Cucurbitaceae	Bitter gourd	LUH 100498
2	<i>Hunteria umbellata</i> (K. Schum) Hallier f.	Apocynaceae	Umbrella Hunteria	LUH 100586

Extraction

The recipe was macerated in 95 % ethanol and allowed to stand for 72 h with constant agitation. The extract was obtained after the macerate was filtered and concentrated (Oiseoghaede *et al.*, 2024, Oiseoghaede *et al.*, 2025).

Qualitative analysis

Phytochemical screening

The extract was tested for the presence of phytochemicals using standard methods (Harbone, 1973, Ayoola *et al.*, 2008).

Quantitative analysis

Estimation of phenolic content

Total phenolic content in the test extract was estimated using Folin-Ciocalteu's reagent (FCR). The experiment was carried based on the protocol established by Oiseoghaede *et al* (2018). Absorbances of the samples (1000 µg/mL) were measured against the blank at 765 nm using a UV-VIS spectrophotometer. A calibration plot of absorbance versus the prepared concentrations of Gallic acid was drawn and total phenolic content of the extracts extrapolated from the curve and expressed in terms of milligrams of gallic acid equivalent per gram of dry weight of extract.

Total flavonoid determination

Total flavonoids of MHF were determined utilizing aluminium chloride method (Oiseoghaede *et al.*, 2018). The absorbance was taken at 510 nm and the results were expressed as mg per gram of dried extract rutin equivalents.

Antioxidant assays

Determination of Antioxidant Activity Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

The ability of the MHF to reduce the absorbance of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical expressed as a violet colour in comparison with Ascorbic acid standard was the basis of this experiment. A slightly modified method employed by Oiseoghaede and Odukoya (2015) was used. The disappearance of violet colour of DPPH was indicative of antioxidant activity. The experiment was blanked with methanol (Oiseoghaede and Odukoya, 2015) and antioxidant activity was expressed as:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

Total antioxidant capacity (Phosphomolybdate determination of total antioxidant capacity)

The total antioxidant capacity of MHF was evaluated by the Phosphomolybdenum method as described by Prieto *et al* (2008). The absorbance was read at 765 nm by UV Spectrophotometer

and antioxidant activity was expressed in number of equivalents of ascorbic acid.

Determination of Antioxidant Activity Using the Ferric Reducing/Antioxidant Power (FRAP) Method

The ability of the MHF to reduce ferric ion (Fe³⁺) to ferrous ion (Fe²⁺) which forms a blue complex with the reagent (Fe²⁺/TPTZ [2,4,6-tri(2-pyridyl)-s-triazine]) resulting in absorbance increase at 593 nm is the basis of this assay. The assay was conducted following the method described by Benzie and Strain (2006) with minor modifications. The result was expressed as a milligram equivalent of Ferrous sulphate (FeSO₄) per dry weight of plant extract in grams (Benzie and Strain, 2006).

Anti-diabetic assays

Inhibition of alpha-amylase enzyme

The reaction was done using a protocol established by Al-Baidhani *et al* (2020). The same procedure was carried out for standard drug Acarbose and values compared with MHF. Percentage inhibition was calculated using the following formular:

$$\% \text{ Inhibition} = \frac{(\text{OD value of control} - \text{OD value of samples}) \times 100\%}{(\text{OD value of control})}$$

OD = Optical density

Inhibition of alpha-glucosidase enzyme

The method of Al-Baidhani *et al* (2020) was adopted to investigate the alpha-glucosidase enzyme inhibitory activity of MHF, using Acarbose as the standard. Percentage of inhibition was calculated using the following formular:

$$\% \text{ Inhibition} = \frac{(\text{OD value of control} - \text{OD value of samples}) \times 100\%}{(\text{OD value of control})}$$

OD = Optical density

Statistical analysis

Data were analysed using Microsoft Excel and One- and Two-way Analysis of variance (ANOVA) with multiple comparisons at 95% significance level as well as Dunnett post hoc analysis (GraphPad Prism 9) [San Diego, CA].

Results

Extract yield

The extract yield after maceration with 95 % ethanol was 9.65 %.

Phytochemical composition of the extract (MHF)

Table 2 shows the phytochemicals composition of the extract. The extract contained reducing sugars, cardiac glycosides, triterpenoids, tannins, steroids, phenolic compounds and

flavonoids with absence of alkaloids, saponins and anthraquinones.

