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Research Article

In Vitro Evaluation of Alpha-Amylase Inhibition and Anti-Glycation Activities of *Aloe barbadensis Miller* Aqueous Extract

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ABSTRACT

Postprandial hyperglycemia is largely driven by the rapid enzymatic breakdown of carbohydrates by alpha-amylase, while non-enzymatic protein glycation leads to advanced glycation end products (AGEs) that accelerate diabetic complications. This study investigated the in vitro antidiabetic potential of *Aloe barbadensis* Miller aqueous extract. The extract was tested for its ability to inhibit alpha-amylase and non-enzymatic haemoglobin glycation. Alpha-amylase inhibition was measured at 0.02-0.08 mg/mL using metformin as the standard, while anti-glycation activity was assessed over the same concentration range using gallic acid as a positive control. Absorbance was measured spectrophotometrically, and percentage inhibition and IC_{50} values were determined. The extract inhibited alpha-amylase in a concentration-dependent manner, showing 75.53% inhibition at 0.02 mg/mL and 53.67% at 0.08 mg/mL, with an IC_{50} of 36.59 mg/mL. Metformin produced 87.18% inhibition at 0.02 mg/mL with an IC_{50} of 0.10 mg/mL. The extract also reduced haemoglobin glycation, producing 51.77% inhibition at 0.02 mg/mL and 41.77% at 0.08 mg/mL, with an IC_{50} of 0.033 mg/mL, compared to gallic acid's 36.35% inhibition and 0.0035 mg/mL IC_{50}. *Aloe barbadensis* aqueous extract exhibited moderate alpha-amylase and anti-glycation activities, supporting its potential as a natural antidiabetic agent. Further studies are needed to isolate its bioactive compounds and confirm their efficacy *in vivo*.

Keywords: *Aloe barbadensis*; Alpha-amylase; Diabetes mellitus; Haemoglobin glycation; IC_{50}; Phytotherapy

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder affecting millions globally and is associated with serious complications including neuropathy, nephropathy, and retinopathy. Current antidiabetic drugs are often costly, have side effects, and may become less effective over time, thus necessitating the search for safer, plant-based alternatives (American Diabetes Association, 2023). One therapeutic strategy involves the inhibition of carbohydrate-digesting enzymes such as alpha-amylase to reduce postprandial hyperglycaemia (Tundis *et al*., 2010). Additionally, the non-enzymatic glycation of haemoglobin contributes to diabetic complications, and its inhibition is considered beneficial in diabetic management (Brownlee, 2001).

Aloe barbadensis Miller (commonly known as Aloe vera) is a succulent plant traditionally used in folk medicine for its anti-inflammatory, antimicrobial and wound-healing properties. Recent studies suggest it may also possess hypoglycaemic effects (Yagi & Takeo, 2003; Hamman, 2008). The aqueous extract of *Aloe barbadensis* Miller is typically obtained from the inner gel of the leaves by homogenization and filtration in water. This extract is known to contain a complex mixture of biologically active constituents including polysaccharides, notably acemannan and glucomannans, phenolic compounds such as aloin and aloe-emodin, vitamins A, C, E, B_{12}, and folic acid, enzymes, minerals, and amino acids (Choudhary *et al*., 2014; Yagi & Takeo, 2003). The high polysaccharide

content contributes to its immunomodulatory, antioxidant and anti-inflammatory properties, while the phenolic compounds are responsible for its antimicrobial, laxative, and possible antidiabetic activities (Boudreau & Beland, 2006).

Several pharmacological studies have highlighted the antidiabetic potential of *Aloe barbadensis* aqueous extracts. The plant has been reported to lower blood glucose levels, improve glucose tolerance, and enhance insulin sensitivity in experimental animal models (Can *et al*., 2015; Rajasekaran *et al*., 2006). These effects have been attributed to its ability to modulate carbohydrate metabolism, improve pancreatic β-cell function, and enhance peripheral glucose uptake.

Despite its long history of use, the specific mechanisms by which *Aloe barbadensis* aqueous extract exerts its antidiabetic actions such as inhibition of carbohydrate hydrolyzing enzymes like alpha-amylase and prevention of non-enzymatic glycation are still being explored.

Therefore, this study investigated the in vitro alpha-amylase inhibitory and anti-glycation activities of *Aloe barbadensis* Miller aqueous extract to evaluate its possible application as a natural antidiabetic agent.

Hence, the study determined the inhibitory effect of aqueous extract of *barbadensis miller* leave extract on alpha amylase activity and haemoglobin glycation.

MATERIAL AND METHODS

Reagents

All reagents used for the experiment are of standard analytical grade. Gallic acid and metformin are the standard drugs used for the experiment.

1% w/v starch, 2% w/v glucose, 3-5 dinitrosalicylic acid reagent, 1% w/v α-amylase in sodium phosphate buffer, pH 6.9, 0.06% w/v haemoglobin solution added to 0.02% w/v gentamycin.

Sample Collection and Preparation of Plant Extract

Barbadensis miller (*Aloe vera)* plant was collected from botanical garden in Kaduna Polytechnic, and it was identified by a Botanist in the Department of Applied Biology, Kaduna Polytechnic, Kaduna. The Gel was squeezed out of the leaves and then the leaves sample was washed and dried at room temperature and was grinded into powdered form using mortar and pestle. 50g of the powdered leaf was weighed and poured into extraction bottle; 500ml of distilled water was added. The mixture was kept for 12 hours with constant agitation at 30minutes intervals. The extract was filtered using funnel and filter paper, the filtrate was concentrated in a vacuum space. The semi-solid extract obtained was kept in a refrigerator for further use.

Alpha Amylase Inhibition Assay

Alpha amylase inhibition assay was used with some modifications. Different concentrations of the sample i.e. 0.02mg/ml, 0.04mg/ml, 0.06mg/ml and 0.08mg/ml were prepared. Different concentrations of the standard drug i.e. metformin was also prepared as above. To 1ml of α-amylase added to 1% w/v in sodium phosphate buffer pH 6.9, 1ml each of different concentrations of the sample solution (1mg/ml) was added and incubated at 37^{o}c for 3 to 15 minutes, and then 1ml of 1% starch solution (1% w/v in sodium phosphate buffer pH 6.9) was added into the mixture and incubated for 15minutes. Then 1ml of 3,5-dinitrosalicylic acid colour reagent was added and placed in a thermoregulatory water bath at 85^{o}c for 5 to 10 minutes, then it was cooled at room temperature and the absorbance was taken at 540nm using UV/visible spectrophotometer. The same procedure was carried out for different concentrations of the standard drug i.e. metformin and the test were carried out in triplicate. Absorbance of the control is the absorbance of all the reagents without the sample or standard drug. Percentage inhibition was calculated using this formula.

% inhibition = (absorbance of control-absorbance of sample)/ (absorbance of control) ×100

Non-Enzymatic Glycation of Haemoglobin Assay

Non-enzymatic glycosylation of haemoglobin assay was determined with slight modification. To 1ml of 0.06% haemoglobin, 5ul of 0.02% gentimycin was added, 1ml each of different concentrations of the extracts was added and 1ml of 0.2% glucose solution was added and then was mix and incubated for 72 hours at 37^{0}C in a dark environment. The degree of glycosylation was measured at 443nm using UV/Visible spectrophotometer after the incubation period (72 hours). The same procedure was carried out for different concentrations of the standard drug i.e. Gallic Acid and the tests were carried out in triplicate. Absorbance of the control is the absorbance of all the reagents without the sample or standard drug. The test was carried out in triplicate. Percentage (%) inhibition was determined using this formula.

% inhibition = $\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ Control} \times 100$

RESULTS

Alpha-Amylase Inhibitory Assay

The aqueous extract of *Aloe barbadensis* exhibited a concentration-dependent inhibition of alpha-amylase activity. At 0.02 mg/mL, the extract showed 75.53% inhibition compared to metformin's 87.18%. The inhibitory effect decreased as concentration increased,

with 53.67% inhibition at 0.08 mg/mL. IC_{50} values: *Aloe barbadensis* = 36.59 mg/mL; Metformin = 0.10 mg/mL.

Non-Enzymatic Hemoglobin Glycation

The extract also inhibited hemoglobin glycation in a concentration-dependent manner. Maximum inhibition (51.77%) was recorded at 0.02 mg/mL compared to gallic acid (36.35%). Inhibition declined to 41.77% at 0.08 mg/mL. IC_{50} values: *Aloe barbadensis* = 0.033 mg/mL; Gallic acid = 0.0035 mg/Ml

Table 1. Percentage Inhibition of the Extracts at Different Concentration on Alpha Amylase

	Concentration	Absorbance	% Inhibition
Sample	0.02	0.519±0.023	75.53
	0.04	0.772±0.018	67.02
	0.06	0.817±0.002	67.33
	0.08	1.212±0.0065	53.67
Metformin	0.02	0.272±0.024	87.18
	0.04	0.661±0.033	71.76
	0.06	0.818±0.023	67.29
	0.08	1.082±0.141	60.42

Values are expressed as mean ±SEM. Values are significant at P<0.5 compared to the positive control (Metformin)