Table 2: Phytochemicals composition of MHF

Test	Method	Presence
Alkaloids	Mayer's Test	-
	Dragendorff's Test	-
	Wagner's Test	-
Saponins	Frothing Test	-
Reducing Sugar	Fehling's Test	++
Cardiac glycosides	Killer Killani's Test	++
Triterpenoids	Liebermaan – Burchard	++
Steroids	Salkowki Test	++
Tannins	Ferric Chloride Test	+
Phenolic Compounds	Lead acetate Test	+++
Flavonoids	Shinoda's Test	+
Anthraquinone	Borntragers Test	-

Note: Heavily detected: + + +; Moderately detected: + +; Slightly detected: +; Not detected: -

Total phenolic and total flavonoid contents

The total phenolic and flavonoid contents of GHF were 25.36 ± 0.19 and 77.51 ± 0.51 mg/g of dry extract respectively. The total phenolic content was determined using the calibration plot (Figure 1), $y = 0.0093x - 0.0063$, $R^2 = 0.9994$ while total flavonoid content was determined using the calibration plot (Figure 2), $y = 0.0041x + 0.0494$, $R^2 = 0.9999$.

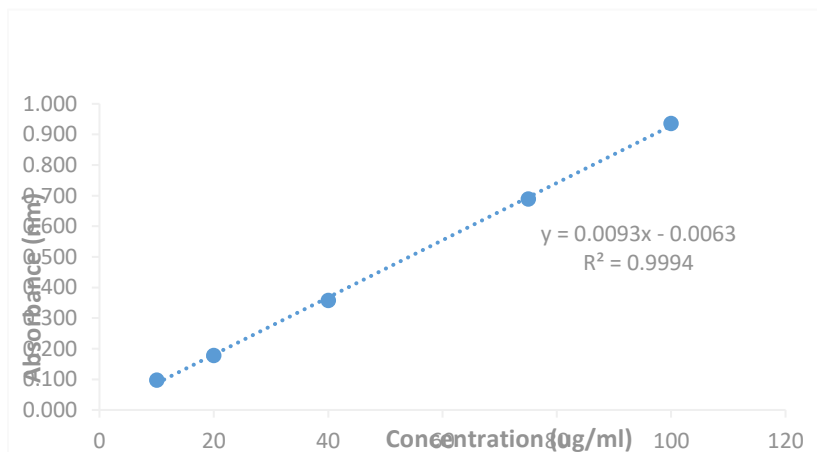


Figure 1: Calibration plot of Total phenolics (mg/g Gallic acid equivalent)

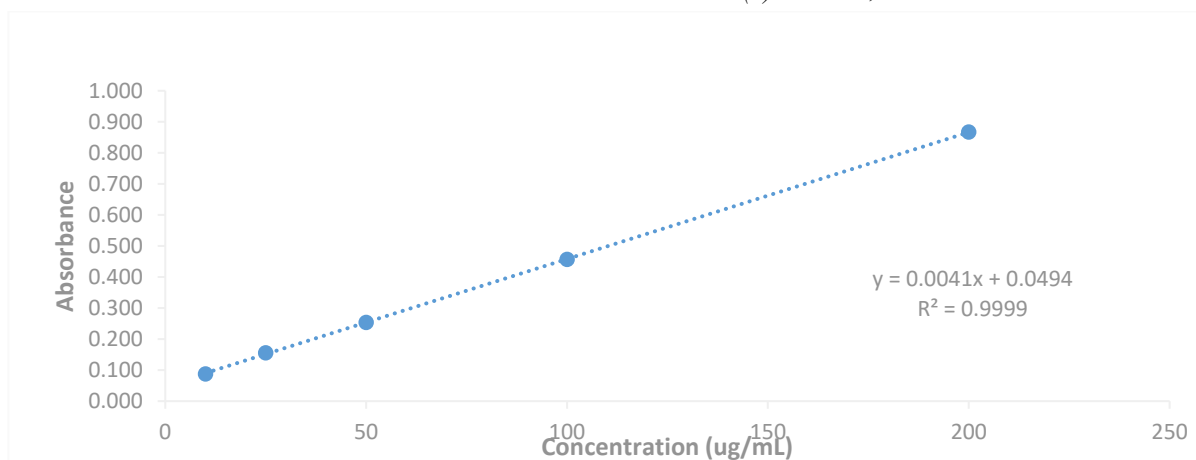


Figure 2: Calibration plot of Total flavonoids (mg/g Rutin equivalent)

Antioxidant assays

The extract had a peak DPPH inhibition of 91.91 ± 0.11 %, peak TAC of 35.52 ± 0.32 mg/g of dry extract ascorbic acid equivalent and peak FRAP of 15.93 ± 0.29 $\mu\text{M Fe}^{2+}/\text{ml}$ (Table 3). TAC was estimated using the calibration curve, $y = 0.0082x + 0.0055$, $R^2 = 0.998$ (Figure 3) while FRAP was estimated using the calibration curve, $y = 0.0073x + 0.1512$, $R^2 = 0.9812$ (Figure 4).

Table 3: Antioxidant activities of the formulation

Concentration ($\mu\text{g/ml}$)	10	25	50	100	200
DPPH inhibition					
MHF	22.51 ± 0.23 ****	37.90 ± 0.16 ****	53.86 ± 0.23 ****	67.15 ± 0.16 ****	91.91 ± 0.11^d
Ascorbic acid	66.55 ± 0.04	89.21 ± 0.06	91.84 ± 0.07	93.00 ± 0.04	93.18 ± 0.07
Total antioxidant capacity (mg/g Ascorbic acid equivalent)					
Concentration (mg/ml)					
MHF	35.52 ± 0.32 ****				
Quercetin	167.42 ± 6.48				
Ferric reducing antioxidant power ($\mu\text{M Fe}^{2+}/\text{ml}$ of extract)					
Concentration (mg/ml)					
MHF	15.93 ± 0.29 ****				
Quercetin	174.60 ± 1.33				

**** P < 0.0001 compared to Ascorbic acid and Quercetin

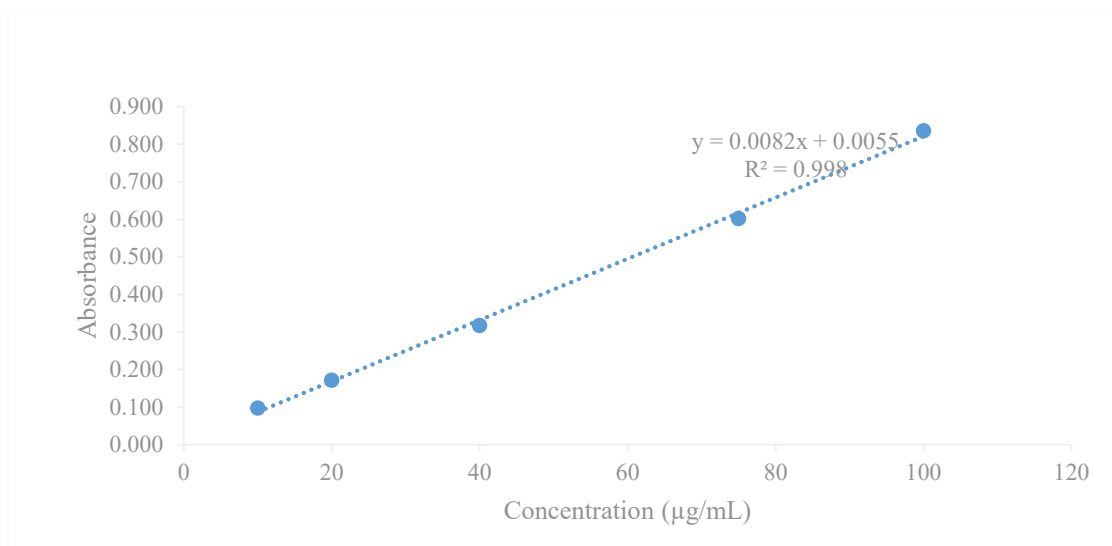


Figure 3: Calibration plot of Total antioxidant capacity (mg/g ascorbic acid equivalent)