Table 2. IC_{50} Value of *Aloe barbadensis miller* aqueous extract inhibitory effect on Alpha amylase

SAMPLE	IC_{50} Value (mg/ml)
Metformin	0.10
Aloe barbadensis miller	36.59

Values are expressed as mean ± SEM. Values are significant at $p < 0.05$ compared to the positive control (*Metformin).*

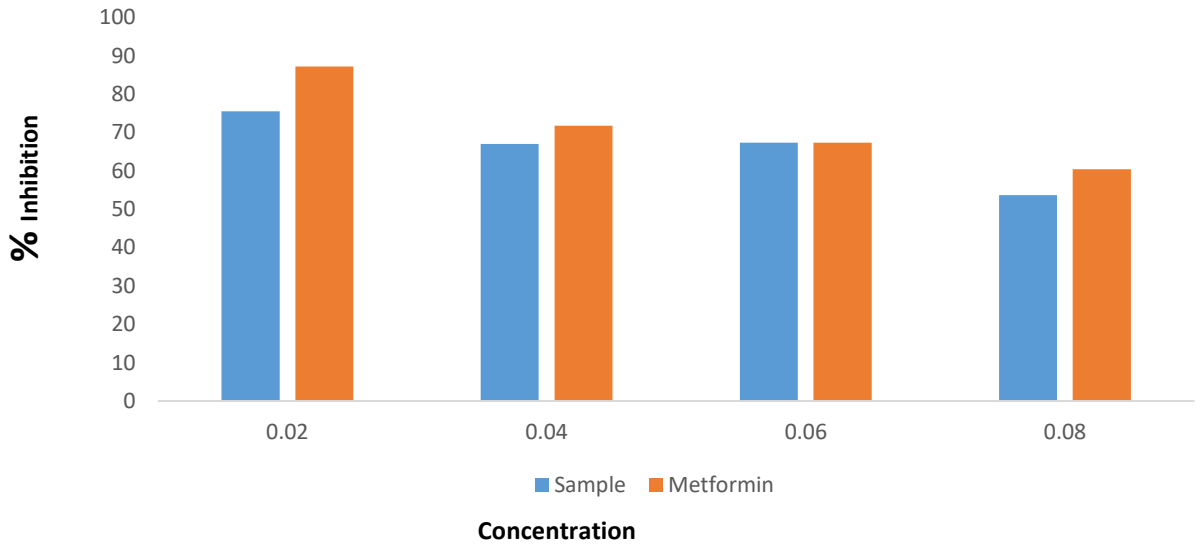

Figure 1. Variation in the % inhibition of the sample and the standard drug at different concentrations on α-amylase activity

Table 3. Percentage Inhibition of the Extracts at Different Concentration on Haemoglobin Glycation

	Concentration	Absorbance	% Inhibition
Gallic Acid	0.02	1.350±0.072	36.35
	0.04	1.525±0.048	34.86
	0.06	2.153±0.047	13.91
	0.08	2.738±0.262	3.39
Sample	0.02	1.023±0.042	51.77
	0.04	1.212±0.037	48.22
	0.06	1.308±0.032	47.70
	0.08	1.592±0.285	41.77

Values are expressed as mean ±SEM. Values are significant at P<0.5 compared to the positive control (Gallic Acid)

Table 4. IC_{50} Value of *Barbadensis miller* aqueous extract inhibitory effect on Non-enzymatic Haemoglobin Glycation

SAMPLE	IC_{50} Value (mg/ml)
Gallic acid	0.0035
Aloe barbadensis miller	0.033

Values are expressed as mean ± SEM. Values are significant at p<0.05 compared to the positive control (Gallic acid)

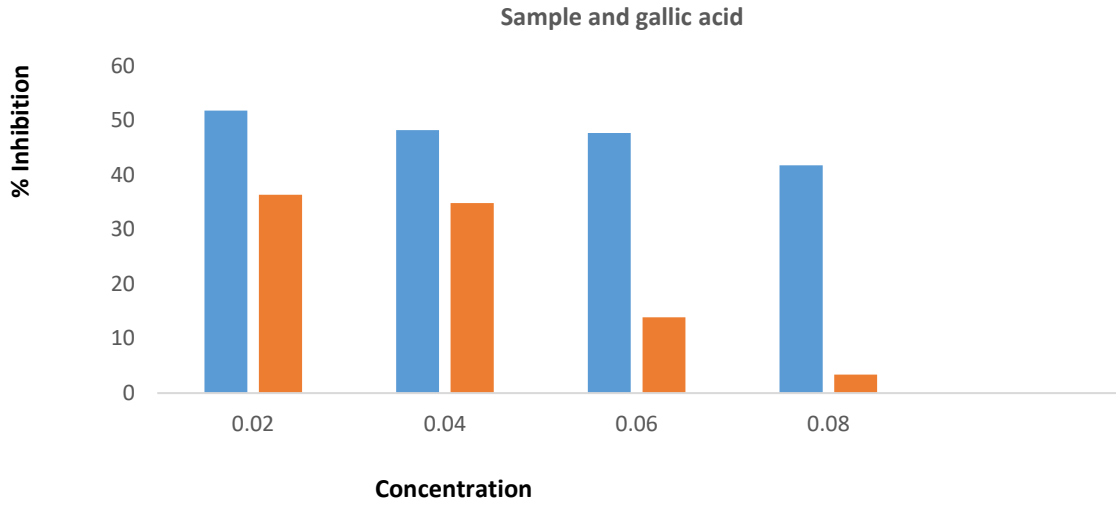

Figure 2. Variation in the % inhibition of the sample and the standard drug at different concentrations on haemoglobin glycation

DISCUSSION

The present study demonstrates that the aqueous extract of *Aloe barbadensis* Miller possesses moderate in vitro inhibitory activity against both alpha-amylase and non-enzymatic hemoglobin glycation, two key pathways in the management of diabetes mellitus and its complications.

The observed concentration-dependent inhibition of alpha-amylase suggests that constituents within the *Aloe vera* extract can interact with this enzyme, potentially slowing the breakdown of complex carbohydrates into absorbable sugars and thereby reducing postprandial hyperglycemia. However, the inhibitory potency (IC_{50} = 36.59 mg/mL) was significantly lower than that of the standard drug metformin (IC_{50} = 0.10 mg/mL). This considerable difference in efficacy is expected, as metformin is a potent synthetic drug, while plant extracts are complex mixtures. Our findings are consistent with other studies on medicinal plants. For instance, an aqueous extract of *Morus alba* (white mulberry) leaves, a well-known antidiabetic plant, was reported to have an IC_{50} of 12.93 µg/mL for alpha-amylase inhibition, which is considerably more potent than the *Aloe* extract found here (Memon *et al*., 2010).

Similarly, a methanolic extract of *Ocimum basilicum* (sweet basil) exhibited an IC_{50} of 0.41 mg/mL (Tadhani *et al*., 2007). The relatively high IC_{50} value for *Aloe* suggests that while it has inhibitory activity, it may not be potent enough as a stand-alone therapy but could be valuable as part of a polyherbal formulation or complementary approach.

More notably, the extract demonstrated promising anti-glycation activity, inhibiting the formation of advanced glycation end-products (AGEs) with an IC_{50} of 0.033 mg/mL, which is only an order of magnitude less potent than the standard gallic acid (IC_{50} = 0.0035 mg/mL). The formation of AGEs is a critical pathological event in diabetes, contributing to long-term complications like nephropathy, retinopathy, and neuropathy (Brownlee, 2001). The anti-glycation activity of *Aloe vera* is likely attributable to its rich content of antioxidant compounds, such as phenolic constituents (e.g., aloin, aloe-emodin) and polysaccharides (acemannan), which can scavenge free radicals and trap reactive carbonyl species involved in the glycation process (Yagi & Takeo, 2003; Habeeb *et al*., 2007). This aligns with recent work by Pothuraju *et al*. (2023), who highlighted the anti-glycation and antioxidant potential of various herbal extracts in managing diabetic complications. Furthermore, a study by Pandey and Mishra (2020) on *Aloe vera* gel extract confirmed its significant ability to inhibit AGE formation in a bovine serum albumin (BSA)-glucose model, attributing the effect to its high flavonoid and phenolic acid content.

The divergence in efficacy between the two assays—moderate anti-glycation but weaker alpha-amylase inhibition—underscores the multi-targeted nature of plant extracts. It suggests that the primary antidiabetic benefit of *Aloe barbadensis* may lie more in mitigating the oxidative stress and downstream complications of diabetes rather than in powerfully suppressing carbohydrate digestion. This supports traditional uses of *Aloe vera* for wound healing and anti-inflammatory purposes, which are processes closely linked to oxidative stress and glycation.

CONCLUSION

This study demonstrated that *Aloe barbadensis* Miller aqueous extract possesses moderate alpha-amylase inhibitory and anti-glycation activities in vitro. These results suggest its potential as a complementary therapy for managing diabetes and its complications. Future studies should focus on isolating active phytoconstituents, determining their mechanisms of action, and validating their antidiabetic effects in in vivo models and clinical trials

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