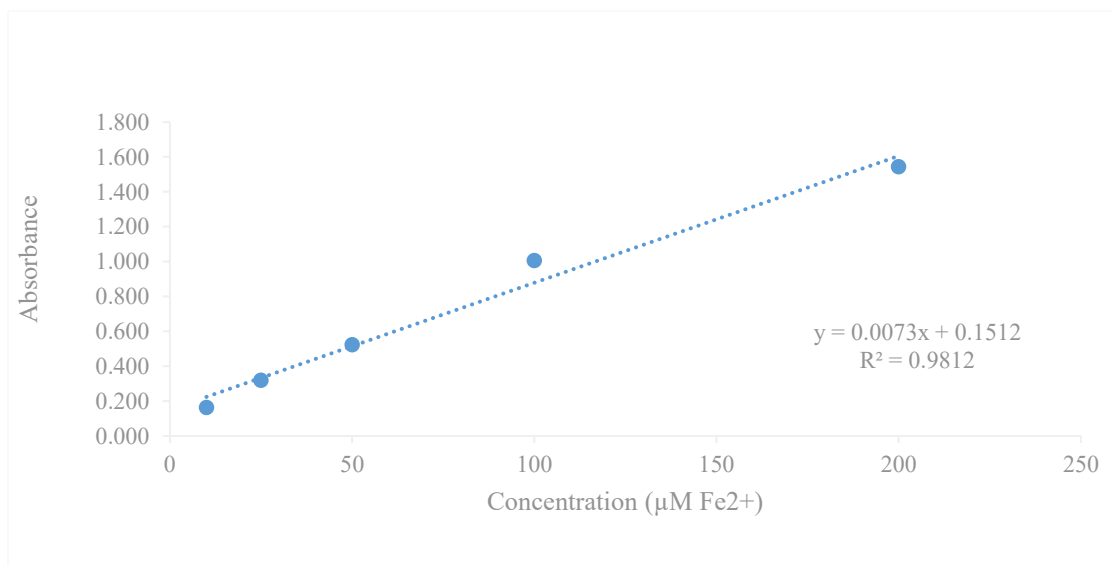


Figure 4: Calibration plot of Ferric reducing antioxidant power (µM Fe²⁺/ml of extract)

Antidiabetic assays

Alpha-amylase and Alpha-glucosidase inhibition

GHF had significant alpha-amylase and alpha-glucosidase inhibition with peak inhibition at 90.60 ± 0.21 % and 91.72 ± 0.20 % respectively (Table 4).

Table 4. *In vitro* antidiabetic activity of MHF

Concentration ($\mu\text{g/mL}$)	100	200	500	750	1000
Alpha-amylase inhibition MHF	49.84 \pm 0.60****	56.12 \pm 0.24****	73.66 \pm 0.17****	88.31 \pm 0.16**	90.60 \pm 0.21
Acarbose	74.68 \pm 0.17	88.31 \pm 0.04	91.39 \pm 0.08	91.94 \pm 0.00	92.06 \pm 0.12
Alpha-glucosidase inhibition MHF	45.52 \pm 0.23****	57.79 \pm 1.80****	76.82 \pm 0.23****	88.45 \pm 0.15	91.72 \pm 0.20
Acarbose	77.93 \pm 0.08	85.83 \pm 0.14	89.40 \pm 0.11	90.88 \pm 0.07	91.41 \pm 0.10

** P < 0.01, **** P < 0.0001 compared to Ascorbic acid

Discussion

The findings from this study differ from previous studies which reported that *Hunteria umbellata* and *Momordica charantia* contained alkaloids (Ali *et al.*, 2019; Abiola and Sunday, 2021). The MHF formulation had no saponins. The results were not consistent with findings from other studies that identified saponins in *Momordica charantia* and *Hunteria umbellata* had saponins (Ali *et al.*, 2019, Abiola and Sunday, 2021). The other identified secondary metabolites in the extract were previously reported in *Momordica charantia* and *Hunteria umbellata* (Ali *et al.*, 2019, Abiola and Sunday, 2021, Salisu *et al.*, 2024). The formulation also had triterpenoids as reported in literature (Shodehinde *et al.*, 2016, Ali *et al.*, 2019, Niwoye *et al.*, 2019, Abiola and Sunday, 2021, Salisu *et al.*, 2024). The total flavonoid content of MHF was higher than that of *Momordica charantia* leaf ethanol extract (Shodehinde *et al.*, 2016) and *Hunteria umbellata* leaf ethanol extract (Niwoye *et al.*, 2019), indicating additive effect. The high DPPH radical inhibition of MHF can be attributed majorly to *Momordica charantia* (Pham *et al.*, 2019). The TAC of MHF was higher than that reported for *Momordica charantia* leaf extract (Shodehinde *et al.*, 2016). This suggests that *Hunteria umbellata* may have had a synergistic activity on the activity of the MHF. There seem to be antagonistic effect on the FRAP activity of MHF, since its activity was lower than the values reported for *Momordica charantia* extract (Amira *et al.*, 2013). The significant alpha-amylase and alpha-glucosidase inhibitory activity of MHF compared to Acarbose ($P < 0.05$), and higher than that reported for *Momordica charantia* alone (Poovitha and Parani, 2016) suggests that the combination of both plants may have a synergistic activity in diabetes control and may have an advantage in the management of the disorder

Conclusion

The results revealed that the combination of *Momordica charantia* and *Hunteria umbellata* (MHF) had enhanced alpha-amylase and alpha-glucosidase activity which can be explored in the management of diabetes. This study validates the ethnobotanical use of this formulation in managing diabetes.

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